Effect of High Intensity Ultrasound on Crystallization Behavior and Functional Properties of Lipids

Yubin ye

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EFFECT OF HIGH INTENSITY ULTRASOUND ON CRYSTALLIZATION BEHAVIOR AND FUNCTIONAL PROPERTIES OF LIPIDS

by

Yubin Ye

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

2015
ABSTRACT

Effect of High Intensity Ultrasound on Crystallization Behavior and Functional Properties of Lipids

by

Yubin Ye, Doctor of Philosophy
Utah State University, 2015

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

The effects of high intensity ultrasound (HIU) on the crystallization behavior and functional properties of shortenings were evaluated. HIU was applied to different shortenings such as interesterified soybean oil (IESBO), multi-purpose commercial shortening, palm oil, and palm stearin. The functional properties measured include crystal morphology, solid fat content, melting profile, viscoelastic properties, hardness, and polymorphism. Different experimental set-ups were evaluated including a static batch system, a temperature cycling design, and flow cell system. Results showed that HIU generated harder material on IESBO, commercial shortening, and palm oil with more uniform and smaller crystal size, sharper melting profile, and higher elasticity. No chemical changes on triacylglycerol (TAG) and fatty acids were observed on IESBO under the sonication conditions used in this dissertation. Application of HIU maintained the texture of the commercial shortening that was subjected to temperature fluctuations, especially when HIU was applied before changes in temperature occurred. When
sonication was applied in a flow-cell system lower power levels applied in a continuous manner was proved to be the most effective at inducing crystallization of palm oil. Research also showed that pulse irradiation of sonication and higher flow rates could be used to decrease the thermal effects generated by higher power levels of HIU. In addition, HIU was used in a highly saturated fat (palm stearin) at low power levels with long durations to delay lipid crystallization and generate a softer material. All the research findings suggest the great potential use of HIU in shortening production and food processing to improve the texture and its stability, as well as other functional properties.
Effect of High Intensity Ultrasound on Crystallization Behavior and Functional Properties of Lipids

Yubin Ye

The elimination of *trans*-fatty acids from food formulations has resulted in the search of new lipids and novel processing conditions that can provide optimum functional properties in fats while providing good nutritional properties. High intensity ultrasound (HIU) has been extensively studied on sonocrystallization of lipids since early 2000. Sonocrystallization refers to the induction of crystallization by HIU without generating any chemical changes. This dissertation aims to provide information on the effects of HIU on crystallization behavior and physical properties of different shortenings based on previous sonocrystallization research. Results in this dissertation showed that HIU successfully induced the crystallization of shortenings and generated harder and more elastic materials with smaller crystals and promoted the formation of a stable polymorphic form. The long-term goal of this dissertation is to provide a novel and additional processing tool to the food industry that can be used in combination with other processing techniques to improve the functional properties of shortenings while maintaining the nutritional value of edible lipids (i.e., replacing or elimination of *trans*-fatty acids).
This dissertation is dedicated to my beloved grandparents

MING CAI       AND      JINCUI ZHANG
ACKNOWLEDGMENTS

First and foremost I would like to thank my advisor, Dr. Silvana Martini. I appreciate all her contributions of time, thoughts, and funding to make my Ph.D. experience productive and inspiring. I’d also like to thank the other members of my committee, Dr. Karin Allen, Dr. Marie Walsh, Dr. Bob Ward, and Dr. David Britt, for their professionalism, insights, and support. Assistances from Dr. Megan Tippetts, Mr. Chin Yiap Tan, Mr. Sarbojeet Jana, Ms. Haoyuan Zhong, Ms. Yinyin Heng, and Ms. Jiwon Lee are sincerely appreciated.

I’d like to thank my parents, and my parents-in-law for all their love and encouragement. Most of all, my loving and supportive wife, Dr. Shanying Gui, and our beloved coming daughter Gloria are so appreciated. Thank you.

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<tr>
<td>AOCS</td>
<td>American oil chemists’ society</td>
</tr>
<tr>
<td>ADM</td>
<td>Archer Daniels Midland</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>CHD</td>
<td>Coronary heart disease</td>
</tr>
<tr>
<td>DTG</td>
<td>Differential thermogravimetric analysis</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
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<tr>
<td>FAME</td>
<td>Fatty acid methyl esters</td>
</tr>
<tr>
<td>G'</td>
<td>Storage modulus</td>
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<tr>
<td>G''</td>
<td>Loss modulus</td>
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<tr>
<td>HIU</td>
<td>High intensity ultrasound</td>
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<tr>
<td>ΔH</td>
<td>Enthalpy variation</td>
</tr>
<tr>
<td>HDL</td>
<td>High density lipoprotein</td>
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<tr>
<td>IESBO</td>
<td>Interesterified soybean oil</td>
</tr>
<tr>
<td>k</td>
<td>Avrami constant</td>
</tr>
<tr>
<td>n</td>
<td>Avrami exponent</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>PLM</td>
<td>Polarized light microscopy</td>
</tr>
<tr>
<td>PHO</td>
<td>Partially hydrogenated oil</td>
</tr>
<tr>
<td>SFC</td>
<td>Solid fat content</td>
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<tr>
<td>SFC&lt;sub&gt;∞&lt;/sub&gt;</td>
<td>Equilibrium solid fat content</td>
</tr>
<tr>
<td>T&lt;sub&gt;c&lt;/sub&gt;</td>
<td>Crystallization temperature</td>
</tr>
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<td>T&lt;sub&gt;p&lt;/sub&gt;</td>
<td>Peak temperature</td>
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<td>T&lt;sub&gt;on&lt;/sub&gt;</td>
<td>Onset temperature</td>
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<td>TAG</td>
<td>Triacylglycerol diffraction</td>
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<td>XRD</td>
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**Additional Terms:**
- ADM: Archer Daniels Midland
- ANOVA: Analysis of Variance
- CHD: Coronary Heart Disease
- DTG: Differential Thermogravimetric Analysis
- DSC: Differential Scanning Calorimetry
- FAME: Fatty Acid Methyl Esters
- G': Storage Modulus
- G'': Loss Modulus
- HIU: High Intensity Ultrasound
- ΔH: Enthalpy Variation
- HDL: High Density Lipoprotein
- IESBO: Interesterified Soybean Oil
- k: Avrami Constant
- n: Avrami Exponent
CHAPTER 1
INTRODUCTION

Lipids are major constituents of foods and play a vital role in providing nutritional and functional properties to foods. Lipids usually refer to fats and oils, with the definition of fats as materials that are solid at ambient temperature and oils as the ones that are liquid at ambient temperature.\(^1\) Shortenings act to lubricate, weaken, or shorten the structure of food components to provide desirable textural properties to food products, they are the tailored fat systems with operated nutritional and functional properties.\(^2\) Shortenings are important in food processing and production since they provide different consumer demands such as mouthfeel, texture, flavor, and melting behavior.\(^3\)

Animal fats (lard) were the first lipids used as shortenings in food production in nineteenth century.\(^4,5\) However, the use of animal fats in food products has been limited due to nutritional concerns related to their high content of saturated fatty acid and cholesterol and high costs associated with their production.\(^3\) Gradually the North American food industry has reduced or replaced the use of animal fat with other sources of lipids, such as vegetable oils. However, due to the liquid characteristics at room temperature, vegetable oils lack texture and plasticity to provide desirable physical properties in the final food product. To overcome this limitation, different processing technologies have been developed and used, including hydrogenation, fractionation, interesterification, and blending of different oils or oil fractions.\(^3\) Partial hydrogenation was one of the important processing techniques used in the food industry since the first hydrogenated shortening “Crisco” was introduced in 1911\(^6\) to improve the functional
properties of vegetable oils. However, research has shown a strong correlation between
trans-isomers generated by the hydrogenation process with incidence of coronary heart
disease.\textsuperscript{7-13} Food producers have been challenged to find replacements for trans-fats that
have optimal functional properties and improved nutritional profiles. Current trans-fat
solutions include the use of tropical oils (such as palm oil or palm based oil), blends of
fully hydrogenated oils with unsaturated oils, interesterification, and fractionation.\textsuperscript{14-16}

Researchers are still dedicated to developing new techniques and methods to additionally
improve the functionalities of vegetable oils to improve food quality. Food producers are
seeking novel lipid sources low in saturated fatty acids and free of trans-fats and/or new
processing techniques that can generate appropriate functional properties, such as texture,
melting and solidification, crystal network formation, and viscoelasticity. There are close
links between functional properties described previously and the crystallization properties
such as crystal structure (polymorphism), shape and rate of crystallization in the crystal
containing systems. It is thus important to control crystallization to achieve desirable
functional properties. Considerable interest has been shown in the early 21\textsuperscript{st} century in
the use of high intensity ultrasound on the crystallization kinetics of edible lipids.\textsuperscript{17-19} It
has been demonstrated that HIU can play a key role in controlling the nucleation and
crystallization of lipid systems. As crystallization properties are directly related to the
functional properties of lipids, it is necessary to evaluate the use and control of HIU on
edible oils and its effect on changes in functional properties.

High intensity ultrasound or power ultrasound, are invasive techniques that use
acoustic waves operating at low frequency (20 to 100 kHz) and high power (10 to 10,000
W cm\textsuperscript{-2}) levels to purposely change the properties of materials. HIU has been extensively
used in several applications such as to induce crystallization of organic and inorganic molecules (sonocrystallization),\textsuperscript{20, 21} to induce chemical reactions (sonochemistry),\textsuperscript{22, 23} and to disrupt cells.\textsuperscript{24, 25} In the food science area, many studies show the significant effect of high intensity ultrasound on the crystallization behavior of food components. Chow \textit{et al.} used HIU to modify the primary and secondary nucleation of ice and sucrose;\textsuperscript{26, 27} Patel \textit{et al.} showed that HIU can control the crystallization process of lactose during the nucleation phase.\textsuperscript{28} Early studies in lipid systems were performed by Sato and coworkers using HIU in cocoa butter and pure triacylglycerols\textsuperscript{17, 18} and showed that HIU induces lipid crystallization as evidenced by shorter induction times when the samples were crystallized in the presence of ultrasound. These studies were followed by Patrick \textit{et al.} who evaluated the effect of ultrasonic intensity on the palm oil crystal structures.\textsuperscript{19} Previous work in our laboratory\textsuperscript{29, 30} shows that HIU can be used as a novel processing tool to modify the functional properties of anhydrous milk fat, palm kernel oil and all-purpose shortening.

Even though there is a substantial body of information regarding the effect of HIU’s on the crystallization of different molecules, most of the research has been in aqueous systems\textsuperscript{26-28} and very little research has been performed on the effect of HIU on the crystallization kinetics, polymorphism, and rheological behavior of lipids, particularly of commercial shortenings. There is a strong scientific and commercial need to fill this knowledge gap and explore new applications of HIU on the processing of lipids. In addition, HIU is not a standardized technology yet and therefore needs to be developed and scaled up for every new application.\textsuperscript{31} Many important questions remain to be answered. For instance, it is still unknown the complex physicochemical mechanism of
HIU and the optimum sonication conditions such as power level (intensity), duration, frequency and sonication tips (including size and shape) needed to achieve a specific results. The interaction between HIU and other processing conditions such as temperature and cooling rate must be also evaluated. Currently, none of these aspects are clearly understood. Thus, the purpose of this dissertation was to optimize the process of ultrasound application in different shortening systems, and to evaluate the effects of ultrasound on the physical and functional properties of these shortenings.

**Hypothesis**

High intensity ultrasound can be used as an advanced and innovative technology to change the crystallization behavior of shortenings and modify their functional properties.

**Objectives**

Evaluate the effect of HIU on the crystallization behavior and functional properties of shortenings and understand the physicochemical mechanisms responsible for this behavior.

1. Modify the functional properties of interesterified soybean oil (IESBO) by optimizing the use of HIU to change the crystallization behavior
   a. Optimize HIU application conditions: power levels and application time for IESBO crystallized at different temperatures
   b. Evaluate the functional properties of IESBO crystalline network formed: crystal morphology, crystal size, thermal behavior, polymorphism, and texture.
2. Evaluate chemical changes in sonicated IESBO quantified as changes in triacylglycerol and fatty acids content for IESBO samples before and after sonication.

3. Determine the effect of HIU on the crystallization behavior and functionalities of a palm oil based shortening during temperature cycling.
   a. Optimization of HIU application conditions: tip size and irradiation duration at a constant temperature.
   b. Evaluation of the functional properties for the shortening after temperature cycling at two cooling rates.

4. Evaluate the effects of HIU on crystallization behavior and functional properties of palm oil crystallized using a flow cell.
   a. Determine the crystallization rate of palm oil under different power levels and irradiation types (continuous vs. pulse) in the flow cell system.
   b. Evaluate the functional properties of palm oil: crystal size, polymorphism, thermal behavior, and elasticity.

5. Evaluate the effect of HIU on the crystallization behavior of high saturated fats.

**Rationale and significance**

Shortenings must possess nutritional properties with low content of saturated fats and be free of *trans*-fatty acids but at the same time provide appropriate functional properties including texture, mouthfeel, and flavor to foods. Current methods for producing shortenings include the use of tropical oils, interesterification, blending of different oils or oil fractions, and fractionation. Food scientists are continuously seeking new lipid sources and novel processing methods to improve the nutritional and functional
properties of shortenings. The research presented in this dissertation evaluated the use of HIU to modify the functional properties of lipids by changing their crystallization behavior. Results from these studies will provide scientific support of the market accessibility of HIU technique in the edible oil processing. This dissertation provides evidence of the effect that processing conditions have in changing molecular organization in the lipid network that will ultimately result in different functional properties of the material. The long term goal of this dissertation is to provide a novel and additional processing tool to the food industry that can be used in combination with other processing techniques to improve the functional properties of shortenings while maintaining the nutritional value of edible lipids.

References


CHAPTER 2
LITERATURE REVIEW

Introduction

This literature review will commence with an overview of semi-solid lipids (shortenings) commonly used in the food industry and with a description of changes experienced by this industry in response to people’s health concerns. The term “trans-fat” will be specifically illustrated by its important role in the development of processing methods. Next, high intensity ultrasound or power ultrasound will be discussed and introduced as a new processing technology to the change of crystallization behavior and functional properties of fats. Other applications of HIU in food systems will be also discussed. Crystallization of fats will be discussed in detail including the basic principles of crystallization behavior (i.e., primary and secondary nucleation, crystal growth) and how these events affect the functional properties of lipid materials. Lasting, a more focused look at different functional properties (i.e., crystal morphology, polymorphism, thermal behavior and elasticity) will be presented; the methods of analysis for measuring parameters related to functional properties will also be addressed.

Shortenings

Shortenings are commercially prepared edible fats used in frying, cooking, baking, and as an ingredient in fillings, icings, and other confectionery items.¹ Their name originates from their original use in bakery products. The term “shortening” refers to the ability of a fat to lubricate, weaken, or shorten the structure of food components (i.e., gluten and starch particles) so that they function in a characteristic way to provide
desirable textural properties to a food product.\textsuperscript{2,3} There are a number of categories of shortening which are determined by the functional requirements or the end use of the product: All-purpose shortening, fluid shortening, icing shortening, pie crust shortening,\textsuperscript{4} cake shortening,\textsuperscript{5} and confectionary fats.\textsuperscript{6} Being one of the most flexible (due to its broad functionalities) basic food ingredients, the use of shortenings is expected to continue growing. As reported by the Institute of Shortening and Edible Oils, there are 22,202 million pounds of shortenings shipped in US in 2004/05.\textsuperscript{7,8}

Shortenings are tailored fat systems whose nutritional and functional properties have been manipulated to provide desirable consistency and ensure high quality end products.\textsuperscript{2} Different types of shortenings in different product applications are listed below in Table 2-1. For example, in baking, shortenings contribute to the quality of the finished product by imparting a creamy texture and rich flavor, tenderness, uniform aeration, moisture retention and size expansion.\textsuperscript{1,4} Three main factors are responsible for delivering the quality properties mentioned above: (a) ratio of solid/liquid phase; (b) plasticity; and (c) oxidative stability.\textsuperscript{2} In addition to these main factors, other properties of the crystalline network formed during shortening production can affect their functional properties.\textsuperscript{9} Some of these characteristics include the size and the shape of the crystal aggregates, distribution of size, and the crystallization kinetics.\textsuperscript{10} Early in 1965, Haighton indicated that the permanent tridimensional fat crystal networks contribute to 60 – 80 \% of the total hardness.\textsuperscript{11} Narine and Marangoni summarized and studied the elastic properties of fat crystal network suggesting and these properties depend integrally on the nature of the microstructure.\textsuperscript{12} Herrera \textit{et al.} investigated the effect of processing conditions on the physical properties of milk fat system, and found larger crystal size and
structures had a more elastic behavior with higher elastic modulus $E'$.\textsuperscript{13} In addition, polymorphism is another essential factor that affects the specific properties of the crystallization of fats and lipids.\textsuperscript{14}

Table 2-1: Different types of shortenings and their product applications based on the physical properties\textsuperscript{2}

<table>
<thead>
<tr>
<th>Shortening types</th>
<th>Physical characteristics</th>
<th>Product application</th>
</tr>
</thead>
<tbody>
<tr>
<td>All-purpose shortening</td>
<td>Creamy, IV 65~80, add emulsifier</td>
<td>Icing, cakes</td>
</tr>
<tr>
<td>Fluid shortening</td>
<td>Liquid, low SFC at room temperature, add emulsifier for preventing crystallization</td>
<td>Bakery</td>
</tr>
<tr>
<td>Cake shortening</td>
<td>Beta prime crystals</td>
<td>Cakes, bakery</td>
</tr>
<tr>
<td>Icing shortening</td>
<td>Beta prime crystals, high melting high SFC</td>
<td>Icing</td>
</tr>
<tr>
<td>Filler fat shortening</td>
<td>Steep SFC profile, stable crystal network without oiling out</td>
<td>Confectionary, snack</td>
</tr>
<tr>
<td>Bread shortening</td>
<td>Wide plastic range at room temperature</td>
<td>Bread, pizza</td>
</tr>
<tr>
<td>Frying shortening</td>
<td>High oxidation stability, proper SFC at room temperature</td>
<td>Chips, coated doughnuts</td>
</tr>
<tr>
<td>Pie crust shortening</td>
<td>Provide lubricity and tenderness, grainy crystal structure</td>
<td>Pie crust</td>
</tr>
<tr>
<td>Confectioner’s shortening</td>
<td>Short plastic range</td>
<td>Chocolate, coated snack</td>
</tr>
</tbody>
</table>

The crystallization behavior of lipids such as crystallization rate, crystal sizes and their network, and crystal morphology are directly influenced by polymorphism. For example, the $\beta'$ polymorph is usually the most functional in fat products, due to its small crystal size and thin, needle shaped morphology. Thus, fat products with other polymorphs other than $\beta'$ might generate different macroscopic elastic constant and
hardness of the fat network.\textsuperscript{15, 16} Therefore, when characterizing physical and functional properties of shortenings these variables must be carefully evaluated.

**Chronology of shortening**

Attempts to find butter replacements started in Europe during the middle of the nineteenth century due to increasing prices for butter.\textsuperscript{4, 17} French chemist Mege Mouries produced the first butter substitute by using tallow, which was proved to be a good raw material to gain desirable consistency and mouth melting behavior.\textsuperscript{4} Lard (pig fat) was then introduced to use as a primary shortening agent due to the easy processing requirements to obtain a consistent and reproducible product.\textsuperscript{18} In the middle and later of nineteenth century, when the world demand for edible fats increased, special attention was paid to the development of substitutes for lard.\textsuperscript{17} By the late 20\textsuperscript{th} century, the use of animal fats in food products had been limited due to health concerns associated with the high content of saturated fats and cholesterol in animal fats, as well as the high costs associated with lard production.\textsuperscript{8, 19} The development of lard alternatives became an important issue.

At the end of nineteenth century, new techniques for refining fats and oils were developed, which made the use of vegetable oils such as soybean, corn, coconut, palm, and palm kernel oil possible to be used.\textsuperscript{3, 20} Vegetable oils usually have lower content of saturated fats than animal fats and are free of cholesterol. However, due to the liquid characteristics at room temperature, vegetable oils lack of texture and plasticity to provide desirable physical properties in the final food product. It is therefore important to improve the functional properties of vegetable oils by using proper processing conditions. In 1911, the *Procter and Gamble Company* introduced its hydrogenated shortening
“Crisco” on the market, and since then, hydrogenation has been employed to produce shortenings. However, research has shown a strong correlation between trans-isomers generated by the hydrogenation process and incidence of coronary heart disease. Therefore, food producers have been challenged to eliminate or reduce trans-fats from food formulations. Current solutions include the use of tropical oils (such as palm oil or palm-based oil), blending of fully hydrogenated oils with unsaturated oils, interesterification, and fractionation. All these solutions were applied based on the balance of the nutritional quality and functional properties of the shortenings. Even though the food industry has successfully eliminated trans-fats from most of their formulations, there is still a substantial amount of effort to search for alternative lipid sources and/or novel processing conditions to produce healthier oils with appropriate functional properties.

Trans-fat

Overview of trans-fat

Lipids usually referred to fats and oils, with the definition of fats as materials that are solid at ambient temperature and oils as the ones that are liquid at ambient temperature. Triacylglycerols are the main constituents of fats and oils. A triacylglycerol or triglyceride (TAG) is an ester derived from glycerol and three fatty acids (Figure 2-1a). Fatty acids can be either saturated or unsaturated (Figure 2-1b). Saturated fatty acids are saturated with hydrogens and have no double bonds. All
unsaturated fatty acids have at least one carbon-carbon double bond. Two configurations are usually formed: \textit{cis} and \textit{trans} (Figure 2-1b). In the \textit{cis} configuration, the two carbon moieties are on the same side of the double bond and in the \textit{trans} configuration they are on the opposite sides. Fatty acids in the \textit{cis} configuration represent the majority of fatty acids found in nature; however, a small amount of \textit{trans} fatty acids can also be found naturally such as conjugated linoleic acid in dairy products. The main source of \textit{trans} fatty acids is represented by those generated through industrial processes.\textsuperscript{2, 33} In the lipid industry, small amounts of \textit{trans} fatty acids can be generated from the refining of edible oils due to the high temperature used during the deodorization process.\textsuperscript{33} However, the

\textbf{Figure 2-1a}: An example of TAG: left part of glycerol, right part from top to bottom: palmitic acid, oleic acid, linoleic acid.

\textbf{Figure 2-1b}: Two dimensional representation of saturated fatty acid (stearic acid), and the representation of unsaturated fatty acid (oleic acid) in \textit{cis} and \textit{trans} isomerization. (Reuse of this work was granted by Wolfgang Schaefer, the copyright holder of this work)
major source of trans-fats is found in margarine and shortenings production, where partial hydrogenation has been used to convert liquid oils (i.e., vegetable oil) into semi-solid or solid fats for different food applications.\textsuperscript{2,33,34} During hydrogenation, unsaturated fatty acids are transformed into saturated fatty acids by adding hydrogen to the double bonds, while at the same time, trans-fatty acids are generated due to the isomerization of cis configuration.\textsuperscript{2,33}

Many studies have shown strong positive correlations between the intake of trans-fats and the increase risk of coronary heart disease (CHD).\textsuperscript{21-27} Researchers showed that trans-fats lead to an increase in the ratio of total cholesterol to high density lipoprotein (HDL), and a raise in plasma triglyceride concentration.\textsuperscript{35,36} It has also been reported that trans-fatty acids have a negative impact on the level of lipoprotein, increasing the risk of breast and colon cancer, diabetes and obesity, and interfering with n-3 and n-6 fatty acid metabolism.\textsuperscript{36,37} These findings resulted in health concerns by consumers and food producers regarding trans-fat consumption. In the early 2000’s industry and regulatory agencies worked together to minimize trans-fat intake. In 1999, the USA Food and Drug Administration (FDA) proposed a rule on trans-fat labeling, requiring companies to declare the content of trans-fat (if 0.5 g or more per serving) on the Nutrition Facts label of food products in a separate line below saturated fatty acids, and this was finally issued in 2006. Recently in 2013, FDA also issued a Federal Register notice with its preliminary determination that partial hydrogenated oils (PHOs) will be no longer “generally recognized as safe” (GRAS) since PHOs are the major dietary source of artificial trans-fat in processed food.\textsuperscript{38} Industry has responded to these regulations by finding alternative ways to minimize trans-fat content in food products. Lipids with reduced levels of trans-
and saturated fatty acids can be used as alternatives to trans-fats but they lack certain functional properties that are important to ensure food quality. Some of the physical properties that must be improved include texture, melting profile, and amount of solids. Food industries are looking for lipid alternatives that have low contents of saturated fatty acids, that are free of trans-fats and that can provide appropriate functional properties to the shortening. Current process approaches include: the use of natural tropical oils (i.e., coconut, palm kernel oils) and the use of blended oils (blending of fully hydrogenated oils with unsaturated oils), fractionation, and interesterification. In addition, genetic modifications of edible oil seeds were also used to achieve important quality improvements. Food scientists continue to search for new technologies that will improve the nutritional profile of lipids, such as decrease the saturated fatty acid composition and zero trans-fat, while at the same time maintaining functional properties required by the food producer, for example desired texture and oxidation stability.

**Processing methods**

**Hydrogenation**

Vegetable oils used as sources of shortenings are too soft in texture due to the liquid nature, while on the other hand saturated fats are too hard. Therefore, the use of liquid oils and lipids that are high in saturated fats are limited to certain applications. In general, a shortening is a lipid material that can be used for a broad range of products and therefore should have specific functional properties. One of the first processes used to achieve these functional properties in vegetable oils was the use of hydrogenation. Hydrogenation is a chemical process in which hydrogen gas reacts with oils to increase
their oxidative and thermal stability by converting liquid components to semisolid fractions (Figure 2-2). The melting and crystalline characteristics developed are essential for formulating shortenings with specific desirable physical and functional properties.1. Hydrogenation can be divided by the degree of saturation level: full hydrogenation and partial hydrogenation. Fully hydrogenated oils are obtained when all the double bonds are saturated; otherwise the oil is referred to as partially hydrogenated oil.4, 18 Thus, hydrogenation can therefore be defined as a process which imparts oxidative stability to oils and increase the shelf life. Hydrogenation is the most widely used and practical method of modifying fats and oils, since the reaction conditions can be changed to tailor the functional properties of the shortening to specific applications. Reaction conditions that affect the hydrogenation process and consequently the final product, are the temperature of the oil mixture, hydrogen gas pressure, catalyst activity, catalyst concentration, agitation of the mixture, and the duration of the reaction.3, 4, 18

Figure 2-2: An example of hydrogenation reaction: the addition of hydrogen to TAG LLO to form TAG SOO. (Permission to copy and distribute this work was granted by the copyright holder, see appendix)
Depending on the conditions applied during this process, hydrogenation can be selective or non-selective. Selective hydrogenation favors the acids containing active methylene groups while non-selective hydrogenation does not have a preferred target. Normally hydrogenation is done under less selective conditions. Under non-selective hydrogenation, lower temperatures and higher hydrogen pressures are applied in the presence of nickel as a catalyst. Hydrogenation is currently used to modify and stabilize all types of vegetable oils. As described in the previous section, trans-fat can be generated by the process of hydrogenation, especially during partial hydrogenation. Food producers face big challenges to use hydrogenation process as consumers’ health concerns increase and as new requirements regarding trans-fats use are developed by regulatory agencies.

**Blending**

Blending of different fats and oils is another way to produce a vegetable shortening with specific functional properties. Depending on the application of the shortening in the food system, vegetable shortenings with certain functional properties, usually indicated with solid fat content (SFC) profile, are needed. An oil with a steep SFC profile as a function of temperature typically has a very narrow plastic range, whereas a fat system with a flat SFC profile typically has a wide plastic range. That is to say, the plastic range is important in applications such as cakes, where the shortenings must maintain its network structure during creaming so that the air can be incorporated in the batter. In order to achieve the desired plastic properties of the shortening, fat sources used for blending must be carefully selected. One of the most important characteristic of the fat that must be taken into account is the chemical composition of the fat in terms of
distribution of fatty acids on each TAG molecule and the quantity of each TAG species.\textsuperscript{2} For example, tri-saturated TAG (i.e., SSS, SSP, PPP) can provide a strong fat crystal network (being solid at room temperature), while the di-saturated (i.e., SSO, PPO) and mono-saturated (i.e., SOO, OOP) TAGs provide both lubricity and structure.\textsuperscript{5} Another important factor to consider when blending fats is the crystalline structure or the polymorphism stability of the final product. The addition of palm oil to hydrogenated oils have been studied in detail.\textsuperscript{44-46} In vegetable shortenings, the $\beta'$ crystal is desired because it imparts a smooth, creamy texture, contributing to a fine texture in baked products.\textsuperscript{43} Palm oil is a $\beta'$ tending fat, hydrogenated canola and soybean oils tend to crystalize in the $\beta$ form.\textsuperscript{5} In order to produce a vegetable shortening with smooth texture and appropriate crystal form, palm oil is therefore incorporated into hydrogenated canola and soybean oils, which can delay or prevent the generation of the $\beta$ form.\textsuperscript{42, 45, 46}

\textit{Interesterification}

Interesterification is the process of rearranging fatty acid distributions within and/or between TAGs on a glycerol backbone.\textsuperscript{47, 48} Two types of interesterification are available: chemical and enzymatic (Figure 2-3).\textsuperscript{49} Chemical interesterification produces a complete positional randomization of acyl groups in TAG, by using chemical catalyst (i.e., sodium methoxide), while enzymatic interesterification usually uses lipases as the catalyst with lower temperature and fewer by-products.\textsuperscript{50} That is to say, chemical interesterification is more randomized and a wide range of TAG is obtained, while enzymatic interesterification is more specific on tailor TAG.\textsuperscript{51, 52} This process is
generally used to modify the melting and crystallization properties of the lipid while maintaining their nutritional quality. Interesterification results in the formation of new TAGs that may not have existed in the original fat. Many studies have been performed on chemical and enzymatic interesterification of TAGs and evaluated the effect that this process has on the hardness and spreadability of fat systems. Rousseau et al. reported that chemical interesterification resulted in a significant increase in hardness index of restructured butterfat-canola oil blends, and that the treated oil consisted solely of β’ crystals while the non-interesterified blends showed a mix of both β and β’ forms. Marangoni’s group also indicated that the hardness index of lard-canola oil increased after interesterification. Kurashige et al. reported that enzymatic interesterification lowered the SFC of palm-soybean and palm-canola oil blends. They proposed that enzymatic interesterification is a useful treatment to improve the fluidity of blends of
palm and canola oil. The physical properties of the interesterified oils were also influenced by the ratio of the original oils that were used. Lee et al. produced interesterified plastic fats with fully hydrogenated soybean oil, extra virgin olive oil and palm stearin in different weight ratios. Harder and more brittle texture, higher melting temperature, higher SFC, smaller crystal size and more elastic characteristics were observed in the produced fats containing a higher content of palm stearin and lower content of olive oil. Nowadays, interesterification is regularly used to process palm, palm kernel, and coconut oils for use in various types of confectionery, margarine, cooking and frying fats, and as blends with lauric oils in reduced-calorie spreads.

Fractionation

Fractionation, or the fractional crystallization of edible oils, is one of the principal modification processes used to make appropriate use of fats and oils in today’s range of fat-containing products. Fractionation is a purely physical process to produce fractionated oils for different purposes. It is broadly used in palm oil, palm kernel oil and coconut oil. Typically, there are three types of fractionation process: dry fractionation, solvent fractionation, and Lanza fractionation. Dry fractionation is a process in which two or more components with different melting points are cooled and separated based on their solubility or crystallization at different temperatures. Solvent fractionation is a process to separate fractions of fats and oils by dissolving the triglyceride in a solvent (i.e., hexane). Lanza or detergent fractionation is a process to use surfactant solution to transfer the crystallized material from the oil phase to an aqueous phase in order to facilitate subsequent separation. Fractionation process was used to extend or add values to the natural fats and oils, as a consequence of their limited
application in their original forms. Palm oil is probably the most flexible of vegetable oils in terms of its TAGs composition and hence its functionality. \(^{61}\) It is fractionated in multistage giving rise to several applications. For example, palm olein can be extensively used as cooking oil; palm super olein as salad oil and frying oil; the palm-mid fraction as component of cocoa butter equivalent and the palm stearin as the shortening production in other processing methods. \(^{62}\) Dr. Sato also noted that there is an increasing necessity to further develop the fractionation technology to meet the following market demands: (a) development of fats that are free of trans-fatty acids; (b) providing new fats for confectionery products; and (c) to maintain better functional properties of physically refined vegetable oils. \(^{14}\)

**High intensity ultrasound (HIU)**

*Overview of HIU*

“Sound” can be defined as a waveform of density variations in an elastic medium that propagates away from a source. \(^{63}\) Ultrasound technology is a discipline that studies sound waves at frequencies above the threshold of human hearing (frequencies above 16 kHz). \(^{64, 65}\) Ultrasonic applications can be divided into low and high intensity categories. \(^{66}\) Typical low-intensity (frequencies 1-10 MHz) applications are nondestructive (power ~ 1W cm\(^{-2}\)) testing of materials, medical diagnosis and livestock judging. \(^{8, 67}\) In food application, low-intensity ultrasound is commonly used as an analytical technique to provide information on the physicochemical properties of food such as firmness, ripeness, sugar content and acidity. \(^{68}\) High intensity (frequencies 20-100 kHz, power 10-10,000 W cm\(^{-2}\)) applications are used to produce an effect on a system to alter its properties either physically or chemically. \(^{69}\) Medical therapy, atomization of liquids,
cleaning, and disruption of biological cells, welding of plastics and metals and homogenization or mixing materials are some of the applications of high intensity ultrasound.\textsuperscript{8}

\textbf{Basic mechanism}

When high intensity ultrasonic waves are applied to a material, they induce cavitation. Acoustic cavitation can be defined as the formation of bubbles in liquids in response to the acoustic pressure field.\textsuperscript{8} The effects of ultrasound on liquid systems are mainly related to the cavitation phenomenon.\textsuperscript{63, 67} Like any other sound wave ultrasound is transmitted via waves which alternately compress and stretch the molecular structure of the medium through which they propagate (Figure 2-4).\textsuperscript{70} Cavitation bubbles can be generated from gas nuclei existing within the fluid when the power of the ultrasound is high enough and the rarefaction cycle exceeds the interaction forces of the liquid molecules. The bubbles form throughout the liquid medium and grow over a period of a few cycles.\textsuperscript{71} The bubbles increase and decrease in size as the wave cycle continues and when enough power is administered to the system bubbles become unstable and finally violently collapse.\textsuperscript{71, 72}

Martini \textit{et al.} explained the bubbles formation phenomenon as two types of cavitation: inertial and non-inertial.\textsuperscript{71} Inertial cavitation involves large scale variation in bubble size relative to the equilibrium size over a time scale of a few acoustic cycles. Rapid bubble growth usually terminates in a collapse of varying degrees of violence. Non-inertial or stable cavitation on the other hand associates small-amplitude oscillations of the bubble radius around an equilibrium radius. Non-inertial cavitation in most
Figure 2-4: Ultrasonic cavitation phenomenon: the rarefaction cycle of the wave and the change in size of bubbles during wave cycles.\(^7^0\) (Reproduced with kind permission from Elsevier Copyright Clearance Center under specific conditions, see appendix.)

instances produces little bubble growth over a time scale of thousands of acoustic cycles.\(^7^3\) As previously described, bubbles created will violently collapse as a result of two types of cavitation, especially the inertial cavitation. The collapse of the bubbles leads to energy accumulations in localized spots, generating high temperature, pressure, and shear forces.\(^7^4\) The collapse of the bubbles provides energy to take effects on the ultrasound-irradiated systems. For example, cavitation has an effect in sonocrystallization,\(^7^5,\)\(^7^6\) by inducing and promoting crystallization.
Uses of HIU in food systems

The use of ultrasound within the food industry has been a subject of research and development for many years. Some of its application are described: HIU is used for the degassing of liquid foods; induction of oxidation reactions in fermented products such as wines, whiskey and spirits; the extraction of sugars, proteins and other compounds; the inactivation of enzymes and microorganisms, and the induction of crystallization process. The application of ultrasound to crystallization is commonly described as sonocrystallization. Sonocrystallization refers to the phenomena associated with the induction or modification of the crystallization behavior of different materials. Sonocrystallization does not chemically modify individual molecules.

There is a rich literature on the uses of sonocrystallization in food systems, and most of them were performed in an aqueous media. Chow et al. used HIU to modify the primary and secondary nucleation of ice and sucrose while Patel et al. used HIU to control lactose crystallization during the nucleation phase, and they showed that ultrasound can help in the recovery of lactose crystals from lactose solutions. Similar results were found by Dhumal et al. when investigating the role of ultrasound as a seeding method. They found that application of ultrasound resulted in rapid and complete crystallization with rod-shaped fine crystals and narrow particle size distribution. Sun and Li investigated the role of ultrasound in the improvement of the freezing rate of the potato tissues as the quality of the frozen foods depends on the size of the ice crystals.

Early studies on lipid sonocrystallization were performed by Patrick et al. who evaluated the effect of ultrasonic intensity on palm oil crystalline structures. In addition, Sato and coworkers showed that HIU promotes the formation of a stable polymorphic
form in lipid systems composed of tripalmitin or cocoa butter. Different polymorphic lipid networks were obtained when trilaurin, tricaprin, and trimyristin were crystallized under the influence of HIU. The capability of HIU to promote a stable polymorphism in cocoa butter for confectionery applications has received special attention. Previous research in our laboratory showed that HIU can be used to induce the crystallization of fats such as anhydrous milk fat and an all-purpose shortening. The crystallization cell diagram was shown below:

![Crystallization cell diagram](image-url)

**Figure 2-5:** Crystallization cell diagram that was used in our research (Reproduced with kind permission from Springer based on the License Agreement)

Even though there is a substantial body of information on HIU’s effect on the crystallization of different molecules, most of the research has been in aqueous systems and very little research was performed in edible lipids. The literature offers little information on the effect of HIU on the crystallization kinetics, polymorphism and
rheological behavior of lipids, particularly of shortenings. There is a strong scientific and commercial need to fill this gap and explore new applications of HIU on the processing of lipids.

**Crystallization of lipid**

Controlling lipid crystallization is important in many food products to achieve a high quality product. During lipid crystallization experiments, a crystalline network is formed that has specific characteristics such as crystal number, size distribution, polymorph, and dispersion of the crystalline phase. In this dissertation, the crystallization behavior of lipid is mainly driven by TAG crystallization; however, other minor components such as free fatty acids, phospholipids, glycolipids, sterols and even impurities, can also play an important role in crystallization behavior. Lipid crystallization usually encompasses three distinct events: generation of supercooling, nucleation, and crystal growth.

**Supercooling and Nucleation**

The first step in a crystallization process is the generation of a driving force. Two major driving forces are discussed: supersaturation and supercooling. In the case of solute-mediated crystallization supersaturation is the main driving force for crystallization to occur. Supersaturation is defined as a state of a solution that contains more of the dissolved material than could be dissolved by the solvent under normal circumstances. Crystallization requires a solute concentration greater than the concentration of the saturated solution. The concentration-temperature curve (Figure 2-6) helps understanding of this phenomenon.
As Figure 2-6 shows, the solid line represents the solubility or saturation line, which indicates the saturated concentrations at different temperatures. Above this line, the system is supersaturated. However, in the metastable zone, crystallization is possible but might still need other external factors such as agitation or seeding. Above the dotted line (upper-limit of the metastable zone), the system is in the unstable zone, which means the crystallization occurs spontaneously. In this case, supersaturation is the first step before crystallization occurs. From the figure, as temperature decreases, the concentration needed for supersaturation status decreases. That is to say, for a specific concentration of solution, lower temperature generates higher supersaturation status. When crystallization occurs from the melt, supercooling is the driving force for crystallization. Supercooling is usually defined as the difference between the temperature of crystallization and the equilibrium melting temperature ($\Delta T = T_m - T_c$, where $\Delta T$ is the supercooling, $T_m$ is the melting temperature, $T_c$ is the crystallization temperature). That is to say, the lower the crystallization temperature is, the higher the driving force (supercooling) for crystallization.
A crystal nucleus is the smallest crystal that can exist in a solution under certain temperature and concentration. Generally, the formation of nuclei or the crystal centers from the liquid phase refers to nucleation process. In order to form nuclei, a free energy barrier must be overcome with the generation of sufficient supercooling. Once a certain amount of supercooling is achieved nucleation occurs with an associated release of energy. In that case, the free energy barrier is referred as the maximum free energy. There is a critical size for a stable nucleus at this maximum free energy. Any nucleus formed above this critical size can continue to grow, whereas clusters smaller than the critical size can potentially disperse into the liquid state.

Nucleation is usually classified as primary and secondary nucleation. Primary nucleation involves the formation of a crystal in a solution or liquid system containing no existing crystals. Primary nucleation may occur either homogeneously or heterogeneously. Homogeneous nucleation occurs in the absence of any foreign particles, which is an ideal situation; while heterogeneous nucleation occurs in practical systems when nuclei develop on the surface of solid impurities that exist in the liquid phase. Secondary nucleation involves the production of new crystals in a system containing pre-existing crystals under conditions such as fragmentation where regular nucleation will not occur. Secondary nucleation occurs when crystals in a subcooled system generate new nuclei, generally because of contacts between two crystals or between a crystal and an outside surface. Secondary nucleation may also occur whenever microscopic crystalline elements are separated from an existing crystal surface.
Crystal growth

As discussed above, once nuclei have formed, they continue to grow by the incorporation of other TAG molecules at proper place on the growing crystal surface. The incorporation of a new TAG molecules into an existing crystal lattice depends on the probability of it having the correct configuration at the correct site on a crystal surface. Crystal growth will continue as long as there is a driving force for crystallization to occur. Thus, the rate of growth in lipid systems is directly proportional to supercooling and viscosity in the system. Higher supercooling usually leads to faster crystal growth; while higher viscosity in the liquid oil system often delays the crystal growth as the molecular diffusion is reduced. However, crystal growth will eventually terminate when the system reaches phase equilibrium or the entire system is crystallized.

Functional properties

Crystal morphology

To study and control crystallization, it is important to quantify crystallization kinetics, number, shape and size of the crystals generated, polymorph of crystals as well as the network interactions among the crystalline elements. The morphology of fat crystals plays an important role in the final properties of fat products, such as margarines, butter and shortenings. Product attributes such as spreadability and hardness are determined by the individual fat crystals (crystal morphology) and the way in which these crystals interact to form clusters and networks (product morphology). In this dissertation, we mainly focused on the amount, shape and size (or size distribution) of the crystals which refer to the morphology of crystals. Stern and Cmolik showed that
rheological properties of margarines partially depended on the shape and amount of fat
crystals.\textsuperscript{102} Shi \textit{et al.} developed the concept that fat crystal network characteristics were
derived from “spherulites” of well-ordered needle-like crystals, linked together by liquid
or semi-solid bridging lipids.\textsuperscript{10} Studies also showed the effect of processing conditions on
the crystal morphology and functional properties changes. Herrera \textit{et al.} stated that higher
agitation and faster cooling rate generated smaller size of crystals, whereas at higher
crystallization temperature, larger crystals with broader crystal size distribution were
found.\textsuperscript{103} Studies by Martini \textit{et al} evaluated the effects of cooling rate, crystallization
temperature and blending ratios on the crystal morphology of blends of high-melting
fraction of milk fat in sunflower oil. Results showed that slow cooling rate generated
more regular boundary crystals with larger sizes; and higher content of sunflower oil led
to broader crystal size distribution.\textsuperscript{104, 105} Further studies by Martini \textit{et al} on the adding of
sucrose esters in the high melting milk fat fraction showed more transparent crystals with
smaller sizes were found.\textsuperscript{106} Thus, the number, size and shape of the fat crystals and
larger clusters will define the physical and functional properties of the fat. Determination
of these morphological properties is very significant to evaluate the overall functionality
of the fat system.

\textbf{Solid Fat Content (SFC)}

Solid fat content (SFC) greatly influences the suitability of oils and fats for a
particular application. SFC refers to the amount of fat crystals (usually related to
hardness) in the blends, it is responsible for many product characteristics including
general appearance, ease of packing, organoleptic properties, ease of spreading, and oil
exudation.\textsuperscript{107} The SFC is crucial to the texture of various food products such as chocolate,
butter, margarine and shortenings. Depending on the application of the fats and oils in the food system, lipids with certain functional properties, usually indicated with solid fat content (SFC) profile, are needed. While an oil with a steeper SFC profile as a function of temperature typically has a very narrow plastic range whereas a fat system with a flat SFC profile typically has a wide plastic range. For example, cocoa butter has a very high SFC at low or room temperatures and then melts very sharply over a narrow temperature range (32-35°C) which is close to the mouth temperature. This fast melting of cocoa butter results in the typical cooling sensation experienced by consumers. This characteristic gives the standard for the modification and production of cocoa butter equivalents and substitutes from vegetable oils. Many shortening functions are described by the industry in terms of the SFC at specific temperature. For example, all-purpose shortenings require 23% of SFC at 10 °C and maintains 11% at 40 °C, while a good filler fat shortening has 44% of SFC at 10°C and sharply decreases to approximately zero at 40 °C. On the other hand, frying shortenings used in coated doughnuts require intermediate SFC at temperature range of 80 to 90 °F. If the SFC is too high, there will be poor adhesion of powdered sugar coatings; while if the SFC is too low, glazes will not stick and there will be too much adhesion of powdered sugar. The use of SFC profiles to characterize the usefulness of shortenings represents the most practical means of selecting fat feedstock for industrial application; at the same time, the measurement of SFC by pulsed NMR is relatively quick and easy. Even though SFC is often used to predict physical properties of fats and taken as evaluating indicator in food industry, many studies also found that SFC alone is unreliable to predict hardness or even overall functionalities of fats, in this case, other physical properties listed in the following
sections must be quantified.

**Melting profile**

It is generally known that vegetable oils are complex materials from the chemical point of view with a wide TAG distribution and different polymorphic transitions, resulting in a complex melting and crystallization behavior. Therefore, it is not surprising that instead of the sharp melting point that is associated with crystalline pure materials, fat crystal networks in shortening systems demonstrate melting ranges rather than melting points.

The melting point and the melting ranges are obtained through the melting profile of the fat crystals. The melting profile analysis consists of a continuous measurement and recording of some structural parameters of the fat crystals while the temperature of is raised at a constant rate. Several parameters can be calculated from the melting profiles such as the peak temperature ($T_p$), onset temperature ($T_{on}$), and enthalpy. These analyses provide information about: (a) the amount of solids generated through the melting enthalpy values, (b) the solid/liquid ratio of the crystal network formed as a function of temperature during melting, and (c) the melting range of the lipid network. Timms used thermal profile of milk fat to identify the three endothermic peaks corresponding to three fractions of milk fat. Polymorphism can also be obtained under considerable rate of cooling and each polymorphic form can be identified using melting profiles. An early study by Suzuki *et al.* determined the two polymorphism peaks and melting temperature of oleic acid through the thermal dynamic curves.

The information provided by the thermal profile such as the melting point / range, polymorph transition temperature, and polymorphisms are very important for the study of
the mechanical properties of the fat system. For example, cocoa butter has a sharp melting range (32-35 °C) which makes it desirable for the production of chocolate and confectionery; deep frying shortening are usually present on the surface of foods, a high melting point can cause a greasy or waxy taste in mouth, thus, snack food are usually fried in a low melting point fat. In recent years, differential scanning calorimetry (DSC) has been used to evaluate melting properties of edible fats. DSC provides a more detailed analysis of the melting behavior than either the slip melting point or solid fat content measurement. A very interesting use of DSC is to evaluate the thermal profile of trans-fat and a corresponding trans-free fat. Foubert et al. found that the crystallization of the trans-free fat is much faster compared to the trans-fat, which is comprised with the fact that trans fatty acids have a higher melting point than their corresponding cis-isomers.

Different processing conditions can affect melting profiles. A study conducted by Martini et al. showed the effect of cooling rate on the melting profile of a fat blends sample. A slow cooling rate showed the fractionations of the TAG as two peaks in the melting curve, whereas under fast cooling rate, only one peak with a smaller shoulder was generated. Melting profiles can also be affected by the addition of emulsifiers, depending on the emulsifiers and shortenings. Martini et al. studied the addition of saturated mono- and diacylglycerols (s-MDG) emulsifier to shortening blends 1 (which is a blend of palm oil, palm olein, palm stearin, palm kernel oil, and soybean oil) resulted in a broader peak, while adding emulsifier sorbitan tristearate (STS) to blends 4 (a blend of palm oil, palm stearin, palm olein, palm kernel oil, and sunflower oil) led to a sharp narrow melting peak. Herrera et al. showed the effect of cooling rate on thermal
behavior of a milk fat system. The study showed that at slow cooling rates, the melting temperature range of the main peak was broader; whereas, at fast cooling rate, a sharper high-melting peak was found. Also, slowly crystallized samples had slightly higher melting enthalpies and ending temperatures.13

**Polymorphism**

Edible lipids can crystallize in different crystalline lattices or polymorphic forms. The most common polymorphs of TAGs are: α, β and β’ form (Figure 2-7b).14 Among the three main polymorphic forms, generally, β is the most stable with highest melting point, β’ is less stable with intermediate melting point and α is the least stable form with lowest melting point due to the descending order of $\Delta G^\#$ (Figure 2-8). Lipids exhibit monotropic polymorphism, where unstable forms are the first to crystallize in a

![Figure 2-7: A triacylglycerol molecule (a), polymorphism and subcell structure (b), chain length structure (c) and unit cell structure of tricaprin β form (d).](https://example.com/figure27.png) (Reproduced with kind permission from Elsevier).
Figure 2-8: Schematic illustration of activation free energy for nucleation ($\Delta G^\#$) of polymorphic forms of $\alpha$, $\beta'$, and $\beta$ of TAGs\textsuperscript{122}. (Reproduced with kind permission from John Wiley and Sons)

subcooled or supersaturated system because of the lower energy state\textsuperscript{122}. Subsequent transformation occurs from the least stable $\alpha$ form to the most stable $\beta$ form as storage time goes by (through tempering) or temperature change\textsuperscript{93} since $\beta$ form has the lowest free energy status while $\alpha$ form maintains the highest level (Figure 2-8).

The above described polymorphic transformation is one type of transformation and named as melt-mediated transformation.\textsuperscript{122} The polymorphic forms present in lipid crystals affect the macroscopic physical properties of the system tremendously. For example, the texture of ice cream is produced by a network of partially coalesced $\alpha$ form fat crystals and ice crystals that surround air bubbles to form discontinuous foams.\textsuperscript{123, 124} The small needle like $\beta'$ form crystals impart food plasticity that is desirable in shortenings and margarines.\textsuperscript{2} Confectionary fats such as cocoa butter and cocoa butter equivalents require tempering to gain $\beta$ form.\textsuperscript{6} However, polymorphism can be difficult to control fully, for which reason the industry has been looking for ways to improve the situation such as stabilizing a certain phase, or delay and even prevent the transitions into
another phase. The transition of polymorphs is often accompanied by product
deterioration such as fat bloom in chocolate or sandiness in spreads. In this case, it is very
important to determine and control the polymorphism of the crystals to study the
functional properties of the fat or other food systems. The three main polymorphic forms
present in fats can be identified by the short spacing (d) of the crystals determined by X-
ray diffraction (XRD). The XRD AOCS method (AOCS Official Method Cj 2-95) states
that α form shows an XRD peak at d = 4.15 Å, and the short spacing of β’ form appears
close to 4.2 and 3.8 Å, while β form shows a single strong spacing at 4.6 Å.

Viscoelasticity

Viscoelasticity is the property of materials that exhibit both visous and elastic
characteristics when undergoing deformation. In this dissertation, viscoelasticity refers to
the rheological properties measured by an oscillatory rheometer, including viscosity, G’,
G” and tanδ. Viscosity is a measure of viscous properties, which refers to its resistance to
gradual deformation by shear stress or tensile stress. G’, or storage modulus, is the
parameter to describe the elastic characteristic (solid-like) of a material: higher G’ means
more solid-like behavior. G”, or loss modulus, is the parameter that describes the viscous
(liquid-like) behavior of a material: higher G” represents more liquid-like behavior. tanδ
is the ratio of G” to G’, it can be from infinity (in a perfect liquid system when G’ is
close to zero) to a very small value (i.e., a high viscosity system). For shortenings, the
physicochemical, textural and viscoelastic properties might change during storage, which
is mainly due to changes in crystals shape and size and changes in the crystal network.
That is to say, when studying crystalline materials, viscoelasticity should be carefully
considered. As for shortentings, plasticity is a highly important property to study. The
correct rheological evaluation provides plasticity of the semi-solid food stuff, such as margarines and shortenings. A good plastic fat is characterized by proper viscoelasticity and provides the firmness, spreadability, consistency, storage stability and good sensory properties.\(^5^4\)

Viscoelastic properties in fats have been extensively studied to explain changes in the rheological properties with different processing methods. Marangoni and Rousseau reported that the G’ of all lard-canola oil blends increased as a result of chemical interesterification.\(^5^4\) Shukla et al. extracted a milk fat fraction enriched with high melting TAG and recombined it into butter. This high melting butter exhibited higher consistency with comparable viscoelastic properties at 32 °C with those of market butter at 22 °C.\(^1^2^7\) A comprehensive study of effect of processing conditions on rheological properties of milk fat was carried out by Herrera et al. in 2000.\(^1^2^8\) They investigated the effects of cooling and agitation rates, crystallization temperature, chemical composition, and time of storage on the storage (G’) and loss moduli (G’’) changes. For example, both G’ and G’’ were higher for samples crystallized at slow cooling rate, and decreased with agitation rate.

**Hardness**

The hardness of a fat or shortening is an important property that strongly influences the perceived texture of a food product.\(^1^2^9\) Depending on the end use, most shortenings require proper hardness. The hardness of the fat in the formulation can partially reflect the solid content of the fat. For example, deficient solids content may result in oil separation, whereas excessive solids can cause hardness or brittleness instead of the desired viscous flow.\(^1\) In the production of shortenings, most vegetable oils are too
soft because of their liquid nature, while fully saturated fats are too hard. To get a desirable property of the shortening, different processing methods were used, such as hydrogenation, blending of different oils or oil fractions, interesterification and fractionation, use of additives such as emulsifiers and waxes.\textsuperscript{2, 130, 131} Hardness provides a quick method to evaluate the physical property of the fat system, however, hardness itself cannot represent the overall functional properties of fats, since other factors such as polymorphism, crystal size and shapes can affect the final hardness,\textsuperscript{12, 132} and it is important to comprehensively consider all the parameters.

\textit{Chemical composition}

Chemically, fats and oils are mainly composed of a wide variety of TAGs which in turn are composed of a different types of fatty acids esterified to a glycerol molecule. Natural oils exhibit a wide range of physical properties which are influenced by the degree of unsaturation, the length of the carbon chain, the isomeric form of the fatty acids, the molecular configurations of the TAG molecules, and the polymorphic state of the fat.\textsuperscript{2, 122} As the diversity of TAGs, fatty acids, and the degree of saturation, physical properties including melting point, crystallization behavior, polymorphism and overall functionalities were tremendously different. TAG molecules affect crystallization kinetics as the attachment of TAG molecules to the surface of growing nuclei is significantly influenced by the carbon number and arrangement of TAG molecules.\textsuperscript{133} TAG carbon number and diversity, TAG structure, and the fatty acid chain length and diversity were reported to influence the stability of a $\beta'$ polymorph by Yap \textit{et al.}\textsuperscript{46} Palmitic acid was reported to affect the stability of the polymorphic forms significantly. Vegetable oils containing 10% of palmitic acid tend to crystallize in the $\beta$ form, whereas those having 20%
of palmitic acid have the tendency to crystallize in the β’ form. Different TAG and fatty acids composition lead to different functional properties of fats and oils. This characteristic also provides the development of different processing methods, such as hydrogenation, blending, and interesterification. In the shortening formulation and blending production, fluid margarines are formulated by blending a high portion of unsaturated TAGs and a low proportion of tri-saturated TAGs; di-saturated and mono-saturated TAGs are used to provide both lubricity and structure, such as the production of pastry shortenings. All in all, to study the original chemical compositions of the shortening system can help us better understand overall functional properties of the product.

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CHAPTER 3

USING HIGH INTENSITY ULTRASOUND AS A TOOL TO CHANGE THE
FUNCTIONAL PROPERTIES OF INTERESTERIFIED SOYBEAN OIL

Abstract

High intensity ultrasound (HIU) was used to change the crystallization behavior, generate small crystals and improve the texture of a low saturated shortening (interesterified soybean oil). Samples were crystallized at different temperatures (26, 28, 30, and 32 °C) without and with the application of HIU. Different acoustic power levels (110, 72, 61, 54, 44 W) were used. Results show that higher acoustic powers had a greater effect on crystal size reduction, induced crystallization, and generated harder, more elastic and viscous materials. These effects were more significant when HIU was applied in the presence of crystals and when the sample was crystallized at 32 °C.

Introduction

The Institute of Shortening and Edible Oils reports an 85% increase in the consumption of shortenings in the last 10 years. During this period the food industry has gradually replaced the use of animal fat with modified vegetable oils. Even though vegetable oils have been used for decades, the lack of texture and plasticity of some vegetable oils has limited their use in food products. To overcome this limitation, different processing technologies, together with new varieties of vegetable oils have been

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developed to provide shortenings with a wide range of functional properties that can be
used by food producers. During the early stages of shortening production, partial
hydrogenation was used to improve the functional properties of vegetable oils.
Depending on the degree of hydrogenation, shortenings with different solid/liquid ratios
can be obtained in an easy and economical manner that can be used for specific
applications in the food industry. However, research has shown a strong correlation
between trans-isomers generated by the hydrogenation process with incidence of
coronary heart disease.2-8 This finding, together with the trend of adopting healthier
lifestyles, has encouraged food manufacturers to produce food products with little or zero
trans-fatty acid content. In addition, since 2006 the FDA requires declaring trans-fatty
acid content on food labels, with 0.5 grams of trans-fatty acid per serving to be labeled as
“no trans-fat” [21 CFR 101.9(C)(2)(ii)]. Interesterified oils,9-13 semisolid oils such as
palm and coconut, and lipid blends have been used to replace trans-fats in food
formulations. The use of low saturated shortenings in food formulations is one potential
solution to replace trans-fats; however, these shortenings are usually too soft for some
food applications leading to low functionality and quality of products.14 To find a balance
between nutritional and functional properties, different processing conditions can be used
to improve the physicochemical characteristics and functionality of shortenings and meet
consumers’ expectations.15

High intensity ultrasound (HIU) has been used in the food industry as an efficient
tool for large scale commercial applications, such as emulsification, homogenization,
extraction, crystallization and viscosity alteration.16 There is a myriad of literature
describing the use of HIU to induce or generate crystallization (sonocrystallization) in
aqueous systems. To name a few studies performed in the food science area, Dr. Malcom Povey’s group described the use of ultrasonic crystallization to control the size and rate of ice crystals formation in frozen foods.\textsuperscript{17} In addition, this same researcher further proved that HIU can modify the primary and secondary nucleation of ice;\textsuperscript{18} while Patel et al.\textsuperscript{19} showed that the use of power ultrasound can control the crystallization process of lactose during the nucleation phase. In 2007, McCausland showed that HIU can be used to produce crystalline materials for pharmaceutical uses.\textsuperscript{20} In general, previous research has shown that HIU can affect crystallization processes by either affecting crystal nucleation, controlling the rate of crystal growth, promoting the formation of small and even-sized crystals, and preventing fouling of surfaces by the newly formed crystals.\textsuperscript{21-23} Early work by Sato’s group in lipid systems has shown that HIU induced the crystallization of tripalmitin and cocoa butter and also promoted the formation of $\beta'$ and $\beta$ crystals.\textsuperscript{24,25} Previous research in our laboratory has shown that HIU can induce the crystallization of other fats such as anhydrous milk fat,\textsuperscript{26} palm kernel oil, and an all-purpose shortening.\textsuperscript{27} The literature provides significant information on the effect of HIU on the crystallization behavior of different molecules in aqueous systems; however, there is very little information on the effect of HIU on the crystallization kinetics, polymorphism and rheological behavior of lipids, shortenings in particular.

The objective of this work was to evaluate the effect of HIU on the crystallization behavior and functional properties of a low saturated shortening (interesterified soybean oil). Different sonication and processing conditions were tested and the functional properties of the material, such as crystallization behavior, microstructure, melting profile, texture, viscoelasticity, and polymorphism were measured.
Materials and Methods

Materials: A low saturated fat shortening consisting of interesterified soybean oil (IESBO) was used in this research. The shortening (product N° 76-240-0) was provided by Archer-Daniels-Midland (Decatur, IL, USA) and produced by interesterification of liquid soybean oil and a fully hydrogenated hard stock. The chemical composition of the IESBO is described in the results section.

Melting point determination: The melting point of IESBO was determined by AOCS official Method Cc 1-25.

TAG chemical composition: Triacylglycerol (TAG) chemical composition was determined using AOCS official Method Ce 5b-89. Composition was expressed as the percentage of equivalent carbon number (ECN) molecular entities as described in the method.

Fatty acid methyl esters chemical composition: Fatty acid methyl esters (FAMEs) of all samples were analyzed by gas chromatography on a Shimadzu 2010 GC equipped with a flame ionization detector (Shimadzu, Columbia, MD). Fatty acid methyl esters (FAME) were prepared as described by O’Fallon et al. with slight modifications. Samples were melted at 60 °C for 15 min; 40 µL of melted oil sample was then placed into a 16 X 125 mm screw-cap Pyrex culture tube to which 6.3 mL MeOH and 0.7 mL of 10N KOH in water were added. After vortex-mixed, the tube was then incubated in a 55 °C water bath for 1.5 h with auto shaking to properly permeate, dissolve and hydrolyze the sample. After cooling down the samples to lower temperature in a bucket with ice water, 0.58 mL of 24 N H2SO4 was added. The tube was vortex-mixed and
incubated in a water bath at 55 °C with auto shaking for 1.5 h. Two mL of hexane was added and the tube was vortex-mixed before being centrifuged for 10 min at 1,000 × g in a tabletop centrifuge. The hexane layer (upper phase), which contains the FAME, was placed into a GC vial and the vial was capped and placed at -20 °C until GC analysis.

Fatty acid methyl esters were separated using a DB-225 fused-silica capillary column (20 m x 0.18 mm i.d. x 0.2 µm film thickness, Agilent Technologies, Palo Alto, CA). Injection was done in the split mode. One µL was introduced into the injector at 250 °C, with a split ratio of 30:1. The carrier gas was hydrogen at a linear velocity of 61.2 cm/sec. The oven temperature program was as follows: 35 °C held for 0.38 min, 33.21 °C/min to 195 °C, 3.99 °C/min to 205 °C, and 10.63 °C/min to 230°C followed by a 5 min hold. The detector temperature was 250°C with an air flow of 400 ml/min, and hydrogen flow at 39 ml/min. Peaks were identified by retention time overlay with authentic FAME standards (Nu-Chek Prep, Elysian, MN).

**Crystallization and HIU application:** IESBO was heated to 80 °C and kept at this temperature for 30 min to allow complete melting of the triacylglycerols. The melted lipid sample was then placed in a thermostated crystallization cell as described elsewhere \(^{26, 29}\) which was set at different crystallization temperatures (\(T_c = 26, 28, 30,\) and \(32 °C\)). IESBO was crystallized at a cooling rate of 5°C/min with agitation using a magnetic stirrer (200 rpm) to increase the heat transfer between the sample and external circulating water. Different crystallization experiments were performed during this research. The first set of experiments was performed to establish the acoustic power needed to achieve a greater effect in crystal size reduction during crystallization. For these sets of experiments, HIU was applied after 10 min into the crystallization
experiments which corresponded to the moment when the sample reached crystallization
temperature. Before HIU was applied, agitation was stopped to avoid dissolution of
bubbles generated during the sonication process. HIU was applied for 10 s (Misonix S-
3000 sonicator, Misonix Inc., NY) using different acoustic power (110, 72, 61, 54, 44 W).
The sonicator operates at an acoustic frequency of 20 kHz and a microtip of 3.2 mm
diameter was used. After sonication, the sample was kept at crystallization temperature
for 90 min to allow complete crystallization. This was demonstrated by the lack of
crystal change as observed in the polarized light microscope. The second set of
experiments consisted of applying HIU using 110 W of acoustic power for 10 s. HIU
was applied at different time points during the crystallization process as can be seen in
Table 3-1. Crystal formation and morphology during crystallization was evaluated using
polarized light microscopy. After holding the sample in the crystallization cell for 90
min, the functional properties of the shortenings were measured using the techniques

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<th>Nomenclature</th>
<th>HIU condition</th>
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<td>10/wo</td>
<td>Sample reached $T_c$ at 10 min (agitation stopped). No HIU is applied</td>
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<td>10/10</td>
<td>Sample reached $T_c$ at 10 min (agitation stopped). HIU is applied at 10 min</td>
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<tr>
<td>10/13</td>
<td>Sample reached $T_c$ at 10 min (agitation stopped). HIU is applied at 13 min</td>
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<td>10/20</td>
<td>Sample reached $T_c$ at 10 min (agitation stopped). HIU is applied at 20 min</td>
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</table>
described below. In addition, functional properties were measured after tempering the samples for 48h at 5 and 25 °C. Samples crystallized without HIU application were used as control groups.

**Polarized light microscopy measurements (PLM):** Crystal morphology was recorded during crystallization. A drop of lipid sample was taken from the crystallization cell at different times and placed between a slide and cover-slide to evaluate crystals’ microstructure during crystallization using a polarized light microscope (PLM, Olympus CX 31, Tokyo, Japan) with a digital camera attached. A total 200 X magnification was used.

**Thermal behavior measurements:** A differential scanning calorimeter (DSC, DSC 2910, TA Instrument, USA) was used to evaluate the melting behavior of the crystallized material. After 90 min in the crystallization cell, 5-15 mg of sample was placed in a hermetic aluminum pan for DSC use. The sample was heated to 80 °C with a ramp rate of 5 °C/min to evaluate the melting behavior of crystallized material. Through this procedure, the melting enthalpy (ΔH) was determined. This evaluation provided information about the amount of solids generated during crystallization. The melting profile of the samples was quantified by integrating the melting endotherms at specific temperatures and the percentage of solid fat at different temperatures during melting was calculated. Melting profiles and melting enthalpy values were also determined for samples tempered at 5 °C for 48 h.

**Texture profile analysis:** The hardness of the lipid network formed was measured by texture profile analysis (TPA) using a Texture Analyzer (Model TA. XT Plus, Texture Technologies Corp. USA). After samples were crystallized for 90 min, crystallized
material was placed in a polystyrene assay tube and left at 5 °C for 48 h to allow complete crystallization. Just before performing the TPA analysis, samples were taken out of the assay tube and cut to a 3/4 in height for TPA analysis.29

Rheology measurements: A TA Instruments AR-G2 Magnetic Bearing Rheometer (TA Instruments, AR-G2) was used to evaluate the viscoelastic properties of the material. Flow procedure was performed by steady state flow step to evaluate samples’ zero-rate viscosity. Oscillatory tests were performed by strain sweep step to obtain viscoelastic parameters such as the storage (G’) and loss (G’”) modulus. The experiments were carried out using a parallel plate geometry (40 mm diameter). For steady state flow step, the shear rate (1/s) was controlled from 1.000E-4 to 100.0; for the strain sweep step, a constant frequency of 1 Hz (6.28 rad/s) was used and strain values was set from 0.0008% to 10%. The viscoelastic behavior of the samples was measured after 90 min crystallization and after tempering the material at 5 °C and 25 °C for 48 h. Different tempering conditions were tested to evaluate how these affect the viscoelastic properties of the material, and therefore its functional properties.

X-Ray diffraction measurement (XRD): The polymorphic form of the IESBO crystallized at 32 °C with and without the application of HIU was evaluated using XRD Philips X’Pert 3040 MPD (PANalytical, Almelo, The Netherlands) diffractometer system with a single PW3050/00 (theta/2-theta) goniometer. The x-ray source was Cu k-alpha radiation with a PW3123/00 Monochromator detector system. The software controlling the system is X’Pert  Data Collector (v2.0e) (PANalytical, Almelo, The Netherlands). Samples were crystallized as previously described and tempered for 48 h at 25 and 5 °C before XRD determination.
**Statistical analysis:** Samples were run in triplicate and data reported are mean values and standard deviations. Significant differences (P < 0.05) were evaluated using a two-way ANOVA by the GraphPad Prism software, version 4.00 for Windows (GraphPad Software, San Diego, CA, USA).

**Results and Discussion**

The TAG chemical composition of the IESBO sample is shown in Table 3-2. Chemical composition was expressed as percentage of equivalent carbon number (ECN); where ECN = CN-2n; CN is the TAG carbon number and n is the number of unsaturations. The IESBO sample was composed of approximately 50% of TAG of 48 ECN. The entities integrated in this group include OOO, SOL, and SSLn, where O refers to oleic acid, S, refers to stearic acid, L refers to linoleic acid, and Ln refers to linolenic acid. The second most abundant group was TAG with 46 ECN which include OOL, POL and SLL with a total of approximately 25%. TAG with 44 ECN constituted approximately 15% of the sample and represent TAG such as OLL and PLL. Approximately 8% of the sample was composed of TAG with ECN of 42 which include TAG entities such as LLL and OLLn. Finally, approximately 2.2% of the sample was composed of TAG with lower ECN (38 and 40) which represented TAG such as LnLnL and LLLn, respectively.

Table 3-3 presents the fatty acid methyl ester composition of the IESBO. The major component of this shortening was linoleic acid (C18:2) with approximately 42% of the total fat. The second major component was stearic acid (C18:0) with approximately 22% and the third major component was oleic acid (C18:1) with approximately 17% of
Table 3-2: TAG chemical composition of the IESBO expressed as the equivalent carbon number (ECN). ECN = CN – 2n, where CN is the total carbon number of the TAG and n is the total number of unsaturations.

<table>
<thead>
<tr>
<th>ECN</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>0.16 ± 0.04</td>
</tr>
<tr>
<td>40</td>
<td>2.10 ± 0.03</td>
</tr>
<tr>
<td>42</td>
<td>8.25 ± 0.12</td>
</tr>
<tr>
<td>44</td>
<td>14.86 ± 1.54</td>
</tr>
<tr>
<td>46</td>
<td>24.77 ± 0.56</td>
</tr>
<tr>
<td>48</td>
<td>49.86 ± 2.23</td>
</tr>
</tbody>
</table>

Table 3-3: Fatty acid methyl ester composition of the IESBO.

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14:0</td>
<td>0.08 ± 0.00</td>
</tr>
<tr>
<td>C16:0</td>
<td>10.58 ± 0.01</td>
</tr>
<tr>
<td>C16:1</td>
<td>0.08 ± 0.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>21.59 ± 0.24</td>
</tr>
<tr>
<td>C18:1</td>
<td>17.32 ± 0.01</td>
</tr>
<tr>
<td>C18:2</td>
<td>41.55 ± 0.15</td>
</tr>
<tr>
<td>C18:3</td>
<td>6.55 ± 0.04</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.39 ± 0.00</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.17 ± 0.00</td>
</tr>
<tr>
<td>C20:2</td>
<td>0.03 ± 0.00</td>
</tr>
<tr>
<td>C20:3</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>C22:0</td>
<td>1.51 ± 0.05</td>
</tr>
<tr>
<td>C22:4</td>
<td>0.02 ± 0.00</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
</tr>
</tbody>
</table>
the total fat. Palmitic acid (C16:0) constituted only 11% of the total fatty acid content. The total amount of saturated fat in this shortening was approximately 34%.

As described in the Materials and Methods section, lipid samples were crystallized without and with different acoustic power levels at different crystallization temperatures. The melting point of the IESBO sample was 33.4 ± 0.5 °C and therefore, the crystallization temperatures (Tc) chosen in these experiments were 26, 28, 30 and 32 °C.

Figure 3-1 shows the morphology of IESBO crystals formed at initial steps of crystallization for 26, 28, 30 and 32 °C, with different acoustic power level when HIU was applied at 10 min. Pictures shown in Figure 3-1 for samples crystallized at 26-30 °C were taken at 15 min after the sample was placed in the crystallization cell, while pictures shown for the sample crystallized at 32 °C were taken at 20 min. As expected, Figure 3-1 shows that when samples were crystallized without HIU the amount of crystals decreased as Tc increased due to a decrease in the driving force (supercooling) for crystallization. In addition, crystallization at lower Tc generated smaller crystals. Figure 3-1 also shows an evident induction in the crystallization and the generation of smaller crystals as a consequence of HIU application. Higher power levels had a greater effect on crystallization behavior by inducing crystallization and by generating more and smaller crystals. This behavior was observed for all Tc tested. For all Tc, samples reached crystallization temperature at 10 min after the sample was placed in the crystallization cell. Samples crystallized at 26 and 28 °C started to crystallize before the sample reached crystallization temperature, while samples crystallized at 30 and 32 °C started to crystallize at 13 and 20 min, respectively. Since HIU was applied 10 min after
Figure 3-1. Microstructure of initial lipid crystals obtained when samples were crystallized at 26, 28, 30 and 32 °C without HIU and when HIU was applied at 10 min with different HIU powers (HIU with acoustic power 101 W, 72 W, 61 W, 54 W and 44 W). Pictures were taken at 15 min for samples crystallized at 26 °C, 28 °C and 30 °C and at 20 min for samples crystallized at 32 °C.

the sample was placed in the crystallization cell, some crystals or nuclei were already present in the media when HIU was applied for samples crystallized at 26 °C and 28 °C. On the other hand, when samples were crystallized at 30 and 32 °C and HIU was applied at 10 min no crystals were present during sonication. This suggests that HIU induces
crystallization in samples crystallized at 30 and 32 °C by inducing primary nucleation in the system.

Figure 3-2 shows the microstructure of crystals after 90 min at different crystallization temperatures with different HIU power level application. The effect of HIU in inducing crystallization was maintained during the 90 min of the experiments as shown in Figure 3-2 where smaller and more crystals are observed still after 90 min at $T_c$. This effect was more significant when higher crystallization temperatures were used ($T_c$.

**Figure 3-2.** Microstructure of lipid crystals obtained after 90 min at crystallization temperature when samples were crystallized at 26, 28, 30 and 32 °C without HIU and when HIU was applied at 10 min with different HIU powers (HIU with acoustic power 101 W, 72 W, 61 W, 54 W and 44 W).
= 30 and 32 °C). These results suggest that HIU also affects crystal growth in the system.

It is apparent from the results shown in Figure 3-1 and 3-2 that the higher the acoustic power level, the greater the effect that ultrasound played on samples’ crystallization behavior, especially on induction of crystallization and crystal size reduction. Based on these results, the highest acoustic power (101 W) was used to further evaluate the effect of HIU on the crystallization behavior and functional properties of IESBO. Data discussed above suggest that HIU induces crystallization by promoting primary nucleation when samples are crystallized at high temperatures (30 and 32 °C). When the sample is crystallized at lower temperatures (26 and 28 °C) and HIU is applied in the presence of crystals, HIU might have an effect on secondary nucleation by breaking up the growing crystals and creating more nuclei in the media. This might be a consequence of the high shear forces generated through the sonication. Therefore, additional processing conditions were tested to explore the effect of HIU on lipid crystallization when sonication is applied in the presence or absence of crystals at different Tc. The new processing conditions consisted of: (a) applying HIU when sample reached crystallization temperature (Tc) and no crystals are observed; and (b) when the first crystals were observed (fc). For samples crystallized at 32 °C, Tc was reached at 10 min and the first crystals were observed at approximately 20 min. Therefore, HIU was applied at 10 min (when the sample reached Tc) and at 20 min (when the first crystals were observed). When samples were crystallized at 30 °C, Tc was reached at 10 min and the first crystals were observed at 13 min. Therefore, HIU was applied at 10 min (sample reached Tc) and at 13 min (when first crystals were observed). In this case, Tc condition is too close in time to fc conditions and therefore, a third condition was used for this
sample to evaluate the effect of HIU before crystallization occurs and also at a time significantly distant from the moment at which crystallization occurs. To achieve this goal, the third processing condition consisted of stopping agitation at 5 min and immediately applying HIU. When agitation was stopped at 5 min, crystallization started at approximately 10 min. This last condition was also used for samples crystallized at 28 and 26 °C. The nomenclature used to identify these processing conditions is summarized in Table 3-1.

Figure 3-3 compares the microstructure obtained after 90 min into the crystallization process when samples were crystallized at 30 and 32 °C and HIU was applied at Tc and fc. The Tc condition always corresponds to HIU being applied at 10 min into the crystallization process, while fc corresponds to HIU being applied at 13 min for samples crystallized at 30 °C (10/13 in Table 3-1) and 20 min for samples crystallized at 32 °C (10/20 in Table 3-1). Micrographs presented in this Figure show that HIU is more

![Figure 3-3](image)

Figure 3-3. Microstructure of crystals obtained after 90 min crystallization, HIU (101 W) applied at different moments: Tc indicates that HIU was applied at the moment when the sample reaches crystallization temperature; fc indicates that HIU was applied at the
moment when the first crystals were observed. Agitation was stopped at T_c (10 min),
HIU applied at f_c (for 30 °C, f_c = 13 min; for 32 °C, f_c = 20 min).
efficient in reducing crystal size when it is applied using the f_c condition; that is, when
HIU is applied in the presence of crystals. These results suggest that the effect of HIU on
secondary nucleation is more significant than the one observed for primary nucleation.
The crystal size reduction observed when T_c condition is used (no crystals observed)
suggests that primary nucleation is affected by acoustic waves, while the crystal size
reduction observed when f_c condition is used suggests that HIU affects secondary
nucleation events. The effect of HIU on the morphology of the crystals is maintained
even after 90 min of processing, suggesting that crystal growth might be also affected.

Figure 3-4 shows the microstructure of crystals obtained after 90 min into the
crystallization process when agitation was stopped at 5 min and HIU immediately applied
for sample crystallized at 26, 28, and 30 °C. When samples were crystallized without the
use of HIU, the induction times of crystallization were 12, 14, and 15 min for samples
crystallized at 26, 28, and 30 °C, respectively. It is evident from these pictures that a
reduction in crystal size is obtained as a consequence of HIU application, especially when
samples are crystallized at low T_c (26 °C). However, as previously discussed, this is a
**Figure 3-4.** Microstructure of crystals obtained after 90 min crystallization: Agitation was stopped at 5 min and HIU (101 W) was applied at 5 min. A less effective way to reduce the size of crystals than when HIU is applied when crystals are present in the medium (Figure 3-2).

Previous research on sonocrystallization in aqueous systems suggests that the induction in crystallization caused by acoustic waves is due to several factors: a) the generation of cavitation, b) shear forces caused by the acoustic waves. Data described above suggest that in lipid systems HIU is more effective when applied in the presence of crystals. This increased effect observed with the presence of crystals can be explained by a combining effect of cavitation and shear forces caused by the acoustic waves. In the presence of crystals, shear forces might break some of the existing crystals, creating new nuclei and resulting in the generation of more and smaller crystals in the network. Similarly, when no crystals are present and HIU is applied, only cavitation events are responsible for inducing crystallization. That is, small bubbles generated during the sonication act as nuclei inducing crystallization in the system.

In general, lipid crystal networks with small crystal sizes result in harder materials. Therefore, we expect that this crystal size reduction caused by HIU will generate harder materials in terms of texture and viscoelastic properties. Figure 3-5 shows the hardness of samples crystallized at different temperatures without and with the application of HIU when samples were crystallized under the conditions described previously. As described in the materials and methods section, hardness was measured with TPA after tempering the crystallized sample for 48 h at 5 °C to allow complete crystallization and the formation of stable structures. Hardness of samples crystallized
without HIU application which agitation was stopped at 5 min (5/wo) was higher (26 and 28 °C) or equal (30 °C) than samples where agitation was stopped at 10 min (10/wo).

**Figure 3-5.** Hardness (expressed in gram-force) of the samples determined by TPA, samples crystallized at 26 °C (A), 28 °C (B), 30 °C (C) and 32 °C (D) were stored for 48 h at 5 °C.

For samples crystallized at 26 °C, HIU always generated a significantly harder material (p < 0.05). Interestingly, the same behavior was not observed for samples crystallized at 28 and 30 °C where even though HIU generated harder materials when agitation was stopped at 10 min, the differences were not statistically significant. When samples were crystallized at 32 °C, the hardness of sonicated samples was significantly higher (p < 0.05)
for all conditions tested and the harder texture was observed for samples where HIU was applied under $f_c$ conditions (10/20). This finding is in accordance to the microstructure observed and reported in Figure 3-5 where significantly smaller crystals were observed for the $f_c$ condition. The texture observed in these measurements is driven by the microstructure of the crystal network formed where smaller crystals are usually associated with harder materials. Crystals formed during the crystallization experiments for 90 min and crystals formed during the tempering at 5 °C are responsible for these textural properties. Micrographs reported in this study show that HIU induces crystallization in the samples; however, the long term effect of sonication during tempering at lower temperatures is unknown. That is, for samples crystallized at intermediate temperatures (28 and 30 °C) crystallization might continue during the tempering step and therefore, bigger crystals are obtained without showing the consequent increase in sample texture. For low crystallization temperatures, 26 °C, the high supercooling and HIU application resulted in very fast crystallization during the first 90 min allowing samples to reach equilibrium during this time. Therefore, no additional crystallization occurs during storage at 5 °C and differences in microstructure and therefore texture are observed after 48 h of storage at 5 °C. Similarly, when samples were crystallized at high temperatures, 32 °C, HIU induced crystallization but the low supercooling in the system did not allow for further crystallization to occur. When the samples are stored at lower temperatures, the liquid material in the sample crystallizes rapidly forming several small crystals. These combined effects result in a harder crystal network.
The thermal behavior of the crystallized material after 90 min at $T_c$ and after tempering for 48h at 5 °C was evaluated using DSC, as described in Material and Methods. Figure 3-6 shows the melting profile of the samples crystallized at different crystallization temperatures with and without HIU application after 90 min (Figure 3-6 A-D) into the crystallization process and after tempering for 48h at 5 °C (Figure 3-6 E-F). Figure 3-6 A-D show a single melting peak characterized by a peak temperature of $43.2 \pm 1.5$ °C. When samples where crystallized with HIU the melting peaks became broader with an evident shoulder at lower temperatures. This is more evident for samples that were crystallized with HIU under the $f_c$ condition. These findings suggest that HIU might induce a slight fractionation of the crystals network. As expected, after tempering the samples at 5 °C for 48 h, two melting peaks were observed (Figure 3-6 E-H). The first melting peak on the left shows the secondary crystallization after tempering with a melting temperature of $19.4 \pm 1.6$ °C. The second melting peak represents crystals formed during the 90 min crystallization process and the re-crystallization or crystal growth during tempering with a melting temperature of $43.3 \pm 2.1$ °C. The fractionation described for the melting profiles observed after 90 min at $T_c$ (Figure 3-6 A-D) is still observed after tempering. Melting enthalpies calculated from the endotherm observed after 90 min into the crystallization process values were not significantly different among samples crystallized with and without HIU application. Enthalpy values for samples crystallized at 26 °C without HIU and with HIU (10/10) were $4.39 \pm 0.64$ and $4.36 \pm 0.42$ J/g. Similar results were obtained for samples crystallized at 28 °C with enthalpy values of $4.19 \pm 0.60$ and $4.62 \pm 1.41$ J/g for samples crystallized with HIU (10/10) and without HIU, respectively. Enthalpy values for samples crystallized at 30 °C were slightly lower
with values of $3.78 \pm 1.13$, $4.81 \pm 1.44$, and $3.35 \pm 0.45$ J/g for samples crystallized without HIU, with HIU (10/10) and with HIU (10/13). When samples were crystallized at $32^\circ$C enthalpy values were $4.35 \pm 0.95$, $5.56 \pm 1.18$ and $5.76 \pm 0.88$ J/g, for samples
Figure 3-6. DSC melting profiles of the samples crystallized at different crystallization temperatures with and without HIU application after 90 min (A to D) into the crystallization process and after tempering for 48h at 5 °C (E to H).
crystallized without HIU and with HIU applied at 10 min (10/10) and 20 min (10/20), respectively. Enthalpy values were also calculated after samples were tempered for 48 h at 5 °C. The second endotherm was used to calculate this parameter. As previously described, no significant differences were found for the enthalpy values among samples with and without HIU application. Enthalpy values for samples crystallized at 26 °C were 6.96 ± 0.61 and 7.19 ± 1.28 J/g for samples crystallized without HIU and with HIU, respectively. Enthalpy values for samples crystallized at 28 °C were 6.64 ± 0.89 and 7.39 ± 0.71 J/g for samples crystallized with HIU and without HIU, respectively. Similarly, enthalpy values for samples crystallized at 30 °C were 6.31 ± 1.34, 6.86 ± 0.47, and 5.84 ± 0.94 J/g for samples crystallized without HIU, with HIU (10/10) and with HIU (10/13). Finally, when samples were crystallized at 32 °C enthalpy values were 5.66 ± 0.74, 5.71 ± 1.49 and 5.67 ± 0.67 J/g for samples crystallized without HIU and with HIU applied at 10 min (10/10) and 20 min (10/20), respectively. This data suggests that further crystallization occurred during the tempering at 5 °C for 48 h as evidenced by the slighter enthalpy values reported. However, no differences were found in the enthalpy values of the samples crystallized with and without HIU in any of the conditions used before and after tempering. These results suggest that the effect of HIU on the crystallization of IESBO is mainly on the generation of smaller crystals but not on the amount of crystallized material.

Figure 3-7 shows the amount of solids remaining at a specific temperature (% solid) as the sample is being heated in the DSC. This figure aids in the quantification of the melting profile of crystals formed during crystallization. Figure 3-7 A-D shows the % solid for the crystal network formed after 90 min at Tc; while Figure 3-7 E-H shows
Figure 3-7. Percentage of remaining solid material as a function of temperature for samples crystallized at different conditions after 90 min (A to D) and after tempering for 48 h at 5 °C (E to H).
the % solid for the crystal network formed after tempering for 48h at 5 °C. Samples crystallized at 30 and 32 °C with HIU had a steeper melting profile. This effect is more evident for the f_c conditions (10/13 and 10/20), while the effect seems to be lost after tempering for 48h at 5 °C for the samples crystallized at 30 °C (Figure 3-7 G) and it is still present for the sample crystallized at 32 °C (Figure 3-7 H). Similar results were reported by Suzuki et al. (28) in other lipid systems such as anhydrous milk fat and an all-purpose shortening. The steeper melting profiles observed as a consequence of HIU can be a result of the smaller crystals formed during sonication, and therefore a faster melting rate, or to the fractionation reported in Figure 3-6.

Tables 3-4 to 3-7 show the viscoelastic properties of samples crystallized with and without HIU application after 90 min into the crystallization process and after tempering for 48h at 25 and 5 °C. In general, higher storage modulus (G’) values were obtained when agitation was stopped at 10 min and samples were crystallized with HIU, especially when acoustic waves were applied when the first crystals were observed. Similar tendencies to the ones observed for G’ were observed for the loss modulus (G’”) values. Table 3-4 shows the storage (G’) and loss modulus (G’”) values for samples crystallized at different temperatures with and without the use of HIU of the crystal network formed after 90 min at T_c. When HIU was applied at 10 min (10/10) and samples were crystallized at 26, 28, 32 °C, both G’ and G’” increase consistently with a larger increase in G’ compared to that observed for G’”. The biggest differences in G’ were observed for samples crystallized at 26 and 32 °C in accordance with the previously discussed TPA data, where smaller differences in hardness were observed for samples crystallized at 28 and 30 °C (Figure 3-5). At 26 °C, G’ increases from 391.5 Pa to 1,839 Pa; while G’”
Table 3-4: Viscoelastic parameters \((G', \text{ and } G'')\) of IESBO crystallized at different temperatures (26, 28, 30, and 32 °C) for 90 min. N/A: Not applicable. Table shows mean values and standard deviations. Superscripts with the same letter within a column indicates that values are not significantly different \((\alpha = 0.05)\).

<table>
<thead>
<tr>
<th></th>
<th>26 °C</th>
<th>28 °C</th>
<th>30 °C</th>
<th>32 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(G') (Pa)</td>
<td>(G'') (Pa)</td>
<td>(G') (Pa)</td>
<td>(G'') (Pa)</td>
</tr>
<tr>
<td>10/wo</td>
<td>391.5 ± 27.0(^a)</td>
<td>48.8 ± 4.9(^a)</td>
<td>470.9 ± 307.0(^ab)</td>
<td>47.5 ± 19.5(^a)</td>
</tr>
<tr>
<td>10/10</td>
<td>1839.0 ± 684.5(^b)</td>
<td>176.6 ± 23.1(^b)</td>
<td>1564.0 ± 19.8(^bc)</td>
<td>154.3 ± 2.6(^b)</td>
</tr>
<tr>
<td>10/13</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10/20</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5/wo</td>
<td>248.7 ± 82.7(^a)</td>
<td>31.9 ± 3.8(^a)</td>
<td>1809.5 ± 413.6(^c)</td>
<td>125.0 ± 22.5(^b)</td>
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<tr>
<td>5/5</td>
<td>315.1 ± 55.6(^a)</td>
<td>36.5 ± 5.3(^a)</td>
<td>535.9 ± 129.4(^b)</td>
<td>50.0 ± 1.9(^a)</td>
</tr>
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Table 3-5: Viscoelastic parameters \((G', \text{ and } G'')\) of IESBO crystallized at different temperatures (26, 28, 30, and 32 °C) for 90 min and tempered at 5 °C for 48 h. N/A: Not applicable. Table shows mean values and standard deviations. Superscripts with the same letter within a column indicates that values are not significantly different \((\alpha = 0.05)\).

<table>
<thead>
<tr>
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<th>28 °C</th>
<th>30 °C</th>
<th>32 °C</th>
</tr>
</thead>
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<tr>
<td></td>
<td>(G') (x 10^5 Pa)</td>
<td>(G'') (x 10^5 Pa)</td>
<td>(G') (x 10^5 Pa)</td>
<td>(G'') (x 10^5 Pa)</td>
</tr>
<tr>
<td>10/wo</td>
<td>2.6 ± 2.5(^bc)</td>
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<td>9.0 ± 0.3(^ab)</td>
<td>1.3 ± 0.6(^a)</td>
</tr>
<tr>
<td>10/10</td>
<td>11.5 ± 0.2(^b)</td>
<td>1.84 ± 0.03(^b)</td>
<td>10.8 ± 1.0(^a)</td>
<td>1.8 ± 0.1(^a)</td>
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<td>N/A</td>
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</tr>
<tr>
<td>10/20</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5/wo</td>
<td>7.4 ± 1.1(^bc)</td>
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<td>6.0 ± 1.1(^b)</td>
<td>0.9 ± 0.3(^a)</td>
</tr>
<tr>
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<td>4.8 ± 0.5(^c)</td>
<td>0.7 ± 0.1(^a)</td>
<td>11.59 ± 0.01(^a)</td>
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</tbody>
</table>
**Table 3-6:** Viscoelastic parameters (G', and G'') of IESBO crystallized at different temperatures (26, 28, 30, and 32 °C) for 90 min and tempered at 25 °C for 48 h. N/A: Not applicable. Table shows mean values and standard deviations. Superscripts with the same letter within a column indicates that values are not significantly different (α = 0.05).

<table>
<thead>
<tr>
<th></th>
<th>26 °C</th>
<th>28 °C</th>
<th>30 °C</th>
<th>32 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G' (Pa)</td>
<td>G'' (Pa)</td>
<td>G' (Pa)</td>
<td>G'' (Pa)</td>
</tr>
<tr>
<td>10/wo</td>
<td>39.4 ± 2.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.6 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>43.6 ± 28.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.8 ± 3.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10/10</td>
<td>2595.5 ± 234.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>297.1 ± 50.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>532.2 ± 83.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>109.1 ± 9.0&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10/13</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10/20</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5/wo</td>
<td>61.6 ± 34.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.5 ± 3.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>56.8 ± 14.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>5/5</td>
<td>117.0 ± 31.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>28.5 ± 7.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.6 ± 3.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>11.0 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

**Table 3-7:** Zero-rate viscosity values (Pa·s x 10<sup>6</sup>) of IESBO crystallized at different temperatures (26, 28, 30, and 32 °C) for 90 min and tempered at 25 °C for 48 h. N/A: Not applicable. Table shows mean values and standard deviations. Superscripts with the same letter within a column indicates that values are not significantly different (α = 0.05).

<table>
<thead>
<tr>
<th></th>
<th>26 °C</th>
<th>28 °C</th>
<th>30 °C</th>
<th>32 °C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>90 min</td>
<td>48 h @ 25 °C</td>
<td>90 min</td>
<td>48 h @ 25 °C</td>
</tr>
<tr>
<td>10/wo</td>
<td>0.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.12 ± 0.03&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.6 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3.8 ± 0.9&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>10/10</td>
<td>2.3 ± 0.3&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.8 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.1 ± 0.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>1.0 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>10/13</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>10/20</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>5/wo</td>
<td>0.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.4 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.26 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.3 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>5/5</td>
<td>0.71 ± 0.02&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.02 ± 0.05&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.47 ± 0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.7 ± 0.1&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
increases from 48.8 Pa to 176.7. When HIU was applied at 20 min at 32 °C, the most significant increase in G’ and G” was observed. Values of G’ increased from 195 Pa to 4,229 Pa, with more than 20 times increase; and G” increases 7 times from 20 Pa to 160 Pa. The value of G’ for HIU applied at 20 min is significantly larger than that for HIU applied at 10 min (P < 0.05), this data also supports the hypothesis that HIU is more effective when it is applied in the presence of crystals.

Table 3-5 shows the variations of G’ and G” of samples with and without HIU application after tempering the samples for 48h at 5 °C. Tempering samples for 48h at 5 °C increases G’ values significantly compared to the values obtained after 90 min into the crystallization process. This is an expected result since crystallization of low melting triacylglycerols occurs at this lower temperature and therefore harder materials are expected. As described for data presented in Table 3-4, G’ and G” values were higher when samples were crystallized with HIU, especially when 26 and 32 °C were used as crystallization temperatures. Interestingly, during tempering at 5 °C, samples crystallized at 30 °C with HIU application showed slightly higher elastic properties (higher G’) than the sample crystallized at the same temperature but without HIU. These data are in accordance with the hardness values discussed in Figure 3-5 and suggest that different re-crystallization mechanisms occur during the tempering process when HIU is applied at this Tc.

Shortening producers use different tempering profiles before the product reaches the consumer. Therefore, a second tempering procedure was tested to evaluate the effect of sonication on the viscoelastic properties of the material. Table 3-6 shows viscoelastic parameters (G’ and G”) for samples tempered for 48 h at 25 °C. In general, G’ and G”
values were lower than the ones reported in Table 3-4 and 3-5, suggesting that crystal reorganization occurs during tempering with the generation of less solid-like structures. Nevertheless, significant differences between sonicated and non-sonicated samples still exist. It is interesting to note that the biggest differences were found in samples that were crystallized using HIU with significantly higher values observed for samples crystallized at 26, 28, and 32 °C when agitation was stopped at 10 min and HIU was applied in the presence of crystals. No significant differences were found in the samples where agitation was stopped at 5 min in any of the conditions tested. Based on these observations, we proposed that HIU might have the function of stabilizing small lipid crystals when stored at room temperature, which is good evidence for industry production of lipid-based food.

Table 3-7 shows the zero-rate viscosity of samples after 90 min into the crystallization process and after tempering at 25 °C for 48 h. Viscosity of samples tempered at 5 °C was not measured since these were too hard to perform this type of measurement. Viscosity values show tendencies similar to those described for G’ and G”, suggesting that HIU application generated crystal networks with higher viscosity. As previously discussed, the effect of HIU in increasing sample viscosity was more significant when samples crystallized at 26, 28, and 32 °C for 90 min and when HIU was applied in the presence of crystals. It is interesting to note that the viscosity of some of the samples increased during tempering at 25 °C for 48 h. In general, samples with high viscosity values after 90 min (samples crystallized with HIU application in the presence of crystals) showed a slight decrease in viscosity with tempering, while those samples with low viscosity values after 90 min showed an increased viscosity after tempering.
For example, values of viscosity for samples crystallized without HIU at 28 °C increased after tempering. Similarly, samples crystallized at 32 °C with HIU applied at 10 min increased significantly after tempering compared to the values after 90 min crystallization. On the other hand, samples crystallized at 30 °C with HIU applied when the first crystals were observed (10/13) had a significantly lower viscosity after tempering for 48 h at 25 °C. These results suggest that different mechanisms of re-crystallization occur during tempering of samples treated with different HIU conditions.

XRD was performed in samples crystallized at 32 °C with and without the use of HIU after 90 min into the crystallization process and after tempering for 48h at 5 and 25 °C. Only these conditions were tested since they are the ones that show more effect of sonication on the crystallization behavior of the IESBO. In all cases, the XRD patterns were characteristic of the $\beta'$-form with two strong signals at 3.8 and 4.3 Å. No signal at 4.6 Å, characteristic of the $\beta$-form, was found. This finding suggests that changes observed in this study are not due to different polymorphic forms generated by sonication and/or storage.

In summary, this research shows that HIU can modify the crystallization behavior of a low saturated shortening (IESBO). Specific changes include the induction in the onset of crystallization, reduction of crystal size and the generation of harder and more elastic materials. The effectiveness of this novel technology depends on the acoustic power used and on several processing conditions, such as crystallization temperature and application time. HIU induces primary and secondary nucleation and might also promote crystal growth. In addition, results show that HIU is more efficient when it is applied in the presence of crystals or nuclei. These results suggest that HIU can be used as an
additional tool to improve the crystal network of lipids with low contents of saturated fatty acids, by modifying the physicochemical characteristics of the material such as texture and viscoelasticity. HIU can not only induce the crystallization and promote the growth of crystals, but also has the function of stabilizing the crystals formed as evidenced in the tempering experiments.

References


(20) McCausland, L. J. Production of crystalline materials by using high intensity ultrasound. 2007.


Abstract

The effect of power ultrasound on physical and chemical properties of low saturated shortening (interesterified soybean oil; IESBO) was investigated. IESBO was crystallized at 32 °C and sonicated for 10 sec with acoustic power of 101 W as described in chapter 3. After sonication, solid fractions of the samples were filtered and tested for melting behavior and chemical composition and compared to those of non sonicated IESBO to determine physical and chemical changes originated as a consequence of sonication. Application of HIU affected the crystals size and interconnections, the melting behavior, but did not affect its chemical composition in terms of TAGs and FAMEs.

Introduction

In Chapter 3, the effect of HIU on the crystallization behavior of interesterified soybean oil (IESBO) was discussed. In short, HIU generated a harder material by forming smaller crystals without changing the amount of crystalline material in the sample. Crystallization temperature, power intensity of ultrasound, and sonication time affected the physical characteristics of the sonicated fats. The effect of HIU in changing the crystallization behavior of lipids is mediated by the formation of cavities. Several studies investigated the chemical composition and off-flavors of fat samples processed
with HIU. Patrick et al. proposed that ultrasound generates off-flavors in sunflower oil.\textsuperscript{1} Chemat et al. also suggested that HIU generates off-flavors and slight chemical composition changes, such as hexanal and hept-2-enal in the sonicated oils.\textsuperscript{2,3} In this case, it is important to study the effect of HIU on both physical and chemical properties after HIU was applied. It is necessary to point out that the studies mentioned above used ultrasound for long periods of time (0.5 to 30 min at 150 W) to show off-flavors generation and other chemical compositions change in the lipid system, it cannot represent the actual conditions used in our studies (10 sec at 110 W) to change the physical properties of lipids.

The objective of this research is to quantify the effect of HIU on the physical and chemical properties of IESBO samples. Results in melting behavior, TAG compositions, and fatty acid methyl esters chemical composition will be discussed.

**Material and Methods**

**Crystallization experiments**

IESBO was heated to 80 °C and kept at this temperature for 30 min to allow complete melting of the triacylglycerols. The melted lipid sample was then placed in a thermostated crystallization cell as described in Chapter 3 which was set at crystallization temperature of 32 °C. IESBO was crystallized at a cooling rate of 5°C/min with agitation using a magnetic stirrer (200 rpm) to increase the heat transfer between the sample and external circulating water. HIU was applied for 10 s (Misonix S-3000 sonicator, Misonix Inc., NY) using 110W of acoustic power at 20 min into the crystallization experiments. The sonicator operates at an acoustic frequency of 20 kHz.
and a micro-tip of 3.2 mm diameter was used. Before HIU was applied, agitation was stopped at 10 min to avoid dissolution of bubbles generated during the sonication process. After sonication, the sample was kept at crystallization temperature for 90 min to allow complete crystallization. This was demonstrated by the lack of crystal change as observed in the polarized light microscope. After 90 min of crystallization, IESBO crystals were separated from the liquid oil by filtration under vacuum. The melting profile, TAG and FAME chemical composition were determined in the crystals (solid fractions) of the IESBO samples crystallized with and without HIU.

**Physical properties of the solid fractions**

*Scanning electron microscopy (SEM)*

Scanning electron microscopy (SEM) was used to determine the crystals image for IESBO solid fractions with and without HIU. After 90 min at crystallization temperature, crystals were filtered and spread lightly on a glass coverslip with a stainless spatula followed by a layer of 15-nm gold coating before introduced into the Hitachi S4000 scanning electron microscope. The images were obtained at an acceleration voltage of 20 kV.

*Melting profile*

A differential scanning calorimeter (DSC, DSC 2910, TA Instrument, USA) was used to evaluate the melting behavior of the crystalline material. After 90 min in the crystallization cell, 5-15 mg of solid fractions of IESBO was placed in a hermetic aluminum pan for DSC use. The sample was heated to 80 °C with a ramp rate of 5 °C/min to evaluate the melting behavior of crystallized material. Through this procedure,
the onset temperature ($T_{on}$), peak temperature ($T_p$), and melting enthalpy ($\Delta H$) were determined.

**Chemical properties of the solid fractions**

*TAG chemical composition*

Triacylglycerol (TAG) chemical composition was determined using AOCS official Method Ce 5b-89. Composition was expressed as the percentage of equivalent carbon number (ECN) molecular entities as described in the method.

*Fatty acid methyl esters (FAME) chemical composition*

FAME was analyzed using AOCS official methods: Fatty Acids-Full Omega 9,6&3 & Trans %W/W (AOCS) - AOCS Ce 2-66 AOCS Ce 1-62.

**Results and Discussion**

*Scanning Electron Microscopy*

Figure 4-1 shows SEM of IESBO crystals morphology for IESBO crystallized at 32 °C with and without HIU. Results showed the formation of smaller crystals and a more interconnected crystalline lattice in the sonicated samples. This was the same as the results we found in the whole IESBO samples as described in Chapter 3.
Melting behavior

The melting profile of the IESBO solid fractions crystallized with and without HIU is shown in Figure 4-2. A sharper melting profile for the sample crystallized with HIU is observed. The melting profile observed for the solid fractions of the sonicated sample is much narrower than the ones observed for the non-sonicated one. Table 4-1 confirms this observation where narrower and sharper melting profiles are represented by a higher $T_{on}$ for the sonicated sample and similar $T_p$ values. The enthalpy values for samples with HIU application are significantly ($P < 0.05$) higher than the ones for non-sonicated samples.
Figure 4-2: Melting profile of the IESBO solid fractions crystallized with and without HIU.

Table 4-1: Melting behavior of the IESBO solid fractions crystallized with and without HIU. Values in the same row with the same superscript are not significantly different ($\alpha = 0.05$).

<table>
<thead>
<tr>
<th>Melting Parameter</th>
<th>Wo HIU</th>
<th>With HIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{on}$ (°C)</td>
<td>$44.8 \pm 0.1^a$</td>
<td>$49.4 \pm 0.7^b$</td>
</tr>
<tr>
<td>$T_p$ (°C)</td>
<td>$52.1 \pm 0.5^a$</td>
<td>$53.1 \pm 0.1^a$</td>
</tr>
<tr>
<td>$\Delta H$ (J/g)</td>
<td>$27.8 \pm 1.2^a$</td>
<td>$39.2 \pm 2.4^b$</td>
</tr>
</tbody>
</table>

Chapter 3 indicated that both samples with and without HIU generated the $\beta'$ polymorphic form, which means the subcell structure is the same for both samples, therefore, the sharper melting profile with higher enthalpy generation might be due to other reasons. Sato et al. suggested that melting behavior might be affected by the atomic level of crystal structures in term of methyl chain end-stacking, subcell packing, and glycerol conformations. Our results suggest that the application of HIU might change
those crystal structures described by Sato et al. and lead to the change in melting behaviors. The sharper melting profile generated by HIU could either be a result of crystal re-arrangement at the atomic/molecular level, and/or the changes in chemical compositions (TAGs or fatty acids). Therefore, analysis on chemical compositions is required to further determine the effect of HIU on changes of melting profiles.

**TAG chemical composition**

The TAG chemical composition of the IESBO solid fractions is shown in Table 4-2. The chemical composition was expressed as percentage of ECN, where ECN = CN - 2\(n\); CN is the TAG carbon number, and \(n\) is the number of unsaturations. No significant difference (\(\alpha = 0.05\)) was found for the TAG compositions between solid fractions with and without HIU. This indicates that the “disruptive” effect of HIU did not lead to any TAG changes, such as breakdown of carbon chain. When compared to the TAG chemical composition of IESBO whole sample in Chapter 3, the only difference that was found is in the composition of TAGs with 48 ECN (whole sample : solid fractions 50% : 45%). TAG of 48 ECN includes OOO, SOL, and SSLn, where O refers to oleic acid, S refers to stearic acid, L refers to linoleic acid, and Ln refers to linolenic acid.

The 5% difference of TAG group with 48 ECN between whole sample and solid fractions might come from the OOO TAG which has the lowest melting point among other TAGs. Other TAG groups with different ECN were already discussed in Chapter 3.

**FAME chemical composition**

Table 4-3 represents the FAME composition of IESBO solid fractions with and without HIU. No significant difference (\(P > 0.05\)) was found between samples with and
Table 4-2: TAG chemical composition (expressed as the ECN) of the IESBO solid fractions with and without HIU; ECN = CN – 2n, where CN is the total carbon number of the TAG and n is the total number of unsaturations. No significant difference ($\alpha = 0.05$) was found for the TAG compositions between two solid fractions.

<table>
<thead>
<tr>
<th>ECN</th>
<th>Wo HIU</th>
<th>With HIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>0.13 ± 0.06</td>
<td>0.15 ± 0.02</td>
</tr>
<tr>
<td>40</td>
<td>2.43 ± 0.52</td>
<td>2.13 ± 0.49</td>
</tr>
<tr>
<td>42</td>
<td>9.15 ± 1.68</td>
<td>8.54 ± 1.47</td>
</tr>
<tr>
<td>44</td>
<td>15.55 ± 2.73</td>
<td>14.92 ± 1.58</td>
</tr>
<tr>
<td>46</td>
<td>23.62 ± 0.93</td>
<td>25.35 ± 0.80</td>
</tr>
<tr>
<td>48</td>
<td>44.81 ± 4.10</td>
<td>44.84 ± 4.18</td>
</tr>
</tbody>
</table>

Table 4-3: Fatty Acid Methyl Esters (FAME) compositions of the IESBO solid fractions with and without HIU were reported. Table below only reports the fatty acid composition which is above 0.1%. No significant difference ($\alpha = 0.05$) was found for the FAME compositions between two solid fractions.

<table>
<thead>
<tr>
<th>FAME</th>
<th>Wo HIU</th>
<th>With HIU</th>
</tr>
</thead>
<tbody>
<tr>
<td>C16:0</td>
<td>11.62 ± 0.01</td>
<td>11.84 ± 0.00</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.17 ± 0.00</td>
<td>0.17 ± 0.00</td>
</tr>
<tr>
<td>C18:0</td>
<td>28.14 ± 0.02</td>
<td>28.94 ± 0.01</td>
</tr>
<tr>
<td>C18:1</td>
<td>14.41 ± 0.04</td>
<td>14.09 ± 0.01</td>
</tr>
<tr>
<td>C18:2</td>
<td>32.97 ± 0.01</td>
<td>31.74 ± 0.04</td>
</tr>
<tr>
<td>C18:3</td>
<td>5.04 ± 0.00</td>
<td>4.79 ± 0.01</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.44 ± 0.00</td>
<td>0.45 ± 0.00</td>
</tr>
<tr>
<td>C20:1</td>
<td>0.54 ± 0.00</td>
<td>0.52 ± 0.00</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.37 ± 0.00</td>
<td>0.38 ± 0.00</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.12 ± 0.00</td>
<td>0.12 ± 0.00</td>
</tr>
<tr>
<td>Total</td>
<td>94.08 ± 0.08</td>
<td>93.30 ± 0.04</td>
</tr>
</tbody>
</table>
without sonication. Results indicate no changes on the fatty acids level, such as breakdown of fatty acid carbon chains, and saturation on unsaturated fatty acids.

To summarize the TAGs and FAMEs chemical composition analysis, conclusions can be made that HIU used in Chapter 3 did not change the chemical composition of the samples and therefore, changes observed in the melting behavior of the samples as a consequence of sonication are due to the different crystallization behavior of the same molecular entities, such as the crystals size, crystal arrangement, and crystal aggregates interconnections.

**Conclusion**

This research shows that HIU can change crystal morphology by generating smaller crystals and a more interconnected lattice network. Melting behavior of the sample was also affected by HIU through the generation of a more uniform crystal structure that results in a sharper melting profile. Studies on TAG and FAME chemical compositions showed no difference before and after HIU application. It would be the best if off-flavors can be determined in our study, however, due to the limit of instrument access, no measurements were carried out. Further studies on off-flavors can be done to better understand the principles of changes that HIU applied to the lipid system. Overall, this research shows that HIU does not induce any chemical change in the material when applied under the conditions used in these experiments and that these conditions are indeed able to change the physical properties of the sample. Therefore, changes observed in the physical and functional properties of the samples as a consequence of sonication
are due to the different crystallization behavior of the same molecular entities and not to chemical changes.

References


CHAPTER 5
APPLICATION OF HIGH INTENSITY ULTRASOUND TO A ZERO-TRANS SHORTENING DURING TEMPERATURE CYCLING UNDER DIFFERENT COOLING RATES

Abstract

The objective of this work was to evaluate the effect of high intensity ultrasound (HIU) on the physical properties of a commercial shortening crystallized at a constant temperature and during temperature cycling at 2 different cooling rates (0.5 and 1 °C/min). Different ultrasound power levels and different durations were evaluated during crystallization at a constant temperature and the best conditions were used to evaluate the effect of HIU during temperature cycling. The physical properties tested were crystal microstructure, viscoelasticity, and melting profile. Results show that HIU is more efficient at changing crystal microstructure when used at 20 °C using a 1/2” tip. No difference was found on the microstructure of the crystals formed when different durations of ultrasound exposure were tested. A significant increase (p < 0.05) was observed in the storage modulus (G’) of the lipid exposed to temperature fluctuations with the use of HIU. The G’ values increased from 662.6 ± 176.8 Pa (no HIU applied) to 3,365.5 ± 426.4 Pa (with HIU applied, 0.5°C/min) and from 354.4 ± 49.7 Pa (no HIU

1 Springer and original publisher /Journal of American Oil Chemists’ Society, Volume 91, July 2014, pp 1155-1169, Application of High Intensity Ultrasound to a Zero-trans Shortening During Temperature Cycling at Different Cooling Rates, Ye, Y.; Tan, C. Y.; Kim, D. A.; Martini, S., original copyright notice is given to the publication in which the material was originally published, by adding; with kind permission from Springer Science and Business Media.
applied) to $1,249.0 \pm 19.8$ Pa (with HIU applied, $1 \, ^\circ\text{C/min}$).

**Introduction**

The type, amount, and morphology of lipid crystals formed during processing determine the physical and sensory properties of fat-based foods [1, 2]. Hence, it is of great importance to understand and control lipid crystallization to optimize overall product quality [3, 4]. Common processing parameters that can be used to control lipid crystallization include the use of different crystallization temperatures, or supercoolings [5, 6], the addition of seed crystals [7, 8] to the system, the use of emulsifiers [9, 10], and the use of different cooling rates [11]. Significant amount of research has been performed to find new, effective, and innovative ways to control lipid crystallization processes. Power ultrasound (or high intensity ultrasound, HIU) has been used to control crystallization since the early 1900’s where this technique was used to enhance crystallization in supersaturated thiosulphate solutions [5]. Since then HIU has been used to induce crystallization of organic molecules [6, 12, 13] with the formation of crystals with a narrower crystal size distribution and a more uniform shape compared to conventional seeding. In addition, several research have reported the use of HIU to control crystallization in different systems related to food processing such as water [14], sugar [15, 16], and lipids [17-19]. In particular, HIU was recently used to induce the crystallization in edible lipids. Early work by Higaki et al. [20] showed that HIU induced the crystallization of tripalmitin as evidenced by a shorter induction time when the samples were crystallized in the presence of ultrasound and promoted the formation of $\beta$ crystals, especially at high crystallization temperatures. For example, induction times of
the samples were reduced from 100 s to approximately 28 s for the non-sonicated and sonicated samples, respectively. In addition, these authors showed that ultrasound promoted the crystallization of cocoa butter in its most stable polymorphic form (form V). Patrick et al. [21] used HIU to induce the crystallization of palm oil under non-isothermal conditions. When palm oil was cooled without the use of ultrasound, crystallization occurred during cooling when the sample reached 26 °C. An induction in the crystallization was observed when HIU was applied and the first crystals were formed at 36 °C. These authors also reported the generation of smaller crystals as a consequence of sonication. Recently Chen et al. [22] showed that HIU decreased the induction time of crystallization in palm oil, especially when crystallized at high temperatures (induction times decreased from 21 min to 8 min for the non-sonicated and sonicated samples, respectively) and also increased the crystallization rate of the fat as measured by SFC values. Previous research in our laboratory has extended the use of HIU in other edible fats such as anhydrous milk fat [17], an all-purpose shortening [19], and an interesterified soybean oil [18]. In all cases, depending on the HIU power and processing conditions used, harder and more elastic materials are obtained with the use of HIU. In particular, results reported by Ye et al. [18] show that HIU generated smaller crystals and formed more elastic crystalline networks. As an example, G’ values of the non-sonicated and sonicated samples were 195 Pa and 4,200 Pa, respectively. These differences in crystal morphologies and on viscoelastic properties were maintained even after tempering the samples under different conditions (48 h at 25 °C or 5 °C). These results suggest that HIU could be used to generate a crystalline network that will “resist” temperature fluctuations that might occur during the processing or transportation of the shortening. Even though a
significant amount of studies report the use of HIU to change the crystallization behavior of edible lipids in terms of their microstructure, polymorphism, and physical properties, the effect of using different processing conditions such as tip size, HIU short exposure, and temperature fluctuations was not explored.

The aim of this work was to evaluate the use of HIU in a commercial shortening during crystallization at a constant temperature and during temperature cycling. Different power levels (generated by different tip sizes) and different ultrasound durations were tested to optimize processing conditions during crystallization at a constant temperature (without temperature cycling). The effect of HIU on samples subjected to temperature cycling was evaluated using fast (1 °C/min) and slow (0.5 °C/min) cooling rates. Different physical and functional properties of the shortening, such as microstructure, melting profiles, and viscoelasticity were measured. The novelty of this research is to compare the effect of HIU on lipid crystallization as affected by specific processing conditions such as tip size, HIU short exposure times, and temperature fluctuation.

Material and Methods

Sample: A multi-purpose shortening with no trans-fats was used for these experiments (IOI Group, Loders Croklaan, Channahon, IL). The sample is a bakery shortening based on palm oil and palm oil fractions with approximately 49.9 ± 0.3% of saturated fatty acids, 40.1 ± 0.5% of mono-unsaturated fatty acids, and 9.6 ± 0.3% of poly-unsaturated fatty acids. The melting point of the sample was determined using AOCS Official Method Cc 1-25.

Developing insonation conditions: Different insonation conditions (power levels
and durations) were tested in the shortening crystallized at $T_c = 20^\circ$C and $30^\circ$C. One hundred grams of sample was melted in the microwave and left in an oven at 75-80°C for 30 min to completely erase crystal memory. Melted samples were placed in a double-walled crystallization cell [17-19] which was attached to a water bath to allow for temperature control. The water bath was set at two specific crystallization temperatures ($T_c = 20^\circ$C or $30^\circ$C). After placing the samples in the crystallization cell, temperature decreased exponentially (data not shown) from approximately $70^\circ$C to $T_c$. The temperature in the samples was recorded during the entire experiment using a thermocouple. Samples were crystallized under agitation using an impeller set at 100 rpm for a total time of 60 min. Time zero corresponds to the moment that the sample is placed in the crystallization cell.

Based on previous research in our laboratory [18], HIU was applied at 5 min for samples crystallized at $20^\circ$C and at 20 min for samples crystallized at $30^\circ$C. These times correspond to the onset of crystallization for each $T_c$, where a slight turbidity is observed in the sample by the naked eye. HIU (20 kHz) was applied using a 1/2” and a 1/8” horn to achieve different power levels (108 µm and 216 µm tip amplitude, respectively, higher tip amplitude indicates higher power level). Acoustic power levels generated by these tips were calculated as described by Martini et al. [23, 24]. Ultrasound was applied using a Misonix S-3000 generator (Misonix Inc., NY) for 10, 5, and 2.5 seconds.

**Temperature cycling experiments:** Temperature cycles were designed to simulate temperature fluctuations that could occur during shortening production and/or distribution. In addition to temperature cycles, two different cooling rates (slow cooling rate $= 0.5$ °C/min and fast cooling rate $= 1$ °C/min) were also tested. Figure 5-1 shows the
temperature cycling used in these experiments and the moment at which HIU was applied to the sample. The following steps provide further explanation:

1. \( t = 0 \) to 60 min: 100 g of melted (\( T \sim 70 ^\circ C \)) sample crystallized at \( T_c = 20 ^\circ C \) for 60 min (without HIU or with HIU applied at 5 min)

2. \( t= 60 \) min to 70 min: increase the water bath temperature from 20 \( ^\circ C \) to 30 \( ^\circ C \)

3. \( t= 70 \) min to 100 min: isothermal at 30 \( ^\circ C \) for 30 min

4. \( t= 100 \) min to 120 min (or 110min for 1 \( ^\circ C/\)min): Cooling from 30 \( ^\circ C \) to 20 \( ^\circ C \) (0.5 \( ^\circ C/\)min).

5. \( t= 120 \) min to 180min (or from 110 min to 170 min for 1 \( ^\circ C/\)min cooling rate): isothermal at 20 \( ^\circ C \) for 60 min (similar to step 1, without HIU or with HIU applied at 125 min / 115 min for 1 \( ^\circ C/\)min cooling rate)

**Microstructure:** Crystal morphology was recorded during crystallization. A drop of lipid sample was taken from the crystallization cell at different time points and placed between a slide and cover-slide, that were kept at crystallization temperature in an incubator, to evaluate the microstructure variation using a polarized light microscope (PLM, Olympus BX 41, America Inc., Melville, N.Y., U.S.A.) equipped with a digital camera (Lumenera Scientific, Infinity 2, Ottawa, Ontario, Canada). A 20x magnification objective was used. Pictures were taken as soon as the sample was placed in the slide and cover slide to avoid morphology changes during this process.
Figure 5-1: Temperature cycling used in this research. The number tagged in each point represents the time (min); the arrows indicate the moment that HIU was applied; and the numbers (1 ~ 5) show the steps of temperature cycling as described in the text.

**Image Analysis on PLM pictures**: All the PLM pictures were analyzed by ImageJ (ImageJ 1.47 bundled with 64-bit Java for Windows). Quantification of cluster morphology was performed using the total area covered by the clusters and the average cluster area (µm²). The cluster area distribution was also reported. Image analysis was performed using several pictures taken for each time point and from at least 2 independent runs. A threshold of 5 µm² was used.

**Solid Fat Content (SFC)**: The SFC of the shortening during crystallization was measured by a NMR 120 Minispec NMR analyzer (Bruker, Germany) using AOCS direct method [25]. During early stage of the crystallization process, samples were loaded from
the crystallization cell into the NMR tube using a Pasteur pipette and the SFC was measured as soon as possible every minute. At later stages of the crystallization process (after 30 min), SFC was measured every 5 min. Before loading the sample, NMR tubes were kept at crystallization temperature in an incubator.

**Thermal behavior:** The thermal behavior of the crystalline network was analyzed using a differential scanning calorimeter (DSC, Q20 TA Instruments, New Castle, DE). After the whole crystallization process, 5 to 15 mg of sample was placed into a hermetic aluminum pan. Samples were then heated from $T_c$ to 80 °C at 5 °C/min. Onset ($T_{on}$) and peak ($T_p$) temperatures, and melting enthalpy were calculated from the melting profile by using the TA Instruments Universal Analysis 2000 (TA Instruments, DE). Peak temperatures (°C) represent the temperature at which the melting peak reaches its minimum, while onset temperatures (°C) are defined as the temperature at which the first deviation from the baseline is observed, which corresponds to the first melting processes in the sample. Melting enthalpies (J/g) are calculated through integration of the area under the melting peak and are used to quantify the amount of solid fat present in the crystalline network.

**Viscoelastic properties:** The viscoelastic properties of the crystallized material were evaluated by using a AR-G2 rheometer (TA Instruments, New Castle, DE). A strain sweep from 0.0008 to 10% with a constant frequency of 1 Hz (6.28 rad/s) was used to evaluate the elastic ($G'$) and viscous ($G''$) behavior of the samples. This strain sweep corresponds to the linear viscoelastic region of the samples tested. The viscoelastic properties of the samples were measured after the crystallization process (60 min for the constant temperature experiments, 170 min [1 °C/min cooling rate] or 180 min
[0.5 °C/min cooling rate] for the temperature cycling experiments) and after tempering the samples for 48 h at 25 °C. Viscoelasticity was measured in duplicate in two independent runs.

Statistical Analysis: Crystallization experiments were performed in duplicate and physicochemical properties were measured in duplicate. Significant differences (P < 0.05) were evaluated using Two-way ANOVA using GraphPad Prism software, version 6.00 for Windows, GraphPad Software, San Diego, CA, USA.

Results and Discussion

Sample characteristics: Sample melting point was 37.3 ± 0.5 °C and therefore crystallization temperatures of 20 and 30 °C were used in our experimental design. These two temperatures were chosen to generate enough supercooling in the sample and therefore induce sample crystallization. In addition, they represent commonly crystallization temperatures used for shortening formulation (T_c = 20 °C) with similar temperature cycles experienced by the sample (T_c = 20 and 30 °C) within an industrial process.

Section I: Evaluate the effect of HIU application to the lipid system

Effect of power level on lipid microstructure: Previous research in our laboratory [17-19] has shown that the application of HIU for 10 sec using a 1/8” tip (216 µm tip amplitude) can change the crystallization behavior of lipids. However, the use of a larger tip and therefore a lower power level was never explored. The first part of this research was to explore the effect of tip size (1/8” vs. 1/2”), and therefore acoustic power, on the crystallization behavior of the shortening. The acoustic power level obtained with the
1/8” tip was 53.9 ± 9.1 W when the ultrasound was applied at the moment when first crystals were observed (20 min at Tc = 30 °C); while the 1/2” tip generated an acoustic power of 99.2 ± 3.0 W. Acoustic power levels were calculated by measuring the increase in temperature generated during sonication [23]. Results show that acoustic power levels obtained with the 1/8” tip are lower than the ones obtained with the 1/2” tip even though the tip amplitude is higher. This is probably due to the large volume used (100 mL) in our experiments. Commercial sonicators like the one used in this research suggest using 1/8” tips with a maximum volume of 15 mL to ensure homogeneous transmission of the acoustic power. It is possible that when larger volumes are used the acoustic power is dissipated resulting in an overall lower power.

Figure 5-2 shows the microstructure of the sample crystallized at 30 °C sonicated for 10 sec using the 1/8” and 1/2” tips. HIU was applied after some crystals were observed in the sample (20 min into the crystallization process) to allow for a significant effect of the ultrasound on the crystallization behavior of the samples [18]. The size of the clusters was quantified with the total area covered by clusters and by the average area of each cluster. No significant difference (p > 0.05) was found between the total area of the clusters as a function of sonication condition. However, as expected, significantly higher (p < 0.05) total area was observed at 60 min compared to the area observed at 25 min. When the average cluster area is analyzed a significant decrease (p < 0.05) was observed as a consequence of sonication after 25 min into the crystallization process. Average cluster areas decreased from 2,797.5 ± 751.5 µm² to 533.8 ± 142.1 and 251.7 ± 49.4 µm² for the non-sonicated and the samples sonicated with the 1/8” and 1/2” tip, respectively (Figure 5-2). No significant difference (p > 0.05) was observed between the
cluster areas of samples sonicated with the smaller (1/8") and larger (1/2") tip.

Differences in cluster areas between the sonicated and non-sonicated samples were not observed (p > 0.05) after 60 min into the crystallization process. Data shown in Figure 5-2 suggest that HIU helps in the formation of smaller clusters which is evident at early stages of the crystallization process and this effect is lost at later stages.

Figure 5-2: Microstructure of crystals obtained for the shortening crystallized at 30 °C and sonicated using a 1/8” and 1/2” tip. HIU was applied after 20 min into the crystallization process for 10 sec. Pictures were taken under the PLM at different time (25 and 60 min) during the crystallization process. Results from image analysis (total area and average area and standard deviations) are also reported.
When samples were crystallized at 20 °C, smaller clusters were observed compared to the sample crystallized at 30 °C due to the higher supercooling (Figure 5-2 and 5-3). As previously described, HIU was applied when the first crystals were observed in the media which corresponds to a time of 5 min into the crystallization experiment. It is evident from Figure 5-3 that HIU promotes crystallization as evidenced by a significant increase in the total area of clusters. The total area of clusters increased significantly (p < 0.05) from 9,233.2 ± 2367.2 µm² to 28,590.1 ± 1270.1 µm² and 32,168.3 ± 439.7 µm² when 1/8” and 1/2” tips ultrasound were used suggesting a promotion of crystallization when HIU is used. It is interesting to note that average cluster areas obtained with HIU were larger than the control, and this difference was statistically significant (p < 0.05) for samples sonicated with the 1/8” tip. This higher cluster area might be a consequence of more agglomeration between clusters resulting in larger areas. However, it is evident from Figure 5-3 that more and smaller clusters are formed as a consequence of sonication. The total area covered by the clusters significantly increased (p < 0.05) after 60 min into the crystallization process only for the samples crystallized without HIU with values increasing from 9,233.2 ± 2367.2 to 27,616.2 ± 545.7 µm² for 15 and 60 min, respectively. Similarly, an increase in cluster area was observed as a function of time for the sample crystallized without HIU, while a decrease in cluster area was observed for samples crystallized with the 1/8” tip and no difference was observed for the samples crystallized with the 1/2” tip.

Results presented in Figures 5-2 and 5-3 suggest that HIU is more efficient at generating smaller clusters when it is applied using the 1/2” tip and when the sample is crystallized at 20 °C. To further evaluate the effect of sonication on the promotion of
crystallization the SFC as a function of time was measured for the conditions reported in Figure 5-3. SFC measurements were only performed at 20 °C since PLM pictures shown in Figure 5-2 suggest that ultrasound is not very efficient at affecting the crystallization behavior of the shortening crystallized at 30 °C and therefore no differences in the SFC as a function of sonication is expected.

**Figure 5-3:** Microstructure of crystals obtained for the shortening crystallized at 20 °C and sonicated using a 1/8” and 1/2” tip. HIU was applied after 5 min into the crystallization process for 10 sec. Pictures were taken under the PLM at different time (15 and 60 min) during the crystallization process. Results from image analysis (total area and average area and standard deviations) are also reported. For scale bar please refer to Figure 5-2.
Results shown in Figure 5-3 would suggest that HIU promotes crystallization, especially when the 1/2” tip is used since no significant differences (p > 0.05) are found between the total cluster area obtained at 15 and 60 min of crystallization. However, this fact is not corroborated by the SFC measured and reported in Figure 5-4 since SFC values were not affected by sonication (Figure 5-4). It is important to note here that p-NMR is not very sensitive to small changes in SFC values with sensitivity in the order of 1%. We suggest that physical changes induced by the application of HIU happen at the molecular level and are mainly related to microstructural changes. The effect of acoustic waves on lipids strongly depends on the type of material sonicated (chemical composition of the lipid) and on the processing conditions used (crystallization temperature, agitation, etc.) since several authors have reported an increase in SFC as a consequence of sonication [19, 22]. Previous research [26, 27] on sonocrystallization in aqueous systems

![Figure 5-4: Solid fat content (%) of the shortening crystallized at 20 °C and sonicated using 1/8” and 1/2” tips.](image)
suggests that the effect of acoustic waves on crystallization events is due to the generation of: a) cavitation, and b) shear forces as a consequence of the acoustic waves applied to the system. In the absence of crystals, the cavitation induced by ultrasound plays a major role in inducing nucleation [28, 29]. That is, small bubbles generated during the insonation act as nuclei inducing the crystallization in the system. In the presence of crystals, shear forces might break some of the existing crystals creating new nuclei, which is similar to adding seed crystals. This induction of the nucleation process may result in the generation of more and smaller crystals in the network. It is probable that the combination of cavitation and shear force is responsible for promoting crystallization in lipid systems.

**Effect of ultrasound duration on lipid morphology:** The effect of ultrasound duration on lipid crystallization was tested using the 1/2” tip for the shortening crystallized at 20 °C. Figure 5-5 shows crystal morphology of the shortening obtained as a function of time when ultrasound was applied for 10, 5 and 2.5 sec. No significant differences (p > 0.05) were found in the total cluster area as a function of sonication time at early (15 min) and advanced stages of the crystallization process (60 min). Compared to the 10 sec sonication condition significantly larger average cluster areas (p < 0.05) were observed at early stages of the crystallization process (15 min) when samples were sonicated for shorter periods of time (5 and 2.5 sec), but no significant difference was observed between the 5 and 2.5 sec sonication times. Average cluster areas for the samples sonicated for 10 sec were 200.8 ± 10.5 µm² and average cluster areas for sample sonicated for 5 and 2.5 sec were 430.2 ± 0.1 µm² and 357.1 ± 39.8 µm², respectively (Figure 5-5). No significant differences were found in the total area and average area of
these three sonication conditions after 60 min into the crystallization process suggesting that applying HIU for 2.5 sec produces similar results compared to HIU applied for 10 sec after 60 min into the crystallization process.

**Figure 5-5:** Microstructure of crystals obtained for the shortening crystallized at 20 °C without HIU application and with HIU applied for 10, 5, and 2.5 sec using a 1/2” tip. HIU was applied after 5 min into the crystallization process. Results from image analysis (total area and average area and standard deviations) are also reported. For scale bar please refer to Figure 5-2.
**Effect of HIU on viscoelastic properties of the material:** Considering the effect of tip types on the crystallization behavior of the sample (Figure 5-2 and Figure 5-3), we decided to continue the experiments using the 1/2” tip and crystallize the sample at 20 °C. In general, lipid crystal networks with smaller clusters result in harder and more elastic materials [23]. Consequently, we expect that the smaller clusters formed after sonication will generate more elastic materials. Figure 5-6 shows the viscoelastic properties (G’ and G’”) of samples without and with HIU applied (20 °C, 1/2” for 10 sec) after 60 min into crystallization process and after tempering for 48 h at 25 °C. As expected, both G’ and G’” significantly increased (p < 0.05) when HIU was used. G’ value increased more than 5 times compared to the sample without HIU applied (from 8.4 ± 3.0 Pa to 47.3 ± 9.6 Pa), while G’” increased approximately 3 times (from 11.3 Pa ± 2.8 Pa to 34.1 Pa ± 5.2 Pa).

*Figure 5-6:* G’ and G’” (Pa) measurements for the shortening crystallized at 20 °C with and without the application of HIU. Samples were taken after 60 min at 20 °C and after tempering for 48 h at 25 °C. HIU was applied after 5 min into the crystallization process using a 1/2” tip for 10 sec. The error bar shows the standard deviation of two independent replicate runs.
29.9 ± 3.0 Pa). Interestingly, after tempering for 48h at 25 °C, G’ values for both groups increased significantly (α = 0.05). For samples crystallized without the use of ultrasound, G’ value increased from 8.4 ± 3.0 Pa to 12,905.0 ± 445.0 Pa; while even a more significant increase in G’ values was found for the sample crystallized with HIU, which increased from 47.3 ± 9.6 Pa to 25,410.0 ± 4,836.0 Pa. This increase in G’ values suggest that some type of re-crystallization, which might include the formation of different polymorphic forms, occurs during tempering resulting in a more elastic material.

Effect of HIU on melting behavior of the material: The thermal behavior of the samples after 60 min into the crystallization process and after tempering for 48 h at 25 °C was determined by using DSC. Table 5-1 shows the melting parameters of the shortening crystallized at 20 °C with and without the application of HIU. No significant differences (α = 0.05) were found on the enthalpies and T_p values as a function of HIU application after 60 min of crystallization process and after 48 h tempering. A significant decrease (p

<table>
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<tr>
<th>Melting parameter</th>
<th>wo HIU</th>
<th>HIU @ 5 min 10 s</th>
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<tr>
<td><strong>60 min</strong></td>
<td></td>
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<tr>
<td>T_on (°C)</td>
<td>28.1 ± 0.1&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>26.6 ± 0.1&lt;sup&gt;b,A&lt;/sup&gt;</td>
</tr>
<tr>
<td>T_p (°C)</td>
<td>35.7 ± 0.3&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>35.0 ± 0.2&lt;sup&gt;a,B&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔH (J/g)</td>
<td>14.7 ± 0.7&lt;sup&gt;a,C&lt;/sup&gt;</td>
<td>15.0 ± 0.1&lt;sup&gt;a,C&lt;/sup&gt;</td>
</tr>
<tr>
<td><strong>48h @ 25 °C</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_on (°C)</td>
<td>28.4 ± 0.1&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>28.5 ± 0.2&lt;sup&gt;a,D&lt;/sup&gt;</td>
</tr>
<tr>
<td>T_p (°C)</td>
<td>36.2 ± 0.2&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>36.6 ± 0.1&lt;sup&gt;a,E&lt;/sup&gt;</td>
</tr>
<tr>
<td>ΔH (J/g)</td>
<td>17.7 ± 0.3&lt;sup&gt;a,D&lt;/sup&gt;</td>
<td>18.2 ± 0.7&lt;sup&gt;a,F&lt;/sup&gt;</td>
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< 0.05) in $T_{on}$ was found after 60 min crystallization when ultrasound was applied. This might suggest that lower melting point triacylglycerols are being crystallized as a consequence of sonication. Tempering resulted in a significant increase (p < 0.05) of melting enthalpies for both conditions tested (without and with sonication) suggesting that samples continue to crystallize during tempering. In addition, tempering resulted in a significant increase (p < 0.05) of all melting parameters including $T_{on}$, $T_p$, and enthalpy of the sonicated samples suggesting that molecular re-organization occurred during tempering. These results support the increase of $G'$ described in the previous section suggesting that this increase in the elasticity of the material during tempering is a consequence of the presence of more crystalline material (higher enthalpy) and molecular re-organization (higher $T_{on}$ and $T_p$ values).

**Section II: Temperature Cycling Experiment**

Based on the data described above regarding HIU power levels (1/8 vs. 1/2” tip) and duration (10, 5, and 2.5 sec.), the following conditions were chosen to perform the cycling experiment: samples were crystallized at 20 °C and HIU was applied using the 1/2” tip at 5 min into the crystallization process for a period of 2.5 sec. The objective of using 2.5 sec instead of 10 sec is to mimic the exposure time that would happen in an in-line system during shortening production. As previously discussed the application of HIU for 2.5 sec does not significantly affect the crystallization behavior of the fat compared to a 10 sec application time (Figure 5-5).

Since very little effect of HIU was observed in samples crystallized at 30 °C (Figure 5-2), HIU was applied when the sample is crystallized at 20 °C. In the cycling
experiments (Figure 5-1), step 1 and step 5 are the two isothermal steps at 20 °C, which means HIU could be applied at both or one of these two steps. Therefore, when HIU was applied in both steps a “with-with” HIU label was used. When HIU was not applied in any of the steps, a “wo-wo” label was used. If HIU was used in step 1 but not used in step 5, a “with-wo” label was used and lastly if HIU was applied only in step 5 a “wo-with” label was used.

Effect of sonication on lipid microstructure during temperature cycling – slow cooling rate: Figure 5-7 shows the morphology of crystals obtained when the shortening was crystallized under temperature cycling as described above using slow cooling rate (0.5 °C/min) during step 4 without ultrasound and when ultrasound was applied for 2.5 sec at different moments during the temperature cycling (step 1 and/or step 5). To better understand the effect of HIU, a set of pictures of the shortening crystallized at 20 °C with no temperature cycling and no HIU application were taken as the control. Since during the first 60 min, pictures are the same as the ones reported in Figure 5-3, pictures of crystals obtained only after 120 min are reported in Figure 5-7. No significant differences (p > 0.05) were found in total area and cluster average area between the samples crystallized without temperature cycling and the ones crystallized under temperature fluctuation without the use of HIU (wo-wo). However, a tendency of larger clusters (bigger cluster areas) is observed in the samples crystallized under temperature fluctuations. This could be a consequence of partial melting of smaller crystals due to the increase of temperature from 20 to 30 °C. No significant differences were found in the average cluster areas between the different treatments after 120 min. However, a tendency of smaller clusters is observed for samples crystallized under the “with-wo” and
Figure 5-7: Microstructure of crystals obtained for the shortening crystallized during temperature cycling and after tempering 48 h at 25 °C. HIU was applied at 5 min and/or at 125 min using slow cooling rate (0.5 °C/min). Time point 120 min shows the finish of step 4 which cools down the temperature from 30 to 20 °C, time point 180 min indicates the final stage of the whole temperature cycling. Results from image analysis (total area and average area and standard deviations) are also reported. For
“with-with” conditions. This is somehow expected since at this point these two conditions are the same since HIU is applied for the second time at 125 min. A “cloud” of smaller crystals can be seed in Figure 5-7 in these two conditions, which is not present in the other conditions. After HIU is applied for the second time (Figure 5-7, 180 min), significantly larger cluster areas (p < 0.05) are observed as a consequence of sonication at 125 min (compare “wo-wo” and “wo-with”; and “with-wo” and “with-with” conditions at 180 min). These differences are maintained after tempering at 25 °C for 48 h but only statistically significantly for the “with-wo” and “with-with” conditions. These results suggest that the application of HIU during the second cooling step promotes crystal growth and crystal re-organization as evidenced by the increase in cluster total area and the change in percentage of different size clusters.

**Effect of sonication on lipid microstructure during temperature cycling – fast cooling rate:** Figure 5-8 shows pictures of crystals obtained for the shortening crystallized under temperature cycling. In this case a fast cooling rate of 1 °C/min was used to decrease the temperature from 30 to 20 °C during the cycling experiments. As described before, smaller cluster areas (p < 0.05) are observed when samples are crystallized with HIU during early stages of the crystallization process (“with-wo” and “with-with” conditions compared to the “wo-wo” and “wo-with” conditions) at 110 min. Considering that HIU is applied at 115 min for the second time, “with-wo” and “with-with” conditions are similar (p > 0.05) at this time point (110 min). After applying HIU for the second time (at 115 min) no significant differences (p > 0.05) are observed in the average cluster areas (Figure 5-8, 170 min); however significantly (p < 0.05) larger
Figure 5-8: Microstructure of crystals obtained for the shortening crystallized during temperature cycling and after tempering for 48 h at 25 °C. HIU was applied at 5 min and/or at 115 min using fast cooling rate (1 °C/min). Time point 110 min shows the finish of step 4 which cools down the temperature from 30 to 20 °C, time point 170 min indicates the final stage of the whole temperature cycling. Results from image analysis (total area and average area and standard deviations) are also reported. For scale bar please refer to Figure 5-2.
cluster areas are observed after tempering for 48 h in the sonicated samples (compare cluster areas of “wo-wo” and “wo-with”; and “with-wo” and “with-with”). These results suggest that HIU promotes the re-organization and growth of clusters during storage. This might be a consequence of the generation of nuclei in the system that eventually grow during storage or they agglomerate with the existing ones contributing to an overall crystal growth.

Results reported in Figures 5-7 and 5-8 suggest that in general, when HIU is applied at early stages of the crystallization (“with-wo” and “with-with” conditions) a reduction in cluster areas is observed. However, when HIU is applied during the second cooling step (“wo-with” and “with-with”) an increase in cluster area is observed for slowly cooled samples (Figure 5-7, 180 min) but no changes in cluster areas is observed for the rapidly cooled samples (Figure 5-8, 170 min). After 48 h of tempering at 25 °C a decrease in cluster areas is observed for the slowly cooled samples while an increase in cluster areas is observed for the rapidly cooled samples (Figure 5-7 and 5-8, respectively).

**Evaluation of viscoelastic properties:** To further evaluate the effect that sonication has during temperature cycling experiments on the elasticity of the material, the viscoelastic properties of the samples sonicated in the first and second cooling steps (“with-wo” and “with-with”) were evaluated. The viscoelastic properties of “wo-wo” and of samples that were not subjected to temperature fluctuations were used as control. Table 5-2 shows the $G'$ and $G''$ values of the crystal networks obtained at the end of the temperature cycling experiments and after tempering. Both $G'$ and $G''$ values significantly increased ($\alpha = 0.05$) as a result of HIU application when crystallized using
Table 5-2: $G'$ and $G''$ (Pa) measurements obtained after the shortening has been subjected to temperature cycling and after tempering 48 h at 25 °C. The table shows mean values and standard deviations of 2 independent replicates. Lower case superscripts with the same letter indicate that values are not significantly different ($\alpha=0.05$) within a row. Upper case superscripts with the same letter indicate that values are not significantly different ($\alpha=0.05$) as a function of storage time.

<table>
<thead>
<tr>
<th>Viscoelastic Parameters</th>
<th>No cycling</th>
<th>Slow cooling wo-wo HIU</th>
<th>Slow cooling with-wo HIU</th>
<th>Slow cooling with-with HIU</th>
<th>Fast cooling wo-wo HIU</th>
<th>Fast cooling with-wo HIU</th>
<th>Fast cooling with-with HIU</th>
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<tr>
<td>180 min</td>
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<tr>
<td>$G'$ (Pa)</td>
<td>24.5 ± 5.9&lt;sup&gt;aA&lt;/sup&gt;</td>
<td>662.6 ± 176.9&lt;sup&gt;B,A&lt;/sup&gt;</td>
<td>617.5 ± 40.4&lt;sup&gt;B,A&lt;/sup&gt;</td>
<td>3,365.5 ± 426.4&lt;sup&gt;C,A&lt;/sup&gt;</td>
<td>354.4 ± 49.7&lt;sup&gt;dA&lt;/sup&gt;</td>
<td>255.8 ± 41.6&lt;sup&gt;dA&lt;/sup&gt;</td>
<td>1,249 ± 19.8&lt;sup&gt;cA&lt;/sup&gt;</td>
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<tr>
<td>$G''$ (Pa)</td>
<td>16.6 ± 1.1&lt;sup&gt;B,B&lt;/sup&gt;</td>
<td>143.6 ± 28.4&lt;sup&gt;B,B&lt;/sup&gt;</td>
<td>138.7 ± 9.1&lt;sup&gt;B,B&lt;/sup&gt;</td>
<td>541.4 ± 55.8&lt;sup&gt;B&lt;/sup&gt;</td>
<td>82.4 ± 3.1&lt;sup&gt;c,dB&lt;/sup&gt;</td>
<td>74.9 ± 7.9&lt;sup&gt;c,B&lt;/sup&gt;</td>
<td>232.0 ± 6.0&lt;sup&gt;c,B&lt;/sup&gt;</td>
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<td>48h @ 25 °C</td>
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<td>$G'$ (Pa)</td>
<td>26,245.0 ± 2227.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,499.5 ± 85.6&lt;sup&gt;C&lt;/sup&gt;</td>
<td>1,916.0 ± 140.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>3,876.0 ± 147.1&lt;sup&gt;c,A&lt;/sup&gt;</td>
<td>1,287.5 ± 185.9&lt;sup&gt;c&lt;/sup&gt;</td>
<td>1,917.0 ± 36.8&lt;sup&gt;c,C&lt;/sup&gt;</td>
<td>1,910.5 ± 226.9&lt;sup&gt;c,C&lt;/sup&gt;</td>
</tr>
<tr>
<td>$G''$ (Pa)</td>
<td>4,115.5 ± 1386.6&lt;sup&gt;c,D&lt;/sup&gt;</td>
<td>255.3 ± 32.7&lt;sup&gt;D&lt;/sup&gt;</td>
<td>354.8 ± 15.6&lt;sup&gt;c,D&lt;/sup&gt;</td>
<td>561.7 ± 26.6&lt;sup&gt;d,B&lt;/sup&gt;</td>
<td>212.7 ± 6.9&lt;sup&gt;b,D&lt;/sup&gt;</td>
<td>367.6 ± 17.2&lt;sup&gt;c,D&lt;/sup&gt;</td>
<td>363.6 ± 34.4&lt;sup&gt;c,D&lt;/sup&gt;</td>
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</table>
the “with-with” condition for both cooling rates tested. Even after the tempering, samples crystallized using the “with-with” condition at slow cooling rate show statistically higher (p < 0.05) elastic values. For samples crystallized using the “with-wo” condition it is interesting to find that G’ and G” values decreased slightly when compared to the control (“wo-wo”). However, the tempering provided enough time for the “with-wo” sample to generate higher (p < 0.05) G’ and G” values than the “wo-wo” sample, but still lower than the “with-with” sample only at slow cooling rate. When analyzing the effect of tempering at 25 °C it can be observed that G’ values did not change significantly for the samples crystallized using the “with-with” condition. G’ values changed from 3,365.5 ± 426.4 Pa to 3,876.0 ± 147.1 Pa for the slow cooling rate and from 1,249.0 ± 19.8 Pa to 1,910.5 ± 226.9 for the fast cooling rate. Samples crystallized using the other conditions (“wo-wo” and “with-wo”) have lower G’ values which significantly increase after tempering (e.g. from 617.5 ± 40.4 Pa to 1,916.0 ± 140.0 Pa for the “with-wo” sample at slow cooling rate). This suggests that the structure of the crystalline network formed as a consequence of the dual application of HIU (“with-with”) does not change significantly as a consequence of tempering. While for other conditions, continuous crystallization of crystals structure is likely to happen during the tempering. A very interesting result showed by this data is that samples with no temperature cycling have the lowest G’ and G” values at the end of the crystallization experiment. The low G’ and G” values for the no cycling group might be explained by a slow crystallization at 20 °C for this sample, which can be evidenced by a significant increase in G’ after tempering. Temperature fluctuations might change the crystallization behavior of the sample resulting in larger G’ and G” values for all the temperature cycling groups. This is supported by the T_on and T_p.
values shown in Table 5-3 and Table 5-4 where $T_{on}$ and $T_p$ values for samples crystallized without temperature cycling were significantly lower ($p < 0.05$) than the ones obtained in samples crystallized under temperature cycling for both cooling rates (see discussion in next section). Even though the $G'$ value of the samples crystallized without temperature cycling was lower after the first 180 min, this value significantly increased after tempering the sample for 48 h at 25 °C. These results suggest that the sample continues to crystallize during the tempering process generating the hardest material with the highest $G'$ values (From 24.5 ± 5.9 Pa to 26,245.0 ± 2,227.0 Pa). When comparing different cooling rates, the effect of HIU on the slow-cooled samples is greater compared to the effect in the fast cooled ones. The $G'$ value for samples obtained under slow cooling rate increased by a 5-fold from 662.6 ± 176.8 Pa (“wo-wo”) to 3,365.5 ± 426.4 Pa (“with-with”), while at fast cooling rate a 2-fold increase from 354.4 ± 49.7 Pa to 1,249.0 ± 19.8 Pa is observed. From the $G'$ values indicated above, we might notice that samples under slow cooling rate show a more elastic behavior than the samples under fast cooling rate, even after 48 h of tempering at 25 °C (only for “with-with” condition). This is in agreement with previous research in milk fat model system reported by Herrera et al. [30], which shows higher storage modulus in samples crystallized at slow cooling rate.

**Evaluation of melting parameters:** Table 5-3 and Table 5-4 summarize the DSC melting parameters of the crystal networks obtained for the shortening crystallized without and with HIU under temperature cycling conditions (0.5 and 1 °C/min, respectively). The melting parameters of the samples with no temperature cycling were also determined as the control. In Table 5-3, during the 3 h-crystallization, samples’ onset temperature ($T_{on}$) and peak temperature ($T_p$) significantly ($p < 0.05$) increased with
Table 5-3: Melting parameters of the shortening crystallized under specific temperature cycling using slow cooling rate (0.5 °C/min). The data was collected at 180 min and after tempering for 48 h at 25 °C. Data reported are mean values and standard deviations of 2 independent runs. Lower case superscripts with the same letter indicate that values are not significantly different (α= 0.05) within a row. Upper case subscripts with the same letter indicate that values are not significantly different (α= 0.05) as a function of storage time.

<table>
<thead>
<tr>
<th>Melting Parameter</th>
<th>No cycling</th>
<th>wo-wo HIU</th>
<th>with-wo HIU</th>
<th>with-with HIU</th>
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<tr>
<td><strong>180 min</strong></td>
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<tr>
<td>T&lt;sub&gt;on&lt;/sub&gt; (°C)</td>
<td>27.3 ± 0.1&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>30.2 ± 0.2&lt;sup&gt;b,A&lt;/sup&gt;</td>
<td>29.5 ± 0.8&lt;sup&gt;b,A&lt;/sup&gt;</td>
<td>30.0 ± 0.8&lt;sup&gt;b,A&lt;/sup&gt;</td>
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<tr>
<td>T&lt;sub&gt;p&lt;/sub&gt; (°C)</td>
<td>34.7 ± 0.0&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>37.7 ± 0.0&lt;sup&gt;b,B&lt;/sup&gt;</td>
<td>38.4 ± 0.2&lt;sup&gt;b,B&lt;/sup&gt;</td>
<td>37.3 ± 0.4&lt;sup&gt;b,B&lt;/sup&gt;</td>
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<td>ΔH (J/g)</td>
<td>15.1 ± 0.5&lt;sup&gt;a,C&lt;/sup&gt;</td>
<td>12.3 ± 0.9&lt;sup&gt;b,C&lt;/sup&gt;</td>
<td>12.8 ± 1.3&lt;sup&gt;b,C&lt;/sup&gt;</td>
<td>11.9 ± 0.1&lt;sup&gt;b,C&lt;/sup&gt;</td>
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<tr>
<td><strong>48h @ 25 °C</strong></td>
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<tr>
<td>T&lt;sub&gt;on&lt;/sub&gt; (°C)</td>
<td>27.7 ± 0.0&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>29.1 ± 0.8&lt;sup&gt;a,b,A&lt;/sup&gt;</td>
<td>28.0 ± 0.8&lt;sup&gt;a,b,A&lt;/sup&gt;</td>
<td>30.1 ± 0.6&lt;sup&gt;b,A&lt;/sup&gt;</td>
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<tr>
<td>T&lt;sub&gt;p&lt;/sub&gt; (°C)</td>
<td>35.7 ± 0.4&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>34.6 ± 0.0&lt;sup&gt;a,D&lt;/sup&gt;</td>
<td>36.8 ± 1.8&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>36.0 ± 1.1&lt;sup&gt;a,B&lt;/sup&gt;</td>
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<tr>
<td>ΔH (J/g)</td>
<td>21.0 ± 0.1&lt;sup&gt;a,D&lt;/sup&gt;</td>
<td>12.7 ± 1.6&lt;sup&gt;b,C&lt;/sup&gt;</td>
<td>17.9 ± 0.3&lt;sup&gt;c,D&lt;/sup&gt;</td>
<td>11.6 ± 0.9&lt;sup&gt;b,C&lt;/sup&gt;</td>
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Table 5-4: Melting parameters of the shortening crystallized under specific temperature cycling using fast cooling rate (1 °C/min). The data was collected at 170 min and after tempering for 48 h at 25 °C. Data reported are mean values and standard deviations of 2 independent runs. Lower case superscripts with the same letter indicate that values are not significantly different (α= 0.05) within a row. Upper case subscripts with the same letter indicate that values are not significantly different (α= 0.05) as a function of storage time.

<table>
<thead>
<tr>
<th>Melting Parameter</th>
<th>No cycling</th>
<th>wo-wo HIU</th>
<th>with-wo HIU</th>
<th>with-with HIU</th>
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<tr>
<td><strong>180 min</strong></td>
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<tr>
<td>T&lt;sub&gt;on&lt;/sub&gt; (°C)</td>
<td>27.3 ± 0.1&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>30.0 ± 0.4&lt;sup&gt;b,A&lt;/sup&gt;</td>
<td>29.2 ± 0.5&lt;sup&gt;b,A&lt;/sup&gt;</td>
<td>30.7 ± 0.0&lt;sup&gt;b,A&lt;/sup&gt;</td>
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<tr>
<td>T&lt;sub&gt;p&lt;/sub&gt; (°C)</td>
<td>34.7 ± 0.0&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>37.3 ± 0.5&lt;sup&gt;b,B&lt;/sup&gt;</td>
<td>38.3 ± 0.2&lt;sup&gt;b,B&lt;/sup&gt;</td>
<td>37.5 ± 0.1&lt;sup&gt;b,B&lt;/sup&gt;</td>
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<tr>
<td>ΔH (J/g)</td>
<td>15.1 ± 0.5&lt;sup&gt;a,C&lt;/sup&gt;</td>
<td>12.1 ± 0.1&lt;sup&gt;b,C&lt;/sup&gt;</td>
<td>12.5 ± 0.3&lt;sup&gt;b,C&lt;/sup&gt;</td>
<td>11.6 ± 0.8&lt;sup&gt;b,C&lt;/sup&gt;</td>
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<td><strong>48h @ 25°C</strong></td>
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<tr>
<td>T&lt;sub&gt;on&lt;/sub&gt; (°C)</td>
<td>27.7 ± 0.0&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>29.4 ± 0.6&lt;sup&gt;b,c,A&lt;/sup&gt;</td>
<td>28.0 ± 1.0&lt;sup&gt;a,b,A&lt;/sup&gt;</td>
<td>30.2 ± 0.9&lt;sup&gt;c,A&lt;/sup&gt;</td>
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<tr>
<td>T&lt;sub&gt;p&lt;/sub&gt; (°C)</td>
<td>35.7 ± 0.4&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>35.2 ± 0.8&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>36.9 ± 1.7&lt;sup&gt;a,B&lt;/sup&gt;</td>
<td>36.2 ± 1.5&lt;sup&gt;a,B&lt;/sup&gt;</td>
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<tr>
<td>ΔH (J/g)</td>
<td>21.0 ± 0.1&lt;sup&gt;a,D&lt;/sup&gt;</td>
<td>13.0 ± 0.1&lt;sup&gt;b,C&lt;/sup&gt;</td>
<td>17.0 ± 1.1&lt;sup&gt;c,D&lt;/sup&gt;</td>
<td>11.9 ± 1.0&lt;sup&gt;b,C&lt;/sup&gt;</td>
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temperature cycling, which indicates that some degree of molecular re-organization or re-crystallization might occur during temperature changes. These changes in $T_p$ and $T_{on}$ values might also be a consequence of the formation of different polymorphic forms. In addition, $T_{on}$ and $T_p$ values are not statistically different among the temperature cycling groups suggesting that HIU does not affect the melting behavior of the samples subjected to temperature cycles. The melting enthalpies decreased significantly ($p < 0.05$) with temperature cycling whether or not HIU was applied. Enthalpy values were not affected by the sonication condition. It is interesting to note that even though there is no difference in the enthalpy values between the sonicated and non-sonicated samples, which means that these samples have the same amount of crystallized material, higher $G'$ values were obtained for the “with-with” condition. Similar to the discussion in the previous section, the higher $G'$ values observed in the “with-with” condition might be a consequence of molecular re-organization induced by sonication. After 48 h tempering, the enthalpy of the control group (no temperature cycling) increased significantly from $15.5 \pm 0.0$ J/g to $21.0 \pm 0.1$ J/g, which contributes to the higher $G'$ value reported in Table 5-2. The same tendency of enthalpy change after tempering was only found for the “with-wo” group. In addition, tempering shifted the $T_p$ of all the temperature cycling groups to the lower temperatures (“wo-wo” from $37.7 \, ^\circ C$ to $34.6 \, ^\circ C$, “with-wo” from $38.4 \, ^\circ C$ to $36.8 \, ^\circ C$, and “with-with” from $37.3 \, ^\circ C$ to $36.0 \, ^\circ C$) suggesting that lower melting point triacylglycerols are crystallizing (especially for the “with-wo” group) or that some type of molecular re-organization takes place during tempering. However, “with-with” HIU seems to fight against this shifting to keep as much higher melting point fractions (formed after temperature cycling) as possible and make those fractions more stable. This is further
supported by the smallest change on the $T_{on}$ values for this group. From the results shown in Table 5-4 similar tendencies to the ones reported in Table 5-3 were observed. Thus, when combining the results in Table 5-2, 5-3 and 5-4, we can conclude that HIU performs better in generating higher elastic properties at slow cooling rate than that at fast cooling rate.

**Conclusions**

The effect of the HIU on microstructure, melting behavior and viscoelasticity of a commercial shortening was determined in this research. Results from this research show that the effectiveness of HIU towards the modification of the physical properties of the shortening depends on the size of the tip used, the crystallization temperatures and temperature fluctuations experienced by the sample, but are not affected by the duration of the sonication. In this research HIU was more efficient when the sample was crystallized at lower crystallization temperatures and when HIU was applied with a larger tip (1/2” vs. 1/8” tip). In addition, HIU was helpful at increasing the elasticity of the samples that have been subjected to temperature fluctuations. This effect was more evident when the samples were cooled using a slow cooling rate. Changes in the viscoelasticity of the samples as a consequence of sonication are probably due to changes in molecular organization of the crystalline network since HIU did not increase the amount of crystalline material formed. All the results above suggest the potential use of HIU in the maintenance and improvement of texture stability of the oil during production and transportation.
References


27. Suslick KS (1988), Ultrasound: its chemical, physical, and biological effects, VCH, New York


CHAPTER 6
APPLICATION OF HIGH INTENSITY ULTRASOUND TO PALM OIL IN A CONTINUOUS SYSTEM

Abstract
High intensity ultrasound (HIU) was used in a continuous system to change the crystallization behavior of palm oil. Different power levels (75, 110, and 180W) and pulse duration (continuous application, 5, 10 and 15 sec pulses) were used to optimize sonication conditions. Results showed that HIU applied at low power level (75W) was the most efficient condition at inducing palm oil crystallization at 35 °C generating a crystalline network with higher solid fat content (SFC), higher elasticity, and sharper melting profile after 60 min of crystallization. Changes in elasticity observed as a consequence of sonication were maintained after tempering the samples at 25 °C for 24 h, but were lost after tempering at 5 °C. No significant differences (α = 0.05) were observed in SFC values of the sonicated and non-sonicated samples after tempering, while the sharper melting behavior observed in the sonicated sample was maintained after tempering.

Introduction
Power ultrasound, or high intensity ultrasound (HIU), are invasive techniques that use acoustic waves operating at low frequency (20 to 100 kHz) and high power (10 to 10,000 W cm\(^{-2}\)) levels. These acoustic waves that travel through the material induce the formation of cavities.\(^1\) Cavitation is associated with high localized temperatures, high shear forces, and high pressures that lead to several physicochemical changes in the material.\(^2\) Some of these changes include the generation of chemical reactions (sonochemistry),\(^3,4\) changes in crystallization behavior...
sonocrystallization), 5, 6 and changes in physical properties of materials. 7-9 In particular, sonocrystallization is defined as the phenomena associated with the induction or modification of the crystallization behavior of different materials without generating any chemical changes. 10

Sonocrystallization techniques have been used in food applications since the early 2000’s. Chow et al. used HIU to modify the primary and secondary nucleation of ice and sucrose, 11, 12 while Patel et al. showed that power ultrasound can be used to control the crystallization process of lactose during the nucleation phase. 13 Early studies in lipid systems were performed by Patrick et al. who evaluated the effect of ultrasonic intensity on palm oil crystalline structures. 14 Sato and coworkers used HIU in cocoa butter and pure triacylglycerols 15, 16 and showed that HIU induces lipid crystallization as evidenced by shorter induction times in sonicated samples. In addition, these authors showed that HIU promotes the formation of a stable polymorphic form, especially at high crystallization temperatures.

Previous work in our laboratory 17-20 and other research groups 21 indicates that HIU can be used as a novel processing tool to modify the functional properties of lipids. HIU has the ability to change the crystallization behavior of lipids and therefore their functional properties such as texture, thermal behavior; solid/liquid ratio and crystal size. All previous studies about lipid sonocrystallization have been performed in a batch with volumes between 5 mL and 100 mL. 16-21 The effect of HIU on the crystallization of lipids in a continuous system has never been investigated.

The purpose of this research is to evaluate the use of HIU in a continuous system which will be a more realistic representation of an industrial setting. The overall objective of this research is to optimize the processing conditions (power level and sonication pulse) needed to use
HIU in a continuous system to modify the physical properties (microstructure, melting profiles, polymorphism, and viscoelasticity) of palm oil.

**Material and Methods**

**Material**

Refined, bleached, and deodorized palm oil provided by Archer Daniels Midland (ADM Oils, Oils Division, IL, US) was used in this experiment. The melting point of the palm oil is 37.2 ± 0.4 °C (AOCS Official Method Cc 1-25). The chemical composition of the sample consisted of 48% of saturated fatty acids, 40% of monounsaturated fatty acids, 10% of polyunsaturated fatty acids, and 2% of trans-fatty acids according to the product data sheet provided by ADM.

**Sample preparation and continuous system**

The continuous system consisted of a flow cell connected to a sonicator and an external water bath (Figure 6-1). Five liters of palm oil were placed in a 6 Lt Erlenmeyer flask and melted in a 70 °C water bath to completely erase crystal memory. The flask was then placed in a 40 °C water bath to allow for temperature stabilization. Once the sample reached approximately 42 °C it was pumped into the flow cell at 7.3 ml/sec using a peristaltic pump (261 ml, the retention of the sample in the cell was 36 sec) and collected in NMR tubes and beakers. The flow cell was connected to a water bath filled with an ethylene glycol water mixture (3:1 v:v) and set at -18 °C. This low temperature in the water bath was necessary to cool the sample in the flow cell from 42 °C to 35 °C. A diagram of the flow cell used is shown in Figure 6-1.
Figure 6-1: Flow cell system used to evaluate the effect of HIU on the crystallization behavior of palm oil.

**Ultrasound application**

HIU (20 kHz ± 1 kHz operation frequency) was generated using a UIP1500hd equipment (Hielscher Ultrasound Technology, NJ, USA) with a 3.8 cm² frontal area sonication tip. HIU was applied 1 min after starting the pumping of the sample to ensure that the cell was completely filled with the sample and to stabilize the temperature of the sample. Previous research in our laboratory\textsuperscript{17,19} and in other research groups\textsuperscript{21} showed that the effect of HIU is more significant when samples are crystallized at low supercoolings. Considering that the melting point of our sample is 37.2 ± 0.4 °C, a crystallization temperature of 35 °C was chosen for our experiments. The effects of ultrasound using different power levels was evaluated in this study using 75 W (the lowest power setting that can be achieved by our ultrasound generator), 110 W (middle setting) and 180 W (safety maximum power setting recommended by the manufacturer of the sonicator).
Sonication was applied in a continuous manner and using 5, 10, and 15 sec pulses durations. A continuous duration indicates sonication continuously for 5 min while sonication using 5 (or 10, and 15) sec pulse duration means that HIU was applied every 5 (or 10, and 15) sec for a duration of 5 (or 10, and 15) sec. For example, 5 sec pulse duration means HIU was applied every 5 sec with the duration of 5 sec. Sample was pumped into the system for 5 min from the start of the sonication. Control samples (samples crystallized without the use of HIU) were collected at the beginning of the experiment before the application of HIU. Samples were collected every minute during the crystallization process in different tubes (one tube per minute). The first tube always consisted of non-sonicated sample (control). Solid fat content and crystal morphology were monitored in these samples as a function of storage time at 35 °C. Samples were kept at 35 °C in an incubator for 60 min to allow complete crystallization and then rapidly cooled to 25 and 5 °C and tempered for 24 h at these temperatures. The objective of this step is to evaluate if the effect of sonication is maintained through the tempering process.

**Solid fat content (SFC)**

SFC values of palm oil during and after crystallization at 35 °C was measured using a NMR 120 Minispec NMR analyzer (Bruker, Germany) following AOCS direct method Cd 16b-93. Samples were collected at the exit of the flow cell and loaded into the NMR tube every minute and kept at 35 °C to allow complete crystallization. SFC was measured as soon as the sample was taken out of the flow cell (time zero) and every 5 min for 30 min and every 10 min until the end of crystallization (60 min). The crystallization curves were fitted to the Avrami equation (eq. 1) by least squares nonlinear regression.22

\[
\frac{SFC(t)}{SFC_{\infty}} = 1 - EXP \left(-kt^n\right) \tag{1}
\]
where SFC(t) and SFC∞ are the SFC (%) at time t and the maximum SFC after crystallization is completed, respectively. The SFC data fitted to this model was used to determine the rate constant of crystallization (k) at a particular temperature, and the mechanism of nucleation and crystal growth through the exponent, n. \(^{23}\)

**Microstructure**

Crystal morphology was recorded during crystallization using a polarized light microscope (PLM-Olympus BX 41 America Inc., Melville, N.Y., U.S.A.) with a digital camera (Lumenera Scientific, Infinity 2, Ottawa, Ontario, Canada) attached and a thermostatized stage to allow for temperature control during the measurement. The pipette, slides and slides covers were tempered at 35 °C in the incubator. A 20x magnification objective was used.

**Crystal polymorphism by X-ray diffraction (XRD)**

After 60 min of crystallization, samples were filtered under vacuum for 2 min and crystals were collected to perform XRD measurement. The same filtration treatment was performed in samples tempered at 25 and 5 °C for 24 h. Samples were filtrated to separate as many crystals as possible from the liquid phase, minimize the signal of the liquid fat in the XRD, and therefore obtain a better resolution. The polymorphic forms of fat crystals were determined by Philips Analytical X’Pert Pro X-ray Diffraction system (PW 3040/60 Console). The X-ray generator used a high power ceramic diffraction X-ray tube with Copper anode (PW 3373/00 Ceramic Tube Cu LFF), with voltage at 45 kV and current 40 mA. Samples were scanned from 3° to 30° (2θ scale) at a rate of 1°/min at ambient temperature. The data was collected using X’Pert Data Collector and analyzed by X’Pert HighScore PANalytical software.
**Melting profile**

The thermal behavior of the crystallized material was evaluated using a differential scanning calorimeter (DSC-TA Instruments Q20). Five to fifteen mg of the crystallized material was placed in a hermetic aluminum pan and heated from \( T_c \) (35, 25, or 5 °C) to 80 °C at 5 °C/min to evaluate its melting behavior. Melting parameters such as onset temperature (\( T_{on} \); temperature at which the sample starts melting), peak temperature (\( T_p \); temperature at which the melting peak reaches its maximum), and melting enthalpy (energy required for melting) were recorded. The melting enthalpy was used to evaluate the amount of crystallized material and the solid/liquid ratio in the lipid network. These analyses provided information about: a) the amount of solids generated as a consequence of HIU (induction or delay of the crystallization) through the melting enthalpy values, b) the solid/liquid ratio of the crystal network formed as a function of temperature during melting, c) the melting range of the lipid network (through \( T_p \) and \( T_{on} \)).

**Viscoelastic properties**

A TA Instruments AR-G2 Magnetic Bearing Rheometer (TA Instruments, AR-G2) was used to evaluate the viscoelastic properties of the material. Oscillatory tests were performed by strain sweep step to obtain viscoelastic parameters such as the storage modulus (\( G' \)). The experiments were carried out using a parallel plate geometry (40 mm diameter) with a temperature controlled peltier plate. The temperature of the plate was set at 35, 25, or 5 °C. For the strain sweep step, a constant frequency of 1 Hz (6.28 rad/s) was used, and strain values were set from 0.0008 to 10%.

**Statistical analysis**

Crystallization experiments were performed in duplicate and physical properties were
measured in duplicate. Significant differences (\( \alpha = 0.05 \)) were evaluated using Two-way ANOVA using GraphPad Prism software, version 6.00 for Windows, GraphPad Software, San Diego, CA, USA.

Results and Discussion

**Optimizing the process condition**

Figures 6-2, 3 and 4 show the crystallization behavior of palm oil measured by SFC as a function of time when different power levels of ultrasound were applied using different pulse types. Figure 6-2 shows SFC changes when samples were sonicated using 75 W of power using a continuous sonication (Figure 6-2a), a 5 sec pulse (Figure 6-2b), a 10 sec pulse (Figure 6-2c) and a 15 sec pulse (Figure 6-2d). When HIU is applied in a continuous manner SFC values increased rapidly after 10 min; while SFC values increased after 20 min in the control samples (Figure 6-2a). Figures 6-2a-d show no difference in crystallization between the modes of sonication (continuous vs. pulses) with onset of crystallization observed at 20 min for the non-sonicated samples and 10 min for the sonicated ones and final SFC values of 3.3 and 4.3 % in average, respectively. Avrami analysis (\( R^2 \) values between 0.96 and 0.99) showed 2 order of magnitude higher \( k \) values for the sonicated samples compared to the non-sonicated ones with very little change in \( n \) values (Table 6-1). This data shows that sonication indeed increased the crystallization rate of palm oil. Figure 6-3a shows SFC values obtained when samples were sonicated at 110W on a continuous manner. This sonication condition did not induce lipid crystallization as shown by the similar SFC obtained between tubes 1-5 min and the control tube and the similar values of \( k \) (Table 6-1). However, when sonication was applied using 5, 10, and 15
Figure 6-2: SFC of palm oil without (control, open round symbols) and with (filled symbols) HIU during the first 60 min crystallization process at 35°C. 75 W power level was used in a continuous manner (a), and using pulses of different durations (5, 10 and 15 sec, b, c, and d, respectively)

sec pulses, sonication seems to be somehow efficient at inducing crystallization (Figures 6-3b-d) and \( k \) values were 1 or 2 order of magnitude higher in the sonicated samples compared to the non-sonicated ones. \( R^2 \) values obtained from the Avrami fitting for these samples ranged between 0.96 and 0.99. When samples were sonicated using 180 W of power in a continuous manner (Figure 6-4a) HIU delayed crystallization after 2 minutes of sonication (see data in Figure 6-4a, tube 2 min to 5 min). This delay in crystallization was so significant that data obtained for tubes 2-5 did not converge to the Avrami equation. Similar to the data discussed in Figure 6-3, HIU was able to induce crystallization when applied at 180W using 5, 10, and 15 sec pulses (Figure 6-4b-d) but higher \( k \) values were consistently obtained only for samples sonicated using 5 sec pulses (Table 6-1). Avrami \( R^2 \) values ranged from 0.93 and 0.99.
Table 6-1: Avrami constant ($k$, min$^{-m}$), Avrami exponent ($n$) and Equilibrium or maximum SFC (SFC$\infty$, %) obtained from the Avrami fitting for samples crystallized without and with different power levels (75 W, 110 W and 180 W) of ultrasound.

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Subscript "c" indicate the control sample, subscript "1-5" indicate the samples collected at each minute during sonication. $R^2$ values for Avrami fitting were all above 0.96 except for the samples collected from 2 to 5 min under 180 W sonication in a continuous manner where data did not fit the Avrami model; *SFC$\infty$ values are significantly different ($P < 0.05$) from the control sample.
Figure 6-3: SFC of palm oil without (control, open round symbols) and with (filled symbols) HIU during the first 60 min crystallization process at 35°C. 110 W power level was used in a continuous manner (a), and using pulses of different durations (5, 10 and 15 sec, b, c, and d, respectively)

Figure 6-4: SFC of palm oil without (control, open round symbols) and with (filled symbols) HIU during the first 60 min crystallization process at 35°C. 180 W power level was used in a continuous manner (a), and using pulses of different durations (5, 10 and 15 sec, b, c, and d, respectively)
Data obtained from the Avrami analysis shows some variation in $n$ values between the sonicated and non-sonicated samples (Table 6-1). $n$ reflects details regarding nucleation and crystal growth mechanisms. Many studies discussed the values of $n$ for various types of nucleation and growth of crystal.$^{24,25}$ For example, an $n$ value of 3 indicates plate-like growth from sporadic nuclei or spherulitic growth from instantaneous nuclei; while an $n$ of 2 corresponds to needle-like growth from sporadic nuclei or plate-like growth from instantaneous nuclei. For the control samples, $n$ values were close to 3, while after sonication, the $n$ values decreased to close to 2. This suggests a change in the type of nucleation and growth of crystals after HIU was applied. As discussed by Meng et al., lower value of $n$ and higher values of $k$ are associated with an increased rate of crystallization and a more instantaneous nucleation process with a shorter induction time.$^{25}$ Table 6-1 also shows the SFC$_\infty$ calculated using the Avrami fit with significantly ($P > 0.05$) higher SFC$_\infty$ values obtained for the samples sonicated using 75W of power.

It is very likely that the lower efficiency of HIU observed at higher power levels is due to temperature increases obtained during sonication. When samples were sonicated in a continuous manner, a linear temperature increase was observed as a function of time with values of 0.6491 °C/min ($R^2 = 0.8879$) for 75 W, 1.463 °C/min ($R^2 = 0.9671$) for 110 W and 1.847 °C/min ($R^2 = 0.9869$) for 180 W. The total temperature increase after 5 min sonication are 3.2 °C, 7.3 °C and 9.2 °C for the 75 W, 110 W and 180 W, respectively. The above results indicate that even though higher power levels of ultrasound can generate more cavitation in the system and therefore more positive effects on crystallization as described in previous research,$^{19-21}$ it can also generate higher temperatures which counteract cavitation events by melting the crystals that are being formed. In this experimental set-up, the thermal effect of higher power levels of HIU played an important role in delaying lipid crystallization (Figure 6-3a and 6-4a). In addition, greater
temperature increases were obtained for the continuous application compared to the pulsed ones for samples sonicated at higher power levels (110 W and 180 W). Temperature increases observed over the 5 min sonication using 110W for 5, 10 and 15 sec were 6.1, 4.9, and 6.2 °C, respectively, while temperature increases observed for the 180W sonication condition were 5.8, 6.1, and 4.9 °C for the 5, 10 and 15 sec pulses, respectively. The lower temperature increase for pulse sonication types with higher power levels explain the induction in crystallization when pulse sonication applied in Figure 6-3b-d and Figure 6-4b-d. In summary, higher power levels of HIU generate more heat and when the temperature is high enough a delay in crystallization might occur. To overcome this disadvantage, pulse sonication of sonication can be applied to induce crystallization by generating less heat. Temperature increases as a function of time can be found in Figure 6-1 of the supporting information available at http://pubs.acs.org.

**Physical properties of sonicated samples (75 W continuous)**

Results described in the previous section demonstrate the sonication using 75 W is the most efficient at inducing crystallization and at generating more solids in the crystalline network formed. Since no differences were found on SFC values as a function of sonication mode (continuous vs. pulse) the continuous mode was chosen to continue our experiments of physical characterization of the sonicated samples. The continuous condition was chosen over the pulse ones since it would be an easier method to implement in a processing plant. To perform the physical characterization, samples were collected at the exit of the flow cell as described before and mixed together as the “sample with HIU.” A control sample was always taken before the sonication. Physical properties such as crystal microstructure, viscoelasticity, melting profile, and polymorphism were evaluated on these samples.
Microstructure of the crystals

Figure 6-5 shows the microstructure of the palm oil samples crystallized in the flow cell with and without HIU using 75W of power in a continuous manner. After inducing the crystallization in the flow cell, samples were kept at 35 °C in an incubator for 60 min to allow for complete crystallization and tempered for 24 h at 25 °C and 5 °C. PLM pictures shown in Figure 6-5 demonstrate that HIU significantly affects the formation of crystals in different size and shape compared to the non-sonicated samples. Big round crystals (indicated with arrow B in the picture wo_60min) were observed after 60 min at 35 °C in samples crystallized without HIU. A continuous growth of size for this big round crystal was observed after 24 h of tempering at 25 °C (approximately 80 µm in diameter). Arrow A in the picture shows an example of a crystal with a different morphology characterized by needle-like spherulites which are usually associated with β’ crystals. Crystals continued to grow and formed an interconnected crystalline network of big spherulites after tempering at 5 °C for 24 h, which makes the identification of individual crystal or cluster very difficult. When HIU was applied to the samples, the amount of crystal clusters increased and the crystal aggregates generated a more uniform size distribution with smaller crystals, especially after 24 h tempering. Similar results were found in the research of Chen et al. on palm oil who showed different crystal morphology of palm oil crystallized with and without sonication. It is also interesting to observe that crystal size and morphology did not change substantially in the sonicated samples as a function of tempering. There might be several reasons which can cause this phenomena: (a) the greater number of crystals generated after HIU application act as nuclei for the secondary crystallization to occur and therefore the solid mass deposited over each crystal is lower compared to the non-sonicated samples were fewer crystals are present and therefore more solid mass is deposited in each crystal resulting in crystal growth;
Figure 6-5: Microstructure of palm oil crystallized without and with HIU at 35°C for 60min, and tempered at 25°C for 24 h and 5°C for 24 h. In the pictures (wo_60min and wo_25°C24h) two different morphologies were indicated (A and B)

(b) the solid/liquid ratio for sonicated samples is higher than that of non-sonicated samples, which means less liquid oil could be used to crystallize in sonicated samples during tempering; and (c) high pressures and high shear forces generated during sonication destroyed the regular shape of the crystal clusters and irregular or rough cluster shape might prevent the orderly arrangement of TAG molecules into the crystalline lattice. Changes observed in crystal morphology as a consequence of sonication can be attributed to the formation of different molecular compounds or to the presence of different polymorphic forms. Therefore, X-ray diffraction measurements were performed to evaluate the polymorphic forms generated during sonication and tempering.

**Crystal polymorphism**

The polymorphism of crystals obtained after crystallizing the samples with and without HIU at 35 °C for 60 min was determined with X-ray. This measurement was also performed in the same samples tempered for 24 h at 25 and 5 °C. Signals at short spacings of 3.7, 3.8, 3.9, 4.45, and 4.6 Å were identified. All conditions tested showed a strong signal at 4.6 Å indicating the
presence of β crystals. No strong peaks were observed at 4.2 Å indicating that β’ crystals were not present in these samples (X-ray diffractograms can be found in Figure 2 of the supporting information available at http://pubs.acs.org).

**Viscoelastic properties**

Figure 6-6 shows the comparison of the storage modulus (G’) and the corresponding SFC measurements between samples crystallized with and without HIU application. Samples were taken after 60 min of crystallization at 35 °C and after 24 h of tempering at 25 and 5 °C. As expected, sonicated samples crystallized for 60 min at 35 °C had significantly (P < 0.05) higher G’ and SFC values than that of non-sonicated ones. After 24 h of tempering at 25 °C, G’ values for both samples (sonicated and non-sonicated ones) increased as a result of ongoing crystallization during tempering (Figure 6-5) due to the exposure to a higher supercooling. Sonicated samples maintained a higher G’ after tempering (P < 0.05), while SFC values showed no statistical difference (P > 0.05). Changes observed in G’ and SFC values after tempering at 25 °C might be due to different phenomena: (a) increased interaction between crystal cluster in smaller crystals (Figure 6-5) leading to a higher G’; (b) SFC of the non-sonicated samples increased from 2.4 to 9.1 % in average during tempering while SFC of the sonicated samples increased only from 4.5 to 8.3 %; this means that HIU induced the crystallization during the first 60 min and therefore there is not much left to crystallize during the tempering as the sample reached the thermodynamic equilibrium. When samples were tempered at 5 °C for 24 h, SFC values for both samples increased dramatically to the same level, and it is very interesting to observe that the G’ values obtained for samples without HIU application are significantly higher than the ones obtained for the sonicated samples. The difference in crystal size and shape (Figure 6-5) and cluster
interconnections might contribute to the different $G'$ values observed after tempering at 5 °C for 24 h.

**Figure 6-6**: Storage modulus ($G'$) and corresponding SFC values of palm oil crystallized without and with HIU at 35 °C for 60 min (a,d), and after tempering at 25°C for 24 h (b,e) and 5°C for 24 h (c,f)

**Melting profile**

The melting profile of palm oil crystallized with and without HIU at 35 °C and stored at different tempering conditions (25 °C and 5 °C for 24 h) are shown in Figure 6-7. Only one melting peak was observed for both samples (with and without HIU) crystallized at 35 °C for 60 min (Figure 6-7a). Samples crystallized with HIU showed a sharper and deeper but narrower melting curve as compared to the non-sonicated sample. The peak temperature of the sample decreased from 48.4 ± 3.1 °C to 41.2 ± 0.1 °C when HIU was applied (Table 6-2). As expected, after tempering at 25 °C for 24h (Figure 6-7b), two melting peaks were observed for both samples (with and without HIU). The melting peak observed at lower temperatures can be attributed to
the secondary crystallization that occurred during tempering. Three peaks were observed for samples tempered at 5 °C for 24h (Figure 6-7c), of which the two lower melting peaks can be attributed to the secondary crystallization that occurs during the tempering at 5 °C. Table 6-2 shows the melting parameters of palm oil crystallized with and without HIU application. Melting
Table 6-2: Melting parameters of palm oil crystallized with and without sonication. The data was collected at 60 min and after tempering for 24 h at 25°C and 5°C. Data reported are mean values and standard deviations of 2 independent runs. Upper case subscripts with the same letter indicate that values are not significantly different (α= 0.05) in the same row. N/A means that the value could not be calculated since it fell outside the temperature range of the DSC run.

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<td>N/A</td>
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Parameters reported for the tempered samples correspond to the highest melting peak since this peak represents the crystals formed during the sonication experiment. The peak temperature (Tp) for the non-sonicated samples crystallized for 60 min at 35 °C was significantly (P < 0.05) higher than the sonicated samples. Same results were maintained after tempering at 25 °C and 5 °C for 24h. The reason for a shifting to lower peak temperature after sonication might be due to the different crystal microstructure and to different particle size distribution of the crystals. Enthalpy (ΔH) values of samples crystallized with HIU and kept 60 min at 35 °C were significantly (P < 0.05) higher than the ones obtained in the non-sonicated samples. After tempering at 25 °C and 5 °C, no difference was found for the enthalpy between with and without HIU samples for the highest melting fraction. These results are in accordance to previously discussed data related to SFC values (Figure 6-6).

Figure 6-8 shows the melting profile of the crystalline networks generated in this study. The melting profile is reported as the amount of solids remaining at a specific temperature (% solid) as the sample is being heated in the DSC. Only the highest melting fractionation was studied. Palm oil crystallized with HIU showed a lower % solid at any given temperature when
Figure 6-8: Melting profile of palm oil samples expressed as the percentage of solids remaining at a specific temperature during DSC melting. Melting profile of sonicated and non-sonicated samples are indicated with filled and open symbols, respectively. Samples crystallized at (a) 35 °C for 60 min, and tempered at (b) 25 °C for 24 h, and (c) 5 °C for 24 hours. The same results were maintained after tempering at 25 and 5 °C. The sonicated sample showed a steeper and sharper melting profile, which can be explained by the smaller size of crystals generated by HIU and as a consequence to a faster melting rate. Similar results were reported by Suzuki et al. \(^{18}\) and Ye et al. \(^{19}\) in other lipid systems such as anhydrous milk fat and interesterified soybean oil.
This study showed that HIU can be used in a continuous system to promote the crystallization of palm oil and to change its functional properties. Results obtained in this study provide scientific support to implement HIU as an additional processing tool in the edible oil industry. The importance of optimizing the sonication conditions is highlighted in this research noting that the amount of heat generated during sonication can contra rest the effect of cavitation. Future work on this area to support the hypothesis that HIU can be used as an additional processing tool to modify the functional properties of edible lipids in industrial settings could include changing the flow rate of the pumped oil and the crystallization temperature in the system. The impact of implementing this additional tool in the edible oil industry is not only to change the physical properties of the lipid but also to induce lipid crystallization, reduce processing times and ultimately reducing processing costs.

**Supporting Information Available:** Temperature changes observed in the samples as a function of time during 5 min sonication process for all the power levels and sonication durations are shown in Figure 1 of the supporting information. In addition, X-ray diffractograms obtained for the samples studied in this research are shown in Figure 2 of the supporting information. This material is available free of charge via the Internet at [http://pubs.acs.org](http://pubs.acs.org). All above supporting information is also included in the Appendix.

**References**


CHAPTER 7
APPLICATION OF HIU ON PALM OIL IN A FLOW CELL SYSTEM USING A HIGH FLOW RATE

Introduction

The use of HIU in a continuous system (flow cell) to change the crystallization behavior of palm oil was described in Chapter 6. Different power levels (75, 110, and 180 W) and pulse durations (continuous application, 5, 10 and 15 sec pulses) were used to optimize the sonication conditions. Results showed that the application of HIU at lower power level (75W) in a continuous manner was the most efficient sonication condition at generating higher crystallization rate, higher elasticity, and sharper melting profile. Higher power levels (110 W and 180 W) in continuous manner showed a delay of crystallization as the temperature change and the increase rate during sonication was much higher. The flow rate of palm oil into the flow cell system used in Chapter 6 is 7.3 ml/sec, a higher flow rate might be able to help decrease the effect of temperature increase when HIU was applied in a continuous mode, as the retention time for the samples in the flow cell must be shorter when compared with the samples with slow flow rate. This chapter provides further studies on Chapter 6 and aims to determine the changes in the physical properties of palm oil when a higher flow rate (20 ml/sec) was applied in the flow cell system. Physical properties such SFC, crystal microstructure, and melting profile will be discussed.
Material and Methods

Materials used in this research are exactly the same as the ones described in Chapter 6. In this chapter, a higher flow rate (20 ml/sec) was used to pump the palm oil into the flow cell system. As described in Chapter 6, a continuous sonication was chosen over the pulse ones since it would be an easier method to implement on a processing plant. Therefore, this chapter will only investigate the effect of continuous sonication using different power levels of HIU (75 W and 110 W) with a higher flow rate. Six and a half liters of palm oil were placed in the original oil-shipping bucket and melted in a 70 °C water bath to completely erase crystal memory. The bucket was then placed in a 40 °C water bath to allow for temperature stabilization. Once the sample reached approximately 40 °C it was pumped at 20 ml/sec using a peristaltic pump into the flow cell. The residence time of the sample in the cell is 13.1 sec. HIU was applied 20 sec after starting the pumping of the sample to ensure that the cell was completely filled with the sample and to stabilize the temperature of the exiting sample. HIU was applied using different power levels (75 W and 110 W) in continuous manner. SFC was measured as a function of time and the data was fitted using the Avrami model (Chapter 6, eq. 1), temperature increase rate, crystal morphology, and melting profile were measured according to the methods described in Chapter 6.

Results and Discussion

Crystallization kinetics

Figure 7-1 shows SFC changes when samples were sonicated using 75 W (Figure 7-1A) and 110 W (Figure 7-1B) of power levels in a continuous manner.
When HIU is applied at 75 W, SFC values increased rapidly after 10 min to a final SFC value of 4.75% on average. While for control samples, SFC values increased after 15 min and with an equilibrium SFC of 3.22%. These are similar to the results showed in Chapter 6 when a slow flow rate was used. Avrami analysis ($R^2$ values between 0.96 and 0.99) showed one order of magnitude higher $k$ values for the sonicated samples compared to the non-sonicated ones with very little change in $n$ values (Table 7-1). This data shows that sonication indeed increased the crystallization rate of palm oil.
Table 7-1. Avrami constant ($k$, min$^{-n}$), Avrami exponent ($n$) and Equilibrium or maximum SFC (SFC$_{\infty}$, %) of the Avrami fitting functions for the samples crystallized without and with different power levels (75 W and 110 W) of ultrasound.

<table>
<thead>
<tr>
<th>Power</th>
<th>$k_c$</th>
<th>$k_1$</th>
<th>$k_2$</th>
<th>$k_3$</th>
<th>$k_4$</th>
<th>$k_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>75W</td>
<td>2.17E-06</td>
<td>3.85E-05</td>
<td>1.64E-05</td>
<td>1.86E-05</td>
<td>1.03E-05</td>
<td>1.10E-05</td>
</tr>
<tr>
<td>110W</td>
<td>2.01E-06</td>
<td>1.12E-06</td>
<td>1.60E-06</td>
<td>1.52E-06</td>
<td>1.78E-06</td>
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<th>Power</th>
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<th>$n_2$</th>
<th>$n_3$</th>
<th>$n_4$</th>
<th>$n_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>75W</td>
<td>3.53</td>
<td>2.91</td>
<td>3.12</td>
<td>3.07</td>
<td>3.32</td>
<td>3.37</td>
</tr>
<tr>
<td>110W</td>
<td>3.58</td>
<td>3.76</td>
<td>3.65</td>
<td>3.68</td>
<td>3.66</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SFC$_{\infty}$</th>
<th>SFC$_{\infty}$</th>
<th>SFC$_{\infty}$</th>
<th>SFC$_{\infty}$</th>
<th>SFC$_{\infty}$</th>
<th>SFC$_{\infty}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>75W</td>
<td>3.22$^a$</td>
<td>4.84$^b$</td>
<td>4.85$^b$</td>
<td>4.68$^b$</td>
<td>4.78$^b$</td>
</tr>
<tr>
<td>110W</td>
<td>4.70$^b$</td>
<td>4.45$^b$</td>
<td>4.63$^b$</td>
<td>4.53$^b$</td>
<td>4.87$^b$</td>
</tr>
</tbody>
</table>

Subscript “c” indicates the control sample, subscript “1-5” indicate the samples collected at each minute during sonication.

$R^2$ values for Avrami fitting were all above 0.96

$^{ab}$Values with same superscript letter in SFC$_{\infty}$ indicate no significant difference ($\alpha = 0.05$)

In Chapter 6, application of 75 W HIU in a continuous manner leads to 2 order of magnitude higher $k$ values for the sonicated samples, this might be due to the longer exposure of the sample to HIU when a slow flow rate is used. When HIU is applied at 110 W, sonicated samples still have the advantage of higher SFCs during 60 min crystallization. However, the crystallization rates ($k$ values) for sonicated samples are similar to the control sample (Table 7-1), even though a slightly faster of crystallization was observed in the SFC kinetics. Similar to 75 W, the application of HIU with 110 W also generated more crystalline material with higher equilibrium SFC value of 4.62% in average. Results obtained with 110 W power level of HIU are significantly different from the ones reported in Chapter 6 where a delay in crystallization was found. This is probably because of the short retention of samples in the flow cell for the higher flow.
rate, which makes the temperature increase under HIU slower than that with slow flow rate. Figure 7-2 compares the temperature increase for the samples sonicated at 75 W and 110 W.

![Figure 7-2](image.jpg)

**Figure 7-2.** Temperature changes of the samples as a function of time during 5 min sonication process for both power levels (75 W and 110 W). Linear regression was fitted to compare the temperature-time profile for different power levels in continuous manner.

Results showed that the temperature increase rate is 0.5473 ($R^2 = 0.93$) and 0.7791 ($R^2 = 0.77$) for 75 W and 110 W, respectively. Whereas, a higher temperature increase rate was found in Chapter 6 with the slow flow rate (0.6491 for 75 W, and 1.463 for 110 W). As discussed in Chapter 6, thermal effects generated by high power levels of HIU melted the crystals and delayed crystallization, pulse sonications of HIU can help to decrease the thermal effects.

Results reported in this chapter provide another option to counteract the temperature increase generated by sonication by using higher flow rate when higher
power level of HIU is used. However, as described before, high flow rate also leads to short exposure of HIU on samples, which decreases the crystallization-inducing effects as well. It is important to balance the effects of cavitation which leads to induction of crystallization and the thermal effects which counteracts cavitation effect by delaying crystallization when applying HIU into the fat system.

**Microstructure of crystals**

PLM pictures shown in Figure 7-3 demonstrate that HIU significantly affect the formation of crystals in different size and shape compared to the non-sonicated samples. Same crystal morphologies were found as in Chapter 6. Big round crystals (indicated with B in the picture wo_60min) and needle-like spherulites (indicated with A in the picture wo_60min) were also observed after 60 min at 35 °C in samples crystallized without HIU. When HIU was applied to the samples, the amount of crystal clusters increased and the crystal aggregates generated a more uniform size distribution with smaller sizes especially when HIU was applied using 75 W and after 60 min of crystallization and 24 h of tempering at 25 °C.

**Melting behavior**

Similar to the results showed in Chapter 6, only the highest melting point fraction (peak) of the thermal profile is evaluated in this chapter. Table 7-2 shows the melting parameters of the palm oil crystallized with (75 W and 110 W) and without HIU at 35 °C for 60 min and stored at different tempering conditions (25 °C and 5 °C for 24h).
Figure 7-3. Microstructure of palm oil without and with HIU at different tempering conditions: 35°C for 60 min, 25°C for 24 h and 5°C for 24 h. In the picture (wo_60min) two different morphologies were indicated (A and B).

When samples are crystallized using HIU at 75 W power level, the peak temperature ($T_p$) for the non-sonicated samples crystallized for 60 min at 35 °C was significantly ($P < 0.05$) higher than the sonicated samples. The peak temperature of the sample decreased from 46.0 ± 0.8 °C to 40.9 ± 0.1 °C when HIU was applied. The same results were maintained after tempering at 25 °C and 5 °C for 24 h. The reason for a shifting to lower peak temperature after sonication might be due to the smaller particle size distribution of the crystals as described in Chapter 6. Enthalpy ($\Delta H$) values of samples crystallized with HIU and kept 60 min at 35 °C were significantly ($P < 0.05$) higher than the ones obtained in the non-sonicated samples. After tempering at 25 °C, the difference was maintained,
Table 7-2. Melting parameters of palm oil crystallized with and without sonication at different power levels (75 W and 110 W). The data was collected at 60 min and after tempering for 24 h at 25°C and 5°C. Data reported are mean values and standard deviations of 2 independent runs. Upper case subscripts with the same letter indicate that values are not significantly different ($\alpha=0.05$) in the same row. N/A means that the value could not be calculated since it fell outside the temperature range of the DSC run.

<table>
<thead>
<tr>
<th></th>
<th>60 min</th>
<th>25 °C 24 h</th>
<th>5 °C 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>75 W</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{on}$ (°C)</td>
<td>N/A</td>
<td>38.0 ± 0.6$^A$</td>
<td>39.4 ± 1.1$^B$</td>
</tr>
<tr>
<td>$T_p$ (°C)</td>
<td>46.0 ± 0.8$^A$</td>
<td>40.9 ± 0.1$^B$</td>
<td>47.3 ± 0.1$^A$</td>
</tr>
<tr>
<td>$\Delta H$ (J/g)</td>
<td>7.0 ± 0.5$^A$</td>
<td>11.1 ± 0.7$^B$</td>
<td>4.9 ± 0.7$^C$</td>
</tr>
<tr>
<td><strong>110 W</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{on}$ (°C)</td>
<td>N/A</td>
<td>N/A</td>
<td>39.4 ± 1.1$^A$</td>
</tr>
<tr>
<td>$T_p$ (°C)</td>
<td>46.0 ± 0.8$^A$</td>
<td>41.3 ± 0.2$^B$</td>
<td>47.3 ± 0.1$^A$</td>
</tr>
<tr>
<td>$\Delta H$ (J/g)</td>
<td>7.0 ± 0.5$^A$</td>
<td>9.2 ± 1.1$^B$</td>
<td>4.9 ± 0.7$^C$</td>
</tr>
</tbody>
</table>

whereas after tempering at 5 °C, no difference was found for the enthalpy between with and without HIU samples for the highest melting fraction. Similar results were found when power level of 110 W HIU was applied.

**Conclusion**

This study investigated the use of HIU in a continuous flow cell system with a high flow rate (20 ml/sec) to change the physical properties of palm oil. Two power levels of HIU was applied (75 W and 110 W). Application of 75W power level of HIU showed a faster crystallization rate with significantly higher final SFC values, while application of 110 W power level of HIU suggested a slight induction of crystallization kinetics, and generated a higher equilibrium SFC value. Temperature increase rate for
both power levels of HIU application was slower than that with slow flow rate. Similar
results on crystal morphology and melting profile were obtained when compared with the
samples with slow flow rate. Results in this study suggest the option of decreasing the
thermal effects that are generated by high power level of HIU by increasing the sample
flow rate, however, crystallization induction was decreased as well due to the short
exposure of samples to HIU. To obtain the best HIU application conditions, factors such
as HIU power levels, sonication durations, and sample flow rate need to be
comprehensively considered.
CHAPTER 8

A PRELIMINARY STUDY ON THE EFFECT OF HIGH INTENSITY ULTRASOUND ON CRYSTALLIZATION BEHAVIOR OF PALM STEARIN

Introduction

Shortenings are plastic fats composed of two phases: a solid crystalline phase and a liquid phase. Fat crystals present in shortening form a network that holds the liquid oil imparting plasticity to the product.¹ The consistency and functional properties of a fat depend mainly on three factors: solid/liquid ratio, plasticity, and oxidative stability.² In addition to these main factors, other properties of the crystalline network formed during shortening production can affect their functional properties ³ including size and the shape of the crystal aggregates, distribution of size, the crystallization kinetics,⁴ and polymorphism.⁵

Palm stearins are produced from palm oil by fractionation. The use of palm stearins in food products is limited due to their high melting points (44 ~ 56 °C) and high amount of solids which results in a hard and brittle product.⁶ However, palm stearin can be interesterified or blended with other fats to obtain a more plastic shortening.⁷, ⁸ Specific uses of palm stearin is in the production of fluid margarines where a small proportion of high melting fats such as palm stearin (or high melting palm stearin) is blended with a high proportion of unsaturated TAGs.² The proportion of palm stearin and unsaturated oil can be changed to obtain desired functional properties. However, the use of unsaturated fats increases the cost of the product and decreases its oxidative stability.
Therefore, it is necessary to explore different options that can decrease the hardness of the palm stearin to reduce the amount of unsaturated oil added.

As described in previous chapters, many studies have been performed to evaluate the effect of high intensity ultrasound on lipid crystallization.\textsuperscript{9-14} Those studies suggested that HIU can be used to induce crystallization to generate smaller crystals and harder material in texture. However, some other studies showed that HIU can also be used to delay crystallization. HIU has been used as a tool on the liquefaction of candied honey.\textsuperscript{15, 16} These studies showed that HIU was more efficient in liquefying honey than the conventional heating treatment. Authors report that HIU was able to melt D-glucose crystals and inhibit re-crystallization. Martini \textit{et al.} studied the effect of HIU on crystallization behavior of anhydrous milk fat (AMF). They found that when crystallization temperature is low (at 22 °C), HIU delayed the crystallization from 16 min to 24 min as fewer crystals were observed in the polarized light microscopy image.\textsuperscript{13}

Based on these previous studies the objective of this research is to determine the use of HIU to delay the crystallization of a commercial palm stearin product. Sonication conditions were optimized with different power levels and sonication durations when palm stearin was crystallized at different crystallization temperatures. Physical properties such as solid fat content, microstructure, melting profile, and elastic properties were measured.

\textbf{Material and Methods}

\textit{Material}

Refined, bleached, and deodorized palm stearin (55\% ~ 65\% palmitic acid content, iodine value is 30.0 ~ 40.0) provided by Archer Daniels Midland Company (ADM Oils,
Oils Division, IL, US) was used in this experiment. The melting point of the palm stearin is $51.4 \pm 0.5 \, ^\circ\text{C}$, which was determined using AOCS Official Method Cc 1-25.

**Sample preparation**

One hundred grams of sample was melted in the microwave and left in an oven at 75-80 °C for 30 min to completely erase crystal memory. Melted samples were then placed in a double-walled crystallization cell which was attached to a water bath to allow for temperature control. The water bath was set at two specific crystallization temperatures ($T_c = 46 \, ^\circ\text{C}$ or $49 \, ^\circ\text{C}$). After placing the samples in the crystallization cell the sample temperature decreased exponentially from approximately 70 °C to $T_c$. The temperature in the samples was recorded during the entire experiment using a thermocouple. Samples were crystallized under agitation using an impeller set at 100 rpm for a total time of 60 min. Time zero corresponds to the moment that the sample is placed in the crystallization cell.

**HIU application**

Ultrasound was applied using a Misonix S-3000 generator (Misonix Inc., NY). Different sonication conditions were tested in the shortening crystallized at $T_c = 46 \, ^\circ\text{C}$ (used as a high supercooling condition) and 49 °C (used as a low supercooling condition). HIU (20 kHz) was applied using a 1/2” sonication horn with different power levels (18 W, 27 W, and 36 W) and different durations ($T_c = 49 \, ^\circ\text{C}$: 10 sec and 30 sec; $T_c = 46 \, ^\circ\text{C}$: 30 sec and 60 sec). The HIU application durations for $T_c = 49 \, ^\circ\text{C}$ were tested for samples crystallized at 46 °C, a decision on extending of sonication of HIU for $T_c = 46 \, ^\circ\text{C}$ was
made based on the results. HIU was applied when the sample showed a significant amount of crystals as will be discussed in the “results” section.

**Solid fat content (SFC)**

The SFC of the sample during crystallization was measured by a NMR 120 Minispec NMR analyzer (Bruker, Germany) using AOCS method Cd 16b-93. During the first 10 min of the crystallization process, samples were loaded from the crystallization cell into the NMR tube using a Pasteur pipette and the SFC was measured as soon as possible every 2 min. After that, SFC was measured every 5 min. Before loading the sample, NMR tubes were kept at crystallization temperature in an incubator. The crystallization curves were fitted to the Avrami equation (eq. 1) by least squares nonlinear regression:

$$\frac{SFC(t)}{SFC_\infty} = 1 - EXP\left(-kt^n\right)$$

where SFC($t$) and SFC$_\infty$ are the SFC (%) at time $t$ and the maximum SFC after crystallization is completed, respectively. The SFC data fitted to this model was used to determine the rate constant of crystallization ($k$) at a particular temperature, and the mechanism of nucleation and crystal growth through the exponent, $n$.

**Microstructure**

Crystal morphology was recorded during crystallization. A drop of lipid sample was taken from the crystallization cell at different time points and placed between a slide and cover-slide, which were kept at crystallization temperature in an incubator, to evaluate the microstructure variation using a polarized light microscope (PLM, Olympus BX 41, America Inc., Melville, NY, USA) equipped with a digital camera (Lumenera...
Pictures were taken as soon as the sample was placed in the slide and cover slide to avoid morphology changes during this process. A thermostatized stage was attached to the microscope to allow for temperature control during the measurement.

**Thermal profile**

The thermal behavior of the crystalline network was analyzed using a differential scanning calorimeter (DSC, Q20 TA Instruments, New Castle, DE). After the whole crystallization process, 5 to 15 mg of sample was placed into a hermetic aluminum pan. Samples were then heated from $T_c$ to 80 °C at 5 °C/min. Onset ($T_{on}$) and peak ($T_p$) temperatures, and melting enthalpy were calculated from the melting profile by using the TA Instruments Universal Analysis 2000 (TA Instruments, DE). Peak temperatures (°C) represent the temperature at which the melting peak reaches its minimum, while onset temperatures (°C) are defined as the temperature at which the first deviation from the baseline is observed, which corresponds to the first melting processes in the sample. Melting enthalpies (J/g) are calculated through integration of the area under the melting peak and are used to quantify the amount of solid fat present in the crystalline network.

**Elastic properties**

The elastic properties of the crystallized material were evaluated by using AR-G2 rheometer (TA Instruments, New Castle, DE). A strain sweep from 0.0008 to 10% with a constant frequency of 1 Hz (6.28 rad/s) was used to evaluate the storage modulus ($G'$) of the samples. This strain sweep corresponds to the linear elastic region of the samples tested. The elastic properties of the samples were measured after the crystallization
process (60 min) and after tempering the samples for 48 h at 25 °C. The elastic values were measured in duplicate in two independent runs.

Statistical analysis

Crystallization experiments were performed in duplicate and physical properties were measured in duplicate. Significant differences (P < 0.05) were evaluated using Two-way ANOVA using GraphPad Prism software, version 6.00 for Windows, GraphPad Software, San Diego, CA, USA.

Results and Discussion

The crystallization behavior of palm stearin at 46 and 49 °C was evaluated by measuring the solid fat content as a function of time (Figure 8-1). Data was well fitted to the Avrami equation with R² values of 0.996 and 0.987 for 46 °C and 49 °C, respectively. As Figure 8-1 shows, the crystallization rate for samples at 46 °C is much faster than that of 49 °C (k = 4.4 x 10⁻⁵ and 3.3 x 10⁻⁷ for 46 °C and 49 °C, respectively). This is due to the higher supercooling generated at 46 °C resulting in a higher driving force for crystallization. The two arrows (A and B) shown in Figure 8-1 indicate the mid-point (half value of maximum SFC) for both kinetic curves. Based on previous studies, our hypothesis is that a significant amount of crystals must be present in the sample when HIU is applied to obtain a delay in the crystallization. Therefore, the moment corresponding to the mid-point of the curve was chosen for HIU application (25 min for samples crystallized at 46 °C and 40 min for samples crystallized at 49 °C).
Figure 8-1: Solid fat content of palm stearin as a function of time at $T_c = 46 \, ^\circ C$ (dotted line) and $49 \, ^\circ C$ (solid line); two arrows (A and B) indicate the point at which half SFC was obtained for both kinetic curves.

*Low supercooling ($T_c = 49 \, ^\circ C$)*

Three HIU power levels (18 W, 27 W and 36 W) with two durations (10 sec and 30 sec) were used in samples crystallized at $49 \, ^\circ C$. HIU was applied at 40 min as discussed in the previous section (Figure 8-1). Sample temperature was recorded during the crystallization process (Figure 8-2) showing an exponential decreased from approximately $70 \, ^\circ C$ to $49 \, ^\circ C$ in approximately 12 min.

Arrow A in Figure 8-2 indicates the time that crystals were observed at approximately 18 min which is evidenced by a slight increase in temperature due to the exothermic nature of the crystallization process. A slight temperature increase ($\Delta T = 1.8 \sim 2.1 \, ^\circ C$) was also observed when HIU was applied at 40 min. As soon as HIU application was stopped sample temperature returned to $T_c$ quickly and remained at $T_c$ until the end of the crystallization process.
Figure 8-2: Temperature profile as a function of time during the crystallization process; Arrow A indicates the moment when crystals were observed; “HIU @ 40 min” represents the moment when HIU was applied (40 min); ΔT suggests the temperature increase as a consequence of HIU application.

Microstructure

PLM was used to evaluate changes in crystal microstructure obtained under different conditions. According to the preliminary results (not shown), three conditions were chosen as the conditions for further evaluation: 18 W for 30 sec, 27 W for 10 sec, and 36 W for 10 sec. Figure 8-3 shows the microstructure of the palm stearin crystals obtained when samples were crystallized with and without HIU application. PLM pictures were taken every 5 min from 45 min to the end of the crystallization process. Results showed that all HIU conditions slightly delayed crystallization, especially within the 10 min (between 40 min and 50 min) after HIU was applied and generated slightly bigger crystals. The sample without HIU application showed a more interconnected crystalline network while individual crystals with fewer interconnections were observed in the sonicated samples.
Figure 8-3: Microstructure of palm stearin crystals obtained without and with HIU application at 49 °C.

\[ 166 \]

\[ \text{Elastic properties} \]

Figure 8-4 shows the \( G' \) of samples crystallized without and with HIU application after 60 min into crystallization process and after tempering for 48 h at 25 °C. Two HIU conditions (18 W for 30 sec, and 27 W for 10 sec) significantly \( (P < 0.05) \) decreased the \( G' \) values compared to the control group \( (G' = 1.23 \times 10^5, 2.88 \times 10^4, \text{and} 2.01 \times 10^4 \text{ for samples without HIU, with 18 W for 30 sec, and 27 W for 10 sec, respectively}) \); whereas samples sonicated at 36 W for 10 sec maintained the same elastic texture as the samples without HIU. At latter stage of the crystallization, the high viscosity delays or prevents the inertial cavitation when proper power of HIU was applied.
After tempering for 48 h at 25 °C, all the samples continue to crystallize as expected, due to the low tempering temperature and the effect of sonication is lost. Samples sonicated at 36 W for 10 sec resulted in the highest G’ value compared to other groups. HIU might contribute to this phenomenon. We hypothesized that after HIU was applied at 36 W, the large crystals were broken down to generate some fragments or small crystals (even though they might not been visible in microscopy pictures). Those fragments act as the new nuclei for samples to continue with secondary crystallization during tempering with the generation of small crystals and therefore a harder material. Since samples crystallized with HIU at 36 W for 10 sec generated the same G’ value than
samples crystallized without HIU, and since the main objective of this research is to
obtained a softer crystalline network no further studies were performed on this sonication
crystallization condition.

**Thermal profile**

The thermal behavior of samples crystallized for 60 min at 49 °C and tempered
for 48 h at 25 °C are shown in Table 8-1. No difference was found on any of the melting
parameters among the conditions tested.

Same enthalpy values generated after HIU application indicates that HIU neither
delayed nor accelerated crystallization and therefore did not affect the amount of
crystalline material generated. Other factors such as shape and size of the crystals and the
arrangement of the crystal aggregates might contribute to the decrease of elastic
properties discussed in Figure 8-4 for these two sonication conditions (18 W for 30 sec,
and 27 W for 10 sec).

**High supercooling (T<sub>c</sub> = 46 °C)**

Based on the results found at low a supercooling (49 °C) section samples
crystallized the sonication condition of 36 W for 10 sec was not tested at T<sub>c</sub> = 46 °C.
Since the formation of a softer texture was not observed at low supercoolings we don’t
expect to see differences at high supercoolings. After preliminary screening, two HIU
conditions were chosen for further evaluation: 18 W for 60 sec and 27 W for 30 sec. As
previously discussed samples crystallize faster when processed at 46 °C compared to
49 °C and SFC values reached a plateau in a shorter time (Figure 8-1). Therefore,
crystallization runs were stopped at 40 min instead of the 60 min duration used for the samples crystallized at 49 °C. HIU was applied at 25 min into the crystallization process.

Table 8-1: Melting parameters (Ton, Tp, and ΔH) for samples crystallized with and without HIU application at 49 °C for 60 min and tempering at 25 °C for 48 h.

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<th>27W for 10s</th>
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<tr>
<td>Ton (°C)</td>
<td>51.9 ± 0.2</td>
<td>51.2 ± 0.1</td>
<td>51.5 ± 0.2</td>
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<tr>
<td>Tp (°C)</td>
<td>56.0 ± 0.0</td>
<td>55.6 ± 0.3</td>
<td>55.5 ± 0.3</td>
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<tr>
<td>ΔH (J/g)</td>
<td>27.4 ± 0.4</td>
<td>27.5 ± 0.0</td>
<td>26.4 ± 0.7</td>
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<th>48hrs @ 25 °C</th>
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<th>18W for 30s</th>
<th>27W for 10s</th>
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<tr>
<td>Ton (°C)</td>
<td>50.7 ± 0.1</td>
<td>50.2 ± 0.1</td>
<td>51.1 ± 0.1</td>
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<tr>
<td>Tp (°C)</td>
<td>55.9 ± 0.2</td>
<td>55.6 ± 0.1</td>
<td>55.7 ± 0.1</td>
<td></td>
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<tr>
<td>ΔH (J/g)</td>
<td>62.9 ± 0.5</td>
<td>62.8 ± 0.6</td>
<td>60.6 ± 2.1</td>
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</table>

Microstructure

Crystal morphology was evaluated using PLM images as shown in Figure 8-5. No evident difference was found within 5 min of HIU application. Slightly more “black spaces” are observed in the sonicated samples which suggests a slightly delay of crystallization. Crystal microstructures did not show any differences after 30 min into the crystallization process (data not shown).

Figure 8-5: Microstructure of palm stearin without and with HIU application at 46 °C.
Elastic property

Figure 8-6 shows the storage modulus (G’) of palm stearin crystallized with and without HIU at 46 °C. Similar to the discussion of samples crystallized at 49 °C, G’ values were significantly lower after 40 min crystallization process in the sonicated samples compared to the samples crystallized without ultrasound. After tempering the samples for 48 h at 25 °C, G’ values for sonicated samples are significantly (P < 0.05) higher than the ones obtained for the non-sonicated ones. This might be due to a delay in crystallization and the formation of fewer crystals as a consequence of sonication. This

![Figure 8-6](image)

**Figure 8-6:** Storage modulus (G’) of palm stearin crystallized with and without HIU application after 60 min into the crystallization at 46 °C and 48 h tempering at 25 °C. Values with same letter (a, b) indicate no significant difference (α = 0.05).
was not confirmed by the PLM images but it is corroborated by the lower enthalpy values obtained in the sonicated samples after 40 min at $T_c$. That is, samples crystallized without the use of HIU were almost completely crystallized after 40 min at $T_c$, and therefore less liquid fat is available to crystallize at 25 °C. On the other hand, crystallization was delayed in the sonicated sample and fewer crystals were generated during the 40 min at $T_c$ (Table 8-2), and therefore more liquid material is available to crystallize when placed at 25 °C generating a harder material.

<table>
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<th>40min @ 46 °C</th>
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<th>18W for 60s</th>
<th>27W for 30s</th>
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<tbody>
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<td>$T_{on}$ (°C)</td>
<td>52.2 ± 1.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>48.7 ± 0.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>49.6 ± 0.3&lt;sup&gt;ab&lt;/sup&gt;</td>
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<tr>
<td>$T_p$ (°C)</td>
<td>55.2 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>55.0 ± 0.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>54.7 ± 0.6&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>$\Delta H$ (J/g)</td>
<td>46.7 ± 1.2&lt;sup&gt;a,A&lt;/sup&gt;</td>
<td>43.1 ± 1.2&lt;sup&gt;b,A&lt;/sup&gt;</td>
<td>42.5 ± 0.7&lt;sup&gt;b,A&lt;/sup&gt;</td>
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</table>

| 48hrs @ 25 °C |
|---------------|--------|-------------|-------------|
| $T_{on}$ (°C)    | 49.9 ± 1.2<sup>a</sup>| 48.5 ± 1.9<sup>a</sup> | 48.6 ± 0.4<sup>a</sup> |
| $T_p$ (°C)      | 55.2 ± 0.3<sup>a</sup>| 55.3 ± 0.0<sup>a</sup> | 55.4 ± 0.0<sup>a</sup> |
| $\Delta H$ (J/g)| 46.3 ± 1.0<sup>a,A</sup> | 48.7 ± 1.5<sup>a,B</sup> | 54.1 ± 0.8<sup>b,B</sup> |

<sup>ab</sup>Values in the same row with same superscripts in lower case indicate no significant difference ($\alpha = 0.05$).

<sup>AB</sup>Enthalpy values before and after tempering show same superscript in upper case indicate no significant difference ($\alpha = 0.05$).

**Thermal profile**

Table 8-2 shows the thermal profile of the samples crystallized at 46 °C for 40 min and subsequent tempering at 25 °C for 48 h. Different from samples crystallized at 49 °C, some statistical differences were found between samples crystallized with and without HIU. After 40 min of crystallization, samples with HIU applied at 18 W for 60 sec had a significantly lower ($P < 0.05$) $T_{on}$. The peak temperatures for all the conditions
were not significantly different (P > 0.05). Significant (P < 0.05) decrease of enthalpy values were found after HIU was applied, which indicate less crystalline material was formed with the application of HIU. After tempering for 48 h at 25 °C, both $T_{on}$ and $T_p$ show no difference among all the conditions. Samples with HIU applied at 27 W for 30 sec had a higher (P < 0.05) enthalpy value than the other two groups of samples. When comparing the enthalpy values before and after tempering, samples without HIU application remain the same, whereas, samples with HIU application both increased significantly (P < 0.05). This result confirms the conclusion that HIU delays the crystallization which causes more “liquid” fat to be crystallized during tempering.

**Additional experiment**

Martini *et al.* investigated the effect of HIU on crystallization behavior of anhydrous milk fat (AMF). They showed that when crystallization temperature is low (i.e. 22 °C), HIU delayed the crystallization from 16 min to 24 min as fewer crystals were observed in PLM pictures. They proposed that under high supercooling, the system is more viscous in nature, and the bubbles generated by HIU are non-inertial, which cannot collapse to promote crystallization. In addition, these authors explain that the temperature increase observed during sonication melts some crystals generating a delay in the crystallization process.\(^{13}\)

Based on this hypothesis we decided to test a higher power level of ultrasound at early stage of the crystallization process under high supercooling condition. HIU was applied at 75 W for 10 sec and 20 sec to the palm stearin crystallized at 46 °C. HIU was applied at 15 min which is the moment that SFC starts to increase (Figure 8-1). Preliminary tests were carried out and no differences were found for the data obtained
using the 10 sec condition and therefore only the 20 sec data is reported. Figure 8-7 shows the microstructure of the samples crystallized without HIU and sonicated for 20 sec using 75W of power.

![Figure 8-7: Microstructure of palm stearin crystallized at 46 °C, HIU was applied at 15 min with 75 W for 20 sec.](image)

After HIU was applied at 15 min, a significant delay of crystallization was observed in the PLM pictures as evidenced by fewer crystals formed at a specific time point (20 min to 25 min).

This result was similar to the findings described by Martini et al. However, after 40 min at Tc no difference was found in the PLM pictures nor in the elastic and
thermal properties for sonicated and non-sonicated samples. The high supercooling ($T_c = 46 \, ^\circ C$) might be the major contribution for this result. In order to make more significant effects, different conditions such as higher crystallization temperature with higher power level of HIU, together with longer sonication time need to be tested in the future work.

**Conclusion**

This study provided preliminary discussion on the effect of HIU on crystallization behavior of palm stearin. Different power levels of HIU with different sonication durations were performed in the palm stearin system under two supercoolings. An innovative idea and method was developed to use HIU to delay crystallization and/or generate softer material (for samples crystallized at 46 °C, crystallization was delayed and lower G’ values were obtained; whereas, at 49 °C, no delay of crystallization but softer material was gained). Further studies are needed to optimize the HIU application conditions. The polymorphism analysis might also be required to better understand the effect of HIU on crystallization behavior.

**References**


CHAPTER 9
CONCLUSION

High intensity ultrasound (HIU) has been extensively used in several applications such as to induce crystallization of organic and inorganic molecules (sonocrystallization),\(^1,\,^2\) to induce chemical reactions (sonochemistry),\(^3,\,^4\) and to disrupt cells.\(^5,\,^6\) However, there has been little research on the effect of HIU on the crystallization behavior of food-related materials. Some of the initial food-related studies were performed in the early 2000. Ultrasound was used in ice crystallization to improve freezing operations of food systems,\(^7,\,^8\) followed by studies in lactose and sucrose crystallization.\(^9,\,^{10}\) Studies on sonocrystallization of lipids were pioneered by Sato’s group,\(^11,\,^{12}\) followed by several further work.\(^13-15\)

Based on previous research and work, this dissertation provides information on the effects of HIU on the crystallization behavior and physical properties of different shortenings. In the first set of experiment, HIU was used in a low saturated IESBO shortening to change the crystallization behavior and generate small crystals and a harder material (Reviewed in Chapter 3). Results in this chapter indicate that HIU is more efficient in inducing crystallization when appropriate higher power was used in a system subjected to low supercooling. In addition, harder, more elastic, and viscous materials were generated when HIU was applied in the presence of crystals at early stages of crystallization. Subsequent chemical composition analysis was carried out to confirm that the effect of HIU on IESBO is mainly focused on sonocrystallization but without
changing the triacylglycerol (TAG) and fatty acids composition (Chapter 4). In the second set of experiments, a multi-purpose commercial shortening was used in a temperature cycling system (Chapter 5). HIU was shown to maintain the texture of the shortening, especially when HIU was applied before temperature fluctuation. Larger sonication tip size (1/2") was proved to be more effective in changing crystallization microstructure in a slow cooling process. The study in Chapter 5 mimics a temperature cycling system to temperature variations that might occur in an industrial setting during shortening production and/or during distribution. The application of HIU in a continuous system (flow cell) was evaluated in Chapter 6. The objective of this study was to determine the effect of HIU in lipid crystallization in an in-line system. Results showed that low power level (75 W) of HIU with continuous sonication leads to the generation of a crystalline network with higher SFC, higher elasticity, and sharper melting profile. This research showed that pulse sonication of sonication and higher flow rate (reviewed in Chapter 7) of palm oil into the flow cell could be two options to decrease the thermal effects generated by higher power levels of HIU. Studies of this flow cell system offered great potential of ultrasound as a processing tool in a real in-line shortening production. Previous studies of sonocrystallization of lipids \textsuperscript{11,13-15} and the chapters discussed above use HIU to induce lipid crystallization; however, some of other studies also showed the use of HIU to delay crystallization.\textsuperscript{14,16,17} Chapter 8 reviewed the application of HIU in palm stearin to delay crystallization and generate softer material. Lower power levels with long durations of HIU application was used in the latter stage of crystallization. This study provides the option to decrease the hardness of the palm stearin to reduce the
amount of unsaturated oil added in the blending formulations, as the use of unsaturated oil increases the cost of the product and decreases its oxidative stability.

Figure 9-1: Summary of the results based on HIU principles

Figure 9-1 summarized the overall effects of HIU on physical and chemical properties of fats and oils which were described in this dissertation. As mentioned before, HIU can be controlled through several parameters: power levels, sonication durations, sonication time (when to apply during the crystallization process), ultrasound tip types and sizes, and the oil sample size. By controlling different parameters, different applications of HIU can be achieved. Looking back to Chapter 2, Table 2-1 listed different types of shortenings. Taking cake and icing shortenings for example, both shortenings require desirable beta prime polymorphism which might be controlled by using HIU. Similar results can also be achieved in chocolate production which also needs specific polymorphic cocoa butter crystals. In both Chapter 3 and Chapter 7, same results
were found that HIU can generate steeper SFC melting profile. This characteristic can be applied in the filler fat shortening and confectioner’s shortening as shown in Table 2-1.

As HIU can be used to control crystallization, potential use of HIU to produce a wide range of texture (from soft to hard) oil products might be achieved. This can help to most effectively use one type of oil so that the value of which can be maximized. A successful example could be the use of fractionation technique in palm products.

Much effort and work have been done to date to suggest the potential use of HIU as a processing tool to improve functional properties of lipids and final products. However, research in the sonocrystallization of lipids is still at its initial stage, and much work needs to be done to further explore it. For example, HIU is not a standardized technology and therefore must be developed and scaled up for every new application.\textsuperscript{18}

Many important questions remain to be answered. It is not clear for example, the role of cavitation or bubbles play in the growth of crystals during sonication at the molecular level; cavitation dynamics and threshold must be quantified to further understand the role of inertial and non-inertial bubbles in inducing crystallization. In addition, the effect of HIU on polymorphism of some commonly used industry fats, such as palm oil, palm kernel oil, and cocoa butter, requires further investigations. It is important to combine and balance HIU and other processing methods to consistently generate the required polymorphism and functional property for a specific food product (i.e., chocolate). At last, the effects of HIU on the final product quality are yet to be established, such as the overall physical and sensory properties, the shelf life, and oxidative stability.

In summary, research presented in this dissertation evaluated the use of HIU to modify the functional properties of lipids by changing their crystallization behavior.
Results from these studies will provide scientific support of the market accessibility of HIU technique in the edible oil processing. This dissertation provides evidence of the effect that processing conditions have in changing molecular organization in the lipid network that will ultimately result in different functional properties of the material. The long term goal of this dissertation is to provide a novel and additional processing tool to the food industry that can be used in combination with other processing techniques to improve the functional properties of shortenings while maintaining the nutritional value of edible lipids, and finally improve the product quality in terms of product performance, consumer acceptability and shelf life.

References


APPENDIX
Supporting information for Chapter 6

**Figure 1:** Temperature change of palm oil samples as a function of time during 5 min sonication process for all the power levels and irradiation durations. Linear regression was fitted to compare the temperature-time profile for different power levels in continuous manner (a), and the difference (continuous vs. pulse) temperature increase with different power levels (75W, 110W and 180W, b, c and d, respectively)
Figure 2: X-ray diffractograms of palm oil samples crystallized without (a) and with (b) HIU (75W, continuous sonication) at 35 °C for 60 min and after tempering at 25°C and 5 °C for 24 h
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Author: Yubin Ye, Ashwini Wagh, Silvana Martini

Publication: Journal of Agricultural and Food Chemistry

Publisher: American Chemical Society

Date: Oct 1, 2011

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CURRICULUM VITAE

YUBIN YE

Education

May 2010 – (Expected in Dec. 2014) GPA: 3.88 / 4.00
PhD in Food Science, Department of Nutrition, Dietetics, and Food Sciences, Utah State University (USU), Logan, Utah, USA

Sep 2005 – July 2009 GPA: Top 15%
Bachelor of Engineering, College of Food Science & Nutritional Engineering, China Agricultural University (CAU), Beijing

Work & Research Experience

- May 2010 – Present Graduate Research Assistant, Martini’s Lab, USU

Lipid Crystallization & Ultrasound Research (Research experience_4 years)
The objective of this research is to use high intensity ultrasound (HIU) in edible lipids to change their physical properties. These lipids with improved physical properties could be used to replace trans fats in foods. The outcomes of my research are:

1. Provided evidence that HIU can change the physical properties of edible lipids in a batch system (USDA-funded project)
2. Proved that lipid texture can be improved and maintained during temperature fluctuations by using HIU (Mondelēz-funded project)
3. Conducted HIU experiments in a continuous system (flow cell system) (Utah Agricultural Experiment Station-funded project)

Sensory Evaluation (Development experience_3 years)
1. Lead the design, execution, data analysis and interpretation of consumer sensory panels (qualitative and quantitative)
   - Lead the sensory team to recruit 120 participants from public to execute the test
   - Conduct tests for different food products: Cookie, Cheese, Beef, Whey protein drink, Peach, Yogurt, Milk, Energy drink, Mineral stick pack and soft gels
2. Train students/employees on experimental design and software using
   - Introduce the use of software for sensory evaluation to employees
   - Mentor students to setup a test: questionnaire design, screen sample, data analysis
3. Teaching assistant of the Sensory Evaluation Class
4. Statistical knowledge: Linear Regression, SAS software using, SIMS2000 sensory software, ANOVA test

- **June 2007 – July 2009** Undergraduate Research Assistant, Functional Dairy Lab, CAU
  - Research focused on natural anti-oxidants extraction and application
  - Gain lab skills and experience, prepare for graduate school

**Regulatory Compliance Certificates**

- HACCP
- Good Manufacturing Practices (GMP)
- Safe Quality Food (SQF)
- Advanced Sanitation
- Employee Based Food Safety
- Statistical Process Control (SPC)

**Technical Skills**

- **Professionally trained in Sensory Evaluation:** SIMS2000, Experimental Design
- **Statistics Analysis:** Linear Regression, SAS, Graphpad Prism, ANOVA test, Excel
- **Familiar with techniques applied in R&D laboratories:** pH, Aw, Temperature
- **Formally trained for analytical laboratory skills:** HPLC, GC, X-ray, Enzyme kinetics,
- **Familiar with analytical tools:** Polarized Light Microscopy (PLM), X-ray Diffraction, SEM, Image Analysis, Differential Scanning Calorimeter (DSC), Rheometer, Nuclear Magnetic Resonance (NMR), Texture Profile Analyzer (TPA)

**Awards**

- 2014 First Place in IFT Bonneville Section Student Research Poster Competition
- 2012 Dr. Niranjan R. Gandhi and Mrs. Josephine N. Gandhi Scholarship – USU
- 2012 College of Agriculture Travel Award – Utah State University
- 2011 Third place in IFT Bonneville Section Student Research Poster Competition
- 2008 “Exceptional Volunteer” of Wrestling Gymnasium in 2008 Beijing Olympics
- 2007 Mars China Scholarship – China Agricultural University

**Services at University**

- June 2010 – Present Food Science Club, USU
- Nov 2007 – Nov 2008 Team leader, Food and Culture Association, CAU
- Sep 2005 & June 2009 Freshman and Senior Spokesman, respectively, CAU

**Publications**

[1] Ye, Y., Martini, S.* Application of high intensity ultrasound to palm oil in a continuous system (flow cell). Journal of Agriculture and Food Chemistry (submitted)


**Presentations**

**Oral Presentation**
- April 28 – May 1, 2013  **104th AOCS Annual Meeting & Expo, Montreal, QC, Canada**  
  Ye, Y., Tan, C. Y., Kim, D. A., Martini, S.* Application of power ultrasound to a zero-trans shortening during temperature cycling under different cooling rates
  1) Ye, Y., Marini, S. Changing the microstructure of a high saturated shortening using power ultrasound
  2) Alberto, J.R., Ye, Y., Martini, S., et al. *Polymorphic Behavior of Sunflower Oil Stearins (Co-author)*

**Poster Presentation**
- May 1-4, 2011  **102nd AOCS Annual Meeting & Expo, Cincinnati, OH, US**  
  Ye, Y., Marini, S. Using High Intensity Ultrasound as a tool to change the functional properties of Interesterified Soybean Oil
- May 4 – May 7, 2014  **105th AOCS Annual Meeting & Expo, San Antonio, TX, US**  
  Ye, Y., Martini, S. Application of HIU to palm oil in a continuous system (flow cell)
- April 8, 2014  **IFT/AACT Utah Food and Candy Expo 2014**  
  Ye, Y., Martini, S Effects of HIU on the crystallization behavior of palm oil in a
continuous system

- April 9, 2013  **IFT/AACT Utah Food and Candy Expo 2013**
  Ye, Y., Martini, S The influence of ultrasound related variables on the crystallization behavior of shortenings

  Martini, S., Ye, Y., Doyle, T. Monitoring bubble and crystal formation in lipid systems using ultrasound spectroscopy during high intensity insonation

  Ye, Y., Wagh, A., Allen, K., Martini, S. High Intensity Ultrasound as a novel technique to change the microstructure and textural properties of fats

- April 5, 2011  **IFT/AACT Utah Food and Candy Expo 2011**
  Ye, Y., Martini, S Using HIU as a tool to change the crystallization behavior of interesterified soybean oil