COMPARATIVE ASSESSMENT OF THE THERMOMECHANICAL PROPERTIES OF HIGH OLEIC PALM OIL AND ACYLGLYCEROL EMULSIFICATION TO STANDARD PALM OIL

By

VICTOR CEDENO SANCHEZ

A THESIS PRESENTED TO THE GRADUATE SCHOOL OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE

UNIVERSITY OF FLORIDA

2020
To Martha, Pablo and Hector, always close by despite the distance
ACKNOWLEDGMENTS

I would like to acknowledge the guidance, support and infinite patience to my committee chair, Dr. Andrew MacIntosh. The conclusion of this project would have not been possible without his impetus to make the best researchers out of his mentees. I would like to thank my committee members Dr. Victoria Miller and Dr. Liwei Gu for their valuable help and insight on the project. I would also like to acknowledge the work of Scarlett Godinez in the PCCL laboratory in the Chemistry department, who helps was of extreme importance to explore new dimensions of the project. Also, special thanks to the FSHN department for their unconditional help during these two years.

A very special acknowledgment goes to Dr. Gloria Cagampang, whose tireless desire to push the knowledge on High Oleic Palm Oil brought us all together. Also, my deepest gratitude to Carlos Corredor for believing in the power of science as a transformative force and Thin Oil Products for their unquestionable support throughout the degree.

Additionally, I have to express my deepest appreciation to all my lab mates, which later turned into friends, to whom I wish the best in their professional endeavors, especially to the R.A.T.A.S. committee. Last but not least, to my family and friends in the diaspora, that somehow contributed in some way or another to the project and were always an infinite source of inspiration, motivation, and laughs.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>ACKNOWLEDGMENTS</th>
<th>..........................................................</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>LIST OF TABLES</td>
<td>........................................................................</td>
<td>7</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>........................................................................</td>
<td>8</td>
</tr>
<tr>
<td>LIST OF ABBREVIATIONS</td>
<td>........................................................................</td>
<td>9</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>........................................................................</td>
<td>10</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>........................................................................</td>
<td>12</td>
</tr>
<tr>
<td>2 OBJECTIVE</td>
<td>........................................................................</td>
<td>14</td>
</tr>
<tr>
<td>3 LITERATURE REVIEW</td>
<td>........................................................................</td>
<td>16</td>
</tr>
<tr>
<td>3.1 Fats and Oils</td>
<td>........................................................................</td>
<td>16</td>
</tr>
<tr>
<td>3.2 Palm Oil</td>
<td>........................................................................</td>
<td>18</td>
</tr>
<tr>
<td>3.3 High Oleic Palm Oil</td>
<td>........................................................................</td>
<td>21</td>
</tr>
<tr>
<td>3.4 Fat Crystallization</td>
<td>........................................................................</td>
<td>22</td>
</tr>
<tr>
<td>3.4.1 Cooling Rate</td>
<td>........................................................................</td>
<td>23</td>
</tr>
<tr>
<td>3.4.2 Shear Rate</td>
<td>........................................................................</td>
<td>24</td>
</tr>
<tr>
<td>3.4.3 Minor Components</td>
<td>........................................................................</td>
<td>24</td>
</tr>
<tr>
<td>3.5 Fat Rheology</td>
<td>........................................................................</td>
<td>26</td>
</tr>
<tr>
<td>3.6 Fat Crystal Networks Characterization</td>
<td>........................................................................</td>
<td>27</td>
</tr>
<tr>
<td>3.6.1 Thermal Analysis by Differential Scanning Calorimetry (DSC)</td>
<td>...........................................</td>
<td>27</td>
</tr>
<tr>
<td>3.6.2 Polymorphism by X-Ray Diffraction</td>
<td>........................................................................</td>
<td>28</td>
</tr>
<tr>
<td>3.6.3 Microstructure by Microscopy</td>
<td>........................................................................</td>
<td>29</td>
</tr>
<tr>
<td>3.6.4 Types of Shortening and Manufacturing</td>
<td>......................................................................</td>
<td>29</td>
</tr>
<tr>
<td>4 THERMAL AND RHEOLOGICAL COMPARISON OF STANDARD PALM OIL TO HIGHER CONTENT OF OLEIC-ACID VARIETIES</td>
<td>............................................</td>
<td>31</td>
</tr>
<tr>
<td>4.1 Background</td>
<td>........................................................................</td>
<td>31</td>
</tr>
<tr>
<td>4.2 Materials and Methods</td>
<td>........................................................................</td>
<td>35</td>
</tr>
<tr>
<td>4.2.1 Materials</td>
<td>........................................................................</td>
<td>35</td>
</tr>
<tr>
<td>4.2.2 Determination of Fatty Acid Methyl Esters (FAME) by GC-FID</td>
<td>.......................................</td>
<td>35</td>
</tr>
<tr>
<td>4.2.3 Thermal Analysis by DSC</td>
<td>........................................................................</td>
<td>35</td>
</tr>
<tr>
<td>4.2.4 Determination of Dynamic Viscosity ((\eta))</td>
<td>........................................</td>
<td>36</td>
</tr>
<tr>
<td>4.2.5 Determination of Shear Stress by Oscillatory Strain Sweep Test</td>
<td>............................</td>
<td>37</td>
</tr>
<tr>
<td>4.2.6 Texture Analysis</td>
<td>........................................................................</td>
<td>37</td>
</tr>
<tr>
<td>4.2.7 Darkfield Microscopy</td>
<td>........................................................................</td>
<td>38</td>
</tr>
</tbody>
</table>
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-1</td>
<td>Total fatty acid composition (% w/w) of HOPO and SPO</td>
</tr>
<tr>
<td>4-2</td>
<td>Hardness and penetration values for HOPO and SPO at different temperatures (n=4)</td>
</tr>
<tr>
<td>5-1</td>
<td>Crystallization temperatures for MAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)</td>
</tr>
<tr>
<td>5-2</td>
<td>Melting temperatures for MAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)</td>
</tr>
<tr>
<td>5-3</td>
<td>Crystallization temperatures for DAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)</td>
</tr>
<tr>
<td>5-4</td>
<td>Melting temperatures for DAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)</td>
</tr>
<tr>
<td>5-5</td>
<td>Hardness and Penetration Force (PF) at different concentration and temperatures for MAGs oleogels</td>
</tr>
<tr>
<td>5-6</td>
<td>Hardness and Penetration Force (PF) at different concentration and temperatures for DAGs oleogels</td>
</tr>
</tbody>
</table>
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-1</td>
<td>Palm oil refining process</td>
<td>20</td>
</tr>
<tr>
<td>3-2</td>
<td>Schematic aggregation of triglycerides into a crystal network</td>
<td>23</td>
</tr>
<tr>
<td>3-3</td>
<td>Common emulsifier used in the food industry</td>
<td>26</td>
</tr>
<tr>
<td>3-4</td>
<td>Discovery series HR-1 Rheometer, PCCL at the University of Florida</td>
<td>27</td>
</tr>
<tr>
<td>3-5</td>
<td>Differential Scanning Calorimeter (DSC) Q1000 TA instruments</td>
<td>28</td>
</tr>
<tr>
<td>4-1</td>
<td>Thermogram for SPO and HOPO at 20°C/min.</td>
<td>40</td>
</tr>
<tr>
<td>4-2</td>
<td>Viscosity of HOPO at shear rates of 0.1, 1, 10 and 100 s⁻¹.</td>
<td>42</td>
</tr>
<tr>
<td>4-3</td>
<td>Viscosity of MPO at shear rates of 0.1, 1, 10 and 100 s⁻¹.</td>
<td>43</td>
</tr>
<tr>
<td>4-4</td>
<td>Storage modulus (G') vs oscillation strain of SPO and HOPO at 5°C</td>
<td>44</td>
</tr>
<tr>
<td>4-5</td>
<td>Oscillatory stress vs oscillation strain of SPO and HOPO at 5°C</td>
<td>44</td>
</tr>
<tr>
<td>4-6</td>
<td>Storage modulus (G') vs oscillation strain of SPO and HOPO at 10°C</td>
<td>45</td>
</tr>
<tr>
<td>4-7</td>
<td>Oscillatory stress vs oscillation strain of SPO and HOPO at 10°C</td>
<td>45</td>
</tr>
<tr>
<td>4-8</td>
<td>Storage modulus (G') vs oscillation strain of SPO and HOPO at 20°C</td>
<td>46</td>
</tr>
<tr>
<td>4-9</td>
<td>Oscillatory stress vs oscillation strain of SPO and HOPO at 20°C</td>
<td>46</td>
</tr>
<tr>
<td>4-10</td>
<td>Penetration test over time</td>
<td>48</td>
</tr>
<tr>
<td>4-11</td>
<td>Microstructure of HOPO and SPO (in duplicate) at 10°C, 15°C and 20°C.</td>
<td>51</td>
</tr>
<tr>
<td>5-1</td>
<td>Thermogram for HOPO (black) and MAGs at 3%, 5%, 7% and 10%.</td>
<td>59</td>
</tr>
<tr>
<td>5-2</td>
<td>Thermogram for HOPO (black) and DAGs at 3%, 5%, 7% and 10%.</td>
<td>63</td>
</tr>
<tr>
<td>5-3</td>
<td>Hardness of MAGs oleogels</td>
<td>66</td>
</tr>
<tr>
<td>5-4</td>
<td>Hardness of DAGs oleogels</td>
<td>67</td>
</tr>
</tbody>
</table>
LIST OF ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>AHA</td>
<td>American Heart Association</td>
</tr>
<tr>
<td>CVD</td>
<td>Cardiovascular Disease</td>
</tr>
<tr>
<td>DAGs</td>
<td>Diglycerides</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential Scanning Calorimetry</td>
</tr>
<tr>
<td>FAME</td>
<td>Fatty acid methyl ester</td>
</tr>
<tr>
<td>FFA</td>
<td>Free fatty acid</td>
</tr>
<tr>
<td>Ha</td>
<td>Hardness</td>
</tr>
<tr>
<td>HOPO</td>
<td>High Oleic Palm Oil</td>
</tr>
<tr>
<td>MAGs</td>
<td>Monoglycerides</td>
</tr>
<tr>
<td>PF</td>
<td>Penetration force</td>
</tr>
<tr>
<td>SPO</td>
<td>Standard Palm Oil</td>
</tr>
<tr>
<td>TA</td>
<td>Texture analysis</td>
</tr>
<tr>
<td>TAGs</td>
<td>Triglycerides</td>
</tr>
</tbody>
</table>
COMPARATIVE ASSESSMENT OF THE THERMOMECHANICAL PROPERTIES OF HIGH OLEIC PALM OIL AND ACYLGLYCEROL EMULSIFICATION TO STANDARD PALM OIL

By

Victor Cedeno Sanchez

December 2020

Chair: Andrew J. MacIntosh
Major: Food Science and Human Nutrition

Standard Palm Oil (SPO) is widely used as an ingredient due to its physical properties, however, the saturated fatty acid (SFA) content is higher than recommended by the AHA. Palm hybrids yielding oil with <35% SFA and >50% oleic acid was recently defined as “palm oil with a higher content of oleic acid” (HOPO) in the 2019 Codex Alimentarius.

This study first compared the crystallization of HOPO to SPO via thermal analysis, shear and oscillatory rheology, microscopy and firmness tests. Second, assessed the thermal and mechanical changes by commercial emulsifiers. HOPO’s higher content of oleic acid (53.9%), decreased the crystallization temperatures by ~8˚C. HOPO and SPO were the hardest at 5˚C, but HOPO fractured instead of deforming, as SPO did, and the viscoelastic behavior of both oils were similar. The storage modulus of HOPO dramatically decreased at higher temperatures. Viscosity profiles were similar between the oils at higher shears, however, crystallization started at 6-8˚C in HOPO for higher shears. HOPO microstructure had well-defined spherulites at 20˚C but irregular shapes at 15˚C and 10˚C.
Monoglycerides promoted a new nucleation stage in HOPO. The strongest oleogel had a hardness of 3.34 kg, using 10% w/w monoglyceride at a Tc of 5°C. Diglycerides shifted the first crystallization of HOPO by 4°C with every addition but had less effect on the hardness, maxing at 2.51 kg, using 10% w/w diglyceride at Tc of 5°C.

Knowing the crystallization of HOPO and the effect of emulsifiers, it is possible to tailor it for new food applications.
CHAPTER 1
INTRODUCTION

Edible fats and oils play a key role in the human diet, providing energy, essential fatty acids and acting as a delivery matrix for phytonutrients. Besides the nutritional benefits, fats enhance the sensory attributes of foods, such as the texture and mouthfeel of finished products. These physical characteristics depend on the three-dimensional crystal network organization and polymorphic state of the fat, which in turn, is directly affected by the triglyceride composition and thermomechanical treatment (Campos, Narine, & Marangoni, 2002).

Palm oil is one of the most important commodities and the most consumed vegetable oil in the world, with 72.27 MMT produced annually (Statista, 2019). The standard palm oil (SPO) tree grows near the equator and tropical climates, being Indonesia the biggest producer with 42.5 MMT, followed by Malaysia with 18.5 MMT, Thailand with 2.8 MMT and Colombia with 1.53 MMT (Statista, 2019).

The interspecific hybrid *Elaeis oleifera X Elaeis guineensis* palm is of major interest due its unique fatty acid profile, rich in monosaturated fatty acids (Mozzon, Pacetti, Lucci, Balzano, & Frega, 2013) and phytonutrient (Lucci, Pacetti, Frega, & Mozzon, 2015). The interspecific hybrid palm crude oil -referred as High Oleic Palm Oil (HOPO) in this document- is naturally rich in oleic acid, with 53.5%-55.25%, 12 points above the standard palm oil (*Elaeis guineensis*), and most of it (64.7%-66%) is in the sn-2 position of the triglyceride.

Research on the optimal ripening of the fruit, the triglyceride (TAG) composition, fatty acid (FA) profile, and phytonutrients available in the Crude Palm Oil from of the interspecific hybrid is available, however, there is currently no research on the
crystallization kinetics of the refined oil and its products, as found by the authors. It is unknown by the field the changes in rheological properties under different thermomechanical treatments or how does HOPO respond to the addition of emulsifiers. The lack of information limits the industrial use and application of a potentially more nutritious vegetable oil.
CHAPTER 2
OBJECTIVE

The main objective of this research was to describe the crystallization dynamics of High Oleic Palm Oil and assess changes induced by emulsifiers. This was completed via two studies on HOPO, the first comparing it to Standard Palm Oil, and the second assessing the thermomechanical effect of emulsifiers on the formulation of HOPO-based oleogels.

The following thesis is constructed as an encompassing literature review with two following independent studies. Chapter 3, the literature review, with a detailed background information for the research and gives the foundations for the two subsequent studies. Chapter 4 is a study that compares thermal, and rheological differences between High Oleic Palm Oil and Standard Palm Oil. We hypothesized that the lower concentration of saturated fatty acids in HOPO would decrease the crystallization and melting temperatures, as well as reduce viscosity and hardness at cold storage (5°C, 10°C and 15°C) and room temperature conditions (20°C). Additionally, the temperature dependence of the viscosity will decrease at higher shear rates for HOPO. This study was written as a manuscript for publication with its own introduction, methodologies, results, and conclusions specifically related to the SPO and HOPO oil thermomechanical characterization.

The same structure was used for Chapter 4, were the effect of two commercially available categories of emulsifiers on the thermal and mechanical properties of HOPO is explored. We hypothesized that the addition of commercially available mono- and diglycerides to HOPO would increase the thermal stability and hardness at higher
crystallization temperatures. These changes in crystallization will be dependent upon the concentration and type of emulsifier.

The final general conclusions chapter relates the results of both studies to assess the crystallization dynamics of HOPO specifically with respect of the objectives of the study.
Edible fats and oils play a key role in the human diet, providing energy, essential fatty acids and acting as a delivery matrix for phytonutrients. The most abundant structure in any oil are triglycerides (TAGs), a naturally occurring structure in which glycerol acts as a backbone for a variety of fatty acids (FA) (Damodaran & Parkin, 2017). Fatty acids are carboxylic acids with an unbranched aliphatic chain with a length between 4 and 28 carbons. Depending upon the degree of saturation, FA could be classified as saturated, monounsaturated (only 1 unsaturation) and polyunsaturated (2 or more unsaturation). The length of the fatty acid carbon chain, the cis-trans isomerism of the double bonds and the degree saturation will affect the physical characteristics of the oil.

Oils rich in saturated fatty acids tend to be solid at room temperature because the fatty acid chain easily stack together increasing the melting point. Because the major force keeping the structure together are Van der Wall forces, shorter chains will have a lower melting point that longer ones (Damodaran & Parkin, 2017). Natural sources high in saturated fatty acids include palm oil, palm kernel oil, cocoa butter, coconut oil and lard.

Fats are of great interest for food applications, however, the American Heart Association (AHA) recommends consuming a maximum of 13g a day as well as the dietary guidelines (U.S. Department of Health and Human services & Agriculture, 2015). Evidence suggest a correlation between saturated fatty acids and an increase in low
density lipoprotein (LDL) in blood, a risk factor for cardiovascular disease (Mensink, Zock, Kester, & Katan, 2003) (Mozaffarian, Micha, & Wallace, 2010).

Monosaturated fatty acids will greatly differ in melting point, depending upon which isomer is more abundant, cis- or trans-. For example, the melting point of cis-9-Octadecenoic acid, commonly referred as oleic acid, is 13-14°C but the melting point of trans-9-Octadecenoic acid, referred as elaidic acid, is 45°C. Both of this FAs are 18 carbon long but greatly differ in physical properties.

*Trans*- fatty acids will have a much higher melting point due the stacking of the fatty acid chains, making them of great interest for room temperature food applications. *Trans*-fats are found in nature, especially in animal tissues, however, they are industrially produced by the hydrogenation of polyunsaturated fatty acids, receiving the name of partially hydrogenated oils (PHO). Consumption of PHOs increased the level of LDL cholesterol in the blood, which in turn, increases the risk of cardiovascular disease (CVD)(Oh, Hu, Manson, Stampfer, & Willett, 2005; Souza et al., 2015; Willett et al., 1993). Because of these findings, the FDA removed PHOs from the "Generally Recognized as Safe" (GRAS) list of ingredients in 2015, by 2018 manufacturers cannot add PHOs to new food products and by 2020, all PHO should be substituted(FDA, 2018b).

The most abundant monosaturated fatty acid in nature is oleic acid, that unlike the *trans* isomer, is linked with a reduction in the risk of coronary heart disease, through the modification of plasma lipids and lipoprotein concentrations (Berglund et al., 2007)(Schwingshackl et al., 2018). Given this characteristic, the FDA approved a qualified health claim for high oleic oils (>70%), that aid in the reduced risk of coronary
heart disease, in oils such as sunflower, safflower, canola, olive and algal oil (FDA, 2018a).

Polyunsaturated fatty acids (PFA) have more than one unsaturation in the cis-isomers, which reduces the stacking of the fatty acid chain, reducing the melting point and are prone to self-oxidation (Damodaran & Parkin, 2017). PFAs provide the body with essential FAs that promote brain development in kids, stabilize cell membranes, and randomized test have shown that the consumption of PFA reduce the incidence of CVD (Mozaffarian et al., 2010)

As mentioned before, triglycerides (TAG) are the most abundant structure found in oils and are categorized depending on the saturation of the fatty acid chain and location in the molecule. When the three FAs are saturated, the TAG is totally saturated (SSS); when one FA is unsaturated, it could be symmetrical (SUS) or asymmetrical (SSU) (Wiedermann & Brook, 1978). If two fatty acids are unsaturated, the triglyceride is disaturated whereas the opposite would be SUU or USU, and at last, when all the structures are unsaturated, it is referred as UUU. The importance of TAGs reside in that they act as a moisture barrier and provide structure (SSS and SSU), lubricity (UUS) or are of nutritional importance (UUU) (Wiedermann & Brook, 1978)

When similar fatty acid profiles differ in the triglyceride composition, there are changes in the solid fat content (SFC) curve and the crystals size, and post-crystallization hardening increases with a higher saturated TAGs content (Veerle De Graef, Vereecken, Smith, Bhaggan, & Dewettinck, 2012)

3.2 Palm Oil

Palm oil is one of the most important crops and the most consumed vegetable oil in the world with 72.27 MMT, followed by soybean with 56.62 MMT (Statista, 2019). The
palm oil (PO) tree grows near the equator in tropical climates, being Indonesia the biggest producer with 42.5 MMT, followed by Malaysia with 18.5 MMT, Thailand with 2.8 MMT and Colombia with 1.53 MMT (Statista, 2019). Because of the high yield of the crop, there is a growing interest in PO in Central and South America and in Africa (Woittiez, van Wijk, Slingerland, van Noordwijk, & Giller, 2017), to supply the local and regional demand for edible oils.

The oil palm takes 2-3 years to mature and start producing fresh fruit bunches (where fruits are found) and will produce a yearly harvest for the next 25 years, or until the palm is too high for the farmers to reach the fruit (Lai Oi-Ming, 2012b). Once the fruit is ripe, the fresh bunch is collected and transferred to a processing facility, where it will be sterilized to denaturalize lipases, stripped to separate the fruit from the bunch, digested with steam to soften the fruit, and pressed to obtain Crude Oil (Lai Oi-Ming, 2012a). Then it will be screened, clarified, purified and dried until it becomes crude palm oil (CPO), a process with an efficiency of 20% (Lai Oi-Ming, 2012a). CPO is already a product ready to consume, however, it is further processed for consumers acceptance or obtaining higher added value products. CPO can undergo physical or chemical refining, being the first chosen for most of processing facilities because is cheaper and preferred by consumers. CPO is physically refined, degummed to remove waxes, bleached to remove color and deodorized using steam (Figure 3-1), to obtain Refined, Bleached, Deodorized (RBD) Palm oil (PO) (Mba, Dumont, & Ngadi, 2015). RBD PO is of great interest for the industry, fractionated into olein, the liquid fractions; or stearin, the solid fraction, by physical or chemical methods (Mba et al., 2015).
There are two major species of palm oil, *Elaeis oleifera* (American palm) and *Elaeis guineensis* (African palm). However, the FAO recognized the oil from the mesocarp of the fruit from the *Elaeis guineensis* as “Palm Oil” (Joint FAO/Who Codex Alimentarius Commission, 2019).

The fatty acid composition of palm oil consists of almost 50% saturated fatty acids (as palmitic acid (16:0) with 43.7%, and stearic acid (18:0) with 4.75%), monosaturated fatty acids as oleic acid (18:1) with 40.0% and polyunsaturated fatty acids, distributed in linoleic acid (18:2) with 10.5%; myristic acid (14:0) lauric acid (12:0) and linolenic acid (18:3) are found in lower concentrations (Prada, Ayala-Diaz, Delgado, Ruiz-Romero, & Romero, 2011). The fatty acid profile of the PO makes it malleable for room temperature applications and very stable, an ideal ingredient for shortenings and margarine (Aini & Miskandar, 2007)(Omar et al., 2015).

![Palm oil refining process](image)

Figure 3-1. Palm oil refining process (Mba et al., 2015)
3.3 High Oleic Palm Oil

The naturally occurring varieties of oil palm are the American Palm *Elaeis Oleifera* (O) and African Palm *Elaeis Guineensis* (G), however, during the 1990s (Zapata Munevar, 2010) selective breeding of these two palms lead to the development of the “interspecific hybrid OxG” in Colombia and Malaysia. It was later found that the strain resistant strain to the bud rot diseases that affected several regions. The same author comments on how the commercial plantations of this strain started on 1998, with an oil yield of 1,000 monthly tons and an expected 150,000 monthly tons by 2020 (Zapata Munevar, 2010).

The oil produced by the hybrid was added to the Codex Alimentarius Commission as “palm oil with a higher content of Oleic Acid (OxG)” to the Standard for Named Vegetable Oils (Joint FAO/Who Codex Alimentarius Commission, 2019), referred in this document as High Oleic Palm Oil (HOPO). Published values from manufacturers and researchers, have quantified oleic acid in values of 53.5-55.2% against the commonly found in PO of 41.5% , which means that the HOPO has approximately 10% less saturated fatty acids than PO (Mozzon et al., 2013). Given the nutritional benefits of oleic acid and the higher content of phytonutrients (Lucci et al., 2015), HOPO is of great interest for potential food product applications, since high oleic oils have been introduced in the market as *trans*-fat replacers due its high stability and prolonged shelf life (Liu, 2014).

High oleic fractions of palm oil are available; however, it is not obtained from the interspecific hybrid. HOPO is processed in the same manner as regular palm oil, and is commercialized as refined, bleached and deodorized (RBD).
3.4 Fat Crystallization

The functionality of fats relies on their physical characteristics, given by the inner arrangement of the molecular structure. This arrangement is called the crystalline structure, and in fats will be mainly affected by triglycerides’ size (chain length), distribution, composition, the presence of minor components, and crystallization conditions (Campos et al., 2002). The first stage of crystallization consists of supercooling, in which the temperature of the oil is dropped below the melting temperature of the solid phase for the highest melting TAG fraction, this decreases the solubility and molecules starts to associate (Damodaran & Parkin, 2017). Figure 3-2. describes the subsequent formation of a 3D crystal network, adapted from (Tran & Rousseau, 2016), that forms a crystal aggregates. Once the TAGs associate and form stable nuclei (1), the prolongation of this event creates lamellae (2), which in turn grow into crystal nanoplates (CNP) with a size between 0.4-200 nm (3). As fats are mainly nonpolar molecules, Van Der Walls forces hold the CNP in aggregates (4-5), which will grow intro crystallites (6) and crystals (7) until a 3D network is visible under the microscope (8) (Tran & Rousseau, 2016).

As crystal grow and aggregate, it is possible to identify three main types alpha (α), beta prime (β’) and beta (β). The first has the lowest melting point and is consider the least stable from all the forms and will quickly transition into β’ (Chawla, P.; deMan, J. M.; and Smith, 1990). As α and β’ are meta-stable structures, they will tend to β over long periods (Damodaran & Parkin, 2017), but ideally, not before the consumption or use of the fat ingredient. Each of these structures will have different mechanical properties, ideal for different applications. β’ crystals are small, well dispersed in the matrix and capacity to entrap liquid oil (low melting point triglycerides). β crystal will tend
to be bigger, with a poor capacity to entrap liquid oil (low melting point triglycerides) and will exhibit a “crumbly” or brittle structure (Damodaran & Parkin, 2017). Different sources of fats will induce different polymorphs. The effect of external and internal factors is described below.

Figure 3-2. Schematic aggregation of triglycerides into a crystal network (Tran & Rousseau, 2016)

3.4.1 Cooling Rate

The change in temperature from the melted oil to the target crystallization temperature over a period of time is the cooling rate, and will alter how TAGs solidify and aggregate, changings crystalline structures, therefore, the mechanical and thermal properties (Campos et al., 2002; Mazzanti, Marangoni, & Idziak, 2005; Vuillequez, Koza, Youssef, Bridier, & Saiter, 2010). Under fast cooling, lard and milk fat were harder than when cooled slowly, also showing smaller and more evenly distributed crystal under the first condition. The effect of cooling rate on PO crystallization was studied by Vuillequez et al. (2010), using two different cooling rates, 0.5°C/min and 50°C/min, and
described smaller crystals with the increase of the cooling rate, but the same spherulitic type of crystal independent of the cooling rate. According to the authors, the best solid fat content was obtained with a cooling rate of 3 °C/min (Vuillequez et al., 2010). Zhang et al. (2013) reviewed the thermograms at different cooling rates and identified peaks at similar temperatures with a much smaller size, suggesting changes in the mechanism of nucleation when the cooling rate changed (Zhang et al., 2013).

### 3.4.2 Shear Rate

Shear rate is described as the average change in velocity experienced by one layer of fluid passing over an adjacent layer (Krishna, Swathi, Hemalatha, & Mohan, 2019). Shear will induce nucleation and will facilitate the homogeneous dispersion of crystals (Wesdorp et al., 1990), as well as improve the heat transfer in the system. Utilizing x-ray diffraction, Mazzanti, et al. at different cooling rates identified that shear induced the transition from alpha to β’ crystallization in palm oil, (Mazzanti et al., 2005). Shear also modified the microstructure of palm oil, Tarabukina et al. (2009) identified that 3D networks are formed below shear rates of 30 s⁻¹, spherulite suspension below 300 s⁻¹ and aggregation does not occur above this point (Tarabukina, Jego, Haudin, Navard, & Peuvrel-Disdier, 2009). The type of crystallite form and interaction within them will also affect the viscosity, storage modulus and hardness of the final product (Moelants et al., 2019). This principle is applied in the scrapped surface heat exchanger (SSHE), the main instrument used to manufacture functional fats and shortenings in an industrial setting (Rao & Hartel, 2006).

### 3.4.3 Minor Components

Fats and oil are mainly composed by triglycerides (TAGs), followed by naturally occurring components, including monoglycerides (MAGs), diglycerides (DAGs), free
fatty acids (FFA), tocotrienols, carotenes and impurities. The presence of this minor compounds will modify the physical characteristics of the oils and in some cases, will be added to achieve a desired functionality (Smith, Bhaggan, Talbot, & Van Malssen, 2011). The addition of minor components and additives have been vastly studied due their uses as nucleating agents, crystallization regulators, polymorph stability and modification of physical properties (de Oliveira, Ribeiro, & Kieckbusch, 2015)(Smith et al., 2011)(Maruyama et al., 2014).

Monoglycerides (MAGs) are ingredients of great interest because of nucleation promotion and early crystallization (Marangoni Alejandro G., 2018). Another remarkable characteristic is that the hydroxide groups of MAGs interact with each other, while the aliphatic chain interacts with the fatty acids, stabilizing the system (Marangoni Alejandro G., 2018). The use of MAGs with a low melting point oil results into an oleogel, which will be affected by processing conditions. When shear is applied during crystallization, the resulting oleogel is softer (Da Pieve, Calligaris, Co, Nicoli, & Marangoni, 2010), whereas under rapid cooling and higher concentrations it is harder and more thermally stable (Giacomozzi, Palla, Carrín, & Martini, 2019).

DAGs are also ingredients of interests, and when being used with 90% purity, at concentrations as low as 5% significantly decreases nucleation rates and concentrations up to 10% are β’ stabilizing agent on palm oil (Saberi, Lai, & Toro-Vázquez, 2011). The emulsifier of interest of this study, Trancendim 130 produced by Corbion, is 67% DAGs and 27% TAGs, and was used on sunflower oil, under different cooling and shearing rates, suggesting a 10% as the optimal value for the best viscoelastic properties (Tavernier, Norton, Rimaux, Lazidis, & Dewettinck, 2019).
Figure 3-3. Common emulsifier used in the food industry A) Monostearin, a common monoglyceride and B) Glyceryl 1,3-dipalmitate, a common diglyceride (National Center for Biotechnology Information, 2020b, 2020a).

3.5 Fat Rheology

Humans perceive different textural characteristics than can be quantified with rheology techniques. Fats are composed by a liquid fraction entrapped in a solid matrix (Rogers, Tang, Ahmadi, & Marangoni, 2008), behaving as a viscoelastic material, therefore, fats behave as elastic materials until a certain force is applied and flows as a viscous fluid (Peyronel, Laredo, & Marangoni, 2011) described by yield stress rheology.

Shear rheology has been utilized to describe the first and second crystallization of palm oil by notable jumps in viscosity, at a constant cooling rate (Tarabukina et al., 2009). Similar results were obtained by (Moelants et al., 2019) that also identified an increase in the storage modulus ($G'$) at lower shear rates. Hardness is an additional parameter tested by a penetration test, that gives information on the firmness and adhesiveness of the oil. This test is used in industrial settings as a quality parameter for commercial fats and margarines by the ease of use, repeatability and correlates to the consumer experience (Glibowski, Zarzycki, Krzepkowska, Glibowski, & Zarzycki, 2008).
3.6 Fat Crystal Networks Characterization

3.6.1 Thermal Analysis by Differential Scanning Calorimetry (DSC)

A tool commonly used in the study of thermal transitions of different material is the Differential Scanning Calorimetry (DSC). By precisely controlling the heating and/or cooling rate of a sample, it can identify the temperatures at which different material will experience changes in the structure. It is widely used in material science to identify melting points and glass transition temperatures for polymers; however, many food matrixes behave as higher polymers, including proteins, starches and fats (Farkas & Mohácsi-Farkas, 1996). Phase transitions are identified by deviations from the baseline...
as sensible heat requirement, whereas crystalline transitions occur as energy peaks. SPO’s characteristic thermogram consists of two characteristics phase transition zones observed for both the crystallization and melting stage that represent the solid (stearin) and liquid (olein) fractions (Tan & Che Man, 2000).

3.6.2 Polymorphism by X-Ray Diffraction

The polymorph of the fat will directly affect the melting point and the rheological properties, therefore making it relevant in terms of functionality and potential applications (Marangoni & Wesdorp, 2012).

In X-ray diffraction, long and short spacing will be detected and provide different information. Long spacings are observed in the 2θ range of 1-15˚, corresponding to the planes formed by the methyl end groups of the triglycerides and are dependent on the chain length and the angle of tilt of the component fatty acids. Short spacings in the range of 16-25˚, corresponding to the cross-sectional packing of the hydrocarbon chains and are independent of chain length (deMan, 1992). Larsson reported the peaks intensity for each crystal structure, such as 4.15 Å for α, 4.20 and 3.80 Å for β' and if
different to the previous one, β (Larsson, 1966), later adopted by the American Oil Chemists Society as the identification of crystals.

### 3.6.3 Microstructure by Microscopy

Fat crystals are observable under a microscope; however, two techniques are commonly used. Polarized light microscopy (PLM) relies on the birefringent characteristic of the fat, whereas darkfield microscopy uses the reflective characteristic of the fat. The shape of the fat crystal clusters can be spherulitic, feather-like, blade or needle-shaped. The size of fat crystal clusters can vary from several micrometers to more than 200 μm (Tang & Marangoni, 2006). The qualitative and quantitative characteristics of the fat microstructure can be done using fractal dimensions, correlating structure to physical properties.

### 3.6.4 Types of Shortening and Manufacturing

Shortenings used to be defined as solid fats at room temperature, which can be used to give foods a crumbly and crisp texture such as pastry, given its name by the effect of “shortening” the gluten strands, however, modern definitions depict shortenings as a fat product formulated processed for a desired functionality (Aini & Miskandar, 2007). Among these functional characteristics are texture and mouthfeel of the final product due the structural integrity, lubrication, and in case of necessary, the incorporation of air. Works as a heat transfer medium when cooking and prolongs the shelf life of the food product (Ghotra, Dyal, & Narine, 2002).

Shortening manufacturers have a fairly similar process but might differ in the formulation of the blend and the quantity of additives such as antioxidants, emulsifiers or inert gases. First, the temperature of the blend should be above than highest melting component, then, this melted blend would enter a scrapped surface heat exchanger.
(SSHE) unit, which is a tubular concentric heat exchanger with a shaft provided with blades that will spin removing any incipient crystal from the cooled inner surface and assuring a homogeneous mix and cooling, at a very low temperature. From the SSHE, the cooled blend could be introduced to another SSHE with a different temperature or to a tempering tank, avoiding clumping and crystal stabilization. The last step consists of the storage, that could be cooled or room temperature, depending upon the post-crystallization expected from the manufacturer (Ramli, Lin, Yoo, Idris, & Sahri, 2008).

Shortenings can be classified by origin (animal or vegetable), delivery form (plasticized, liquid or flakes/beds) or final use. In the latter category, shortenings are categorized upon diversity of applications, such as all-purpose, fluid, cake, icing, filler, bread or frying (Ghotra et al., 2002).
CHAPTER 4
THERMAL AND RHEOLOGICAL COMPARISON OF STANDARD PALM OIL TO HIGHER CONTENT OF OLEIC-ACID VARIETIES

This chapter was written as a paper for submission to the Journal of American Society of Oil Chemist (JAOCS) after committee approval.

4.1 Background

Edible fats and oils play a key role in the human diet, providing energy, essential fatty acids for cellular development and delivery of liposoluble phytonutrients. As an ingredient, fats improve the sensory attributes of foods, such as texture and mouthfeel. Currently, the most consumed fat in the world is Standard Palm Oil (SPO), from the species *Elaeis guineensis*, largely due to its versatility as a food ingredient (Aini & Miskandar, 2007), and highest yield per hectare when compared to any other major oil-bearing crop. The typical saturated fatty acid content in SPO (~50%) is responsible for its physical properties, however, it is higher than the recommended intake by the AHA (Sacks et al., 2017). The same entity recommends the consumption of alternative oils that are higher in mono- and polyunsaturated fatty acids which have been shown to reduce the incidence of cardiovascular disease (Berglund et al., 2007; Sacks et al., 2017; Schwingshackl et al., 2018).

In 2019, the Codex Alimentarius included a new definition for a “palm oil with a higher content of oleic acid” in the standard for named vegetable oils. Grown primarily in Colombia, Ecuador and Brazil, this oil is obtained from the interspecific hybrid *Elaeis oleifera* × *Elaeis guineensis* palm (Joint FAO/Who Codex Alimentarius Comission, 2019). This interspecific hybrid palm oil (HOPO) is of major interest to consumers due to the higher concentration of monounsaturated oleic acid (53.5%-55.25% of total FA) than SPO (37% of total SA), resulting is a lower concentration of saturated fatty acids for
HOPO (30%). Furthermore, most of the oleic acid in HOPO (64.7%-66%) is in the $sn$-2 position of the triglyceride, where it is most likely to be absorbed by the body (Mozzon et al., 2013). HOPO also has higher reported concentrations of tocotrienols (Lucci et al., 2015) and carotenoids (Mozzon, Pacetti, Frega, & Lucci, 2015) when compared to SPO, which are nutrients of interest for proper bodily functions.

The position of the fatty acid chain in the triglycerides not only affects the way it is absorbed by the body, but also the physical properties of the fat. HOPO has less totally saturated triglycerides (1.6%) (an important structuring agent for room temperature applications) than SPO (8.5%) (Mozzon et al., 2013). The total disaturated triacylglycerols, another high melting point fraction, is lower in HOPO than SPO (33.3% against 49.2%) (Mozzon et al., 2013).

The crystallization process of fats and oils is dependent on both intrinsic and extrinsic factors. Intrinsic factors include: the fatty acid profile, triglyceride composition and presence of minor components naturally occurring, such as free fatty acids, monoglycerides and diglycerides or impurities. Extrinsic factors include the cooling rate, degree of undercooling, annealing time and shear modification of the crystalline structure. The effect of these factors can be studied by thermal, rheological or optical techniques; however, it should be noted that the concentration of fatty acids (a critical parameter in crystallization) varies in commercial oils with growing and seasonal effects. The focus of this study was to use several common methods to compare the properties of SPO to HOPO.

A common study of the crystallization kinetics response to temperature changes is by using Differential Scanning Calorimetry (DSC). Under constant cooling/heating
rates, it is possible to identify when the oil undergoes exothermic or endothermic processes, directly correlated to phase or crystalline transitions. Phase transitions are identified by deviations from the baseline heat requirement. SPO’s characteristic thermogram consists of two phase transition zones for the crystallization and melting stages (Tan & Che Man, 2000).

Specific crystallization temperatures (identified in the thermograms) and adequate shear rate are key operating conditions required to initiate the crystallization process of semi-solid industrial fats. Undesired characteristics may develop under inadequate shearing and cooling rates, including graininess, hardness or phase separation. Decrease in temperature will induce the saturation of the higher melting oil fractions promoting early crystallization, immediately increasing the viscosity of the system. Shear rheology has been utilized to describe the first and second crystallization of palm oil by notable jumps in viscosity, at a constant cooling rate (Tarabukina et. al., 2009). The same group noticed lower viscosity plateaus at higher shear rates at constant cooling rates, mainly due the type of crystalline aggregate form. Similar results were obtained by Moelants et al. (2019) who also identified an increase in the storage modulus (G’) at lower shear rates.

Yield stress is an additional viscosity parameter that helps to describe viscoelastic properties, that in the case of a fat, is a key characteristic at different crystallization temperatures. It also describes the mechanical characteristics of the fat at various crystallization conditions. A very common technique is the penetration test, which gives information on the firmness and adhesiveness of the oil. This test is used in
industrial settings as a quality parameter for commercial fats and margarine due to its ease of use and repeatability of the results (Glibowski et al., 2008).

Numerous researchers have correlated the physical properties of fats to the macrocrystalline structure (Campos et al., 2002; Litwinenko, Rojas, Gerschenson, & Marangoni, 2002). Microscopy is an optical technique to characterize the fat crystals in terms of size, shape and abundance, that will affect the mechanical properties and mouthfeel of final products. The main microstructures that fat forms during crystallization are spherulites, feather-like or needle-shaped structures, depending upon the fatty profile, cooling rate and shear, among other parameters. The ideal spreadable fat consists of small (<30µm) and largely dispersed crystals (Herrera, 1998). Other techniques include pulsed nuclear magnetic resonance (pNMR), a technique to quantify the solid fat content (SFC) that greatly affects the texture of the fat at different temperatures and x-ray diffraction, that describes the type of polymorph of the fat for the same purpose.

Currently, there is limited information on the crystallization kinetics and physical properties of HOPO and its products (Mozzon, Foligni, & Mannozzi, 2020). The objective of this study was to compare the thermal and rheological behavior of HOPO with SPO to support its use as a low-SFA alternative to SPO in food applications. First, the composition of fatty acids was determined via FAME analysis for both oils, and the crystallization transition temperatures were assessed using DSC. Texture analysis was conducted near common crystallization temperatures as determined by the DSC to better contrast the physical properties of the oils at different temperatures. Next, the rheological properties of the oils were determined using a strain sweep test and a
cooling test under constant shear to assess the changes of viscosity with the crystalline structure. Finally, polarized light microscopy was used to describe the microstructure and correlate to the previous analyses. This will allow to establish difference between HOPO and SPO at different temperatures and thermo-mechanical regimes.

4.2 Materials and Methods

4.2.1 Materials

Refined Bleached and deodorized high oleic palm oil (HOPO) and Refined Bleached and deodorized standard palm oil (SPO) from interspecific hybrid *E. Guineensis* and *E. Oleifera* OxG and *E. Guineensis*, respectively, were kindly provided by Thin Oil Product (Florida). Additionally, a standard palm oil from Malaysia was kindly provided by Catania Oils (MA, USA) for the dynamic viscosity test. All reagents and solvents were of analytical or HPLC grade, obtained from Sigma Aldrich (St. Louis, MO).

4.2.2 Determination of Fatty Acid Methyl Esters (FAME) by GC-FID

The fatty acid composition was determined by Gas Chromatography-Flame Ionized Detector of Fatty Acid Methyl Esters according to AOCS method Ce1b-89, using an Agilent Auto System GC-FID equipped with a wall coated open tubular capillary column. The reference used was Supelco S37 with 37 reference fatty acids. Due to discrepancies between obtained values and those obtained by the suppliers, an additional FAME of HOPO was conducted by a third-party, Diversified Laboratories Inc. (VA, USA) using the same method (AOCS method Ce1b-89).

4.2.3 Thermal Analysis by DSC

In the thermal analysis, the samples were heated until the crystal memory was erased (70°C) to precisely controlled the cooling and heating rates to identify the
temperatures at which phase transitions occurred. A Differential Scanning Calorimeter (DSC) Q1000 (TA Instruments) was used to assess the thermomechanical properties of both oils. Samples between 10-14 mg were weighed into aluminum pans to the nearest 0.1 mg and tightly sealed. An empty hermetically sealed aluminum pan was used as a baseline. The experiment was conducted in a controlled Helium atmosphere chamber. Samples followed the temperature program 70°C isotherm for 5 min, cooled down at a rate of 5°C/min to -70°C and heated at the same rate to 70°C. The chosen temperatures encompass the range where physical changes are more likely to occur in an industrial setting. The same procedure was done at heating/cooling rates of 10°C/min, 15°C/min and 20°C/min as described by Zhang et al. (2013). Each temperature assay was run in triplicate. A TA Universal Analysis software was used for analysis and presentation of results.

4.2.4 Determination of Dynamic Viscosity (η)

Dynamic viscosity was measured using a Discovery series HR-1 rheometer (DE, USA). The liquid oil at 70°C was contained in a concentric cylinder (cup and bob setup) to reduce measuring errors at initial low viscosities and maintain a constant fat thickness (the gap between the cup and bob) during cooling. This setup also minimized condensate contamination due low temperatures. The samples were heated at 70°C for 10 minutes to erase the crystal memory and subsequently cooled to 10°C as described by Tarabukina et al. (2009) at the maximum cooling rate of the instrument, 3°C/min. The shear rates selected were 0.1, 1, 10 and 100 s⁻¹. The same experiment was repeated to a minimum temperature of 0°C for HOPO due the liquid-like behavior at 10°C.
4.2.5 Determination of Shear Stress by Oscillatory Strain Sweep Test

An oscillatory strain sweep test was used to describe and compare the viscoelastic properties of HOPO and SPO at various temperatures. A Discovery series HR-1 Rheometer (DE, USA) equipped with parallel plates was used to perform oscillatory strain sweeps from 0.0001-100% strain at constant at a constant frequency of 1Hz and constant temperatures of 20°C, 10°C and 5°C. These temperatures were selected after the DSC results as key crystallization events. This test also identified the shear stress required for the structure to yield and behave as a liquid.

4.2.6 Texture Analysis

Samples were melted at 80°C in a water bath, placed in glass vials (n=4) and then transferred to a water bath at 45°C, 40°C, 35°C and 30°C. Upon equilibrium, samples were transferred to another water baths at 20°C, 15°C, 10°C and 5°C, respectively, and conditioned for 24 h. These two water baths were used to achieve a 25°C difference and simulate similar cooling rates for all the crystallization temperatures. This procedure was intended to create fat samples under identical cooling and shear conditions (0s⁻¹), carefully controlling the crystallization by temperature changes until equilibrium was reached. These samples were then assessed examining the effect of temperature on the different fats by taking the samples out of the water bath and immediately performing the test. A puncture test was performed using a texture analyzer TA-XTplus (Stable Micro Systems, Scarsdale, NY, USA) equipped with a 6-mm diameter cylindrical probe at room temperature. Hardness (maximum positive value) and penetration force (area under curve) were measured when the probe compressed the sample, and adhesiveness (area for the negative
force) when the probe returns to the starting position. The test was replicated in 4 different vials and the results were amalgamated.

4.2.7 Darkfield Microscopy

A modified AmScope microscope (USA) equipped with a darkfield filter was used to describe the morphology and size of fat crystals. Most of the direct light was blocked by the filter, forming a scattered halo of light reflected by the crystals, making them self-luminous. The illuminated crystals against the dark background, improved the visibility and resolution of the structures. To prepare the samples, HOPO and SPO were completely melted and heated to 70˚C, then, 10 µL of the melted oil were placed in a pre-heated slide at 65˚C and covered with a coverslip at the same temperature, that would rest in a cooling device at 20˚C, 15˚C, 10˚C and 5˚C in a dry air chamber to prevent condensation. This provided equal cooling rates for each sample which was then held isothermally for 24h before observation.

Disclaimer: Due to the technical expertise required to operate the rheometer, the determination of dynamic viscosity and shear stress by oscillatory strain sweep experiments, were performed by Scarlett Godinez (Ph.D. candidate, University of Florida) in the Polymer Chemistry Characterization Lab (PCCL) at the University of Florida.

4.3 Results and Discussion

4.3.1 Fatty Acid Profile Analysis

The fatty acid profile for SPO and HOPO is shown in Table 4-1 for palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acid, representing more than 95% of the fatty acids. Those under 1% w/w were not reported for visualization purposes.
<table>
<thead>
<tr>
<th></th>
<th>C16:0 (%)</th>
<th>C18:0 (%)</th>
<th>C18:1 (%)</th>
<th>C18:2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard Palm Oil¹</td>
<td>31.32 ±0.77</td>
<td>5.22 ± 0.10</td>
<td>49.54 ± 0.68</td>
<td>11.28 ± 0.02</td>
</tr>
<tr>
<td>Standard Palm Oil²</td>
<td>43.52 ±0.54</td>
<td>4.66 ± 0.62</td>
<td>40.15 ± 1.08</td>
<td>9.49 ± 0.55</td>
</tr>
<tr>
<td>High Oleic Palm Oil¹</td>
<td>21.87 ±0.30</td>
<td>2.86 ± 0.03</td>
<td>59.26 ± 0.51</td>
<td>12.45 ± 0.12</td>
</tr>
<tr>
<td>High Oleic Palm Oil²</td>
<td>27.8</td>
<td>2.8</td>
<td>55.5</td>
<td>11.9</td>
</tr>
<tr>
<td>High Oleic Palm Oil³</td>
<td>29.92 ±1.27</td>
<td>2.89 ± 0.16</td>
<td>53.92 ± 1.21</td>
<td>11.6 ± 0.29</td>
</tr>
</tbody>
</table>

¹UF results ²Third-party lab (n=1) ³Historic values from supplier

The fatty acid profile of HOPO done on campus showed a higher content of oleic and linoleic acid, and lower content of palmitic acid from previously reported values (54.6% for oleic acid, 11.02% for linoleic acid and 28.3% palmitic acid by Mozzon et al. (2013)). Due to these inconsistencies, HOPO was tested by a third-party laboratory resulting in values of oleic acid (C18:1) of 55.54%, linoleic acid (C18:2) of 11.94%, palmitic acid (C16:0) of 27.84% and stearic acid (C18:0) of 2.84%. These third-party values were closer to the historic fatty acid profile for HOPO and SPO and similar to literature values, as seen in Table 4-1.

In general, HOPO had a lower concentration of palmitic and stearic fatty acids when compared to SPO (the most representative saturated fatty acids found in palm oil). Consequently, HOPO had a considerably higher content of oleic acid, the monosaturated fatty acid of nutritional interest and a higher concentration of linoleic acid when compared to SPO. The differences in the fatty acid profile will have a major effect in the thermal properties of the oil, including the melting and crystallization points for industrial applications.
4.3.2 Differential Scanning Calorimetry

The crystallization (up) and melting (down) curves obtained by the DSC are shown in Figure 4-1 thermograms. The first cycle started at $T=70^\circ C$ before cooled to $-70^\circ C$ at a constant rate following a counterclockwise motion, to be heated back to $70^\circ C$.

![Figure 4-1. Thermogram for SPO and HOPO at 20°C/min.](image)

Figure 4-1. Thermogram for SPO and HOPO at 20°C/min.

The SPO and HOPO exhibited onset crystallization temperatures ($T_{on}$) of 16.45°C and 7.9°C respectively. These are the points at which the first fractions start to crystallize, which peaked at 14.05°C and 5.81°C (1A and 1B, respectively). This was followed by an additional crystallization onset temperature ($T_{on2}$) of 4.64 and -1.27, these peaked at -1.18°C and -8.11°C (2A and 2B, respectively). The main difference between the thermograms was the height and position of the first peak in HOPO, which is related to the lower concentration of saturated fatty acids fractions. Most of the
following work is going to focus on temperatures above 0°C, in the common range of food industry applications. Possibly useful for other industries, another shape discrepancy was observed for HOPO at -5°C (B*), not observed in SPO. Notably, HOPO’s peak temperatures are shifted by 7°C from SPO’s values.

The melting profile of the thermogram showed complete melting at 29.5°C for HOPO and 37.36°C for SPO. The transition of crystal structure in the SPO melting peak has commonly been reported to occur at 34.5°C (Man, Haryati, Ghazali, & Asbi, 1999).

The shape discrepancy observed in HOPO’s (Fig. 4-1, 4B) melting curve suggests the lack of high melting point fully saturated triglycerides. This is present in SPO (Fig. 4-1, 4A), described as the second melting stage that resembles the characteristic plateau from 14.02 °C to 37°C. Thermograms for HOPO haven’t been published at the time of the research. Based on the onset temperature and peak crystallization points of both oils, the mechanical and rheological properties were tested at 20°C, 15°C, 10°C and 5°C.

4.3.3 Viscosity measurement by Shear Rheology

The viscosity in terms of temperature and shear rates (0.1, 1, 10 and 100 s⁻¹) are shown in Figure 4-2, for HOPO, and in Figure 4-3, for SPO. Prior to t=1000 the changes in viscosity were small and omitted for visualization purposes. As expected, the shear rheology analysis showed increased viscosity with the decrease of temperature, likely explained by the development of the crystalline structure.

At very low shears, fats will crystallize as in an essentially static environment, forming larger crystalline structure that result in increased viscosity. When HOPO was subjected to a 0.1 s⁻¹ shear while cooling (Fig. 4-2.), crystallization initiated at a higher temperature than the onset identified in the DSC. It is plausible that when nucleation
starts to occur at very low shears, weak networks start to form that would not exist under a higher shear, were the unstable crystalline formations are destroyed, and stable nucleation sites are promoted (Tran & Rousseau, 2016).

![Figure 4-2. Viscosity of HOPO at shear rates of 0.1, 1, 10 and 100 s⁻¹.](image)

At higher shears, viscosity considerably increased near 7.8°C, similar to the onset temperature. Viscosity of HOPO at 1s⁻¹ plateaus an order of magnitude higher to shears of 10s⁻¹ and 100 s⁻¹, that performed the same way. Induction time for SPO was shorter and crystallization started to occur at 20°C, higher than the onset temperature, with noticeable viscosity differences between shear rates. The viscosity of HOPO at shear rates of 10s⁻¹ and 100 s⁻¹ resembled SPO at 100 s⁻¹.
Higher shear rates give less time for larger crystalline structures to grow, creating fat aggregate suspensions. Viscosity profiles were similar at different shear rates under isothermal conditions, with a shift in the induction time, being between 1380 and 1440s (11.16°C and 8.48°C) for HOPO and between 1130 and 1160 s (18.9°C and 17.7°C) for SPO, suggesting a great effect of the solid fat for early nucleation and crystal growth.

4.3.4 Yield Stress

The viscoelasticity of HOPO and SPO was tested with an oscillatory amplitude strain sweep test. The linear viscoelastic region (LVR) corresponds to the segment where the slope is near 0 for the storage modulus ($G'$). The change in slope suggest
that the maximum stress the structure is allowed to tolerate before yielding and behaving as a liquid.

**Figure 4-4.** Storage modulus ($G'$) vs oscillation strain of SPO and HOPO at 5°C

**Figure 4-5.** Oscillatory stress vs oscillation strain of SPO and HOPO at 5°C

HOPO and SPO exhibit similar viscoelastic behavior at 5°C as observed in Fig. 4-4, and a yield points similar in scale. At 5°C, past the first crystallization peak
fats have been solidified, therefore, solid-like behavior is expected for both oils. At lower temperatures, the SFC is higher which will improve the viscoelastic behavior of the fat.

Figure 4-6. Storage modulus (G’) vs oscillation strain of SPO and HOPO at 10°C

![Storage modulus (G’) vs oscillation strain of SPO and HOPO at 10°C](image)

Figure 4-7. Oscillatory stress vs oscillation strain of SPO and HOPO at 10°C

![Oscillatory stress vs oscillation strain of SPO and HOPO at 10°C](image)

The largest observed difference in storage modulus behavior was at 20°C (Fig. 4-6). The discrepancy between HOPO and SPO behavior was 10 times larger than that
seen at 10°C and 100 times than that of 5°C. With an increase in temperature, the solid fat content in the oil decreases, reducing its capacity as a structuring agent responsible of the plastic-like behavior.

![Storage modulus (G') vs oscillation strain of SPO and HOPO at 20°C](image1)

**Figure 4-8.** Storage modulus (G’) vs oscillation strain of SPO and HOPO at 20°C

![Oscillatory stress vs oscillation strain of SPO and HOPO at 20°C](image2)

**Figure 4-9.** Oscillatory stress vs oscillation strain of SPO and HOPO at 20°C
The oscillatory stress response observed at HOPO at 20˚C suggests an almost liquid-like behavior, noticeable in the lack of VLR in the storage modulus and the proportional response of the oscillation stress to oscillatory strain (Fig. 4-9)

4.3.5 **Hardness, Penetration Force and Adhesiveness**

The puncture test complements the rheological study of the oils, by examining the hardness, penetration force, and adhesiveness of the oils at set crystallization temperatures. The 24h conditioning method allows for the formation and stabilization of the crystals, imitating the conditions at which a fat product would be found for consumption.

The hardness of the fats increased with lower crystallization temperatures likely due to the amount solid fat content (SFC) in suspension. At 5˚C, SPO exhibited plastic deformation, characterized by the shape of the response to the axial force applied by the probe, with a maximum hardness of 5.06 kg. The fat behaved as a semisolid, deforming without showing an ultimate yield point. At 5˚C, HOPO behaved as a true plastic, with a linear response to the probe until it fractured at the maximum hardness of 2.495 kg (Fig. 4-10.C). At 5˚C, HOPO was less hard or strong to SPO.

The most noticeable increase in hardness between sample temperatures was between 10 and 5˚C for both oils. According to the DSC, at this temperature, SPO has completely undergone the first crystallization, whereas HOPO has passed its peak crystallization point, but not fully completed the transition. At 15 and 20˚C, SPO showed an initial plastic behavior, however, the hardness plateaus at values around 0.8 and 0.2kg respectively, mainly because an oily boundary layer formed by the friction of the probe with SPO slightly decrease the hardness measurement.
Figure 4-10. Penetration test over time. A) SPO hardness. B) HOPO hardness. C) SPO adhesiveness. D) HOPO adhesiveness
Figure 4-10. Continued
At 10˚C, HOPO exhibited small deformations before reaching a plateau between 0.16 and 0.20 kg, by the same effect described before. At 15˚C, the hardness values were barely higher than the threshold detected by the instrument between 0.018 and 0.036 kg. The hardness values are lower in HOPO, possibly due low SFC and probable small and dispersed crystalline structures. It was not possible to measure HOPO’s hardness at 20 ºC, due the liquid behavior at this temperature.

The adhesiveness of SPO decreases with the increase in temperature, so did HOPO. With the increase in temperature, HOPO behaved more like a liquid so the characteristic adhesiveness of fats is lost. At 5 ºC, both oils had similar adhesiveness values.

Table 4-2. Hardness and penetration values for HOPO and SPO at different temperatures (n=4)

<table>
<thead>
<tr>
<th>Temp (˚C)</th>
<th>High Oleic Palm Oil</th>
<th>Palm Oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (kg)</td>
<td>PF (kg.s)</td>
</tr>
<tr>
<td>5</td>
<td>2.495 ± 0.182</td>
<td>6054 ± 594.8</td>
</tr>
<tr>
<td>10</td>
<td>0.224 ± 0.022</td>
<td>688.5 ± 39.94</td>
</tr>
<tr>
<td>15</td>
<td>0.038 ± 0.003</td>
<td>112.0 ± 12.92</td>
</tr>
<tr>
<td>20</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Even when similar phase transitions occur 7˚C apart in the thermograms, the hardness of HOPO is not similar to SPO at 7˚C of difference, therefore, the solid fat at the given temperatures has a major role in the hardness of the oil due the TAG composition.
4.3.6 Microstructure

To have a better understanding of the mechanical properties of fats, it is necessary to characterize the crystalline microstructure. In darkfield microscopy, the fat crystals will appear white and the oil in the background, black. The samples were crystallized at 10, 15 and 20°C for 24h and images were taken in duplicate (Figure 4-11).

Figure 4-11. Microstructure of HOPO and SPO (in duplicate) at 10°C, 15°C and 20°C.
At 20°C, spherulites start to form in HOPO, with an average size of 30 μm. The morphology and size of the spherulite under these conditions suggest that HOPO crystallizes in a β polymorph (Chawla, P.; deMan, J. M.; and Smith, 1990). At 15 and 10 °C, irregular shapes were prevalent, dominating the microstructure and forming layers on top of each other, making it difficult to accurately measure the size of the crystals or describe the morphology.

In the case of SPO, the microstructure is dominated by small, and regular spherulite crystals at all temperatures, increasing the packing as the temperature drop. At lower temperatures, the presence of small needle-like structures appears that will grow into new spherulites or will attach to an existing structure. Crystallization below room temperature and different cooling rates, other polymorphs including α and β’ tending have been observed for regular palm (Kawamura, 1979).

4.4 Chapter Summary

A comparison between the rheological and thermal characteristics between standard palm oil and the high oleic variety was established and related to the fatty acid composition. The FAME analysis showed significant differences in the monounsaturated FA, mainly in the oleic acid content which is higher in HOPO (53.92% vs 40.15%) and the saturated fatty acids, represented as palmitic acid (29.92% vs 43.52%).

The effect of the FA profile on the melting/cooling behavior during DSC analysis was observed for both oils. The thermograms had similar shapes, however, a ~8 C offset in the crystallization and melting profiles were identified. Crystallization started at 7.9°C in HOPO, whereas it occurs at 16.45 °C in SPO. HOPO completely melted at 29°C, but SPO at 37°C. These findings are important to establish the optimal crystallization temperatures for HOPO and how this will affect mechanical and
rheological characteristics. Major crystallization events occurred at 16.45°C, 14.05°C, 4.64°C and -1.18°C in SPO and were consistent with the literature. The corresponding values for HOPO were 7.9°C, 5.81°C, -1.27 and -8.11°C, which offset the limits the maximum usable temperature of HOPO.

Texture analysis for the oils near major crystallization temperatures found the highest hardness value was 2.495 ± 0.182 kg at 5°C for HOPO 5.064 ± 0.546 at 5°C for SPO, with the difference that SPO deformed as the force was applied, while HOPO was more brittle, breaking at the max hardness. The hardness is directly correlated with the sweep test, that exhibited the same viscoelastic behavior for both oil at 5°C but a dramatic drop in storage modulus for HOPO as the temperature increased. Under different cooling regimes, viscosity profiles were similar between the oils at higher shear, however, there was a shift in the induction time of near 4 minutes for the crystallization, occurring around 6-8°C in HOPO for high shear, 10°C below SPO.

The microstructure of HOPO was temperature-sensitive, with well-defined spherulites at 20°C but irregular and hard to define structures at 15°C and 10°C. The microstructure of SPO did not experience major structural changes with the decrease of temperature.

A better understanding of the physical properties of HOPO suggests the use in low-temperature applications as a more nutritious alternative to oils with higher saturated fatty acid concentrations, and how the processing conditions play a key role in the use of HOPO as an ingredient.
CHAPTER 5
EFFECT OF EMULSIFIER ADDITION TO THE FUNCTIONALIZATION OF A HIGH
OLEIC PALM OIL BASED SHORTENING

5.1 Background

Vegetable oils and other fats in various configurations should make up to 20-35% of the daily calories intakes consumed in the American diet (Sacks et al., 2017). In 2015, a ban on partially hydrogenated oils (PHO) led to the introduction of high saturated fatty alternatives (SFA) for food products produced or imported to the United States. Under adequate processing conditions, SFA alternatives had similar mechanical properties to PHOs in food applications (Verle De Graef, Fubert, Smith, Cain, & Dewettinck, 2007). However, negative health effects have been also associated with SFA. The consumption of oils rich in mono and polyunsaturated fatty acids are recommended by major health institutions, but they present a challenge for food developers because these oils are typically liquid at room temperature or have poor mechanical performance. To widen the application of more nutritious liquid oils and improve room temperature applications, studies have been conducted to create a stable fat matrix at room temperature. This was achieved by using low molecular weight structuring agents (emulsifiers) and crystallization techniques that promote entrapping the liquid oil. These configurations are referred to as oleogels and have experienced a surge in the study over the last 20 years (Da Pieve et al., 2010; Giacomozzi et al., 2019; Marangoni Alejandro G., 2018) as a potential alternative to oils high in SFA.

Commonly found low SFAs have been studied for potential oleogel applications, including the high oleic varieties with better oxidative stability. However, there is limited information on the use of palm oil with a higher content of oleic acid (HOPO) in oleogels. HOPO has a higher concentration of monounsaturated oleic acid (53.5%-

54
55.25% of total FA) than regular palm oil (37% of total FA), resulting in a lower concentration of saturated fatty acids for HOPO (30%). This characteristic makes it semisolid at room temperature. The addition of a gelling agent could broaden the applications of HOPO.

Many studied structuring agents have been used depending upon availability, cost, additional health benefits and/or gelling properties. The molecular structures of monoglycerides (MAGs) makes them appealing gelling agent (Marangoni Alejandro G., 2018). The hydrophilic head of the MAGs are stabilized by hydrogen bonds, whereas the affinity of the hydrophobic tail with the triglycerides stabilizes the structure and promoting crystallization (López-Martínez et al., 2014). A similar principle applies for diglycerides (DAGs), that improve oil retention by the additional aliphatic chain in the glycerol backbone. DAGs are also of great interest because of low absorption in animal models (Kondo, Hase, Murase, & Tokimitsu, 2003).

The processing conditions in oleogel manufacturing are as important to the stability of the oleogel as the ingredients. Critical processing parameters include shearing, crystallization temperature and conditioning. Additionally, high-intensity ultrasound (HIU) induced crystallization has been studied to increase oil retention in oleogels. Shear has been observed to weaken cod liver oleogels stabilized with monoglycerides (Da Pieve et al., 2010), mainly due the rupture of the gel-like structure formed. Giacomozzi (2019) stabilized a high oleic sunflower oil with monoglycerides, identifying that higher cooling rates and higher monoglyceride content improve viscoelastic and thermal properties of the oil (Giacomozzi et al., 2019). Commercial DAGs have been used with sunflower oil at different shearing and cooling rates,
resulting in more brittle structures for DAGs mixtures under shear (Tavernier et al., 2019).

For oleogels to replace existing shortenings and other high saturated fatty acids ingredients, the physical characteristics need to be comparable. It is possible to identify the optimal concentration of the structuring agent that modifies the crystallization temperature and melting point by thermal analysis using differential calorimetry analysis (DSC). To compare mechanical properties, a puncture test is a fast and reliable technique to assess changes in the hardness and spreadability of the oleogel. Other techniques such as oscillatory rheology, oil-binding capacity and microscopy are also tools that describe the performance and characteristics of the oleogel at different operation conditions.

To the knowledge of the authors, changes in the behavior of HOPO by the addition of emulsifiers have not been studied. HOPO has approx. 2.5% w/w naturally occurring DAGs, which slow down the nucleation stage in regular palm oil and a low concentration of fully saturated triglycerides (1.5%) (Mozzon et al., 2013).

The objective of this project is to study the effect of mono and diglycerides on the mechanical and thermal properties of HOPO oleogels.

5.2 Materials and Methods

5.2.1 Material and Reagents

Refined Bleached and deodorized high oleic palm oil (HOPO) from the interspecific hybrid *E. Guineensis* and *E. Oleifera* OxG was provided by Thin Oil Product (Florida, USA). The crude oil was obtained by mechanical press extraction. Commercially available emulsifiers Alphadim 90 PBK and Trancendim 130 were donated by Corbion Ingredients (Kansas, USA). Alphadim 90 PBK (referred as MAG) is
95% monoglycerides and Trancendim 130 (referred as DAG) is 67% diglyceride, 27% triglycerides and 6% monoglyceride.

The particular emulsifiers for this study were chosen in collaboration with an industry expert (Dr. Gloria Cagampang) and the insight from Corbion, emulsifiers and ingredients manufacturer.

5.2.2 Oleogel Preparation

To prepare the samples, HOPO was mixed with Alphadim 90 PBK or Trancendim 130 to make 3%, 5%, 7% and 10% w/w. dispersions of each emulsifier, for a total of 8 different samples: MAG_3%, MAG_5%, MAG_7%, MAG_10%, DAG_3%, DAG_5%, DAG_7% and DAG_10%. For the shortening preparation, HOPO was completely heated under constant stirring (>75 °C) to induce all solids to melted and crystal memory erased. At 75 °C, each emulsifier was added until the dispersion was clear and further stirred for 10 minutes. The cooling of samples was critical for textural analysis, described in section 5.2.4.

5.2.3 Differential Scanning Calorimetry

A Differential Scanning Calorimeter (DSC) Q2000 (TA Instruments) was used to assess the thermomechanical properties of both oils. Samples between 6-9 mg were weighed into aluminum pans to the nearest 0.1 mg and hermetically sealed. An empty hermetically sealed aluminum pan was used as a baseline. All the samples followed the AOCS Cj 1-94, 2009 method described by Maruyama et al. (2014), described as 80˚C isotherm for 10 min, cooled down at a rate of 10˚C/min to -60˚C and heated at a rate of 10˚C/min to 80˚C. All the samples underwent the same heating/cooling program and were ran in duplicate. A TA Universal Analysis software was used for analysis and presentation of results.
5.2.4 Textural Analysis

Oleogels were melted at 80°C in a water bath, placed in glass vials (n=4) and then transferred to a water bath at 45°C, 40°C, 35°C and 30°C to simulate the 25°C change under similar conditions. Upon equilibrium, samples were transferred to another water baths at 20°C, 15°C, 10°C and 5°C, respectively, and conditioned for 24 h. This procedure was intended to create fat samples under identical cooling and shear conditions (0s-1), carefully controlling the crystallization by temperature changes until equilibrium was reached. These samples were then assessed examining the effect of temperature on the different fats by taking the samples out of the water bath and immediately performing the test. A puncture test was performed using a texture analyzer TA-XTplus (Stable Micro Systems, Scarsdale, NY, USA) equipped with a 6-mm diameter cylindrical probe at room temperature. Hardness (maximum positive value) and penetration force (area under the curve) were measured when the probe compressed the sample and adhesiveness (area for the negative force) when the probe returns to the starting position. The test was performed 4 times, ANOVA 2-ways was used to confirm the separation of means and Tukey to separate the means and identify differences between treatments (crystallization temperature and emulsifier concentration).

5.2.5 Statistical Analysis

Two-way Anova was used to identify differences between means for the textural analysis, that were separated using Tukey’s test. The level of significance for all tests was 0.05. Statistical analyses were performed with GraphPad Prism 8.0 (GraphPad Software, Inc., San Diego, CA).
5.3 Results and Discussion

5.3.1 Differential Scanning Calorimetry for MAG Oleogels

The mixtures of MAGs and HOPO were precisely heated and cooled within the DSC. The key crystallization onset/offset temperatures, as well as the phase transitions, were identified for each oleogel. The addition of mono and diglycerides at different concentrations changed the crystallization and melting profiles of HOPO. With the addition of the emulsifier rich in monoglycerides (MAGs), a peak that previously did not existent in HOPO appears as observed in Figure 5-1 (The new peak is at the top right of the cooling curve, labeled “1A”).

Figure 5-1. Thermogram for HOPO (black) and MAGs at 3%, 5%, 7% and 10%.

With the increase in MAGs, the onset temperature increased as well, suggesting promotion of early nucleation within the system. It is worth noting that the energy
required to crystalize the first peak correlated with the concentration of MAGs, however, there is a considerable change in intensity from 5% to 7% w/w. The standard first peak observed in HOPO (labeled in Fig. 5-2. as “2A”) was modified by the addition of the emulsifier, changing the shape, onset temperature and peak crystallization energy for every new concentration of MAGs. The onset temperature of the peak “3A” for HOPO was not affected by the presence of the monoglyceride (Table 5.1), as the onset and peak temperatures are in the same position of the thermograms as in the oleogels. On a macro-scale, this behavior would be described as the oil fraction suspended in the oleogel.

The presence of MAGs affected the melting profile of the low melting point fraction by increasing the onset temperature of the first peak (observed on the left bottom referred to “4”), initially at -4.98°C, to -4.24°C at 3% MAGs. The onset temperature in the presence of MAGs was -4.31°C ± 0.07.

The resolution of the two events identified in HOPO as “4A”, increased with the addition of MAGs. This is a behavior typically reported for SPO (ref), related to the melting of β’ crystals, followed by the alpha crystals. This suggests a crystalline transition or two different polymorphs coexisting between -2.8°C and 0.6°C. To confirm the transition of structures or two polymorphs coexisting, a further study of X-ray diffraction at the same temperature range and heating rate would be necessary.

After the first melting event, the high melting point fraction of HOPO (referred as “5A” in the bottom center) disappeared in the presence of MAGs. With the increase of MAGs, there are more intermediate transitions, that also increased the melting point, from HOPO at 35°C to 53°C when MAGs were at 10%.
Table 5-1. Crystallization temperatures for MAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)

<table>
<thead>
<tr>
<th>MAGs</th>
<th>$T_{on1A}$ (°C)</th>
<th>$T_{peak1A}$ (°C)</th>
<th>$T_{on2A}$ (°C)</th>
<th>$T_{peak2A}$ (°C)</th>
<th>$T_{on3A}$ (°C)</th>
<th>$T_{peak3A}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-</td>
<td>9.39</td>
<td>7.15</td>
<td>-2.14</td>
<td>-7.8</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>31.74</td>
<td>30.14</td>
<td>12.34</td>
<td>7.16</td>
<td>-1.67</td>
<td>-7.42</td>
</tr>
<tr>
<td>5</td>
<td>39.78</td>
<td>38.27</td>
<td>11.98</td>
<td>9.37</td>
<td>-1.68</td>
<td>-7.27</td>
</tr>
<tr>
<td>7</td>
<td>41.52</td>
<td>40.18</td>
<td>12.26</td>
<td>10.08</td>
<td>-1.72</td>
<td>-7.48</td>
</tr>
<tr>
<td>10</td>
<td>45.92</td>
<td>44.19</td>
<td>11.63</td>
<td>10.17</td>
<td>-1.78</td>
<td>-7.06</td>
</tr>
</tbody>
</table>

Table 5-2. Melting temperatures for MAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)

<table>
<thead>
<tr>
<th>MAGs</th>
<th>$T_{on4A}$ (°C)</th>
<th>$T_{pk4A,1}$ (°C)</th>
<th>$T_{peak4A,2}$ (°C)</th>
<th>$T_{offset 4A}$ (°C)</th>
<th>$T_{offset5A}$ (°C)</th>
<th>$T_{peak5A}$ (°C)</th>
<th>$T_{offset2}$</th>
<th>$T_{peak}$ (°C)</th>
<th>$T_{melt}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-4.98</td>
<td>-3.63</td>
<td>-0.13</td>
<td>2.47</td>
<td>4.7</td>
<td>7.02</td>
<td>-</td>
<td>28.64</td>
<td>35.64</td>
</tr>
<tr>
<td>3</td>
<td>-4.24</td>
<td>-2.8</td>
<td>0.55</td>
<td>3.37</td>
<td>9.38</td>
<td>11.99</td>
<td>28.42</td>
<td>40.82</td>
<td>47.59</td>
</tr>
<tr>
<td>5</td>
<td>-4.31</td>
<td>-2.93</td>
<td>0.57</td>
<td>3.45</td>
<td>10.03</td>
<td>12.37</td>
<td>28.26</td>
<td>47.42</td>
<td>52.94</td>
</tr>
<tr>
<td>7</td>
<td>-4.3</td>
<td>-2.63</td>
<td>0.67</td>
<td>3.34</td>
<td>10.01</td>
<td>12.14</td>
<td>13.17</td>
<td>49.23</td>
<td>50.84</td>
</tr>
<tr>
<td>10</td>
<td>-4.41</td>
<td>-2.94</td>
<td>0.49</td>
<td>3.04</td>
<td>10.41</td>
<td>11.61</td>
<td>12.43</td>
<td>52.15</td>
<td>53.38</td>
</tr>
</tbody>
</table>
5.3.2 Differential Scanning Calorimetry for DAG Oleogels

The presence of an emulsifier rich in fully saturated diglycerides (63%) (DAGs), modified the first crystallization event of HOPO, as seen in Fig. 5-2. Unlike the addition of MAGs, the addition of DAGs moved the first crystallization peak (labeled as “1B” from right to left) without creating a new peak. This resulted in peaks at higher temperatures and energy requirements that was exacerbated with increasing concentrations. Contrary to MAGs inducing an independent crystallization, DAGs displaced the initial event observed in HOPO.

With the addition of DAGs, the first onset melting point decreased by 0.5˚C. Following the change in the onset temperature, the previously described melting peaks for HOPO “5A” was obscured with the increase in DAGs, suggesting that DAGs regulates the polymorphic changes. The presence of DAGs delayed the offset melting point. At 35.64˚C, HOPO was completely melted, increasing to 44.3˚C at 3% and 48.97˚C at 10%.

The diglycerides naturally found in HOPO vary in the degree of saturation. 1,3-PalmiticOleic (0.59% w/w) and 1,3-OleicOleic (0.48% w/w) are the two most abundant diglycerides structures as reported by (Lucci et al., 2015). This might explain that regardless the initial concentration of DAGs in HOPO (2.5% w/w), the addition of saturated diglycerides modified the existing crystallization curve, as opposed to forming a new peak as was observed with the addition of MAGs.
Figure 5-2. Thermogram for HOPO (black) and DAGs at 3%, 5%, 7% and 10%.
Table 5-3. Crystallization temperatures for DAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)

<table>
<thead>
<tr>
<th>DAGs (%)</th>
<th>T&lt;sub&gt;on1B&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;peak1B&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;on2B&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;peak2B&lt;/sub&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>9.39</td>
<td>7.15</td>
<td>-2.14</td>
<td>-7.8</td>
</tr>
<tr>
<td>3</td>
<td>19.86</td>
<td>16.06</td>
<td>-1.45</td>
<td>-7.71</td>
</tr>
<tr>
<td>5</td>
<td>23.37</td>
<td>20.59</td>
<td>-1.48</td>
<td>-7.63</td>
</tr>
<tr>
<td>7</td>
<td>26.95</td>
<td>23.63</td>
<td>-1.34</td>
<td>-7.71</td>
</tr>
<tr>
<td>10</td>
<td>30.96</td>
<td>28.68</td>
<td>-1.46</td>
<td>-7.8</td>
</tr>
</tbody>
</table>

Table 5-4. Melting temperatures for DAGs Oleogels at 0%, 3%, 5%, 7% and 10% (w/w)

<table>
<thead>
<tr>
<th>DAGs (%)</th>
<th>T&lt;sub&gt;on3B&lt;/sub&gt;</th>
<th>T&lt;sub&gt;peak3B,1&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;peak3B,2&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;offset3B&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;5A&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;peak2,1&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;peak2,2&lt;/sub&gt; (°C)</th>
<th>T&lt;sub&gt;melt&lt;/sub&gt; (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>-4.98</td>
<td>-3.63</td>
<td>-0.13</td>
<td>2.47</td>
<td>4.7</td>
<td>7.02</td>
<td>28.64</td>
<td>35.64</td>
</tr>
<tr>
<td>3</td>
<td>-4.58</td>
<td>-3.16</td>
<td>0.37</td>
<td>2.96</td>
<td>8.24</td>
<td>31.24</td>
<td>39.92</td>
<td>44.3</td>
</tr>
<tr>
<td>5</td>
<td>-4.88</td>
<td>-3.16</td>
<td>-0.6</td>
<td>312</td>
<td>6.46</td>
<td>32.45</td>
<td>43.05</td>
<td>43.94</td>
</tr>
<tr>
<td>7</td>
<td>-5.16</td>
<td>-2.83</td>
<td>-0.51</td>
<td>3.28</td>
<td>6.59</td>
<td>35.85</td>
<td>45.31</td>
<td>46.61</td>
</tr>
<tr>
<td>10</td>
<td>-5.17</td>
<td>-3.32</td>
<td>0.07</td>
<td>2.96</td>
<td>6.45</td>
<td>34.4</td>
<td>47.94</td>
<td>48.97</td>
</tr>
</tbody>
</table>
5.3.3 Textural Analysis

Based on the DSC results and common operation temperatures in industrials settings, texture analysis was performed at 15, 10 and 5°C. To achieve consistent results, the oils were conditioned at the test temperatures for 24h to promote a uniform gel structure. The hardness of both types of oleogels increased when a higher concentration of emulsifier was used. The temperature also had a major effect on the hardness, with higher firmness values at lower temperatures (Figure 5-3 and Figure 5-4).

The highest hardness was achieved using MAGs at a concentration of 10% at 5°C, with the highest penetration force (Table 5-5). All the oleogel using MAGs had a higher hardness than HOPO at any temperature, implying that the emulsifier improved the dispersion of solid fats within the liquid oil strengthening the structure. There was not a significant difference between the hardness values of MAGs between 5% and 7% at 5 °C and 10 °C.

The highest hardness of the DAG-HOPO oleogel was identified at 10% emulsifier and 5°C for crystallization temperature. There was not a significant difference in hardness at 5°C between 0% and 3% of emulsifier and between 5% and 7%; however, at higher temperatures, the hardness differences become significantly different. There is a considerable increase in hardness between 10°C and 5°C, suggesting a higher concentration of solid fat at colder temperatures. When compared with the thermogram in Figure 5-2, all first crystallization events have already fully developed at 10°C, therefore, at 5°C there should be a major crystal aggregation than at higher temperatures. Microstructure observations should confirm this hypothesis.
Figure 5-3. Hardness of MAGs oleogels A) 0%, 3%, 5%, 7% and 10% at 5°C, 10°C and 15°C, 10°C and 5°C. B) Zoom of the same graph at 10°C and 15°C.
Figure 5-4. Hardness of DAGs oleogels A) 0%, 3%, 5%, 7% and 10% at 5°C, 10°C and 15°C, 10°C and 5°C. B) Zoom of the same graph at 10°C and 15°C.
### Table 5-5. Hardness and Penetration Force (PF) at different concentration and temperatures for MAGs oleogels

<table>
<thead>
<tr>
<th>Temp (˚C)</th>
<th>MAG 3%</th>
<th>MAG 5%</th>
<th>MAG 7%</th>
<th>MAG 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (kg)</td>
<td>PF (kg.s)</td>
<td>Hardness (kg)</td>
<td>PF (kg.s)</td>
</tr>
<tr>
<td>5</td>
<td>3.243 ± 0.201</td>
<td>7871 ± 577.8</td>
<td>3.685 ± 0.310</td>
<td>10100 ± 828.5</td>
</tr>
<tr>
<td>10</td>
<td>0.673 ± 0.069</td>
<td>2191 ± 165.5</td>
<td>1.029 ± 0.117</td>
<td>3167 ± 342.5</td>
</tr>
<tr>
<td>15</td>
<td>0.127 ± 0.014</td>
<td>418.9 ± 47.45</td>
<td>0.365 ± 0.058</td>
<td>1120 ± 180.7</td>
</tr>
</tbody>
</table>

### Table 5-6. Hardness and Penetration Force (PF) at different concentration and temperatures for DAGs oleogels

<table>
<thead>
<tr>
<th>Temp (˚C)</th>
<th>DAG 3%</th>
<th>DAG 5%</th>
<th>DAG 7%</th>
<th>DAG 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hardness (kg)</td>
<td>PF (kg.s)</td>
<td>Hardness (kg)</td>
<td>PF (kg.s)</td>
</tr>
<tr>
<td>5</td>
<td>2.510 ± 0.295</td>
<td>7388 ± 448.6</td>
<td>3.073 ± 0.160</td>
<td>9552 ± 501.6</td>
</tr>
<tr>
<td>10</td>
<td>0.124 ± 0.006</td>
<td>388.2 ± 13.60</td>
<td>0.218 ± 0.019</td>
<td>672.7 ± 75.61</td>
</tr>
<tr>
<td>15</td>
<td>0.024 ± 0.003</td>
<td>62.87 ± 10.57</td>
<td>0.0573 ± 0.007</td>
<td>154.7 ± 29.22</td>
</tr>
</tbody>
</table>
5.4 Chapter Summary

The use of mono and diglycerides changed the thermal and mechanical properties of HOPO. The addition of commercially available saturated monoglycerides promoted an early nucleation stage that did not exist in HOPO previously. The increase in concentration also accelerated the aforementioned nucleation stage by roughly 2°C with every mass percentage in emulsifier increase. The hardness of the oleogel generally increased with the addition of emulsifier, but at 5°C, the concentration has less of an effect. The strongest oleogel had a hardness of 3.34kg, using 10% w/w monoglyceride at a crystallization temperature of 5°C, indicating a much stronger network.

The commercially available saturated diglyceride also had a major effect on the thermal and mechanical characteristics of HOPO, by dominating the initial crystallization stage of HOPO. The onset crystallization temperature increased with the addition of DAGs, promoting the early crystallization of the high melting fraction of HOPO. DAGs had less of an effect on the hardness of the oleogel, with the maximum hardness at 2.51 kg, using 10% w/w diglyceride at a crystallization temperature of 5°C. However, the effect of temperature was greater than in the MAGs oleogel. The addition of a commercially available emulsifier is an option to increase the range of applications of HOPO, by incorporating the liquid and solid phase of the oil creating stable oleogels of industrial interest.

5.5 Future Work

The current work opens the possibility for further study and characterization of HOPO in the presence of emulsifiers. By including oil binding capacity (OBC), it would be possible to quantify how much of the liquid oil is bounded with the solid matrix, and
how the crystallization temperature and concentration of emulsifier will optimize this characteristic. The description of the microstructure by Polarized Light Microscopy is another study that would show the assembly of the crystalline structures at different temperatures, which will suggest new fields of applications. These two additional assessments will be added to the current study prior to publication.

Other aspects of interest include the addition of mono and diglycerides at different ratios to HOPO, to resemble a commercial shortening. These ratios could be mathematically optimized for a desired hardness and storage modulus for particular applications.
CHAPTER 6
GENERAL CONCLUSIONS

This work successfully compared the thermal and rheological characteristics of high oleic palm oil to the standard counterpart, including the modification of HOPO with the use of emulsifiers.

As hypothesized due to the higher concentration of oleic acid in HOPO (53.92\% vs 40.15\%) and the lower content of saturated fatty acids, the thermograms had similar shapes, however, a ~8 C offset in the crystallization and melting profiles. Static crystallization started occurring at 7.9˚C in HOPO, while at 16.45˚C in SPO. HOPO completely melted at 29˚C, but SPO at 37˚C. Near the main crystallization events, hardness value was evaluated obtaining the highest value as 2.495 ± 0.182 kg at 5˚C for HOPO 5.064 ± 0.546 at 5 C for SPO, with the difference that SPO deformed as the force was applied, while HOPO was more brittle, breaking at the max hardness. This confirmed the initial hypothesis that SPO would be harder than HOPO, and this was true at every assessed temperature. The hardness was directly correlated with the sweep test, that exhibited the same viscoelastic behavior for both oils at 5˚C but a dramatic drop in storage modulus for HOPO as the temperature increased.

Following the hypothesis, the viscosity decreased at higher shear rates, however, viscosity profiles were similar between the oils at higher shear. The critical shear for HOPO was lower than SPO, situated below 10s^{-1}. There was a shift in the crystallization temperature, occurring around 6-8˚C in HOPO for high shear, 10˚C below SPO. These findings suggest the use of HOPO for low-temperature applications that require malleability at lower temperatures, including margarine or pourable shortenings.
These mechanical and thermal characteristics of HOPO with emulsifiers were successfully assessed as hypothesized. The addition of both emulsifiers increased the temperature of the first crystallization events but did not affect the second crystallization. The presence of MAGs promoted a new early nucleation stage whereas DAGs displaced the first peak in HOPO to a higher temperature but did not create a new one. While both emulsifiers improved the thermal stability of HOPO, only MAGs increased the hardness at all temperatures tested at all the concentration.

With better knowledge of the crystallization dynamics of HOPO and the effect of emulsifiers commercially available, it is possible to tailor fat products for different food applications, especially low saturated fatty acid, trans-free alternatives. An example would be utilizing emulsifiers at low concentrations to create margarine and shortenings with good spreadability and high melting point. Other applications would include the use of HOPO based oleogels in bakery or emulsified meat products.
LIST OF REFERENCES


73


78


BIOGRAPHICAL SKETCH

Victor Cedeño was born and raised in Caracas, Venezuela, where he grew up with his parents Martha Sanchez and Pablo Cedeño, and his little brother, Hector. He moved to Colombia to pursue a Bachelor of Science in chemical engineering from Universidad de La Sabana, in 2013. Victor was awarded a scholarship to research high Oleic Palm oil, reason why he continued his studies at the University of Florida, completing his Master of Science in food science and human nutrition in 2020.