ENZYMATIC INTERESTERIFICATION OF COCONUT AND HIGH OLEIC SUNFLOWER OILS FOR EDIBLE FILM APPLICATION AND SENSORY EVALUATION

by

MARIA A MOORE

(Under the Direction of Casimir C. Akoh)

ABSTRACT

Blends (60:40, 70:30 and 80:20 (w/w)) of coconut (CO) and high oleic sunflower oils (HOSO) were interesterified using immobilized enzyme, Lipozyme® TL IM, to increase the oleic acid content at the *sn*-2 position. The 60:40 interesterified product (IP6) was used to further investigate the use of IP in edible films based on significant difference in tensile strength, elongation break, and oleic acid content at the *sn*-2 position. The IP6 was produced on a large scale and fortified with natural tocopherols to prevent lipid oxidation. The emulsion edible film was then coated on a granola bar and monitored over a five-day period. Consumer tests (n=80) showed that there was a difference between the control and test sample and overall liking of 50% of the population was 8.0 on a 9-point hedonic scale. The product has commercial potential in the future as a granola bar binder, especially after minor modifications.

INDEX WORDS: Coconut oil, High oleic sunflower oil, structured lipid, enzymatic interesterification, natural antioxidants, emulsion edible film, granola bars, sensory evaluation

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MARIA A MOORE

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MARIA A MOORE

Major Professor:	Casimir C Akoh
Committee:	William L Kerr Gabriela Sanchez-Brambila

Electronic Version Approved:

Suzanne Barbour
Dean of the Graduate School
The University of Georgia
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DEDICATION

I would like to dedicate my thesis to my dad, Robert Michael Moore Jr, who taught me that "Opportunities come once," who forever has and will be the biggest inspiration in my life. May you forever rest in peace.

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CHAPTER 1

INTRODUCTION

The edible coating ingredients market was valued at ~$3 million in 2016. Sugars and syrups dominate the top of the ingredient sales for edible coatings at 36%. Edible coatings have dominated the market especially in bakery products, ready to eat cereals, snack and nutritional bars, and several other areas with the largest being confectionaries (Grandview Research 2017). Edible coatings can contain a variety of components varying from carbohydrates, to proteins and lipids. Edible coatings can consist of a single, bi-layer or emulsion film depending on the intended use (Galus and Kadrinska 2015). Vegetable oils are commonly used in films due to the fact they are low-cost, non-toxic, non-depletable and non-volatile. Vegetable oils can help provide a moisture barrier to products. However, they are prone to oxidation. Oils rich in saturated and monounsaturated fats can be used since they are less prone to oxidation than polyunsaturated fatty acids. In addition to the vegetable oils being highest in saturated and monounsaturated fatty acids, they have natural tocopherols which help prevent oxidation.

Two vegetable oils: coconut and high oleic sunflower oils can be used in edible film making. Coconut oil contains a high percentage (61-69%) of medium chain fatty acids (MCFAs) which contain between 8-12 carbons (Prasanth-Kumar and Gopala Krishna 2015). High oleic sunflower oil, unlike coconut oil contains a high percentage (75-90.7 mol%) of oleic acid (FAO 2001). A structured lipid (SL) is a lipid that has been chemically or enzymatically modified from its natural biosynthetic form to improve nutritional or physical properties (Akoh and Kim 2017). SLs have been used in the past for single layer edible film development (Sellappan and

Akoh 2000) but not used in emulsion edible films. Coconut oil interesterified with high oleic sunflower oil will increase the oleic acid content at the *sn*-2 position of the SL. This is desirable because long chain fatty acids (LCFAs) have increased digestibility at the *sn*-2 position.

After interesterification, free fatty acids (FFAs) are formed and need to be reduced to less than 0.1% to prevent lipid oxidation. One method to reducing FFAs is using short-path distillation (SPD) which has been used to reducing FFAs successfully in the past. Although SPD successfully reduces the amount of FFAs, tocopherols present are typically destroyed by high temperatures which increases the chance for lipid oxidation (Zou and Akoh 2013; Pande and others 2012). Tocopherols are a type of antioxidant which can fortify an oil after SPD to help retard lipid oxidation (Augustyniak and others 2010).

Nutritional bars have increased in popularity and can be categorized into three different groupings: breakfast bars, granola bars and nutrition/health bars (Bakery and Snacks 2016). According to Wyatts (2017), consumers are looking for snacks which provide hunger satisfaction, meet daily nutritional goals and provide energy/fuel. However, more than 90% of consumers choose a snack based on taste. Sensory evaluation of consumer acceptance of a product is crucial in understanding if the granola bar will do well in the marketplace.

This thesis includes five chapters. The first chapter is an introduction that includes objectives to this research. The second chapter is a literature review of related topics including coconut oil, high oleic sunflower oil, structured lipids, enzymatic interesterification, edible films, mechanical properties, lipid oxidation, natural antioxidants, puncture testing, nutritional bars and sensory evaluation. The third chapter includes the enzymatic interesterification of three blends (60:40, 70:30, 80:20 (w/w)) coconut: high oleic sunflower oils in order to increase the oleic acid at the *sn*-2 position. The three SLs were compared to starting substrates to determine the best oil

for emulsion edible film use. The fourth chapter includes the large-scale reaction of the 60:40 coconut: high oleic sunflower oil which includes the fortification with natural tocopherols to prevent lipid oxidation. In addition, this chapter includes the use of the scaled-up SL in an edible emulsion film on a commercial granola bar. The coated granola bar was evaluated over a five-day period at two different temperatures. Finally, the coated granola bar was tested using two different sensory tests: a discriminative, paired-comparison test and an affective, consumer acceptance hedonic test. The fifth and last chapter presents the conclusion of the entire research along with future possible work.

The objectives of this research were:

1. To develop a structured lipid from coconut and high oleic sunflower oils using enzymatic interesterification in order to increase the percentage of oleic acid at the *sn*-2 position of coconut oil and to use that developed structured lipid in an emulsion edible film
2. To fortify a large scale produced structured lipid from coconut and high oleic sunflower oils with natural tocopherols to prevent their rancidity as well as use the structured lipid in an emulsion edible film to coat a granola bar and test the physical and sensory properties of the coated granola bar

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CHAPTER 2

LITERATURE REVIEW

Coconut Oil

Coconut oil is a popular oil which is considered a functional food that has a wide range of uses from cosmetics to cooking. Some specific uses include: hair conditioning, pain relief for ulcers and wounds, sunscreen, and a flavor carrier (Rockridge 2003). Coconut oil is composed mostly of medium chain saturated fatty acids (approx. 61%) in the form of triacyclglycerols (TAG). The coconut oil fatty acid composition used for this study can be found in **Table 2.1**. Lauric acid is the predominant fatty acid in coconut oil (46.91 ± 0.27 mol%). Coconut oil also contains (80.53 ± 4.92 mol% lauric acid) at the *sn*-2 position, and (30.11 ± 2.58 mol%) at the *sn*-1,3 positions (Moore and Akoh 2017). Orsavova and others (2015) found that the major fatty acids in coconut oil were 47.7 mol% lauric, 19.9 mol% myrstic, 6.2 mol% oleic, and 7.6 mol% caprylic acids. According to Marina and others (2009) the predominant TAG molecular species for coconut oil are LaLaLa (26.19%), CLaLa (21.56 %), LaLaM (18.49%), CLaC (15.44%), LaMM (10.56%), LaMP (3.81%), and LaCCp (2.09%), also found in **Table 2.2**. The TAG molecular species for this study is comparable to past data **Table 2.2**. Coconut oil contains mostly medium chain TAGs and when medium chain fatty acids (MCFA) occupy the *sn*-1 and *sn*-3 positions, they are more easily digested because they are more water soluble and rapidly hydrolyzed by lipase than at the *sn*-2 position (Dayrit 2015).

According to Harris and others (2017), virgin coconut oil had mostly neutral effects on cardiovascular disease (CVD) and body composition. Several studies have concluded that in

countries in which coconut oil consumption is high, there is an overall lower risk of CVD (Riberio 2017). In addition to CVD importance, monolaurin and lauric acid have been shown to have antimicrobial properties and are specifically, very active against gram positive bacteria (Dayrit 2015).

High Oleic Sunflower Oil

High oleic sunflower oil (HOSO) is derived from high oleic acid-bearing seeds. According to the FAO codex standards for fats and oils from vegetable sources, HOSO can contain between 75-90.7 mol% oleic acid. The fatty acid profile of HOSO for this study is included in **Table 2.3** which contained 86.38 ± 0.52 mol% oleic acid. According to the FAO (2001), HOSO must contain no less than 75% oleic acid as a percentage of total fatty acids. Medium chain fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs) are more readily digested at the *sn*-2 position (Karupaiah and Sundram 2007). HOSO contains approximately 92.81 ± 1.10 mol% at the *sn*-2 position and therefore would be advantageous to use in an interesterification reaction. The most predominant TAG species is OOO (96.14 %).

Structured Lipids

Structured lipids (SLs) are lipids that have been modified either chemically or enzymatically from their natural form for specific nutritional and physical properties. Modification of TAGs can change the melting point, iodine value, crystallization profile, saponification, and oxidative stability. Potential uses for SLs in foods exist in products such as margarines, improving melting properties, cocoa butter substitutes, confectionaries, and soft candies, in the form of reduced- or low-calorie fats, baking chips, baked goods, snack foods and even dairy products (Akoh and Kim 2017). During digestion *sn*-1 and *sn*-3 positions in TAG species are cleaved by lipases and result in free fatty acids (FFAs) and *sn*-2 monoacylglycerols.

Long chain fatty acids (LCFAs) require a protein-mediated process while *sn*-2 monoacylglyerols are absorbed by passive diffusion. The metabolic rate is also different depending on stereospecificity. LCFAs located on the *sn*-1 and *sn*-3 positions are reassembled via the phosphatidic pathway into new TAG structures. Short and medium chain fatty acids are solubilized in intestinal fluids and absorbed into the portal system. They will form complexes with albumin to be used for oxidation in the liver (Karupaiah and Sundram 2007).

Enzymatic Interesterification

Interesterification reactions can occur either chemically or enzymatically. The development of industrial applications for enzyme use have been slow due to cost; however, recovery and several reuses of the enzyme could help decrease the cost. Certainly, enzymatic interesterification is a safe process, the reaction can only yield randomized products. Enzymatic interesterification also results in improved the quality of oils compared to chemical interesterification by offering better retention of tocopherols and tocotrienols and increased oxidative stability (Gibon 2011). **Figure 2.1** explains the reaction scheme of two TAG molecules undergoing enzymatic interesterification using a *sn*-1,3 specific enzyme and the six possible products produced.

Enzymatic reactions using lipases can be classified into two major groups, *sn*-1,3-specific and nonspecific lipases. An example of a *sn*-1,3 specific lipase is *Rhizomucor miehei* and a nonspecific lipase is *Candida rugosa* (Kim and Akoh 2015). *Rhizomucor miehei* was used in the interesterification reaction of coconut oil, safflower oil, soybean oil and tripalmitin to simulate human milk fat (HMF) (Maduko and others 2007). In recent research, Lipozyme TL IM® has been used for *sn*-1,3 specific interesterification reactions (Ifeduba and others 2016, Li and others 2015, Pande and others 2012, and Yamaguchi and others 2004).

Edible Films

Edible films and coatings have been around for centuries especially using waxes to coat various fruits for aesthetic purposes. Today, the popularity of edible films used in products has grown exponentially with the annual revenue greater than $100 million. Some commercially available examples of edible coatings are Freshseel™ and Opta Glaze™ (Pavlath and Orts 2009). Possible components of edible films can include proteins, carbohydrates, and lipids. Lipids are useful in reducing water transmission; polysaccharides can be used for oxygen and gas transmission, and proteins can provide mechanical structure and stability. Emulsion films have become more popular from an economic stand point and have commercial appeal. Emulsions by protein or fat and carbohydrate components allow for direct adhesion and form a hydrophobic layer or coating at the surface of the product (Pavlath and Orts 2009). Emulsion films can contain hydrocolloidal materials which consist of alginates, carrageenan, carboxymethycellulose, gum Arabic etc. Hydrocolloids are used in films to help control the texture, flavor and shelf-life. The texture is improved by the hydrocolloids acting as an emulsifier, gelling agent, or thickener (Skurtys and others 2010).

Emulsion films have been studied using various components reported by the following research papers (Binsi and others 2013; Gounga and others 2007; Monedero and others 2009; Han and others 2006; Bravin and others 2006; Won-Seok and Han 2001). Binsi and others (2013) studied edible films formed with chitosan and virgin coconut oil. They concluded the optimal oil to chitosan ratio was 0.5 to 1 mL/g chitosan and that this film could be used for packing dry as well as moist foods. Han and others (2006) studied the use of pea starch edible films containing a beeswax emulsion. They concluded the addition of a hydrophobic material increased water resistant of hydrophilic films when more than 30% beeswax was incorporated. In

a different study conducted by Bravin and others (2006), they studied an emulsion film composed of corn starch, methylcellulose, and soybean oil. They concluded in their study that crackers which had a low a_w coated with the edible coating could become an integral part of the food to reduce the hydration kinetics in a high a_w environment. Edible films have a good potential and should be further researched to become an integral part of the food to reduce hydration kinetics.

Mechanical Properties

Typically, the mechanical resistance of hydrocolloidal films are studied based on three parameters: tensile strength, Young's modulus, and percent of elongation at break. Tensile strength is calculated by dividing the maximum load on the film before failure by the initial cross-sectional area. The elongation at break is expressed as the percentage of change in the original length of the film before it breaks and describes the measure of the amount of elasticity. Young's modulus is a measure of stiffness determined by the slope of the stress-strain curve during the tensile test (Skurty and others 2010).

According to American Society for Testing Materials and Standards (ASTM), the mechanical properties help to determine differences among edible films. For example, Oses and others (2009) determined differences in glycerol content in edible films by measuring elongation at break (EB), tensile strength (TS), and Young's modulus (YM). Edible films containing 30% glycerol compared to 0% had a significantly lower tensile strength and a higher elongation at break due to the added plasticity. Pea starch films with varying beeswax concentrations (10, 20, 30, and 40%) tested EB, TS, and YM to help determine if a difference existed among the various concentrations (Han and others 2006). The mechanical properties were not significantly impacted until the beeswax content was above 20-30% of the pea starch concentration which the

Young's modulus or elastic modulus, tensile strength and elongation of break decreased in each prospective test (Han and others 2006). Other research has been conducted using the same testing methods to determine the plasticity of edible films (Carpine and others 2015; Fernandez and others 2007). Although research into understanding mechanical properties has been conducted using various oils, there is little information of edible films containing SLs that has been reported.

Lipid Oxidation

Lipid oxidation is a major concern of the food industry because reactions occur autocatalytically through free radicals. Products from lipid oxidation are attributed to the unpleasant, rancid odor. The unpleasant, rancid like odors of oils are attributed to lipid oxidation can be detected by humans at parts per million and even parts per billion thresholds. There are several flavors and aromas associated with volatile compounds resulting from lipid oxidation such as butanoic acid, propanoic acid, pentanal, hexanal and several others (Brewer 2011). It is reported that such volatile compounds also have the possibility to initiate oxidative chain processes (Yanishlieva and Marinova 2003). Lipid oxidation increases with the degree of unsaturation. **Figure 2.2** shows three phases: initiation, propagation, and termination of lipid oxidation.

There are several factors such as enzymes, metalloproteins, light, high processing temperatures and irradiation which can promote lipid oxidation (Barden and Decker 2016). Structured lipids are extremely prone to lipid oxidation due to being exposed to high temperatures during free fatty acid removal (Zou and Akoh 2013). SLs containing tocopherols degrade quickly because α-tocopherol decreases in antioxidant activity above 100 °C and δ-tocopherol above 150 °C (Brewer 2011). SLs can be fortified with synthetic and natural

antioxidants to restore them to levels present before short path distillation which exposes such antioxidants to high temperatures.

Lipid oxidation in low-moisture foods, which are defined as foods that have a water activity below 0.5, are primarily susceptible to lipid oxidation. The reason low-moisture foods are susceptible to lipid oxidation is thought to be due to water acting as a protective barrier to fats. Lipid oxidation is less likely to occur in saturated fatty acids such as those found in grain-based desserts and snacks which are some of the top contributors of saturated fat in the American diet. Lipid oxidation of these products is often determined by sensory evaluation and defined as when consumers can detect rancidity of a product (Barden and Decker 2016). Some fatty acid decomposition products and free radicals can cause additional food quality losses such as degradation of vitamins, loss of color and changes in protein functionality. In order to prevent lipid oxidation in foods, methods such as removing oxygen from packaging can be used to combat the problem. Another factor which increases lipid oxidation is water activity, which is highest between (0.6-0.8) and (0.0-0.2) (Vieira and others 2015). Water activity should be kept between 0.2-0.6 to avoid lipid oxidation.

Natural Antioxidants

Antioxidants are chemical compounds that provide a defense mechanism which helps protect unsaturated fatty acids from a free radical attack and act as inhibitors of lipid oxidation. There are two types of inhibitors that can retard the free radical chain process of autoxidation: chain breaking inhibitors and preventive inhibitors (Yanishlieva and Marinova 2003). An example of a chain breaking inhibitor is antioxidants. The antioxidant gains a free radical and the antioxidant inhibits oxidation because it can delocalize the free radical. There are several different natural antioxidant sources such as vitamin C, vitamin E, carotenoids, and polyphenols:

flavonoids (Rietjens and others 2002). The effectiveness of these free radical scavengers depends on chemical properties and physical location within a food system (Vieira and others 2015). Currently there are three primary methods used for the routine quality control and measurement of antioxidant capacity: oxygen radical absorbing capacity (ORAC), Folin-Ciocalteu phenolics assay (F-C) and Trolox equivalent antioxidant capacity (TEAC) (Augustyniak and others 2010). In the last decade, there has been an increasing trend to switch from using synthetic antioxidants to antioxidants from natural sources.

Vitamin E is a term to describe a family of tocopherols which includes α, β, γ, and δ-tocopherols and can be used as a natural antioxidant source. Vitamin E has three distinctive domains which are described as: the functional domain, which is responsible for the antioxidant activity, the signaling domain, which is comprised of the aromatic rings; and the hydrophobic domain, which is responsible for docking the agents (Augustyniak and others 2010). Vitamin E has multiple functions and can have an antioxidant, neutral or pro-oxidant effect. The prooxidant action of vitamin E can be found in **Figure 2.3**. Because of the dual functionality of vitamin E, it has been recommended not to increase α-tocopherol levels to significantly high levels in products already containing α-tocopherol. Increasing α-tocopherol levels so that it unbalances the cell network can cause α-tocopherol radicals to form which can be toxic (Rietjens and others 2002). Taghvaei and Jafari (2015) reviewed the effects of adding natural tocopherols instead of their synthetic counterparts in edible oils. In the conclusions of Taghvaei and Jafari's work (2015) the natural tocopherols had more antioxidant activity and thermal stability than their synthetic counterparts.

Puncture Testing

There are several different machines used to test the physical properties of foods such as a Texturometer and Instron used for measuring the texture profile of materials. Since food products vary, the choice of test will be dependent on the food product. Some physical tests have multiple uses, the profile analysis (TPA) given by the texturometer can measure fracturability, cohesiveness, adhesiveness, springiness, hardness, gumminess, and chewiness. The Instron measures multiple physical properties such as compression, tensile, harness, and impact testing is known by Instron Texture Profile (ITP). There are also physical tests that have specific uses such as the Tarr-Baker Jelly tester used to determine the firmness of pectin jellies or the Bailey Shortometer used to determine the snapping properties of baked goods (Aramouni and Deschenes 2015). A Bailey Shortometer test is a specific test developed as a type of three-point bend test and the set up can be seen in **Figure 2.4.**

A three-point bend test has multiple names and is often used to evaluate hardness of products. The three-point bend test is best used for food in a uniform shape- bar or sheet. The test method is set up to have a bar or sheet resting on two beams and the third beam moves down to "snap" the bar between the other two beams (Bourne 1982). Woody (2013) used a three-point bend test to study the differences of several commercial cookie products compared to the sensory evaluation of the products. The study concluded that although the method is recommended by the American Institute of baking for measurement of texture, the results suggested that a linear relationship did not exist between the three-point bend method and sensory evaluation. Another study conducted by Inglett and others (2015) looked at the physical properties using a three-point bend test of gluten free sugar cookies made from amaranth-oat. The test was successful in helping to determine differences among hardness in different cookie types and agrees with past

research. Kim and others (2012) determined that a three-point bending test helped to determine the perception of hardness using sensory analysis. There was strong correlation that confirmed the sensory perception of hardness of brittle solid foods is based on the stress required to initiate and propagate a crack in food. Three-point bend test is a helpful tool in determining the hardness of a brittle food over time to help determine sensory quality of a product.

Nutritional Bars

Granola bars have increased in popularity in recent years due to their nutritional value as well as the fact that they are a convenient and easy to eat snack. Consumers are most concerned with calories, fat, protein and fiber when it comes to nutritional bars (Mahanna and others 2009). Recent research has studied the use of cassava flour as a base for a well-balanced nutritional cereal bar (Silvia and others 2012). Another study conducted by Trier and Johnston (2012), investigated if the nutritional bars made a caloric difference on a healthy young adult's diet. Recruited panelists were given one of two test foods, a high protein bar or two high carbohydrate bars. The researchers concluded that the nutritional bars were a unique opportunity to influence nutrient status without promoting excessive energy intakes. Padmashree and others (2013) prepared flax/oat nutty bars to determine shelf stability in different packages. The packaging significantly impacted the shelf-stability of the bar. The bar was stable for 12 months packaged in premier flexible packaging and MP packed samples compared to 6 months in polypropylene. Granola bars are one of the most popular breakfast items and should be studied to add functional ingredients.

Sensory Evaluation

Sensory evaluation is a scientific discipline used to evoke, measure, analyze, and interpret reactions to food characteristics as they are perceived by the senses (Stone and others

2012). There are three classes of testing: difference, descriptive, and affective testing. Difference testing answers the simple question, if there is a perceivable difference between two products. The three most commonly used tests are: a paired comparison test, triangle test and a duo-trio test. Statistics of a paired comparison test are simple to analyze by counting correct and incorrect answers and then referring to a table provided to determine significance (Lawless and Heyman 2010). A paired comparison can be directional and offer two choices which creates a limitation because panelists may be unaware what constitutes a difference. Modification to the test such as including the words same and different can eliminate the issues of directionality and semantics. Most panelists expect a difference and therefore four answer choices should be presented AB, BA, AA and BB. A paired comparison/same difference test should be utilized when answering simple questions and limited exposure to the product is preferable (Stone and others 2012).

Another type of test is quantitative descriptive analysis (QDA) which is used to quantify perceived intensities of the sensory characteristics of the product. One advantage is that a fewer number of panelists are needed such as 10-12 well-trained individuals (Laweless and Heyman 2010). Another version of QDA is the Spectrum™ QDA method in which panelists learn reference samples with number values on an absolute intensity scale (Meilaard and others 2016). QDA has been used in some past research to study the effects of structured lipid use in products. A canola oil/caprylic acid structured lipid was developed for a nutritional beverage and analyzed using QDA for acceptance (Osborn and others 2003). The panelists helped to identify differences in foaminess which may have been a result of the acidolysis reaction. Further investigation into measuring physical properties with machinery could help verify the differences in foaminess. Using both mechanical and human sensory properties helps to detect differences present in products.

One of the most common testing methods utilized is a consumer acceptance test which determines how well consumers like a product. As well as likeability, a consumer acceptance test asks about the products specific sensory attributes using just-about-right scale. Consumer tests are conducted for various reasons such as product maintenance, product improvement/optimization, development of new products, assessment of market potential, category review/benchmarking, support for advertising claims, and uncovering consumer needs (Meilaard and others 2016). Recently, a consumer acceptance test conducted by Biatek and others (2016) tested a new cereal bar offered to children. The researchers found the nutritional bars had improved nutritional value, safety and high acceptability amongst school children (Biatek and others 2016).

There are several different ways to analyze sensory data which include ANOVA, principle components analysis (PCA), correlation analysis, and cluster analysis which study variables of equal status. PCA is often used to summarize many variables into just the most important variables. Most often however, PCA is used in descriptive analysis. Another method is correlation analysis which looks to determine the strength of the linear relationships between two variables. Correlation analysis can be used most effectively to determine relationships between two different types of test methods such as sensory and instrumental analysis. Another method is cluster analysis, which can use hierarchical or nonhierarchical methods to determine cluster groups (Meilgaard and others 2016). Hierarchical clustering has been recently used to help evaluate and discriminate IMS fingerprints of apple essences by identifying cluster groups based on batches and manufacturers (Min and others 2017). Hierarchical clustering can be a helpful tool and therefore is utilized in this work to correlate consumer data while a t-test of means helped to determine if there was a significant difference among clusters.

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Table 2.1 Fatty Acid Composition (mol %) of Coconut Oil[ab]			
Fatty Acid	Total	*sn*-2	*sn*-1,3
C8:0	8.18 ± 0.04	1.98 ± 0.30	11.25 ± 0.32
C10:0	6.19 ± 0.02	2.48 ± 1.05	8.05 ± 0.57
C12:0	46.91 ± 0.27	80.53 ± 4.92	30.11 ± 2.58
C14: 0	17.66 ± 0.05	6.36 ± 1.90	23.31 ± 0.99
C16:0	8.89 ± 0.05	1.77 ± 0.31	12.47 ± 0.37
C18:0	2.58 ± 0.15	ND	3.88 ± 0.58
C18:1n9	6.91 ± 0.29	5.50 ± 1.67	7.62 ± 1.16
C18:2n6	2.08 ± 0.32	1.37 ± 0.29	2.43 ± 0.86

ND = Not Detected

[a]Trace amounts of C6:0, C18:3n6, and C18:3n3 found

[b]Data was obtained from Moore and Akoh (2017)

Table 2.2 TAG Molecular Species (%)

	CO[a]	RBDCO[b]
CpCpLa	0.24 ± 0.16	1.24
LaCCp	2.09 ± 1.09	3.53
CLaC	15.44 ± 0.46	13.15
CLaLa	21.56 ± 0.75	17.33
LaLaLa	26.19 ± 3.13	21.95
LaLaM	18.49 ± 0.88	17.18
LaMM	10.56 ± 1.42	10.19
LaMO	ND	2.11
LaMP	3.81 ± 1.78	5.8
LaOO	0.51 ± 0.33	1.39
LaPP	0.77 ± 0.55	1.59

[a]Data obtained from Moore and Akoh (2017)

[b]Data obtained from Marina and others (2009)

Cp caprylic, La lauric, C capric, M myristic, O oleic, P palmitic

Table 2.3 Fatty Acid Composition (mol %) of High Oleic Sunflower Oil[ab]

Fatty Acid	Total	*sn*-2	*sn*-1,3
C16:0	3.57 ± 0.03	1.47 ± 0.36	4.62 ± 0.32
C18:0	2.56 ± 0.23	0.39 ± 0.10	3.64 ± 0.72
C18:1n9	86.38 ± 0.52	92.81 ± 1.10	83.15 ± 1.22
C18:2n6	4.59 ± 0.12	4.64 ± 0.08	4.56 ± 0.53
C22:1	1.27 ± 0.06	ND	1.90 ± 0.36

ND = Not Detected

Mean ± SD of (n = 3)

[a]Trace amounts of C14:0, C16:1, C18:3n6, C20:1, C18:3n3 and C24:1 found

[b]Data obtained from Moore and Akoh (2017)

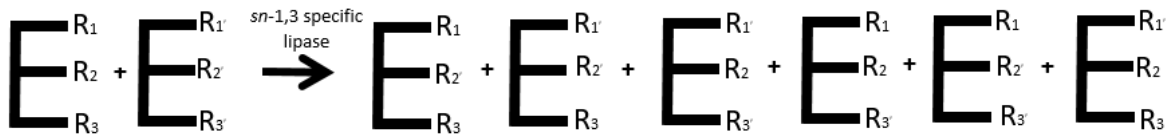

Figure 2.1 Scheme using a *sn*-1,3 specific lipase for enzymatic interesterification of two TAG molecules and their potential structured lipid products

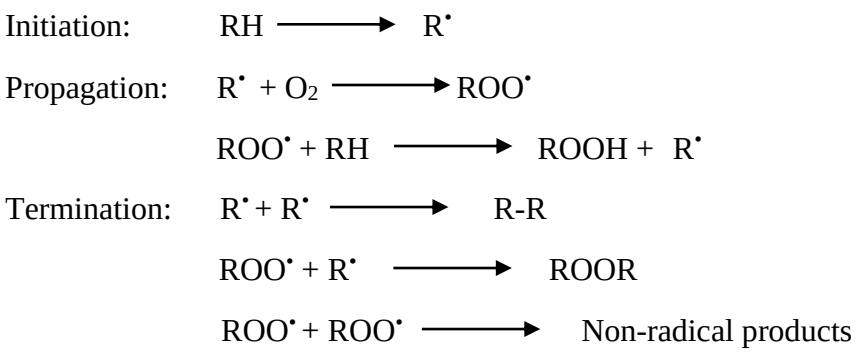

Figure 2.2 Auto-catalytic process consisting of a free radical chain mechanism. Image was recreated from Taghvaei and Jafari (2015).

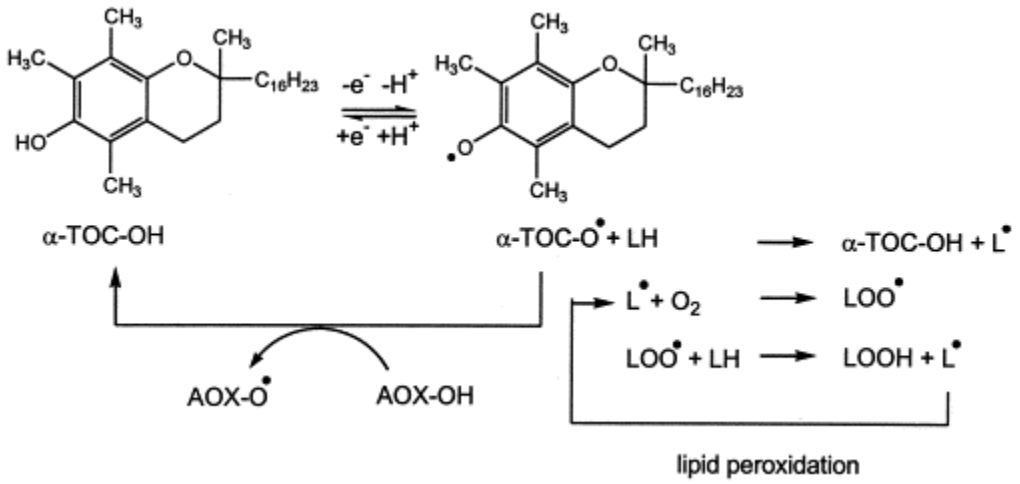

Figure 2.3 Pro-oxidant action of vitamin E. Image was recreated from Rietijens and others (2002).

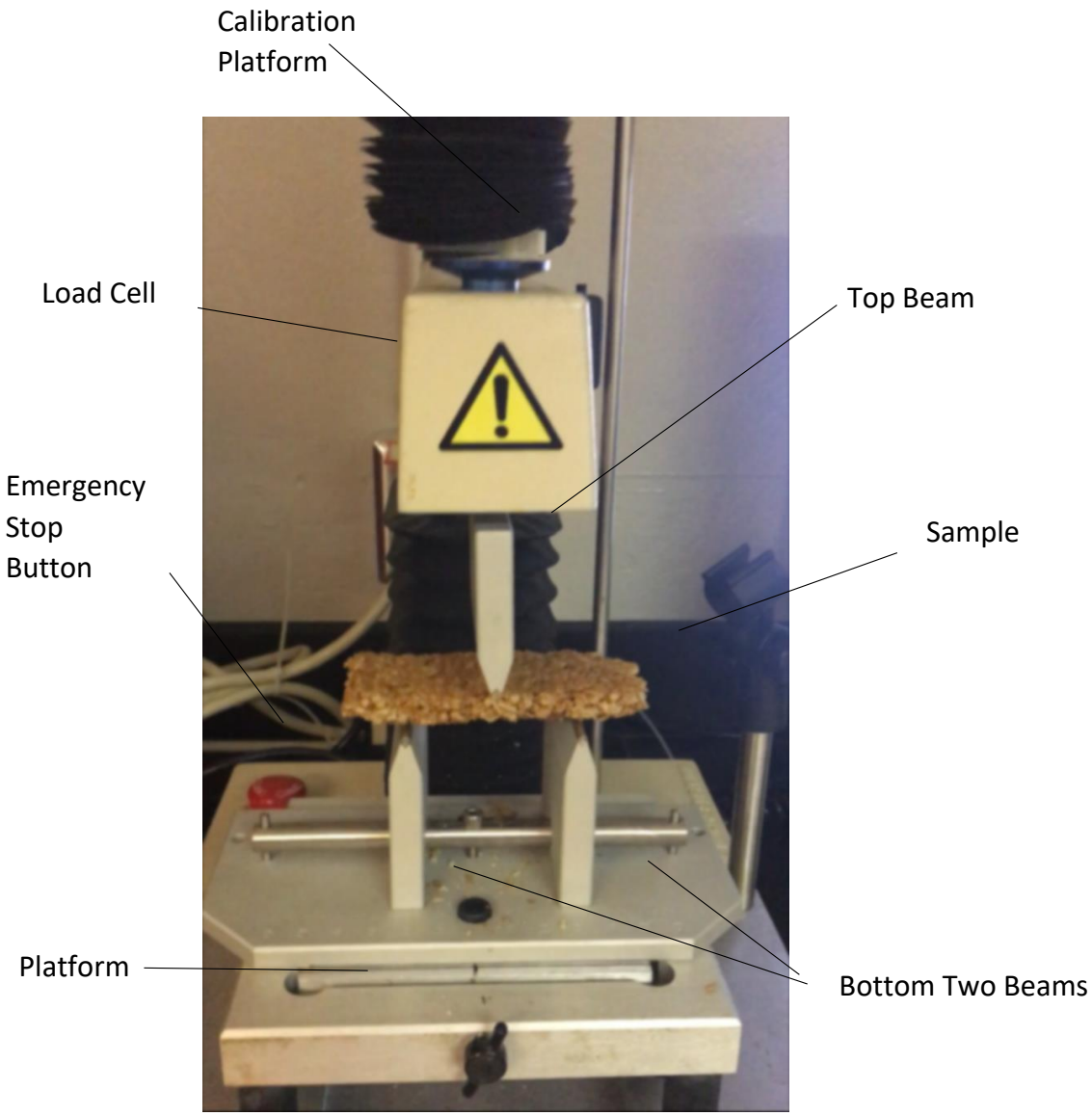

Figure 2.4 Texture analyzer set up with a three-point bend test.

CHAPTER 3

ENZYMATIC INTERESTERIFICATION OF COCONUT AND HIGH OLEIC SUNFLOWER OILS FOR EDIBLE FILM APPLICATION[1]

[1]Moore MA and Akoh CC. (2017). Journal of the American Oil Chemists' Society 94:567-576. Reprinted here with the permission from Springer.

Abstract

Blends (60:40, 70:30 and 80:20 (w/w)) of coconut (CO) and high oleic sunflower oils (HOSO) were interesterified using immobilized enzyme, Lipozyme® TL IM (Novozymes North America Inc., Franklinton, NC, USA). The structured lipids (SLs) referred to as interesterified products (IPs) IP60:40, IP70:30, and IP80:20, were compared to CO and HOSO to determine the best SL for application in an edible film. IPs were compared based on fatty acid profile, TAG molecular species, melting profile, moisture vapor permeability, mechanical properties, film transparency, density, and thickness. Interesterification increased oleic acid content at the *sn*-2 position of IPs. CO had 5.50 ± 1.67 mol% oleic acid at the *sn*-2 position, and when interesterified with HOSO (92.81 ± 1.10 mol% oleic acid) the amount of oleic acid significantly increased ($p<0.05$) at the *sn*-2 position for IP60:40, IP70:30, and IP80:20 (33.86 ± 1.55, 27.34 ± 1.20, 20.61 ± 1.50 mol%), respectively. There was no significant difference between SLs, HOSO, and CO for water vapor permeability and density when applied to emulsion edible films. The HOSO film was significantly different (1.43 ± 0.27 $AUmm^{-1}$) from the rest of the SLs and CO for film transparency. IP60:40 (2.20 ± 0.22 $AUmm^{-1}$) decreased the opacity and was significantly different from HOSO and IP80:20 (2.88 ± 0.08 $AUmm^{-1}$). Tensile strength of IP60:40 was 0.39 ± 0.17 MPa which was significantly different from IP70:30, IP80:20, and HOSO. The elongation at break was significantly different for HOSO and IP60:40. IP60:40 could be used to further investigate the use of SL in edible film for sports nutrition products.

Keywords: Enzymatic interesterification, composite edible film, coconut oil, high oleic sunflower oil

Introduction

The use of edible films and coatings in food products has seen an increase in the industry. There are multiple types of edible coatings such as mono-, bi-layer, and composite (emulsion) films. Composite or emulsion films can contain a carbohydrate or protein component and a lipid component. The lipids incorporated into these films can range from animal and plant waxes, to vegetable oils, and to fatty acids (Galus and Kadrinska 2015). Lipid incorporation into films improves water vapor barrier properties which lacks in both carbohydrate and protein films. Unsaturated fatty acids contribute a lower melting temperature and improved moisture barrier to the composite film. Fernandez and others (2007) reported that unsaturated fatty acids were attributed to a reduced surface tension compared to saturated fatty acids. In addition, their study discovered that unsaturated fatty acids were more mobile than saturated fatty acids although they did not affect the flexibility of the whey protein isolate films but reduced slightly the tensile strength (Fernandez and others 2007).

Polysaccharides are advantageous in edible film development because they provide structural stability and ability to slow down oxygen transmission. Specifically, maltodextrins have been used in film formulation at water soluble concentrations up to 70% (w/v). Maltodextrins can slightly reduce the brittleness due to relatively low molecular weight and slightly hygroscopic properties. Typically, maltodextrins are added at 10-20% (w/v) and help to improve adhesion of the film (Embuscado and Huber 2009).

Structured lipids (SLs) are lipids that have been chemically or enzymatically modified from their natural biosynthetic form (Akoh and Kim 2008). SLs which contain medium-chain fatty acids (MCFAs) may provide a faster hydrolysis and absorption due to the lower number of carbon atoms as well as a greater water solubility in comparison to long chain fatty acids (LCFAs)

(Jensen and Jensen 1992). LCFAs are more easily absorbed at the *sn*-2 position and typically MCFAs are often targeted at the *sn*-1,3 positions of a triacylglycerol (Jandack and others 1987). The objective of this work was to interesterify coconut oil with high oleic sunflower oil using Lipozyme® TL IM lipase derived from *Thermomyces lanuginosus* for potential use in an edible film. Coconut oil was used as the main substrate because it contains a high content of MCFAs whereas high oleic sunflower oil was selected because it contains a large percentage of oleic acid at the *sn*-2 position. The medium-long-medium chain (MLM) structure was selected for sports nutritional purposes. There has been little previous work utilizing SLs for the development of edible films (Sellappan and Akoh 2000). In order to determine the best SL formulation, three ratios of coconut:high oleic sunflower oils were tested. The test methods to differentiate the three blends include fatty acid profile, melting profiles, mechanical properties, water vapor permeability, light transmission, film translucency and film thickness.

Materials and Methods

Materials

Frymax sun supreme deep frying oil was donated by Stratas Foods (Memphis, TN). RBD 76 °F (24.44 °C) melting coconut oil was donated by ADM (Chicago, IL). Immobilized lipase, Lipozyme® TL IM (*sn*-1,3 specific *Thermomyces lanuginosus* lipase with a specific activity of 442.0 IUN/g as specified by manufacturer) was obtained from Novozymes North America (Franklinton, NC). Supelco 37 component FAME mix and porcine pancreatic lipase were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO), while heptadecanoic acid (C17:0) was purchased from Tokyo Chemical Industry Co. Ltd. (Tokyo, Japan). TAG standard mixes (GLC reference standard 437 and 570) were purchased from Nu-Chek Prep, Inc. (Elysian, MN). Other chemicals and solvents were purchased from Fisher Scientific (Norcross, GA), Sigma-

Aldrich Chemical Co., and J. T. Baker Chemicals (Center Valley, PA). Film formula ingredients such as maltodextrin STAR-DRI® 1 was donated by Tate & Lyle (Decatur, IL), Dixie sugar was purchased from Publix (Athens, GA), carrageenan and locus bean gums were both donated by Ingredient Solutions Inc. (Waldo, ME), and glycerol was purchased from Hoefer Inc. (San Francisco, CA).

Determination of Enzyme Load

The time course of interesterification of one blend 70:30 (w/w) coconut: high oleic sunflower oil was determined by weighing 50 g of sample and placing into an Erlenmeyer flask. One milliliter (in duplicate) of sample was removed to represent the starting oil (substrate) composition and stored in an amber vial in a freezer at -20 ºC until RP-HPLC analysis. Then, lipase (Lipozyme® TL IM) was added at three different concentrations (10, 5, and 2.5 %, w/w) of substrates. The flask was closed with a rubber stopper to prevent water from entering the flask. The reaction took place in a shaking water bath at 60 ºC for 8 h with shaking at 200 rpm. One mL (in duplicate) of oil was sampled every 30 min, and the samples were immediately filtered through anhydrous sodium sulfate column to remove any trace of water and the biocatalyst. The samples were then placed in amber capped vials and stored in a freezer at -20 ºC until RP-HPLC analysis to monitor the decrease in triolein as a means of determining the reaction time.

Gram-Scale Interesterification

The time course of interesterification of coconut and high oleic sunflower oils of three different blends (60:40, 70:30, and 80:20 (w/w)) were determined by weighing 50 g of sample and placing into an Erlenmeyer flask. Duplicate sampling was done as described above. The interesterified products (IP 60:40, 70:30, 80:20) were then placed in amber capped vials and stored in a freezer

at -20 ºC until RP-HPLC analysis to monitor the decrease in triolein and to determine the reaction time.

Large-Scale Interesterification

Two hundred grams of each blend (60:40, 70:30, 80:20 (w/w)) were weighed out. The interesterified products of the blends are designated as (IP 60:40, IP 70:30, and IP 80:20, respectively). All of the interesterification reactions were solvent-free and they occurred in a 1-L (10 cm inner diameter and 18 cm long) stirred-batch reactor under vacuum with a circulating water bath at 60 ºC and mixing at 200 rpm for (3, 2, 2 h, respectively). During the reaction, the reactor was covered with aluminum foil to reduce exposure to light. Each reaction used Lipozyme® TL IM at 10% (w/w) weight of substrates. At the end of the reaction, the enzyme was removed by vacuum filtration with a Buchner funnel. The enzyme was washed with hexane to recover any remaining product, and the hexane solution was pooled with the structured lipid in the filtrate. After filtration, the SLs were placed in amber Nalgene bottles and were flushed with nitrogen before being stored at -80 ºC in a freezer. Approximately 5 g of each SL was kept for analysis of free fatty acid (FFA) percentage before short-path distillation.

Short-Path Distillation

Short-path distillation was used to remove excess FFAs from large-scale synthesis of SLs (i.e., IPs). Short-path distillation was performed using a KDL-4 (UIC Inc., Joliet, IL) unit under the following conditions: holding temperature of 50 ºC, feeding rate of approximately 100 mL/h, heating oil temperature of 185 ºC, coolant temperature of 30-35 ºC, and vacuum of < 100 mTorr or <13.33 Pa. The IP 60:40, IP 70:30, and IP 80:20 were passed through the short-path distillation once. After short-path distillation, the FFA content as lauric acid equivalents was determined according to AOCS Official Method Ac 5-40 (2011). The percent yield was

calculated with Eq. (1) with the initial and final values representing the weight and FFA amount before and after short-path distillation. The SLs were analyzed for their fatty acid profile and positional analysis as described below. Eq. (1)

$$\text{Percent yield (\%)} = \frac{(\text{Final weight (g)})(1\text{-Final FFA\%})}{(\text{Initial weight (g)})(1\text{-Initial FFA\%})} \text{ x } 100$$

Determination of Fatty Acid Profiles

Total fatty acid (FA) percentages were determined by weighing 0.1 g of sample into separate Teflon-lined screw capped test tubes and 1 mL of 20 mg/mL C17:0 in hexane as internal standard. The lipid samples were then converted into fatty acid methyl esters (FAME) following the AOAC Official Method 996.01, Section E (Satchitanadam and others 2001) with minor modifications as previously described (Alvarex and Akoh 2015). An external standard, Supelco 37 component FAME mix, was used for identification of fatty acids (FAs) after GC separation. Samples were analyzed in triplicate and the average and standard deviation were reported.

Fatty Acids Positional Analysis

Positional analysis (*sn*-2 and *sn*-1,3) of high oleic sunflower and coconut oil was conducted along with each of the physical blends (PBs) and interesterified products (IPs) for the ratios (60:40, 70:30, 80:20 (w/w) coconut:high oleic sunflower oils). The analysis was performed according to a modified version of the method described by Ifeduba and Akoh (2013) which used pancreatic lipase. One modification made was extracting with 2 mL of diethyl ether instead of 4 mL. Samples were analyzed in triplicate and the average and standard deviation were reported.

GC Analysis

Total and positional FAs were analyzed as FAME on an Agilent Technology 6890 N gas chromatograph (Agilent Technologies, Santa Clara, CA) equipped with a Supelco SP-2560

capillary GC column (100 m x 0.25 mm, 0.20 µm film, Sigma-Aldrich Co., St. Louis, MO). Injection of 1 µL of the sample was made at split ratio of 50:1 for total FAs analysis and 5:1 for positional FAs analysis. Helium was the carrier gas at a flow rate of 1.1 mL/min. The detector temperature was set at 250 ºC. The oven was held at 140 ºC for 5 min, then increased to 240 ºC at 4 ºC/min and held isothermally for 17 min. The relative FA content was calculated as mol %. Samples were analyzed in triplicate. The average and standard deviations were reported. The *sn*-1,3 position was calculated using the following formula Eq. (2)

$$\frac{(3 \text{ x } (\text{Average of total fatty acid composition}) - (\text{Average of } sn\text{-2 position}))}{2}$$

TAG Molecular Species

Analysis of TAG molecular species of CO and HOSO, in addition to IPs and PBs of ratios (60:40, 70:30, and 80:20 (w/w)) were performed by reversed-phase HPLC on a Agilent 1100 HPLC system (Agilent Technologies Inc., Santa Clara, CA) equipped with a Sedex Model 55 evaporative light scattering detector (ELSD) (Richard Scientific Novato, CA) and a 4 mm x 250 mm, 5 µm particle size, Ultrasphere C18 analytical column (Beckman Coulter Inc., Pasadena, CA). Samples were diluted in chloroform to a concentration of ~5 mg/mL for the IPs, PBs and CO. The HOSO was diluted in chloroform to a concentration of ~1 mg/mL. Sample injection volume was 20 µL. The column temperature was set at 30 ºC. The mobile phase was acetonitrile (A) and acetone (B). The flow rate was set at 1 mL/min. Gradient elution began with 65% B at 0 min to 95% B at 55 min, and was followed by 5 min post-run at 65% B. The drift tube temperature for ELSD was set at 86 ºC, and the nebulizer gas pressure was 3.0 bar. The retention times of TAG species depended on polarity and equivalent carbon number (ECN). ECN is defined as C_N-2n, where C_N is the number of carbon atoms in the TAG excluding the three carbon atoms of glycerol, and n is the number of double bonds. For peak identification, the

retention times of sample TAG species were compared with TAG standards of known ECN, namely, tricaprylin (24), tricaprin (30), trilaurin (36), trimyristin (42), tirpalmitin (48), triolein (48), tristearin (54), and triarachidin (60). Samples were analyzed in triplicate and average values reported.

Melting Profiles

The melting profiles of each of the IPs, CO and HOSO were determined using a 204 F1 Phoenix DSC (NETZSCH Instruments North America, Burlington, MA) following AOCS Official Method Cj 1-94 (2011). Nitrogen was used as the protective gas (purge).

Emulsion Film Formulation

The emulsion film was dispersed by gelatinizing 10% (w/v) maltodextrin-1 DE in water at 70 ºC at 600 rpm for 30 minutes. The oil (HOSO, IP 60:40, IP 70:30, IP 80:20 or CO) at 15%, carrageen (5%) and locus bean gum (3.5%) were homogenized using a Polytron® homogenizer PT 10/35 fitted with a PTA7 generator (Brinkmann Instruments Inc., Westbury, NY) at max speed 8 m/s and then heated at 70 ºC for 5 minutes. The sugar (10%) and glycerol (15%) were then added to the oil mixture and mixed at 600 rpm using a VWR (Henry Troemner LLC, Thorofare, NJ). Finally, the (60%) maltodextrin solution was added and mixed at 600 rpm and then homogenized using the Polytron® at max speed 8 m/s for 5 minutes. The emulsion was then diluted to 50:50 in distilled water. The films were cast onto glass plates (7.5 cm x 11.5 cm) and left to dry at 25 ºC for 24 h.

Mechanical Properties of Edible Films

Mechanical properties such as tensile strength and elongation break were measured using a Ta-XT2i Texture Analyzer (Texture Technologies Corp., Scarsdale, NY). The settings of the texture analyzer were determined based on a method provided by Tang and others (2005).

Film Thickness and Density

The film thickness (FT) was measured using a handheld caliper/micrometer Bel-Art-SP Scienceware (Wayne, NJ), to the nearest 0.001 mm. The mean thickness was calculated from measurements taken randomly at ten points at various locations on each film sample. Density was calculated directly from the film weight and dimensions according to (Tang and others 2005) Eq. (3)

$\rho = m/ (AxFT)$

where ρ is density which is equal to the m which is the mass (g), A is the film area (1 cm^2) and FT is the film thickness (cm). The film density was expressed as the average of three independent determinations.

Water Vapor Permeability

The water vapor permeability (WVP) was gravimetrically measured according to protocol B of ASTM 96-95 (2016) with adaptations proposed specifically for edible films (Tang and others 2015). Circular aluminum cups, with a diameter of 5 cm and a depth of 1.7 cm, were used. Distilled water (15 mL) was placed in each test cup, to expose the lower film face to a high relative humidity (RH). The film surface exposed was 3 cm in diameter. The film samples were mounted and the upper film face was exposed to a RH (50 ± 1%) at a temperature of 27.5 ºC. The weight loss of the total cup was monitored over a 72 h period, with weights recorded at 24 h intervals. The WVP (g mm m^{-2} d^{-1} kPa^{-1}) of the film was calculated as follows Eq. (4)

$WVP = (\Delta W \times FT)/(S \times \Delta p)$

Where ΔW is the weight loss of the cup per day (g d^{-1}) (i.e. slope of the linear behavior), FT (mm), S is the area of the exposed film (m^2) and Δp is the vapor pressure differential across the test film (kPa). Samples were analyzed in triplicate and the standard deviation was calculated.

Light Transmission and Film Transparency

A modified method from (Ramos and others 2012) was conducted to determine the ultraviolet (UV) and visible light barrier properties of the dried films at selected wavelengths (in the 300-800 nm range), using a UV-1601 visible Spectrophotometer (Shimadzu Corp., Columbia, MD). The films were cut into rectangles of 35 mm x 8 mm (length, width) and placed into cuvettes. Film's transmittance was determined at wavelengths of 300 to 800 nm in order to determine the barrier effect against UV and visible light. The transparency was measured at 600 nm and was calculated Eq. (5)

$$\text{Transparency} = A_{600} / FT$$

Where A_{600} is the absorbance at 600 nm and FT is the film thickness (mm). Three sample strips of each of the five total variables (HOSO, IP 60:40, IP 70:30, IP 80:20, and CO) were averaged and the standard deviation reported.

Statistical Analysis

Analyses were completed in triplicate or duplicate. All statistical analyses were conducted with the SAS software package (SAS Institute Inc., Cary, NC). Duncan's multiple range test was performed to determine the significance of difference at $p \leq 0.05$**.**

Results and Discussion

Total and Positional Fatty Acid Profiles and Determination of Reaction Parameters

Coconut and high oleic sunflower oils were characterized because they were the starting substrates in this study. The fatty acid profiles are shown in **Table 3.1**. The total and positional analyses of the fatty acid composition of high oleic sunflower oil were in agreement with previous studies (Ifeduba and others 2016). The fatty acid profile of coconut oil was in compliance with the manufacture's certificate of analysis. The major fatty acids in coconut oil

were caprylic (8.18 ± 0.04 mol%), capric (6.19 ± 0.02 mol%), lauric (46.91 ± 0.27 mol%), myristic (17.66 ± 0.05 mol%), palmitic (8.89 ± 0.05 mol%), and oleic (6.91 ± 0.29 mol%) acids. The *sn*-2 position of coconut oil had high amounts of lauric (80.53 ± 4.92 mol%), myrisitc (6.36 ± 1.90 mol%), and oleic (5.50 ± 1.67 mol%) acids. The amount of oleic acid at the *sn*-2 position of coconut oil was low, therefore high oleic sunflower oil was added in the interesterification reaction to increase the amount of oleic acid at the *sn*-2 position. The major fatty acid in high oleic sunflower oil was oleic acid (86.38 ± 0.52 mol%). The major fatty acid at the *sn*-2 position is oleic acid (92.81 ± 1.10 mol%). The *sn*-1,3 positions of coconut oil had high amounts of caprylic (11.25 ± 0.32 mol%), capric (8.05 ± 0.57 mol%), lauric (30.11 ± 2.58 mol%), myristic (23.31 ± 0.99 mol%), palmitic (12.47 ± 0.37 mol%) acids. The *sn*-1,3 positions of high oleic sunflower oil had high amount of oleic acid (83.15 ± 1.22 mol%).

A preliminary determination of the percentage of enzyme to be used in the interesterification reaction was carried out using a time course. The time course interesterification for triolein disappearance when CO was reacted with HOSO was performed to determine the reaction equilibrium. **Figure 3.1** describes the reaction equilibrium which was 7 h for 2.5%, 4.5 h for 5% and 2 h for 10% enzyme. We chose 10% enzyme for the small-scale reaction due to the shortest amount of time and cost. In addition, a time course consumption of triolein was constructed to compare the three different ratios 80:20, 70:30, and 60:40 using 10% (w/w) enzyme. **Figure 3.2** describes the reaction equilibrium which was 2 h for 80:20 and 70:30 and 3 h for the 60:40. The three ratios were then tested on a larger scale to determine which would be the best blend for an edible coating based on chemical and physical characteristics. Such include the total and positional fatty acid profiles, mechanical properties, density, thickness and light properties.

Comparisons of the total, *sn*-2 and *sn*-1,3 composition of the IPs and the PBs were conducted and data is shown in **Table 3.2**. The results for the *sn*-2 oleic acid between physical and interesterified blends differed significantly for each of the three blends. The PB 80:20 was (13.90 ± 2.71 mol%) whilst the IP 80:20 was (20.61 ± 1.50 mol%). The PB 70:30 was (18.52 ± 0.50 mol%) compared to the IP 70:30 (27.34 ± 1.20 mol%). Finally, the PB 60:40 was (24.29 ± 3.94 mol%) and IP 60:40 (33.86 ± 1.55 mol%). Therefore, interesterification resulted in increased oleic acid amount at the *sn*-2 position of the interesterified products. The results for the *sn*-1,3 lauric acid between phyiscal and interesterified blends also differed significantly for each of the three blends. The PB 80:20 was (22.79 ± 1.08 mol%) and the IP was (29.83 ± 0.76 mol%). The PB 70:30 was (16.52 ± 0.65 mol%) and the IP was (24.78 ± 1.31 mol%). The PB 60:40 was (9.75 ± 1.19 mol%) and the IP was (21.66 ± 0.71 mol%). The product reaction yields for the 80:20, 70:30 and 60:40 were 88.50, 93.73, and 94.57%, respectively.

TAG Molecular Species and Melting Profile

The relative TAG molecular species in CO, HOSO, PBs and IPs of 80:20, 70:30, and 60:40 were determined in order to follow the change in TAG species due to enzymatic reactions (**Table 3.3**). The TAGs shown are not representative of stereochemical configuration. The peak determinations were based on elution time of TAG standards, equivalent carbon number (ECN), and published works on coconut and high oleic sunflower oils (Ifeduba and others 2016, Dayrit 2015). The major TAG species found in HOSO was OOO at (96.34 ± 1.80 %). In CO, the major TAG species were LaMM (10.56 ± 1.42 %), LaLaM (18.49 ± 0.88 %), LaLaLa (26.19 ± 3.13 %), LaCLa (21.56 ± 2.10 %), and CLaC (15.44 ± 0.46 %). In the PB 60:40, the predominant TAG species were CLaC (9.48 ± 0.61 %), LaCLa (15.02 ± 0.29 %), LaLaLa (18.36 ± 0.51), LaLaM (10.63 ± 1.61 %), and OOO (40.62 ± 2.65 %). Whereas for the IP 60:40, the

predominant TAG species were LaOC (14.67 ± 1.26 %), LaLaM (12.02 ± 1.60 %), LaOLa (27.01 ±1.48 %), LaMP (12.14 ± 0.44 %), and LaOO (18.26 ± 0.30 %). The predominant TAG species for PB 70:30 were CLaC (11.42 ± 0.09 %), LaCLa (17.90 ± 0.54 %), LaLaLa (21.40 ± 0.38 %), LaLaM (13.34 ± 0.08 %), and OOO (27.84 ± 0.83 %). Whereas the predominant TAG species for the IP 70:30 were LaOC (17.32 ± 0.10 %), LaLaM (14.92 ± 0.32 %), LaOLa (26.80 ± 0.32 %), LaMP (13.12 ± 0.08 %), and LaOM (12.64 ± 0.36 %). In the PB 80:20, CLaC (13.80 ± 1.33 %), LaCLa (20.27 ± 0.98 %), LaLaLa (24.77 ± 0.26 %), LaLaM (15.06 ± 0.54 %), and OOO (16.80 ±2.60 %) were the dominant TAG species whereas for the IP 80:20 LaCLa (8.07 ± 0.64 %), CLaM (23.55 ± 2.44 %), LaLaM (18.97 ± 0.27 %), and LaOLa (26.66 ± 1.47 %) were the predominant species.

The melting profile of (HOSO, IP 60:40, IP 70:30, IP 80:20, and CO) was determined using DSC. The melting onset temperature for HOSO was -8.4 °C, IP 60:40 11.5 °C, IP 70:30 16.6 °C, and IP 80:20 18.5 °C, and CO was 24.6 °C. The melting profile was as expected with the IP products having melting temperatures between the two starting oils. Clearly, enzymatic interesterification of CO and HOSO resulted in new TAG molecular species and melting behavior which may affect the physical properties of the products and their application in edible film.

Film Thickness and Density

Film thickness and density for each of the interesterified products and initial oils can be found in **Table 3.4**. The thickness for the films varied from 0.43 ± 0.03 mm to 0.47 ± 0.04 mm. The thickness of the film was greater than that of previously reported films. In Binisi *et al.* (2013), the emulsion film comprised of chitosan and virgin coconut oil had a thickness of 0.075 mm thickness which is thinner than the film thickness we achieved. The film thickness could be

reduced by adding less film initially onto each plate which would help improve all parameters as the film thickness affects multiple parameters. The density has no significant difference ($p \leq 0.05$) among lipid types. The density ranged slightly higher from 1.53 to 1.75 (g cm^{-3}) than that found by Ramos and others (2012) which had density values ranging from (1.16 to 1.29 g cm^{-3}).

Film Light Transmission and Opacity

The results of opacity experiments with the three structured lipids (IP 60:40, IP 70:30, and IP 80:20) and starting substrates are presented in **Table 3.4**. The HOSO film was significantly less opaque than the other films (1.43 ± 0.27 AU/mm). However, the IP 80:20 (2.88 ± 0.08 AU/mm) film was significantly different from the HOSO film and the IP 60:40 film but not IP 70:30 and CO. The structured lipid with the least opacity was IP 60:40 (2.20 ± 0.22 Au/mm) which was significantly different $p \leq 0.05$ from IP 80:20 (2.88 ± 0.08 AU/mm) and HOSO (1.43 ± 0.27 AU/mm). The concentration of oil in the emulsion film was greater than that in previous research conducted by Bisni and others (2013) who reported 0.58 and 2.83 opacity index. The opacity differences may be due to various amounts of saturated fatty acids present in CO, IP 60:40, IP 70:30, and IP 80:20 compared to HOSO.

Film light transmission can be found in **Figure 3.3**. Light transmission indicates how much light can penetrate the film barrier. The HOSO film had the highest light transmission and the coconut oil had the lowest light transmission. The UV/Light barrier would be advantageous in protecting the product and has been reported as beneficial in preventing color loss and lipid oxidation (Gounga and others 2007). Therefore, the structured lipids containing saturated fatty acids compared to HOSO would be expected to provide barrier to light transmission and reduce lipid oxidation and color loss when used in edible film.

Water Vapor Permeability

The water vapor permeability (WVP) of the films with the starting substrates and the structured lipids were determined. The WVP was not significantly different from one film to the other (**Table 3.5**). The WVP for a film with HOSO was 3.76 ± 0.23 (g mm d^{-1} m^{-2} kPa^{-1}). While the range for all structrured lipids was 3.48 to 3.73 g mm d^{-1} m^{-2} kPa^{-1}. Monedero and others (2009) found similar results of no significant difference ($p \leq 0.05$) between oleic acid and beeswax addition to an edible film. Whereas, Binisi *et al*. [18] found WVP to be between 0.41 ± 0.02 for the control to 0.08 ± 0.004 g mm/ m^2 d kPa after adding virgin coconut oil at 1.5 mL/g chitosan. The WVP recorded by Ramos and others (2013) was higher, ranging from 10.1 ± 0.20 to 13.4 ± 0.41 g mm/ m^2 d kPa than that found by other researchers that included a lipid component. The interaction between the maltodextrin and lipid component impacts the WVP in addition to the thickness of the film.

Mechanical Film Characteristics

Tensile strength (TS) and elongation at break (**Table 3.5**) were determined for all structured lipids and the starting substrates. TS (MPa) for IP 60:40 was significantly higher at $p \leq 0.05$ (0.39 ± 0.17 MPa) than HOSO, IP 70:30, and IP 80:20, but was not significantly different from CO. According to Binsi and others (2013), the film containing 1.5 mL/g chitosan of virgin coconut oil had a TS of 57.1 ± 1.53 MPa whereas the control had a TS of 81.7 ± 1.10 MPa. The TS is affected by the amount of plasticizers present in the film as well as the type of oil or fatty acids present.

The elongation at break (EB) for HOSO was significantly different ($p \leq 0.05$) with a value of 29.60 ± 2.67% from IP 60:40, IP 70:30, and CO. IP 60:40 was significantly different at a value of 19.46 ± 1.22 % from IP 80:20 and HOSO. EB in films with virgin coconut oil and

chitosan had EB values between 10.21 and 39.72% which increased as more oil was added (Binsi 2013). Conversely, the EB of each of the emulsion films tested was within the ranges described by other researchers. Ramos and others (2012) used whey protein isolate and glycerol in an edible film that resulted in a EB between 10 and 20%. Monedero and others (2009) incorporated an oleic acid:beeswax mixture into soy protein isolate films. Oleic acid had a greater plasticizing effect than beeswax, therefore increasing the EB. Furthermore, studies showed the addition of a lipid component to the film helped to increase the EB value. As the percentage of oleic acid increased, the films EB increased.

Conclusion

Structured lipids were designed for use in an edible film application and their properties compared. The enzyme was successful in increasing oleic acid at the *sn*-2 position of the TAGs. The structured lipids were helpful in reducing the opacity of CO in a film use and this is advantageous. The IP 60:40 provided the strongest TS although it had the weakest EB. The IP 60:40 contained the most oleic acid (33.86 ± 1.55%) at the *sn*-2 position and also resulted in a fairly translucent product. The IP 60:40 will be used to continue further research on the use of SLs in edible films to prepare sports nutrition products in our laboratory.

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Table 3.1 Total and positional fatty acid composition (mol %) of coconut and high oleic sunflower oils

Fatty acid	Coconut oil[a]			High oleic sunflower oil[b]		
	Total	*sn*-2	*sn*-1,3	Total	*sn*-2	*sn*-1,3
C 8:0	8.18 ± 0.04	1.98 ± 0.30	11.25 ± 0.32	ND	ND	ND
C 10:0	6.19 ± 0.02	2.48 ± 1.05	8.05 ± 0.57	ND	ND	ND
C 12:0	46.91 ± 0.27	80.53 ± 4.92	30.11 ± 2.58	ND	ND	ND
C 14: 0	17.66 ± 0.05	6.36 ± 1.90	23.31 ± 0.99	ND	ND	ND
C 16:0	8.89 ± 0.05	1.77 ± 0.31	12.47 ± 0.37	3.57 ± 0.03	1.47 ± 0.36	4.62 ± 0.32
C 18:0	2.58 ± 0.15	ND	3.88 ± 0.58	2.56 ± 0.23	0.39 ± 0.10	3.64 ± 0.72
C18:1n9	6.91 ± 0.29	5.50 ± 1.67	7.62 ± 1.16	86.38 ± 0.52	92.81 ± 1.10	83.15 ± 1.22
C 18:2n6	2.08 ± 0.32	1.37 ± 0.29	2.43 ± 0.86	4.59 ± 0.12	4.64 ± 0.08	4.56 ± 0.53
C 22:1	ND[c]	ND	ND	1.27 ± 0.06	ND	1.90 ± 0.36

Values are mean ± SD (n = 3)

[a]Trace amounts of C6:0, C18:3n6, and C18:3n3

[b]Trace amounts C14:0, C16:1n7, C18:3n6, C20:1, C18:3n3, C24:1

[c]ND Not Detected

Table 3.2 Total and positional fatty acid composition (mol %) of three physical blends and interesterfied products

Fatty acid	Total			*sn*-2			*sn*-1,3		
	60:40*	70:30*	80:20*	60:40	70:30	80:20	60:40	70:30	80:20
C8:0 PB	4.46 ± 0.08^{g}	5.46 ± 0.04^{e}	6.12 ± 0.05^{i}	0.73 ± 0.17^{kl}	1.02 ± 0.20^{kl}	ND	6.59 ± 0.44	7.65 ± 0.29	9.18 ± 0.32
C8:0 IP	4.53 ± 0.03^{g}	5.32 ± 0.20^{e}	5.33 ± 0.13^{j}	2.53 ± 0.35^{hijk}	2.57 ± 0.16^{ij}	2.43 ± 0.47^{gh}	5.57 ± 0.31	6.66 ± 0.67	6.71 ± 0.60
C10:0 PB	3.51 ± 0.04^{h}	4.23 ± 0.03^{f}	4.71 ± 0.03^{k}	2.76 ± 0.46^{ghij}	3.09 ± 0.35^{hi}	2.95 ± 0.28^{gh}	3.88 ± 0.34	4.80 ± 0.28	5.58 ± 0.29
C10:0 IP	3.56 ± 0.02^{h}	4.19 ± 0.11^{f}	4.51 ± 0.05^{k}	3.54 ± 0.95^{gh}	2.64 ± 0.92^{ij}	3.50 ± 0.08^{gh}	3.58 ± 0.51	4.97 ± 0.68	5.02 ± 0.32
C12:0 PB	27.00 ± 0.19^{d}	32.52 ± 0.13^{a}	35.91 ± 0.23^{b}	61.50 ± 2.82^{a}	64.53 ± 0.99^{a}	62.13 ± 1.60^{a}	9.75 ± 1.19	16.52 ± 0.65	22.79 ± 1.08
C12:0 IP	27.99 ± 0.11^{c}	32.60 ± 0.44^{a}	36.77 ± 0.11^{a}	40.67 ± 1.01^{b}	48.26 ± 1.70^{b}	50.66 ± 0.91^{b}	21.66 ± 0.71	24.78 ± 1.31	29.83 ± 0.76
C14:0 PB	10.26 ± 0.06^{e}	12.37 ± 0.02^{c}	13.58 ± 0.11^{f}	6.52 ± 0.26^{f}	7.35 ± 0.35^{f}	11.55 ± 4.83^{de}	12.14 ± 0.39	14.88 ± 0.25	14.61 ± 2.46
C14:0 IP	10.53 ± 0.03^{e}	12.25 ± 0.01^{c}	14.29 ± 0.04^{e}	8.87 ± 0.14^{e}	9.70 ± 0.10^{e}	10.80 ± 0.19^{e}	11.37 ± 0.27	13.52 ± 0.15	16.04 ± 0.31
C16:0 PB	6.72 ± 0.01^{f}	7.31 ± 0.01^{d}	7.65 ± 0.05^{h}	1.12 ± 0.11^{jkl}	1.76 ± 0.11^{jk}	3.90 ± 0.73^{fg}	9.51 ± 0.15	10.12 ± 0.13	9.55 ± 0.48
C16:0 IP	6.66 ± 0.11^{f}	7.12 ± 0.01^{d}	7.96 ± 0.01^{g}	4.23 ± 0.09^{gh}	4.24 ± 0.27^{gh}	5.02 ± 0.18^{fg}	7.85 ± 0.51	8.60 ± 0.51	9.46 ± 0.17
C18:0 PB	2.72 ± 0.12^{j}	2.68 ± 0.10^{g}	2.82 ± 0.31^{l}	ND	ND	ND	4.07 ± 0.52	4.02 ± 0.46	4.23 ± 0.84
C18:0 IP	3.13 ± 0.15^{i}	2.98 ± 0.09^{gh}	3.06 ± 0.25^{l}	1.47 ± 0.44^{ijkl}	0.15 ± 0.25^{l}	ND	3.96 ± 0.62	4.39 ± 0.47	4.59 ± 0.75
C18:1n9 PB	39.65 ± 0.58^{a}	30.73 ± 0.13^{b}	24.82 ± 0.46^{c}	24.29 ± 3.94^{d}	18.52 ± 0.50^{d}	13.90 ± 2.71^{d}	47.38 ± 1.80	36.84 ± 0.57	30.28 ± 2.69
C18:1n9 IP	38.51 ± 0.13^{b}	30.98 ± 0.53^{b}	23.96 ± 0.53^{d}	33.86 ± 1.55^{c}	27.34 ± 1.20^{c}	20.61 ± 1.50^{c}	40.83 ± 0.93	32.80 ± 1.25	25.66 ± 0.90
C18:2n6 PB	3.60 ± 0.05^{h}	3.29 ± 0.06^{g}	2.98 ± 0.10^{l}	3.09 ± 0.12^{ghi}	3.73 ± 0.80^{hi}	5.57 ± 3.51^{fg}	3.85 ± 0.33	3.07 ± 0.47	1.68 ± 1.82
C18:2n6 IP	3.31 ± 0.07^{hi}	3.14 ± 0.02^{g}	2.97 ± 0.04^{l}	4.58 ± 0.07^{h}	5.11 ± 0.67^{g}	6.99 ± 0.68^{fg}	2.67 ± 0.39	2.16 ± 0.40	0.96 ± 0.45

ND = Not Detected

*Trace amounts found of C6:0, C18:3n6, C20:1n9, C18;3n3, C22:1, and C24:0

Values are mean ± SD (n = 3)

Different letters between, physical blend (PB) and interesterified product (IP) of the same blend indicate significant difference

Table 3.3 Relative percentage (%) of peak areas of triacylglycerol (TAG) molecular species based on equivalent carbon number (ECN) of high oleic sunflower oil (HOSO), coconut oil (CO), physical blend (PB) and interesterified products (IP)

TAG Species[a]	ECN (DB)[b]	CO	PB 60:40	IP 60:40	PB 70:30	IP 70:30	PB 80:20	IP 80:20	HOSO
CyCyLa	28	0.24 ± 0.16	ND	ND	ND	ND	ND	ND	ND
CCyC	28	ND	ND	0.06 ± 0.02	ND	ND	ND	ND	ND
LaCCy	30	2.09 ± 1.09	0.74 ±0.05	ND	0.81 ± 0.07	ND	0.98 ± 0.13	1.10 ± 0.19	ND
CyOCy	32 (1)	ND	ND	0.24 ± 0.05	ND	0.26 ± 0.08	ND	ND	ND
CyLaLa	32	ND	ND	2.08 ± 0.21	ND	1.47 ± 0.10	ND	ND	ND
CLaC	32	15.44 ± 0.46	9.58 ± 0.61	ND	11.42 ± 0.09	ND	13.80 ± 1.33	4.71 ± 0.05	ND
LaCLa	34	21.56 ± 2.10	15.02 ± 0.29	ND	17.90 ± 0.54	ND	20.27 ± 0.98	8.07 ± 0.64	ND
CyOLa	36(1)	ND	ND	3.01 ± 0.38	ND	4.05 ± 0.27	ND	ND	ND
CLaM	36	ND	ND	5.25 ± 0.19	ND	6.17 ± 0.27	ND	23.55 ± 2.44	ND
LaLaLa	36	26.19 ± 3.13	18.36 ± 0.51	ND	21.40 ± 0.38	ND	24.77 ± 0.26	ND	ND
LaOC	38(1)	ND	ND	14.67 ± 1.26	ND	17.32 ± 0.10	ND	ND	ND
LaLaM	38	18.49 ± 0.88	10.63 ± 1.61	12.02 ± 1.60	13.34 ± 0.08	14.91 ± 0.30	15.06 ± 0.54	18.97 ± 0.27	ND
LaOLa	40(1)	ND	ND	27.01 ± 1.48	ND	26.80 ± 0.32	ND	26.66 ± 1.47	ND
LaMM	40	10.56 ± 1.42	3.96 ± 0.24	ND	6.06 ± 0.63	ND	6.82 ± 0.33	ND	ND
LaOM	42(1)	ND	ND	ND	ND	13.12 ± 0.10	ND	6.60 ± 0.61	ND
LaMP	42	3.81 ± 1.78	1.1 ± 0.08	12.14 ± 0.44	1.22 ± 0.29	ND	1.44 ± 0.13	3.29 ± 0.79	ND
LaOP	44 (1)	ND	ND	ND	ND	1.14 ± 0.16	ND	3.35 ± 0.49	ND
LaOO	44 (2)	0.51 ± 0.33	ND	18.26 ± 0.30	ND	12.64 ± 0.36	ND	ND	ND
LaPP	44	0.77 ± 0.55	ND	ND	ND	ND	ND	2.75 ± 0.31	ND
OLO+LOP	46(4), 46(3)	ND	ND	2.78 ± 0.25	ND	0.94 ± 0.07	ND	0.94 ± 0.15	2.04 ± 1.56
LaOS	46(1)	ND	ND	1.78 ± 0.37	ND	0.89 ± 0.01	ND	ND	ND
OOO	48(3)	ND	40.62 ± 2.65	0.70 ± 0.22	27.84 ± 0.83	0.38 ± 0.16	16.80 ± 2.60	ND	96.34 ± 1.80
POS+PPS	50	ND	ND	ND	ND	ND	ND	ND	1.62 ± 0.65

Values are mean ± SD (n = 3)
ND not detected
Cy caprylic acid (C8:0), C capric acid (C10:0), La lauric acid (C12:0), M myristic acid (C14:0), P palmitic acid (C16:0), S stearic acid (C18:0), O oleic acid (C18:1), L linoleic acid (C18:2)

[a]TAG species do not reflect stereochemical configuration
[b]Equivalent carbon number (ECN) = TC - (2x DB); TC is total carbon number of acyl groups and DB is total number of double bonds in parenthesis

Table 3.4 Film thickness, density, and opacity

Sample	Thickness (mm)*	Density (g/cm^3)[#]	Opacity (A600/mm)[#]
HOSO	0.47 ± 0.04^{a}	1.69 ± 0.10^{a}	1.43 ± 0.27^{c}
IP 60:40	0.46 ± 0.02^{a}	1.75 ± 0.16^{a}	2.20 ± 0.22^{b}
IP 70:30	0.47 ± 0.03^{a}	1.64 ± 0.20^{a}	2.64 ± 0.53^{ab}
IP 80:20	0.45 ± 0.01^{a}	1.61 ± 0.13^{a}	2.88 ± 0.08^{a}
CO	0.43 ± 0.03^{a}	1.53 ± 0.14^{a}	2.54 ± 0.10^{ab}

*Mean ± SD (n = 10)

[#]Mean ± SD (n = 3)

HOSO high oleic sunflower oil, interesterified product (IP) 60:40 CO:HOSO SL, IP 70:30 CO:HOSO SL, IP 80:20 CO:HOSO SL, CO coconut oil

Table 3.5 Tensile strength (TS), elongation break (EB), and water vapor permeability (WVP) of test films

	Tensile strength (Mpa)	Elongation break (%)	Water vapor permeability (g mm d^{-1} m^{-2} kPa^{-1})
HOSO	0.26 ± 0.02^{b}	29.60 ± 2.67^{a}	3.76 ± 0.23^{a}
IP 60:40	0.39 ± 0.17^{a}	19.46 ± 1.22^{c}	3.50 ± 0.13^{a}
IP 70:30	0.19 ± 0.02^{b}	23.73 ± 2.47^{bc}	3.48 ± 0.36^{a}
IP 80:20	0.16 ± 0.01^{b}	26.02 ± 0.35^{ab}	3.73 ± 0.17^{a}
CO	0.26 ± 0.09^{ab}	22.83 ± 2.96^{bc}	3.70 ± 0.60^{a}

*Mean ± SD (n = 3)
HOSO high oleic sunflower oil, interesterified product (IP) 60:40 CO:HOSO SL, IP 70:30 CO:HOSO SL, IP 80:20 CO:HOSO SL, CO coconut oil

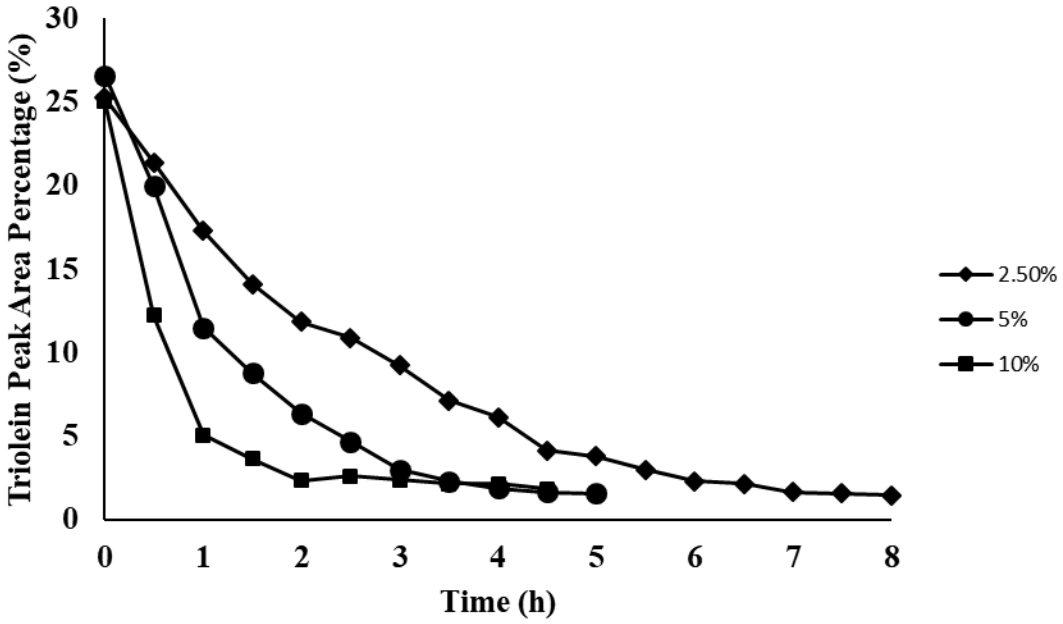

Figure 3.1 Time course showing reduction in triolein (TO) during enzymatic interesterification of coconut and high oleic sunflower oil blend 70:30 (w/w)

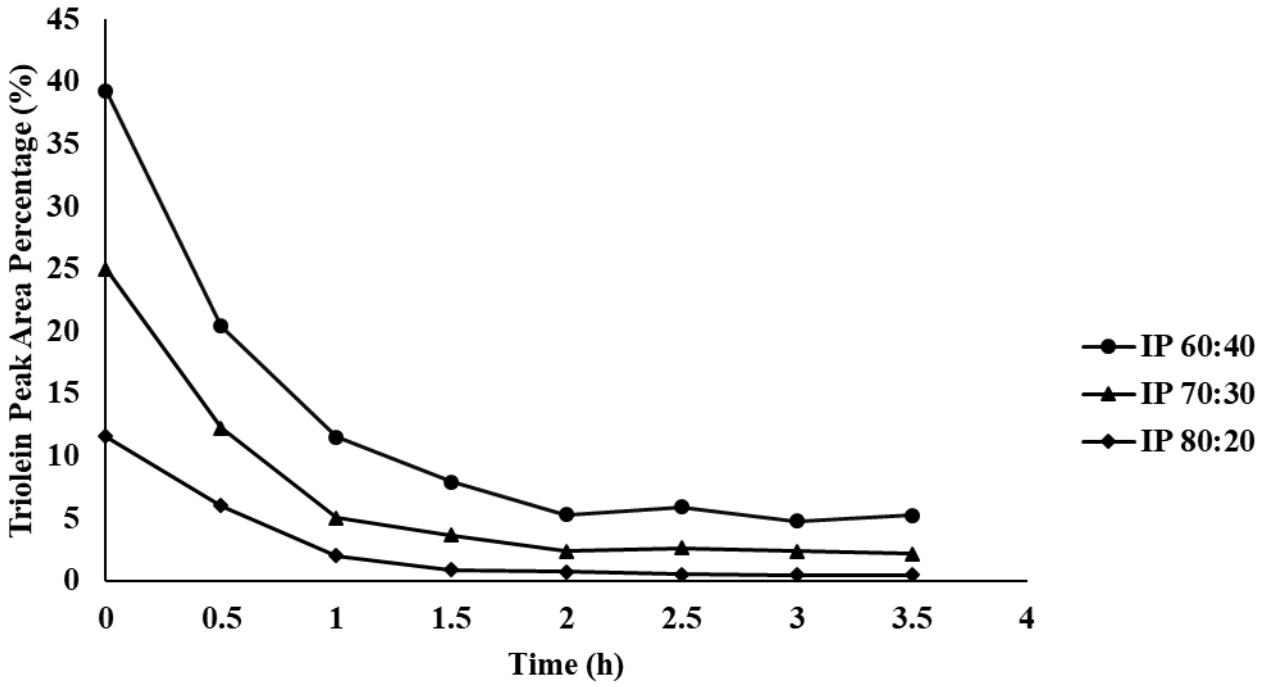

Figure 3.2 Time course showing reduction in triolein (TO) during enzymatic interesterification of coconut and high oleic sunflower oils for three interesterified products (IP)

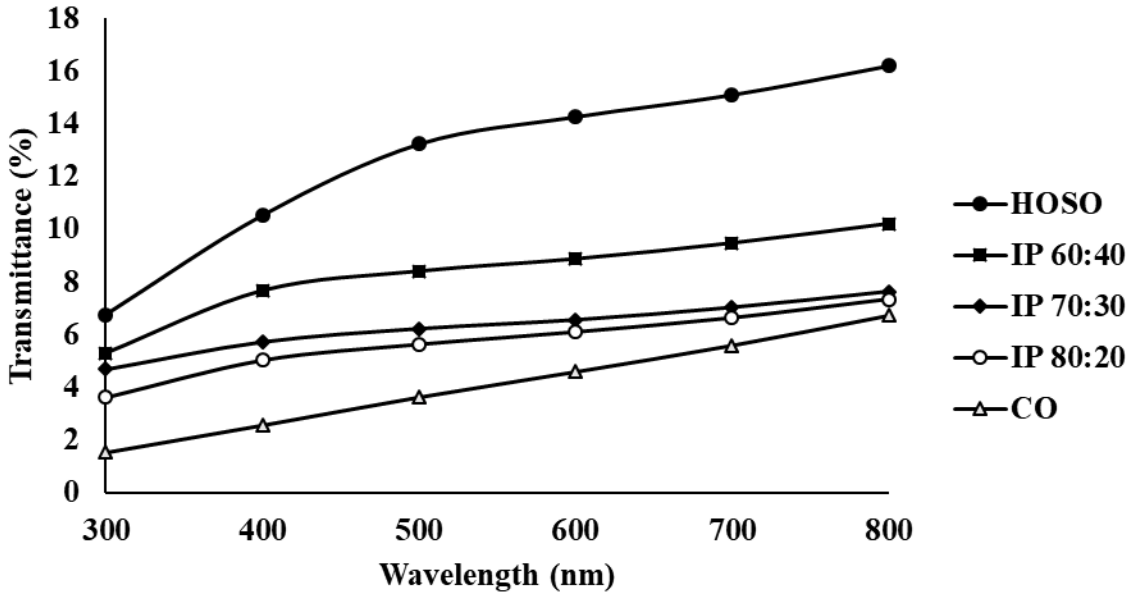

Figure 3.3 Light transmittance changes at different wavelengths ranging from 300-800 nm, high oleic sunflower oil (HOSO), interesterified products (IP), coconut oil (CO)

CHAPTER 4

CHEMICAL, PHYSICAL, AND SENSORY PROPERTIES OF A GRANOLA BAR WITH AN EDIBLE COATING CONTAINING A COCONUT: HIGH OLEIC SUNFLOWER OIL STRUCTURED LIPID [1]

[1] Moore MA, Brambila G, Akoh CC. To be submitted to the Journal of American Oil Chemists Society

Abstract

Coconut and high oleic sunflower oils were enzymatically interesterified to increase the oleic acid content at the *sn*-2 position of coconut oil. The structured lipid (SL) referred to as interesterified product (IP6) was characterized and contained (30.46 ± 1.54 mol% oleic acid) at the *sn*-2 position. Tocopherol concentrations were monitored and fortified with Novatol™ 4-80, (> 50% α-tocopherol), after short-path distillation (SPD). Fortification of the IP6 provided oxidative stability comparable to the physical blend. The final IP6 was formulated into an edible film that was used to encapsulate commercially purchased granola bars. Control (commercially purchased bars) and coated bars were analyzed for lipid oxidation (OSI), water absorption properties, three-point bend test, and paired-comparison test (n = 80). Minimal lipid oxidation, water absorption and bend were observed in the coated bar. The coated bar, at 25 °C and 50% relative humidity (RH), maintained the same hardness over a five-day period compared to the control which lost hardness due to moisture absorption. Consumer tests (n=80) showed that there was a difference between the control and coated bar. A consumer liking and acceptance test (n = 100) was used to determine the overall liking of the coated product. The overall liking of 50% of the population was 8.0 (like very much) on a 9-point hedonic scale and results indicated the addition of an IP6 did not change the purchase intent of consumers. The product has commercial potential in the future, especially after certain minor modifications such as adjusting the appearance.

Keywords: Structured lipid, granola bars, sensory evaluation, tocopherols, oxidation

Introduction

Medium-chain triacylglycerols (MCTs) are triacylglycerols whose fatty acids have an aliphatic tail of 6–12 carbon atoms and provide about 10 percent fewer calories than long-chain triacylglycerols (LCTs). MCTs are more rapidly absorbed by the body thus, more quickly metabolized as energy (Medium Chain Triglycerides). Structured lipids (SLs) are tailor-made fats that have been enzymatically or chemically modified to improve nutritional and physical properties (Akoh and Kim 2017). SL, referred to as interesterified product (IP), applications have been studied in the past, however, few studies have looked at the incorporation of IPs into edible films or evaluated sensorial properties (Osborn and others 2003; Jennings and others 2010).

To date, low-moisture snacks account for a nutritionally significant proportion of saturated fat in the diet, making these foods a key target for improving consumer health while providing new research challenges. These challenges include creating healthier nutritional profiles by incorporating more unsaturated fatty acids, which are generally perceived to be more heart-healthy, and removing synthetic antioxidants and hydrogenated oils from food products (Barden and Decker 2016). Conversely, problems exist maintaining oxidative stability when replacing saturated fats with unsaturated fats.

Application of edible films to low-moisture foods plays an important role in preventing moisture migration and maintaining the integrity and stability of food products while providing some nutritional benefit. Granola bars, a low-moisture food, are an excellent vessel choice. Granola bars have seen a 7% increase in consumer demand in recent years according to an IRI marketing research survey; Nature Valley granola brand was the number one brand of granola bars sold in 2015 (Bakery and Snacks 2016). Trier and Johnston (2012) conducted a research study looking at college student's intake of nutrition bars and concluded that granola bars present

a unique opportunity to influence the college student's nutritional status; when purchasing granola bars, consumers are looking for bars with increased health and nutritional benefits (Mahanna and others 2009). The use of edible films containing IPs in this category of products such as granola or breakfast bars, offer the opportunity to provide a vessel for additional nutritional benefits to consumers as well as to improve physical properties and shelf life.

Investigating consumer's preferences and product acceptance is critical in determining success of a product in the market. There are two main types of consumer testing analytical and affective testing. A paired-comparison test is an analytical testing method used to determine if two samples are the same or different. Another type consumer acceptance testing is an affective testing method which determines consumer liking and overall acceptance. Typical questions can range from a 5-point to 9-point hedonic scale or can even ask questions about just-about-right (JAR) scale, purchase intent, and preference questions. Each question helps to understand the consumers perception of a product and how likely a consumer is willing to purchase a product (Stone and others 2012).

The aim of this study was to interesterify coconut (CO) and high oleic sunflower oils (HOSO) to increase the oleic acid content at the *sn*-2 position of coconut oil. The enzymatic product, referred to as interesterified product 60:40 (IP6), was analyzed for fatty acid profile by GC, triacylglycerol (TAG) molecular species and tocopherol concentrations by HPLC, thermal characteristics by DSC, oxidative stability index (OSI) and total oxidation (TOTOX). The IP6 was fortified with Novatol™ 4-80, after SPD, the OSI was determined and compared to the physical blend of CO and HOSO. The fortified IP6 was then used in an edible film encapsulating a granola bar. Oxidative stability of the control and coated granola bars was tested by determining the OSI at 110 °C, as well as the physical properties at 25 and 50 °C. For consumer

evaluation, a paired comparison sensory test was performed to determine if consumers could detect a difference between the control and the coated granola bar; overall likeability test to determine consumer acceptability, just-about-right (JAR) to determine sweetness level, 5-point bitterness to see if film was bitter, 5-point scale on various emotions and 5-point scale purchase intent. Statistical analysis was utilized to determine significant difference at $p < 0.05$.

Materials and Methods

Materials

Frymax Sun Supreme Deep Frying (high oleic sunflower oil) oil was donated by Stratas Foods (Memphis, TN, USA). RBD 76 °F (24.44 °C melting point) coconut oil and Novatol™ 4-80 (natural tocopherols) were donated by ADM (Decatur, IL, USA). Novozymes North America (Franklinton, NC, USA) provided the Lipozyme® TL IM (*sn*-1,3 specific *Thermomyces lanuginosus* lipase with a specific activity of 442.0 IUN/g as specified by manufacturer). Heptadecanoic acid (C17:0) was purchased from Tokyo Chemical Co. Ltd. (Tokyo, Japan) and TAG standard mixes (GLC reference standard 437 and 570) were purchased from Nu-Check Prep Inc. (Elysian, MN, USA). Porcine pancreatic lipase and Supelco 37 FAME mix were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Other chemical and solvents were purchased from J.T. Baker Chemicals (Center Valley, PA, USA), Fisher Scientific (Norcross, GA, USA) and Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Maltodextrin STAR-DRI® 1 was donated by Tate & Lyle (Decatur, IL, USA) and used for film formula. Dixie sugar and Nature Valley Granola bars were purchased from Publix and Walmart (Athens, GA, USA). Carrageenan and locust bean gum were both donated by Ingredient Solutions Inc. (Waldo, ME, USA) and glycerol was purchased from Hoefer Inc. (San Francisco, CA, USA).

IP Synthesis and Characterization

One thousand grams of the 60:40 (w/w) (coconut oil: high oleic sunflower oil) blend was weighed out and designated as physical blend (PB6). The solvent-free interesterification reaction occurred in a 1-L (10 cm inner diameter and 18 cm long) stirred-batch reactor under vacuum with a circulating water bath at 65 ºC and mixing at 200 rpm for 6.25 h. During the reaction, the reactor was covered in aluminum foil to reduce light exposure. Lipozyme® TL IM lipase at 2.5% (w/w) of substrates was used based on previous studies (Moore and Akoh 2017). When the reaction was complete, the enzyme was removed by vacuum filtration with a Buchner funnel and then washed with hexane to recover any remaining product. After filtration, the IP6 was placed in an amber Nalgene bottle and was flushed with nitrogen before being stored at -80 ºC in a freezer. Approximately 5 g of IP6 was kept for analysis of free fatty acid (FFA) percentage before SPD. The product was purified by SPD using a KDL-4 (UIC, Inc., Joliet, IL, USA). The oil was passed through the distillation apparatus one time under the following conditions: holding temperature, 50 ºC; feeding rate of approximately 100 mL/h; heating oil temperature, 185 ºC; coolant temperature, 30-35 ºC; pressure, < 100 mTorr or <13.33 Pa. After SPD, the FFA content as lauric acid equivalents was determined AOCS Official Method Ac 5-40 (2011a). IP6 was then characterized by fatty acid analysis and TAG molecular species

IP6 was converted to fatty acid methyl esters (FAME) and analyzed with an Agilent 6890 N gas chromatography system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a Supelco SP-2560 capillary column (100 m × 0.25 mm ID, 0.20 μm film) (Sigma-Aldrich Co. St. Louis, MO, U.S.A.) and a flame ionization detector. Protocols for total and *sn*-2 positional distribution of fatty acids were previously described by Moore and Akoh (2017).

Analysis of TAG molecular species of the IP6 product was performed on an Agilent 1260 Infinity HPLC system (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a Sedex Model 85 evaporative light scattering detector (ELSD) (Sedere, Alfortville, France) and a Ultrasphere C18 analytical column (4 mm × 250 mm, 5 μm particle size) (Beckman Coulter Inc., Pasadena, CA, USA). Samples were analyzed in triplicate according to the method described by Ifeduba and Akoh (2014).

Thermal Behavior

The melting profiles of the IP6 and emulsion film were determined using a 204 F1 Phoenix DSC (NETZSCH Instruments North America, Burlington, MA, USA) following AOCS Official Method Cj 1-94 (2011b). Nitrogen was used as the purge and protective gases.

Tocopherol Analysis

Tocopherols were identified and quantified in HOSO, CO, IP6, interesterified product fortified (IPF) 6, IP 60:40 before short path distillation, high oleic sunflower oil fortified (HOSOF) and physical blend 6 (PB6) using a HPLC system. An isocratic mobile phase of 0.85% (v/v) isopropanol in hexane, degassed through sonification, was used at a flow rate of 1.0 mL/min. A Shimadzu LC-6A pump equipped with an RF-10AXL fluorescence detector (Shimadzu Corp., Columbia, MD, USA), a Spectra Series As100 autosampler (Thermo Speparation Products, Inc., San Jose, CA, USA), a LiChrosorb Si 60 column (4 mm, 250 mm, 5 μm particle size), (Hiber Fertigs€aule RT, Merck, Darmstadt, Germany) and Agilent Chemstation software were used for the analyses. The samples were prepared as described by Zou and Akoh (2013). Tocopherol standards were prepared according to Lee and others (1998) and measured on a Shimadzu model UV-1601 UV-vis spectrophotometer with a quartz cuvette. Extinction coefficients (E_{1cm} 1%) were 71.0, 86.4, 92.8, and 91.2, respectively for α, β, γ, and δ tocopherols, respectively. For α, β,

γ, and δ tocopherols, the maximum wavelengths were 294, 297, 298, and 298, respectively [14]. The following equation was used to determine percent purity: Eq. (1)

$$\% \text{ purity} = \frac{\text{Actual Concentration}}{\text{Theoretical Concentration}}$$

The purities of α-, β-, γ-, and δ-tocopherols were found to be 93.3, 95.6, 96.8, and 95.0%, respectively. Stock solutions of α-, β-, γ-, and δ-tocopherols had concentrations of 2.068, 2.264, 2.0, 5.14 mg/mL, respectively. The stock solutions were diluted with the mobile phase containing 0.01% butylated hydroxytoluene (BHT) for the daily working standard. Standards and samples were injected at a volume of 20 μL into the HPLC.

Coating Application

Commercially purchased granola bars (Nature Valley brand, General Mills, Minneapolis, MN, U.S.A.) were used as the delivery food system. The granola bars were coated by dipping one side in the emulsion film developed in Moore and Akoh (2017). The edible emulsion film consisted of IP6, maltodextrin, glycerol, sugar and gums. The subsequent backside was then coated after the first side had dried. The film was then scraped, with a spatula to create an even coat and remove any excess film.

Oxidative Stability

Approximately 2 g of samples PB6, IP6 and IP6F were weighed into test tubes, closed and placed into an oven at 65 °C. Individual samples were removed at intervals to measure the peroxide (PV) and *p*-Anisidine values (*p*-AV) over 18 days. PV was measured according to the AOCS official method Cd 8b-90 (2011c) while *p*-AV was determined according to AOCS official method Cd18-90 (2011d). The total oxidation (TOTOX) value was calculated using the following equation: Eq. (2)

$$\text{TOTOX value} = 2(\text{PV}) + p\text{-AV}$$

The oil stability index (OSI) of PB6, IP6, IP6F, granola bars, and coated granola bars with IP6 were done in triplicate and were determined using an Oxidative Stability Instrument (Omnion Inc., Rockland, MA, USA) according to the AOCS official method Cd 12b-92 (2011).

Moisture Absorption Study

Samples (control and coated granola bars) were placed in a chamber at 25 and 50 °C at 50% relative humidity (RH) for 72-h. Samples were weighed every eight-hours during the 72-h period. The displacement in weight was then plotted.

Physical Properties

The control and coated granola bars were stored for five days at 25 and 50 °C both at 50% RH. Each day the hardness of the granola bars was analyzed using a three-point bend test. A TA-XT2 Texture Analyzer equipped three-point bending rig was utilized for the test. The span between the supports was 50 mm and the rupture test distance was 4.0 mm. The load cell was 25 kg. The pre-test speed was 1.00 mm/s, test speed 3.0 mm/s and post-test speed 10 mm/s. The trigger return was set to 20 mm. Five samples per day were analyzed.

Sensory Evaluation

After obtaining approval for human subject studies from the University of Georgia Institutional Review Board (IRB), sensory tests were carried out in the sensory facility at UGA. Panelists for the consumer tests were recruited from the university campus. Test subjects between the ages of 18-65 were selected randomly, however, those who were sensitive to food allergies, or pregnant, were excluded from participation. In addition, each participant was asked to fill out a questionnaire for demographic information including gender, age, race, annual income, and physical activity level. Also, consumers were asked if they knew what a structured lipid was, if

they considered themselves a regular consumer of energy/granola bars and how often they consumed energy/granola bars.

Paired-Comparison Sensory Test

A paired-comparison test was conducted (n=80) to help determine if a difference existed between the coated granola bars and the commercial granola bars. Each panelist received one of the following combinations: AA, AB, BA and BB. Each sample presentation was represented in an equal amount of 20 times. Samples were prepared the day before. The control (A) was a commercial granola bar and sample (B) was the commercial granola bar coated with the edible emulsion film containing the IP6. The control granola bars were taken out of the packaging prior to preparing for the test. The control and test samples were then cut into one-inch pieces. Then one piece was placed into a sample cup given a three-digit code. Water cups, in addition to unsalted crackers were given to consumers to cleanse their pallets in-between tasting of samples. Consumers were shown to separate sensory booths under traditional white lighting to conduct the test after signing a consent form and pre-questionnaire. Consumers were then asked to identify if the samples given were the same or different. The chi-squared hypothesis (H_o) was that there is no difference in the number of correct and incorrect responses and the alternative hypothesis (H_a) was that there is a difference in the number of correct and incorrect responses. The results were statistically analyzed using a chi-squared analysis.

Consumer Acceptance Test

A series of acceptance tests were conducted (n=100) in the sensory laboratory at UGA. A total of 100 consumers were recruited and were each asked the following series of acceptance test questions about the coated granola bar product:

A) Overall liking test (9-point scale): Consumers were asked their overall liking from 1-dislike extremely to 9-like extremely on several attributes such as appearance, texture, chewiness, sweetness, overall taste, and overall liking.

B) 5-Point intensity test: Consumers were asked to identify the intensity of bitterness association with the product to see if the edible coating added bitterness to the product by assessing on a 5-point scale from none to very strong.

C) 3-Point JAR: Consumers were asked to identify the sweetness of the product on a 3-point JAR scale, not enough, just-about-right or too much to help identify if the coating was adding additional sweetness.

D) Emotions 5-point scale: Consumers were asked if they associated several emotions (active, energetic, good, happy, pleased, satisfied, guilty, and disappointed) from 1-not at all to 5-extremely when consuming the product.

E) 5-Point scale purchase intent: Consumers were asked a on a 5-point scale the purchase intent of the product (1-definetly would not buy to 5- definitely would buy) before and after learning what a SL/IP was.

Each consumer received two pieces of one-inch width of the coated granola bar, water, and unsalted crackers. Statistical analysis of sensory data was analyzed using JMP Pro 13 (SAS Institute Inc., Cary, NC, USA) by hierarchical clustering for quantitative data then a one-way ANOVA using a student's t-test at a significance level of $p < 0.05$ to determine if there was a significant difference among clusters.

Results and Discussion

Structured Lipid Characterization

The total and positional fatty acid profile of the IP6 are shown in **Table 4.1**. The oleic acid content at the *sn*-2 position was (30.46 ± 1.54 mol%) and the content of lauric acid was (44.73 ± 1.01 mol%). After interesterifying CO with HOSO, the total fatty acid profile was similar to the data reported by (Moore and Akoh 2017). SPD reduced the FFA % to 0.06% and the product reaction yield was 94.02%.

The major TAG species found in the large-scale reaction were CyOLa (3.25 ± 0.26%), CLaM (6.96 ± 0.15%), LaOC (17.93 ± 0.67%), LaLaM (14.00 ± 0.80%), LaOLa (25.14 ± 0.95%), LaMP (11.47 ± 0.79%), LaOO (13.78 ± 0.38%), and LaOP (1.66 ± 0.10%) where Cy = caprylic, O = oleic, La = lauric, C = capric, M = myristic, and P = palmitic. The major TAG species found in the large-scale reaction were similar to those reported in Moore and Akoh (2017) with some exceptions. There was a decrease in LaOO from (18.26 ± 0.30%) to (13.78 ± 0.38%) but an increase in LaOC (14.67 ± 1.26%) to (17.93 ± 0.67%) in the current paper. The increase in medium-long-medium (MLM) chain TAG content was advantageous because the MCFA are rapidly absorbed and LCFA at the *sn*-2 position are nutritionally advantageous (Kennedy 1991).

Thermal Behavior

The melting profile of the IP6 and edible film were determined using a differential scanning calorimeter (DSC). The melting onset for IP6 was 15.17 °C and the melting completion was 20.83 °C. The melting point was slightly different than what was reported before (Moore and Akoh 2017) and this can be attributed to a slightly different fatty acid profile and TAG species. The melting characteristics of the edible film showed two main peaks. The first peak had a melting onset of 119.13 °C and a melting completion of 123.03 °C and the other peak had a

melting onset of 110.95 °C and a melting completion of 113.45 °C. The melting point of the film ingredients, locust bean gum is 80 °C and maltodextrin has a melting temperature of 67-69 °C. The addition of glycerol and water to edible films at different amounts impacts the melting onset and the thermal transitions (Hernandez-Izquierdo 2008). Both glycerol and water were added to the film coating and as a result, the melting temperature of the ingredients was modified.

Tocopherol Addition

Tocopherol content of the CO, HOSO, PB6, HOSOF, IP6, and IP6 before and after SPD and IP6F were measured and results are in **Table 4.2**. HOSO contained mostly α-tocopherol (2.37 ± 0.22 mg α-tocopherol/g oil), whereas coconut oil contained only (0.21 ± 0.01 mg of α-tocopherol/g oil). CO does not contain many tocopherols since the fatty acid composition is mostly saturated fatty acids. There were three other tocopherols (β, δ, and γ) found but in minimal amounts. The PB6 contained (1.09 ± 0.05 mg α-tocopherol/g oil) which is reasonable balance between the amounts of α-tocopherol found in CO and HOSO. The α-tocopherol content of IP6 after SPD decreased to 0.31 ± 0.08 mg α-tocopherol/g oil. Akoh and Moussata (2001) and Zou and Akoh (2013) suggested tocopherol loss could be attributed to further processing such as SPD. The Novatol™ 4-80, a natural tocopherols supplement, from ADM was too concentrated and contained 546 mg α-tocopherol/g oil and therefore was diluted with HOSO. After SPD, the IP6 was fortified with the HOSOF, which contained 40.24 mg α-tocopherol/g oil, to increase the α-tocopherol content 1.19 ± 0.26 α-tocopherol/g oil.

Oxidative Stability

Oxidative stability was determined by OSI and TOTOX values. The data for PB6, IP6, and IP6F data are shown in **Figure 4.1**. Between days 9 and 15 of storage, lipid oxidation started earlier in IP6 than IP6F and PB6. Also, the OSI values of PB6 (41.48 h) and IP6F (63.37 h) were

significantly higher than that of the IP6 (7.72 h). Due to SPD, IP6 contained less tocopherols and therefore, was more likely to oxidize faster than PB6 and IP6F. Past research has also shown that the oxidative stability of IP6 is lower than their physical blend counter parts (Ifeduba and others 2016; Zou and Akoh 2013). The addition of tocopherols to IP6 helped improve the oxidative stability of the IP6F. Both the IP6F and PB6 were stable over the 18-day period of incubation at 65 °C. However, IP6F had an OSI value of 63.37 h which was higher than the PB6 which had an OSI value of 41.48 h. The OSI of the control granola bar and the granola bar with film were also determined. The granola bar had an OSI value of 63.4 h and the granola bar with film had a statistically the same value of 61.95 h. The addition of the edible film to the granola bar did not decrease the oxidative stability of the granola bar.

Physical Properties

A texture analyzer using a three-point bend test protocol determined the snap of granola bars stored over five-days and can be found in **Figure 4.2.** The control bars started with a breaking force of about 2000 g and Woody (2003) found a similar snap in a sugar cookie of 2263.6 g. In addition, the sugar cookie and control granola bar had similar water activity (0.2,0.25), respectively. The control bars stored at 25 °C and 50% RH saw an increase in force then decreased dramatically after day three. The coated bars at the same temperature experienced relatively a stable force over the five-day period. The coated bars at 50 °C increased in force over the five-day period while the control bars decreased in force. Both control and coated bars at 25 °C had a lower gradient on days 3-5 than the gradients for bars at 50 °C. The force needed to break the bars is associated with the bars' hardness. Since the granola bars are crunchy to begin with, monitoring hardness over time is important to see if the addition of the edible coating altered the hardness. The coated granola bar stored at 25 °C over the five-day period had

decreased hardness to begin with, but the control bar at the same temperature had the same force as the coated bar by the end of the five-day period. The coated granola bars stored at 50 °C increased in hardness over the five-day period compared to the control. Matthews and Dawson (1963) found that the larger the percentage of fat added to pastries the lower the break strength needed. The coated bars contained additional fat in the form of IPs, which may contribute to the lower break strength or force.

Water Absorption Study

The water absorption for both the control granola bar and the granola bar with film were observed for 72 h at two temperatures 25 and 50 °C. Samples were taken at 8-h intervals to observe change in weight. The control bars gained weight over time for the first 40 h the two temperatures were significantly different seen in **Figure 4.3.** Over time the coated bars lost weight this could be attributed to the film reaching equilibrium. Sellappan and Akoh (2000) observed less moisture or less weight gain in IP coated crackers indicating that the coating lipid can serve as moisture barrier in foods.

Paired-Comparison Sensory Test

A paired-comparison test was conducted to determine if consumers could detect a difference amongst a control sample and test sample. There was a total of 40 matched pairs and 40 unmatched pairs. Demographics of the panelists can be found in **Table 4.3**. Panelists were asked if the samples were the same or different and the results are shown found in **Table 4.4**. Of the panelists who received matched pairs, 16 correctly identified the samples while 24 panelists incorrectly guessed the samples were different when in fact they were the same. Of the panelists who received unmatched pairs, 36 correctly identified the sample while only 6 panelists believed the samples to be the same that were in fact different. A chi-squared analysis was conducted and

the x^2_{cal} value of 6.26 was greater than the chi-squared table value (O'Mahony 1986) of 5.41 at $p \leq 0.02$. Therefore, the null hypothesis that there was a difference in the number of correct and incorrect responses (i.e., samples are different) was rejected.

Consumer Acceptance Test

The consumer demographic data is shown in **Table 4.3**.

A) Overall liking (OL): data was analyzed by hierarchical cluster method, which determined that there were only three clusters (**Table 4.5**). The OL and attributes between clusters were significantly different ($p \leq 0.05$). Cluster 1 had an OL value of 6.5, and cluster 2 with an OL of 4.5, were different ($p \leq 0.05$), but showed similar trends in the liking order of attributes. The two clusters ranked chewiness, texture, and appearance from lowest to highest liking, respectively. Cluster 3 ranked appearance the lowest even though the OL score was 8.0. Sweetness and overall taste had the highest rating scores for all clusters, which could imply that sweetness may have the greatest influence on taste and liking of the product. Cluster 3's appearance rating, which was the lowest rated attribute, may be improved by drying the bars with a different method. In a similar study, Mahanna and Lee (2010) consumers were asked to rate 11 different brands of granola bars including several lab-made bars on a 9-point hedonic. Commercial granola bars such as Fiber One and Curves had higher acceptability scores (7.5,7.4), respectively. When compared to this study cluster 3 had a higher acceptability score (8.0). However, Go Lean Bars, male, female and overall had lower appearance scores (5.7, 3.4, 5.8, 3.4), respectively compared to the appearance scores of both clusters 1 and 3 (6.06,6.96), respectively.

B) The 5-Point intensity for bitterness showed that there was no strong or very strong bitterness and 78% of the consumers responded that there was no bitterness associated with the product indicating the addition of the film did not add any bitterness to the product.

C) 3- Point JAR for sweetness: According to the test results in this test, more than 80% of consumers responded that the level of sweetness perceived was just about right. Therefore, there was no additional sweetness perceived from the addition of the edible film.

D) Results for the emotions 5-point scale can be found in **Table 4.6**. Cluster 3 was not statistically different ($p \leq 0.05$) than clusters 1 and 2 on feeling guilty about consuming the product. Consumers in cluster 3 solicited an overall satisfied reaction from the product which correlates with their responses that they liked the coated product to a high degree. Whereas, consumers in cluster 2 were least pleased and satisfied with the product with a score of 2.0 compared to consumers in Cluster 3 which was significantly different having a score of 4.06 and 4.15 out of 5.

E) **Figure 4.4** describes the level of intent of purchase before knowing the product contained SL/IP and after being given a description that a SL/IP was a modified oil using an enzyme. Consumers did not change their mind based upon the knowledge of the addition of a SL/IP to the SL/IP-coated product at a significance level of $p \leq 0.05$. Cluster 2 was the least likely to purchase the product while the other two clusters were more likely to purchase the product while the other two clusters were more likely to purchase the product, although not significantly. Mahanna and others (2009) studied consumer expectations of food bars in which some of the most important factors were healthy, high fiber, high protein, and contains whole grains/oats. The current product contained whole grains and oats and consumers were

not told whether the product contained high fiber or protein which may have changed the purchase intent had certain claims been revealed.

Conclusion

Enzymatic interesterification successfully incorporated more oleic acid (30.46 ± 1.54 mol%) at the *sn*-2 position. Fortification of IP6 containing the natural tocopherol source provided oxidative stability comparable to the PB6. The coated granola bar was more stable over a five-day period when held at 25 °C than the commercial granola bar. About 85% of consumers liked the product moderately or better with 50% who liked the product very much (8.0). Overall, consumers did not change their purchase intent based on knowledge of adding SL/IP to products nor influence consumer's decision negatively. The shelf-life stability of the granola bar is promising with the addition of the edible emulsion film.

Acknowledgments

We thank Stratas foods for providing the high oleic sunflower oil and ADM for providing the coconut oil and Novatol™ 4-80. This research was supported in part by Food Science Research fund from the University of Georgia.

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Tables

Table 4.1 Total and positional fatty acid composition (mol%) of the interesterfied product 60:40 (IP6)

Fatty Acid	Total	*sn*-2	*sn*-1,3
C8:0	3.94 ± 0.03	2.66 ± 0.40	4.46 ± 0.33
C10:0	3.45 ± 0.10	3.40 ± 0.70	3.47 ± 0.58
C12:0	27.38 ± 0.06	44.73 ± 1.01	18.71 ± 0.63
C14:0	10.92 ± 0.05	8.71 ± 0.73	12.03 ± 0.48
C16:0	7.02 ± 0.05	4.12 ± 0.31	8.51 ± 0.36
C18:0	3.17 ± 0.02	0.48 ± 0.84	4.26 ± 0.82
C18:1n9	39.78 ± 0.03	30.46 ± 1.54	44.44 ± 0.82
C18:2n6	3.07 ± 0.04	5.47 ± 2.22	1.88 ± 1.15

Values are mean ± SD (n = 3)

*Trace amounts found of C6:0, C18:3n6, C20:1n9, C18;3n3, C22:1, and C24:1

Table 4.2 Analysis of tocopherol content (mg tocopherol /g oil) of starting material high oleic sunflower oil (HOSO) and coconut oil (CO), HOSO fortified with Novatol™ 4-80, Novatol™ 4-80, physical blend 60:40 (PB6), interesterified product (IP) before short path distillation, after short path distillation (SPD) and after fortification (F).

Tocopherol Analysis (mg/g) oil

Sample	α-tocopherol	β-tocopherol	γ -tocopherol	δ-tocopherol
HOSO	2.37 ± 0.22	0.20 ± 0.01	0.23 ± 0.01	0.11 ± 0.01
CO	0.21 ± 0.01	0.13 ± 0.01	0.26 ± 0.26	0.13 ± 0.01
HOSOF	40.24 ± 2.78	3.23 ± 0.13	9.27 ± 0.56	8.24 ± 0.48
Novatol™ 4-80[a]	546	20	98	45
PB6	1.09 ± 0.05	0.17 ± 0.01	0.21 ± 0.01	0.12 ± 0.01
IP6 After SPD	0.31 ± 0.08	0.14 ± 0.01	0.12 ± 0.01	0.09 ± 0.01
IP6 Before SPD	1.02 ± 0.07	0.19 ± 0.03	0.23 ± 0.02	0.11 ± 0.01
IP6F	1.19 ± 0.26	0.15 ± 0.01	0.30 ± 0.05	0.19 ± 0.02

[a] Novatol™ 4-80 provided by ADM specification sheet

Table 4.3 Demographic questions for consumer acceptance tests and paired-comparison tests.

		Consumer Acceptance Test				Paired-Comparison Test
Item %		Total	Cluster 1	Cluster 2	Cluster 3	Total
Adults (n)		100	35	12	48	80
Gender	Female	59	54.3	83.3	54.2	54
	Male	41	45.7	16.7	45.8	46
Age (y)	18-25	61	63.9	58.3	62.5	62.5
	26-35	20	19.4	8.3	20.8	20
	36-45	9	2.8	16.7	12.5	7.5
	46-55	1	2.8	0	0	2.5
	56-65	9	11.1	16.7	4.2	7.5
Race	White American	59	66.7	58.3	56.3	53.2
	Black American	9	5.6	0	12.5	7.8
	Hispanic	1	0	0	0	0
	Asian	26	22.2	33.3	27.1	35.1
	Other	5	5.6	8.3	4.2	3.9
Income ($)	<15,000	48.5	48.6	54.5	47.9	51.9
	15,001-30,000	26.8	25.7	18.2	29.2	24.7
	30,001-50,000	6.2	5.7	0	8.3	5.2
	>50,000	18.6	20	27.3	14.6	18.2
Activity Level	Sedentary	30.3	31.4	41.7	25	38
	Active	60.6	57.1	58.3	64.6	54.4
	Super Active	9.1	11.4	0	10.4	7.6
Do you know what a structured lipid/IP is?	Yes	41	27.8	33.3	29.2	32.5
	Not Sure	29	22.2	25	33.3	37.5
	No	30	50	41.7	37.5	30
Do you consider yourself a regular consumer of granola bars?	Yes	32	30.6	25	35.4	23.8
	Not Sure	8	2.8	16.7	10.4	8.8
		60				
	No		66.7	58.3	54.2	67.5
How often do you eat granola bars?	Everyday	6.3	2.9	9.1	6.5	5.2
	1-2 times per week	38.5	34.3	27.2	47.8	27.3
	1-2 times per month	55.2	62.9	63.6	45.7	67.5

Table 4.4 Paired-comparison test results.

	Matched (AA or BB)	Unmatched (AB or BA)	Total
Same	16	6	22
Different	24	34	58
Total	40	40	80

Table 4.5 Mean ratings[1] of liking of appearance, flavor, texture, chewiness, overall taste and overall liking for consumer clusters[2] of coated granola bar[3]

	Cluster 1 (n = 35, 37%) (p<0.0001)	Cluster 2 (n = 12, 13%) (p<0.0001)	Cluster 3 (n = 48, 50%) (p<0.0001)
Appearance	6.06 ± 1.15[a]	6.06 ± 1.15 [b]	6.96 ± 1.13[c]
Texture	5.58 ± 0.44[a]	3.67 ± 0.45 [b]	7.19 ± 0.30[c]
Chewiness	5.97 ± 0.44[a]	3.83 ± 0.43[b]	7.63 ± 0.29[c]
Sweetness	7.08 ± 0.35[a]	5.17 ± 0.34[b]	8.02 ± 0.24[c]
Overall Taste	7.14 ± 0.31[a]	5.00 ± 0.3[b]	8.06 ± 0.21[c]
Overall Liking	6.75 ± 0.29[a]	4.50 ± 0.28[b]	8.00 ± 0.19[c]

[a-c] Values within a row with different letters are significantly different ($p < 0.05$)

[1] Consumers evaluated appearance, texture, chewiness, sweetness, overall taste, and overall liking on a 9-point hedonic scale (1 = dislike extremely; 9- like extremely)

[2] Clusters represent consumer subgroups that were homogeneous with regards to their overall liking of samples identified by ward hierarchical cluster analysis

[3]Data were analyzed for comparisons between clusters using 1-way ANOVA followed by student's t-test

Table 4.6 Mean ratings[1] of emotions active, energetic, good, happy, please, satisfied, guilty and disappointed for consumer clusters[2] of coated granola bar[3]

	Cluster 1 (n = 35, 37%)		Cluster 2 (n = 12, 13%)		Cluster 3 (n = 48, 50%)	
	Mean ± SD	*p*-value	Mean ± SD	*p*-value	Mean ± SD	*p*-value
Active	1.89 ± 0.26^{b}	0.2572	1.58 ± 0.18^{b}	0.2572	3.08 ± 0.26^{a}	<0.0001
Energetic	1.94 ± 0.20^{b}	0.1354	1.5 ± 0.29^{b}	0.1354	3.15 ± 0.44^{a}	<0.0001
Good	2.78 ± 0.26^{a}	0.0013	1.92 ± 0.25^{b}	<0.0001	3.88 ± 0.17^{c}	<0.0001
Happy	2.36 ± 0.27^{b}	0.0516	1.83 ± 0.18^{b}	0.0516	3.85 ± 0.26^{a}	<0.0001
Pleased	2.94 ± 0.24^{a}	<0.0001	2.00 ± 0.17^{b}	<0.0001	4.06 ± 0.25^{c}	0.0003
Satisfied	2.92 ± 0.25^{a}	<0.0001	2.00 ± 0.17^{b}	<0.0001	4.15 ± 0.26^{c}	<0.0001
Guilty	1.11 ± 0.21^{b}	0.2083	1.58 ± 0.15^{a}	0.1711	1.31 ± 0.22^{ab}	0.035
Disappointed	1.42 ± 0.19^{a}	<0.0001	2.33 ± 0.20^{b}	<0.0001	1.04 ± 0.13^{c}	0.0055

[a-c] Values within a row with different letters are significantly different ($p < 0.05$)

[1] Consumers evaluated emotions, active, energetic, good, happy, pleased, satisfied, guilty, and disappointed on a 5-point hedonic scale (1 = not at all; 5 = extremely)

[2] Clusters represent consumer subgroups that were homogeneous with regards to their overall liking of samples identified by ward hierarchical cluster analysis

[3]Data were analyzed for comparisons between clusters using 1-way ANOVA followed by student's t-test

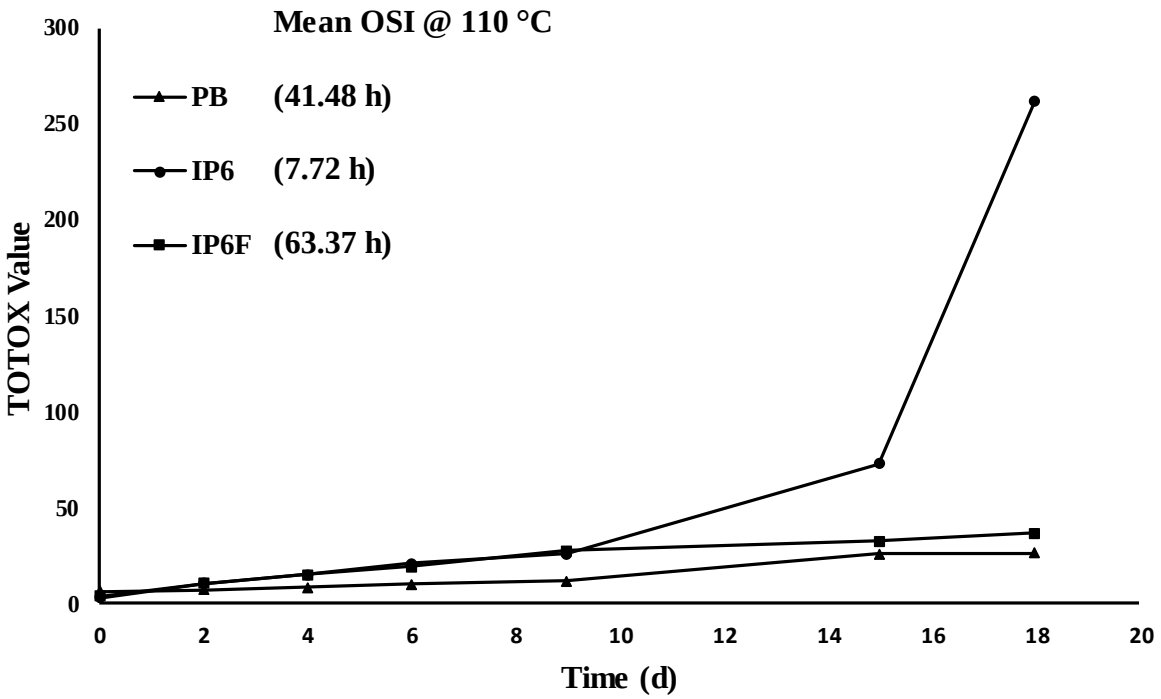

Figure 4.1 Oxidative stability index (OSI) and total oxidation (TOTOX) values during storage of physical blend (PB6), interesterified product 60:40 (IP6), and interesterified product 60:40 fortified (IP6F) during storage.

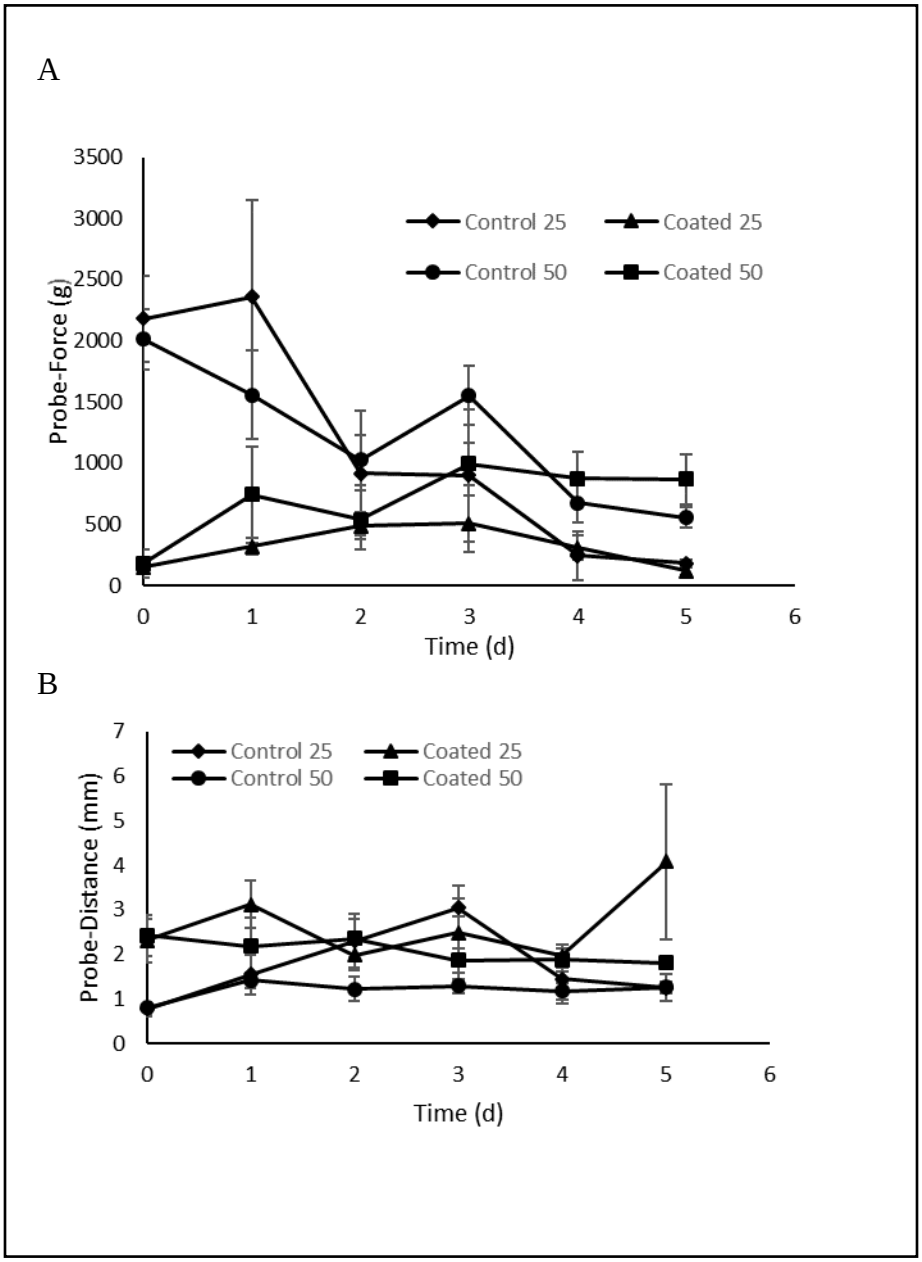

Figure 4.2 Three-point bend test over 5-day period (A) Force (g) (B) Distance (mm) (n = 5).

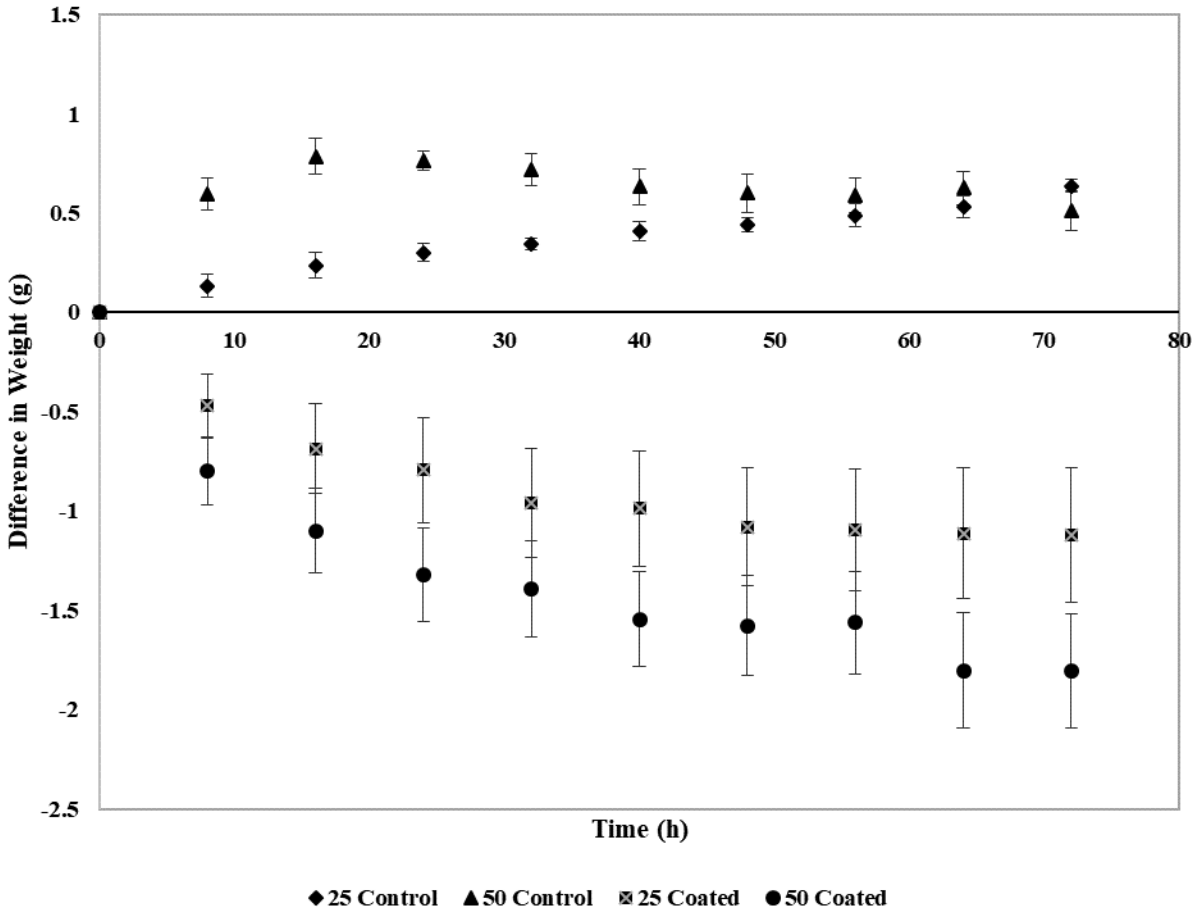

Figure 4.3 Changes in water absorption of both control and coated granola bars at 25 and 50 °C and 50% RH over a 72-h period.

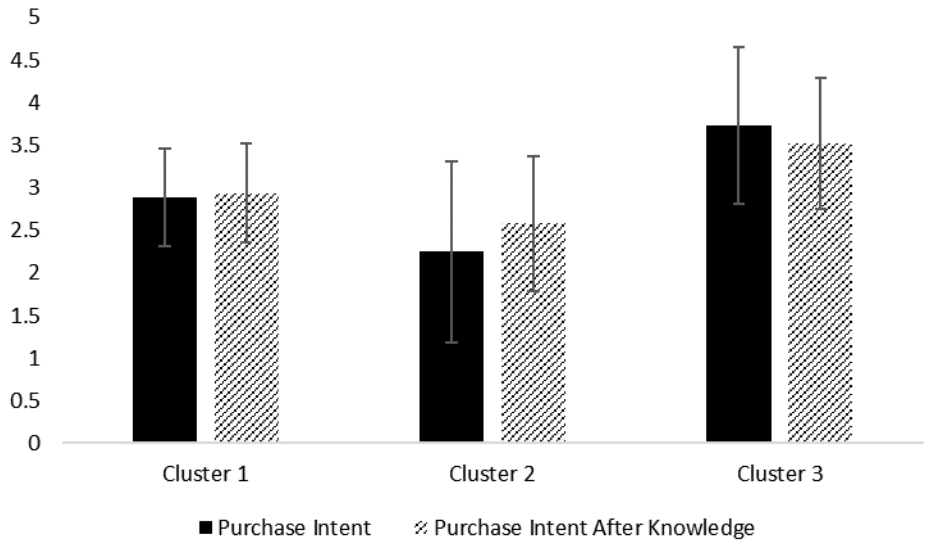

Figure 4.4 Purchase intent of coated granola bars before knowledge of and after the definition of structured lipid/interesterified product (IP) was explained.

CHAPTER 5

CONCLUSIONS

Three different structured lipids (60:40, 70:30, and 80:20 (w/w)) and their physical blends were compared. Lipozyme® TL IM was used as the enzyme for the enzymatic interesterification reactions. Based on fastest reaction time 10% enzyme was selected for use in the first project. The FA profile of the IP using Lipozyme® TL IM compared to the PB significantly increased the oleic acid content of the IP. The IP 60:40 contained the most oleic acid (33.86 ± 1.55%) at the *sn*-2 position. The structured lipids were then used in an edible film application. The SLs helped reduce the opacity of CO in film use. The IP 60:40 had the weakest EB but the strongest TS. and resulted in a translucent product. The IP 60:40 was used for further research.

A large-scale enzymatic interesterification of PB6 was conducted and the fatty acid profile, TAG species and melting profile were determined. The enzymatic interesterification was successful in incorporating oleic acid (30.46 ± 1.54 mol%) at the *sn*-2 position. Tocopherol levels were reduced after SPD and were then fortified in IP6 to increase stability. The IP6F had oxidative stability comparable to the PB6. The granola bar and coated granola bar OSI were measured and had significantly the same values. However, consumer panelists determined that the coated granola bar was significantly different than the control granola bars. Also 85% of consumers liked the product moderately or better with 50% who liked the product very much with a rating of 8.0 on a 9.0 scale. Overall, consumers did not change their purchase intent based

on knowledge of adding SL/IP to products. SL/IP addition in products did not influence consumer's decision negatively.

Future studies could evolve from this research. One possibility is to test various film formulations to see if an increased amount of SL could be used in the film. Varying SL content in films could be useful to see if additional or decreased SL content helped to decrease the water vapor permeability. Another study could look at using the emulsion edible film as a binder for granola based bars. The binder could then be tested and compared to commercially available products. Sensory test such as an in-home test could be used to determine the acceptability of the granola bar with the emulsion edible film. Another possibility would be to test various applications and drying methods to improve the appearance properties of the granola bar. The different applications would include testing different spraying pressures using a pressurized sprayer. Another drying method may include vacuum drying or drying in an environmental chamber. Consumers in this study were most unhappy with the appearance which could be improved and increase the purchase intent of the product. These additional studies could help improve the edible emulsion film for commercial use.

APPENDIX A

UNIVERSITY OF GEORGIA
CONSENT FORM
Sensory Evaluation and Physical Properties of a Granola Bar with an Edible Coating Containing Coconut: High Oleic Sunflower Oil Structured Lipid

Researcher's Statement

We are asking you to take part in a research study. Before you decide to participate in this study, it is important that you understand why the research is being done and what it will involve. This form is designed to give you the information about the study so you can decide whether to be in the study or not. Please take the time to read the following information carefully. Please ask the researcher if there is anything that is not clear or if you need more information. When all your questions have been answered, you can decide if you want to be in the study or not. This process is called "informed consent." A copy of this form will be given to you.

Principal Investigator: *Dr. Gabriela Sanchez-Brambila*
Department of Food Science and Technology
gsbrambila@gmail.com

Purpose of the Study

The study looks to develop an edible film coating using a designer oil for the use on an energy/granola bar. Part of the project is to look for consumer acceptance or preference of the product. You are being asked to participate due to the fact that many consume energy bars. If you are allergic or sensitive (meaning experience discomfort such as upset stomach) to peanuts, treenuts, soy, wheat, milk and coconuts, you cannot participate. You also cannot participate if you are pregnant or not between the ages of 18 and 65.

Study Procedures

If you agree to participate, you will be asked to …

- Read and sign this consent form (approx. 5 mins)
- If you are allergic to any food allergies or sensitive especially: wheat, peanuts, treenuts (specifically coconuts), milk, and soy you will not be allowed to continue the study
- Given samples and instructional ballot to follow- if any questions the researcher will be on hand (approx. 15 mins)
    - Some demographic questions are asked such as age, gender ect.

Risks and discomforts

Samples are manufactured/ prepared according to federal regulations the risk of foodborne illnesses is very small. Only approved food additives shall be used, in legal levels. Although some distaste may occur. The risk is very minor and does not exceed what a panelist will encounter in everyday life. In between each sample, panelists will be asked to cleanse their palate with water and crackers. There is also risk associated with an allergic reaction. You will be specifically asked prior to participation if you have any known allergy or sensitivity to any food compounds may or may not include milk, wheat, soy, peanuts and treenuts such as coconut. In the event that a participant in this study experiences a serious medical problem, emergency personnel will be contacted E911 system. If a person has a less severe issue they will be transported to the University Health Services for treatment. However, the participant, or that of your third-party payer, are not precluded from seeking to collect compensation for injury related to malpractice, fault, or blame on the part of those involved in the research. Food and Drug Allergies that I have include _____.

Benefits

Participants will benefit from the knowledge that their participation was helpful in determining the use of structured lipids in food products.

Incentives for participation

Participants may receive food rewards.

Privacy/Confidentiality

Panelists will be given random numbers to track the samples given but no names will be associated with specific numbers.

Taking part is voluntary

Your involvement in the study is voluntary, and you may choose not to participate or to stop at any time without penalty or loss of benefits to which you are otherwise entitled. If you are a student, your decision to participate or not will have no bearing on your grades or class standing.

If you have questions

The main researchers conducting this study is *Dr. Gabriela Sanchez-Brambilla*, an Adjunct Faculty and Maria Moore, a graduate student at the University of Georgia. Please ask any questions you have now. If you have questions later, you may contact *Dr.Gabriela Sanchez-Brambila* at gsbrambila@gmail.com or at 704.250.1222 *or Ms. Maria Moore at* maria.moore25@uga.edu *or 225-252-7554*. If you have any questions or concerns regarding your rights as a research participant in this study, you may contact the Institutional Review Board (IRB) Chairperson at 706.542.3199 or irb@uga.edu.

Research Subject's Consent to Participate in Research:

To voluntarily agree to take part in this study, you must sign on the line below. Your signature below indicates that you have read or had read to you this entire consent form, and have had all of your questions answered.

_____	_____	_____
Name of Researcher	Signature	Date
_____	_____	_____
Name of Researcher	Signature	Date
_____	_____	_____
Name of Participant	Signature	Date

Please sign both copies, keep one and return one to the researcher.

APPENDIX B

Recruitment Pre-Screening for Consumer Test on Edible Film on Granola Bars

Consumer Number:_____

The sensory lab at University of Georgia Athens campus is conducting acceptance/preference for edible films on granola bars. For this project, we have up to 5 tests over 5 days. Each test will last approximately 25 minutes and you will be compensated with a food reward. Would you be interested? If yes, please answer / verify the following questions:

1. Gender: Male Female
2. Age:
    1) 18-25
    2) 26-35
    3) 36-45
    4) 46-55
    5) 56-65
    6) Older than 65 (Terminate)
3. Are you allergic or sensitive to any food/food ingredients?
        1) Yes (Terminate)
        2) No
4. Are you allergic or sensitive nuts, treenuts, wheat, soy?
        1) Yes (Terminate)
        2) No
5. Are you pregnant?
      1) Yes (Terminate)
        2) No

You are qualified to take this study

The test will now continue which is held in Food Processing Laboratory, Athens, GA.
Thank you so much for your help!

APPENDIX C

Paired- Comparison Test Ballot

1. **Demographic information**

Gender: [ ] Female [ ] Male

Age (years): [ ] 18-25 [ ] 26-35 [ ] 36-45 [ ] 46-55 [ ] 56-65

Race: [ ] White American [ ] Black American [ ] Hispanic [ ] Asian [ ] Other

Annual income: [ ] <15,000 [ ] 15,001-30,000 [ ] 30,001-50,000 [ ] >50,000

Physical Activity level: [ ] Sedentary (just day to day) [ ] Active [ ] Super Active

Do you know what a structured lipid is?

[ ] Yes [ ] Not sure [ ] No

Do you consider yourself a regular consumer of energy bars?

[ ] Yes [ ] Not sure [ ] No

How often do you eat energy bars?

[ ] Everyday [ ] 1-2 times a week [ ] 1-2 times a month

2. **Samples testing**

Please taste the following energy bar samples in the order presented 381 then 950. Between the samples, drink water and eat unsalted crackers to clean your palate.

Are the samples the same or different?

[ ] same [ ] different

1. **Demographic information**

Gender: [ ] Female [ ] Male

Age (years): [ ] 18-25 [ ] 26-35 [ ] 36-45 [ ] 46-55 [ ] 56-65

Race: [ ] White American [ ] Black American [ ] Hispanic [ ] Asian [ ] Other

Annual income: [ ] <15,000 [ ] 15,001-30,000 [ ] 30,001-50,000 [ ] >50,000

Physical Activity level: [ ] Sedentary (just day to day) [ ] Active [ ] Super Active

Do you know what a structured lipid is?

[ ] Yes [ ] Not sure [ ] No

Do you consider yourself a regular consumer of energy bars?

[ ] Yes [ ] Not sure [ ] No

How often do you eat energy bars?

[ ] Everyday [ ] 1-2 times a week [ ] 1-2 times a month

2. **Samples testing**

Please taste the following energy bar samples in the order presented 420 then 124. Between the samples, drink water and eat unsalted crackers to clean your palate.

Are the samples the same or different?

[ ] same [ ] different

1. **Demographic information**

Gender: [ ] Female [ ] Male

Age (years): [ ] 18-25 [ ] 26-35 [ ] 36-45 [ ] 46-55 [ ] 56-65

Race: [ ] White American [ ] Black American [ ] Hispanic [ ] Asian [ ] Other

Annual income: [ ] <15,000 [ ] 15,001-30,000 [ ] 30,001-50,000 [ ] >50,000

Physical Activity level: [ ] Sedentary (just day to day) [ ] Active [ ] Super Active

Do you know what a structured lipid is?

[ ] Yes [ ] Not sure [ ] No

Do you consider yourself a regular consumer of energy bars?

[ ] Yes [ ] Not sure [ ] No

How often do you eat energy bars?

[ ] Everyday [ ] 1-2 times a week [ ] 1-2 times a month

2. **Samples testing**

Please taste the following energy bar samples in the order presented 420 first then 950. Between the samples, drink water and eat unsalted crackers to clean your palate.

Are the samples the same or different?

[ ] same [ ] different

1. **Demographic information**

Gender: [ ] Female [ ] Male

Age (years): [ ] 18-25 [ ] 26-35 [ ] 36-45 [ ] 46-55 [ ] 56-65

Race: [ ] White American [ ] Black American [ ] Hispanic [ ] Asian [ ] Other

Annual income: [ ] <15,000 [ ] 15,001-30,000 [ ] 30,001-50,000 [ ] >50,000

Physical Activity level: [ ] Sedentary (just day to day) [ ] Active [ ] Super Active

Do you know what a structured lipid is?

[ ] Yes [ ] Not sure [ ] No

Do you consider yourself a regular consumer of energy bars?

[ ] Yes [ ] Not sure [ ] No

How often do you eat energy bars?

[ ] Everyday [ ] 1-2 times a week [ ] 1-2 times a month

2. **Samples testing**

Please taste the following energy bar samples in the order presented 381 then 124. Between the samples, drink water and eat unsalted crackers to clean your palate.

Are the samples the same or different?

[ ] same [ ] different

APPENDIX D

Consumer Acceptance Test Ballot

3. Demographic information

Gender: [ ] Female [ ] Male

Age (years): [ ] 18-25 [ ] 26-35 [ ] 36-45 [ ] 46-55 [ ] 56-65 [ ] >65

Race: [ ] White American [ ] Black American [ ] Hispanic [ ] Asian [ ] Other

Annual income: [ ] <15,000 [ ] 15,001-30,000 [ ] 30,001-50,000 [ ] >50,000

Physical Activity level: [ ] Sedentary (just day to day) [ ] Active [ ] Super Active

Do you know what a structured lipid is?

[ ] Yes [ ] Not sure [ ] No

Do you consider yourself a regular consumer of energy bars?

[ ] Yes [ ] Not sure [ ] No

How often do you eat energy/granola bars?

[ ] Everyday [ ] 1-2 times a week [ ] 1-2 times a month

4. Sample testing

Please taste the following granola bar sample presented. Before eating the sample, drink water and eat unsalted crackers to clean your palate.

- How would you **rate** the following attributes of this product?

	Dislike Extremely	Dislike Very much	Dislike Moderately	Dislike Slightly	Neither Like nor Dislike	Like Slightly	Like Moderately	Like Very much	Like Extremely
Appearance	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9
Texture	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9
Chewiness	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9
Sweetness	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9
Overall taste	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9
Overall liking	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5	[ ] 6	[ ] 7	[ ] 8	[ ] 9

Please, rate the **bitterness** of this product

[ ] None [ ] Weak [ ] Moderate [ ] Strong [ ] Very strong

Please rate the sweetness of the product [ ] Not enough [ ] Just about right [ ] Too much

How do you **emotionally feel** about this product?

	Not at all	Slightly	Moderately	Very much	Extremely
Active	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Energetic	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Good	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Happy	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Pleased	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Satisfied	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Guilty	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5
Disappointed	[ ] 1	[ ] 2	[ ] 3	[ ] 4	[ ] 5

How likely will you **purchase** this product?

[ ] 1 Definitely would not buy [ ] 2 Probably would not buy [ ] 3 Maybe/maybe not [ ] 4 Probably would buy [ ] 5 Definitely would buy

How likely will you **purchase** this product if it contains a structured lipid (a modified oil using enzymes)?

[ ] 1 Definitely would not buy [ ] 2 Probably would not buy [ ] 3 Maybe/maybe not [ ] 4 Probably would buy [ ] 5 Definitely would buy