12-2008

Effect of Processing and Formulation Conditions on Physicochemical Characteristics of Food Emulsions

Megan Tippetts
Utah State University

EFFECT OF PROCESSING AND FORMULATION CONDITIONS ON PHYSICOCHEMICAL CHARACTERISTICS OF FOOD EMULSIONS

by

Megan Tippetts

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

MASTER OF SCIENCE

in

Nutrition and Food Sciences

Approved:

_____	_____
Dr. Silvana Martini	Dr. Marie Walsh
Major Professor	Committee Member
_____	_____
Dr. Donald McMahon	Dr. Byron R. Burnham
Committee Member	Dean of Graduate Studies

UTAH STATE UNIVERSTY
Logan, Utah

2008

ABSTRACT

Effect of Processing and Formulation Conditions on Physicochemical Characteristics of Food Emulsions

by

Megan Tippetts, Master of Science

Utah State University

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

The objective of this research was to systematically study the effect of processing conditions on crystallization behavior and destabilization mechanisms of oil-in-water (o/w) emulsions. The effects of oil content (20 and 40 wt %); crystallization temperature (T_c = 10, 5, 0, -5, -10 °C); homogenization conditions, such as high shear (HS), very low pressure homogenization (VLPH), and high pressure homogenization (HPH); and cooling rate (0.2 and 30 °C/min) on both thermal behavior and destabilization mechanisms were analyzed. Docosahexaenoic acid (DHA) was added to VLPH emulsions and its effect on the physicochemical and oxidative stabilities and flavor was studied.

Emulsions with 20% oil were less stable than those with 40% oil with a fast-cooling rate; however, stability increased when the emulsions were cooled slowly. Stability was also affected by oil and droplet size; the smaller the droplet the more stable the system. Smaller droplets (i.e., VLPH, HPH) had an effect on crystallization by delaying the onset of the crystal formation, which was promoted in emulsions with larger

droplets (i.e., HS); 20% o/w emulsion crystallization was delayed more than 40%; and in emulsions crystallized using a slow-cooling rate, the crystal formation was less inhibited (i.e., crystals formed at a higher onset temperature [T_{on}], but at lower T_c) than when using a fast-cooling rate. The formation of lipid crystals either helped stabilize (small droplets) the emulsion and melted in a less fractionated manner or destabilized (big droplets) the emulsion. In addition, fast-cooling rates have greater fractionation than slow-cooling rates.

Due to the greater stability of VLPH emulsions after thawing from being at -10 °C for 3 h, DHA was added to evaluate its effect on flavor (besides the effect on stability) of the emulsion. A descriptive panel was used to evaluate four attributes: oxidized, rancid, fishy, and buttery. The panelists were given samples after 72 h, because contrary to the TBA analysis which showed no significant differences between samples with and without DHA, the fishy smell was evident. The sensory evaluation results showed that there was a significant ($p < 0.05$) difference in fishiness between the VLPH emulsions with and without DHA, and that the odor was repulsive. No significance was seen for rancid and buttery flavors, and only a marginal significance was seen for oxidized.

(134 pages)

ACKNOWLEDGMENTS

I would like to thank Dr. Silvana Martini, Dr. Marie K. Walsh, and Dr. Donald J. McMahon for their support and guidance in this endeavor, by not only helping me through this new adventure, but also by sharing their passion for food science and all it can offer. I would also like to extend my appreciation to the Seely-Hinckley scholarship committee and to Dr. Niranjan R. and Josephine N. Ghandi for their financial support. I am greatly appreciative to my lab mates, Jennifer Thurgood and Andreia Suzuki for their support and aid with my various projects and mostly for their excellent listening skills. I'm very grateful to True Fox for her assistance in expediting my IRB application. And finally, I'm particularly indebted to my mom and dad; my mom for being the best set of "second" eyes a girl could ask for and my dad for "keeping it real."

Megan Tippetts

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS, NOTATIONS, DEFINITIONS

AMF: Anhydrous Milk Fat

BS: Back Scattering

CHD: Coronary Heart Disease

DHA: Docosahexaenoic acid

DSC: Differential Scanning Calorimetry

FDA: Food and Drug Administration

ΔH^c: Enthalpy of Crystallization

ΔH^m: Enthalpy of Melting

HDL: High Density Lipids

HMP: High Melting Point

HPH: High Pressure Homogenization

HS: High Shear

LDL: Low Density Lipids

LMP: Low Melting Point

MDA: Malonaldehyde

MMP: Middle Melting Point

O/W (o/w): oil-in-water

PUFA: Polyunsaturated Fatty Acids

SBO: Soybean Oil

T_c: Crystallization Temperature

T_{on}^c: Onset Crystallization Temperature

T_{on}^m: Onset Melting Temperature

T_p^c: Peak Crystallization Temperature

T_p^m: Peak Melting Temperature

TAG: Triacylglyceride

TBA: Thiobarbituric Acid

TFA: *Trans*-fatty acids or *trans*-fat

VLPH: Very Low Pressure Homogenization

WPI: Whey Protein Isolate

CHAPTER I

INTRODUCTION

Introduction

Since January 1, 2006, the Food and Drug Administration (FDA) requires labeling of *trans*-fat (TFA) on food labels (saturated fats have been labeled since 1993). In December 2006, New York City Board of Health banned the use of TFAs in their restaurants, and the trend is spreading across the country either voluntarily, as in California, or legislatively, as in Chicago. This trend began beyond the US boarders. In January 2003, Canada introduced TFA labeling (the first in the world); in January 2004 the Danish government prohibited the selling of fats and oils containing more than 2% industrially produced TFAs in Denmark. The movement to ban the use of TFAs is in response to research which has found them to be a main contributor to coronary heart disease (CHD) a leading cause of death among Americans today (12.5 million people have CHD and over 500,000 die each year from it) [Stender and Dverberg, 2004; Revealing *trans* fats, 2003; Lueck and Severson, 2006; Barboza, 2007].

Currently, many food products contain high melting point (e.g., hard) fats that impart particular sensory properties to food (i.e., texture, flavor, and smell). In general, these hard fats are formulated using saturated fats or TFAs. Saturated fats are found in nature and are also produced using a chemical process called hydrogenation. Naturally occurring saturated fats (plant fats) can be found in high concentration in tropical oils such as palm oil, palm kernel oil, and coconut oil. Animal fats such as tallow, lard, and milk fat also contain saturated fats; however, their composition of saturated fatty acids varies from those of tropical fats. For example, saturated fats from swine are

approximately 15% palmitic acid and 3% stearic acid [Seerley et al., 1978], while saturated fats from palm oil are about 50% of the fats and are mostly palmitic (44%) and stearic (5%) fatty acids as given by American Palm Oil Co (2004). Most chemically produced fats used for food production are only partially hydrogenated. Partial hydrogenation results in production of TFAs with remarkable physical and chemical properties, making them ideal for industrial-scale food production (e.g., extended shelf-life, solid at room temperature, high flash point, etc.). Although TFAs have clear production advantages to food manufacturers and impart desirable flavor and mouthfeel for consumers (as mentioned before), they have a negative impact on the human cardiovascular system [Ascherio et al., 1999]. Therefore, with current legislation and concern for the impact on health of TFAs, alternatives to partially hydrogenated fats are gaining momentum in the marketplace.

At present, the trend is toward using tropical fats, especially palm oil, and palm kernel oil (imported mainly from Malaysia). In many applications, these are acceptable fat substitutions for partially hydrogenated fats, and the supply is readily available at an acceptable price. However, tropical fats are just a quick fix for food producers concerned about declaring TFAs on their labels; unfortunately, tropical fats contain a high amount of palmitic and lauric fatty acids. Though less severe than TFAs, these saturated fats have anti-nutritional effects on the human body, which are similar to *trans*-fats [Mensink et al., 2003; Simon et al., 1995].

Yet, not all saturated fats are health hazards. Recent human studies indicate that stearic fatty acids decrease the total cholesterol/HDL ratio in blood. A decrease in this ratio is associated with a lower risk of cardiovascular disease in humans (Mensink et al.,

2003). In response to advanced nutritional findings, new strategies need to be developed to identify alternatives for TFAs and harmful saturated fats, and to satisfy the nutritional needs of our society. Possible strategies include: substituting TFAs and tropical fats (high content of palmitic) with dairy and animal fats (lower content of palmitic fatty acids); developing new processing conditions for structuring liquid vegetable oils (e.g., emulsions); and combining these strategies.

A possible replacement for TFA in the formulation of several processed foods is anhydrous milk fat (AMF). AMF can be blended with vegetable oils, such as soybean oil (SBO). For example, a composition of 50:50 AMF to SBO can decrease the undesirable saturated fatty acid (e.g., palmitic acid) content from 33% to 22 % and increase desirable unsaturated fatty acid (i.e., α-linolenic acid) from 3 to 30%. Blends of AMF and SBO have the potential to be used in the formulation of oil-in-water (o/w) food emulsions such as mayonnaise, salad dressings, etc. Understanding the destabilization mechanism of emulsions formulated with AMF and SBO and the emulsion's relationship with respect to processing conditions is crucial to successfully developing or reformulating new food products.

In addition to new ways of creating fats with low saturated fats and zero TFAs, demand is growing among consumers for foods with enhanced nutritive value. Delivering functional foods and nutraceutical products is yet another challenge facing food producers. Various ingredients are of great interest to researchers investigating nutritional alternatives to TFAs and tropical oils. These include: polyunsaturated fats (PUFAs) derived from plant and animal sources (i.e., soybean oil and fish) and milk products (i.e., whey protein).

The purpose of this research is to add to the understanding of how to create and optimize healthier products using locally available commodities (i.e., SBO, AMF, and whey protein). To achieve this objective, an o/w emulsion model system was used. Emulsions are composed of two immiscible phases, for example, water and lipids. Stability and sensory attributes of emulsions (which can be affected by lipid crystallization) are heavily influenced by formulation and processing conditions. In this study, various strategies have been investigated to optimize the physicochemical stability of SBO/AMF o/w emulsions. These strategies have included emulsion formulation, processing conditions of the emulsions, and the addition of beneficial fats such as docosahexaenoic acid (DHA), a polyunsaturated fatty acid.

Understanding the physicochemical characteristics that result from changing the formulation and/or processing conditions of emulsions is useful. However, the application is worthless if the consumer does not approve, and, though consumers desire healthier alternatives, they are unlikely to accept radical sensory changes in food products. Reformulated foods must deliver virtually the same physicochemical and sensory attributes as their counterparts. Consequently, when incorporating DHA in emulsions, a sensory panel is needed to evaluate differences between emulsions with and without DHA to provide an indicator for the level of efficacy achieved by adding a nutritive component and for the possible discovery of off flavors.

Though extensive bibliography can be found relating to the stability of emulsions [Rousseau, 2000; Thanasukarn et al., 2004a, b, 2006; Vanapalli et al., 2002], very little material correlates the physicochemical and structural characteristics of these products with their sensory attributes. Consequently, understanding the mechanical properties that

control the stability of emulsion-type products and the relationship to their sensory attributes is very important to both scientists and food producers.

Hypothesis

The stability of o/w emulsions may be modified by controlling the processing and/or formulation conditions.

Objectives

Objective 1: Understand the destabilization mechanisms of o/w emulsions as affected by processing conditions and formulation.

Objective 2: Formulate healthier emulsions by incorporating DHA:

a) Study the effect of physicochemical and oxidative stability when incorporating DHA into AMF/SBO o/w emulsions.

b) Analyze sensory characteristics (i.e., flavor attributes) of DHA emulsions.

References

Ascherio, A., Katan, M.B., Zock, P.L., Stampfer, M.J. & Willet, W.C. (1999). *Trans* fatty acids and coronary heart disease. The New England Journal of Medicine, 340, 1994-1998.

Barboza, T. (2007). L.A. County can't ban *trans* fats. Los Angeles Times (January 30). Pp. B1.

Frequently asked questions. APOC American Palm Oil Council (2004). American Palm Oil Council (April 8). www.americanpalmoil.com/faq.html.

Lueck, T. J. & Severson, K. (2006). New York bans most *trans* fats in restaurants. New York Times (December 6). http://www.nytimes.com/2006/12/06/nyregion/06fat.html

Mensink, R.P., Zock, P.L., Kester, A.D.M. & Katan, M. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: A meta-analysis of 60 controlled trials. American Journal of Clinical Nutrition, 77, 1146–55.

Revealing *trans* fats (2003). FDA Consumer Magazine, 37 (5), 20-26.

Rousseau, D. (2000). Fat crystals and emulsion stability – A review. Food Research International, 33, 3-14.

Seerley, R.W., Briscoe, J.P. & McCampbell H.C. (1978). A comparison of poultry and animal fat on performance, body composition and tissue lipids of swine. Journal of Animal Science, 46, 1018-1023.

Simon, J.A., Hodgkins, M.L., Browner, W.S., Neuhaus, J.M., Bernert, J.T., Jr. & Hulley, S.B. (1995). Serum fatty acids and the risk of coronary heart disease. American Journal of Epidemiology, 142 (5), 469-76.

Stender, S. & Dverberg, J. (2004). Influence of *trans* fatty acids on health. Annual Nutrition and Metabolism, 48, 61-66.

Thanasukarn, P., Pongsawatmanit, R. & McClements, J.D. (2004a). Influence of emulsifier type on freeze-thaw stability of hydrogenated palm oil-in-water emulsions. Food Hydrocolloids, 18 (6), 1033-1043.

Thanasukarn, P., Pongsawatmanit, R. & McClements, J.D. (2004b). Impact of fat and water crystallization on the stability of hydrogenated palm oil-in-water emulsions stabilized by whey protein isolate. Colloids and Surfaces: Physicochemical and Engineering Aspects, 246 (1-3), 49-59.

Thanasukarn, P., Pongsawatmanit, R. & McClements, J.D. (2006). Impact of fat and water crystallization on the stability of hydrogenated palm oil in water emulsions stabilized by a nonionic surfactant. Journal of Agriculture and Food Chemistry, 54, 3591-3597.

Vanapalli, S. A., Palanuwech, J. & Coupland, J. N. (2002). Influence of fat crystallisation on the stability of flocculated emulsions. Journal of Agriculture and Food Chemistry, 50, 5224-5228.

CHAPTER II

LITERATURE REVIEW

Emulsions

Emulsions are systems which consist of two immiscible phases (e.g., aqueous and lipid) in which one phase is dispersed in another (i.e., continuous phase). The stability of this system usually requires an emulsifier to keep the dispersed phase from separating out of the continuous phase.

Emulsions are a great part of the consumer's diet, but a part about which most consumers are unaware. Products such as butter, margarine, soups, sauces, ice cream, beverages, and salad dressing are all forms of various types of emulsions (i.e., water-in-oil, oil-in-water, etc.). This is quite remarkable considering that emulsions are thermodynamically unstable systems. They have an innate desire to destabilize (i.e., separate) through various mechanisms (e.g., gravitational separation, flocculation, coalescence, phase inversion, etc.) depending on their composition, microstructure, and environment, because at the core they are two substances that are immiscible with each other [Thanasukarn et al., 2004a]. Due to the repellant nature of emulsions, other compounds are used to keep the dispersed phase from separating out of the continuous phase (i.e., emulsifiers and stabilizers). Many studies have been done on various types of emulsions for stability [Thanasukarn et al., 2004a, b, 2006; Vanapalli et al., 2002a, b; Demetriades et al., 1997; Gu et al., 2007], and it has been found that there is too much variability between types of emulsions to create just one model system to represent them all (e.g., o/w emulsions) [Rousseau, 2000].

Oil-in-water Emulsions

O/w emulsions are emulsions in which oil is the dispersed phase in an aqueous continuous phase (e.g., mayonnaise and salad dressings). As these two components are naturally immiscible with each other, another constituent is needed to keep the two phases in a homogenous solution rather than alienating each other and extricating the oil phase away from the aqueous phase. Studies have been done on the stability of these emulsions using various types of emulsifier or other stabilizing components (e.g., whey proteins, gelatin) and on how additional components (e.g., NaCl and sucrose) affect stability [Thanasukarn et al., 2004a, b; Gu et al., 2007] with various types of oils such as hydrogenated palm oil, salmon oil, anhydrous milk fat, sunflower oil, or soybean oil [Hu et al., 2001; Thanasukarn et al., 2006; see Chapter III].

Lipids

Lipids (a.k.a. fats) are an essential part of the diet (e.g., some fats which aren't already made by the body). Besides being an energy source, lipids are imperative for the availability of fat-soluble vitamins (i.e., A, D, E and K) and phytochemicals (e.g.,carotenoids) and contribute to the flavor, odor, and texture of a product. There are two types of lipids, those that are solid at room temperature (which are considered as fats) and those that are liquid at room temperature (which are considered oils) [Insel et al., 2006]. Extensive research has been done with lipids on how they crystallize, which can help (e.g., extend the shelf-life) or hinder (e.g., create a grainy texture) the end product's quality [Martini et al., 2001, 2002a, b; Awad and Sato, 2001; Campbell et al., 2001, 2002, 2004; McClements et al., 1993b]. This crystallization behavior of lipids is strongly dependent on processing conditions (e.g., cooling rate and homogenization). Processing

conditions can affect type, number, and form of crystals, which ultimately affects final characteristics of lipid networks.

Cocoa butter has at least six known polymorphic crystalline forms, with each form having a specific melting range, and in a few cases distinct microstructures [Marangoni and McGauley, 2003]. This leads to selection of a specific polymorphic form for desired attributes such as the right gloss, snap, and texture. An incorrect polymorphic form can detrimentally affect the shelf life, mouthfeel, and consumer acceptance by, for example, causing bloom (i.e., the oil seeps to the surface). Likewise, if margarine is made by rapid cooling, creating a mixture of small crystals, rather than large uniform crystals created by slow cooling, then a smooth texture rather than a grainy texture is obtained.

Since fat is a crystalline material, crystallization conditions specific for each type of fat product determine its morphology and macroscopic properties. Crystal morphology of fats dramatically influences sensory attributes such as taste and mouthfeel of foods containing fats. Also, different chemical compositions affect the crystallization behavior of the fats, both in bulk and in emulsified form. Thus, chemical composition and processing conditions must be optimized to obtain the specific crystal morphology that will result in consumer acceptance of the food product. Crystallization behavior of different fat systems has been studied by a group of researchers (Martini et al., 2001, 2002a, b, c; Martini and Herrera, 2000, 2008; Martini and Marangoni, 2007). Their research describes the crystallization properties of milk fat and milk fat blends with vegetable oil. Their microscopic, macroscopic, and kinetic behaviors, among other characteristics, have also been intensely studied by the same group of researchers (Puppo

et al., 2002; Cerdeira et al., 2003, 2005). Structural changes in this type of system also occur during storage, especially during transport from producer to consumer, altering the flavor and sensory attributes of the product [Martini and Herrera, 2008].

Trans-fat

Trans-fatty acids can be found naturally within dairy fat and muscle of ruminants, though not in high amounts, and often with beneficial health impact (e.g., conjugated linoleic acid) [McGuire and McGuire, 2000]. *Trans*-fatty acids in the human diet became predominantly man-made with the discovery of partial hydrogenation, which converts liquid oils (i.e., vegetable oils) into semi-solid or solid fats. Using a catalyst (e.g., nickel), high heat, and high pressure, the oil is exposed to hydrogen gas. This process alters some of the double bonds from their natural *cis*-configuration to a *trans*-configuration, while other double bonds become saturated (complete hydrogenation makes a saturated oil and not a *trans*-fat). These hydrogenated fats were found to be cheaper (e.g., the cost of margarine and shortening versus butter and lard), to have a longer shelf life, and higher flash points, which made them ideal for replacing butter and lard for storing and deep frying. Initially, hydrogenated fats were considered a healthy alternative to butter and lard, which were high in saturated fat and cholesterol; based on the erroneous assumption that the hydrogenated fats healthier because they were from vegetable sources and not animal [Asherio and Willet, 1997]. Current research shows that *trans*-fat is actually more harmful than saturated fat [Asherio et al., 1999] and is therefore being taken off the market incrementally.

Anhydrous Milk Fat

Anhydrous milk fat (AMF) considered to be pure milk fat made from butter or cream (i.e., AMF contains at least 99.8% milk fat) is known to have a longer shelf-life than butter at -4 °C (6 months versus 6 weeks), because of lower water activity; and takes up less shelf space due to lack of water. These two qualities, in addition to the way AMF is able to keep the texture and flavor of butter in the product, make it an ideal substitute for butter and to use in an oil blend [Bylund, 2003]. AMF consists mainly of triacylglycerols (TAG) at 97-98% with the remaining 2-3% being made up of diacylglycerols, monoacylglycerols, free fatty acids, free sterols, and phospholipids. TAGs are three fatty acids of varying lengths connected to a glycerol. In AMF the TAGs have such diversity that the melting range is quite broad spanning from -40 to 40 °C (Lopez et at., 2001). Due to AMF's wide melting range most studies have focused on fractionating it into high, mid, and low melting point fractions [El-Rahman et al., 1997; Shukla et al., 1994] and to evaluating the effect on various products (e.g., ice cream, butter, margarine, and chocolate). Though studies have been done on the effects of various fractions of AMF (i.e., high melting-point fraction [HMF]) in bulk or in blends [Martini et al., 2001, 2002a, b], there has not been much research on studying non-fractionated AMF blended with other oils [Martini and Tippetts 2008].

Although AMF does have a small portion of naturally occurring *trans*-fatty acids, it has been shown that naturally occurring TFAs do not lower good HDL cholesterol as industrial TFAs do, nor does it increase the number of LDL particles [Chardigny et al., 2008; McGuire and McGuire, 2000]. These benefits and the fact that AMF keeps the flavor and texture of butter in the end product make it a highly functional component.

Soybean Oil (SBO)

Soybean oil, considered a healthy oil consisting mostly of unsaturated fatty acids, is high in α-linolenic acid (a healthy fatty acid) [Kris-Etherton et al., 2002]. It has been a main source of vegetable oil for consumer use. Manipulated by partial hydrogenation (having an initial melting point approximately – 12 °C), it has also become a room-temperature stable margarine. Though, SBO has many beneficial attributes, it does have a few drawbacks. Since it consists of unsaturated fatty acids, it is susceptible to oxidation, which is considered the primary reason for the deterioration of flavor stability [Lee and Min, 1990].

Docosahexaenoic Acid (DHA)

DHA is an omega-3 polyunsaturated fatty acid (PUFA), which consists of a long chain fatty acid with 22 carbons and 6 double bonds, with the first double bond between the third and fourth carbons. DHA is a desirable component to a food system and makes the product a functional food because of the health benefits attributed to omega-3 fatty acids. Studies have attributed proper neural development, the ability of seeing and learning, and the decrease of incidence of cardiovascular disease, some cancers, diabetes, and other diseases to the intake of DHA [SanGiovanni and Chew, 2005; Kolanowski et al., 1999; Fomuso et al., 2002].

Due to the positive impact of DHA to human health, incorporating this essential fatty acid into various food systems has become wide spread, from yogurt (e.g., Breyers Smart yogurt) to chocolate (e.g., Cocoa Tickles™ by Andes Natural LLC.) and energy bars (e.g., Jennie's Omega-3 Energy Bars by Jennies). However, the flavor of the product is important to maintain when adding omega-3 fatty acids. DHA is known to

create an unpleasant off-odor and flavor usually referred to as fishy [Gonzalez-Esquerra and Leeson, 2000; Kolanowski et al., 2001]. Therefore, extensive sensory studies have also been conducted to find ways of incorporating DHA (e.g., by putting it in the feed for animals or adding it to an already made product) and still have an acceptable product [Kolanowski et al., 1999, 2001; Huang et al., 1990; Romans et al., 1995]. Without consumer acceptance, a product will fail despite its health benefits.

This research combines the above three fats to create a more healthful alternative to using *trans*-fats. They are combined in an emulsion, which decreases the amount of fat and yet maintains the necessary sensory and functional properties. The stability was found to also be a factor of how much oil was in the emulsion, with an oil content of 40% being more stable than emulsions with only 20%.

Emulsifiers

Emulsifiers are compounds, which have a hydrophilic head and a hydrophobic tail. When added to a mixture of two substance which normally repel each other (e.g., water and oil), the emulsifier positions itself at the oil and water interface, creating a barrier and thereby decreasing the surface tension, which permits the two phases to continue in a homogenous mixture versus reverting back to two phases. Common food emulsifiers include lecithin, mono- and diglycerides, polyglycerol esters, polysorbates, sucrose esters, and caseinates. Various emulsifiers (e.g., whey protein isolate, Tween 20, sweet whey, β-lactoglobulin, α-lactalbumin, casein) have been studied to determine their effect on the stability of oil-in-water emulsions using a wide range of concentrations (e.g., 0.2, 0.9, 2.0%) to determine the efficacy of each type of emulsifier with the

different oils (e.g., salmon, fish, and palm oil) [Thanasukarn et al., 2004a, b, 2006; Hu et al., 2003; Faraji et al., 2004; Courthaudon and Dickinson, 1999].

Whey Protein Isolate (WPI)

Many by-products from the effluent of cheese manufacturing have come from separating out caseinates and whey protein (either concentrate or isolate). These by-products (i.e., whey protein concentrate and isolate) of cheese manufacturing have also been recognized as good emulsifiers [Demetriades et al., 1997]. Whey protein isolate (WPI) is considered to be greater than 90% protein, with most of the lactose and lipids from the whey having been filtered out. This can be compared to whey protein concentrate which is 35- 80% protein and contains more lactose and ash. WPI is a good way to use a readily available source, increase the nutritive value of the product and take advantage of a good emulsifier. WPI can be used as a protein source (i.e., nutrition bars, energy drinks, etc.). It has specific conditions at which it can function as an emulsifier, Demetriades et al. (1997) showed that when an emulsion gets close to WPI's isoelectric point (~4.8) it tends to become a viscous paste and syneresis occurs (using 2 wt%); flocculation was seen to occur for pH values 4-6. Therefore, the emulsion needs to have a pH of <4 or >6 for WPI to work effectively; this study used an emulsion at pH 7.28, and therefore far enough away to not induce flocculation. Demetriades also found that as ionic strength increased from 0 to100mM the pH range for flocculation broadens, and the ionic strength best for the use of WPI is between 1-25 mM.

Processing Conditions

After the lipid and emulsifier components of an oil-in-water emulsion have been determined, the next step is to determine how to process them. Processing conditions of emulsions are key components to understanding the relationship between lipid crystallization and emulsion stability. Depending on how an emulsion is created its destabilization rate and mechanism can be predicted [Elizalde et al., 1991]. For a model system, it is necessary to understand the impact of homogenization, cooling, tempering, and storage conditions (i.e., will crystallization occur at given temperatures).

Homogenization and Droplet size

Emulsion droplet size is dependent on homogenization conditions. For instance, emulsions made using only shear forces at atmospheric pressure have a droplet size significantly larger than the droplet size of an emulsion made with an increase in pressure. Droplet size limits how much lipid is available to crystallize in a specific area. The smaller the droplet size, the smaller the lipid crystal formed, which gives added stability to the emulsion. If droplet size is small and uniform, then crystallization is more likely to happen homogenously because the fat will be the nucleation site and not from impurities within the emulsion [Coupland, 2002; Rousseau, 2000]. However, if the droplet becomes too small the fat crystals might break the lamella thereby causing instability through coalescence. Finding a droplet size which allows for fat crystallization, but in which crystals will not break through the o/w interface and cause instability, is a desirable attribute and is directly related to the homogenization processing condition [Rousseau, 2000]. Droplet size is also a factor when considering coverage by the emulsifier. For a given level of fat, smaller droplets have a larger surface area and so

more emulsifier is needed to surround the droplets, produce a stable system, and form a barrier against coalescence [Rousseau, 2000]. Also, droplet size has a large impact on effect of perception and release of volatile compounds [Charles et al., 2000], which will affect the perception of desired or undesired flavors within an emulsion.

Cooling Rate and Crystallization

The cooling rate of an emulsion to a crystallization temperature is important due to the different types of crystal formation, which can affect the smoothness or graininess of margarine, the snap and gloss of chocolate, and the spreadability of butter and margarine. When an emulsion is cooled quickly (e.g., quenching), many small crystals form which are considered unstable and yet rigid. If the emulsion is cooled slowly, then larger but fewer lipid crystals form having had time for the TAGs to adjust and fit together in a preferable uniform lattice and are in a more stable form [Campos et al., 2002; Sato, 2001; Martini et al., 2001, 2002b]. It has been suggested that the less stable form is more rigid and therefore unable to bend within its confined barrier (lamella) and thereby puncture the confinement and cause partial coalescence [Coupland, 2002]. Thus, emulsion stability is influenced by crystalline form produced by either a slow or fast-cooling rate. In this research the effect of cooling rate on the stability of emulsions was evaluated. It was seen that the emulsion's stability increased when the cooling rate was decreased from 30 to 0.2 °C/min.

Though, once the emulsion has gone through the cooling process to crystallization temperature the crystals do not stop either forming or changing. The crystal polymorphic form may change over time at a given temperature (e.g., going from α to β' form or vice versa) [Coupland, 2002]. Crystallization of lipids in oil-in-water emulsions has been well

investigated [Coupland, 2002; Rousseau, 2000; McClements et al., 1993a], but there is a lack of studies comparing the same emulsion with various cooling rates, which might be able to show the differences in crystalline forms and/or fractionation and the possibility that these can also change over time when held at a specific crystallization temperature, this influencing stability and sensory attributes.

Part of the analysis of crystallization is done by using a differential scanning calorimeter (DSC), which is able to be programmed to cool and heat a sample using defined conditions (e.g., cooling a sample from 60 to -10 °C at 0.2°C/min, holding it at -10 °C for 3 h and then heating the sample at 5 °C/min to 80 °C). The DSC measures the change in heat flow. If lipid crystallization occurs (an exothermic reaction [indicated by a positive deviation from the baseline]), the DSC is able to detect the point at which crystallization began, also known as the onset temperature (T_{on}^{c}), the temperature (T_{p}^{c}) at which there was a peak difference from the reference pan, and the change in enthalpy (ΔH^{c}), which is the calculated area under the curve from when the heat flow changed between the reference and the sample. The same can be done for melting a crystallized sample (an endothermic reaction [indicated by a negative deviation from the baseline]).

Sensory

When all is said and done, if the product is not approved of by the consumer, then it has all been for naught (or the application might change). Therefore, it is imperative that when incorporating a new ingredient or creating a new product that a significant amount of research goes into discovering if the public will welcome the new addition to the food/ingredient ranks.

DHA is a compound with highly noticeable sensory characteristics, namely a fish odor and off flavor, which occurs for both plant and animal sources. This means that when DHA is added as an ingredient or to the feed of an animal, sensory tests must be done to ensure that this alteration to the product is not noticeable. For example, studies were done on both chicken and bacon, where the animals were fed various treatments of omega-3 enriched feed. Sensory was then done to determine if there was a significant difference in the flavor with varying results dependant on type of meat and degree of DHA [Huang et al., 1990; Romans et al., 1995]. Huang found that chickens can eat omega-3 fatty acids (up to 3% of their feed) without an off-fishy flavor, if it is stabilized with 0.1 % ethoxyquin, which helps prevent rancidity. Romans found that the many on the consumer panel rated 'dislike' on the pork samples, whose feed had been 15% omega-3 fatty acids. The two studies indicate that the level of DHA is important to consumer acceptance.

Sensory studies have also been done on the incorporation of DHA into spreads (i.e., butter, oil, and margarine combination) and it was found that acceptability was found to be at levels that could increase DHA intake by 0.2-0.3% daily [Kolanowski et al., 2001], which gives hope to those trying to create acceptable *trans*-fatty acid replacements fortified with DHA. In this study, the sensory panel was asked if the fishiness attribute was detectable in the samples with the current formulation, which would indicate if the formulation needed more work or not.

References

Ascherio, A. & Willet, W. C. (1997). Health effects of *trans* fatty acids. American Journal of Clinical Nutrition, 66supp, 1006S-10S.

Ascherio, A., Katan, M.B., Zock, P.L., Stampfer, M.J. & Willet, W.C. (1999). *Trans* fatty acids and coronary heart disease. The New England Journal of Medicine, 340, 1994-1998.

Awad, T. & Sato, K. (2001). Effect of hydrophobic emulsifier additives on crystallization behavior of palm mid fraction in oil-in-water emulsion. Journal of American Oil Chemists' Society, 78, 837-842.

Bylund, G. (2003). Anhydrous milk fat and butteroil. In: Dairy Processing Handbook, 2nd edn. Pp 294-296. Lund, Sweden: Tetra Pak.

Campbell, S.D., Goff, D.H. & Rousseau, D. (2001). Relating bulk-fat properties to emulsified systems: characterization of emulsion destabilization by crystallizing fats. In: Crystallization and Solidification Properties of Lipids (edited by N. Widlak, R. Hartel & S. Narine). Pp 176-189. Champaign, IL: AOCS Press.

Campbell S.D., Goff H.D. & Rousseau, D. (2002). Comparison of crystallization properties of a palm stearin/canola oil blend and lard in bulk and emulsified form. Food Research International, 35, 935-944.

Campbell S.D., Goff H.D. & Rousseau, D. (2004). Modeling the nucleation and crystallization kinetics of a palm stearin/canola oil blend and lard in bulk and emulsified form. Journal of American Oil Chemists' Society, 81, 213-219.

Campos, R., Narine, S.S. & Marangoni, A.G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. Food Research International, 35, 971-981.

Cerdeira, M., Martini, S., Hartel, R.W. & Herrera, M.L. (2003). Effect of sucrose ester addition on nucleation and growth behavior of milk fat-sunflower oil blends. Journal of Agriculture and Food Chemistry, 51, 6550-6557.

Cerderia, M., Pastore, V., Vera, L., Martini, S., Candal, R.J. & Herrera, M.L. (2005). Nucleation behavior of blended high-melting fractions of milk fat as affected by emulsifiers. European Journal of Lipid Science and Technology, 107 (12), 877-885.

Chardigny, J.M., Destaillats, F., Malpuech-Brugere, C., Moulin, J., Bauman, D.E., Lock, A.L., Barbano, D.M., Mensink, R.P., Bezelgues, J., Chaoumont, P., Combe, N., Cristiani, I., Joffre, F., German, J.B., Dionisi, F., Boirie, Y. & Sebedio, J. (2008). Do *trans* fatty acids from industrially produced sources and from natural sources have the same effect on cardiovascular disease risk factors in health subjects? Results of the *trans* fatty acids collaboration (*TRANS*FACT) study. American Journal of Clinical Nutrition, 87, 558-66.

Charles, M. Rosselin, V., Beck, L., Sauvageot, F. & Guichard, E. (2000). Flavor release from salad dressings: Sensory and physicochemical approches in relation with the structure. Journal of Agriculture and Food Chemistry, 48, 1810-1816.

Coupland, J.N. (2002). Crystallization in emulsions. Current Opinion in Colloid and Interface Science, 7, 445-450.

Courthaudon, J. & Dickinson, E. (1991). Competitive adsorption of lecithin and β-casein in oil in water emulsions. Journal of Agriculture and Food Chemistry, 39, 1365-1368.

Demetriades K., Coupland, J.N. & McClements D.J. (1997). Physical properties of whey protein stabilized emulsions as related to pH and NaCl. Journal of Food Science, 62, 342-347.

El-Rahman, A.M., Madkor, S.A., Ibrahim, F.S. & Kilara, A. (1997). Physical characteristics of frozen desserts made with cream, anhydrous milk fat, or milk fat fractions. Journal of Dairy Science, 80, 1926-1935.

Elizalde, B.E., Pilosof, A.M.R. & Bartholomai, G.B. (1991). Prediction of emulsion instability from emulsion composition and physicochemical properties of proteins. Journal of Food Science, 56 (1), 116-120.

Faraji, H., McClements, D.J. & Decker, E. A. (2004). Role of continuous phase protein on the oxidative stability of fish oil-in-water emulsions. Journal of Agriculture and Food Chemistry, 2, 4558-4564.

Fomuso, L.B., Corredig, M. & Akoh, C.C. (2002). Effect of emulsifier on oxidation properties of fish oil-based structured lipid emulsions. Journal of Agriculture and Food Chemistry, 50, 2957-2961.

Gonzalez-Esquerra1, R. & Leeson, S. (2000). Effect of feeding hens regular or deodorized menhaden oil on production parameters, yolk fatty acid profile, and sensory quality of eggs. Poultry Science, 79, 1597-1602.

Gu, Y.S., Decker, E.A. & McClements, D.J. (2007). Application of multi-component biopolymer layers to improve the freeze-thaw stability of oil-in-water emulsion: β-Lactoglobulin-ï-carrageenan-gelatin. Journal of Food Engineering, 80, 1246-1254.

Hu, F. B., Manson, J. E. & Willett, W. C. (2001). Types of dietary fat and risk of coronary heart disease: A critical review. Journal of the American College of Nutrition, 20, 1, 5-19.

Huang, Z., Leibovitz, H., Lee, C.M. & Millar, R. (1990). Effect of dietary fish oil on omega-3 fatty acid levels in chicken eggs and thigh flesh. Journal of Agriculture and Food Chemistry, 38, 743-747.

Insel, P., Turner R.E. & Ross, D. (2006). Discovering Nutrition, 2nd edn. Pp174-175. Boston, USA: Jones & Bartlett Publishers.

Kolanowski, W., Swiderski, F. & Berger, S. (1999). Possibilities of fish oil application for food products enrichment with omega-3 PUFA. International Journal of Food Sciences and Nutrition, 50, 39-49.

Kolanowski, W., Swiderski, F., Lis, E. & Berger, S. (2001). Enrichment of spreadable fats with polyunsaturated fatty acids omega-3 using fish oil. International Journal of Food Sciences and Nutrition, 52, 469-476.

Kris-Etherton, P.M., Harris, W.S. & Appel, L.J. (2002). Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease. AHA Circulation, 106, 2747-2757.

Lee, S. & Min, D. (1990). Effects, quenching mechanisms, and kinetics of carotenoids in chlorophyll-sensitized photooxidation of soybean oil. Journal of Agriculture and Food Chemistry, 38, 1630-1634.

Lopez, C., Lesieru, P., Bourgaux, C., Keller, G. & Ollivon, M. (2001). Thermal and structural behavior of milk fat 2: Crystalline forms obtained by slow cooling of cream. Journal of Colloid and Interface Science, 240 (1), 150-161.

Marangoni, A.G. & McGauley, S.E. (2003). Relationship between crystallization behavior and structure in cocoa butter. Crystal Growth and Design, 3 (1), 95-108.

Martini, S., Cerdeira, M. & Herrera, M.L. (2004). Effect of sucrose esters on the crystallization behavior of bulk oil systems. Journal of American Oil Chemists' Society, 81, 209-211.

Martini, S. & Herrera, M.L. (2000). Crystallization behavior of milk fat model systems: Kinetics and polymorphism. In: Research Advances in Oil Chemistry. Pp.1-23. Killipalam, India: Global Research Network.

Martini, S. & Herrera, M.L. (2008) Physical properties of low-*trans*-shortenings as affected by emulsifiers and storage conditions. European Journal of Lipid Science and Technology, 110, 172-182.

Martini, S., Herrera, M.L. & Hartel, R.W. (2001). Effect of cooling rate on nucleation behavior of milk fat-sunflower oil blends. Journal of Agriculture and Food Chemistry, 49(7), 3223-3229.

Martini, S., Herrera, M.L. & Hartel, R.W. (2002a). Effect of processing conditions on microstructure of milk fat fraction/sunflower oil blends. Journal of American Oil Chemists' Society, 79, 1063-1068.

Martini, S., Herrera, M.L. & Hartel, R.W. (2002b). Effect of cooling rate on crystallization behavior of milk fat fraction/sunflower oil blends. Journal of American Oil Chemists' Society, 79,1055-1062.

Martini, S., Puppo, M.C., Hartel, R.W. & Herrera, M.L. (2002c). Effect of sucrose esters and sunflower oil addition on microstructure of a high-melting milk fat fractions. Journal of Food Science, 67, 3412-3418.

Martini S. & Marangoni, A.G. (2007). Microstructure of dairy fat products. In: Structure of Dairy Products (edited by A.Y. Tamime). Pp. 72-103. Oxford, UK: SDT.

Martini, S. & Tippetts, M. (2008). Crystallization behavior and destabilization kinetics of oil in water emulsions. Journal of American Oil Chemists' Society, 85(2), 119-128.

McClements, D.J., Povey, M.J.W. & Dickinson, E. (1993a). Absorption and velocity dispersion due to crystallization and melting of emulsion droplets. Ultrasonics, 31, 433-437.

McClements, D.J., Dungan, S.R., German, J.B., Simoneau, C. & Kinsella, J.E. (1993b). Droplet size and emulsifier type affect crystallization and melting of hydrocarbon-in-water emulsions. Journal of Food Science, 58, 1148-1178.

McGuire, M.A. & McGuire, M.K. (2000). Conjugated linoleic acid (CLA): A ruminant fatty acid with beneficial effects on human health. Journal of Animal Science, 77, 1-8.

Puppo, M.C., Martini, S., Hartel, R.W. & Herrera, M.L. (2002). Effect of sucrose esters on isothermal crystallization and rheological behaviors of blends of high-melting milk fat fraction and sunflower oil. Journal of Food Science, 67, 3419-3426.

Romans, J. R., Wulf, D. M., Johnson, R.C., Libal, G.W. & Costello, W. J. (1995). Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II. Duration of 15% dietary flaxseed. Journal of Animal Science, 73, 1987-1999.

Rousseau, D. (2000) Fat crystals and emulsion stability – a review. Food Research International, 33, 3-14.

SanGiovanni, J.P. & Chew, E.Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Progress in Retinal and Eye Research, 24, 87-138.

Sato, K. (2001). Crystallization behavior of fats and lipids—a review. Chemical Engineering Science, 56, 2255-2265.

Shukla, A., Bhaskar, A.R., Rizvi, S.S.H. & Mulvaney, S.J. (1994). Physicochemical and rheological properties of butter made from supercritically fractionated milk fat. Journal of Dairy Science, 77, 45-54.

Thanasukarn, P., Pongsawatmanit, R. & McClements, J.D. (2004a). Influence of emulsifier type on freeze-thaw stability of hydrogenated palm oil-in-water emulsions. Food Hydrocolloids, 18 (6), 1033-1043.

Thanasukarn, P., Pongsawatmanit, R. & McClements, J.D. (2004b). Impact of fat and water crystallization on the stability of hydrogenated palm oil-in-water emulsions stabilized by whey protein isolate. Colloids and Surfaces: Physicochemical and Engineering Aspects, 246 (1-3), 49-59.

Thanasukarn, P., Pongsawatmanit, R. & McClements, J.D. (2006). Impact of fat and water crystallization on the stability of hydrogenated palm oil in water emulsions stabilized by a nonionic surfactant. Journal of Agriculture and Food Chemistry, 54, 3591-3597.

Vanapalli, S. A., Palanuwech, J. & Coupland, J. N. (2002a). Stability of emulsion to dispersed phase crystallization: Effect of oil type, dispersed phase volume fraction, and cooling rate. Colloids and Surfaces: Physicochemical and Engineering Aspects, 204, 227-237.

Vanapalli, S. A., Palanuwech, J. & Coupland, J. N. (2002b). Influence of fat crystallisation on the stability of flocculated emulsions. Journal of Agriculture and Food Chemistry, 50, 5224-5228.

CHAPTER III

EFFECT OF OIL CONTENT AND PROCESSING CONDITIONS ON THE THERMAL BEHAVIOR AND PHYSICOCHEMICAL STABILITY OF OIL-IN-WATER EMULSIONS[1]

Abstract

The destabilization mechanism of oil-in-water (o/w) emulsions was studied as a function of oil content (20% and 40% o/w), homogenization conditions, and crystallization temperatures (10, 5, 0, -5 and -10 °C). A mixture of anhydrous milk fat and soybean oil was used as the lipid phase and whey protein isolate (1.8 wt % protein) as emulsifier. Crystallization and melting behaviors were analyzed using differential scanning calorimetry. Physicochemical stability was measured with a vertical scan macroscopic analyzer. Emulsions with 20% oil were less stable than 40% oil. For 20% o/w emulsions, the crystallization was delayed and inhibited in emulsions with smaller droplets and promoted in emulsions with larger droplets when compared to 40% o/w emulsions. Depending on the droplet sizes in the emulsion, the formation of lipid crystals (in combination with the emulsifier) either stabilizes (small droplets) or destabilizes (big droplets) the emulsion.

Introduction

Consumer demand for *trans*-fat free products has increased over the years. Since January 2006, the United States requires *trans*-fat information to be included on nutrition

[1] Co-authored by Megan Tippetts and Dr. Silvana Martini. See Appendix A for copyright clearance. We thank the International Journal of Food Science and Technology for permission to publish.

labels. This requirement was a consequence of the association between *trans*-fatty acids, coronary heart disease (CHD), and the increase of undesirable LDL [Hu et al., 2001; Tarrango-Trani et al., 2006; Aro et al., 1997]. Due to the harmful effects of *trans*-fatty acids, healthy lipid alternatives are being sought. For items such as salad dressings, mayonnaise, and baked goods (where *trans*-fat is prominent), an appropriate fat emulsion substitute is desirable. A possible substitute would be anhydrous milk fat (AMF), which is already known as a butter replacement and can easily be used in an oil blend [Bylund, 2003]. AMF is known to be high in stearic acid, which has been shown to have a neutral effect on CHD, unlike other saturated fats which contribute to CHD and increased levels of LDL [Tarrago-Trani et al., 2006; Aro et al., 1997]. Besides fat composition, AMF has good sensory attributes such as flavor and mouthfeel [Kaylegain et al., 1993]. Blending AMF with vegetable oils (i.e., soybean [SBO]), can decrease the amount of saturated fats while maintaining functional and sensory attributes.

Emulsions are thermodynamically unstable systems. Emulsifiers are used to avoid or delay phase separation and to increase emulsion stability. Whey protein has been found to be an effective stabilizing agent [Kiokias et al., 2007; Thanasukarn et al., 2006]. The combination of AMF, SBO, and whey protein creates a blend of nutritive components to substitute for *trans*-fat in foods. By replacing one ingredient (i.e., *trans*-fat) with another, the quality of the product is at risk. Therefore, it's crucial to understand the substitute's attributes and how they change given various processing conditions. Understanding different aspects of emulsion destabilization leads to creating innovative new products and updating ones that are not meeting consumer demands. Studies have been performed on the effect of crystal formation in o/w emulsions [Coupland, 2002].

However, to the best of our knowledge, very few studies provide a systematic approach to address specific effects of processing conditions, such as crystallization temperature, oil content and homogenization on the stability mechanisms and destabilization kinetics of the emulsion [Marquez et al., 2005].

The aim of this research is to study the effect of oil content and processing conditions (homogenization and crystallization temperature) on the physicochemical stability of oil-in-water emulsions.

Materials and Methods

Emulsions: Oil-in-water emulsions were prepared using a 50 wt% blend of SBO in AMF as the oil phase, and a 2 wt% whey protein isolate (Inpro 90: 90% whey protein isolate (WPI) by Vitalus) solution as the aqueous phase. WPI was used as the emulsifier. It was dissolved in water with sodium phosphate dibasic, 7-hydrate, crystal (0.01M $Na_2HPO_4 \cdot 7H_2O$; pH 7.28), and stirred at room temperature to allow complete dissolution of the protein. The solution was filtered through Whatman 1 filter paper to eliminate any micro particles that might be suspended in the solution which will affect the stability/instability of emulsions. Prior to homogenization the lipid phase was heated at ~60 °C to keep the lipids in a liquid state during the emulsion formation. The water phase was kept at the same temperature to avoid a decrease in temperature when mixing the water and oil phase. For formation of the emulsions, the oil phase was added to the water phase for a total of 50g in a 100mL beaker. Two o/w ratios were used: 40:60 and 20:80 (oil-in-water expressed in weight %), which are oil-in-water ratios commonly used in many lipid based foods such as salad dressings.

Homogenization Process: The oil and water phases were mixed using three conditions. The first consisted of a high shear (HS) homogenization process using an Ultra Turrax (IKA T18 basic) at 18,000 rpm for 1 min. The second condition combined HS followed by a high pressure homogenization step using a Microfluidics Microfluidizer Processor (Model M-110S) at 2,530 ± 230 psi (very low pressure homogenization, VLPH). The third condition was the same as VLPH, except with a pressure of 9,430 ± 230 psi (high pressure homogenization, HPH). The microfluidizer coil was kept at 67 °C to avoid lipid crystallization during emulsion formation.

Crystallization Conditions: Emulsions were held for 3 h at various crystallization temperatures (T_c = 10, 5, 0, -5, -10°C) to ensure complete crystallization of the lipid phase. Crystallization and melting behavior were measured using differential scanning calorimetry. The physicochemical stability of the emulsions was measured using a vertical scan macroscopic analyzer.

Differential Scanning Calorimetry (DSC): The DSC was calibrated with Indium at a heating rate of 5 °C/min. Immediately after homogenization (≤ 5 min), emulsion samples (~5-15 mg) were placed in a weighed, heated DSC pan, then sealed, weighed, and placed in the DSC compartment at 60 °C opposite to an empty weighed reference pan. They were cooled at 30 °C/min to T_c (-10, -5, 0, 5, 10 C), held for 3 h at T_c, and then heated at 5 °C/min to 80 °C. Samples were cooled from 60 °C at 30 °C/min to T_c and held 3 h to induce crystallization of the lipid phase. Finally, samples were heated at 5 °C/min to obtain the melting profile of the crystallized fat. The change in enthalpy (ΔH) of the oil phase for the crystallized and melting peaks were calculated based on sample

weight and percent oil; the onset (T_{on}) and peak (T_p) temperatures of the oil phase were also recorded.

Physicochemical stability: The physicochemical stability of the emulsions was studied using a vertical scan macroscopic analyzer (TurbiScan MA 2000, Sandyhook, CT). TurbiScan consists of a reading head moving along a flat-bottomed cylindrical cell while scanning the entire sample height. The reading head consists of a pulsed near-infrared light source and two synchronous detectors. Only the backscattering (BS) detector, which receives the light backscattered by the product (135 °), was used for data due to the emulsion being opaque. The reading head acquires BS data every 40 μm to a maximum height of 80 mm. The profile obtained characterizes the sample's homogeneity, particle concentration, and mean diameter. The parameters are represented by a curve showing the percentage of BS light as a function of the sample height in mm. The acquisition along the product is repeated with programmable frequency obtaining a superimposition of sample fingerprints, which characterize the stability or instability of the sample (e.g., the more identical the readings, the more stable the system). After forming the emulsions, they were immediately placed in an assay tube and in a 60:40 glycerol/water bath kept at T_c. The cooling rate observed under these conditions was of 30 °C/min. The tube was taken out of the water bath at 10 min intervals for the first hour and 15 min intervals for the second 2 h in which BS measurements were performed. After BS measurements (15 sec) the tube was placed again in the water bath. Emulsions' destabilization kinetics was measured by calculating the variation in BS as a function of time at half the maximum of the BS peak value (i.e., the maximum point at which the BS deviates from the initial reading of the emulsion) with respect to the initial reading. That

is, if the maximum BS value obtained is 50%, then the calculations are made at 25% of BS values.

Droplet Size Distribution: Droplet size distributions for all the emulsions were determined using a Beckman Coulter particle characterization equipment (LS20 Version 3.19, Beckman Coulter Inc.). Isolated droplets were measured with this equipment as evidenced by the lack of flocculation when emulsions were observed under a microscope.

Statistical analysis: Experiments were performed in duplicate or triplicate as necessary. Data reported are the mean and standard deviation values calculated from the replicates. Significant differences were analyzed using a two- or one-way ANOVA test, as appropriate, and a Bonferroni post-test ($\alpha = 0.05$). Statistical analysis was performed using Graph Pad software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results and Discussion

Droplet Size Distribution of Emulsions: Droplet sizes [$D_{3,2}$, the diameter of a sphere with the same volume/surface area ratio as the particle of interest] of 20:80 and 40:60 HS, VLPH, and HPH emulsions are summarized in Table 1. No significant differences ($p < 0.05$) were observed between oil contents (i.e., 20:80 vs. 40:60). However, significant differences ($p < 0.001$) between HS and both VLPH and HPH processing conditions were found**.** Significant differences ($p < 0.001$) were also observed between VLPH and HPH droplet sizes. This size difference had an effect on DSC and TurbiScan results, which will be discussed in their respective sections.

Figure 1 shows the distribution of droplet diameters with respect to volume percent (Vol. %) for each oil content and processing condition. HS emulsions (Figures 1a

Table 1: **Droplet Size [$D_{3,2}$] of emulsions (i.e., high shear [HS], very low pressure and high pressure homogenization [VLPH and HPH, respectively]) formulated with anhydrous milk fat and soybean oil**

$D_{[3,2]}$ in Microns (± SE)			
Formulation	**HS**	**VLHP**	**HPH**
20:80	13.42 (1.18)[a]	0.90 (0.06)[b]	0.42 (0.02)[c]
40:60	14.22 (1.92)[a]	0.91 (0.03)[b]	0.47 (0.04)[c]

Note: Values with the same superscript have no significant difference ($p < 0.001$).

and 1d) have the largest diameters; droplets larger than 10μm represent most of the emulsion's volume. Droplet profiles for this processing condition were skewed to the left, meaning the population of smaller droplet sizes represents only a small percentage of the emulsion's volume. VLPH emulsions (Figures 1b and 1e) are represented by a broad droplet-size distribution without a central peak at any given diameter, meaning the volume of the emulsion is represented by different droplet sizes ranging from 0.1 to 10μm. HPH emulsions (Figures 1c and 1f) presented two narrow populations of droplets; the first peak, consisting of smaller droplet sizes, is larger than the second peak, which is made up of bigger droplet sizes (~ 2 μm). These droplet sizes can be compared to typical food emulsions droplet sizes, which range from 0.1 to 50 μm for foods such as milk, mayonnaise, butter and margarine (Shroder et al. 1998).

Changing the oil content volume from 20 to 40 % had little effect on droplet size. Figures 1a and 1d show the differences in droplet sizes observed for 20:80 and 40:60 HS emulsions, respectively. Increasing oil content to 40% produced more droplet diameters less than 10 μm. Emulsions with 20% oil have a sharp peak with a defined tail to the left, while the 40:60 emulsion has a gradual decline to the left with a broader droplet size distribution. This suggests that in the 20:80 emulsion there was a large population at

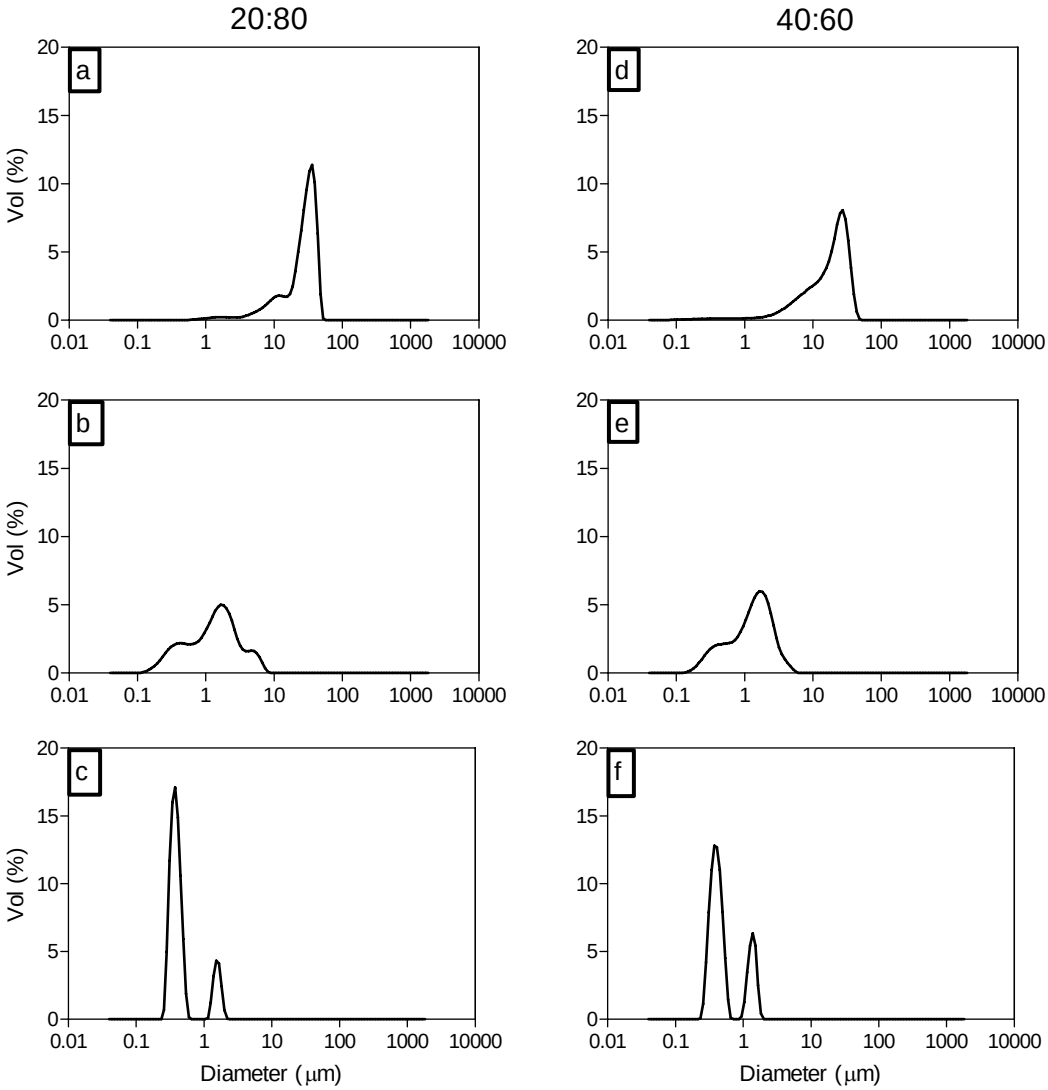

Figure 1: Droplet size distribution of all emulsions. a) 20:80 HS, b) 20:80 VLHP, c) 20:80 HPH, d) 40:60 HS, e) 40:60 VLHP, f) 40:60 HPH

approximately 50 μm and a smaller population of droplets at 10 μm. Differences between VLPH emulsions (Figures 1b and 1e) were also observed; those formulated with a 20% oil phase have a balanced diameter range, including broad tails on both sides of the droplet size distribution. However, only one tail was observed for emulsions with 40% oil, which indicates that the droplet size distribution range is approximately the same.

Lastly, when comparing oil content in HPH emulsions (Figures 1c and 1f), the 20:80 emulsion has about an 80 to 20% ratio of the larger peak (smaller droplets) to the smaller peak (bigger droplets), while the ratio for 40:60 is closer to 67 to 33%. Therefore, although both emulsions have a greater abundance of smaller droplets, there are a greater proportion of bigger droplets in the 40:60 emulsion's volume than in the 20:80. From these results, we can conclude that the droplet size distribution of oil-in-water emulsions depends not only on the processing conditions, but also on the emulsion's oil content. Particular tendencies were not found that could predict droplet-size distribution behavior or range. However, a shift towards smaller droplet sizes occurs as the processing conditions increase in the shear force applied to the emulsion. In addition, given a constant processing condition, an increase in oil phase seemed to decrease the amount of smaller droplets for HS and HPH emulsions. The opposite effect was observed for VLPH, where bigger droplets did not play as important a role in the total volume of the emulsion.

Crystallization: DSC crystallization parameters (i.e., onset [T_{on}^{c}] and peak [T_{p}^{c}] temperatures, and enthalpy [ΔH^{c}]) are given as a function of crystallization temperature (T_c) in Figure 2 for emulsions with 20 and 40% oil phase and homogenized under the

different processing conditions. DSC crystallization profiles (Figure 3a) for 40% HS emulsions exhibited two crystallization peaks for T_c = 0, -5, and -10 °C while 20% HS emulsions showed two crystallization peaks only for -10 and -5 °C. The rest of the processing conditions, and crystallization temperatures showed one crystallization peak. For clarity, data reported in this paper focuses on comparing the first crystallization peak of emulsions. Figures 2a and 2d show the differences in T_{on}^c between HS and both VLPH and HPH emulsions. Data show that differences in droplet size as a function of homogenization process affect T_{on}^c values. That is, the smaller the droplet size the lower the T_{on}^c, indicating a delay in crystallization for emulsions with smaller droplets. For example, HS emulsions have a T_{on}^c around 8 °C, while VLPH emulsions begin to crystallize around 2 °C, which shows that both emulsions crystallize but that VLPH emulsions take a longer time and do not crystallize until reaching a lower temperature. Therefore, with VLPH and HPH emulsions having smaller droplets, greater supercooling (i.e., delaying the T_{on}^c) is needed for the onset of nucleation for crystallization to occur [Hartel, 2001; Vanapalli et al., 2002]. The delay in crystallization would explain why no crystallization was observed at T_c = 10 and 5 °C for VLPH and HPH emulsions, while some crystallization was observed at T_c = 5 °C for HS emulsions.

Significant differences ($p < 0.01$) were found between HS and both VLPH and HPH T_{on}^c and T_p^c between 0 and -10 °C for both 20:80 and 40:60 emulsions. Significant differences ($p < 0.01$) were observed for 20:80 emulsions between VLPH and HPH T_{on}^c between 0, -5 and -10 °C. For T_p^c (Figures 2 b and e), the only significant difference was found at -10 °C for 20:80 ($p < 0.05$). Note that though the difference between T_p^c values is negligible at 0 °C, it increases as T_c decreases, suggesting that processing conditions

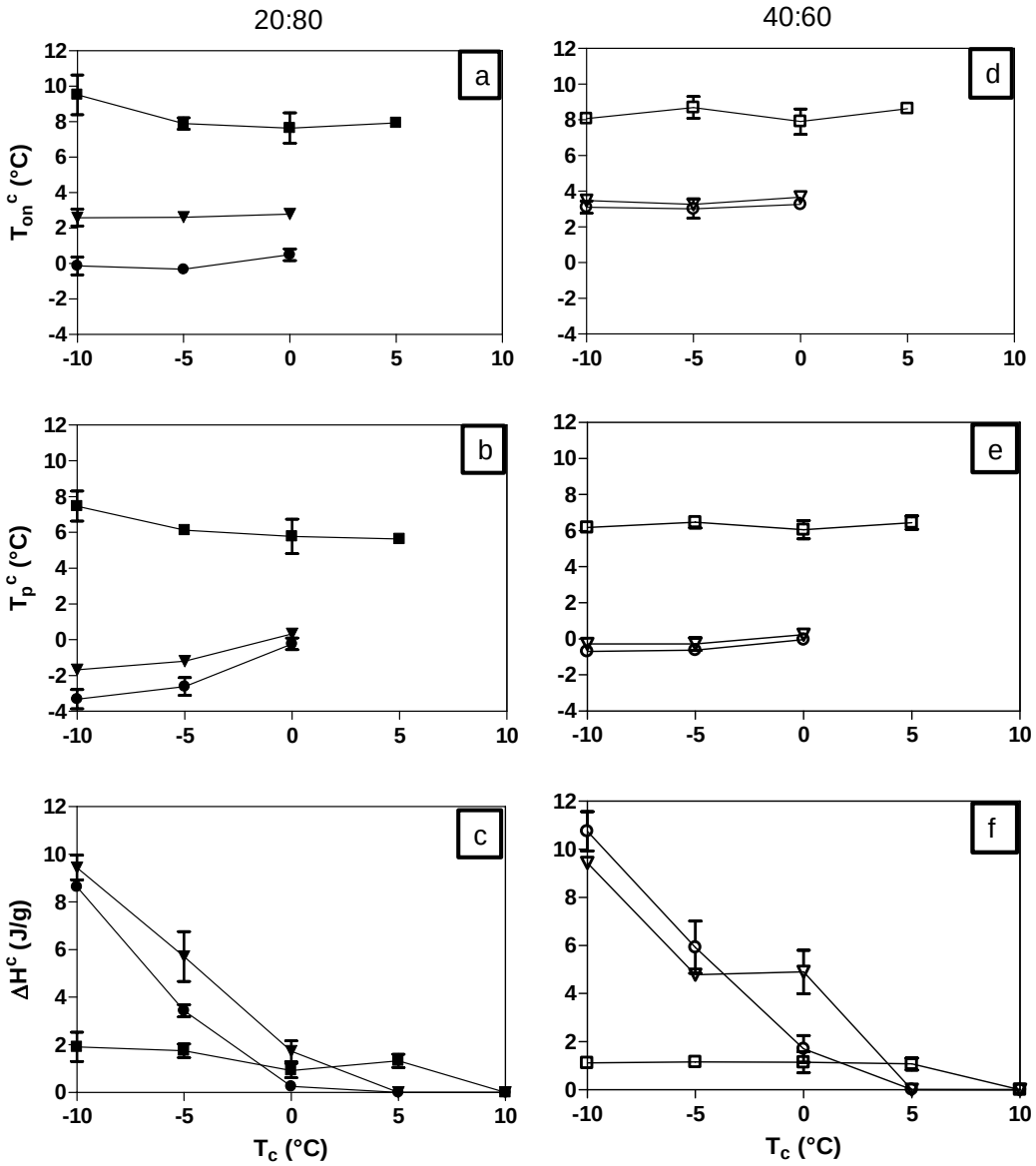

Figure 2: DSC crystallization parameters: onset (T_{on}^{c}) and peak (T_p^c) temperatures, and the change in enthalpy (ΔH^c). 20:80 o/w (a-c) solid symbols, 40:60 o/w (d-f) open symbols. HS ■, VLHP ▼, HPH ●: HS = high shear homogeneization conditions; VLPH = very low pressure homogenization condition; and HPH = high pressure homogenization condition.

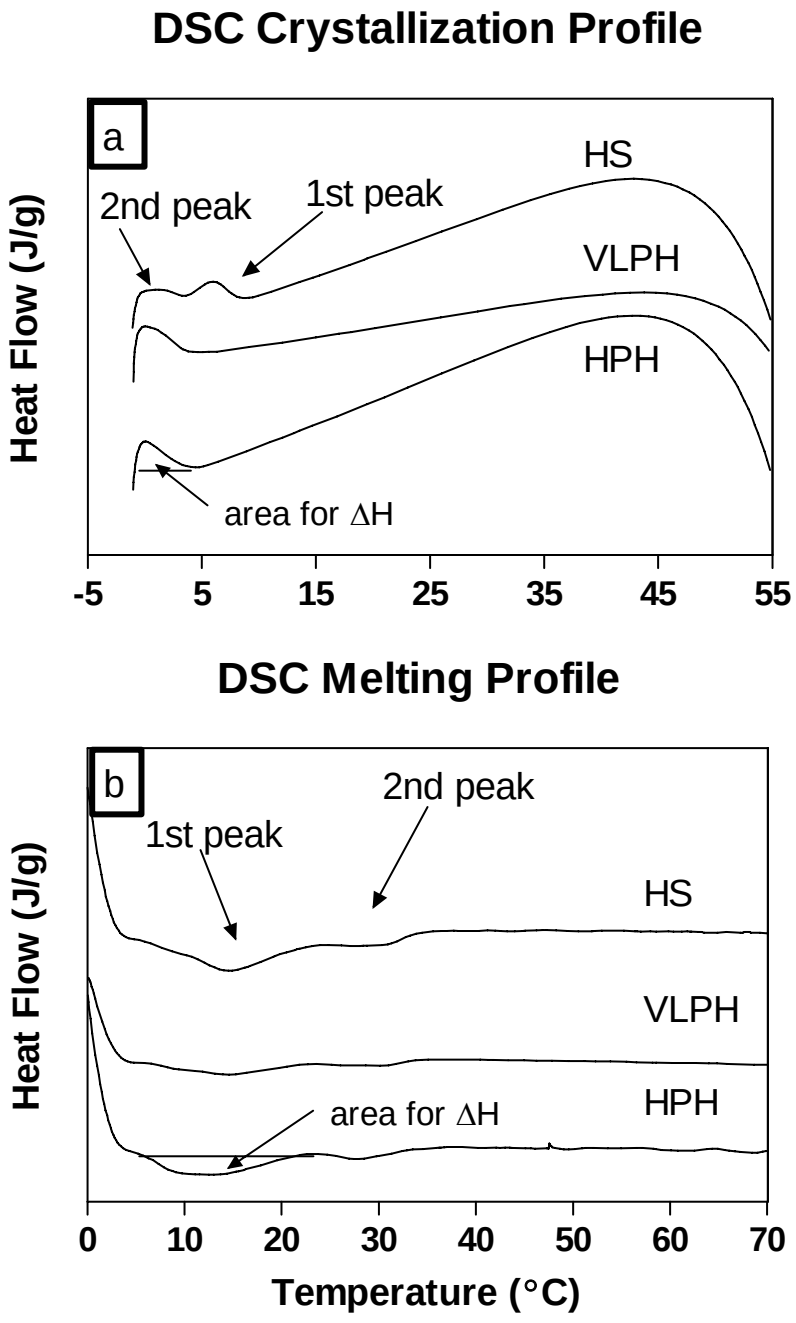

Figure 3: DSC profiles of 40% HS, VLPH and HPH emulsions crystallized at 0 °C; (a) crystallization profiles and (b) melting profiles after being held at T_c for 3h.

(i.e., T_c), affect T_p^c. No significant differences were found in T_{on}^c and T_p^c between 40:60 VLPH and HPH emulsions Also, no significant differences were found between oil contents (i.e., 20:80 vs. 40:60) for T_{on}^c values of HS and VLPH emulsions. Significant differences ($p < 0.01$) were observed between oil contents for HPH emulsions' T_{on}^c and T_p^c ($p < 0.05$) for T_c = -5 and -10 °C with 40:60 HPH having higher T_{on}^c and T_p^c than 20:80. Finally, significant difference ($p < 0.01$) was found between oil contents of VLPH emulsions'; T_p^c at -10 °C, again 40:60 was seen to have a higher temperature. These findings indicate that oil content affects the T_{on}^c and T_p^c. As the amount of the oil phase decreases (i.e., from 40 to 20%) and the droplet size becomes smaller (HS vs. VLPH vs. HPH), the T_{on}^c and T_p^c values decrease, especially for high supercooling (lower T_c) indicating a delay in the crystallization of the lipid phase (Figures 2a-b, 2d-e).

Figures 2c and 2f show ΔH^c values obtained from the DSC crystallization profiles. The ΔH^c values are directly proportional to the amount of fat crystallized, and the fat's chemical composition is consistent throughout all of the emulsions, therefore it is possible to compare the ΔH^c of HS, HPH and VLPH emulsion.

When comparing solely the first crystallization peaks, the HS enthalpies were significantly lower than VLPH and HPH enthalpies ($p < 0.01$) for -5 and -10°C. ΔH^c values for HS emulsions were not significantly affected by either T_c or the amount of oil present in the emulsion. However, by adding the second HS crystallization peak enthalpies to the first (data not shown), HS enthalpy values followed a similar trend when compared to the VLPH and HPH values: ΔH^c values increased in emulsion and T_c = 0 and -10 °C with no significant differences observed between homogenization conditions. No significant differences were found in ΔH^c values between emulsion formulated with

different oil content and processed under different conditions with the exception of 40:60 VLPH emulsion at T_c = 0 °C. A significant difference ($p < 0.05$) was found between the 20:80 and 40:60 VLPH emulsions at 0°C, and between 40:60 VLPH and both HPH and HS ($p < 0.01$) emulsions.

A correlation was found for the ΔH^c values of HPH (both 20 and 40% oil) and 20:80 VLPH emulsions between T_c = 0 and -10°C. The higher the crystallization temperature, the lower the enthalpy observed. Significant differences were found ($p < 0.01$) between various T_c for each type of emulsion. For 40:60 VLPH, no significant difference was found between 0 and -5 °C, but a significant difference ($p < 0.01$) was still observed between -5 and -10 °C. ΔH^c values slightly changed as a function of the amount of oil in the emulsion. Total enthalpies found for 20:80 HS emulsions were slightly higher than those obtained for 40:60. For VLPH homogenization conditions, both 20:80 and 40:60 had similar ΔH^c values. However, for HPH emulsions, ΔH^c values obtained for 20:80 emulsions were lower than those obtained for 40:60. This suggests that for emulsions with big droplets (HS), even if crystallization is neither induced nor delayed by the amount of oil in the emulsion (Figures 2 a and d), crystal growth is promoted for lower oil contents. For emulsions with smaller droplets (VLPH and HPH), crystallization is not only delayed, as evidenced by the lower T_{on}^c values, but also inhibited in emulsions prepared with lower amounts of oil. This delay and inhibition are more evident for the HPH emulsions.

Melting: Two melting peaks were observed for all processing conditions (i.e., HS, VLPH and HPH) and oil content (Figure 3b). Figure 4 shows DSC parameters (i.e., T_{on}^m,

T_p^m and ΔH^m) of the first melting peak as a function of T_c for 20:80 and 40:60 o/w emulsions homogenized under different processing conditions.

No DSC values were recorded for 20:80 HS and 20:80 HPH at -10 °C (Figure 4a-c) in contrast to 20:80 VLPH and all 40:60 emulsions (Figure 4d-f). This is due to a melting peak of ~200 J/g attributed to water crystallization (which has a latent melting heat of 334 J/g when not in an emulsion) that masked the melting of the lipid phase. A possible explanation for the water freezing in the 20:80 HS emulsion is the large-droplet size and low stability (see Figure 6a). The emulsion sample (~15mg) would most likely have separated quickly in the DSC pan giving the separated water time to react independently at T_c (-10 °C). On the other end, with the 20:80 HPH emulsion freezing at -10 °C, a definite uniformity of small droplet sizes was observed (Figure 1f, Table 1) throughout the emulsion. These small droplets might act as nucleation sites for the water to crystallize. 20:80 VLPH emulsions did not freeze since the emulsion is more stable; therefore, the phase separation is delayed. In addition, the droplets are big enough to "fail" as a nucleation site for the water to crystallize.

Figure 4 shows that no melting peaks were observed for 20:80 VLPH emulsions (Figures 4a-c) at T_c = 5 and 10 °C suggesting the lack of crystallization even after 3 h. On the other hand, 40:60 VLPH emulsions did present a melting peak at both 5 and 10 °C indicating that emulsion oil content affects the crystallization behavior of the lipid. In this case, the lower proportion of oil delayed crystallization in the lipid phase to a greater extent, which is in accordance with the crystallization T_{on}^m described previously. The same behavior was observed for HPH samples; however, due to the smaller droplets the

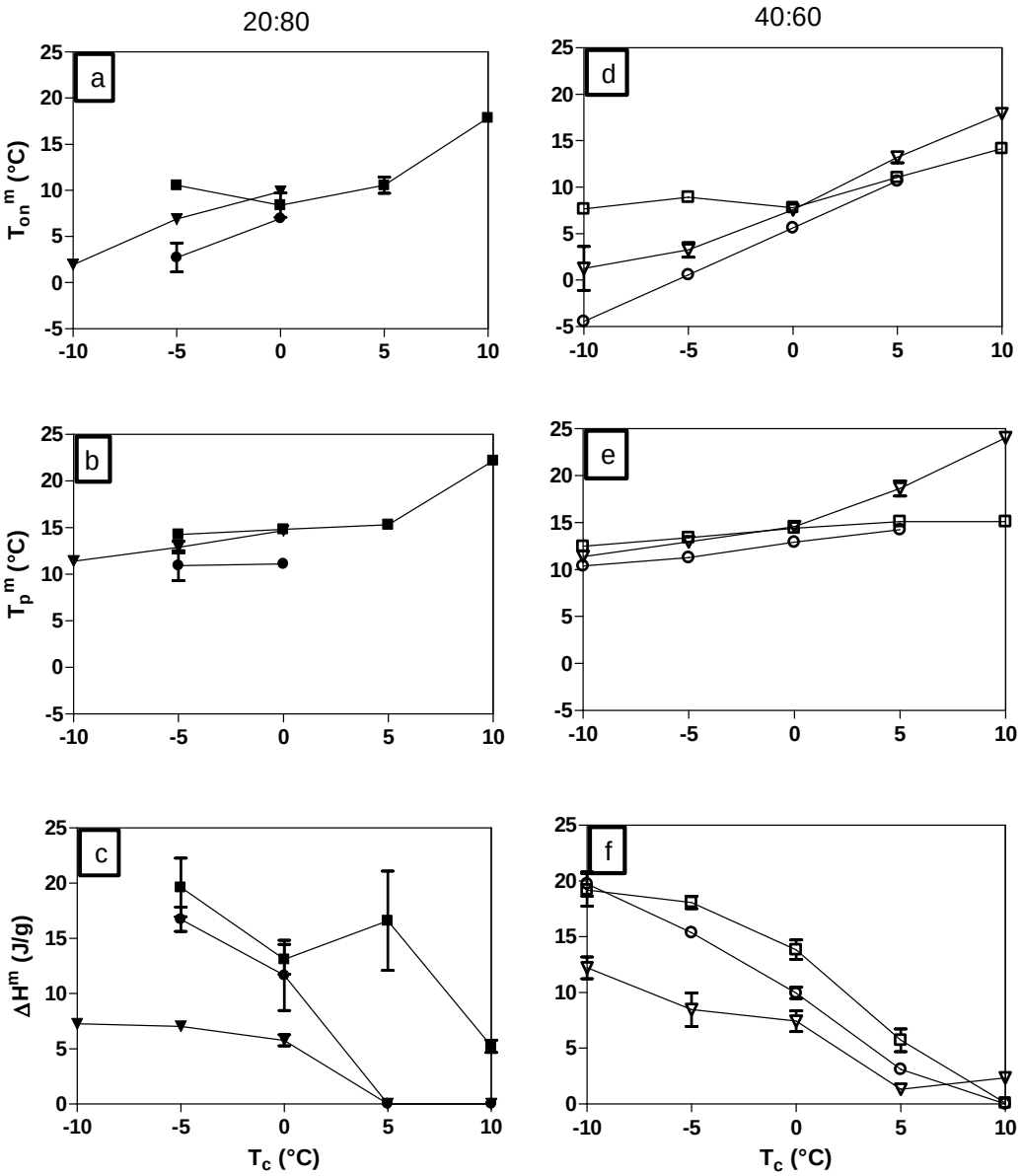

Figure 4: DSC melting parameters: onset (T_{on}^{m}) and peak (T_p^{m}) temperatures, and the change in enthalpy (ΔH^{m}). 20:80 o/w (a-c) solid symbols, 40:60 o/w (d-f) open symbols. HS ■, VLHP ▼, HPH ●. HS = high shear homogeneization conditions; VLPH = very low pressure homogenization condition; and HPH = high pressure homogenization condition

inhibition of crystallization was even more pronounced as evidenced by the lack of a melting peak at 10 °C for the 40:60 emulsions.

A positive correlation between T_{on}^{m} and T_c was observed for 20:80 VLPH emulsions (Figure 4a) showing a significant increase ($p < 0.05$) in T_{on}^{m} values as T_c increases. No difference was found for T_{on}^{m} values in HS emulsions crystallized from 5 to -5 °C and a significantly higher value of T_{on}^{m} was observed when HS emulsions were crystallized at 10 °C. The same behavior was observed for both 20 and 40% oil; however, T_{on}^{m} values at 10 °C were significantly higher for 20:80. These results agree with the previous discussion about how crystal growth is promoted in the lipid phase for emulsions with larger droplet sizes and prepared with lower amount of lipids.

Peak temperatures followed the same pattern (Figures 4b and 4e) observed for T_{on}^{m}. T_{on}^{m} and T_p^{m} for the different oil contents and processing conditions seemed to have the following tendency: in general, the smaller the droplet size, the lower the melting T_{on}^{m} and T_p^{m} values. An exception to this behavior was found at $T_c = 5$ and 10 °C for the 40:60 VLPH emulsion, and at 0 °C for the 20:80 emulsion. Enthalpy values shown in Figures 4 c and f decreased with increasing T_c for all samples and processing conditions. An exception to this behavior is found in 20:80 emulsions homogenized under VLPH conditions. This sample reaches a plateau for $T_c < 0$ °C indicating that the lipid phase is completely crystallized in this sample and no further crystallization occurs even when T_c decreases. Finally, VLPH emulsions had the least amount of enthalpy for both 20:80 and 40:60 emulsions. When comparing the crystallization and melting behavior of the different emulsification conditions (HS, VLPH and HPH), results suggest that the crystallization is delayed and inhibited as the droplet size decreases. Even though

VLPH emulsions had a slightly bigger droplet size than HPH emulsions, the crystallization was delayed in greater proportion in VLPH. This might be due to the broad droplet size distribution found in VLPH emulsions. That is, VLPH emulsions are composed of smaller droplets that even though they do not represent a significant proportion of the emulsion volume (Figure 1 b and e), they are significant in number (data not shown). These small droplets might be responsible for the unexpected inhibition of the crystallization.

The T_{on}^{m}, T_{p}^{m} and enthalpy values obtained from the second melting peak are not shown because differences between processing conditions and oil contents were not significant. When analyzing the total melting enthalpy values (first and second melting peaks) slight differences were found in emulsions with different oil contents and homogenization conditions. Samples formulated with 20% oil and processed under HS conditions resulted in slightly higher enthalpy values when compared with the 40% oil. As described for the crystallization behavior, total enthalpies observed for the VLPH emulsions were not significantly different between 20 and 40% oil emulsions. However, for HPH emulsions the total enthalpy value was higher for emulsions formulated with 40% oil than for those with 20% oil. This suggests that oil content and homogenization conditions are important variables in the crystallization of lipids in emulsions. That is, lowering oil content and decreasing droplet size inhibits lipid crystallization.

Emulsions' Destabilization Profiles: Figure 5 shows the change in backscattering profiles for both oil contents with various types of processing conditions for emulsions crystallized at 0 °C for 3 h. Similar destabilization trends were found in this study for both 20 and 40% oil contents under different homogenization conditions. HS emulsions

were the most unstable (Figures 5a and d). Large clarification peaks were seen at the bottom of the tube (i.e., tube ht of ~10-35 mm for 20:80 HS and ~10-20 mm for 40:60 HS), while creaming peaks were found at the top of the tube (i.e., tube ht of ~35-45 mm for both 20:80 and 40:60 HS). Comparison between Figures 5 a and d show a greater destabilization in 20:80 HS emulsions compared to 40:60 emulsions as indicated by the larger clarification profile at the bottom of the tube, which is narrower and confined for 40:60 HS (Figure 5d). This is a clear indication that the emulsion stability is affected by the emulsion's oil content. Considering the above discussion, the lower stability found in 20:80 emulsions (Figure 5) might be due to the higher amount of crystallized lipid in this system [Campbell et al., 2001; Coupland, 2002]. In general, the destabilization mechanism for VLPH and HPH emulsions involved a sedimentation phenomenon at the bottom of the tube and clarification at the top. The only exception to this rule was VLPH emulsions crystallized at 5 and 10 °C. These emulsions were destabilized through clarification at the bottom of the tube as a result of a creaming mechanism. The lack of crystals in VLPH emulsions at 5 and 10 °C (Figures 2 and 4) indicates a possible decrease in the density of the droplets, which therefore migrate to the surface of the solution resulting in a creaming phenomenon. The oil phase volume also affected stability for VLPH and HPH emulsions (Figures 5b-c, 5e-f). Though both emulsions appear to be similarly stable, there is a greater BS percent difference for 20:80 emulsions than for 40:60 emulsions.

Figure 6 shows the destabilization kinetics at different T_c expressed as the thickness variation of the separating layer as a function of time (i.e., t = 0-180 min) and as the difference from the reference point (i.e., the initial reading at t = 0 min). Figure 6 a

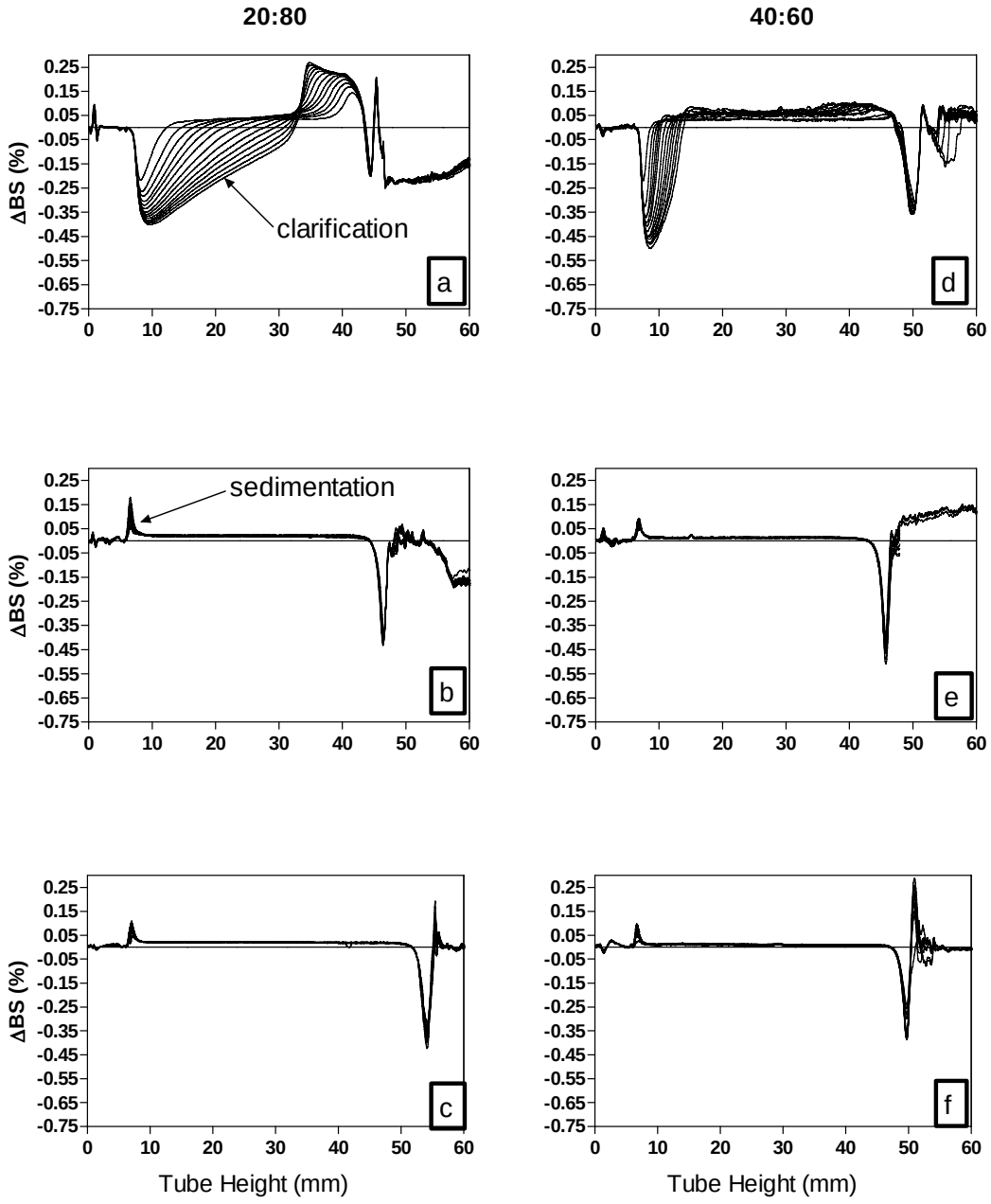

Figure 5: BS profiles of emulsions: the change in BS difference from initial reading as a function of tube height and repeated every 10 min for the first hour and every 15 min for the last 2 h. HS (a & d), VLPH (b & e), HPH (c & f). 20:80 means 20% of oil-in-water emulsions. 40:60 means 40% of oil-in-water emulsions. HS means high shear homogeneization conditions, VLPH means very low pressure homogenization condition, and HPH means high pressure homogenization condition.

and d show the destabilization kinetics for HS emulsions formulated with 20 and 40% oil, respectively. The thickness of the separating layer constantly increased as a function of time for both oil contents. However, it is evident from these figures that 20:80 emulsions were significantly more unstable than 40:60, especially at higher T_c. Also, as expected, the destabilization of the emulsions was faster for higher temperatures. The higher destabilization kinetics observed for 20:80 emulsions can be attributed to more crystallized material present in the oil droplets. Destabilization kinetics for VLPH and HPH emulsions presented greater variability than HS emulsions; however, the thickness of the separating layer is significantly lower when compared to HS emulsions (Figures 6 b, c, e and f). The higher stability in VLPH and HPH emulsions is caused by the smaller droplets.

Several observations can be made from these homogenization conditions. Figures 6 b and e show that VLPH emulsions formulated with 20% oil are more unstable than 40:60 emulsions, especially when crystallized at 0, -5 and -10 °C as evidenced by the higher values of the change in BS observed. When crystallized at -10 °C, emulsions formulated with 20% of oil froze after approximately 10 min at T_c. Although the same behavior was observed for the emulsion with 40% of oil, freezing did not occur until after 60 min at T_c. On the other hand, VLPH emulsions formulated with 20% oil crystallized at 10 and 5 °C were more stable than when the same emulsion is crystallized at other T_c and than 40% oil VLPH emulsions crystallized at the same T_c. This might be due to the lack of crystals in the 20% oil emulsion as evidenced by the DSC parameters discussed above (Figures 4 c and f).

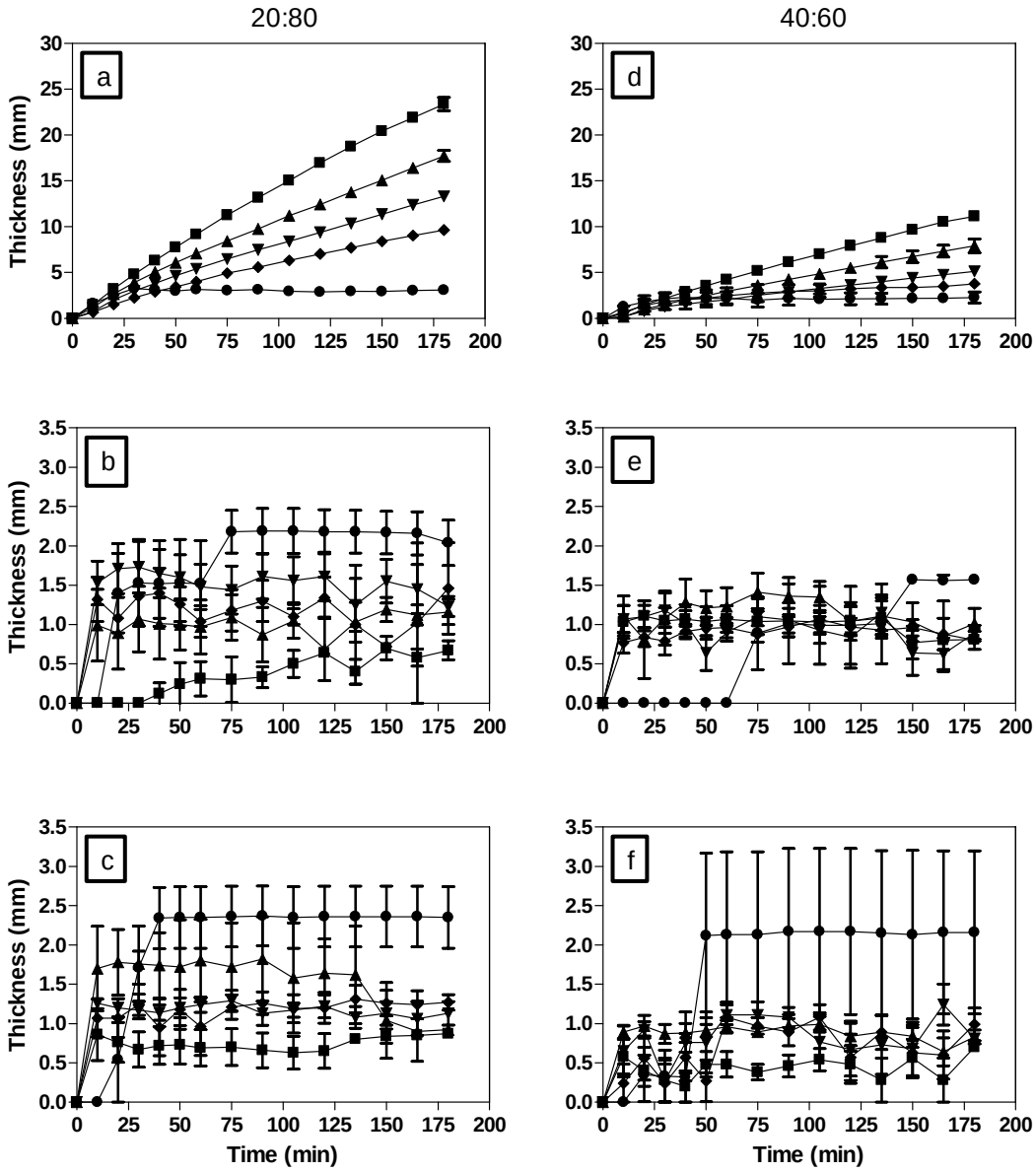

Figure 6: Thickness of separated layer. T_c = ■, 10 °C; ▲, 5 °C; ▼, 0°C; ◆, -5 °C; ●, -10 °C; 20:80 o/w emulsions (a-c) and 40:60 o/w emulsions (d-f); HS (a and d); VLPH (b and e); and HPH (c and f). Error bars are SE. Note: graphs b-c, e-f are at a different scale to show the differences between HPH and VLPH emulsion destabilization, which would have been undifferentiated if on the scale of figures a and d.

Finally, Figures 6c and 6f compare the destabilization kinetics of 20:80 and 40:60 HPH emulsions. For both samples, freezing of water is observed at -10 °C, though for 40:60 it occurs 50 min after being in T_c vs. 20:80, which only took 20 min to freeze. Emulsions formulated with 40% oil are again more stable than emulsions formulated with 20% oil. This is opposite to the expected result since crystallization was induced in the 40% samples as described before, and therefore these emulsions are expected to be less stable. Thus for emulsions stabilized with WPI, important factors that need to be taken into account when evaluating the stability of emulsions are droplet size, lipid crystallization, and the interaction between these variables.

Conclusion

For emulsions prepared with a constant emulsifier the oil content, homogenization conditions, and crystallization temperature affect the destabilization mechanism of emulsions. As expected, larger droplets result in less stable emulsions. However, for same droplet sizes, oil content also plays an important role. A larger lipid phase volume in the emulsion resulted in a more stable system. The increased stability can be explained by the amount of crystallized lipid in the emulsion. For larger droplet sizes, lower amounts of lipid phase result in less stable emulsions due to a higher amount of crystallized material, as evidenced by a higher total melting enthalpy. On the other hand, for emulsions with smaller droplets, the amount of crystallized material increases (higher total melting enthalpy) with the amount of oil in the emulsion suggesting, in this case, that the presence of crystals stabilized the system. Therefore, a combination of droplet size and crystal formation is responsible for the stability of the emulsion. These variables show the importance of understanding the destabilization mechanism. Figure 7

summarizes the effect of processing conditions and emulsion oil content on the stability of oil-in-water emulsions with a constant emulsifier concentration. This summary is independent from the study of the surface coating on the droplet interface. Further research needs to be accomplished on the degree of protein coating in relationship to droplet size. The research would be able to determine if there is enough protein to encapsulate the droplet, which would either help keep the droplets suspended or cause partial coalescence of the droplets. The formation of large fat crystals inside emulsions' droplets results in punctuation of the lamella, inducing partial coalescence in the emulsion (Rousseau, 2000; Coupland, 2002). However, for emulsions with small droplets, only small fat crystals are formed through a homogeneous nucleation (Hartel, 2001), which in combination with the emulsifier, stabilizes the interface of the emulsion droplets (Rousseau, 2001). Therefore, for emulsions stabilized with the same emulsifier, factors that affect the crystallization of fat inside the lipid droplets (such as the droplets sizes) directly affect the stability of the emulsion.

Acknowledgements:

Financial support for this research was provided by the Utah Agricultural Experiment Station (AES-UTA00253), and by NFRG-USU. Approved as paper N° 7927. Ms. Tippetts is a Gandhi Fellowship and a Seely-Hinckley Scholarship recipient. The authors would like to thank Kraft Foods Inc. for donating the AMF.

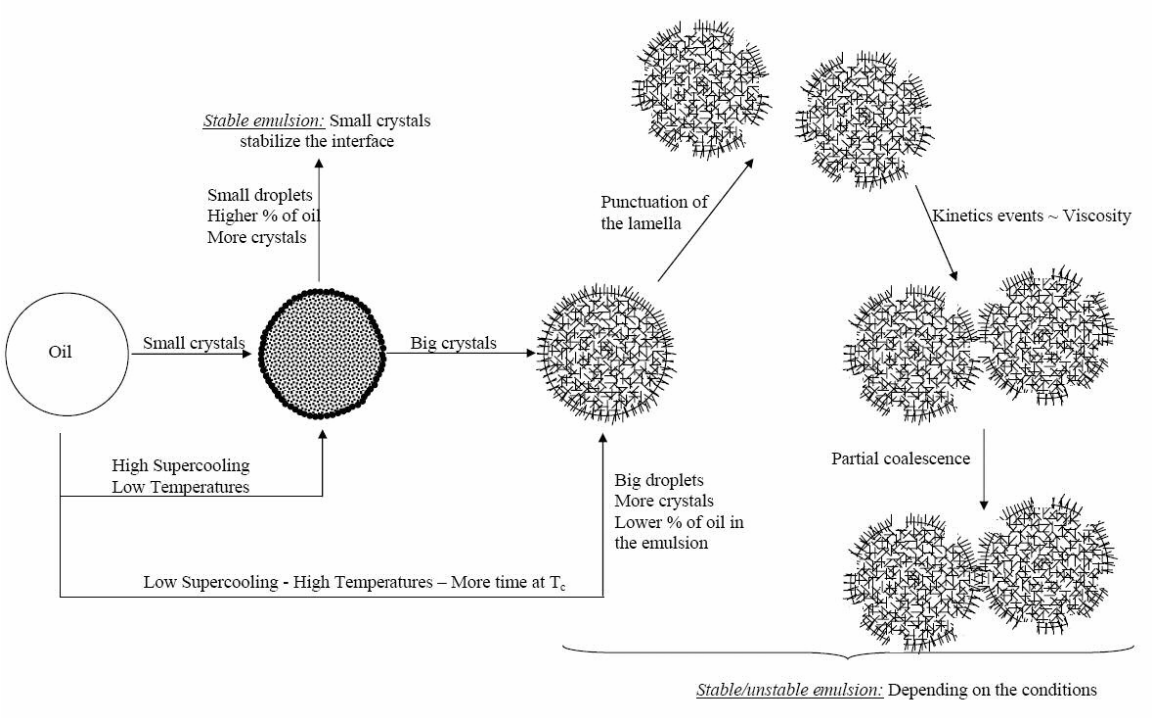

Figure 7: **Proposed destabilization mechanism as a function of processing conditions and oil content for emulsions formulated with a constant emulsifier**

References:

Aro, A., Jauhiainen, M., Partanen, R., Salminen, I. & Mutanen, M. (1997). Stearic acid, *trans* fatty acids, and dairy fat: effects on serum and lipoprotein lipids, apolipoproteins, lipoprotein (a), and lipid *trans*fer proteins in health subjects. American Journal of Clinical Nutrition, 65, 1419-26.

Bylund, G. (2003). Chapter 13: Anhydrous milk fat and butteroil. In: Dairy Processing Handbook, 2nd edn. Pp 294-296. Lund, Sweden: Tetra Pak.

Campbell, S.D., Goff, D.H. & Rousseau, D. (2001). Relating bulk-fat properties to emulsified systems: characterization of emulsion destabilization by crystallizing fats. In: Crystallization and Solidification Properties of Lipids (edited by N. Widlak, R. Hartel & S. Narine). Pp 176-189. Champaign, IL: AOCS Press.

Coupland, J. N. (2002). Crystallization in emulsions. Current Opinion in Colloid and Interface Science, 7, 445-450.

Hartel, R. W. (2001). Chapter 5: Nucleation. In: Crystallization in Foods. Pp. 145-191. Gaithersburg, MD: Aspen Publishers.

Hu, F. B., Manson, J. E., & Willett, W. C. (2001). Types of dietary fat and risk of coronary heart disease: a critical review. Journal of the American College of Nutrition, 20, 1, 5-19.

Kaylegian, K. E., Hartel, R. W. & Lindsay, R. C. (1993). Applications of modified milk fat in food products. Journal of Dairy Science, 76, 1782-1796.

Kiokias, S., Dimakou, C. & Oreopoulou, V. (2007). Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions. Food Chemistry, 105, 94-100.

Marquez, A., Palazolo, G. & Wagner, J. (2005). Creme-like emulsions prepared with soy-milk 1: stability and formulation. Grasas y Aceites, 56 (1), 59-66

Rousseau, D. (2000). Fat crystals and emulsion stability – a review. Food Research International. 33, 3-14.

Tarrago-Trani, M. T., Phillips, K. M., Lemar, L. E., & Holden, J. M. (2006). New and existing oils and fats used in products with reduced *trans*-fatty acid content. Journal of the American Dietetic Association, 106, 867-880.

Thanasukarn, P., Pongsawatmanit, R. & McClements, D. J. (2006). Impact of fat and water crystallization of the stability of hydrogenated palm oil-in-water emulsions stabilized by a nonionic surfactant. Journal of Agriculture and Food Chemistry, 54, 3591-3597.

Vanapalli, S. A., Palanuwech, J. & Coupland, J. N. (2002). Influence of fat crystallization on the stability of flocculated emulsions. Journal of Agriculture and Food Chemistry, 50, 5224-5228.

CHAPTER IV

EFFECT OF COOLING RATE

Abstract

The effect of cooling rate on the destabilization mechanism of oil-in-water (o/w) emulsions was studied as a function of oil content (20% and 40% o/w), homogenization conditions, and crystallization temperatures (10, 5, 0, -5 and -10 °C). The lipid phase was a mixture of anhydrous milk fat and soybean oil with whey protein isolate (1.8 wt % protein) as emulsifier. Differential scanning calorimetry was used to analyze the crystallization and melting behaviors; while a vertical scan macroscopic analyzer measured the physicochemical stability. Emulsions with 20% oil and a slow-cooling rate were more stable than those with 40% oil. The onset of crystallization was promoted in emulsions with slow cooling; and those with 40% oil, crystallization was more promoted than for emulsions with 20% oil.

Introduction

The need to find replacement fats for *trans*-fatty acid (TFA) has become a concern for industry due to the constraints of government on the use of TFAs. Nations around the world (e.g., Denmark, Canada, the USA, etc.) have begun to label food items indicating how much TFA is in a product, and to restrict the use of TFAs in restaurants and the amount of TFAs in oils used for consumption [Stender and Dverberg, 2004; FDA Consumer Magazine, 2003; Lueck and Severson, 2006; Barboza, 2007]. One issue with replacing TFAs is that the desirable properties (e.g., texture, flavor, and shelf-life) are at

risk. Currently, TFAs are being replaced by saturated fats such as coconut oil. Saturated fats are a good substitute in as much as they maintain the quality of the product's texture and flavor; however, this can only be a temporary substitute as these saturated fats are high in palmitic and lauric fatty acids, which are known to contribute to heart disease and cholesterol in a similar manner to TFAs [Mensink et al., 2003; Simon et al., 1995]. Therefore, healthier alternatives must be sought; however, the quality of texture and flavor should not be compromised.

Previous research has been done to systematically show how changing the oil content and homogenization conditions affect an anhydrous milk fat (AMF) and soybean oil (SBO) mixture in an oil-in-water emulsion [see Chapter III]. The next phase of this research was to study the effect of cooling rate on the emulsions for given crystallization temperatures (T_c).

The cooling rate of an emulsion to a crystallization temperature is important due to the different types of crystal formation, which can affect the smoothness or graininess of margarine, the snap and gloss of chocolate, and the spreadability of butter and margarine [Campos et al., 2002]. When an emulsion is cooled quickly (e.g., quenching) then many small crystals form, which are considered unstable and yet rigid. If the emulsion is cooled slowly, then larger but fewer lipid crystals form having had time for the triacylglycerides (TAGs) to adjust and fit together in a preferable uniform lattice and are in a more stable form [Campos et al., 2002; Sato, 2001; Martini et al., 2001, 2002]. It has been suggested that the less stable form is more rigid and therefore unable to bend within its confined barrier (lamella) and thereby puncture the confinement and cause

partial coalescence [Coupland 2002]. Thus, emulsion stability is influenced by crystalline form produced by either a slow or fast- cooling rate.

Though, once the emulsion has gone through the cooling process to a crystallization temperature the crystals do not stop either forming or changing. The crystal form may change over time at a given temperature (e.g., going from α to β' form or vice versa) [Coupland, 2002]. Crystallization of lipids in oil-in-water emulsions has been well investigated [Coupland, 2002; Rousseau, 2000; McClements et al., 1993], but there is a lack of studies comparing the same emulsion with various cooling rates, which might be able to show differences, in crystalline forms and/or fractionation, and the possibility that these over time can also change when held at a specific crystallization temperature, thus influencing stability and sensory attributes. Lopez et al. (2002) did study the effect of cooling rate on milk fat and cream (not a model system) and found that though crystallization of the lipid state did not change significantly; there was a difference in the melting profiles, which showed that the slower the cooling rate the less fractionated the milk-fat fractions. The same was found in this study, though this research also looked at the destabilization of a model system emulsion using a lightscattering device, which will assist in the understanding of crystallization of emulsified systems.

Deciphering the differences in cooling rate could lead to a better understanding of the fractionation of the lipid crystals with anhydrous milk fat (AMF) and soybean oil (SBO) and how that might affect the end product.

Materials and Methods

Emulsion Formulation: The oil phase was a 50 wt% blend of soybean oil (SBO) donated by Bunge Limited (St. Louis, MO) and anhydrous milk fat (AMF) was donated by Kraft Foods Inc. (Chicago, IL). Both lipids were melted by heating to ~60 °C for ≥ 30 min prior to mixing.

The water phase was prepared by dispersing 2.0 wt% whey protein isolate (WPI) (Inpro 90 by Vitalus [Abbotsford, B.C., Canada], which consists of ≥ 92% whey protein, ≤ 3.0% lactose, ≤ 5.0% moisture, ≤ 1.0% fat, and ≤ 3.5% ash) in a 0.01M ($Na_2HPO_4 \cdot 7H_2O$) aqueous solution (pH 7.28). The solution was then filtered (Whatman #1 filter paper) to eliminate any possible undissolved particles that might affect the stability/instability of emulsions. The solution was then heated to ~60 °C for ≥ 30 min prior to homogenization of the two phases.

Oil phase was added to water phase for a total of 50 g in a 100 mL beaker. Two oil-in-water (o/w) ratios were used: 40:60 and 20:80 (o/w expressed in weight %), which are o/w ratios commonly used in many lipid based foods such as salad dressings.

Emulsion Preparation: The phases were homogenized using two methods: very low pressure homogenization and high pressure homogenization. Very low pressure homogenization (VLPH) was done by first mixing the phases with an Ultra Turrax (IKA T18 basic) at 18,000 rpm for 1 min. The mixture was then quickly (less than 2 min) put through a Microfluidics Microfluidizer Processor (Model M-110S, Newton, MA) at 2530 ± 230 psi. The emulsion made only one pass through the microfluidizer. The microfluidizer coil was kept at approximately 60 °C to avoid lipid crystallization during

emulsion formation. High pressure homogenization (HPH) was the same as VLPH, except with a pressure of 9430 ± 230 psi (HPH).

Testing Methods

Differential Scanning Calorimetry (DSC): The crystallization and melting behaviors of the samples were studied by DSC (TA Instruments, 2910, New Castle, DE). Approximately 5 to 15 mg of a sample was placed in a DSC pan soon after homogenization. The DSC pans were kept at approximately 60 °C to avoid cooling the sample prior to analysis. The DSC was calibrated with Indium at a heating rate of 5 °C/min.

Crystallization and melting enthalpies (expressed in units of J/g), with peak and onset temperatures (given in °C), were calculated for all emulsions. Enthalpy comparisons were based on the oil phase only. That is, the enthalpy was increased to represent 100% oil. For example, if a 20:80 sample had a calculated enthalpy of 0.2 J/g then the compared value would be 1 J/g (see equation 1).

Equation 1

$$Oil\ phase\ enthalpy\,(J/g) = \frac{sample\ enthalpy\,(J/g)}{wt.\ fraction\ of\ oil}$$

Fast cooling rate: samples were placed in the DSC chamber at an initial temperature of 60 °C and then cooled at a rate of 30 °C/min to T_c (i.e., 10, 5, 0, -5 and -10 °C) and held there for 3 h. Samples were then heated at 5 °C/min to analyze the melting profile of the crystallized fat. This cooling rate was chosen to reproduce the fast cooling experienced by the emulsions during the physicochemical stability tests (see section below).

Slow cooling rate: the same as program one, with the exception that the samples were cooled at a rate of 0.2 °C/min.

Physicochemical Stability: Five to 7 mL of the emulsions were placed in an test tube designed especially for the TurbiScan 2000 (Sandyhook, CT). The cap of the tube has a notch for tube placement within the equipment to ensure readings were taken at the same spot each time.

Fast cooling rate: An initial reading (i.e., when the sample was still approximately 60 °C) was taken prior to the sample being placed in a water bath thermostatized at a specific T_c. The sample was placed in a water bath thermostatized at T_c. The physicochemical stability of the emulsions was measured during the 3 h at T_c. Measurements were taken every 10 min for the first hour and then after 15 min for the next 2 h. To perform the BS measurement, test tubes with the emulsions were taken from the water bath (set at T_c) and placed in the TurbiScan. After the measurement was taken (40 sec) the assay tube was placed again in the thermostatized water bath.

Slow cooling rate: After the initial reading was taken, the sample was placed in a programmable water bath (Ecoline Lauda E300, Westbury, NY), which was initially set at 60 °C. The waterbath would then cool to T_c at 0.2 °C/min. Readings were taken every 5 °C (25min) until T_c was reached, at which time measurements were taken in accordance with the fast cooling rate.

Back scattering profiles and kinetics were reported in the reference mode, which meant samples could be compared with respect to each other even if they didn't begin with the same amount of sample, initially. The change in the thickness of the

destabilization peak at half its height was used to follow the destabilization kinetics of the emulsions.

Statistical Analysis: Experiments were performed in duplicate or triplicate as necessary. Data reported are the mean and standard deviation values calculated from the replicates. Significant differences were analyzed using a two- or one-way ANOVA test, as appropriate, and Bonferroni post-tests ($\alpha = 0.05$). Statistical analysis was performed using Graph Pad software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com).

Results

DSC Results: Figure 8 shows the crystallization parameters for the slow cooling rate in comparison with their fast cooling rate counterpart for VLPH and HPH emulsions. The most obvious difference is in the lack of data for crystallization temperatures greater than -5 °C for the slow cooling rates of 20:80 HPH and VLPH emulsions (Figure 8A-C) and not for 40:60 emulsions (Figure 8D-F). The T_{on} and T_p (~ 4 and 2 °C, respectively) of -5 and -10 °C (Figure 8A) would suggest that values should also be seen for 0 °C; however, the lack of oil in the system and the sensitivity of the DSC, which just cannot register the slight effect of the lattice forming crystals at that T_c, might be why values are not observed. The fact that crystallization did occur for 40:60 emulsions with a slow-cooling rate would indicate that lipid content does affect crystallization.

In Figures 8A and 8D, which compare the onset temperatures (T_{on}), the difference between fast and slow cooling rates can be observed. For 20:80 emulsions, the

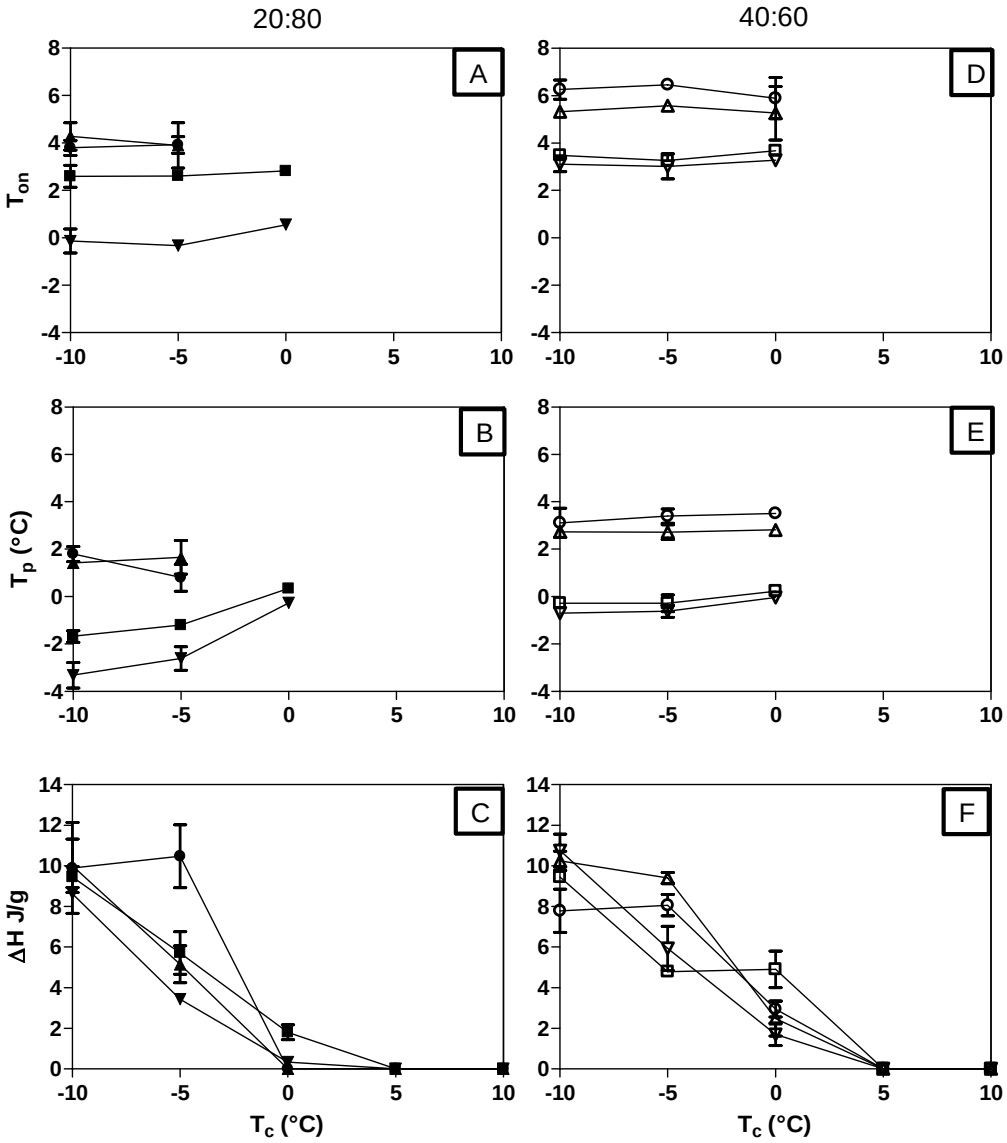

Figure 8: Comparison of crystallization parameters among samples and T_c. Closed symbols are 20/80 emulsions (A-C) and open symbols are for 40/60 emulsions with ■ VLPH fast cooling-rate, ▲ VLPH slow cooling-rate, ▼ HPH fast cooling-rate, and ● HPH slow cooling-rate.

greatest significant difference appears to exist between fast and slow cooling rates for HPH emulsions. The slow-cooling rate has an onset temperature (~4 °C) significantly greater ($p < 0.001$) at -5 and -10 °C than with rapid cooling (~0 °C); 40:60 HPH emulsions also had significant difference ($p < 0.01$) at -5 and -10 °C between cooling rates, with slow-cooling rates slightly higher than fast-cooling rates. No significant differences were found between the 20:80 VLPH fast and slow cooling rates (Figure 8A) and only at -5 °C ($p < 0.05$) for 40:60 VLPH emulsions (Figure 8D). This would indicate that the slow-cooling rate induced the crystallization of the fat. That is by giving molecules time to find the right configuration during the cooling step, the crystals have time to form and develop, whereas with a rapid cooling rate, the temperature had to reach a lower temperature before nucleation would happen (i.e., a forced nucleation based on temperature). Besides, the differences within a given oil content, significant difference ($p < 0.05$) was found for the T_{on} between 20 and 40% oil VLPH emulsion with the slow cooling rate at -5 °C; the significant difference increased ($p < 0.001$) for HPH emulsions with slow-cooling rate for T_c = -5, and -10 °C (Figures 8A and 8D). Therefore, not only does crystallization occur for 40% oil emulsions, but crystallization is induced sooner than for 20% oil emulsions.

Figures 8B and 8E indicate the peak temperatures (T_p^c) of the DSC profiles. For 20:80 emulsions, no significance was found between HPH and VLPH emulsions with the slow cooling rate (Figure 8B); the same was found for 40% oil content (Figure 8E). However, for 20% oil content a significant difference ($p < 0.01$) was found for T_p^c of VLPH emulsions between the two cooling rates at -5 and -10 °C; T_p^c for HPH emulsions also were found to have a significant difference ($p < 0.001$) between cooling rates for the

same T_c (Figure 8B). Emulsions formulated with 40% oil also showed a significant difference ($p < 0.001$) between fast and slow cooling rates for both VLPH and HPH processing conditions at T_c of 0, -5, and -10 °C. Significant difference ($p < 0.01$) was also found between emulsions with the different ratios of oil at -5 and -10 °C for HPH emulsions with a slow cooling rate.

Finally, Figures 8C and 8F show the enthalpies (J/g) of the various emulsions for the two cooling rates. No significance was found for 20% oil emulsions, except between the HPH emulsion with slow cooling ($p < 0.05$) and all other emulsions at -5°C (Figure 8C). On the other hand, 40% oil content had a significant difference ($p < 0.05$) between VLPH emulsions at fast and slow-cooling rates for T_c = -5 °C, and for HPH fast and slow cooling rates at -10 °C. Incidentally, the only significant difference ($p < 0.05$) between 20 and 40% VLPH slow cooling rate was at -5 °C, and no significant difference was found for HPH slow-cooling rate between the two oil ratios. Within 20:80 HPH and VLPH emulsions with a slow-cooling rate, a significant difference ($p < 0.01$) was found between 0 and -10 °C, and for just HPH ($p < 0.01$) between 0 and -5 °C. The same can be seen for 40%, with the addition of VLPH having a significant difference ($p < 0.01$) between 0 and -5 °C. Though, there might be significance within an emulsion between crystallization temperatures it should be noted that there really isn't much significance between cooling rate and emulsion type. This would indicate that the same amount of oil is being crystallized for the various types of emulsions independent of cooling rate.

From this information, the next step is to look at the melting profiles for the various T_c (Figure 9). After the 3 h at T_c, the melting profile would show a better determination of how much lipid crystallized into its various fractions and if it is

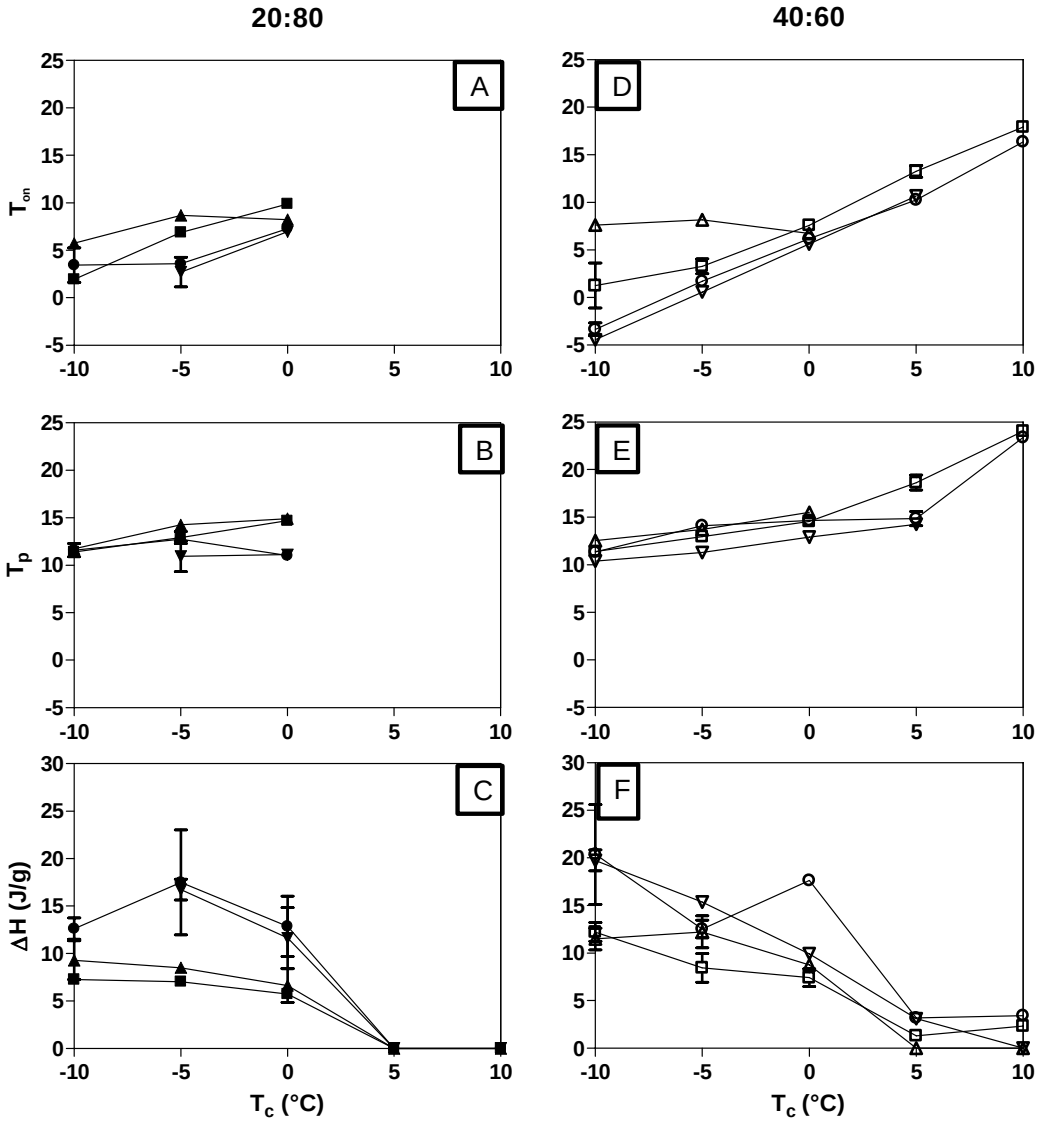

Figure 9: Comparison of melting parameters among samples and T_c. Closed symbols are 20/80 emulsions (A-C) and open symbols are for 40/60 emulsions with ■ VLPH fast cooling-rate, ▲ VLPH slow cooling-rate, ▼ HPH fast cooling-rate, and ● HPH slow cooling-rate.

significant. Though the melting parameters (i.e., T_{on}, T_p, and enthalpy) do not show as much significance between the fast and slow-cooling rates as the crystallization parameters did, there are a few points of note.

First, for the 20:80 emulsions (Figures 9A-C), there are values for T_c of 0 °C for both cooling rates. This data shows that though the crystallization could not be detected while the emulsion was being slowly cooled with time at T_c, crystallization did occur (Figures 9A-B) and there was a significant difference ($p < 0.01$) between the T_{on} for 20:80 VLPH fast-cooling rate and HPH slow-cooling rate at 0 and -5 °C and for T_p at 0 °C; the same significance was found between VLPH slow-cooling rate and HPH fast-cooling rate for -5 °C and for T_p at 0 and -5°C. The VLPH emulsions had a delay in their melting parameters, which would indicate higher melting points for the lipids and thereby show that the lipids are possibly more protected in VLPH emulsion than HPH emulsions, which are dependant on droplet size. Meaning that with the smaller droplets in HPH emulsions, there is more surface area and therefore better heat transfer, which would assist in melting the lipid crystals sooner. The 40:60 emulsions (Figures 9D-E) showed that significant differences ($p < 0.001$) were again seen between VLPH fast-cooling rate and slow-cooling rate HPH emulsions at -10 °C for T_{on} and -5 ° for T_p ($p < 0.05$); however, for VLPH slow-cooling rate and the two cooling rates for HPH emulsions the significant difference was seen at -5 and -10 °C for T_{on} and T_p (for fast HPH only) and just for -10 °C between the two slow-cooling rate emulsions; and for the VLPH slow and HPH fast-cooling rates significance was also seen at 0 and -5 °C for T_{on}. There was no significance for 20:80 emulsions (i.e., T_{on} and T_p) between the cooling rates for VLPH and HPH emulsions. On the other hand, for 40:60 emulsions there is a significant

difference ($p < 0.001$) between the cooling rates for VLPH emulsions at -5 and -10°C. Also, although no significance was found between cooling rates of HPH emulsion for T_{on}, a significant difference ($p < 0.05$) was found between them between 0 and -10 °C for T_p. Finally, a significant difference ($p < 0.05$) for T_{on} was found between slow cooling rates for both ratios of oil at -5 and -10 °C (i.e., 20 VLPH and 40 HPH; 40 VLPH and 20 HPH [at 0 °C for T_p]; and 20 HPH and 40 HPH, but only at -10°C for T_{on} and 0 °C for T_p) .

Interestingly, even though there was some significance for the T_{on} and T_p, there was very little for the change in enthalpy. For 20:80 emulsions the significant difference ($p < 0.05$) was found at -5 °C between VLPH fast and HPH slow-cooling rates and then between the two cooling rates for HPH emulsions at -10 °C ($p < 0.01$). For 40:60 emulsions the significant difference ($p < 0.05$) was found at 0 and -10 °C between the slow-cooling rate for HPH emulsions and both cooling rates for VLPH emulsions; and also between the slow-cooling rate for VLPH emulsions and fast-cooling rate for HPH emulsions at -10 °C. There were no significant differences between slow cooling rates and oil contents. These minor deviations would indicate that the amount of oil crystallized remains independent of emulsion type even after given time to completely crystallize. Also of note, is that the melting of VLPH emulsions is delayed (Figure 9A), meaning higher T_{on}, which is confirmed by lower enthalpy values for 20:80 emulsions (Figure 9C). So, though crystallization is induced in VLPH emulsion, the crystal growth is inhibited leading to the low melting enthalpies.

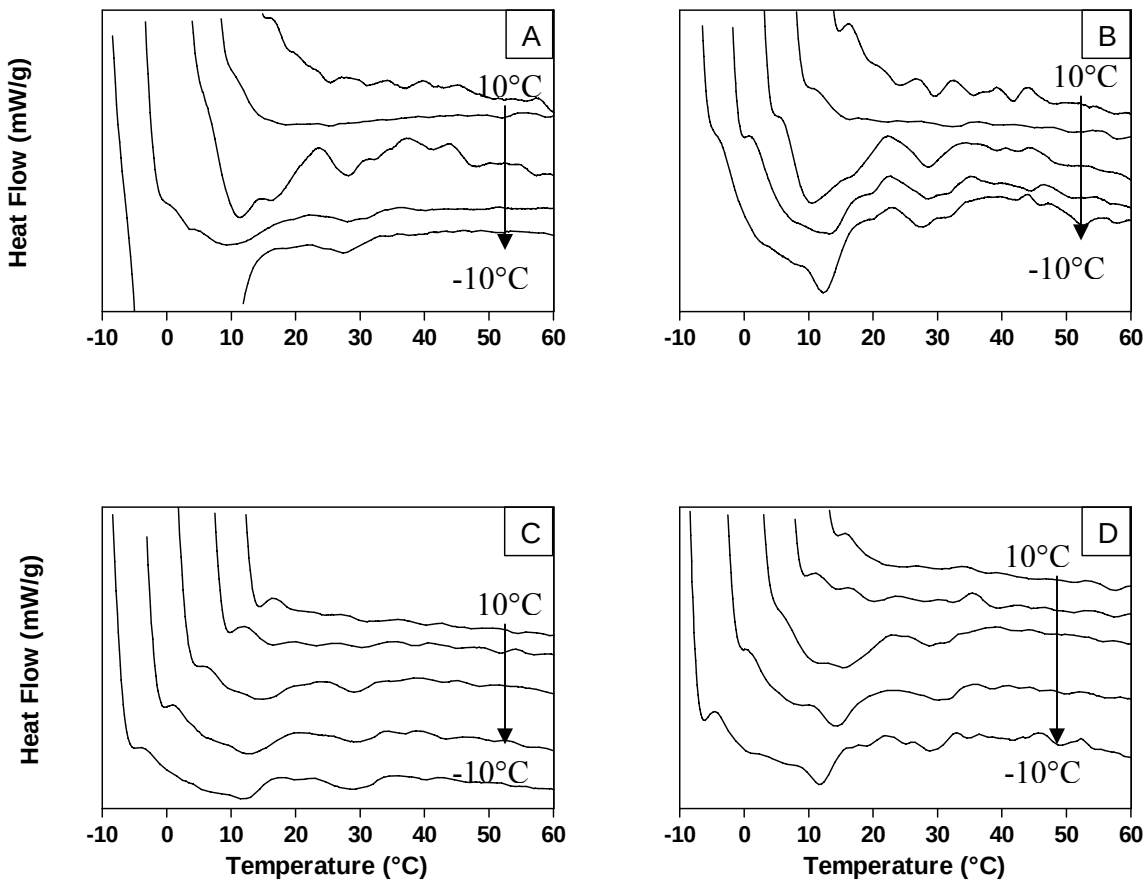

Figure 10: Melting profiles for 20/80 emulsions going from 10 °C to -10 °C in 5 °C intervals: A) HPH fast cooling- rate, B) HPH slow cooling-rate, C) VLPH fast cooling rate and D) VLPH slow cooling-rate for each T_c.

Although, there is no significant difference between the various emulsions for the designated melting parameters, there is some differentiation in their DSC melting profiles. Figure 10 shows 20:80 HPH and VLPH melting profiles for all T_c for both cooling rates. Figure 10A, which has a peak that goes off the edge is when the emulsion froze for HPH at a fast-cooling rate, which is unique, all other emulsion did not show freezing tendencies in the DSC. It is still possible to see that for HPH emulsions between fast and slow cooling rates (Figure 10A and 10B, respectively) that a shoulder in the first melting peak (i.e., the melting slope goes from a steady decline to a dramatic decline, the shoulder, which is especially prominent in Figure 10D) for -10 °C is more prominent as T_c decreases for the slow-cooling rate. This is also seen for VLPH emulsions (Figures 10C and 10D). The fast-cooling rate is broad and less distinctive while the peaks for slow-cooling rates show a distinct peak, which indicates more fractionation.
Figure 11 shows the same profiles except for 40:60 emulsions. It is interesting to note that VLPH emulsions have a slight decline as the crystals melt (see the lower temperatures) than for HPH emulsions and the shoulder, which is the sharp decline of the slope prior to the T_p for the melting profile, is much more noticeable. This difference might indicate what types of crystals are being formed for the two droplet sizes.

In addition to seeing the fractionation in the first melting peak, it is possible to gain a better understanding of the overall fractionation by taking ratios of the second peak to the first peak (i.e., P2:P1). This observation would help to quantify the fractionation which occurred during the 3 h hold at T_c. Table 2 gives the ratios for 20:80 emulsions. No crystallization was observed at 5 and 10 °C and therefore no fractionation occurred for those emulsions. In the end, though little significant difference was seen between the

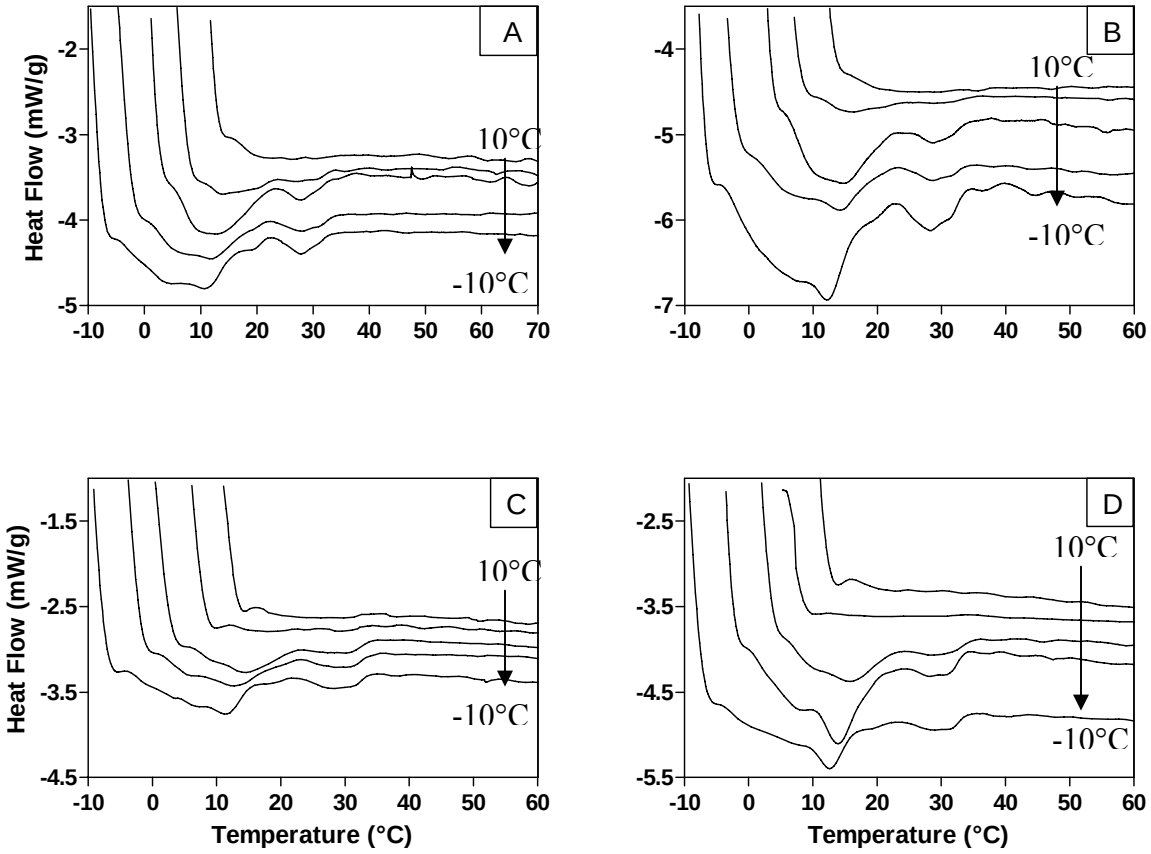

Figure 11: Melting profiles for 40/60 emulsions going from 10 °C to -10 °C in 5 °C intervals: A) HPH fast cooling- rate, B) HPH slow cooling-rate, C) VLPH fast cooling rate, and D) VLPH slow cooling-rate for each T_c.

Table 2: ΔH ratio values of melting peak 2 to peak 1 for 20:80 HPH and VLPH emulsions.

T_c (°C)	20:80 HPH fast	20:80 HPH slow	20:80 VLPH fast	20:80 VLPH slow
10	--	--	--	--
5	--	--	--	--
0	0.25 ± 0.09^{a}	0.25 ± 0.04^{a}	0.37 ± 0.05^{a}	0.34 ± 0.12^{a}
-5	0.19 ± 0.06^{a}	0.21 ± 0.06^{a}	0.33 ± 0.07^{a}	0.22 ± 0.06^{a}
-10	--	0.28 ± 0.20^{ab}	0.45 ± 0.04^{a}	0.22 ± 0.03^{b}

Note: Values with the same superscript have no significant difference ($p < 0.01$), between rows and columns. -- indicates that no crystallization occurred at that T_c.

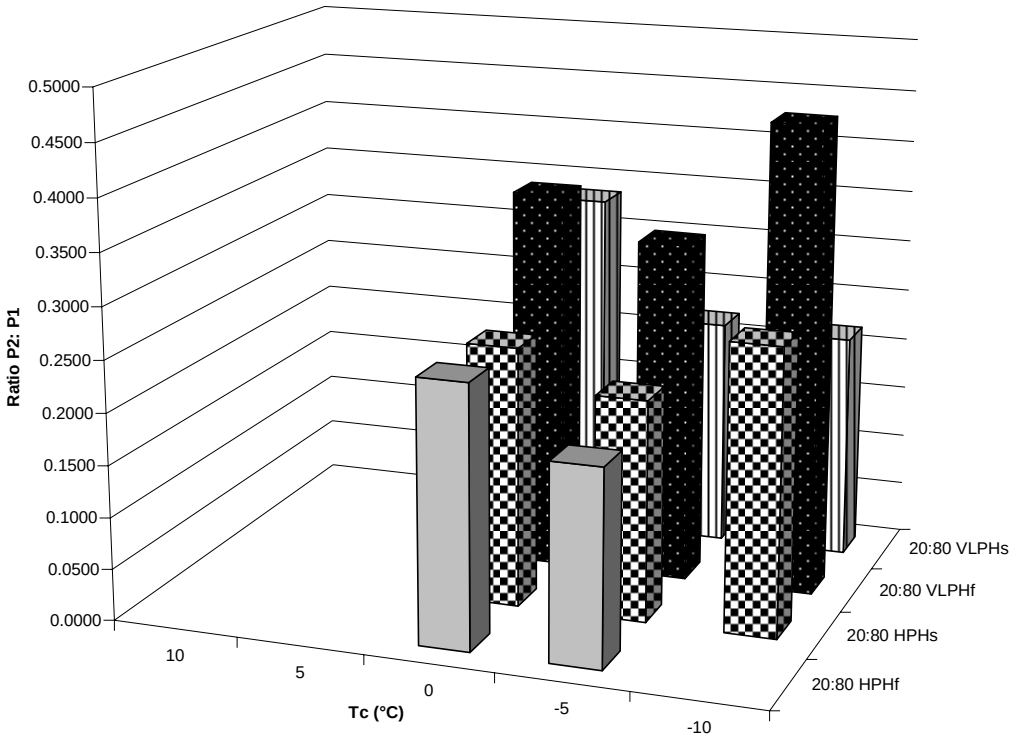

Figure 12: Ratio of enthalpy values of peak 2:peak 1 for 20/80 emulsions at given T_c.

Table 3: ΔH ratio values of melting peak 2 to peak 1 for 40:60 HPH and VLPH emulsions.

T_c (°C)	40:60 HPH fast	40:60 HPH slow	40:60 VLPH fast	40:60 VLPH slow
10	--	0.00	0.00	--
5	0.30 ± 0.01^{b}	0.18 ± 0.06^{ab}	0.54 ± 0.02^{c}	--
0	0.16 ± 0.03^{a}	0.17 ± 0.05^{a}	0.27 ± 0.03^{ab}	0.19 ± 0.03^{a}
-5	0.17 ± 0.01^{a}	0.13 ± 0.02^{a}	0.28 ± 0.04^{b}	0.15 ± 0.04^{a}
-10	0.13 ± 0.01^{a}	0.12 ± 0.05^{a}	0.24 ± 0.01^{ab}	0.20 ± 0.03^{a}

Note: Values with the same superscript have no significant difference ($p < 0.01$), between rows and columns. -- indicates that no crystallization occurred at that T_c; 0.00 means that only one crystallization peak happened.

various emulsions, the VLPH slow-cooling rate emulsions are observed to be less fractionated than its fast-cooling rate counterpart and significantly different at lower T_c (i.e., -10 °C). Interestingly, HPH emulsions do not show significance between cooling rates, though at -10 °C, the results are masked due to the crystallization of water for those emulsions. The only other significance is found between VLPH emulsions at -10 °C. Here, it is possible to see that for VLPH emulsions at a fast cooling rate more crystals consisted of higher melting points (i.e., by having a larger second melting peak) than all the others. Though, not significantly different, VLPH emulsions with a fast-cooling rate are usually higher than the other emulsions for T_c of 0 to -10 °C (between 8 and 50% higher); this would indicate that VLPH emulsions are more fractionated than HPH emulsions. This difference might due to the quick cooling and the size of the droplets.

In comparison, there is a more of a difference when observing 40:60 emulsion values (Table 3 and Figure 13). There are still a few which did not show fractionation (i.e., VLPH emulsions and fast-cooling rate HPH emulsion at 10 °C and slow-cooling rate VLPH emulsions at the additional temperature of 5 °C). Though, the VLPH fast-cooling rate values are similar between the two oil ratios, most 40:60 values are less than those of 20:80, which would indicate that less fractionation is observed for the 40% oil emulsions, especially at lower T_c. The 40:60 VLPH emulsions (as also see with 20:80 emulsions) appear to be more fractionated than HPH emulsion. Again, fast cooling rates appear to be more fractionated than slow cooling rates. At 5 °C, three emulsions have values and the fast-cooling rates are significantly different from each other. VLPH fast-cooling rate is significantly higher than the slow-cooling rate at -5 °C, and though not

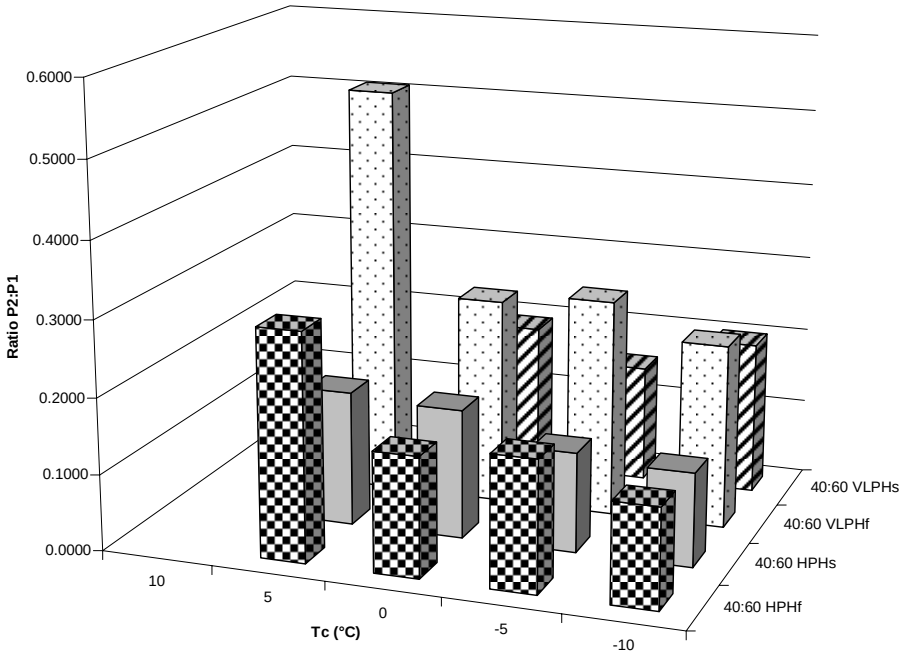

Figure 13: Ratio of enthalpy values of peak 2:peak 1 for 40/60 emulsions at given T_c.

showing significance the values are still a bit higher for the other temperatures, too. Interestingly, there is not a significant difference between HPH emulsions for the two cooling rates.

Physicochemical Stability Results: When a larger sample is observed by light-scattering technique, how does the emulsion destabilizes versus seeing how the emulsions compared by changing the parameters in the DSC. Figure 14 shows the destabilization of the slow-cooling rate samples from the time that the sample reaches T_c. The reference point for these samples is from time zero, which is when the sample has just been made and is still at ~60 °C. The difference between destabilization mechanisms are denoted by positive and negative values. Positive values are representative of sedimentation phenomena, while negative values represent a creaming phenomenon (i.e., there is

clarification at the bottom of the tube). These two types of phenomena are represented in both HPH and VLPH emulsions with the slow-cooling rate. Note that the creaming phenomena has a higher tendency to occur in VLPH emulsions (Figure 14C and 14D) than HPH emulsions (Figures 14A and 14B), though it does occur in both. This can be compared to previous results, which showed for fast-cooling rate of all emulsions had a greater tendency towards sedimentation (Chapter 3, Figure 6), except for 20:80 VLPH emulsions, which were observed to cream at higher temperatures (i.e., 5 and 10 °C).

It is interesting to note that 20:80 emulsion had a higher occurrence of both types of destabilization mechanisms, while 40:60 emulsions were pretty consistent at sedimentation (with one exception as seen in Figure 14B). Comparing the stability of the emulsions, it was observed that for 40:60 emulsions no significant differences were seen between fast and slow-cooling rates. However, for 20:80 emulsions, there appears to be greater stability in emulsions with a slow-cooling rate than the fast-cooling rate. This tendency was found for both HPH and VLPH emulsions (compare Chapter 3, Figure 6 and Figure 14); though for 40:60 VLPH emulsions with a fast-cooling rate, -10 °C appears to be more stable than at slow-cooling rate until it freezes (Chapter 3, Figure 6). By comparing the 40:60 slow-cooling rate emulsions (Figures 14A and 14B), they are extremely similar, except that VLPH emulsions were seen to be slightly more stable (i.e., most values appear to be below 1mm while HPH go above and below 1mm), especially at -10 °C when the HPH emulsions froze. The 20:80 slow-cooling rate emulsions appear to be very similar in how much they destabilize with the most notable difference in that both have a creaming tendency at higher temperatures, but VLPH slow-cooling rate continues

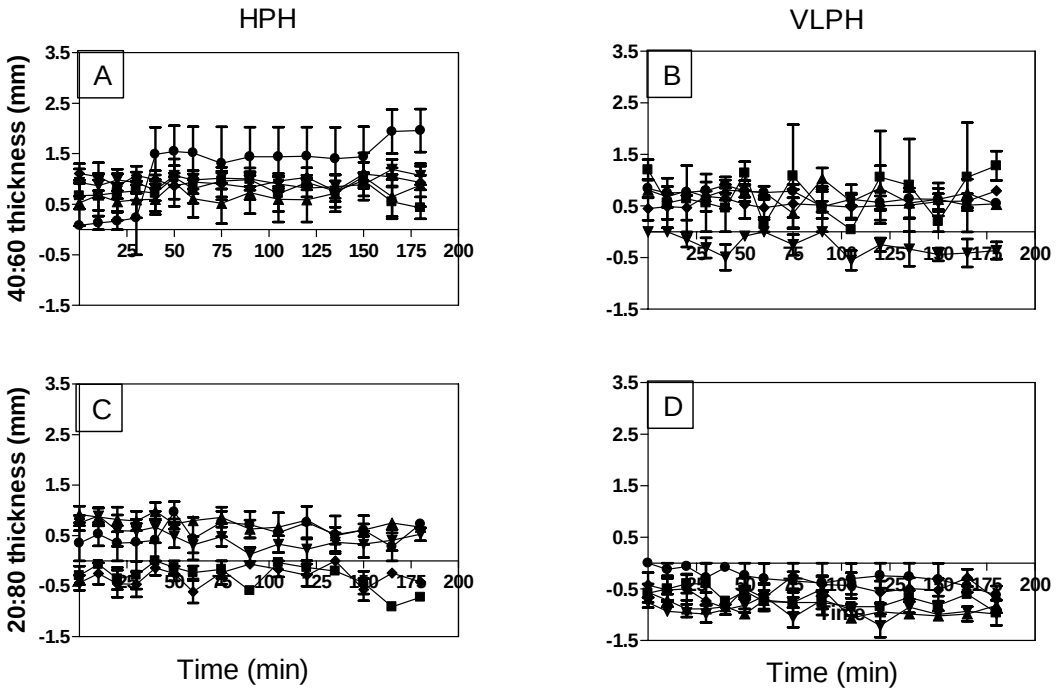

Figure 14: Destabilization mechanisms for slow-cooling rate of 40:60 A) HPH and B) VLPH; and 20:80 C)HPH and D) VLPH emulsions at T_c (■ ,10°C; ▲, 5°C; ▼, 0 °C; ◆, -5°C; ●, -10°C).

with that tendency throughout the different crystallization temperatures while HPH emulsions shift towards a sedimentation tendency.

Discussion

This research has shown that by changing a variety of variables (i.e., oil phase volume, homogenization conditions, crystallization temperature and cooling rate) that an affect to o/w emulsion stability will occur. By having higher oil content (i.e., 40%), it has been shown to increase the stability for emulsions with a fast-cooling rate (i.e., HPH and VLPH emulsions). Interestingly, when the cooling rate is changed, then it is possible to increase the stability of o/w emulsions with a lesser amount of oil (e.g., 20%).

The differences in homogenization conditions have shown that depending on crystallization temperature and cooling rate stability and freezing conditions change. For example, 20:80 HPH emulsions froze at fast-cooling rates in the DSC. This was most likely due to the lack of oil in the emulsion, because the 40:60 emulsions did not freeze. Then, by changing only the cooling-rate, the 20:80 emulsion were not seen to freeze, which would indicate that by being given the time, the crystals were able to form in such a way as to keep suspended within the emulsion, which inhibited the water from crystallizing and thereby breaking the lamella.

However, one can also notice that for HPH emulsions for TurbiScan readings both ratios of oil content froze at -10 °C, which would indicate that quantity of an emulsion also has an effect on its physicochemical properties. This freezing in a larger quantity was also able to show the differences in thawing responses of HPH and VLPH emulsions. Note that VLPH emulsions had a lower likelihood of freezing (<30%) than HPH (>80%). When HPH emulsions thawed they initially showed two phases, the water and oil phase; however, over a period of time at room temperature the AMF and SBO would separate from each other and it was possible to discern all three main components (i.e., WPI solution, AMF and SBO). On the other hand, when VLPH emulsions did happen to freeze, and then left to thaw they remained in an emulsified state. Over time slight clarification occurred, but never to the dramatic separation that occurred for HPH emulsion. The greater physicochemical stability observed for 20:80 emulsions crystallized at slow-cooling rate can be attributed to the induction of crystallization observed by DSC. Oil content is also a factor in emulsion stability, since cooling rate did not significantly affect 40:60 emulsion destabilization kinetics.

The cooling rate also showed that less fractionation occurs with slow-cooling rates than for fast. This corresponds with the idea that similar crystals create a uniform lattice structure. However, fractionation is induced when larger droplets are involved, as in VLPH emulsions, with fast-cooling rates. Also, the more fractionated the system the narrower the distribution of the molecular species and a narrower melting range is observed (i.e., LMP, MMP, and HMP).

When an emulsion is cooled quickly (e.g., quenching) then many small crystals form, which are considered unstable and yet rigid. If the emulsion is cooled slowly, then larger but fewer lipid crystals form having had time for the TAGs to adjust and fit together in a preferable uniform lattice and are in a more stable form [Campos et al., 2002; Sato, 2001; Martini et al., 2001, 2002]. This difference can be seen in the 20:80 VLPH emulsions, which had an increase in stability as the cooling rate decreased from 30 to 0.2 °C. Thus, emulsion stability is influenced by crystalline form produced by either a slow or fast- cooling rate.

References:

Barboza, T. (2007). L.A. County can't ban *trans* fats. Los Angeles Times (January 30). Pp. B1.

Campos, R., Narine, S.S., & Marangoni, A.G. (2002). Effect of cooling rate on the structure and mechanical properties of milk fat and lard. Food Research International, 35, 971-981

Coupland, J.N. (2002). Crystallization in emulsions. Current Opinion in Colloid and Interface Science, 7, 445-450.

Lopez, C., Bourgaux, C., Lesieur, P., Bernadou, S., Keller, G. & Ollivon, M. (2002). Thermal and structural behavior of milk fat 3. Influence of cooling rate and droplet size on cream crystallization. Journal of Colloid and Interface Science, 254, 64–78.

Lueck, T. J. & Severson, K. (2006). New York bans most *trans* fats in restaurants. New York Times (December 6). http://www.nytimes.com/2006/12/06/nyregion/06fat.html

Martini, S., Herrera, M.L. & Hartel, R.W. (2001). Effect of cooling rate on nucleation behavior of milk fat-sunflower oil blends. Journal of Agriculture and Food Chemistry, 49(7), 3223-3229.

Martini, S., Herrera, M.L. & Hartel, R.W. (2002). Effect of cooling rate on crystallization behavior of milk fat fraction/sunflower oil blends. Journal of American Oil Chemists' Society, 79,1055-1062.

McClements, D.J., Povey, M.J.W. & Dickinson, E. (1993). Absorption and velocity dispersion due to crystallization and melting of emulsion droplets. Ultrasonics, 31, 433-437.

Mensink, R.P., Zock, P.L., Keste,r A.D.M. & Katan, M. (2003). Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. American Journal of Clinical Nutrition, 77, 1146–55.

Revealing *trans* fats (2003). FDA Consumer Magazine, 37 (5), 20-26.

Rousseau, D. (2000). Fat crystals and emulsion stability—a review. Food Research International, 33, 3-14.

Sato, K. (2001). Crystallization behavior of fats and lipids—a review. Chemical Engineering Science, 56, 2255-2265.

Simon, J.A., Hodgkins, M.L., Browner, W.S., Neuhaus, J.M., Bernert JT Jr. & Hulley, S.B. (1995). Serum fatty acids and the risk of coronary heart disease. American Journal of Epidemiology, 142(5), 469-76.

Stender, S. & Dverberg, J. (2004). Influence of *trans* fatty acids on health. Annual Nutrition and Metabolism, 48, 61-66.

CHAPTER V

SENSORY ANALYSIS OF VLPH EMULSIONS WITH AND WITHOUT DHA

Abstract

Docosahexaenoic acid (DHA: 1.4 ± 0.2 wt %) was added to oil-in-water (o/w) emulsions, which were made up of a lipid phase (i.e., a mixture of anhydrous milk fat and soybean oil) with whey protein isolate (1.8 wt % protein) as emulsifier. Samples with and without DHA were studied as a function of oil content (20% and 40% o/w) for very low pressure homogenization (2,530 ± 230 psi) at a crystallization temperature of -10 °C. A vertical scan macroscopic analyzer measured the physicochemical stability. The addition of DHA stabilized emulsions with 20% oil, while emulsions with 40% oil became less stable. Thiobarbituric acid (TBA) analysis was done to compare oxidative stability between emulsions, quantitatively. A descriptive panel was used to evaluate the oxidative stability by assessing four attributes: oxidized, rancid, fishy and buttery. The panelists were given samples after 72 h, because contrary to the TBA analysis which showed no significant differences between samples with and without DHA, the fishy smell was evident. The panelists showed that there was a significant ($p < 0.05$) difference in fishiness between the VLPH emulsions with and without DHA, and commented on the odor being repulsive. No significance was seen for rancid and buttery flavors, and only a marginal significance was seen for oxidized. Also, the buttery flavor might be masking the fishy flavor, which would throw off the intensity expressed in the 40% emulsions with DHA.

Introduction

Previous studies by this research group have shown the possibilities of replacing TFAs with anhydrous milk fat and soybean oil mixture in an oil-in-water emulsion. Oil content, homogenization conditions, cooling rates (Chapter IV), and crystallization temperatures have been explored (see Chapter III). From these studies, the desire to create a more functional food application has led to incorporating docosahexaenoic acid (DHA) into the emulsions.

The addition of DHA, an omega-3 polyunsaturated fatty acid (PUFA), was done because it is considered a heart-healthy fatty acid. DHA is a desirable component to a food system and makes the product a functional food because of the health benefits attributed to omega-3 fatty acids. Studies have attributed proper neural development, the ability of seeing and learning, and the decrease of incidence of cardiovascular disease, some cancers, diabetes, and other diseases to the intake of DHA [SanGiovanni and Chew, 2005; Kolanowski et al., 1999; Fomuso et al., 2002].

Due to the positive impact of DHA to human health, incorporating this essential fatty acid into various food systems has become extensive. However, not only is increasing the intake of omega-3 fatty acids important, so is not changing the initial flavor of the product. DHA has an unpleasant off-odor, which is attributed to a fishy smell and flavor. This means that when DHA is added as an ingredient or to the feed of an animal, sensory tests must be done to ensure that this alteration to the product is not noticeable. For example, studies were done on both chicken and bacon, where the animals were fed various treatments of omega-3 enriched feed. Sensory was then done to determine if there was a significant difference in the flavor with varying results

dependant on type of meat and degree of DHA [Huang et al., 1990; Romans et al., 1995]. Huang found that chickens can eat omega-3 fatty acids (up to 3% of their feed) without an off-fishy flavor, if it is stabilized with 0.1 % ethoxyquin, which helps prevent rancidity. Romans found that the many on the consumer panel rated 'dislike' on the pork samples, whose feed had been 15% omega-3 fatty acids. The two studies indicate that the level of DHA is important to consumer acceptance.

Sensory studies have also been done on the incorporation of DHA into spreads (i.e., butter, oil, and margarine combination) and it was found that acceptability was at levels that could increase DHA intake by 0.2-0.3% daily [Kolanowski et al., 2001], which gives hope to those trying to create acceptable functional food products fortified with DHA.

In the end, without consumer acceptance, a product will fail despite its health benefits. However, prior to doing consumer tests, sensory studies are done on products with a panel, who have more training, to characterize various attributes of a new or re-formulated product before it will be tested on the populace. This is done due to the high cost of product development of new food items. Therefore, as this project is a model system and is only in development of being used as either an additional ingredient (e.g., within baked goods such as pastries) or the fundamental process of formulation for a new product (e.g., mayonnaise with this emulsion for its base) then the system is in the initial stages of sensory analysis and a trained descriptive panel is more appropriate than a mass consumer panel.

The descriptive panel can also be used to evaluate attributes which might not always be detected by standard procedures such as thiobarbituric acid (TBA) analysis,

which is a method of determining the oxidation of a substance by detecting levels of malonaldehyde (MDA) concentration [Jayasingh et al., 2002; Tong et al., 2000]. This practice has been slightly altered by including an antioxidant (i.e., butylated hydroxytoluene) in the TBA stock solution to evaluate emulsion systems [McDonald and Hultin, 1987] and has been used for various studies [Dimakou et al., 2007; Alamed et al., 2006; Kiokias et al., 2007] to evaluate highly oxidative samples.

Materials and Methods

Emulsion Formulation: Fifty-gram samples of oil-in-water mixtures were prepared containing 20 and 40 wt% oil. The oil phase was a 50 wt% blend of soybean oil (SBO) donated by Bunge (St. Louis, MO) in anhydrous milk fat (AMF) donated by Kraft (Chicago, IL). Both lipids were melted by heating ≥ 60 °C for ≥ 30 min prior to mixing.

The water phase was prepared by dispersing 2.0 wt% whey protein isolate (WPI) (Inpro 90 by Vitalus [Abbotsford, B.C., Canada], which consists of ≥ 92% whey protein, ≤ 3.0% lactose, ≤ 5.0% moisture, ≤ 1.0% fat, and ≤ 3.5% ash) in a 0.01M (Na_2HPO_4-$7H_2O$) aqueous solution (pH 7.28). The solution was then filtered (Whatman #1 filter paper) to eliminate any possible un-dissolved particles that might affect the stability/instability of emulsions. The solution was then heated to ≥ 60 °C for ≥ 30 min prior to homogenization of the two phases.

Emulsion Preparation: The phases were homogenized using very low pressure homogenization (VLPH). The samples were made by first mixing the phases using an Ultra Turrax (IKA T18 basic, Wilmington, NC) at 18,000 rpm for 1 min. The mixture was then quickly (less than 2 min) put through a Microfluidics Microfluidizer Processor (Model M-110S, Newton, MA) at 2,530 ± 230 psi. The emulsion made only one pass

through the microfluidizer. The microfluidizer coil was kept at approximately 60 °C to avoid lipid crystallization during emulsion formation.

DHA Incorporation: Incorporation of DHA (encapsulated, which is approximately 18% DHA) by Martek (Columbia, MD) was done by adding 2 wt% of the aqueous phase. The addition of DHA happened right before the two phases were mixed. The WPI solution was taken from the oven (~60 °C), DHA was added, and finally the AMF/SBO oil phase was put in and the three components were then combined. This amount of DHA gives between 216-288 mg per 100 g of emulsion. This amount (per 100 g emulsion) is approximately a fifth of what's recommended by the USDA (2007) (i.e., 1480 ± 200 mg), a good starting place for a new product.

Physicochemical Stability: Five to 7 mL of the emulsions were placed in a test tube designed especially for the TurbiScan 2000 (Sandy Hook, CT). The cap of the tube has a notch for tube placement within the equipment to ensure readings were taken at the same spot each time.

An initial reading (i.e., when the sample was still approximately 60 °C) was taken prior to the sample being placed in a water bath thermostatized at a specific T_c. The sample was placed in a water bath thermostatized at T_c. The physicochemical stability of the emulsions was measured during the 3 h at T_c. Measurements were taken every 10 min for the first hour and then after 15 min for the next 2 h. After the 3 h, the samples were refrigerated at ~5 °C, and readings were taken daily up to day 5 and for day 7. To perform the BS measurement, test tubes with the emulsions were taken from the water bath (set at T_c) and placed in the TurbiScan. After the measurement was taken (40 sec) the assay tube was placed again in the thermostatized water bath.

Back scattering profiles and kinetics were reported in the reference mode, which meant samples could be compared with respect to each other even if they did not begin with the same amount of sample, initially. The change in the thickness of the destabilization peak at half its height was used to follow the destabilization kinetics of the emulsions.

Sensory Evaluation: Samples were made consecutively; two samples were formulated with 20% oil, one with and one without DHA; the other two samples were formulated with 40 % oil, one with and one without DHA. After homogenization, they were held at -10 °C for 3 h and then refrigerated for approximately 72 h. Samples of approximately 5-10 ml (i.e., enough sample to coat the tongue and swirl around the mouth) were placed in 0.75 oz containers and refrigerated until the panel tested them.

A descriptive panel was used for this research. The panelists were selected and trained according to general practices as described in Meilgaard et al. (2007). The panel, which consisted of 13 people (6 men and 7 women of ages ranging from early 20s to their 50s), were trained for approximately 20 h using a 7-point scale and a 15-point spectrum scale for determination of flavor intensity. They were asked to sample the above mentioned four VLPH emulsions for the following flavors: buttery, fishy, rancid and oxidized. They were asked to comment initially on the odor of the sample (which had been placed in a 0.75oz plastic container with a lid to allow for any volatile odors to be trapped) and then to rate the flavors and finally to comment on the overall experience for each sample (see Appendix B for questionnaire).

The samples were given in a random order (see Appendix C for sampling plan) and administered to each panelist in an individual booth. The panelists were trained not

to eat anything 30 min prior to sampling. Between samples they were instructed to rinse their mouth with water and to consume an unsalted cracker to cleanse their palates. This was done to inhibit cross contamination of sample flavors. By only having four samples, sensory fatigue should not be an issue. Sensory data was collected using SIMS 2000 (Morristown, NJ) and analyzed using SAS 9.1 TS Level 1M3 XP_PRO platform (Cary, NC).

Thiobarbituric Acid (TBA) Analysis

Analysis with TBA was done on both 20 and 40% oil VLPH samples with and without DHA to have a comparison of oxidative values. Samples were done in triplicate. The first sample was taken at time zero (i.e., prior to being place in the waterbath). Samples were then taken at 4.5 h and then daily up to day 5 and then day 7.

Thiobarbituric acid reactive substances (TBARS) assay was performed as described by Buege and Aust (1978). Duplicate samples of emulsions (0.5 g) were mixed with 2.5 ml of stock solution containing 0.375% TBA, 15% TCA, and 0.25 N HCl. The mixture was heated for 10 min in a boiling water bath to develop a pink color, cooled in tap water, and then centrifuged (Sorvall Instruments, Model RC 5C, DuPont, Wilmington, DE, U.S.A.) at 5500 rpm for 25 min [Jayasingh et al., 2002]. To aid in inhibiting immediate oxidation of the fish oil, butylated hydroxytoluene (BHT) was added to the mixture by 2 wt% BHT in absolute ethanol. Then 3 ml of BHT solution was added per 100 ml of TBA stock solution [McDonald and Hultin, 1987]. The absorbance of the supernatant was measured spectrophotometrically (Spectronic 21D, Milton Roy, Rochester, N.Y., U.S.A.) at 532 nm against a blank that contained all the reagents minus the emulsion. The malonaldehyde (MDA) concentration was calculated using an

extinction coefficient of 1.56×10^5 $M^{-1}cm^{-1}$ [Sinnhuber and Yu, 1958]. The MDA concentration was converted to TBA number (mg MDA/kg sample) as follows [Jayasingh et al., 2002; McDonald and Hultin, 1987]:

Equation 2

$$TBAnr\left(\frac{mg}{kg}\right) = Abs_{532}(sample) * \frac{1M_{MDA}}{1.56*10^5} * \frac{1mole/L}{1M} * \frac{0.003L}{0.5g_{sample}} * \frac{72.07g_{MDA}}{mole_{MDA}} * \frac{1000mg}{g} * \frac{1000g}{kg}$$

$$or$$

$$TBAnr(ppm) = Abs_{532}(sample) * 2.77$$

Microbiology

Total aerobic plate count and coliform tests were done for refrigerated samples to ensure a safe sample for the sensory panel. After 72 h, samples were plated. One milliliter samples were pipetted onto 3M aerobic plate count Petrifilm™ and Coliform Petrifilm™. Samples were then incubated at 32 °C (in compliance with AOAC Official Method 989.10 for dairy products due to the AMF component) for 48 h. After 48 h, the plates were taken out of the incubator and a total plate count occurred immediately or they were placed in a freezer for the plate count to occur within 48 h.

Statistical Analysis

Experiments were performed in duplicate or triplicate as necessary. Data reported are the mean and standard deviation values calculated from the replicates. Significant differences were analyzed using a two- or one-way ANOVA test, as appropriate, and Bonferroni and LSD post-tests ($\alpha = 0.05$). Statistical analysis was performed using Graph

Pad software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA, www.graphpad.com) and SAS 9.1 TS Level 1M3 XP_PRO platform.

Results

Physicochemical Stability: As stated in Chapter IV, HPH emulsions tended to freeze over 80% of the time; VLPH emulsion, on the other hand, froze less than 50% of the time. When HPH emulsions would thaw, the three main components would separate out; however, VLPH emulsions would revert back to a homogenous liquid state with only some clarification occurring at the bottom of the tube, which is why VLPH emulsions were used for analysis of incorporating DHA. Besides analyzing the physicochemical stability of the emulsions with the incorporation of DHA, oxidation was also analyzed due to the sensory properties of DHA (i.e., fishy flavor and odor). Therefore, a standard method for determining the level of oxidation was used (i.e., TBA).

Figure 15 shows the stability for the various VLPH emulsions (done in triplicate) with and without DHA for the two contents of oil. The first observation is that 20:80 without DHA is quite unstable over time and has a high variability in destabilizing. All samples showed that the overall destabilization mechanism was due to creaming. Since this was done after 3 h at -10 °C, which had a sedimentation tendency, the emulsion may sediment during crystallization but over time will clarify and indicate a creaming tendency. The same can be said for the 40:60 emulsions. Interestingly, the 20:80 with DHA was quite stable and consistent and was almost as good as 40:60 without DHA. Even though the differences were not significant, it seems that for the 20% emulsions, DHA addition increased stability, while for 40% emulsions, DHA addition decreased stability. Samples used for the sensory evaluation appeared to all be at the low end of the

standard deviations, for visually there was not a lot of separation for the samples, which were given to judges, though they were vortexed to ensure the judges received homogeneous samples.

TBA: Figure 16 shows that there are no significant differences between samples with or without DHA and with 20 or 40% oil with the exception at 72 h a marginal significant difference ($p < 0.05$) was found between 40% emulsions with and without DHA. This slight significance and the fact that the odor of the emulsions changed to indicate oxidation of the DHA by 72 h gave a reasonable time line to do sensory tests at this point.

Sensory Evaluation: After determining that the samples would be safe for human consumption and the 72 h timeline would be appropriate for the sensory analysis, the samples were given to a descriptive panel. Each judge tasted each sample, which were given in a completely randomized order, twice. That is on two consecutive days, each judge tasted each emulsion once per day. Each attribute can be considered a separate test, which happened to be given at the same time and therefore they were statistically analyzed individually.

The statistical analysis for all of the attributes showed that there were many interactions between judges, rep and emulsion. Due to the assumption that the replicates were not significantly different, measures were taken to eliminate the interactions with replicates and between judge and emulsion. The criteria to take a judge off the panel were to note the standard deviation between replicates up to 50% of the mean value for the judge; then judges with 75% of emulsions with high standard deviations were looked at first and then 50% and so on; judges were taken off one-by-one until the significance

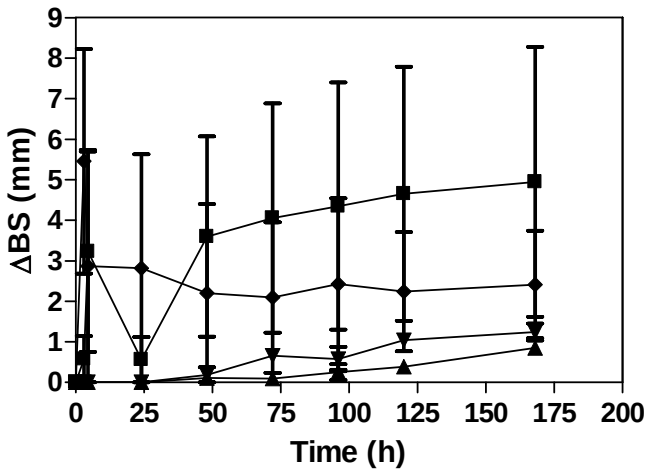

Figure 15: TurbiScan analysis of stability comparing VLPH emulsions. 20:80 ■, without DHA and ▼, with DHA. 40:60 ▲ without DHA and ◆, with DHA

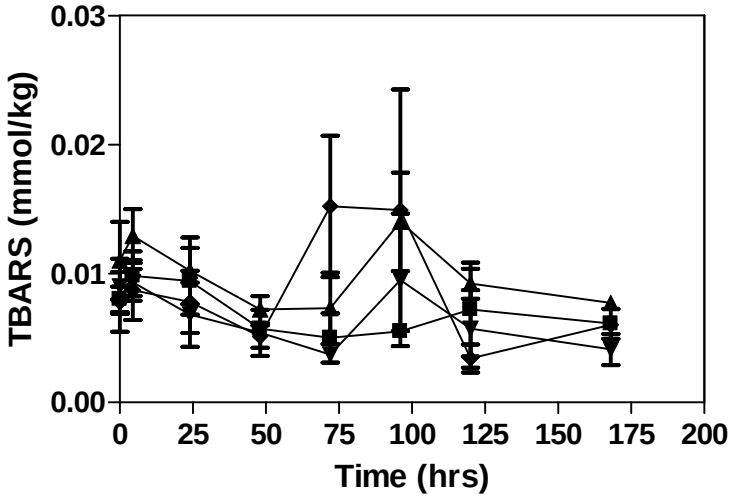

Figure 16: TBA analysis of VLPH emulsions with and without DHA for the duration of a week. 20:80 ■, without DHA; ▲, with DHA. 40:60 emulsions ▼, without DHA; ◆, with DHA.

Table 4: Plate count values for 1 ml of emulsion per Petrifilm™ for both total aerobic plate count (TAC) and coliform plate count (Coliform); C=without DHA; 20 = 20% oil and 40= 40% oil.

	Emulsion			
TAC (cfu/ml)	20 C	20 DHA	40 C	40 DHA
Rep 1	11	9	7	1
Rep 2	9	9	3	1
Average	10	9	5	1
Coliform (cfu/ml)				
Rep 1	0	0	0	0
Rep 2	0	0	0	0
Average	0	0	0	0

between interactions was above 0.05. The judge/emulsion interaction was also noted and if possible was taken to not being significant ($p < 0.05$). The criteria for this interaction were to look at the standard deviations for the judges for each replicate (i.e., all emulsions for the first replicate had a mean and standard deviation; the same for the second replicate). If there was no significant difference this would indicate that the judge was tasting all the samples similarly and therefore the attribute was either not being tasted or not accurately being tasted between samples and the judge was taken off for that attribute (see Appendix D).

The above criteria affected the selection of judges as follows: for oxidized, only one judge was taken off, who had three of the four emulsions with high standard deviations between replicates. Rancid did not have any interactions and therefore all judges were used. Fishy on the other hand was quite difficult to work with even by using the above parameters to disregard certain judges. When it was determined that the interaction between judge and emulsion could not be eliminated, then the data was used which did not have significance with the replicates. This difficulty of analysis would indicate that fishy is a tricky attribute to analyze because of the sensitivity of the

individual even with training. Some have an extremely low tolerance to the flavor, while others could not detect it until high concentrations. Finally, for buttery, six judges were taken off for the interactions to not be a factor, again using the same criteria from above. Table 5 is a summary of the significance found for each attribute. With the judges having significant difference between each other, then it is possible that this significance masks the significance between emulsions. This could lead to saying that there is no difference between emulsions, when in actuality there are differences.

The rating of intensity scale for each attribute was from 1 to 7, with 1 being a rating of "no flavor" and 7 being a rating of "extremely strong flavor," a 4 represented "moderate flavor."

One of the more interesting findings for sensory is the high oxidation values that were observed, and most were high in comparison to the values seen for fishy intensity with only 20:80 with DHA at approximately the same level. The oxidized attribute was on the edge of being marginally significant between the 40:60 emulsions with DHA incorporated when compared to all the other emulsions. Though all of the emulsions are rated between 3, which is "slight flavor" and 4 "moderate flavor." This would indicate that for all samples the oxidized attribute was noticeable, which might be attributed to the soybean oil and the slightly higher ratings for DHA samples might be accounted for by the additional component of DHA.

No significance was found between samples for rancidity; it was present from very slight flavor (i.e., a rating of 2) to slight flavor (i.e., 3). This might not be detrimental to the system depending on how the emulsion is used. If the emulsion was

Table 5: Mean scores (1 = no flavor, 2 = very slight flavor, 3= slight flavor, and 4= moderate flavor) per attribute for (n) judges for each emulsion after 72 h (C=without DHA; 20 = 20% oil and 40= 40% oil).

		Emulsion				Significance			
Attribute	n	20 C	20 DHA	40 C	40 DHA	judge	emulsion	rep	judge*emulsion
Oxidized	12	3.1^{a}	3.5^{ab}	3.2^{a}	3.8^{b}	<0.0001	0.1	0.8	0.1
Rancid	13	2.5^{a}	2.5^{a}	2.5^{a}	2.9^{a}	<0.0001	0.4	0.1	0.1
Fishy	8	2.0^{c}	3.4^{a}	1.8^{c}	2.8^{b}	<0.0001	<0.0001	0.3	<0.0001
Buttery	7	2.8^{a}	2.7^{a}	3.4^{a}	3.4^{a}	<0.0001	0.1	0.4	0.1

Superscripts with the same letter are not significantly different ($p < 0.05$) across rows.

used as a salad dressing the bite of rancidity would probably be masked by the addition of acid (e.g., citric).

The fishy attribute was definitely present in the samples, even those without DHA, which might be accounted for by the oil being oxidized. The fishiness of the samples was highly significantly different, mean scores ranging from 3.4 (a bit higher than "slight flavor") to 1.75 (almost "very slight flavor"). The 20:80 emulsions with DHA had the highest value for fishiness, which was significantly higher than that of the 40:60 emulsions with DHA. The higher flavor rating for 20:80 with DHA might be due to the extra 0.2g of DHA, though a good possibility is due to having less oil to mask the fishiness, or a combination of the two.

The two emulsions without DHA were the ones that were "very slight flavor" and below, which is good due to the lack of DHA in the samples. Some panelists commented on how fishy the samples were, but the combination of all judges' values lowered the overall value of the fishy intensities. This is where the judge/emulsion interaction might be a factor. Depending on the judges sensitivity to fishiness this could alter the results significantly. More training might be advisable for this attribute to see if a better

correlation could be obtained, but then it might just be an off day for the judge for that attribute.

Finally, the buttery flavor was looked at, due to the incorporation of AMF into the emulsions. No significance was seen between samples, all of which were close to a rating of 3 (slight flavor); however, the emulsions with 40% oil did have slightly higher values, with the emulsion without DHA with the highest, indicating that the DHA might mask some of the buttery flavor (though more testing would need to be done to determine if this is true).

Overall, it was found that the fishy attribute contributed the most significance between emulsions, which is not completely surprising due to the additional ingredient; however, the significance between judges for each attribute might be masking some very important differences between emulsions.

In addition to the rating of flavor intensity, the judges were asked to give comments on their impressions prior to tasting the samples and then after having tasted the samples. The samples had lids on the container so that the volatile compounds would be trapped. The judges were instructed to lift the lid and then waft the released compounds towards them. Figure 17 shows an overall picture of the judges' reactions to the emulsions prior to tasting them (see Appendix E for a complete list of pre-taste comments). The three main comments were agreeable, slight smells, and repulsive smells. Most people did smell slightly off odors from the emulsions, with repulsive coming in a close second. Agreeable odor was minimal with only 2 occurrences and both for 20:80 without DHA; on the other hand, 20:80 with DHA had the highest frequency of repulsive smells (in accordance with the fishy ratings). In Figure 18, the slight and

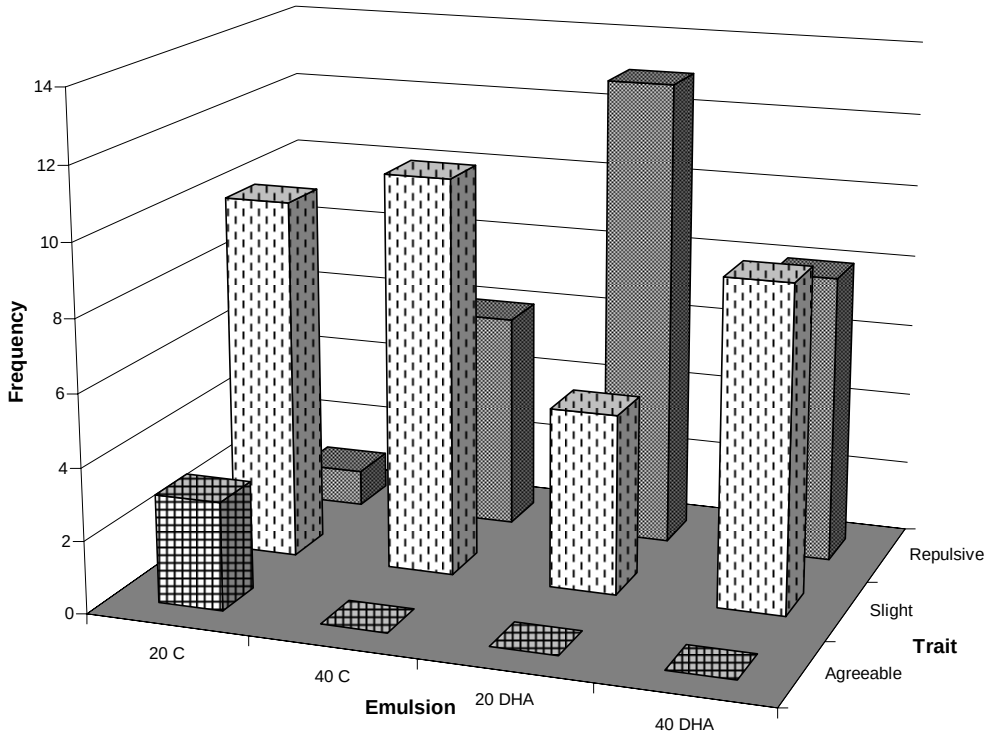

Figure 17: Pre-tasting (olfactory) overall impression of the emulsions prior to tasting. C=without DHA; 20 = 20% oil and 40= 40% oil.

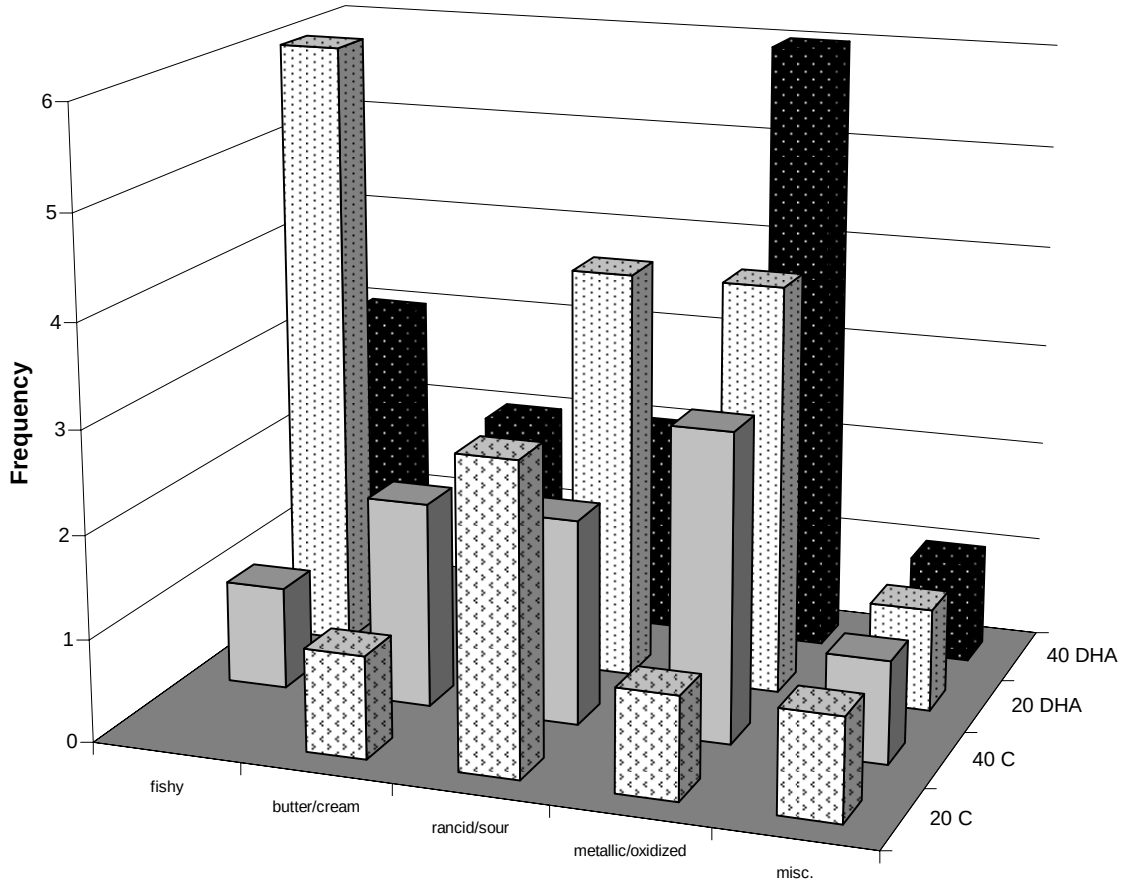

Figure 18: Pre-tasting overall qualities broken down into specific attributes (misc. is salty, musty and cheesy). C=without DHA; 20 = 20% oil and 40= 40% oil.

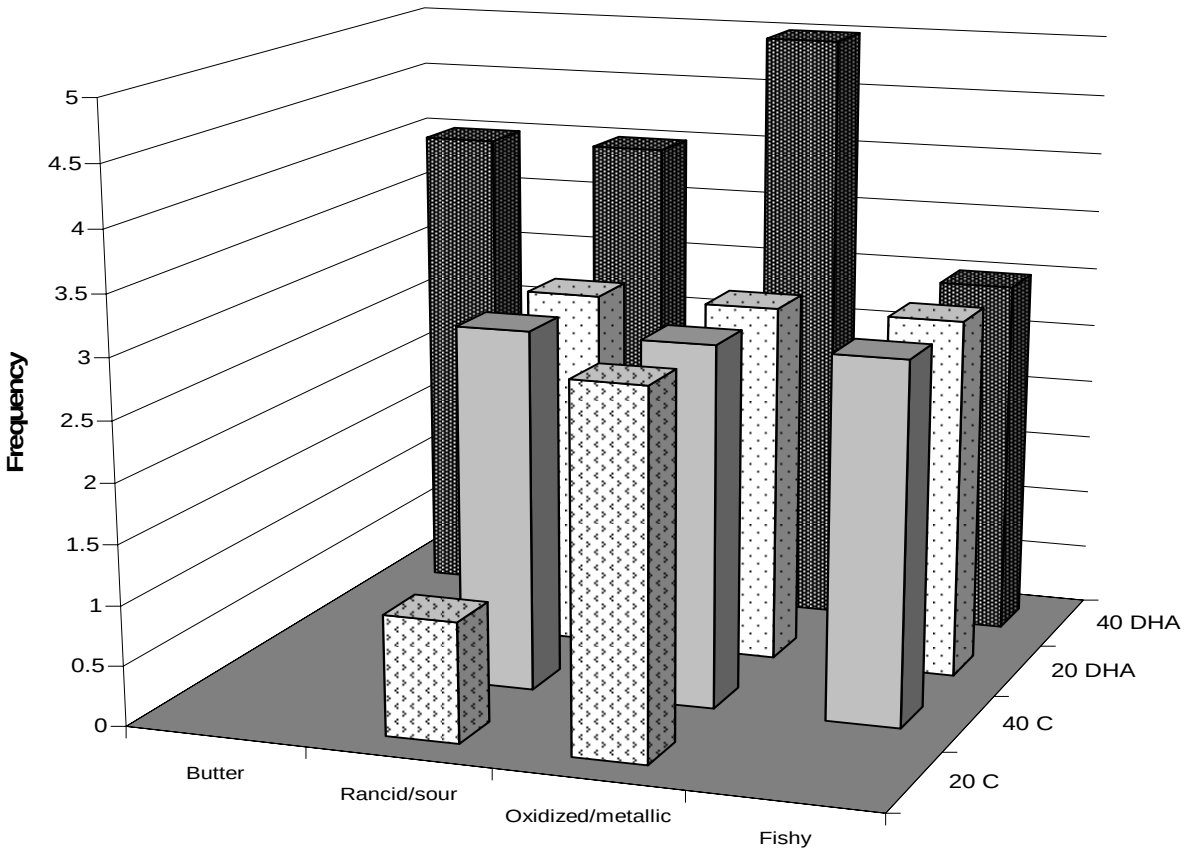

Figure 19: Overall impressions of emulsion for the four basic attributes. 20 and 40 stand for percentage of oil content; C=without DHA; 20 = 20% oil and 40= 40% oil.

repulsive odors are broken down to more specific attributes, though the attributes are combined for the two degrees of odor.

The most obvious and most frequent odor is that of fishy for the samples with DHA, which means prior to even sampling the emulsion the fishiness of the sample was evident and thereby impacted the overall flavor of the sample (which is a combination of taste, olfactory and retro-nasal senses). Next in frequency, were the observations of a metallic/oxidized odor (the judges usually grouped these two smells together, which is why they are grouped here). One judge referred to the odor as wet metal. It was not considered to be a pleasant smell. The rancid and sour smells were also frequent, especially for 20:80 emulsions. And it is not surprising that there was no significant difference between the samples with the consistency of the odor comments between all four samples for the rancid/sour characteristic. The butter/cream odor shows the same relationship with the statistical findings in that there is not a lot of variance between the samples.

The comments based solely on pre-tasting observations seem to mirror the statistical findings, which then leads to consider a pretty good correlation between taste and odor. To make a comparison the observations of the judges after experiencing the samples are seen in Figure 19 for the tested attributes. There are few overall comments for specific attributes, but this might be due to the judge considering the rating of the attributes to be sufficient and then added other off flavors to their overall impression (see Figure 20) (see Appendix F for a complete list of post-taste comments). The comments below would then be considered that for certain judges the attributes were so noticeable

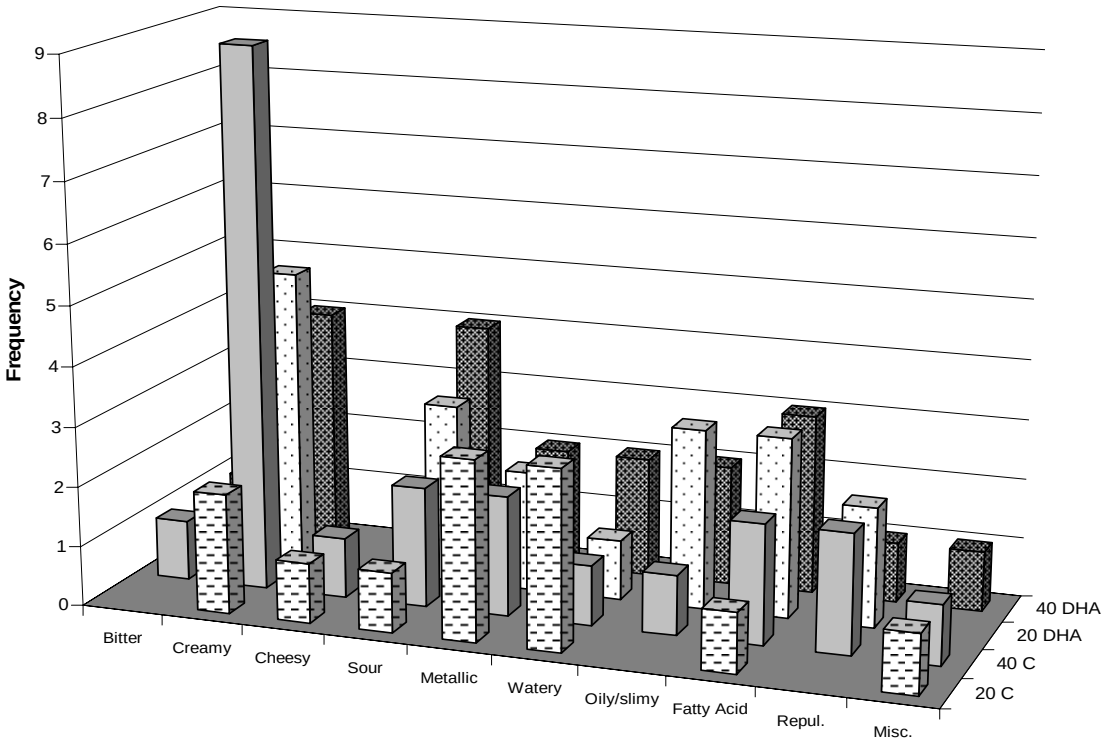

Figure 20: Overall impressions of emulsions broken down by more specific attributes. Miscellaneous (misc.) constitute floral, good, and no defect comments. C=without DHA; 20 = 20% oil and 40= 40% oil..

as to comment on them again in the overall impression section. Sour and metallic were included with rancid and oxidized, respectively, to keep consistent with the previous observations. Fishy is still commented on, and so is oxidized, but rancid is only commented on due to the sour characteristic.

The overall comments have similar qualities as the pre-taste comments such as metallic, sour, creamy and repulsive, however, there are now textural qualities added to the mix such as oily and slimy. Also, creamy should now be considered more of a textural quality rather than a smell, because the sample has been tasted and rolled about on the tongue, which would lead to the use of creamy in context with the feel of the sample rather than an odor. Creamy was the most frequent overall comment and most were for 40:60 without DHA.

It is also interesting to note that some judges distinguished the two phases commenting on either the oiliness of the emulsion or the water feel; one judge actually mentioned very specifically being able to distinguish the two phases. The fatty acid recognition was also a new attribute commented on for the various samples, and one judge mentioned bitter. Finally, there was at least one sample (i.e., 40:60 without DHA) that one judge thought was "good."

Also, it was possible to obtain a general idea of how samples changed with the inclusion of DHA, which was usually a difference of rating of approximately one degree of intensity at the most (see Table 5). The idea that one flavor might mask another is highly likely; for instance, with oxidized and buttery for 40:60 emulsions, the high values of oxidation may have masked the buttery flavor, which then made buttery intensity not significantly different between emulsions with 20 and 40% oil content. Finally, the need

to find a better way to mask the DHA is prominent with the pre and post sampling comments.

Discussion

Two items of note is that the 20:80 with DHA had a higher ratio of DHA to oil content than 40:60, though the concentration of DHA to the water phase was consistent, which might explain why the oxidized and fishy flavor intensities were similar. However, for 40:60 with DHA, oxidized flavor intensity is still high, but the fishy flavor intensity is almost one intensity rating lower (i.e., the difference between 'slight' and 'very slight' flavor), which might be do to the effect of one flavor masking another.

Believing that the buttery flavor is possibly masking some of the fishy flavor in the 40:60 emulsions, it is interesting to see that there is no significant difference between 20:80 and 40:60 emulsions within the buttery attribute. This might be due to the significance in judges or that within an emulsion half the AMF content is less noticeable. Another possibility is that the fishy flavor is also masking some of the buttery flavor, which leads to lower ratings. Therefore, even though there is not a lot of variance between buttery, rancid and oxidized, they all contribute to the overall flavor of the emulsions and how the flavors are interacting with each other.

Though, it is possible that the fishy flavor is masking some of the buttery flavor, which would lower it enough for the 40:60 and 20:80 emulsions to have similar ratings. The point being that the interaction of flavors could help or harm an emulsion for creating a desirable product depending on which flavors were masking alternate flavors or possibly enhancing various attributes.

As for looking at the new component in the emulsions, DHA, even with the high significance between judges, it was still possible to determine that at 72 h the DHA had oxidized to create an undesirable off-flavor to the emulsions (i.e., fishy). This indicates how a useful a sensory panel can be, because the oxidation of the DHA was not detected by TBA analysis, but could be detected by the panel. And it should be noted that no judge particularly liked tasting an o/w emulsion with only AMF, SBO and a WPI solution mixture. However, notwithstanding their dislike of tasting straight o/w samples, the problem is that the DHA oxidizes too quickly. Three days is not a long duration for a primary component to not only volatilize, but that the "functional" part of the food may no longer be bioavailable and therefore void the desired claim. Therefore, future research needs to be done in stabilizing DHA in AMF/SBO o/w emulsions.

Unlike Huang and Romans, a consumer panel was not applicable here due to initial descriptive panelist data, which indicated that more formulation work needed to be accomplished before the step of using the emulsion in a product to offer for public consumption. Huang et al. (1990) found an acceptable level of omega-3 fatty acids to put in the feed for chickens (<3%) and Romans found that 15% omega-3 fatty acids created off-flavors in pork, and Kowlanski (2001) was able to incorporate omega-3 fatty acids into margarine at an acceptable level; therefore, though the initial results indicated that the DHA concentration at this point is high, with possible alterations to the formula there might be an acceptable product that will be also be healthier than the current fats on the market.

References

Alamed, J, McClements, D.J. & Decker, E.A. (2006). Influence of heat processing and calcium ions on the ability of EDTA to inhibit lipid oxidation in oil-in-water emulsions containing omega-3 fatty acids. Food Chemistry, 95, 585-590.

Buege, J. A. & Aust, S. D. (1978). The thiobarbituric acid assay. Methods Enzymol, 52, 306.

Dimakou, C.P., Kiokias, S.N., Tsaprouni, I.V. & Oreopoulou, V. (2007). Effect of processing and storage parameters on the oxidative deterioration of oil-in-water emulsions. Food Biophysics, 2, 38-45.

Fomuso, L.B., Corredig, M. and Akoh, C.C. (2002). Effect of emulsifier on oxidation properties of fish oil-based structured lipid emulsions. Journal of Agriculture and Food Chemistry, 50, 2957-2961.

Huang, Z., Leibovitz, H., Lee, C.M. & Millar, R. (1990). Effect of dietary fish oil on omega-3 fatty acid levels in chicken eggs and thigh flesh. Journal of Agriculture Food Chemistry, 38, 743-747.

Jayasingh, P., Cornforth, D.P., Brennard, C.P., Carpenter, C.F. and Whittier, D.R. (2002). Sensory evaluation of ground beef stored in high-oxygen modified atmosphere packaging. Institute of Food Technologists, 67(9), 3493-3496.

Kiokias, S., Dimakou, C. & Oreopoulou, V. (2007). Effect of heat treatment and droplet size on the oxidative stability of whey protein emulsions. Food Chemistry, 105, 94-100.

Kolanowski, W., Swiderski, F. & Berger, S. (1999). Possibilities of fish oil application for food products enrichment with omega-3 PUFA. International Journal of Food Sciences and Nutrition, 50, 39-49.

Kolanowski, W., Swiderski, F., Lis, E. & Berger, S. (2001). Enrichment of spreadable fats with polyunsaturated fatty acids omega-3 using fish oil. International Journal of Food Sciences and Nutrition, 52, 469-476.

McDonald, R.E. & Hultin, H.O. (1987). Some characteristics of the enzymic lipid peroxidation system in the microsomal fraction of flounder skeletal muscle. Journal of Food Science, 52, 15–21, 27.

Meilgaard, M.C., Civille, G.V. & Carr, B.T. (2007). Sensory Evaluation Techniques, 4th edn. Champaigne, IL: CRC.

Romans, J. R., Wulf, D. M., Johnson, R.C., Libal, G.W. & Costello, W. J. (1995). Effects of ground flaxseed in swine diets on pig performance and on physical and sensory characteristics and omega-3 fatty acid content of pork: II. Duration of 15% dietary flaxseed. Journal of Animal Science, 73, 1987-1999.

SanGiovanni, J.P. & Chew, E.Y. (2005). The role of omega-3 long-chain polyunsaturated fatty acids in health and disease of the retina. Progress in Retinal and Eye Research, 24, 87-138.

Sinnhuber, R. 0. & Yu, T. C. (1958). Characterization of the red pigment formed in the 2-thiobarbituric acid determination of oxidative rancidity. Food Research, 23, 626-433.

Tong, L.M., Sasaki, S., McClements, D.J. & Decker, E.A. (2000). Mechanisms of the Antioxidant Activity of a High Molecular Weight Fraction of Whey. Journal of Agricuture Food Chemistry, 48, 1473-1478.

U.S. Department of Agriculture (USDA), Agricultural Research Service (2007). Nutrient Intakes from Food: Mean Amounts Consumed per Individual, One Day, 2003-2004. www.ars.usda.gov/ba/bhnrc/fsrg.

CHAPTER VI

SUMMARY AND FUTURE RESEARCH

Summary

In summary, this research was able to show the importance of understanding the various parameters (i.e., oil content, crystallization temperature, cooling-rate, and homogenization conditions) have on an emulsion.

By understanding the different effects on the stability of emulsions by the different variables, it might be possible to incorporate the knowledge into formulating actual food products (e.g., a new type of mayonnaise) and being able to determine how they will function under different environmental systems.

This model system has given a good foundation of AMF/SBO in WPI solution for various processing conditions. Given the low freezing rate of VLPH emulsions, -10 °C is probably at the edge of the freezing point for them. It would be interesting to take the emulsions down to a lower temperature and observe if they would remain stable after freezing and thawing to see if they would continue to maintain their homogenized state or if they would reflect the stability of HPH emulsions when placed under harsher conditions.

Future Research

Possible future research would be to continue to do long term physicochemical stability on VLPH emulsion with various amounts of DHA with the addition of an antioxidant. The antioxidant would be needed to hinder the oxidation of both the DHA and the soybean oil, which are both susceptible. Though, the DHA would be the greater

concern due to its fishy flavor and odor, which are highly unacceptable. If a way was found to encapsulate the DHA, but still make it bioavailable, then that would be the direction to head. After which, a descriptive panel could be used to evaluate the attributes to see if the objective was obtained. Later, an acceptability sensory test could be done in a product to see how the emulsion could actually be incorporated into a current food item to replace the *trans*-fat. Also, flavor interaction could be studied to determine which flavors might mask or bring out other flavors.

APPENDICES

Appendix A: Permission to Publish

Permission to Publish Inbox X

Megan Tippetts To whom it may concern, I am writing concerning man Mar 20 (13 days ago)

Megan Tippetts Sally, So sorry for my misunderstanding. The journal titl Mar 26 (8 days ago)

Journals Rights to me show details Mar 27 (7 days ago) | Reply |

Dear Megan,

Thank you for your email. Your permission is below.

Permission is granted for you to use the material below for your thesis/dissertation, subject to the usual acknowledgements and on the understanding that you will reapply for permission if you wish to distribute or publish your thesis/dissertation commercially.

All the best,

Sally

From: Megan Tippetts [mailto:m.tippetts@aggiemail.usu.edu]
Sent: 20 March 2008 19:15
To: clarcs@cla.co.uk; Journals Rights
Cc: chris.smith@chester.ac.uk
Subject: Permission to Publish

To whom it may concern,

I am writing concerning manuscript number: **IJFST-2007-02728.R2; Title: "Effect of oil content and processing conditions on the thermal behaviour and physicochemical stability of oil-in-water emulsions"**

I am first author, and this work was done in conjunction with my Master's research. I would like permission to publish the article within my thesis.

Thank you for your time and consideration of my request.

Appendix B: Descriptive Panel SIMS Instructions and Questionnaire

Questionnaire Code.....: DHA1E (Done also for DHA2E)
Questionnaire Description: Mar 3rd (and for Mar 4th) DHA vs Control 20 vs 40 VLPH
Questionnaire Type.....: Affective (because it gives hedonic scales to work with)
Notes: Use CDP panel

Page Number: 1
Attribute Sequence Number: 1
Attribute Type.....: Instruction Box
Seen With Relative Sample: 1

Instruction:
Please taste the samples from left to right as the computer prompts you. Place the whole sample in your mouth to allow the sample to coat your tongue. After analyzing the sample and rating the intensities of the various attributes, remember to expectorate and rinse your palate with water and eat a cracker.
PLEASE do not talk, nor disturb other panelists during your time in the sensory test.

Attribute Sequence Number: 2
Attribute Type.....: Page Break
Seen With Relative Sample: 1

Page Number: 2
Attribute Sequence Number: 3
Attribute Type.....: Comment
Seen With Relative Sample: none

Comment Type: Required
Question/Instruction:
Please comment on the odor of the sample. Note the intensity of the odor and what your impression of it is (e.g., agreeable, inoffensive, repulsive or something to that effect).

Attribute Sequence Number: 4
Attribute Type.....: Instruction Box
Seen With Relative Sample: none

Instruction:
Please rate the intensity of the following flavor attributes:

Attribute Sequence Number: 5
Attribute Type.....: Hedonic
Seen With Relative Sample: none
Question/Instruction:
Oxidized
Hedonic Labels on Questionnaire are, by Seen Order in Label(n):

Label(1) = No flavor (Ret value: 1)
Label(2) = Very slight flavor (Ret value: 2)
Label(3) = Slight flavor (Ret value: 3)
Label(4) = Moderate flavor (Ret value: 4)
Label(5) = Strong flavor (Ret value: 5)
Label(6) = Very strong flavor (Ret value: 6)
Label(7) = Extremely strong flavor (Ret value: 7)

Hedonic Type: Horizontal
Attribute Sequence Number: 6
Attribute Type.....: Hedonic
Seen With Relative Sample: none
Question/Instruction:
Rancid
Hedonic Labels on Questionnaire are, by Seen Order in Label(n):
Label(1) = No flavor (Ret value: 1)
Label(2) = Very slight flavor (Ret value: 2)
Label(3) = Slight flavor (Ret value: 3)
Label(4) = Moderate flavor (Ret value: 4)
Label(5) = Strong flavor (Ret value: 5)
Label(6) = Very strong flavor (Ret value: 6)
Label(7) = Extremely strong flavor (Ret value: 7)

Hedonic Type: Horizontal
Attribute Sequence Number:
Attribute Type.....: Hedonic
Seen With Relative Sample: none
Question/Instruction:
Fishy
Hedonic Labels on Questionnaire are, by Seen Order in Label(n):
Label(1) = No flavor (Ret value: 1)
Label(2) = Very slight flavor (Ret value: 2)
Label(3) = Slight flavor (Ret value: 3)
Label(4) = Moderate flavor (Ret value: 4)
Label(5) = Strong flavor (Ret value: 5)
Label(6) = Very strong flavor (Ret value: 6)
Label(7) = Extremely strong flavor (Ret value: 7)

Hedonic Type: Horizontal
Attribute Sequence Number: 8
Attribute Type.....: Hedonic
Seen With Relative Sample: none
Question/Instruction:
Buttery
Hedonic Labels on Questionnaire are, by Seen Order in Label(n):

Label(1) = No flavor (Ret value: 1)
Label(2) = Very slight flavor (Ret value: 2)
Label(3) = Slight flavor (Ret value: 3)
Label(4) = Moderate flavor (Ret value: 4)
Label(5) = Strong flavor (Ret value: 5)
Label(6) = Very strong flavor (Ret value: 6)
Label(7) = Extremely strong flavor (Ret value: 7)

Hedonic Type: Horizontal
Attribute Sequence Number: 9
Attribute Type.....: Comment
Seen With Relative Sample: none

Comment Type: Required
Question/Instruction:
Now that you have tasted the sample, please comment on any overall impression that you had (e.g., texture, additional flavors).

Appendix C: Randomized Sampling Plan for Descriptive Panels

ROTATION PLAN: BY SAMPLE SET

Experimental Definition: DHA1E

Rep: 1

Sample Set	PanelistID	Sample Order (Sample#/Sample Code)			
1	0000000006	2-941	3-748	4-165	1-379
2	0000000008	4-423	1-248	3-729	2-549
3	0000001001	3-871	2-137	1-247	4-325
4	0000001002	1-214	4-492	2-789	3-654
5	0000001003	1-693	3-327	4-861	2-261
6	0000001004	4-285	2-381	3-435	1-964
7	0000001005	3-675	1-258	2-317	4-491
8	0000001006	2-217	4-629	1-346	3-894
9	0000001007	4-457	3-978	1-637	2-785
10	0000001008	1-618	2-769	3-369	4-957
11	0000001009	3-794	4-348	2-152	1-498
12	0000001010	2-873	1-683	4-937	3-546
13	0000001011	3-619	1-279	2-592	4-178

ROTATION PLAN: BY SAMPLE SET

Experimental Definition: DHA2E

Rep: 1

Sample Set	PanelistID	Sample Order (Sample#/Sample Code)			
1	0000000006	3-621	2-839	4-387	1-586
2	0000000008	2-732	4-691	1-259	3-842
3	0000001001	4-819	1-467	3-526	2-681
4	0000001002	1-517	3-178	2-679	4-269
5	0000001003	2-546	3-461	4-637	1-736
6	0000001004	3-853	4-735	1-243	2-935
7	0000001005	4-724	1-534	2-416	3-318
8	0000001006	1-968	2-843	3-192	4-451
9	0000001007	4-745	1-478	3-157	2-896
10	0000001008	1-874	3-632	2-179	4-941
11	0000001009	3-289	2-951	4-195	1-495
12	0000001010	2-742	4-127	1-583	3-863
13	0000001011	2-731	4-279	1-572	3-123

Appendix D: Statistical ANOVAs of Sensory Attributes

Table D- 1: ANOVA table for Oxidized Flavor with all 13 judges

Source	df	Mean Square	F	p-value
judge	12	12.70	13.03	<0.0001
emulsion	3	2.11	2.17	0.1091
rep	1	2.16	2.22	0.1451
judge*emulsion	36	1.56	1.60	0.0828
judge*rep	12	2.75	2.82	0.0081
emulsion*rep	3	1.42	1.46	0.2429

Table D- 2: ANOVA table for Oxidized Flavor with 12 judges

Source	df	Mean Square	F	p-value
judge	11	13.30	14.19	<0.0001
emulsion	3	2.38	2.86	0.0519
rep	1	0.09	0.10	0.7538
judge*emulsion	33	1.56	1.66	0.0753
judge*rep	11	1.55	1.65	0.1296
emulsion*rep	3	1.81	1.94	0.1427

Table D- 3: ANOVA table for Rancid Flavor with 13 judges

Source	df	Mean Square	F	p-value
judge	12	14.77	13.51	<0.0001
emulsion	3	0.99	0.90	0.4493
rep	1	3.11	2.85	0.4004
judge*emulsion	36	1.66	1.52	0.4074
judge*rep	12	1.72	1.57	0.1445
emulsion*rep	3	0.63	0.57	0.6355

Table D- 4: ANOVA table for Fishy Flavor with 13

Source	df	Mean Square	F	p-value
judge	12	9.82	19.64	<0.0001
emulsion	3	13.55	27.10	<0.0001
rep	1	3.12	6.23	0.0173
judge*emulsion	36	2.16	4.32	<0.0001
judge*rep	12	2.28	4.56	0.0002
emulsion*rep	3	0.83	1.67	0.1914

Table D- 5: ANOVA table for Fishy Flavor with 8 judges.

Source	df	Mean Square	F	p-value
judge	7	12.66	30.54	<0.0001
emulsion	3	9.35	22.56	<0.0001
rep	1	0.39	0.94	0.3427
judge*emulsion	21	2.52	6.07	<0.0001
judge*rep	7	0.89	2.15	0.0827
emulsion*rep	3	0.39	0.94	0.4378

Table D- 6: ANOVA table for Buttery Flavor with 13 judges

Source	df	Mean Square	F	p-value
judge	12	10.18	15.96	<0.0001
emulsion	3	1.86	2.91	0.0474
rep	1	0.15	0.24	0.6263
judge*emulsion	36	1.33	2.09	0.0151
judge*rep	12	0.86	1.35	0.2339
emulsion*rep	3	0.85	1.33	0.2808

Table D- 7: ANOVA table for Buttery Flavor with 7 judges

Source	df	Mean Square	F	p-value
judge	6	8.24	9.09	<0.0001
emulsion	3	1.95	2.15	0.1291
rep	1	0.64	0.71	0.4108
judge*emulsion	18	1.97	2.17	0.0548
judge*rep	6	1.18	1.31	0.3042
emulsion*rep	3	0.98	1.08	0.384

Appendix E: Pre-taste Comments from Sensory Panel

Raw Data from:
Rep 1

20_80 VLPH Control

Panelist Code	Odor Comment
0000000006	agreeable rancid
0000000008	very odd flavor, fishy
0000001001	Offensive in the rancidity. Didnt really smell anything else.
0000001002	Slight oxidized smell. A little musty. Not bad at all. Fairly light and mild odor.
0000001003	old cheesy smell
0000001004	This smells tiny bit rancid.
0000001005	aggreeable
0000001006	unoffesnive slight smell
0000001007	slightly offensive oxidized and rancid smell, intensity 6
0000001008	creamy, thick
0000001009	I associate the smell with the smell outside after it rains. This sample was the most agreeable, yet still not the most pleasant odor.
0000001010	smelled oily, unoffensive
0000001011	disagreeable

Raw Data from:
Rep 2

20_80 VLPH Control 20_80 VLPH Control

Panelist Code	Odor Comment
0000000006	agreeable
0000000008	aggreable rancid
0000001001	Very slight rancid smell, only slightly offensive
0000001002	slightly detectible, mild smell.
0000001003	VERY slight odor of cream and stale water
0000001004	Unofffensive
0000001005	ok
0000001006	unoffensive slight smell
0000001007	No odor (I do have a cold so this may be a slight problem for my data!)
0000001008	sour smell
0000001009	not a strong odor at all. only very slight fishy odor. just unoffensive.
0000001010	This one doesnt make me want to throw up but is still not a smell that I like.
0000001011	odor mild

Raw Data from:
Rep 1

40_60 VLPH control

Panelist Code	Odor Comment
0000000006	buttery
0000000008	repulsive buttery
0000001001	Slightly rancid odor, not pleasant but not overwhelming.
0000001002	Slight, unoffensive odor.
0000001003	slight odor of cream
0000001004	u
0000001005	repulsive
0000001006	unoffensive.
0000001007	Slightly offensive oxidized and rancid smell. A little salty as well. Intensity 6
0000001008	sharp smell
0000001009	Still getting a fishy odor which is less than pleasant.
0000001010	It is not horrible, but I probably would not pick it out to eat.
0000001011	offensive

Raw Data from:
Rep 2

40_60 VLPH control

Panelist Code	Odor Comment
0000000006	unoffensive fishy and oxidized
0000000008	repulsive butrtery
0000001001	Slight, unoffensive metallic/oxidized odor
0000001002	Very mild and slight odor.
0000001003	smells creamy
0000001004	This sample smells bad
0000001005	ok
0000001006	unoffensive, about the same as last sample
0000001007	No odor (again, I have a cold)
0000001008	no smell at all
0000001009	slightly fishy odor. not quite as bad as the first sample.
0000001010	yuck, dont like
0000001011	mildly offensive

Raw Data from:
Rep 1
20_80 VLPH DHA

Panelist Code	Odor Comment
0000000006	repulsive oxidized
0000000008	repulsive oxidixed flavor
0000001001	The odor of the sample has a fairly strong intensity and is unpleasant. It seems slightly metallic, mixed with something else mroe unpleasant... rancidity?
0000001002	Medium intensity.
0000001003	fishy smell, has a smell of wheat or grass
0000001004	Unoffensive
0000001005	soapy odor
0000001006	repulsive fishy smell
0000001007	unoffensive intensity 10 slightly fishy and sour
0000001008	I can smell fishy, I think its unagreeable
0000001009	the odor is unpleasant. it reminds me of wet metal. The odor is quite intense. (strong)
0000001010	digusting
0000001011	a strong fishy odor, offensive

Raw Data from:
Rep 1
20_80 VLPH DHA

Raw Data from:
Rep 2

Panelist Code	Odor Comment
0000000006	unoffensive oxidized
0000000008	repulsive rancid
0000001001	Slightly offensive rancid odor along with a moderate, unoffensive metallic/oxidized odor
0000001002	Musty-ish, as usual.
0000001003	smells fishy
0000001004	t
0000001005	offensive
0000001006	repulsive, fishy smell
0000001007	slightly fishy and oxidized odor--fishy being the strongest attribute --so repulsive intensity 5
0000001008	smells fishy, not pleasant
0000001009	very fishy smell. easily detected. I dont like the smell.
0000001010	offensive, yuck, nasty.
0000001011	offensive

Raw Data from:
Rep 1
40_60 VLPH DHA

Panelist Code	Odor Comment
0000000006	repulsive cardboardy
0000000008	kind of sour flavour, stale flavor..may be rancid
0000001001	Very strong rancid odor, very repulsive. Metallic/oxodized almost as strong, not as unpleasant.
0000001002	This sample has a mild chive odor to it. Kind of garden-like.
0000001003	old cheesey smell, smells rich & creamy
0000001004	Unoffensive
0000001005	offensive smell
0000001006	unoffensive
0000001007	unoffensive intensity 5 buttery and a bit oxidized
0000001008	creamy smell
0000001009	The fishy smell is very present in this sample. I dont like that smell so I think it is unagreeable.
0000001010	nasty.
0000001011	offensive

Raw Data from:
Rep 1
40_60 VLPH DHA

Raw Data from:
Rep 2

Panelist Code	Odor Comment
0000000006	repulsive
0000000008	aggreable oxidized
0000001001	Strong, offensive rancid smell
0000001002	Light pungant smell.
0000001003	smells metallic
0000001004	this tastes bad
0000001005	offensive smell
0000001006	repulsive, fishy smelling
0000001007	Unoffensive odor, intensity 7 rather buttery and a little oxidized
0000001008	thick, cooked smell
0000001009	slightly metallic odor. also fishy. Not my favorite smell. unoffensive.
0000001010	nasty, smells a little fishy to me.
0000001011	offensive

Appendix F: Post-Taste Comments from Sensory Panel

Raw Data from:
DHA1E

20_80 VLPH Control

Panelist Code	Overall impression
0000000006	rancid
0000000008	fishy flavor
0000001001	Once again, buttery and rancid more detectable in aftertaste.
0000001002	Not horrible tasting, but not something I would probably seek out to buy. Watery. Musty. Mild.
0000001003	smoothe texture, almost had a sour taste to it
0000001004	This one tasted the worst of all the samples. Not terrible just not good
0000001005	fishy
0000001006	Buttery flavor was strongest
0000001007	This was very smooth again and once I tasted it, it reminded me of mozzeralla cheese, although I did not smell that. A slightly sour taste as well.
0000001008	I tasted fatty acid, but not the sweet kind you get in ice cream
0000001009	the most watered down of the samples. Not quite as creamy and thick as the others. Not quite as fishy either.
0000001010	yuck. It tasted like water with rotten fishy ranch dressing in it.
0000001011	it had an almost metalic odor

Raw Data from:
DHA2E

20_80 VLPH Control

Panelist Code	Overall impression
0000000006	no defect
0000000008	rancid
0000001001	Smooth texture, metallic flavor tingles the tongue
0000001002	Palatable, but not gourmet.
0000001003	somewhat oily but pleasant for the most part, creamy with a slight metal taste
0000001004	Texture is ok
0000001005	not good taste
0000001006	unique taste
0000001007	Smoother than first sample in texture, less flavorful-- sour only intensity 2 and overall rather bland/benign taste and smell
0000001008	fatty acid
0000001009	very watery, runny.
0000001010	This sample was not as bad tasting as the other two. Didnt seem to seperate in my mouth as much. And didnt leave such a strong after taste.
0000001011	moderatley disagreeable

Raw Data from:
DHA1E

40_60 VLPH control

Panelist Code	Overall impression
0000000006	smmoth buttery
0000000008	buttery flavor kind of odd taste, repulsive
0000001001	Aftertaste seemed VERY buttery. Rancid was more detectable in odor than taste.
0000001002	Less strong than the preceding samples.
0000001003	very rich and creamy taste to it
0000001004	This tasted good to me!
0000001005	no comments
0000001006	Thick texture, didnt task that bad
0000001007	This tastes very similar to me as the last sample (637)--reminded me of mozzeralla cheese again, but with a slightly more buttery flavor. So I actually like the taste but do not like the smell of these last two samples for some reason!
0000001008	a little bitter
0000001009	This one had more of that metalic taste to it. It was not as thick and creamy as the others=more watered down it seemed.
0000001010	This was very creamy and almost reminded me of ranch dressing that had been sitting out a few weeks.
0000001011	texture oily

Raw Data from:
DHA2E

40_60 VLPH control

Panelist Code	Overall impression
0000000006	fishy and cardboardy
0000000008	buttery
0000001001	Smooth texture
0000001002	Had a lot of fatty-acid feel to it.
0000001003	creamy, smooth; not terribly oily
0000001004	Texture is good
0000001005	oxidised
0000001006	fishy was noticable stronger
0000001007	This was the least flavorful to me. It has a smooth oily texture and the strongest taste is the aftertaste (which was a mix of sour and rancid).
0000001008	creamy, fatty acid
0000001009	creamier (thicker) had a cardboardy taste.
0000001010	very creamy, but the flavor is almost unbearable.
0000001011	almost tasted metallic

Raw Data from:
DHA1E

20_80 VLPH DHA

Panelist Code	Overall impression
0000000006	oxidized
0000000008	bitter after taste
0000001001	somewhats creamy, very unpleasant, tingles the tongue (oxidized?), like rotting milk? The combination of rancid and metallic is really gross!
0000001002	The strongest of all the samples. Almost has a bland powdered sugar icing taste to it.
0000001003	creamy, coating texture; an oily taste to it, almost slimy
0000001004	Texture was good taste was good also
0000001005	no comments
0000001006	Absolutely horrendous taste. Worst of the four samples. Wasnt sure what to make of the flavor. Unique taste.
0000001007	Smooth texture again. I tasted a lot of sour in this one!
0000001008	Fishy, fatty acid, oxidized
0000001009	texture was creamy. Im sensing some metalic taste as well. The fishy was the strongest flavor
0000001010	I wouldnt eat this either. It is has to strong of a nasty after taste. It is almost as though the water and cream have seperated and so it is two textures in my mouth with rotten ranch dressing.
0000001011	oily and fishy, icky

Raw Data from:
DHA2E

20_80 VLPH DHA

Panelist Code	Overall impression
0000000006	oxidized
0000000008	very slight fishy too
0000001001	Fatty texture, fatty acid flavor
0000001002	Very similar to other samples.
0000001003	disgusting
0000001004	there is a very strange flavor that I am not sure of
0000001005	not good
0000001006	fishy was strong and sample had a spoiled taste
0000001007	Smooth texture, not many additional flavors detected (maybe sour = 1) b/c fishy was overwhelming in this one to me!
0000001008	slightly oxidized, fatty acid
0000001009	fishy taste dominated my senses. it was not so creamy, kind of watery texture. I also noticed a metallic flavor.
0000001010	Has the consistency of whipping cream except with a really bad taste that lingers for a really long time. Like the rest of the day. Still reminds me of old/rotten ranch dressing.
0000001011	oily with a very strong fish flavor

Raw Data from:
DHA1E

Panelist Code	Overall impression
0000000006	oxidized
0000000008	it is having a buttery texture. like fatty acid that has oxidized.
0000001001	Seemed to have the most of the above flavors at stronger intensities than other samples. VERY unpleasant, especially the strength of the rancid flavor.
0000001002	Has a mild buttery flavor and a fatty acid feel to it. Not terrrible, but not super yummy. has a mild lingering aftertaste.
0000001003	very creamy taste and a watery/oily texture to it
0000001004	Not really bad tasting but different. Maybe a little metallic???
0000001005	worst sample
0000001006	Much stonger flavor than 217. had a very slight floral taste
0000001007	very smooth texture
0000001008	I didnt taste any oxidized, just buttery and fatty acid
0000001009	texture was thick. there was more of an oxidized flavor with this one.
0000001010	Tasted like a buttery fish dipped in rotten milk
0000001011	texture, slimy

Raw Data from:
DHA2E

Panelist Code	Overall impression
0000000006	oxidized
0000000008	bitter aftertaste
0000001001	Strong fatty acid flavor, somewhat thick fatty texture
0000001002	Seemed a little stronger than the last sample.
0000001003	very thick and pastey; had a bit of zing to it
0000001004	Texture is fine
0000001005	cardboardy taste
0000001006	this sample makes me gag. It was more of a spoiled fishy taste.
0000001007	Smooth texture and rather sour taste
0000001008	a little bit frothy, fatty acid
0000001009	grainy texture. very thick and not a pleasant sensation on the tongue. very cardboardy
0000001010	There is a seperation of tastes for me. One is watery and the other creamy or oily. Together they just dont seem to mix.
0000001011	again, almost metallic, very fishy