Effect of Soy Lecithin Concentration on Formulating Dairy Emulsions Through Ultrasound Treatment

Collette Kernyuy Nyuydze
South Dakota State University

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EFFECT OF SOY LECITHIN CONCENTRATION ON FORMULATING DAIRY
EMULSIONS THROUGH ULTRASOUND TREATMENT

BY

COLLETTE KERNYUY NYUYDZE

A thesis submitted in partial fulfilment of the requirement for the
Master of Science
Major in Biological Sciences
Specialization in Dairy Science
South Dakota State University
2020
THESIS ACCEPTANCE PAGE

Collette Kernuy Nyuydze

This thesis is approved as a creditable and independent investigation by a candidate for the master’s degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Sergio Martinez Monteagudo
Advisor

Department Head

Dean, Graduate School
This thesis is dedicated to the Lord Almighty for His guidance, strength, and protection.

To my family for their love and support, and to my late mum Nyuydze Relindis.
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# ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
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<tbody>
<tr>
<td>GRAS</td>
<td>Generally recognized as safe</td>
</tr>
<tr>
<td>HLB</td>
<td>Hydrophilic-lipophilic balance</td>
</tr>
<tr>
<td>HPH</td>
<td>High-pressure homogenization</td>
</tr>
<tr>
<td>Ia</td>
<td>Acoustic intensity</td>
</tr>
<tr>
<td>IgE</td>
<td>Immunoglobulin E</td>
</tr>
<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>O/W</td>
<td>Oil-in-water</td>
</tr>
<tr>
<td>Pa</td>
<td>Acoustic power</td>
</tr>
<tr>
<td>PA</td>
<td>Phosphatic acid</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphotidylcholine</td>
</tr>
<tr>
<td>PE</td>
<td>Phosphotylinositol</td>
</tr>
<tr>
<td>Sa</td>
<td>Surface area</td>
</tr>
<tr>
<td>SDS</td>
<td>Sodium dodecyl sulfate</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscope</td>
</tr>
<tr>
<td>TS</td>
<td>Total solids</td>
</tr>
<tr>
<td>WPC</td>
<td>Whey protein concentrate</td>
</tr>
<tr>
<td>WPI</td>
<td>Whey protein isolate</td>
</tr>
</tbody>
</table>
ABSTRACT

EFFECT OF SOY LECITHIN CONCENTRATION ON FORMULATING DAIRY EMULSIONS THROUGH ULTRASOUND TREATMENT

COLLETTE KERNUY NYUYDZE

2020

The ability of high intensity ultrasound to produce stable emulsions without the addition of surfactant was evaluated in a dairy-based formulation. The formulation consisted of protein (4.33 ± 0.05%, whey protein concentrates (WPC80), carbohydrates (21.52 ± 0.75%, sucrose and maltodextrin), oil (2.90 ± 0.05%, soybean oil), and surfactant (0.05%). Pre-emulsions formulated with either 0, 0.025, and 0.05% of soy lecithin were treated for 5 min at an acoustic intensity of either 42.58 ± 2.98, 56.83 ± 3.01, or 70.48 ± 2.97 W cm\(^{-2}\). The stability of the emulsions was evaluated through particle size, dynamic rheology, gel electrophoresis, and microstructure. In general, the particle size decreased with the acoustic intensity (397 to 230 nm), regardless of the concentration of soy lecithin. Dynamic rheology (strain and frequency sweeps) showed an improved stability of the emulsions treated at 56.83 ± 3.01 and 70.48 ± 2.97 W cm\(^{-2}\) without the addition of soy lecithin, displaying a distinctive viscoelastic region and a behavior of weak gel. During 21 days of storage at 4°C, the particle size slightly increased (470-500 nm), while the mechanical spectra remained essentially unchanged. High intensity ultrasound offers opportunities for reducing surfactants in dairy-based formulations.

Keywords: Ultrasound, dairy-based emulsions, soy lecithin, dynamic rheology
Chapter 1

Introduction and objectives

1.1. Significance of the research

The increasing demand for clean label products is driving manufacturers in the food and dairy industry towards the use of ingredients and processing methods that are aimed at satisfying this demand. This project will focus on characterizing the temperature change of different material compositions to obtain acoustic intensities at different ultrasound treatments. During ultrasound treatments, sound is transmitted through the liquid media that results in expansion and compression cycles. The expansion cycle generates small bubbles in the liquid that grow and violently implode at threshold levels. This results in high temperatures and pressures in the bubbles (Chaudhari et al., 2015).

This work will also present the process of formulation of emulsions and the factors responsible for their destabilization. It will focus on the use of stabilizers to ensure stability of these emulsions. Additionally, the demand by consumers for use of natural additives rather than synthetic additives in food products will be presented. Furthermore, the work will focus on the potential use of plant-based natural emulsifier (soy lecithin) as a clean label trend. The technologies used in the preparation of emulsions will follow suit. Finally, ultrasound technology will be addressed as a potential technology in the formation of stable emulsions by investigating their stability with storage.
Nowadays, there is an increasing demand by health-conscious consumers for food products made with natural ingredients. According to a survey by market researchers Innova Market Insights, 50% and 72% of European and US consumers respectively agree that a product’s ingredient list must be simple and understandable (Food business report, 2015).

Several products in the food and dairy industry rely on emulsification process. This process requires surfactants or emulsifiers to ensure stability and increase the shelf-life of the products. Emulsifiers can either be synthetic or natural, and with the clean trend, manufacturers are moving towards natural ingredients and their use in food formulations to satisfy consumers (Ozturk and McClements, 2016).

Soy lecithin is one of the most commonly used natural emulsifier because of its availability, excellent emulsifying properties, taste and color (Cherry and Kramer, 1989). It is amphiphilic in nature, readily available and the cost is low. The mechanical energy required for emulsion formation can be provided by rotor-stator systems, high-pressure systems, and ultrasound. Research is carried out on food preparation technologies that minimize the use additives, while at the same time, maintain the quality of the food product.

Ultrasound is a promising technology in food processing and preservation as it increases the shelf life of the product (Chemat et al., 2011). The technology is non-destructive in which the interaction of the acoustic energy with the food occurs mainly
through a liquid medium, as cavitation, physical and chemical actions of the ultrasound play an important role in food quality during its transformation (Gallo et al., 2018). During ultrasound treatment, acoustic cavitation is generated that results in growth and violent collapse of bubbles leading to increase in the temperature and pressure of the medium (Ashokkumar and Mason, 2007). This can have different impacts on the physical and functional properties of materials. In dairy products, ultrasound can impact the conformation of dairy proteins and cause their aggregation. When ultrasound was used to study its effect on whey protein isolate (WPI) and whey protein isolate (WPC) solutions, the turbidity of WPC solution decreased when higher frequencies were used due to aggregation (Zisu et al., 2011). They also reported a different observation for WPI treated solutions which could be attributed to difference in compositions. Some studies have also shown shear forces induced by ultrasound can reduce the viscosities of starch solutions and result smaller size particles (Iida et al., 2008, Zuo et al., 2009).

Ultrasound has potential in food processing, particularly in the formulation of emulsion (Aslan and Dogan, 2018). Commercially available food products, such as sauces, infant formula, chocolate, condiments, spreads, salad dressings and desserts are all food emulsions. Due to consumers’ demands, studies have been carried out on ultrasound preparation of emulsions with no emulsifiers. In research carried out on the impact of ultrasound on emulsifier free emulsions, the results show the possibility of eliminating food additives like emulsifiers in preparation of food emulsions (Aslan and Dogan, 2018).

**Hypothesis**
1. \( H_0 \): The use of ultrasound will produce stable emulsions with minimized soy lecithin emulsifier.

\( H_1 \): Ultrasound will not produce stable emulsions with minimized soy lecithin emulsifier.

2. \( H_0 \): Soy lecithin concentration will have a significant impact on emulsion stability.

\( H_1 \): Soy lecithin concentration will not have a significant impact on emulsion stability

1.2. References


Chapter 2.

Literature review

This chapter will bring an insight on the formation of emulsions, factors associated with their instability and the use of emulsifiers to prevent destabilization of emulsions. Since there is growing concerns on additives used in the production of food products, manufacturers are moving towards natural additives. The literature review will focus on processing technologies used in production of emulsions. It will then address the potential of ultrasound in production of emulsions with or without emulsifiers.

2.1. Emulsification

Emulsification is the dispersion of at least two immiscible liquids, usually oil and water and are classified as oil-in-water or water-in-oil emulsions. In the formation of emulsions, oil, aqueous phase (water), stabilizers (reduce interfacial tension) and mechanical energy provided by rotor-stator systems or high-pressure homogenizers are required (Cucheval and Chow, 2008). A common practice during the formation of emulsions is to dissolve the components separately in their soluble phases before mixing them together (Young, 1988). Properties of emulsions such as stability, rheology, and their industrial uses are dependent on temperature and composition, as well as the particle size distribution (Leal-Calderon et al., 2007). Many other factors play a critical role during this process, including the type and concentration of emulsifier and relative viscosities of the dispersed/continuous phases, (Lee et al., 2013).
Homogenization breaks the particles into smaller droplets and is influenced by the pressure, number of homogenization steps and emulsifier concentration as instigated by (El Kinawy et al., 2012). In their study, it was seen that high-pressure homogenizers with flat valve at a pressure of 200 bar, one homogenization stage and low emulsifier concentration (Tween 400 of 3%) produced emulsions with mean droplet size of 1.7 µm. Increasing the pressure to 500 bar with 2 to 3 homogenization stages resulted in a mean droplet size of 600 nm and further increase in emulsifier concentration (7%) decreased the mean droplet size to 200 nm. Some restrictions associated with emulsion formation are the preparation of edible emulsions, need for stability of the emulsions over an extended time period, and microbial safety after extended storage (Dalgleish, 2001).

Emulsions are thermodynamically unstable due to the presence of high surface free energy between the oil and water phase but operationally stable when they are slow to changes that result in separation of these two phases (Pearce and Kinsella, 1978). Moreover, during emulsification, the concentration of components and processing parameters (amount of surfactant and mechanical device) can affect the distribution and structure of the emulsion hence, affecting its stability (Bos et al., 1997). Physical and chemical changes are responsible for emulsion breakdown. The physicochemical mechanisms that cause instability in emulsions are presented in a number of characteristics that can be grouped as flocculation, creaming, coalescence and Oswald ripening (Fredrick et al., 2010). Factors responsible for these destabilization mechanisms are nature and concentration of emulsifier or stabilizer used, the pH of the system, ionic strength, temperature, parameters of homogenization and the interaction of the dispersed with
continuous phase (Maphosa and Jideani, 2018). It is however difficult to differentiate these mechanisms but understanding which of the mechanisms occurs in a particular system, can result in effective strategies to improve their stability (McClements, 2015).

2.2. Mechanisms of emulsion instability

2.2.1. Flocculation

Flocculation is the process of droplet aggregation without any rupture of the stabilizing layer at the oil-water interface (Adams et al., 2007). Flocculation is thought to occur due to insufficient stabilizer during emulsification. Van der Waals forces, centrifugation, Brownian forces, electrostatic and steric forces contribute to flocculation (Maphosa and Jideani, 2018). However, it can also result from the presence of excess surfactant in the continuous phase due to depletion effect (Khan, 2011). Figure 1.1 exemplifies the formation of droplet aggregates through flocculation mechanism.

![Flocculation mechanism](image)

Figure 2.1. Schematic diagram of the flocculation mechanism. Adapted from (Bouyer et al., 2012).
In addition, the surfactant type and additional interactions between the absorbed surfactant film also affect flocculation (Damodaran, 2005). This process causes two effects that are responsible for the instability of emulsions:

i) increase in droplet size that enhances the rate of creaming

ii) increase in the probability of coalescence (Borwankar et al., 1992).

Flocculation may be advantageous or detrimental to the quality of the emulsion depending on the nature of the product by accelerating gravitational separation and creation of desirable texture when controlled. Flocculation that occurs due to hydrophobic interactions may be prevented by the addition of sufficient emulsifier to complete cover droplet surfaces or an emulsifier that does not result in surface hydrophobicity (McClements, 2015).

2.2.2. Creaming

Creaming is the separation of oil from the water phase due to density difference between the dispersed and continuous phases (Costa et al., 2019). Figure 1.2 illustrates the breakdown of emulsion by creaming where the layer of oil droplets rises to the top of the water layer. When severe, it leads to a cream layer at the top and serum at the bottom. Creaming can ultimately lead to decrease in acceptability of some food products (McClements, 2015) and the process is influenced by the particle size, concentration, rheology and the state of aggregation. Creaming can be reduced by increasing the viscosity
of the continuous phase. Upward creaming occurs in O/W emulsions due to lower density of disperse to the continuous phase and vice versa for W/O emulsions (Khan, 2011).

![Diagram of creaming mechanism](image)

Figure 2.2. Schematic diagram of the creaming mechanism. Adapted from (Bouyer et al., 2012)

2.2.3. Coalescence

Coalescence is the principal cause of emulsion instability and occurs when two droplets in contact forms a bridge between them, merging to form one larger drop that may eventually result in phase separation of the emulsion (Chen et al., 2013). The average droplet size reduces with and ultimately reduces the stability of the emulsion. This has been reported by (Ivanov et al., 1999, Binks et al., 2000). Coalescence is irreversible and usually follows flocculation. Figure 1.3 shows the schematic illustration of coalescence where small dispersed droplets accumulate to form a larger droplet. The merging of the droplets results in entrapment of the thin film between the droplets in the continuous phase. The process is thus facilitated by flocculation rate, low oil, viscosities of the two phases, surfactant concentration at the interphase and high temperature (Raya et al., 2020).
The energy input and device design are responsible for the balance between droplet distribution and coalescence (Jafari et al., 2007). This mechanism can be prevented by the use of surface-active materials, such as stabilizers and proteins through electrostatic and steric interaction forces that slow down the drainage of the intervening continuous film when two drops come together (Mohan and Narsimhan, 1997). In a study conducted on coalescence of emulsions stabilized with whey protein isolate (WPI) or sodium dodecyl sulfate (SDS) with high hydrophilic-lipophilic balance, the researchers concluded that coalescence is affected by the stabilizer concentration and external force acting on emulsions (van Aken and Zoet, 2000). Also, it is well known that the faster an emulsifier molecule adsorbs at the interface of newly formed emulsion droplets, the smaller the particles produced and the lower the probability of coalescence (Schröder et al., 1998).

![Figure 2.3](image)

*Figure 2.3. Schematic diagram of the coalescence mechanism. Adapted from (Bouyer et al., 2012)*

### 2.2.4. Oswald ripening

Oswald ripening is the growth of an emulsion droplet at the expense of a smaller one and is characterized by the diffusion of molecules of the disperse phase from small to large particles (Fredrick et al., 2010). *Figure 1.4* demonstrates emulsion instability by
Oswald ripening. In this process, the internal pressure between smaller and larger droplets leads to transport of dispersed phase by diffusion (Bergenståhl, 2015). This results from shrinking and disappearance of the smaller droplets at the expense of growth of larger droplets. The driving force for this is significant solubility of the dispersed phase in the continuous phase (Dickinson, 2009).

![Figure 2.4](image.png)  

Figure 2.4. Schematic diagram of the Oswald ripening mechanism. Adapted from (Bouyer et al., 2012).

2.3. Stability of emulsions

Emulsion stability is the ability of an emulsion to resist changes in its physicochemical properties with time (Hu, 2017). Hence, emulsions require surfactants to remain stable during storage, handling, and use. In addition, different technologies have been used to improve emulsion stability. Surfactants prevent the agglomeration of the dispersed material in the liquid phase to increase their stability. This is by forming a bilayer around each droplet particle.
2.3.1. Emulsifiers/Surfactants

Emulsifiers are food additives which are amphiphilic in nature and reduces the surface tension between mutually insoluble phases, hence facilitating emulsification and increasing emulsion stability (Krog and Sparso, 2004). They create a barrier for coalescence and droplet growth during storage. Therefore, the droplet size produced during homogenization depends on the different characteristics of the emulsifier which must be sufficient to cover the surfaces of the newly formed droplets. The time taken for emulsifier to move from the bulk phase to the droplet surface, probability of emulsifier adsorption, amount of emulsifier, and effectiveness of the emulsifier in protecting droplets against coalescence, is characterized by the emulsion and environmental conditions such as pH, ionic strength, heating and freezing (Guzey and McClements, 2006). Research studies on the influence of environmental stresses on o/w emulsions stabilized by sodium dodecyl sulfate (SDS)-chitosan-pectin membranes have shown good stability for tertiary emulsions over a pH range, thermal treatment and freeze-thaw cycling, (Aoki et al., 2005). This study also shows the probability of improving emulsion stability using multilayered interfacial membranes.

Small-molecular emulsifiers and macromolecules are used in emulsion stabilization (Wahlgren et al., 2015), and these emulsifiers are classified according to their hydrophobic-lipophilic balance (HLB) numbers, which give an indication of the relative affinity of an emulsifier to the oil and aqueous phases. The HLB numbers are expressed based on the molecular weight of hydrophobic components to the molecular weight of the molecule as expressed in Equation (1) (Wahlgren, 2015).
Equation 1.1

\[ HLB = 7 + \sum (\text{hydrophilic group numbers}) - \sum (\text{hydrophobic group numbers}) \]

HLB values are used in selecting an appropriate emulsifier, preparing the emulsion, as well as blending many emulsifiers to obtain a desired HLB value (Robinson and Eskin, 2000). The HLB values of some common surfactants used in the food industry are presented on Table 1.1 with their applications. Surfactants with higher HLB values stabilize oil-in-water emulsions while those with lower HLB values stabilize water-in-oil emulsions (Stauffer et al., 2020). For example, o/w emulsions have HLB values of 2-6 and w/o emulsions are from 10-18 (Walstra, 2005). The setback of this concept is its determination in emulsifiers or their blends, which is experimented in isolation than in a practical environment. This shows the dependence of the concept on the emulsifier balance at the oil-water interface, nature of the oil phase and the additives on the aqueous and oil phases (Boyd et al., 1972).

2.4 Clean label

Consumers are more interested in the amount of ingredients in the food they consume, and this has brought about the consumption of food made with ingredients they are familiar with and perceive as healthy. According to FDA, there is no clear definition of clean label and could be interpreted to mean food formulations with familiar ingredients and no artificial chemicals (Lefferts, 2017). Consumers look at nutritional labels before purchasing the products and usually base their decisions on those made with natural or
organic ingredients. In the US, tracking of clean label products positioned in the market increased from 17% in 2013 to 20% in 2014 (do Nascimento et al., 2018). Natural labeling has declined due to regulatory complexities but however, terms such as pure and simple are used to replaced it.
Table 2. 1. Classification and characteristics of common surfactants

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>HLB value</th>
<th>Common application</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium oleate</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>Polysorbate 60 and 80, sucrose monolaurate</td>
<td>15.0</td>
<td>Solubilizer</td>
</tr>
<tr>
<td>Polysorbate 65</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>Decaglycerol monooleate</td>
<td>14.0</td>
<td>Detergent</td>
</tr>
<tr>
<td>Decaglycerol dioleate</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Polysorbate 65</td>
<td>11</td>
<td>Emulsifier for o/w emulsions</td>
</tr>
<tr>
<td>Hexaglycerol dioleate</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Sorbitan monolaurate</td>
<td>9.0</td>
<td></td>
</tr>
<tr>
<td>Diacetyl tartaric acid esters of monoglycerides (DATEM), soy lecithin</td>
<td>8.6</td>
<td>Wetting agents</td>
</tr>
<tr>
<td>Calcium stearoyl lactylate</td>
<td>5.1</td>
<td></td>
</tr>
<tr>
<td>Sorbitan monostearate</td>
<td>4.7</td>
<td>Emulsifier for w/o emulsions</td>
</tr>
<tr>
<td>Propylene glycerol monolaurate</td>
<td>4.5</td>
<td></td>
</tr>
</tbody>
</table>

Adapted from (Chen, 2015, Yamashita et al., 2017).

The clean label trends driven by consumer health concerns are associated to all-natural ingredients, no artificial ingredients, no artificial preservatives, no high fructose
corn syrup, organic, and no artificial colors (Hutt and Sloan, 2015). Consumers are now moving towards natural alternatives of additives to synthetic ingredients as presented on Table 1.2. The highest percent of consumers with health concerns are the elderly from 60 years and above. Formulation of stable colloidal dispersions require a high concentration of surfactants. The natural surfactants are biopolymers-based emulsifiers such as whey, soy and egg proteins or polysaccharides such as gum Arabic and modified starch (Yang et al., 2013).

Table 2. 2. Consumer concerns for Artificial additives by age.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>% Health concerns</th>
<th>% Preference for natural products</th>
</tr>
</thead>
<tbody>
<tr>
<td>8-34</td>
<td>58</td>
<td>69</td>
</tr>
<tr>
<td>35-49</td>
<td>62</td>
<td>66</td>
</tr>
<tr>
<td>50-64</td>
<td>75</td>
<td>59</td>
</tr>
<tr>
<td>60+</td>
<td>76</td>
<td>58</td>
</tr>
</tbody>
</table>

Adapted from (Hutt, 2015)

Several reports have reported on the use of natural surfactants in emulsion formation. In a study conducted by (Chung et al., 2017) on replacement of synthetic emulsifiers with natural ones in the production of liquid coffee creamer intended for hot coffee, quillaja saponin was used in stabilizing the emulsion preparation. The results showed that the color of the creamer was similar to that of commercial liquid creamer, was stable against droplet aggregation and creaming at pH of 3.5-7.0 and the coffee drinks prepared with the creamer also had similar appearance to those produced with commercial ones. Natural surfactants such as lecithin and various proteins from milk are used for the emulsion preparations in the food industry (Kralova and Sjöblom, 2009).
2.5. Lecithins

Lecithin is a natural emulsifier, extracted from egg yolk, milk, sunflower kernels, rapeseeds, or soybeans for use in the food industry (Ozturk and McClements, 2016). Lecithin is an ingredient considered as GRAS, ‘generally recognized as safe’ according to US Food and drug Administration (Dickinson, 1993). Table 1.3 shows the main soy lecithin phospholipids which are phosphotidylcholine (PC), phosphotidylethanolamine (PE), phosphatidylinositol (PI), phosphatidic acid (PA) and other smaller substances, and their percent compositions.

Table 2.3. Phospholipid composition of lecithins.

<table>
<thead>
<tr>
<th>Phospholipid</th>
<th>% Composition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphotidylcholine</td>
<td>19-21</td>
</tr>
<tr>
<td>Phosphotidylethanolamine</td>
<td>8-20</td>
</tr>
<tr>
<td>Phosphatidylinositol</td>
<td>20-21</td>
</tr>
<tr>
<td>Phosphatide acid and others</td>
<td>5-11</td>
</tr>
</tbody>
</table>

Adapted from Scholfield, 1981.

Lecithin enriched in PC has more oil-in-water emulsifying characteristics whereas those with higher concentrations of PE and PI are preferable for water-in-oil emulsions (Ushikubo and Cunha, 2014). Lecithins are modified enzymatically and chemically to effectively stabilize oil-in-water emulsions (Yang et al., 2013). However, the chemically modified lecithins are used in non-food applications (Whitehurst, 2008). The efficiency of an emulsifier is dependent on its ability to substantially lower the tension at the oil-water interface at given concentrations during emulsification. Ushikubo and Cunha (2014) have reported rapid decrease in interfacial tension of water-in-oil emulsions due to the action of
an emulsifier, resulting in higher stability against gravitational force. On the other hand, hydrophilic-lipophilic balance (HLB) determines their use in emulsions, where low HLB values are suitable for lipophilic emulsions and high values are for hydrophilic emulsions (Claesson et al., 2001, Fiordemondo and Stano, 2007). Contrarily, HLB values do not give significant information on the emulsifying behavior of lecithin but its molecular geometry and intermolecular forces (Fiordemondo and Stano, 2007). The HLB of lecithin is in the 9-10 range, making them good wetting agents (Boyd et al., 1972). To be effective o/w emulsifiers, lecithins can either be used in combination with other surfactants or hydrolyzed chemically or enzymatically to break off one of the hydrocarbon tails, making them hydrophilic and hence capable of stabilizing o/w emulsions.

2.6 Sources of lecithin

The main sources of lecithin are vegetable oils (soybean, cottonseed, corn, sunflower and rapeseed) and animal tissues (egg and bovine brain). The increasing demand for lecithin and remarkable growth in the soybean oil processing industry has made soybeans the main source of commercial lecithin (Joshi et al., 2006), with an annual world production of 130,000 tons (Wendel, 2000).

2.6.1. Soybean

Soybean (Glycine max) is one of the world’s valuable crops which is a good source of food and feed. It had an annual production increase of 4.6% from 1961-2007 that reached an annual production of 217.6 million tons in 2005-07 and a predicted annual increase by 2.2% to 371.3 million tons by 2030 (Masuda and Goldsmith, 2009). Soybean is also a
primary ingredient in most food products, including dairy products and it is a vital source of vegetable oil and proteins. Soybean however is believed to be responsible for 90% of food allergies due to the presence of allergenic proteins (L'Hocine and Boye, 2007).

2.6.2. Soy lecithin

Soy lecithin is mostly used because of its availability, excellent emulsifying properties, taste and color (Cherry and Kramer, 1989). The lecithin content in soybean is about 1.1 – 3.2 % (L'Hocine and Boye, 2007) and soy lecithin is one of the commonly used emulsifiers in the food, feed, pharmaceutical and technical industries (Van Nieuwenhuyzen, 1976). Commercial lecithin is the most important co-product from the oil processing industry due to its functionality and diverse application in the food industry and industrial utility (Szuhaj, 1983). It consists of a mixture of phospholipids (up to 75%) with triglycerides and smaller amounts of other substances (Scholfield, 1981). In addition, it also contains soy protein which is an alternative protein source for those looking for non-animal protein in their diet and lactose intolerant individuals (Hoffman and Falvo, 2004). Despite these advantages, soy proteins are allergenic to protein intolerant consumers (Gu et al., 2001). A study on commercial soybean lecithins helped the researchers to conclude that soy lecithins can introduce hidden allergens in processed foods and monitoring the protein content of these soy lecithins can make it safe for allergic consumers (Müller et al., 1998). The most common commercial grades of lecithin are clarified, fluidized, compounded, hydroxylated, deoiled and fractionated lecithins.
2.6.3. Production of soy lecithin

There are four main stages in the production of phospholipids which are the hydration of phospholipids, separation of lecithin gums, drying and cooling. The production of lecithin is presented on Figure 1.5 and involves the hydration step, characterized by mixing 2-3% of water with the oil at 50-70 °C to form a sludge. The sludge is then centrifuged at 50-70 °C, resulting to 0.25-0.5% phosphatides and lecithin sludge with a 40-50% water content. The lecithin is then dried to < 1% moisture content and cooled to below 50 °C to prevent post darkening. The spray drying of lecithins with wheat protein, soy and milk is to ensure a free-flowing product with good handling and synergistic properties on emulsion stability (van Nieuwenhuyzen and Tomás, 200).

Figure 2.5. Flow diagram of lecithin production. Adapted from (Van Nieuwenhuyzen, 1976).
2.6.4. Application of lecithin

Lecithin is the most versatile and valuable byproduct from the oilseed industry. It is used as an emulsifier, wetting agent, viscosity reduction, release agent and in control of crystallization (List, 2015). In addition, it can be used in the food and non-food applications. In non-food applications, lecithin is used in detergents, pigment dispersing, mold release, animal feed and cosmetics (Szuhaj, 1983).

There is a wide variety of food applications of lecithin as well as different types available in the market. The food applications are in baking and baked foods, disperse fats, antioxidant, chocolate, instant foods, stabilizing agent, margarine and emulsification (Tanno, 2000). The emulsifying property of lecithin drives its demand in food and industrial applications. In emulsification, the phospholipids in lecithin lower the interfacial tension of the oil-water boundaries, resulting in more stable emulsions (Szuhaj et al., 2020). Energy is required in emulsion formation and can be produced from a number of mechanical processes such as high-pressure, membrane, rotor-stator and ultrasonic systems (Schultz et al., 2004a).

2.7. Emulsification methods

2.7.1. High-pressure homogenizer

High-pressure homogenizers are the most common devices used in the food industry to produce finely dispersed emulsion droplets and require a high input of energy. The device consists of pump that compresses the crude emulsion at a pressure of 50 to 2,500 bar (Stang et al., 2001). High pressures homogenization may project some effects on food macromolecules such as fat, proteins and polysaccharides (Innocente et al., 2009).
High pressure homogenization can also be used to improve emulsion stability. In a study by Martinez-Monteagudo a pressure limit of 100-150 MPa showed possibilities of producing stable emulsions with reduced stabilizer concentration (Martínez-Monteagudo et al., 2017a). In addition, a study evaluating the effects of pressure on emulsion characteristics showed pressures up to 350 MPa. In this study, sunflower oil-in-water emulsion (20% oil) was stabilized by whey protein concentrate (85). At pressure of 300 MPa, the combined effects of high pressure and increasing temperature showed changes in conformation of proteins resulting in loss in their emulsifying properties. It was concluded that optimum pressure for this study was 100 MPa (Desrumaux and Marcand, 2002).

2.7.2. Membrane systems

In Emulsification with membrane systems, the dispersed phase is pressed through membrane pores into the continuous phase and there is control of droplet size depending on the membrane choice (Joscelyne and Trägårdh, 2000, Charcosset et al., 2004). Process configuration also plays a role determining the droplet sizes and size distributions of the emulsions. A study to determine the potential use of membrane systems in formation of food-grade emulsions found that increasing transmembrane pressure from critical pressure (5 and 10 kPa), gradually increases the droplet size until they are fully grown and detach from the pore (Spyropouloua et al., 2011). This process offers small stress to products and hence advantageous to stress-sensitive products (Schultz et al., 2004b). Another study investigating the conditions for producing small emulsion droplets with ceramic membranes showed that wall shear stress of 135 Pa, membrane size of 0.1 µm and high
emulsifier concentration (8%) resulted in submicron particles at membrane flux of >100 kgm⁻² h⁻¹ (Joscelyne and Trägårdh, 1999).

2.7.3. Rotor-stator systems

This technology is used in many applications including food processing because high shear enables control of product quality and high energy efficiency. Scholz and Keck conducted a study on production of nanoemulsions with rotor-stator high speed stirring (Scholz and Keck, 2015). They used maximum speed of 36,000 rpm with a processing time of 5 min that resulted in emulsions with 135 nm and narrow size distribution. The studies concluded that droplet sizes produced by high speed stirring are larger when compared to those produced by HPH.

2.7.4. Ultrasound

Ultrasound emulsification is driven primarily by cavitation in which bubbles collapse at or near the oil-water interface, causing disruption and mixing of the two phases to produce fine emulsion droplets (Chandrapala and Leong, 2015). In ultrasound processing, sound waves are transmitted at frequencies higher than the human hearing threshold. These sound waves are transmitted as longitudinal waves in which the deformations are in the direction of wave travel or shear waves, in which waves travel through the material causing deformations normal to the movement of the wave front (Coupland and McClements, 2001). The intensity of the waves determines their use in activation or deactivation of enzymes, homogenization, mixing, emulsification, dispersion, preservation, stabilization, dissolution and crystallization, hydrogenation, meat
tenderization, ripening, aging and oxidation (Gallo et al., 2018). However, this technology can cause changes in the physical, chemical and functional properties of food and can be categorized into low and high-intensity ultrasound (Jambrak et al., 2009).

Research focusing on emulsification of oil by power ultrasound showed that increasing ultrasonic output level, increases the acoustic power and ultimately the rate of size reduction. Sonication of time of 5 min resulted in decreased in droplet size to 0.7µm (Cucheval and Chow, 2008). Since breaking of the interface requires a large amount of energy, it is preferable to prepare a coarse emulsion before applying the acoustic power (Jafari et al., 2008). Research has shown that ultrasound treated post-emulsification milk protein isolate (Driscoll et al., 2001) resulted in smaller emulsion droplets to approximately 20 µm due to arrangement of MPI at the interface during ultrasound treatments (O’Sullivan et al., 2015).

High-power ultrasound has been applied in emulsion formulations with low surfactant concentration. The effects depend on the characteristics of the matrix on which it is applied. The form of energy offered by ultrasound improves the characteristics of high-quality and ensures food safety, while minimizing any negative effects on their sensory characteristics. The technology is non-destructive in which the interaction of the acoustic energy with the food occurs mainly through a liquid medium, since cavitation, physical and chemical actions play an important role in food quality during its transformation (Gallo et al., 2018).
Ultrasound technology can be applied by either replacing conventional methods or used in assisting these conventional methods. Emulsion formation using ultrasound is ensured by using high intensity, low frequency ultrasound. This technology offers many benefits over other technologies, which are centered on its energy efficiency, ease of manipulation, better control of ultrasound variables (de Barros Fernandes et al., 2016). In addition, ultrasound emulsions are more stable, require minimal or no stabilizers, submicron size and narrow size distribution. This process is influenced by parameters such as hydrostatic pressure, gas content, pre-emulsification, viscosity of the continuous phase, oil and water ratio, concentration of the surfactant, position of the probe as regards the liquid-liquid interface, ultrasonic power and exposure time (Chandrapala et al., 2012a). On the contrary, ultrasound can result in detrimental effects on the quality parameters in food such as flavor, color and modifications of other minor compounds (Pingret et al., 2013).

High-intensity ultrasounds that uses ultrasonic transducer probes may result in release of metals in the food product. In studies conducted by (Mawson et al., 2014) to investigate the formation of metallic particulates by a series of transducers at different frequencies (18 kHz to 2 MHz) in water systems, metal leach was observed at values below accepted drinking water limits even after prolonged ultrasonic exposure. In addition, the metallic nanoparticles suggested no serious health implications and hence the feasibility of safely using high frequency transducers in direct contact with food.
2.8. Conclusions

There are several studies that have been conducted on the use ultrasound in food industries in emulsification processes. Recently, more studies are aimed at using this technology in producing clean label products to satisfy the growing consumer demand for less use of additives in food products due to their health concerns. The review has therefore presented studies on the possibility of the use of this technology in production of stable emulsions with no emulsifiers and the potential for commercial applications.

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Chapter 3

Effect of ultrasound treatment on different material compositions

This chapter is focused on investigating the effect of ultrasound on water, skim milk and cream. Ultrasound treatments increase the temperature of the liquid medium which is different for different material compositions. The information from the temperature modelling of water at different amplitudes (50, 60, 70, 80, 90 and 100 %) will be used in estimating the acoustic power and intensities at these different ultrasound conditions. Treatment of the different material compositions will determine their threshold values.

3.1. Introduction

The application of ultrasound in different liquids results in transmission of sound waves (as longitudinal waves) enabling the formation of rapidly growing bubbles that expand during negative pressure excursion and collapse during positive excursion, increasing the temperature, pressure and shear forces of the medium (Jambrak et al., 2009). The propagation of sound through the material depends on the physical and chemical properties (texture and structure) (Mohammadi et al., 2014). The ultrasound power (mechanical energy) is dissipated partly as heat when ultrasound passes through the material, hence the temperature is recorded as a function of time resulting in estimation of power in watt (Jambrak, 2008). The amount negative pressure depends on the type and purity of the liquid which is 1,000 atmospheres for pure water and few atmospheres for tap water (Suslick, 1989).
Speed, impedance, and attenuation are properties that quantitatively describe the propagation of ultrasound through materials (O’Brien Jr, 2007). When ultrasound interacts with gas bubbles, chemical and biochemical effects occur that are used in many applications (Bhangu and Ashokkumar, 2017). This is influenced by acoustic power, frequency, ultrasonic power, viscosity of the medium. Cavitational effects caused by ultrasound treatments result in functional effects in different materials. These effects as well as intensity are dependent on amplitude, pressure, temperature, viscosity, and concentration of solids (Patist and Bates, 2008). In this study, temperature was characterized using different materials (water, milk concentrate and cream) due to cavitation. In one study to determine the effect of ultrasound on skim milk, there was denaturation of whey proteins and formation of soluble whey–whey/whey–casein aggregates leading to interaction with casein micelles and formation of micellar aggregates (Shanmugam et al., 2012).

The main objective of this work is to characterize the temperature change of the different material compositions with the use of ultrasound technology. Water will be used to obtain data on temperature change with increase in time and amplitude. The data obtained from this will be used to estimate the acoustic power and intensities of the different ultrasound conditions. Milk concentrate, and cream will finally be used to study the critical limit that the various materials can withstand before they experience any functional changes at ultrasound amplitudes of 50, 60, 50, 80, 90 and 100 %. 
3.2. Materials and methods

3.2.1. Preparation of model systems

Skimmed milk and raw cream were obtained from the Davis dairy plant at South Dakota State University. Distilled water was used to estimate the acoustic power and subsequently, the acoustic power and intensities at different ultrasound treatments were estimated using the equations 3.1 and 3.2 respectively (O'Sullivan et al., 2014).

\[ P_a = m \cdot c_p \frac{dT}{dt} \]

\[ I_a = \frac{P_a}{S_a} \]

Where \( P_a \) is acoustic power (W), \( S_a \) is the surface area of the ultrasound emitting surface (~ 1 cm\(^2\)), \( m \) is the mass of ultrasound treated solution (g), \( C_p \) is the specific heat capacity of medium (4.18 kj/gK), \( dT/dt \) is the rate of temperature rise with respect to starting time and \( I_a \) is acoustic intensity (W cm\(^{-2}\)).

The skimmed milk was concentrated with the use of an evaporator (Heidolph rotatory evaporator) connected to a refrigerant (VWR Scientific) to different total solid contents of 9.3 ± 0.5, 21.2 ± 0.8, 32.3 ± 0.7, and 39.5 ± 0.7 %. The Raw cream was standardized to different fat contents of 13.2 ± 1.0, 23.2 ± 0.6, 33.7 ± 1.2, and 43.5 ± 0.8 % prior to ultrasound treatment. 250 mL of each sample was ultrasonically treated at 1 or
3 min at an initial temperature of 40°C and amplitudes of 50-100%. The change in temperature was recorded with the use of a thermocouple placed in the sample and connected to a data logger as on Figure 3.1. Distilled water was used in characterization of ΔT and the data was used to estimate acoustic power and subsequently, acoustic intensities.

![Figure 3.1. Schematic diagram of ultrasound modelling](image)

**3.3. Results and discussion**

**3.3.1. Temperature rise**

*Figure 3.2* shows the graph of temperature change with time at ultrasound treatments of 50 to 100% amplitude for water. The temperatures increase with increasing time and amplitude is linear. This trend shows that increasing amplitude results in increase in power transmitted through the sample. This results in rapid formation and breakdown of bubbles that in turn increase the temperature of the samples.
Figure 3.2. Temperature modelling at amplitudes 50, 60, 70, 80, 90 and 100%.

**Figure 3.3.** shows the temperature change with different material compositions. This relates to threshold values that are reached before any physical or chemical changes occur due to ultrasound intensities on different material compositions. The impact of the ultrasound on material composition shows significant differences for samples with 39.5 % total solids and 43.5 % fat content, treated at 100 % amplitude. The effect of sonication can vary depending on the experimental conditions such as acoustic power density, volume of the sample, temperature of the solution, and other factors (Shanmugam et al., 2012). The magnitude at which ultrasound travels through the material is dependent on the intensity applied and type of material treated. Ultrasound effects on the kinetics of mass transport appear only when an acoustic intensity threshold is reached.
3.3. Conclusion

The effects of ultrasound on the model systems prepared by ultrasonication of water and different material compositions became apparent when threshold values were reached before chemical or biochemical changes occurred. Measuring the variation of ultrasound properties, enable generation of information about the properties of the system. Overall, different ultrasound treatments and processing time will result to functional changes in different materials depending on their compositions and threshold limits.
3. References


Chapter 4\(^1\)

**Formulation of dairy emulsions with or without soy lecithin**

4.1. Introduction

Emulsification is a key processing step in the manufacture of a number of dairy and food products, including ice cream, infant formulations, sauces, dressings, soups, mayonnaise, butter, and margarine (Dalgleish, 2001). Emulsification involves the dispersion of two immiscible liquids within a continuous medium, stabilized by the addition of a surfactant and application of mechanical energy (Leong, 2016). The dispersion of immiscible liquids is thermodynamically unstable by nature, and separation of the liquids may occur over time (Piorkowski & McClements, 2014). Destabilization of emulsions is a complex process that includes many phenomena, such as coalescence, flocculation, creaming, and Oswald ripening (Yamashita, Miyahara, & Sakamoto, 2017). The mechanisms involved for emulsion break-up and coalescence are discussed elsewhere (Lee, Niknafs, Hancocks, & Norton, 2013).

Surfactants are used for the formulation of emulsions to perform specific functions, such as lowering the interfacial tension, increasing the viscosity of the continuous medium, and providing stability toward separation (Claesson, Blomberg, & Poptoshev, 2001). Surfactants not only facilitate the formation of new droplets of smaller size during the mechanical treatment (McClements, 2007) but also ensure long-term stability of the emulsions by creating a barrier at the oil/water interface and acting against flocculation and coalescence. The choice of a surfactant depends on its ability to act on the surface of the

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\(^1\) A version of this chapter is currently under review in International Journal of Dairy Technology
lipid droplets, reducing the interfacial tension and protecting the droplets from aggregating during emulsification, storage, and final usage. Characteristics and properties of surfactants commonly used in the formulation of food emulsions are discussed elsewhere (Dalgleish, 2001; McClements, 2007; Wahlgren, Bergenstähl, Nilsson, & Rayner, 2015).

Soy lecithin is one of the most commonly used surfactants during the manufacture of food and pharmaceutical formulations. The industrial manufacture of soy lecithin consists of several steps including hydration of phosphatides, separation, drying, and cooling (van Nieuwenhuyzen & Tomás, 2008). Commercial soy lecithin consists of a mixture of phospholipids (up to 75%) with triglycerides and smaller amounts of other substances (Scholfield, 1981). Lecithin can also be produced from other vegetable sources, such as sunflower kernels and rapeseed (Klang & Valenta, 2011).

Over the past few years, consumers have begun to redefine the desired attributes of food and dairy products. The perception among the consumers regarding “healthy” and “unhealthy” ingredients has driven the food and dairy industry to reformulate their existing portfolio of products with perceived healthy ingredients and free of unfamiliar compounds (Asioli et al., 2017). Concerns on the use of soy lecithin as a surfactant has emerged because it contains a number of IgE-binding proteins, making it a source of hidden allergens (Gu, Beardslee, Zeece, Sarath, & Markwell, 2001). Consequently, the development of emulsification methods that help to reduce the concentration of soy lecithin has gained momentum in recent years (Yan, Park, & Balasubramaniam, 2017).
The preparation of stable emulsions requires high input of mechanical energy to break the liquid interfaces. Common methods for emulsification have been extensively reviewed by Schultz, Wagner, Urban, and Ulrich (2004). In ultrasound processing, sound waves are transmitted through the liquid at frequencies above human hearing threshold (> 16 kHz, resulting in compression and stretching of the molecular spacing leading to cavitation bubbles (Cabrera-Trujillo, Sotelo-Díaz, & Quintanilla-Carvajal, 2016). Upon collapsing, these bubbles release energy in the form of heat, shockwaves, and shearing, that can be put to work for dispersion, mixing, and emulsification (Chandrapala & Leong, 2014). Emulsification through ultrasound is characterized by the collapse of the bubbles at or near the oil-water interface that disrupts and mixes the two phases, hence forming fine droplets (Mason, Chemat, & Ashokkumar, 2015). Ultrasound has been shown to produce stable oil-in-water emulsions over a wide range of oil content, 3-20% v/v (Modarres-Gheisari, Gavagsaz-Ghoachani, Malaki, Safarpour, & Zandi, 2019). Aslan and Dogan (2018) emulsified olive oil (7-15%, v/v) in reconstituted skim milk by the application of ultrasound treatment (24 kHz for 3 min). The resulting emulsions were stable against creaming without the addition of surfactants. Similarly, Kaci et al. (2014) dispersed vegetable oil (5-15%, v/v) in water without the addition of surfactants using high-frequency ultrasound generated by piezoelectric ceramic transducer.

The application of ultrasound treatment (100 W for 8 min) emulsified black seed oil (7%, v/v) in skim milk, and the emulsions were stable for 8 days at 4°C without the addition of surfactants. An investigation on the emulsification of flax seed oil (7-21%, v/v) showed that the ultrasound treatment (20 kHz for up to 8 min) dispersed droplets of oil in
skim milk, and such droplets were stable for 9 days at 4°C. In summary, the literature on the ultrasound emulsification appears to produce stable oil-in-water emulsions with reduced surfactants.

Scientific reports on the influence of soy lecithin on the formation and stability of ultrasound emulsions are scarce. The current investigation aims at evaluating the impact of soy lecithin during formation and stability of oil-in-water ultrasound emulsions. The emulsions were evaluated for rheological behavior, particle size, gel electrophoresis, and microstructure during 21 d of storage at 4°C.

4.2. Materials and Methods

4.2.1. Preparation of formulations

A formulation of industrial interest was used to study the impact of soy lecithin on ultrasound emulsions. The formulation consisted of 4.33 ± 0.05% of protein, 2.90 ± 0.05% of fat, 21.52 ± 0.75 of carbohydrates, and 0-0.05% of surfactant, soy lecithin. Firstly, whey protein concentrate 80 (Milk specialties, Eden Prairie, MN) was dissolved in distilled water for 15 min at 60°C under constant stirring. In a separate beaker, the carbohydrate blend made of 12.9% of granulated sugar (United Sugar Corp. Minneapolis, MN) and 8.6% of maltodextrin (Cargill Incorporated, Minneapolis, MN) were dissolved in distilled water for 15 min at 50-55°C. Then, the protein and carbohydrate blends were mixed. Finally, vegetable oil was added to the mixture followed by the addition of soy lecithin. Different concentrations of soy lecithin were added (0.05, 0.025, and 0%). Then, the whole formulation was mixed for additional 5 min at 50-55°C. The final pH of the formulation
was adjusted to a value of $6.66 \pm 0.04$ with NaOH. Total solids of the final formulation were $32.6 \pm 0.95\%$.

4.2.2. Ultrasound emulsions

Two-hundred and fifty mL of pre-emulsified formulation were ultrasonicated for 5 min using a 20 kHz sonicator (U1P1000hd, Hielscher Ultrasonics, GmbH, Teltow, Germany). Prior to the sonication, the ultrasound horn (21 mm length and 3.0 cm$^2$ of surface area) was immersed about two-third in the pre-emulsified formulation. Peak to peak amplitude was tested at 80, 90, and 100%. The initial temperature ($40^\circ$C) for all treatments was kept constant, and the temperature rise due to ultrasound was recorded using a K-type thermocouple connected to a data logger (Omega Engineering Inc., Stamford, CT). For each amplitude, the acoustic intensity ($I_a$, W cm$^2$) was calculated according to Equation (1) (O’Sullivan, Park, Beevers, Greenwood, & Norton, 2017).

Equation 4.2

$$I_a = \frac{m \cdot C_p \cdot (dT/dt)}{S_a}$$

Where m is the mass of the sample (g), $C_p$ is the heat capacity of the sample (J g$^{-1}$ K$^{-1}$), $dT/dt$ is the rate of temperature rise measured experimentally, and $S_a$ is the surface area of ultrasound emitting surface (cm$^2$). Equation (1) accounts for the energy dissipated as heat during the application of ultrasound (Margulis & Margulis, 2003).
4.2.3. Experimental design

A factorial design consisted of three variables with three levels of each variable was used to study the influence of soy lecithin on the stability of ultrasound emulsions. The studied variables were the concentration of soy lecithin (0.05, 0.025, and 0%), acoustic intensity (42.58 ± 2.98, 56.83 ± 3.01, and 70.48 ± 2.97 W cm\(^{-2}\)), and storage time (0, 7, and 21 d). Experimental runs were conducted in triplicates, and all the figures were plotted using SigmaPlot software V11 for Windows (Systat Software, Inc, Chicago, IL, USA). Comparison of mean values was performed with one-way analysis of variance using Tukey's post hoc (p < 0.05).

4.2.3. Analytical determinations

4.2.1. Composition

The samples were analyzed for pH, total protein, fat, and total solids. The pH was measured in 10 mL of the sample using an Orion Versa Star Pro (Thermo Fisher Scientific, Waltham, MA). The protein content was determined by the Kjeldahl method, while the fat content was measured using the Mojonnier extraction. Total solids (TS) were gravimetrically determined by drying the samples in an oven (Isotemp oven, Iowa, USA) for 15 h at 103°C.

4.2.2. Particle size distribution

Average size and distribution of the formulations were determined by dynamic light scattering using a ZetaSizer Nano ZS (Malvern Instruments Ltd, Cambridge, UK). The guidelines provided by Ma, Yang, Zhao, and Guo (2018b) were followed. Before the
analysis, the formulations were brought to room temperature (25°C) and equilibrated for 20 min. An aliquot of 10 µL was transferred to disposable cuvette (DTS 0012, Sigma-Aldrich, St Louis, MO), and diluted 100x with deionized water to prevent multiple scattering. Then, the cuvettes were placed in the measuring chamber, where the samples were equilibrated for 120 s at 25°C. The analysis was conducted at a scattering angle of 173° and a refractive index of 1.46. Average size and distribution of particles were obtained in percentage of volume as function of droplet diameter in the range of 0.6-6000 nm.

4.2.3. Rheological measurements

An MCR 92 rheometer (Anton Paar, GmbH, Ostfildern, Germany) equipped with a plate and plate geometry (PP25/S, diameter 25mm) was used to characterize the rheological behavior of the formulations at 25°C. Three types of tests were performed: i) strain sweep from 0.01 to 100% at a constant frequency of 10 rad s\(^{-1}\); ii) frequency sweep from 0.1 to 100 rad s\(^{-1}\) at a constant strain of 0.1%; and iii) step rate where the viscosity of the formulations was evaluated at 13, 50 and 100 s\(^{-1}\). Details on the methodology can be found elsewhere (Martínez-Monteagudo et al., 2017).

4.2.4. Gel electrophoresis

The protein profile of the formulation was determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) using the reducing method. Details on the methodology can be found elsewhere (Meletharayil, Patel, & Huppertz, 2015). Two mL of sample were mixed with 20 mL of a chloroform/methanol solution (2:1, v/v). Then, the mixture was placed in a freezer for 1 h at -18°C, and then, centrifugated (Jouan CR412,
Jouan Inc., Winchester, VA, USA) for 15 min at 3600 rpm at 0°C. After centrifugation, the supernatant was discarded, while the pellets were dissolved with phosphate buffer (3 mL, 0.1 N). Five μL of the dissolved pellets were transferred into a test tube followed by the addition of 4.75 μL of 2x Laemmli sample buffer (Bio-Rad, Hercules, CA), and 0.25 μL of 4% 2-mercaptoethanol (Fisher Scientific, Hampton, NH). Then, the tested tubes were capped and heated for 5 min at 90°C. Afterward, an aliquot of 10 μL was loaded into Tris-acrylamide gels (4-15% Mini-Protean TGX precast gels with 10 wells, Bio-Rad), and the gels were run for 1 h at 200 V using Tris/Glycine/SDS Buffer (Bio-Rad). Then, the gels were removed from the cassettes and stained with Bio-safe Coomassie G-250 stain (Bio-Rad). A molecular weight standard (Bio-Rad, USA; Precision Plus ProteinTM Kaleidoscope Standards) was used as reference. Finally, the gels were de-stained with a de-staining solution containing 100 mL of acetic acid, 300 mL of methanol, and 600 mL of distilled water. The gels were scanned using Bio-5000 Microtek (Microtek, Taiwan).

4.2.5. Microstructure

The microstructure of the samples was evaluated with confocal laser scanning electron microscope (CLSM). An Olympus FV1000 inverted confocal laser scanning electron microscope (Olympus America Inc., Center Valley, PA) was used. The samples were stained for fats and proteins whereby 60 μL of each sample was placed in a concave glass slides and stained with fast green (30 μL) and Nile red (10 μL).
4.3. Results and discussions

4.3.1. Particle size distribution

Figure 4.1 shows the average particle size of the ultrasound emulsions formulated with different concentration of soy lecithin. Overall, the particle size decreased with increasing the acoustic intensity, where the lowest values (252, 288, and 406 nm for 0.05, 0.025, and 0% of soy lecithin) were obtained at acoustic intensity of 70.48 ± 2.97 W cm⁻². Similarly, O'Sullivan et al. (2016) reported a reduction in the particle size of ultrasound emulsion with increasing the acoustic intensity.

![Graph showing the effect of acoustic intensity on the mean particle size of the emulsions formulated with different concentration of soy lecithin.](image)

Figure 4.1. Effect of acoustic intensity on the mean particle size of the emulsions formulated with different concentration of soy lecithin.

Acoustic cavitation is thought to be responsible for the reduction of the particle size (Ashokkumar, 2015). During the application of ultrasound, cavities or bubbles are formed
within the liquid and subsequently collapse due to the contraction-expansion cycles (Kaci et al., 2014). Upon collapse, the liquid experiences mechanical effects (shear forces, hot spots, and shockwaves) of different magnitudes that together provides the energy for breakdown of the particles. van Wijngaarden (2016) reviewed the collapsing mechanisms of cavitation bubbles. The concentration of soy lecithin did not show significant differences on the particle size of the ultrasound emulsions. This observation has been exemplified by Aslan and Dogan (2018), who produced ultrasound droplets of 1000 to 200 nm without the addition of emulsifiers. Ultrasound also reduces the volume of proteins and therefore the surface tension, facilitating the droplet break-up during emulsification (O'Sullivan et al., 2016).

4.3.2. Protein profile

Electrophoretic profile obtained by SDS-PAGE for the ultrasound emulsions is given in Figure 4.2. All samples displayed four distinctive bands at about at 75, 50, 15, and 10 kDa corresponding to lactoferrin, bovine serum albumin, β-lactoglobulin, and α-lactalbumin, respectively. The application of ultrasound within the tested acoustic intensities did not significantly impact the primary structure of the proteins. The changes in the structure of proteins induced by ultrasound might result from hydrophobic interactions (non-covalent) rather than peptide cleavage. Similar impact of ultrasound on the whey protein has been reported elsewhere (Chandrapala et al., 2012b, O'Sullivan et al., 2016, Ma et al., 2018a).
Figure 4.2. SDS-PAGE patterns of ultrasound emulsions. (1) Molecular weight standard, (2) 0% of soy lecithin and 42.58 ± 2.11 W cm⁻², (3) 0% of soy lecithin and 56.83 ± 2.53 W cm⁻², (4) 0% of soy lecithin and 70.40 ± 2.13 W cm⁻², (5) 0.025% of soy lecithin and 42.58 ± 2.11 W cm⁻², (6) 0.025% of soy lecithin and 56.83 ± 2.53 W cm⁻², (7) 0.025% of soy lecithin and 70.40 ± 2.13 W cm⁻², (8) 0.05% of soy lecithin and 42.58 ± 2.11 W cm⁻², (9) 0.05% of soy lecithin and 56.83 ± 2.53 W cm⁻², and (10) 0.025% of soy lecithin and 70.40 ± 2.13 W cm⁻².
4.3.3. Strain sweep

Figure 4.3 shows the storage and loss moduli as a function of strain amplitude for the ultrasound emulsions. All samples exhibited a viscoelastic region, where both parameters (G’ and G”) remained constant over a given range of the strain amplitude (Martínez-Monteagudo et al., 2017b). Within the viscoelastic region, the length and relationship between G’ and G” are commonly used to gain insights into the molecular organization of emulsions (Hyun et al., 2002). The length of the viscoelastic region is closely related to emulsion stability since it accounts for the maximum deformation that a sample can withstand without structural failure. Figure 4.3 shows that the length of the viscoelastic region varied with the acoustic intensity and the concentration of soy lecithin. For instance, a relatively short viscoelastic region (~0.01 to 1% strain) was observed in those sample emulsions formulated with 0 and 0.025% of soy lecithin and emulsified at an acoustic intensity of 42.58 ± 2.10 W cm⁻² (Figure 4.3a). Similarly, the emulsions formulated with 0 and 0.05% of soy lecithin at 56.83 ± 2.51 W cm⁻² (Figure 4.3b) displayed a rather short viscoelastic region (~0.02 to 1% strain). Emulsions within this range of viscoelastic range resembled a Type I behavior, strain thinning, according to the classification developed by Hyun et al. (2002). A Type I behavior is characterized by a solid-like behavior (G’>G”), and it has been reported for a number of food emulsions, including protein beverage (Martínez-Monteagudo et al., 2017b), *Lepidium perfoliatum* seed gum solutions (Hesarinejad et al., 2014), salad dressings (Franco et al., 1995), and dark chocolate (van der Vaart et al., 2013).
Figure 4.3. Strain sweep behavior of emulsions subjected to an ultrasound treatment: (a) 42.58 ± 2.11; (b) 56.83 ± 2.53; and (c) 70.40 ± 2.13 W cm⁻². Frequency = 5 rad s⁻¹.
Interestingly, the short viscoelastic region corresponded to the emulsions having larger particle size (Figure 4.1). Contrary, longer viscoelastic regions (>10%) were obtained in emulsions prepared with higher acoustic intensity (56.83 ± 2.51 and 70.40 ± 2.10 W cm$^{-2}$). Emulsions within this region also contained the smallest particle size (Figure 4.1). The strain curve of such emulsions resembled a Type III behavior, strong strain overshoot (Hyun et al., 2002). This type of behavior is characterized by a slight increase at the end of the curve, where concentration of particles occurs due to attractive forces (Franco et al., 1995). A Type III behavior has been reported for gum solutions (Hyun et al., 2002).

**4.3.4. Frequency sweep**

The mechanical spectra of the ultrasound emulsions were evaluated through frequency sweeps (Figure 4.4). Overall, the samples exhibited the typical behavior of a weak gel, where G’ was always higher than G” and both parameters gradually increased with the frequency (Hyun et al., 2002). Such a behavior is characterized by elastic and recoverable deformation (Anvari et al., 2016). The increasing tendency of G’ with the frequency revealed that the structure of the ultrasound emulsions resembles that of a physical gel (Khondkar et al., 2007). The exception to this generalization was observed in those samples containing 0.05% of soy lecithin treated at 56.83 ± 2.51 W cm$^{-2}$ (Figure 4.4, where no clear pattern was observed.
Figure 4.4. Frequency sweep analysis of the ultrasound emulsions: (a) 42.58 ± 2.11; (b) 56.83 ± 2.53; and (c) 70.40 ± 2.13 W cm$^{-2}$. 
The mechanical spectra displayed by the ultrasound emulsions (Figure 4.4) has also been reported for protein beverage (Martínez-Monteagudo et al., 2017b), colloidal gels (Chan and Mohraz, 2012), gum solutions (Anvari et al., 2016), and whey protein solution (Paraskevopoulou et al., 2013). Regardless of the concentration of soy lecithin, the magnitude of $G'$ and $G''$ increased with the acoustic intensity (Figure 4.4c). Shear forces generated during acoustic cavitation may induce the formation of intermolecular networks, providing strength to the gel. However, mechanistic studies are needed to support such claim.

4.3.5. Viscosity

Table 4.1 shows the effect of soy lecithin concentration and acoustic intensity on the viscosity of the ultrasound emulsions. The viscosity of the emulsions treated at 42.58 ± 2.98 and 56.83 ± 3.01 W cm$^{-2}$ ranged from 11 to 16 cP without showing any particular trend, regardless of the shear rate. Contrary, an acoustic intensity of 70.48 ± 2.97 W cm$^{-2}$ imparted viscosity at low, medium, and high shear rate (13, 50, and 100 s$^{-1}$). Within the low shear spectrum, the viscosity increased about 7- and 10-fold as the concentration of soy lecithin increased to 0.025 and 0.05%, respectively. The imparted viscosity was less pronounced at higher shear rates about 6-fold at 100 s$^{-1}$. Martínez-Monteagudo et al. (2017b) reported an 11-fold increment in the viscosity of protein beverage under shear forces of large magnitude. Such changes in the viscosity can be explained by the shear forces acting on the structure of the proteins. Indeed, Ma et al. (2018b) reported that the application of ultrasound disrupts the structure of proteins, changing its functional properties.
Table 4. Effect of acoustic intensity on the viscosity (cP) of the emulsions formulated with different concentration of soy lecithin.

<table>
<thead>
<tr>
<th>Concentration of soy lecithin</th>
<th>Shear rate (s⁻¹)</th>
<th>13</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>42.58 ± 2.11 W cm⁻²</td>
<td>17.49 ± 1.92Aa</td>
<td>15.36 ± 0.95Ba</td>
<td>14.93 ± 0.22Ba</td>
</tr>
<tr>
<td>0.025%</td>
<td>15.21 ± 1.01Aa</td>
<td>13.96 ± 0.67Bb</td>
<td>14.15 ± 0.75Ba</td>
<td></td>
</tr>
<tr>
<td>0.05%</td>
<td>16.96 ± 1.10Aa</td>
<td>12.73 ± 1.05Bb</td>
<td>12.73 ± 1.01Ba</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of soy lecithin</th>
<th>Shear rate (s⁻¹)</th>
<th>13</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>56.83 ± 2.50 W cm⁻²</td>
<td>10.98 ± 1.02Aa</td>
<td>10.51 ± 0.75Aa</td>
<td>10.63 ± 0.89Aa</td>
</tr>
<tr>
<td>0.025%</td>
<td>14.14 ± 0.35Ab</td>
<td>13.02 ± 1.03Ab</td>
<td>11.90 ± 1.11Ba</td>
<td></td>
</tr>
<tr>
<td>0.05%</td>
<td>16.67 ± 1.05Ac</td>
<td>14.76 ± 1.02Bb</td>
<td>14.52 ± 0.85Bb</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Concentration of soy lecithin</th>
<th>Shear rate (s⁻¹)</th>
<th>13</th>
<th>50</th>
<th>100</th>
</tr>
</thead>
<tbody>
<tr>
<td>0%</td>
<td>70.40 ± 2.10 W cm⁻²</td>
<td>8.61 ± 1.02Aa</td>
<td>8.71 ± 0.88Aa</td>
<td>8.96 ± 0.87Aa</td>
</tr>
<tr>
<td>0.025%</td>
<td>62.15 ± 1.85Ab</td>
<td>54.40 ± 1.66Bb</td>
<td>48.56 ± 3.55Cb</td>
<td></td>
</tr>
<tr>
<td>0.05%</td>
<td>86.15 ± 3.14Ac</td>
<td>51.62 ± 2.17Bb</td>
<td>38.90 ± 2.97Cc</td>
<td></td>
</tr>
</tbody>
</table>

Mean ± standard deviation (n = 3) within each row with different letters (A–C) are significantly different (P<0.05) according to Tukey test. Mean ± standard deviation within each column with different letters (a–c) are significantly different (P<0.05) according to Tukey test.

4.3.6. Storage study

Additional set of experiments were conducted to evaluate the stability of the ultrasound over 21 d of storage at 4°C. The droplet size plays a critical role in the physical stability of the emulsions. Figure 4.5 shows the changes in the particle size of the ultrasound emulsions. An increasing tendency was observed in the particle size during
storage up to 21 d. The magnitude of the increment was influenced by the concentration of soy lecithin and acoustic intensity. At low acoustic intensity ($42.58 \pm 2.98$ W cm$^{-2}$, Figure 4.5a), the largest particle size was obtained in samples without the addition of soy lecithin, reaching values up to 800 nm after 21 d. The formation of larger particles might be due to coalescence of fat droplets (Anandan et al., 2017). Similar behavior but less pronounced was observed in samples treated at $56.83 \pm 3.01$ W cm$^{-2}$, (Figure 4.5b), where the largest particles varied 640-790 nm after 21 d. Contrary, emulsions having a relatively small particle size (470 to 521 nm) were obtained at an acoustic intensity of $70.48 \pm 2.97$ W cm$^{-2}$ (Figure 4.5c).

The application of ultrasound of $70.48 \pm 2.97$ W cm$^{-2}$ yielded a desired range of droplet size without the addition of surfactant. The shear forces generated during the acoustic cavitation provide the energy for particle break-up and their dispersion. This explanation seems reasonable since the current investigation employed acoustic intensities higher than the threshold value ($2$ W cm$^{-2}$) reported elsewhere (Abismaīl et al., 1999). Emulsions of small droplet sizes are more stable toward coalescence as the diffusion rate is reduced (Kong et al., 2001). The stability of emulsions during storage was also evaluated through strain sweep analysis after 21 d of storage (Figure 4.6). Emulsions treated at $42.58 \pm 2.98$ W cm$^{-2}$ exhibited the shortest viscoelastic region, while the application of higher acoustic intensities ($56.83 \pm 3.01$ and $70.48 \pm 2.97$ W cm$^{-2}$) not only extended the viscoelastic region but also yielded a stronger network in comparison with emulsions of lower acoustic intensity.
Figure 4.5. Changes in the particle size of emulsions subjected to an ultrasound treatment during storage: (a) 42.58 ± 2.11; (b) 56.83 ± 2.53; and (c) 70.40 ± 2.13 W cm⁻².
Remarkably, emulsions prepared without the addition of soy lecithin and treated at 56.83 ± 3.01 W cm⁻² exhibited comparable results than emulsions with added soy lecithin (0.025 and 0.05%) and treated at 70.48 ± 2.97 W cm⁻².

Figure 4.6. Strain sweep for the ultrasound emulsions after 21 d of storage at 4 °C. Frequency = 5 rad s⁻¹.

The frequency analysis of the ultrasound emulsions after 21 d of storage is given in Figure 4.7. During storage, the mechanical spectra of the emulsions remained essentially the same, a weak gel behavior where G' > G'' over a given range of frequency. This behavior is thought to be as a result of the formation of intermolecular networks (Anvari et al., 2016). The gross morphology of the ultrasound emulsions is showed in Figure 4.8.
Figure 4. 7. Frequency sweep analysis of the ultrasound emulsions after 21 d of storage at 4 °C. (a) 42.58 ± 2.11; (b) 56.83 ± 2.53; and (c) 70.40 ± 2.13 W cm².
All emulsions displayed a porous and heterogenous microstructure, where fat droplets and protein can be observed. At 42.58 ± 2.98 W cm\(^{-2}\), the fat droplets were clearly distinguished in the micrograph (Figure 4.8a-c), independently of the concentration of soy lecithin. Contrary, the images that correspond to higher acoustic intensities (56.83 ± 3.01 and 70.48 ± 2.97 W cm\(^{-2}\)) did not display any visible red spots (fat droplets). In summary, the application of ultrasound at 56.83 ± 3.01 and 70.48 ± 2.97 W cm\(^{-2}\) yielded stable emulsions for at least 21 d without the addition of soy lecithin. Improvement of emulsion stability was judged according to the relatively small droplet size (470-521 nm) and dynamic rheological measurements (longer viscoelastic region and weak gel behavior).

4. Conclusions

High power ultrasound at an acoustic intensity of 56-70 W cm\(^{-2}\) produced stable emulsions without the addition of soy lecithin. Ultrasound emulsions were stable for at least 21 d at 4°C, and they exhibited mechanical behavior of a weak gel during storage. The obtained droplet size and dynamic rheology suggested that ultrasound can be used as manufacturing aid for the formulation of dairy-based emulsions. Further implementation at large scale will require additional studies such as evaluation of shelf-life, color, and sensory attributes.
Figure 4.8. Confocal micrograph of ultrasound emulsions 21 d of storage at 4 °C: (a) 42.58 ± 2.11 W cm\(^{-2}\) and 0%, (b) 42.58 ± 2.11 W cm\(^{-2}\) and 0.025%, (c) 42.58 ± 2.11 W cm\(^{-2}\) and 0.05%, (d) 56.83 ± 2.53 W cm\(^{-2}\) and 0%, (e) 56.83 ± 2.53 W cm\(^{-2}\) and 0.025%, (f) 56.83 ± 2.53 W cm\(^{-2}\) and 0.05%, (g) 70.40 ± 2.13 W cm\(^{-2}\) and 0%, (h) 70.40 ± 2.13 W cm\(^{-2}\) and 0.025%, and (i) 70.40 ± 2.13 W cm\(^{-2}\) and 0.05%. The protein matrix is represented in green while the fat phase is represented in red. Scale bar = 500 nm.
References


Chapter 5

Conclusions and future work

5.1. Overall conclusions

The dairy and food industry are working on satisfying consumers demand due to their health concerns by limiting or completely removing artificial additives in food products. A lot of research is carried on novel technologies such as ultrasound to satisfy these consumers demand.

The main objective of the first study was to model different material compositions with ultrasound treatment. water, skimmed milk (varying total solids) and raw cream (varying fat contents) showed the threshold values that can be attained before chemical or biochemical modifications can take place. The data obtained for water was used to determine the acoustic intensities of the system. The objective of this project was to produce emulsions with different soy lecithin concentrations using ultrasound technology and determine their stability during storage (3 weeks). The lecithin concentrations were 0, 0.025 and 0.05% with acoustic intensities of 56 -70 Wcm\(^{-2}\). Stable emulsions were formed with or without soy lecithin concentration with increasing acoustic intensity. No change was observed in the protein structure of the gels and no significant differences in the chemical composition of the emulsions. However, for the rheology properties of the emulsions after 21d of storage, weak gels were observed. Additionally, modification of food properties due to mechanical, physical, chemical, or biochemical changes reduces reaction time and increases reaction yield when mild conditions are used saving energy.
Overall, these studies show the possibility of ultrasound in emulsification processes in food and dairy industries without addition of emulsifiers. Specific combinations can therefore help in the formulation of dairy emulsions without stabilizers.

5.2. Further work

In previous studies, ultrasound prepared emulsions have been reported to give off metallic flavors. Implementation of ultrasound prepared emulsions in a large scale will require additional studies such as evaluation of shelf-life, color, sensory attributes, and flavor compounds to ensure acceptability by consumers. Microbial evaluation should also be carried out as this will give information on the toxicity, quality and subsequently shelf life of the formulations.
Appendix

0%  

42.58 ± 2.98 W cm\(^{-2}\)  

56.83 ± 3.01 W cm\(^{-2}\)  

70.48 ± 2.97 W cm\(^{-2}\)  

0.025%  

42.58 ± 2.98 W cm\(^{-2}\)  

56.83 ± 3.01 W cm\(^{-2}\)  

70.48 ± 2.97 W cm\(^{-2}\)  

0.05%  

42.58 ± 2.98 W cm\(^{-2}\)  

56.83 ± 3.01 W cm\(^{-2}\)  

70.48 ± 2.97 W cm\(^{-2}\)