PHYSICAL AND SENSORY ANALYSIS OF SOY-BASED ICE CREAM FORMULATED WITH HIGH OLEIC LOW LINOLENIC SOYBEAN OIL

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled

PHYSICAL AND SENSORY ANALYSIS OF SOY-BASED ICE CREAM FORMULATED WITH HIGH OLEIC LOW LINOLENIC SOYBEAN OIL

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PHYSICAL AND SENSORY ANALYSIS OF SOY-BASED ICE CREAM FORMULATED WITH HIGH OLEIC LOW LINOLENIC SOYBEAN OIL

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ABSTRACT

Due to increased health awareness of dietary fats, consumers are demanding products with healthier oils. A diet high in oleic acid, a monounsaturated omega-9 fatty acid, has been linked to a reduced risk of coronary heart disease, obesity, and insulin resistance. Recently, there has been significant growth in the plant-based frozen dessert market. These products are typically made with palm or coconut oil. SOYLEIC™ soybean oil is an emerging high oleic low linolenic (HOLL) soybean oil.

In this study, the initial objective was to evaluate the performance of cold-pressed SOYLEIC™ soybean oil as a healthy fat source in frozen dessert products. The effect of SOYLEIC™ soybean oil on the physical and sensory properties and consumer acceptance of soy-based ice cream was compared to commercial vegetable oil, commercial high oleic (HO) soybean oil and heavy cream. SOYLEIC™ soybean oil and commercial HO soybean oil resulted in an overrun of ice cream equivalent as cream, but higher than commercial vegetable oil.

A descriptive sensory panel was carried out to determine the differences in sensory characteristics among the four soy-based ice creams. There was no significant difference in flavor attributes between ice creams prepared with SOYLEIC™ soybean oil and cream, showing less painty odor as well as the aftertaste than commercial vegetable oil. However, ice cream formulated with SOYLEIC™ soybean oil had lower mouth coating than heavy cream. The consumer acceptance study showed that ice cream containing SOYLEIC™ soybean oil and cream received higher overall and flavor likings than those with commercial vegetable oil and commercial HO soybean oil. Proprietary SOYLEIC™ soybean oil has potential to be used in formulating plant-based ice creams.

In order to improve the texture properties of ice cream made from liquid oil, heat-set SPI gel particles were used to structure oil into a high internal phase emulsion (HIPE) gel and the HIPE containing high fraction of oil was used to formulate ice cream. The HIPE was successfully fabricated using SPI gel particles via a two-step method and exhibited a gel-like texture. Stability test showed that the HIPE was stable against thermal treatment at 90 ºC for 30 min, but prone to freeze-thawing treatment. Ice cream formulated with the HIPE showed better meltdown resistance compared to the ice creams prepared using liquid oil. This study provided an insight into a novel utilization of HIPE as a solid fat substitute in ice cream.

Chapter 1 INTRODUCTION

1.1 Background

Frozen dessert is a broad term that covers all sorts of desserts meant to be eaten while frozen (Goff, 2000). Ice cream is one of the most popular frozen desserts in the United States. According to the International Dairy Foods Association (International Dairy Foods Association, 2022), Americans eat 20 pounds of ice cream each year on average. Typical ice creams are formulated using dairy solids and milk fat (Marshall et al., 2003). Due to innovations in sources of raw material and advances in technology, variations of ice cream have been developed. Ice cream formulations are not just limited to using dairy ingredients (Goff, 2006; ReportLinker, 2022). Plant-based frozen desserts (or so-called plant-based ice creams) are a current rapidly growing trend. The global market size of plant-based frozen dessert was valued at $2.6 billion in 2021 and forecasted to expand at a CAGR of 34.50% in the period of 2022-2029 (Data Bridge, 2022).

Plant-based ice creams are typically made with palm or coconut oil, which are high in saturated fatty acids (Goff, 2006). Saturated fats have been linked to a number of adverse effects on health problems, including heart disease, obesity, insulin resistance, etc. (Dhaka et al., 2011; Elson & Alfin-Slater, 1992). Therefore, heathy sources of fat for ice cream production are needed. Vegetable oils consisting of high unsaturated fatty acid profile are believed to be healthier (Vieira et al., 2015). SOYLEIC™ soybean oil is an emerging high oleic low linolenic (HOLL) soybean oil, patented by the Missouri Soybean Merchandising Council (MSMC). Oleic acid is a monounsaturated omega-9 fatty acid naturally existing in many different food sources (List, 2022) and has received a qualified health claim from FDA (FDA, 2018) regarding consumption of high oleic oils to a reduced risk of heart disease. In addition, being monounsaturated, oleic acid has relatively high resistance to oxidative degradation compared to polyunsaturated fatty acids (Napolitano et al., 2018). SOYLEIC^{TM} soybean oil contains more than 75% oleic acid. With all these advantages, SOYLEIC^{TM} soybean oil has potential to produce ice cream with a better nutritional profile and extended shelf-life. The results from this study could provide useful information for the development of commercial products with SOYLEIC^{TM} soybean oil.

The investigations on the application of HOLL soybean oil in ice cream formulation are still limited. Previous studies have reported unsatisfactory quality of ice cream made using high oleic oil in terms of lower overrun when compared to that made based on coconut oil (Marín-Suárez et al. 2016). There are still technological limitations of utilizing vegetable oils in ice cream due to their liquid form and slow crystallization rate (Marín‐Suárez et al., 2016; Sung & Goff, 2010). Technologies to structure liquid oil into solid form have gained great attention. Oleogel, a relatively novel technology, has been studied as a solid fat replacer in various food systems (Pușcaș et al., 2020). However, the practical use of oleogels in food products is limited for safety concern and lack of knowledge. Like oleogel, High internal phase emulsions (HIPEs) also exhibit a solid like structure and contain high volume fraction of oil, which can be facilely fabricated using food grade proteins and polysaccharides (Bascuas et al., 2021). Current studies on HIPEs have been focusing on the applications in dressing, mayonnaise, sauces, dips and spreads (Bai et al., 2021). Investigation of HIPEs as an alternative to solid fat in ice cream is still blank.

In the second part of this PhD project, we aimed to utilize heated SPI gel particles to stabilize HIPE and form a gel-like texture. The application of SPI gel particles stabilized HIPE as a milk fat replacer in soy-based ice cream was investigated in terms of physical, textural and perception properties. This study provided an insight into a novel utilization of HIPE as a solid fat substitute in ice cream.

1.2 Objectives

The objectives of this study were

1) To formulate soy-based ice creams using cold-pressed SOYLEIC^{TM} soybean oil;
2) To determine the effect of cold-pressed SOYLEIC^{TM} soybean oil on physical and textural properties of ice creams in comparison with commercial vegetable oil, commercial high oleic soybean oil and heavy cream;
3) To conduct a descriptive sensory test and a consumer hedonic test to evaluate the effect of cold-pressed SOYLEIC^{TM} soybean oil on sensory characteristics of soy-based ice creams and consumer acceptance;
4) To fabricate high internal phase emulsions (HIPEs) using heated SPI gel particles;
5) To investigate the effect of gel-like SPI gel particles stabilized HIPE on the physical and sensory properties of soy-based ice creams.

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Chapter 2 LITERATURE REVIEW

2.1 Frozen desserts and ice cream

Frozen dessert as a broad term can be used to describe all kinds of desserts that are made by freezing liquids, semi-solids and even solids and are meant to be consumed while frozen. Frozen dessert can be classified into categories based on its mix ingredients, including ice cream, frozen dairy desserts, frozen custard, gelato, non-dairy frozen desserts, sherbets, sorbets, frozen yogurts, mellorine, milkshakes, smoothies, slush, novelties and frozen confection (Goff, 2000; Kilara et al., 2007; Krahl et al., 2016). The popularity of frozen dessert is likely because of its cooling sensation, sweet taste and no preconditioning aroma (Kilara et al., 2007). A report from Statista (2021) has shown the consumption of frozen dessert in all categories has significantly increased during the pandemic. The estimated size of global frozen dessert market accounting for about 69% of the global ready-to-eat food market, is valued at $100.1 billion by the end of 2022, and is expected to expand at a compound annual growth rate (CAGR) of 5.8% between 2022 and 2032. The market is expected to reach to $176 billion in 2032 (Future Market Insights, 2022).

However, frozen desserts in this dissertation are specifically referred to as frozen foods, which are produced by freezing and aerating emulsions containing protein, fat, sweeteners, stabilizers and emulsifiers, including ice cream formulated using dairy ingredients and plant-based frozen dessert using non-dairy ingredients. Factors such as chemical and microbial composition, amount of air incorporated and size of ice crystals and air cells in the main structure determine the physical properties of frozen dessert as well as further sensory quality (Goff, 2000). In the following context, overviews of ice cream and plant-based frozen desserts as well as their microstructure and processing will be discussed.

2.1.1 Ice cream

Ice cream is a popular frozen dairy food worldwide and is the most widely consumed product among frozen desserts (Goff & Hartel, 2013c). The United States, Canada, New Zealand, Australia, Belgium, Finland and Sweden are the major consumer countries of ice cream (Kilara et al., 2007). From the International Dairy Foods Association (2022), the ice cream industry is worth $13 billion, having a big impact on the U.S. economy. Americans on average consume 20 pounds of ice cream each year.

A typical ice cream formulation contains seven categories of ingredients, fat, non-fat milk solids, sweeteners, stabilizers, emulsifiers, water and other additives such as flavors (Kilara & Chandan, 2015; Marshall et al., 2003). However, the compositional standards of ice cream vary from country to country (Kilara et al., 2007; Marshall et al., 2003). According to the U.S. Food and Drug Administration (FDA) standards (21 CFR 135.110), ice cream is defined as “a food produced by freezing, while stirring, a pasteurized mix containing at least 10% milk fat, 20% total milk solids (TMS)” (Marshall et al., 2003). Ice cream must weigh a minimum of 4.5 pound per gallon and contain at least 1.6 pound total solids per gallon (Arbuckle, 2013).

Table 2-1. Typical compositional range for ingredients in ice cream mix (Goff, 1997)

Component	Range (%)
Milkfat	10-16
Nonfat milk solids	9-12
Sugar	9-12
Corn syrup solids	4-6
Stabilizers/emulsifiers	0-0.5
Total solids	36-45
Water	55-64

Based on the fat content, ice cream can be further divided into four different trade grades, super-premium, premium, standard and economy ice cream. As the name suggests, economy grade ice cream is the cheapest product with the minimum fat content and maximum overrun as allowed by law. Premium ice cream typically is high in fat (12-15%) and low in overrun (60-90%) to provide good quality. Super-premium products are characterized by an even higher fat content (15-18%) and lower overrun (25-50%) than premium ice cream and are designed to use high quality ingredients (Kilara et al., 2007; Pszczola, 2002). Detailed information about ice cream of different grades is shown in Table 2-2.

Table 2-2. Composition standard of ice cream

(David, 2014)

	Super-premium	Premium	Standard	Economy
Fat (%)	15-18	12-15	10-12	10
Total solids (%)	>40	38-40	36-38	36%
Overrun (%)	25-50	60-90	100-120	Legal max. ~120
Cost	High	High than average	Average	Low

As work to improve the functional and nutritional quality is ongoing, other variations and changes of ice cream have been developed. For generic use, the term ice cream has also covered products containing non-dairy fats, "light" versions containing lower fat content, ice milk, sherbet and frozen yogurt (Goff, 2006).

Compared to conventional ice cream, the "light" versions are nutritionally altered. Forms of so-called "light" ice cream include reduced fat, light, low fat and nonfat available in ice cream. The International Ice Cream Association (ICA) standardized the fat content as following:

- Reduced fat: 25% less fat than regular ice ream
- Light: 50% less total fat or 33% fewer calories than regular ice cream
- Low fat: no more than 3 g of total fat per serving (half cup)

- Nonfat: less than 0.5 g of total fat per serving (Farooq, 1997; Frye & Kilara, 2015)

Mellorine is an ice cream product made with animal or vegetable derived fats other than milkfat (Kilara et al., 2007). A healthy alternative to ice cream is frozen yogurt which is made by adding yogurt to the ice cream mix (Tamime, 2008). Nowadays, with the increasing demand for healthy food by consumer, innovations in ice cream formulation are urgently needed. The current market of ice cream has gradually shifted to plant-based concepts.

2.1.2 Plant-based frozen desserts

There are a few different nomenclatures used as prefix in front of frozen desserts such as non-dairy, dairy-free, plant-based and vegan. So far, no regulatory definition applies to terms of dairy-free, plant-based and vegan. However, regulatory definition allows milk protein and casein to be used in non-dairy products (Agropur, 2019; Yang et al., 2022). Here, we focus on the frozen desserts formulated using ingredients only from plant origins.

Due to increasing attention to healthy diet and concerns about sustainability of our planet, plant-based diets have gained popularity among consumers. Plant-based diets which are high in protein but fewer in calories are linked to lower blood sugar, blood pressure and total cholesterol levels. Furthermore, the hit by the pandemic has changed people's diet focus (Data Bridge, 2022). Now consumers in a great number shift from dairy ice cream to non-dairy frozen desserts or plant-based frozen desserts (Craig & Brothers, 2022). Such frozen desserts are lactose free so they can be consumed by people with lactose intolerance. The global plant-based frozen dessert market value was projected to be $2.6 billion in 2021. The market size is forecasted to grow at a CAGR of 34.5% in the period of 2022-2029 and surpass $27.9 billion by 2029 (Data Bridge, 2022). The boost of the market has relied on the rapid improvement of technologies, new sources of raw materials, and evolving solutions to taste and texture difficulties in industry (ReportLinker, 2022).

In recent years, more and more plant-based frozen dessert varieties have emerged into the market. Many major ice cream brands like Haagen-Dazs, Ben & Jerry, Breyers, and Talenti, have launched dairy-free ice cream products. Besides, plant-based food manufacturers including So Delicious, Oatly, and Ripple have expanded their product lines from plant-based beverages to various kinds of non-dairy frozen desserts.

The basic formulation of plant-based frozen desserts consists of similar ingredient categories as dairy ice cream: protein solids, fat, sweeteners, stabilizers, emulsifiers, flavor and other additives. Sweeteners, stabilizers and emulsifiers are often the same while plant-based frozen desserts are designed to use plant protein and fat from plant sources including legumes, grains, seeds and nuts. Furthermore, clean label is a key emphasis of plant-based frozen dessert product development (Agropur, 2019).

The main protein solids come from varieties of plant-based milk alternatives such as cashew milk, oat milk, soy milk, coconut milk, rice milk and almond milk, and isolated protein forms such as pea protein concentrate and soy protein isolate. The used of plant-based proteins in plant-based frozen desserts have been associated with off-notes like earthy, beany, nutty, malty and brothy. Common sources of fat in plant-based frozen desserts include coconut oil, coconut cream, palm oil, palm kernel oil, sunflower oil, soybean oil, and avocado oil. Less common sources including emulsions like coconut cream, margarine, and shortening have also been used (Agropur, 2019; Goff, 2006). Dietary oils are made up of triacylglycerol. The ability of triacylglycerols to form a network of crystalline fat is important for the formation of ice cream structure (McClements & Grossmann, 2021). Different oils with different fatty acid compositions and degree of unsaturation have different fat crystallization rates, which could affect the rheological and structural properties of frozen desserts during freezing. Therefore, the use of proper fat is critical to the sensory and physical properties of frozen desserts. A few examples of currently commercialized plant-based frozen desserts are listed in Table 2-3. Coconut oil which has a high melting temperature due to its high degree of saturation, is often used.

In this dissertation, soy milk and soybean oil were used in the formulation of the plant-based frozen dessert model. The first concept of making an ice cream from soy was brought up by Arao Itano from Japan in 1918 and the first commercial soy ice cream was introduced by Jethro Kloss in 1930 (Shurtleff & Aoyagi, 2013). To prevent confusion, the term ice cream will be used interchangeably with frozen dessert in the following context.

Table 2-3. Examples of alternative milk, protein powder and fat used in vanilla flavored plant-based frozen desserts from different commercial products.

Brand	Milk base and protein	Protein content (%)	Fat source	Fat content (%)
Ripple Vanilla Non-dairy Frozen Dessert	Pea protein	1.8	Coconut oil	12.7
Oatly Vanilla Frozen Dessert	Oat milk	<1	Coconut oil, rapeseed oil	11.8
So Delicious Dairy Free Vanilla Bean Coconut Milk Frozen Dessert	Coconut milk	<1	Coconut oil	11.6
So Delicious Creamy Vanilla Soy Milk Frozen Dessert	Soy milk	1.8	Soybean oil	3.6
Favorite Day Non-Dairy Plant Based Vanilla Almond Frozen Dessert	Almond milk, pea protein	1.8	Coconut oil	9.1
Trader Joe's Vanilla Soy Creamy Non-Dairy Frozen Dessert	Soy milk	2.2	Oleic safflower oil	7.5

2.1.3 Manufacturing process

A typical ice cream and frozen dessert manufacturing process starts by blending of ingredients, followed by pasteurization and homogenization, aging, freezing, and hardening (Goff, 2000; Goff & Hartel, 2013b). In the next paragraph, the role of each step will be illustrated.

Pasteurization aims to kill pathogenic bacteria, which is an important biological control to ensure food safety. Following pasteurization, the hot pasteurized mix is passed through a two-stage homogenizer with a pressure of 2,000-2,500 psi on the first stage and 500-1,000 psi on the second stage. Homogenization allows the formation of a stable emulsion by breaking down bulk fat into fine droplets and dispersing them in the mix. Then, the mix is aged by cooling at refrigeration temperature. Several phenomena occurring during ageing include crystallization of fat, full hydration of proteins and stabilizers as well as rearrangement of the surfactant membrane (Goff, 2006; Ruger et al., 2002; Segall & Goff, 2002). The freezing step is a dynamic process, namely freezing while whipping to incorporate air and form small and discrete ice crystal under shear forces. During this step, fat globules destabilize and partially coalesce. About 50% of the water is frozen after freezing (Pei & Schmidt, 2010; Schmidt, 2004; Segall & Goff, 2002). The final step is hardening by further rapidly freezing the remainder of the water in the semi-frozen ice cream (Hartel, 1996; Kilara et al., 2007).

2.1.4 Microstructure

Ice cream is a complicated physicochemical system, containing multiphases. It is equally an emulsion and a foam (Cook & Hartel, 2010; Goff, 2019). Figure 2-1 schematically demonstrates the microstructure of ice cream. The main structural elements consist of fat globules, ice crystals, air cells and an unfrozen continuous serum phase. Air cells are dispersed in the serum phase. In the serum phase, components such as proteins and emulsifiers form a protein/emulsifier layer and coat the fat globules while sugar and stabilizers become freeze concentrated. Ice crystals are embedded in the serum phase (Arbuckle, 2013).

Microstructure of ice cream determines physical properties of ice cream like shape retention, overrun, meltdown, rheological and mechanical properties as well as sensory perception (Goff & Hartel, 2013a; Koxholt et al., 2001). An important structural component of ice cream is the fat network. Fat globules can be either distinctly dispersed or partially coalesced in the serum phase. When milk fat is used in the ice cream mix formulation, milk fat globules tend to destabilize in the serum phase during the freezing stage with the presence of emulsifiers at the fat globule interface to form fat cluster around air cells (Daw & Hartel, 2015; Goff & Hartel, 2013a; Warren & Hartel, 2018). This fat network provides important structural support and smooth sensory perception for ice cream (Goff, 2002).

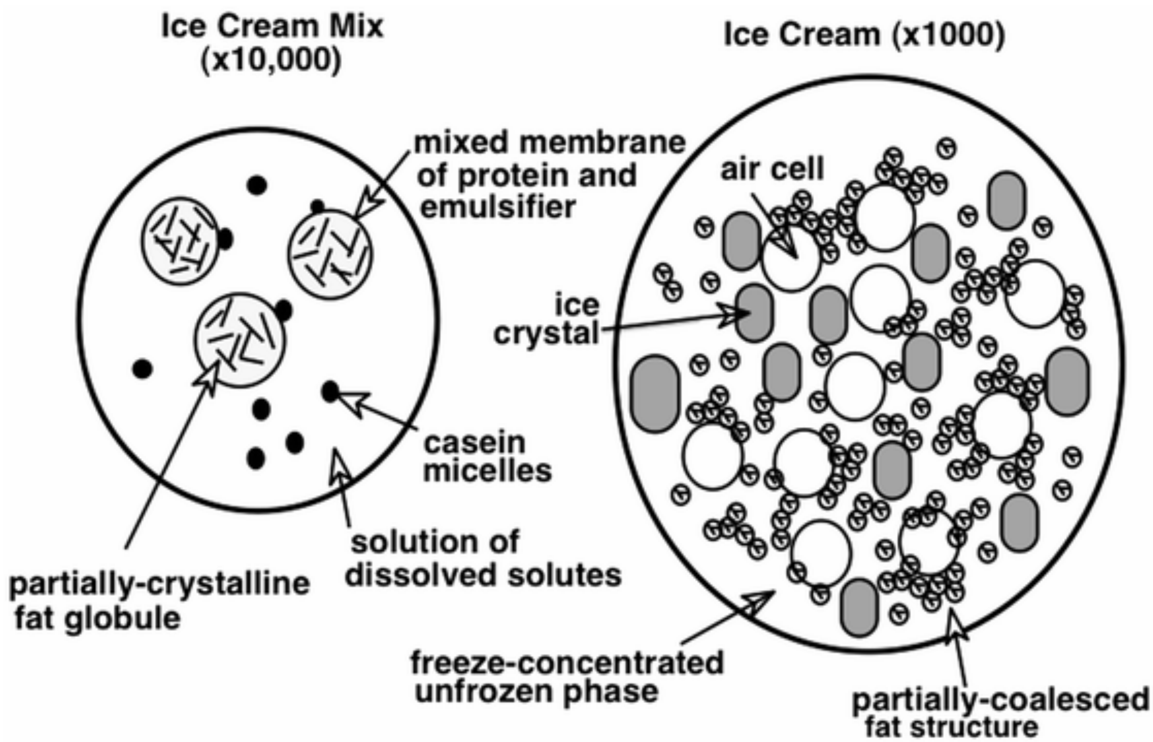

Figure 2-1. A schematic illustration of ice cream microstructure
(Goff & Hartel, 2013a)

2.1.5 Sensory quality of ice cream

Ice creams with satisfactory perception are expected to have firm body, smooth mouthfeel, slow meltdown property and stability (Hammond, 2006), which are associated with the microstructure and physical characteristics of ice cream. The partial coalesced fat network and a certain viscosity of mix and proper amount of air incorporated play important roles to the textural properties of ice cream. The delicate quality of an ice cream is to a great extent related to the small size of ice crystals. Ice crystals are mainly formed during the scraped-surface freezing process. The size of ice crystals is usually in the range of 20-50 μm (Goff, 1997). During storage, the ice crystals in ice cream continually grow and recrystallize trough Ostwald ripening and accretion due to high storage temperatures and temperature fluctuations. In the recrystallization process, the number, size and shape of ice crystals change. Large ice crystals (above 100 μm) give a coarse, grainy and icy mouthfeel of ice cream (Flores & Goff, 1999; Hartel, 1998).

2.2 Soybean oil

Soybean oil is one of the most widely consumed vegetable oils and is crushed from soybean seeds. Soybean seed contains approximately 40-41% protein, 8-24% oil and 35% carbohydrates on the dry seed weight (Medic et al., 2014). It is a leguminous crop, composed of hull, hypocotyl axis and cotyledon cells (Preece et al., 2017). Oil is stored in the cotyledon. 90% of the oilseed production in the United States is extracted from soybeans (ERS USDA, 2022). The soybean oil production for 2021 was about 26.2 billion pounds (Statista, 2022).

Soybean is a nutritious food ingredient, being a good source of protein, dietary fatty acids and bioactive chemcials. The United States is a big producer and exporter of soybean crops all over the world. The “soyfoods” movement started from the United States in 1970s, which promoted the growth of the soyfood industry (Shurtleff & Aoyagi, 2013) The health benefits that soy products can provide include reducing the risks of heart disease, cancer, osteoporosis and anti-aging .

2.2.1 Sensory aspects of soybean oil

Commodity soybean oil consists mainly of five fatty acids, palmitic acid, stearic acid, oleic acid, linoleic acid and linolenic acid, each accounting for 10%, 4%, 18%, 55% and 13% of the oil content, respectively (Clemente & Cahoon, 2009). The utilization of soy in food has been limited in Western countries due to some undesirable flavor called beany or grassy flavor. Studies have shown that the beany flavor is mainly due to the oxidation of polyunsaturated lipids including linoleic acid and linolenic acid by the lipoxygenases in soybean (Yu et al., 2017). Linoleic acid and linolenic acid both contain a cis,cis-1,4-pentadiene moiety which is linked to the formation of hydroperoxides upon oxidation and in turn the production of beany flavor compounds. Off-flavor compounds associated with green beany flavor include hexanal, hexanol, ethyl vinyl ketone, heptanal, 1-penten-3-ol, t-2-hexenal, 2-pentylfuran, 1-pentanol, 1-octen-3-ol, 1-octen-3-one, trans,trans-2,4-decadienal, and trans,trans-2,4-nonadienal (Suratman et al., 2004).

Three major lipoxygenases are found in mature soybean, LOX-1 (lipoxygenase-1), LOX-2 (lipoxygenase-2) and LOX-3 (lipoxygenase-3). Lipoxygenases and polyunsaturated lipids are separated within the cell until soybeans are smashed. While making soy milk or producing soy oil, the cracking and soaking steps make lipoxygenases and polyunsaturated lipids mix and react to form the oxidation products resulting in the off flavors (Yang et al., 2016).

To eliminate the beany flavor of soy, we can seek to remove or inactivate the lipoxygenases. The first approach is to breed lipoxygenase-free soybean variety. Back to 1980s, scientists had started to use genetic tools to breed soybean seeds lacking lipoxygenases. Studies have shown the lipoxygenase-free soybean mutants had significantly lower hydroperoxide and hexanal levels than normal varieties (Furuta et al., 1996). Less beany flavor in soymilk or tofu made from lipoxygenase-null soybeans was confirmed by a sensory panel (Torres-Penaranda, 1999). However, lipoxygenases are non-heme iron-containing proteins in plants and lack of lipoxygenases may impact plant physiological processes. Thus, the effect of knockout of lipoxygenases on storage ability, agronomic character and resistance to biotic and abiotic need to be studied (Wang et al., 2020). Other approaches involve appropriate treatments to reduce the activity of lipoxygenases, for example, thermal treatment (Lv et al., 2011; Yuan et al., 2008), microwave processing (Wang & Toledo, 1987), ultrasound treatment (Islam et al., 2014), and pulsed electric field (PEF) (Li et al., 2008).

Besides a beany flavor, soybean oil may have rancid or oxidized off-flavors during storage or upon heating. Due to the relatively high percentage of the polyunsaturated fatty acids, linoleic acid and linolenic acid, soybean oil is unstable against oxidation. The oxidative breakdown of soybean oil produces beany and rancid odors in food products. Heating facilitates the oxidative reaction. Increasing the content of oleic acid in soybean oil can make oil more resistant to oxidation as oleic acid is relatively slower in oxidation rate. Owing to the high oxidative stability, high oleic oil is a desirable high temperature frying oil. Warner and Gupta (2005) reported better frying stability of high oleic soybean oil with increasing amounts of oleic acid. Although increasing oleic acid content can result in better oxidative stability and reduced rancidity and off flavors, stronger fishy flavor may be developed during frying if the linolenic and linoleic acid levels are not balanced. When the linoleic acid level is lower than that of linolenic acid, fishy flavors are produced from the degradation of linolenic acid. Researchers at the University of Missouri have developed varieties with the high oleic low linolenic (HOLL) seed oil trait utilizing mutations in certain fatty acid desaturase genes in soybean to the alteration of fatty acid profiles in soybean (Pham et al., 2010).

2.2.2 High oleic low linolenic (HOLL) soybean oil

2.2.2.1 Oleic acid

Oleic acid is a monounsaturated omega-9 fatty acid that can be found in many different food sources like oilseeds, animal meat, nuts, vegetables, eggs, milk and algal lipids (List, 2022). Oleic acid is found to be naturally rich in olive oil (about 70%), which is the reason why oleic acid is named after olive oil. The structure of oleic acid is shown in Figure 2-2. It consists of 18 carbon atoms with a *cis*-double bond at the 9-carbon position.

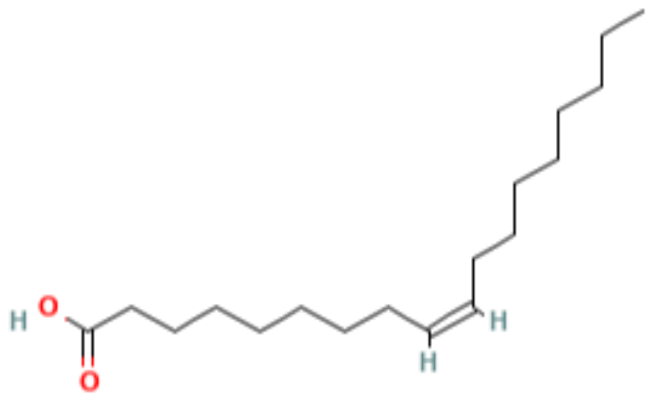

Figure 2-2. Chemical structure of oleic acid
(source: https://pubchem.ncbi.nlm.nih.gov/compound/Oleic-acid)

Oleic acid rich diet has been found to benefit cholesterol metabolism, reduce risks of developing coronary disease, obesity and insulin resistance, and enhance brain function (Herrera et al., 2001; Lopez et al., 2021; Yodice, 1990). Due to its health benefits, oleic acid has received a lot of attention over the past several decades. Oils containing high oleic acid content have become increasingly desirable among consumers from a health perspective.

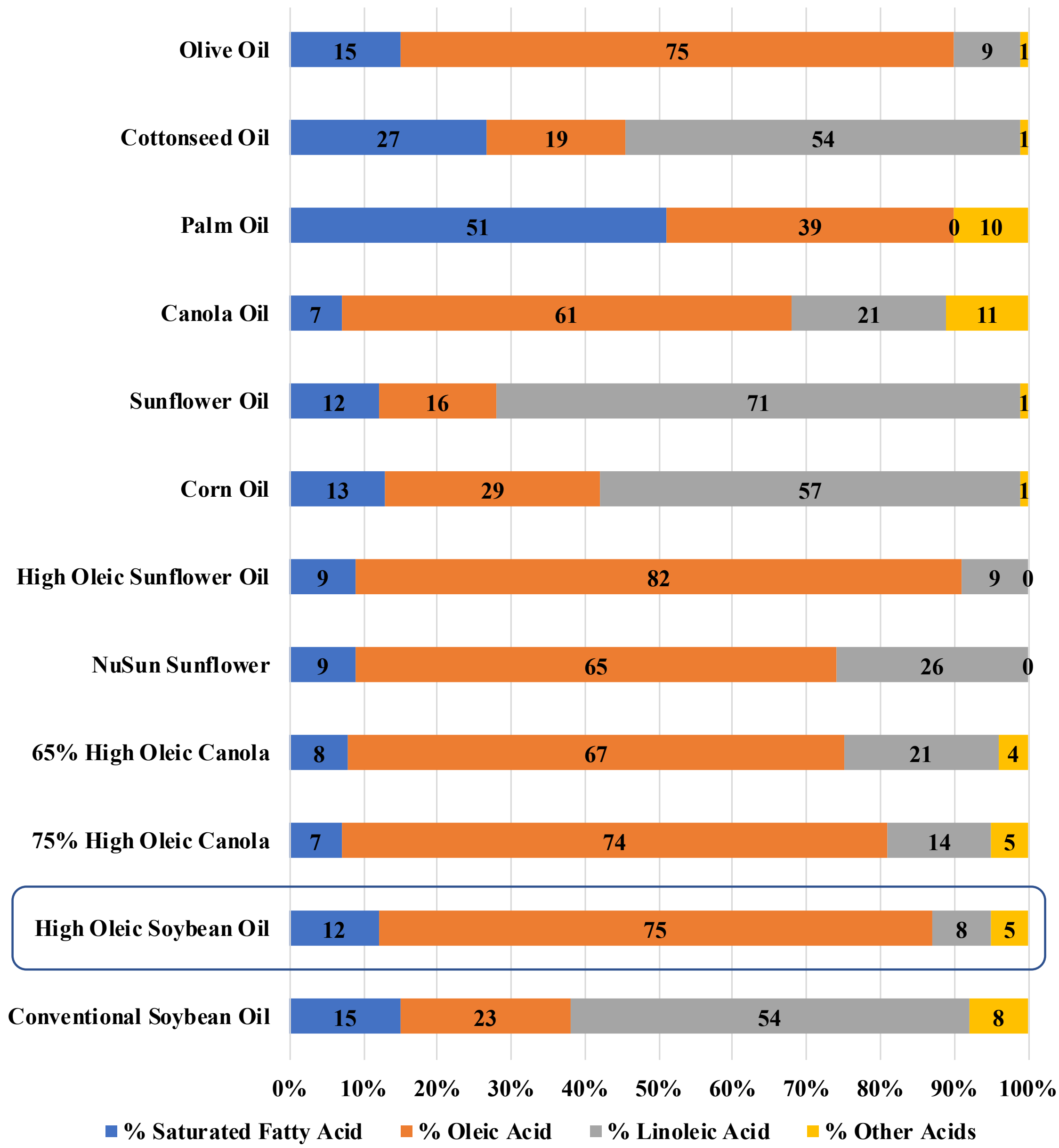

Figure 2-3. Fatty acid compositions of conventional and high oleic versions of vegetables (Source: U.S. High Oleic Soybeans & High Oleic Soybean Oil Sourcing Guide for International Customers)

The FDA (2018) has authorized a qualified health claim relating consumption of high oleic oils to a reduced risk of heart disease. Currently, commercial high oleic oils available in the market include olive oil, high oleic soybean oil, high oleic sunflower oil, high oleic canola oil, high oleic avocado oil, high oleic safflower oil, and high oleic corn oil. Fatty acid compositions of conventional and high oleic version of vegetable oils are shown in Figure 2-3. These healthful oils are used to replace trans-fat and saturated fat in daily diets, food processing and food services (Clemente & Cahoon, 2009; Liu & Iassonova, 2012).

2.2.2.2 HOLL soybean oil

The advances in plant biochemistry and biotechnology have led to the development of special varieties of soybean containing high content of oleic acid and low content of linolenic acid. The typical oleic acid content in HOLL soybean oil is greater than 75% while the polyunsaturated fatty acids, namely linoleic and linolenic acids only account for 6% in total (Pham et al., 2012). Commercializing HOLL soybean oil is expected to promote a gradual replacement of current commodity oils in food production and food services which is the major oil used (Napolitano et al., 2018). There are several advantages of HOLL soybean oil that grant it greater importance in food applications. HOLL soybean oil has been considered a healthier alternative to palm oil, coconut oil and hydrogenated oil which are high in saturated fats. The low saturated fat content of HOLL soybean oil allows it to provide diverse nutritional benefits to foods (List, 2022; Napolitano et al., 2018).

A key technological advantage of HOLL soybean oil in food applications is its improved oxidative stability. Oleic acid has a much lower rate of oxidation than linoleic and linolenic acids (Frankel, 2014). Due to the relatively high percentage of linoleic and linolenic acids, commodity soybean oil is oxidative instable. The oxidative breakdown of soybean oil produces beany and rancid odors in food products. Using HOLL soybean oil in food applications could lead to food products with better flavor of (Warner & Gupta, 2005). Heating facilitates the oxidative reaction in soybean oil. Owing to the high oxidative stability, high oleic oil is a desirable high temperature frying oil. Warner and Gupta (2005) reported better frying stability of high oleic soybean oil with increasing amounts of oleic acid. Moreover, HOLL soybean oil also had longer shelf life. Using HOLL soybean oil is able to extend shelf life of food products.

SOYLEIC is an emerging non-GMO HOLL soybean trait patented by the Missouri Soybean Merchandising Council. In this study, HOLL soybean oil crushed from SOYLEIC soybean was used in the ice cream formulation to investigate its performance as a fat component in frozen desserts.

2.2.3 Cold pressing of oil

Today, oils are usually extracted from a variety of oilseeds in two main ways, solvent extraction and mechanical extraction methods. Each method has its own advantages and disadvantages. Solvent extraction is the most commonly used method (Kemper, 2020), involving steps of comminution, solvent extraction, meal desolventizing, oil separation and recovery of solvent. Hexane is the conventional solvent used due to its high oil extraction efficiency and its availability. However, concerns about cost, health, safety, and environment have been raised regarding using hexane. Researchers are seeking green solvents (Mwaurah et al., 2020; Russin et al., 2011).

Compared to the conventional solvent extraction process, mechanical oil extraction method is currently used for premium oil due to its low yield (Cakaloglu et al., 2018). Heat that is generated during pressing which can have a negative impact on the quality of extracted oils (Russin et al., 2011). Therefore, oils produced by cold pressing techniques are receiving more interest (Cakaloglu et al., 2018).

Cold pressing of oil can be achieved by using expellers, expanders and twin-cold systems. The temperature during pressing may be controlled in the range of 50-60 ºC (Cakaloglu et al., 2018; Leming & Lember, 2005). Mechanical cold pressing provides several advantages over solvent extraction. Throughout the process, no solvent such as hexane is used, making the process more environmentally friendly (Al Juhaimi et al., 2018).

Without the high heat treatment and refining process, cold pressed oil is obtained without any alterations in functionality, color, and flavor while bioactive phytochemicals can be preserved (Chew, 2020). For example, cold pressed soybean oil contains γ- and δ-tocopherols that are known antioxidants. The antioxidants could help preserve foods and prolong the shelf-life of food products. A great variety of constituents including neutral lipids, polar lipids, fibers, phytosterols, phenolic compounds and other bioactive compounds are present in cold pressed oil (Ramadan, 2020). These molecules could provide functional properties to foods. The phospholipids of soybean oil are highly made up of phosphatidylcholine or lecithin. Lecithin has been used for multiple purposes in food systems, such as emulsifier, lubricant, stabilizer, dispersing agent, mixing aid and many more (Szuhaj, 2016).

2.3 Pickering stabilization

2.3.1 Definitions

The effect of Pickering was first identified in emulsion system by Walter Ramsden in 1903 but it was not fully described until 1907 by Spencer Umfreville Pickering. This phenomenon was named after Pickering. In Pickering's work (1907), he summarized that when the oil is forced to break up into small globules, these globules will attract many minute solid particles to form a coating or pellicle of mechanical barrier around them to keep oil globules apart. Pickering particles are solid particles that can be utilized solely to stabilize two immiscible phases either oil/water or air/water by accumulating at the interface of droplets and bubbles (Bon, 2014; Yang et al., 2017).

Numerous organic or inorganic solid particles have been used as Pickering stabilization particles, including silica, clay, hydroxyapatite, magnetic, nanoparticles. Chitosan, cyclodextrin, and carbon nanotube. Some food grade ingredients have also shown Pickering effect in emulsion or foam systems. They include whey protein, soy protein, zein and starch. The advantage of using food grade stabilizers is their low toxicity (Yang et al., 2017). Other forms, including protein aggregates, nanoparticles and microgel particles have also shown Pickering stability effect (Murray, 2020).

In the last several decades, Pickering particles serving as stabilizers have drawn significant attention in many research fields including food technology, material science, oil recovery, cosmetic products and drug deliver (Ortiz et al., 2020). In this research project, the concept of Pickering was applied to stabilizing both emulsion and foam systems. Eventually, we intended to utilize the Pickering effect to stabilize the foam structure within ice cream's microstructure.

2.3.2 Stabilization mechanisms

Pickering phenomenon was first discovered in emulsion and most of the studies in Pickering stabilization were conducted in emulsion systems in the past. In recent twenty years, research focus has expanded into foam systems as foams are important colloidal systems having widely applications in diverse industries (Hunter et al., 2008). The stabilization mechanisms of Pickering effect in emulsions and foams have been investigated extensively. There are many similarities between emulsions and foams in terms of instability mechanisms. Both emulsions and foams are thermodynamically unstable, and they have some common primary instability processes, which are creaming and sedimentation in emulsions and drainage in foams caused by gravity, and coalescence and flocculation. Also, these two systems possess high surface area per volume of the interface (Hunter et al., 2008; Langevin, 2019). Therefore, the basic principle of Pickering stabilization for both emulsions and foams is proposed to be the steric force resulting from a solid particles layer at the interfaces of gas-liquid or oil-liquid, which inhibits droplets or bubbles from coming in contact and coalescing (Du Sorbier et al., 2015; Wu & Ma, 2016).

This theory has been commonly accepted. However, other more complicated mechanisms have been discussed by researchers, depending on the compositions and physicochemical properties of the applied systems, including oil's type and polarity, solid particle's type and hydrophobicity, particle coverage percentage, particle to droplet size ratio (Aveyard et al., 2003). Here, we emphasize important mechanisms that can be compared between emulsion and foam systems.

Differences in the stabilization mechanisms of Pickering also exist between emulsions and foams because of the different underlying physical principles of the two systems (Hunter et al., 2008). In emulsions of low particle coverage, droplet-droplet particle bridging has been observed and considered as a stability mechanism. When oil droplets are not completely coated, particles can attach onto two close droplets at the same time as a bond. This clustering can increase stability (Vignati et al., 2003).

The size of gas bubbles dispersed in foams can be 1,000 times larger than that of the droplets in emulsions. The density difference between gas-liquid is greater than that between oil-liquid. Moreover, gas bubbles are more prone to deformation with individual bubbles changing to a polyhedral shape, which is not a common phenomenon in emulsions (Hunter et al., 2008; Walstra, 1987). The probability of film rupture is higher (Dickinson, 2010). The instability of foams occurs in three main ways:

- Foam drainage caused by gravity
- Disproportionation caused by gas diffusion from smaller bubbles to larger bubbles
- Bubble coalescence caused by thinning and breakages of liquid films (Wang et al., 2016)

Figure 2-5 illustrates these instability processes in foams. When thinking of approaches for foam stabilization, traditional stabilization mechanisms fall into two main directions. One is to increase the elasticity of liquid films to make them more resistant against rupture. The other is to increase the disjoining/conjoining pressure inside liquid films by utilizing steric and/or electrostatic repulsions provided from charged molecules (Denkov et al., 2020).

Like Pickering emulsion, the close-packed particle barrier at the gas-liquid interface can serve as a mechanical barrier to inhibit bubble coalescence and coarsening meanwhile the steric force keeps the bubbles apart (Dickinson, 2010). Disproportionation is an important process for instability in foam systems, induced by capillary pressure difference. Disproportionation can be retarded if a highly packed solid particle inter-network can be formed at the interface or within the liquid-continuous phase (Hunter et al., 2008). However, there is one special type of foam systems in which disproportionation is not a concern. An example is ice cream, where air bubbles are actually embedded in a frozen matrix (Dickinson, 2010).

Compared to emulsion systems, the instability induced by gravity, namely drainage, is more significant. The Pickering stabilization mechanisms for foams are inherently more complex compared to that for emulsions (Hunter et al., 2008). While some particles are absorbed at the bubble surfaces and accumulate to form a dense coating, other particles remaining in the liquid phase can structure themselves and create particle-rich regions between nearby bubble surfaces and inside aqueous thin films to slow down foam drainage (Dickinson, 2015).

Foam stability is the most important characteristic in ice cream. Pickering foam in food applications will be discussed in the next section.

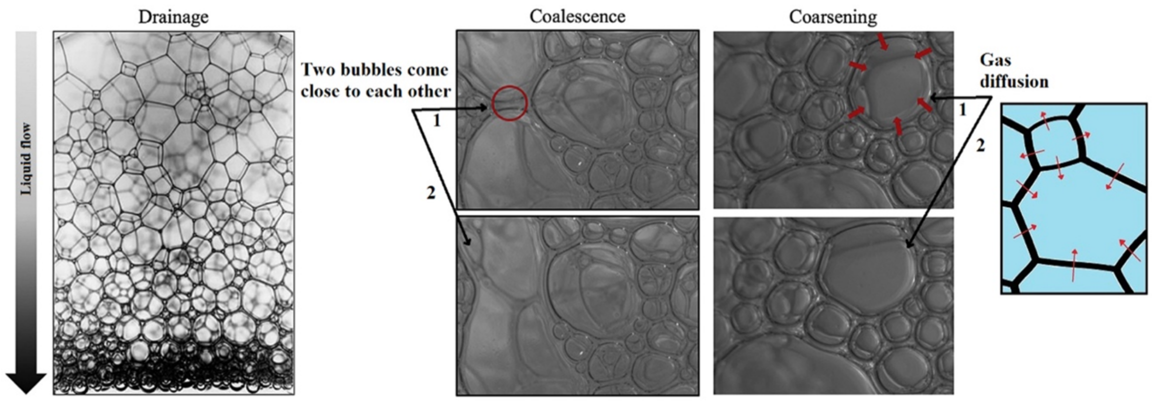

Figure 2-4. Instability process of foams
(Amani et al., 2022)

2.3.3 Pickering foam

Foam is a colloidal system with gas being distributed in a continuous liquid phase. Properties of foam in terms of foamability and stability are gaining more and more interests. Foam is an important component in the food industry as it exists in numerous food structures, for example, ice cream, cakes, and carbonated soda drinks. Herein, Pickering solid particles can be promising stabilizing agents in aerated structures to provide the essential textural characteristics and can provide great value to the food industry (Amani et al., 2022). Much research has been done and many more projects are currently ongoing. Examples of food grade Pickering foam will be discussed in the following content.

Food proteins are good sources for Pickering stabilizers for foams. Casein micellar particles were found to have a Pickering effective in stabilizing foam by Chen et al. (2016). Egg white proteins already possess outstanding foaming ability and stability. However, egg white protein microgels showed superior foaming ability, stability and resistance to bubble disproportionation (X. Li et al., 2020). Long fibrils formed by heated soy glycinin increased foam stability at pH 5 and 7 (Wan et al., 2016). A bacterial Pickering bio-particles which was fabricated by lactic acid bacteria surface modified by oppositely charged milk proteins could stabilize foam better than sole milk protein (Falco et al., 2017). In another study, starch nanocrystals linked rice proteins and pea proteins particles were able to dock at the interface and also form three-dimensional networks in the continuous phase, resulting in stable foam which could last up to 4 days (Wang et al., 2022).

Other than proteins, Quillaja saponin-coated nanodroplets showed improved foamability and stability than Quillaja saponin alone. These particles assembled at the gas–liquid interface in a hierarchical structure under microscope (Chen et al., 2017). Binks et al. (2017) found that calcium carbonate hydrophobized *in situ* with negatively charged emulsifiers could be used to stabilize the foam interface synergistically with either sodium dodecanoate or sodium stearoyl-2-lactylate.

2.3.4 Factors affecting Pickering foam

Pickering foams are multifaceted. Of course, the stabilization of Pickering foams also depends on various aspects, including pH, type of gas, temperature and rheological properties. The presence of other surfactants in the system as well as their properties can cause differences. Nevertheless, how effective solid particles are is determined by various characteristics of the particles themselves, such as particle size, charge, hydrophobicity and concentration (Amani et al., 2022). The last section of the literature review of Pickering foam will focus on the important influencing factors of the solid particles.

Surface hydrophobicity:

Different types of interfaces, gas-liquid and oil-liquid, need particles with different hydrophobicity to attach to the interfaces. Hydrophobicity is determined by affinity to water. For Pickering foams, certain intrinsically hydrophilic and intermediate hydrophobic particles are favored as they have the tendency to stay in water. The surface hydrophobicity of particles can be tuned by altering their surface chemistry in two ways, physisorption (in-situ) and chemisorption (ex-situ) (Amani et al., 2022).

Particle size:

The size of Pickering particles can play an important role and the relationship between particle size and foam stability is more complex. The favorable sizes can range from a few nanometer to 50 microns (Amani et al., 2022), depending on other characteristics of the particles, for example, hydrophobicity. Chen et al. (Chen et al., 2016) pointed out that larger casein micellar aggregates of about 500 nm led to greater foam stability whilst the foam stability was not found to be related to the surface rheological properties. The reason could possibly be that the casein micellar particles were structured within the foam lamellae and plateau borders. However, the size must be controlled within an optimum range as particles that are too large in size lead to bridging and rupturing liquid films. Foams are destabilized instead (Amani et al., 2022). Here the casein micelles are hydrophilic solids. On the other hand, hydrophobic particles are found to stabilize the Pickering foams when they are smaller in size and higher in concentration. This can be explained by the formation of a more completely dense solid-like layer of particles by smaller particles at the interface (Amani et al., 2022).

Concentration:

Ideally, minimum bubble surface coverage should be achieved for sufficient Pickering stability. Chen et al. (Chen et al., 2018) established a distinct relationship between the foam stability and the concentration of aggregates entrapped within the foam lamellae. The more particles up to a certain extent, the more stable is the foam.

Charge:

Charges on particles provide electrostatic repulsive forces which is the basic mechanism for foam stabilization. The addition, surfactants may act synergistically to stabilize foam if the surfactants have similar charge as the particles. (Amani et al., 2022).

2.4 Structured oil

Due to increasing concerns regarding to the adverse effects of *trans* and saturated fats on health conditions, the food industry has banned use of *trans* fat and has been working to reduce saturated fats in foods. Saturated fatty acids (SFAs) from both animals and SFA-rich tropical oils including palm, coconut and palm kernel oils were found to cause an increase in “bad” LDL cholesterol (Elson & Alfin-Slater, 1992). Unsaturated fatty acids are believed to be healthier than the former two types of SFAs (Vieira et al., 2015). *trans* fats are made through partial hydrogenation processes of vegetable oils, by which liquid oils are converted into solid fats. Although *trans* fats are unsaturated fatty acids, they also increase LDL cholesterol and decrease “good” HDL cholesterol (Dhaka et al., 2011). However, *trans* and saturated fats have their own technological advantages in different aspects including quality, shelf life and acceptability. For example, the presence of solid phase provides textural supporting properties and proper mouthfeel to processed foods such as frozen desserts, confectionery products and bakery goods (Vieira et al., 2015); fat shortenings are effective in preventing gluten network development in bakery products due to plasticity and elasticity; solid fats are spreadable (Temkov & Mureșan, 2021). A current challenge in the food industry is to formulate replacers for *trans* and saturated fat using healthful sources of fats without compromising food quality and nutrition. Transforming unsaturated fat into solid-like structured oil without changing its chemical structure as a healthy alternative to solid fats that can mimic the functional properties of solid fats is of great interest.

2.4.1 Oleogel

2.4.1.1 Definitions

As a relatively novel technology, oleogel, also called organogel, has attracted extensive attention attributed to their promising uses in food, pharmaceutical and cosmetic industries. Oleogelation structures liquid oil to form a solid-like or gel-like material using a small amount of oleogelators (Meng et al., 2018; Zhao et al., 2022). Oleogels have been considered as a promising solid fat substitute.

In oleogel structures, the lipid phase technically should be the continuous phase while the structuring agents are dispersed in lipid phase (Scholten, 2019).

Oleogelators can create a three-dimensional network in different structures by self-assembly or crystallization, allowing liquid oil to be trapped within the structure (Zhao et al., 2022). Various types of materials have been investigated for their oleogelation ability and have been successfully used to fabricate oleogels, which included fatty acids and alcohols, waxes and wax ester, monoglycerides, phytosterols, and ceramides. These oleogelators can be further classified into low-molecular weight oleogelators and high-molecular weight oleogelators. Oleogelators in the former category are self-assembled to form a crystal structure for oil bind while those in later category are obtained by creating supramolecular network (Hwang, 2020). Each has its own advantages and disadvantages. The availability, high cost, and safety are the major concerns regarding the oleogelator (Meng et al., 2018). Ethyl-cellulose is the only direct food-grade polymer oleogelator (Davidovich-Pinhas et al., 2016).

One aspect worth noticing here is that the effective dosage of oleogelator to structure oil depends on the properties of the oil. Unrefined oils require a higher amount of oleogelator to form oleogels with similar properties than refined oils (Zhao et al., 2020).

2.4.1.2 Fabrication methods

There are two main strategies for preparing oleogels based on what structure agent is used, direct dispersion of oleogelator in the oil phase and indirect templated system method.

Direct dispersion of oleogelator in the oil phase:

This approach is straightforward. The structuring agent is simply dispersed in the liquid oil under mild agitation while being heated at a temperature above the melting point of the material. Then, the whole mixture is cooled to room temperature to allow gelation. Food grade natural waxes and ethyl-cellulose have been extensively used to prepare oleogels via direct dispersion method (Vélez-Erazo et al., 2022).

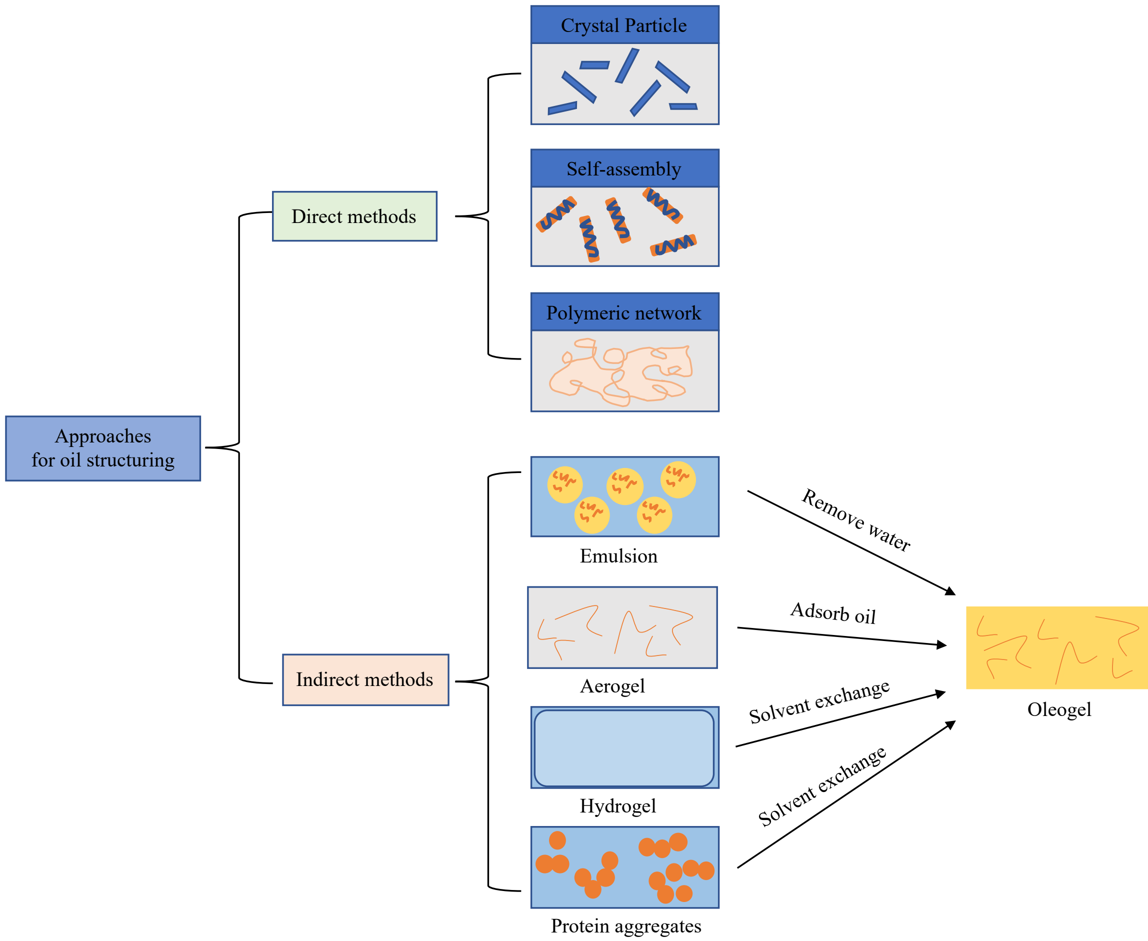

Figure 2-5. Diagram of oleogel fabrication approaches

adapted from (Feichtinger et al., 2022; Vélez-Erazo et al., 2022; Zhao et al., 2022)

Indirect templated system method:

Other than direct methods, novel oleogels can be indirectly fabricated from certain templated systems, including emulsions, hydrogels, aerogels and protein aggregates. In order to obtain oleogel, subsequent steps to remove water or solvent exchange need to be incorporated. With these facile indirect methods, polymers with limited solubility in oil, like proteins, can be used as oil texturants (Feichtinger & Scholten, 2020; Scholten, 2019). Different types of colloidal structures can be used.

Emulsion-templated:

In this emulsion-templated approach, an emulsion is first formed with the presence of proteins as the emulsification agent, followed by removal of water from the aqueous continuous phase. At this point, a semi-solid material with a high oil content is obtained which is called high internal phase emulsion (HIPE). HIPEs cannot be classified as oleogels as they are still an oil-in-water system. In order to obtain oleogel, mechanical shearing is applied to switch the continuous and dispersed phase by breaking down the protein network (Scholten, 2019). Tavernier and others (Tavernier et al., 2017) successfully prepared oleogels with soy protein: κ-carrageenan complexes through this emulsion-templated approach. This study also indicates that hydrophilic proteins have the potential to serve as structuring agents of oleogels. Methyl cellulose and hydroxypropyl and hydroxypropyl methylcellulose are feasible to form oleogel through emulsion template (Espert et al., 2020).

Aerogel templated:

Porous polymer structures can also be used to prepare oleogel by simply absorbing liquid oil into the dried network. Use of aerogel template has been reported. Chen and Zhang (Chen & Zhang, 2020) utilized an aerogel prepared using alginate-protein conjugates formed through Maillard reaction and a freeze-drying process to trap oil in the aerogel network. The oil uptake was up to 10.89 g oil per g aerogel and the oil holding capacity was 40%.

Hydrogel templated:

Hydrogel is another template that can be used for oleogel fabrication. However, the process to obtain oleogel is different from the previous two approaches. Solvent transfer steps are usually incorporated. De Vries and coworkers (De Vries et al., 2015) investigated the use of heat-induced whey protein isolate hydrogels as initial medium for oleogel formation. The internal aqueous phase within the polymer structure was fully replaced by sunflower oil through a stepwise solvent exchange procedure via an intermediate solvent such as acetone or tetrahydrofuran. This method did not cause noticeable interruption to the protein matrix in which oil was homogenously distributed.

In another approach, hydrogel may be converted to alcohol gel first and then undergo supercritical carbon dioxide drying processes to form an aerogel structure, followed by adding oil to finally form oleogel. Manzocco and others (Manzocco et al., 2017) successfully used this approach to make oleogel within a κ-carrageenan polymer network.

Protein aggregates:

Like hydrogel templated approaches, protein aggregates are also able to structure oil using solvent transfer via an intermediate solvent. An extra centrifugation step is needed to create necessary protein matrix to hold oil. Plant-based proteins were also found to be suitable to obtain complete plant-based protein oleogels solely through solvent exchange procedures (Feichtinger et al., 2022).

All examples above have proved that the fabrication of oleogels is not limited to the choice of existing oleogelators for direct dispersion method but from a number of food grade polymers including proteins and polysaccharides via a variety of templates.

2.4.1.3 Applications in food

Oleogel as a solid fat replacer has been widely researched in varieties of food applications including breakfast spreads, confectionary products (chocolate and chocolate pastes, pralines, fillings), pastry (Cookies and biscuits, cakes and baked goods, bread), meat products (sausages, meat patties), dairy products (cream cheese, ice cream), and margarines (Singh et al., 2017). Examples of edible oleogels in some food applications will be provided.

Solid fats such as milk cream play an important role in frozen desserts by providing body and mouthfeel. Using liquid vegetable oil to replace the saturated milk fat in ice cream is challenging. This problem can now possibly be overcome by using oleogel (Zulim Botega et al. 2013). The application of oleogel aims to facilitate the rate of fat destabilization of unsaturated fatty acid and in turn to favor fat partial coalescence and ice cream network structuring. Zulim Botega et al. (2013) reported that oleogel made from 90% high oleic sunflower oil and 10% rice bran wax was successfully incorporated into ice cream and the resulting ice cream possessed higher overrun compared to ice cream made from liquid oil. Oleogel droplets were observed behaving like crystallized fat droplets in ice cream and the level of fat destabilization in oleogel ice cream was similar to that of milkfat ice cream. However, meltdown was still a problem. Nevertheless, this idea of using oleogel to enhance the properties of high oleic oil is still a promising way to produce ice cream with desirable properties. In another study, oleogels were obtained through the process of microfluidization of whey and high oleic palm oil and were found to be a viable replacement for cream in the ice cream formulation (Silva-Avellaneda et al., 2021).

Similarly, baked foods also require a certain level of saturated fats to produce desirable textural properties such as tenderness, moistness and smooth mouthfeel. Baking fat should have appropriate technological properties including good plasticity, solid consistency and presence of solid phase (Demirkesen & Mert, 2020). However, high oleic oil lacks a solid phase. Thus, using high oleic oil to substitute the saturated fat part in baked products also needs assistance from structured oleogel. Onacik-Gür and Żbikowska (2020) found that using oleogel with high oleic rapeseed oil and 5% monoacylglycerols in biscuit short-dough resulted in similar properties to the control biscuits with palm oil. Moreover, cookies baked with high oleic soybean oil and sunflower wax-formed oleogel exhibited even lipid distribution which enabled good air-incorporation and air retention abilities (Zhao et al., 2020). Oleogel obtained via a pulse protein (pea and faba proteins) with xanthan gum-stabilized foam template showed good performance in a cake specific prototype. Cakes prepared using this oleogel as shorting alternative exhibited an equivalent volume as that of the control cake using shortening, although the texture was harder and chewier (Mohanan et al., 2020).

High levels of saturated fats naturally exist in meats. Thus, reduction of saturated fat content in meat products has gained growing interest in industry. Oleogels prepared using monoglycerides, natural waxes, phytosterols and ethylcellulose have been used in processed meat products such as sausages, frankfurters and meat patties (López-Pedrouso et al., 2021).

Surprisingly, oleogels were already tested in the deep-frying processing of instant noodles (Lim et al., 2017). An interesting area to investigate further is the use of oleogel mediums in different frying processes (Singh et al., 2017).

Due to the high levels of lipidic component, oleogels can be an effective delivery vehicle carrying lipid soluble compounds. Future studies of oleogels can move forward to the application of controlled or delayed release of nutraceuticals and pharmaceuticals as well as increasing bioavailability of lipid soluble molecules (Singh et al., 2017).

2.4.2 High internal phase emulsions (HIPEs)

HIPEs have already been mentioned in the previous part of oleogel. Like oleogel, HIPE possesses high oil volume fraction (ϕ), usually exceeding 0.74 and exhibits a solid gel-like texture. However, HIPE cannot be classified into oleogel. In HIPE system, oil is still the dispersed component not the continuous phase. Instead, HIPE can serve as a templated medium for the fabrication of oleogel in the process of indirect emulsion-based approach. HIPE need to undergo further drying or mechanical shearing for complete removal of water to form oleogel structure (Scholten, 2019).

The internal phase volume fractions of HIPEs usually exceed 0.74. Due to the high fractions, oil droplets are often present in polyhedral shapes and closely packed together in between thin films of continuous phase (Bai et al., 2021), shown as Figure 2-6.

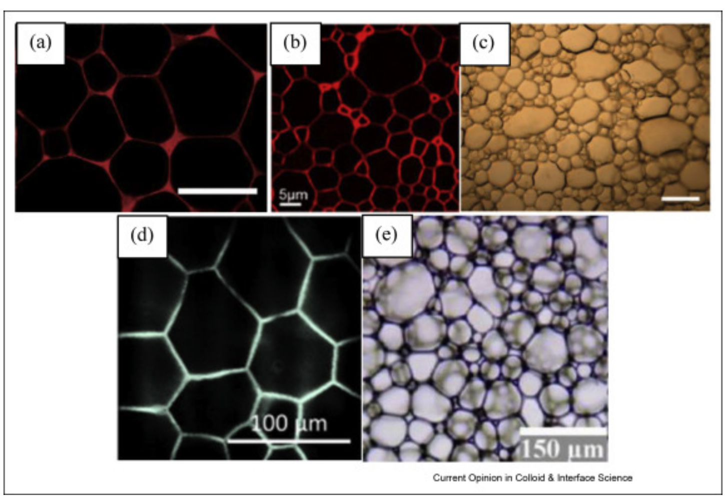

Figure 2-6. Microscopy images of HIPEs
(Rodriguez & Binks, 2021)

As a highly concentrated gelled emulsion system, HIPEs are another promising material providing desired textural properties that come from solid fats. With the ability to form gelled soft texture, HIPEs are suitable for highly viscous or semi-solid emulsified foods. Although HIPEs are still thermodynamically unstable, this sort of specialized emulsions with proper design exhibit good storage stability, being more resistant to breaking down and gravitational separation compared to conventional emulsions. The high internal phase volume fraction gives HIPEs good loading capacity to carry bioactive molecules. The viscoelastic and shear thinning properties of HIPEs allow them to be candidates as edible inks in 3D food printing technology. (Bai et al., 2021).

The typical fabrication method for HIPEs is homogenizing oil in a continuous phase in the presence of an appropriate emulsifying agent. The two liquids having different phases can be homogenized together or the dispersed phase can be gradually added into the continuous phase during shearing (Bai et al., 2021). Proteins, polysaccharides and their complexes or conjugates have been widely used to stabilize HIPEs (Bascuas et al., 2021). In some cases, drying of emulsions is necessary to obtain HIPEs. Here are some examples of HIPEs stabilized by different proteins and polysaccharides. Velez-Erazo and others (Velez-Erazo et al., 2020) successfully used pea protein as emulsifier and xanthan gum or tara gum as stabilizers to prepare HIPEs with ϕ=0.77 and 0.74, respectively. Meat proteins from pork could form HIPEs (ϕ=0.8) with excellent heat, freeze-thaw and storage stabilities in a pH range of 3-11 (R. Li et al., 2020).

Pickering HIPEs are relatively new. The Pickering effect strengthens the stability against coalescence, creaming and Ostwald ripening of HIPEs by providing a coating of packed particles around droplets (Bascuas et al., 2021; Zamani et al., 2018). Heated whey protein microgel Pickering colloids increased stability of the HIPEs (ϕ =0.75) in comparison to non-gelled whey protein isolate (Zamani et al., 2018). Uses of plant-based proteins have also been explored in research of Pickering HIPEs. Pickering HIPEs with ϕ =0.8 were stabilized by soy β-conglycinin (Xu et al., 2019). Complexation of soy protein isolate and chitosan enabled successful formation of Pickering HIPEs (ϕ =0.75) as a mayonnaise model system at low pH of 3 (Huang et al., 2022a). Huang et al. (2022b) also utilized heated soy protein isolate alone to prepare the mayonnaise-type HIPEs. Both of these two mayonnaise analogues HIPEs showed outstanding thermal and freeze-thaw stabilities.

Currently, most studies investigating HIPEs focused on the applications of fat replacer in dressings, mayonnaise, sauces, dips and spreads model systems (Bai et al., 2021). In this study, we extend the investigation on the use of HIPEs in frozen desserts.

2.5 References

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Chapter 3 EFFECT OF COLD-PRESSED HIGH OLEIC LOW LINOLENIC SOYBEAN OIL ON THE PHYSICAL PROPERTIES OF SOY-BASED ICE CREAM

3.1 Introduction

A diet high in oleic acid, a monounsaturated omega-9 fatty acid, has been linked to a reduced risk of coronary heart disease, obesity, and insulin resistance as well as enhanced brain function (Herrera et al., 2001; Perdomo et al., 2015; Tutunchi et al., 2020; Yodice, 1990). The proposed biochemical mechanisms for these effects are modification of cholesterol and lipoprotein concentrations, inhibition of blood coagulation, improvement of glucose homeostasis, anti-inflammation and reduction of oxidative stress (Lopez et al., 2021). The FDA (2018) has authorized a qualified health claim relating consumption of high oleic oils to a reduced risk of heart disease. Currently, various kinds of high oleic oils are commercially available, including olive oil, high oleic soybean oil, high oleic sunflower oil, high oleic canola oil, high oleic avocado oil, high oleic safflower oil, and high oleic corn oil. These oils can be used to replace *trans*-fat and saturated fat in food processing and food services (Liu & Iassonova, 2012). High oleic soybean oil, produced from trait-enhanced soybean varieties, is intended for gradual replacement of current soybean commodity oils in foods (Napolitano et al., 2018).

Recent development in soybean germplasm research has led to deployment of varieties with the high oleic low linolenic (HOLL) seed oil trait. For example, utilizing mutations in certain fatty acid desaturase genes in soybean can lead to the alteration of fatty acid profiles in soybean (Pham et al., 2010). Those efforts includes the non-transgenic high oleic trait which has been awarded the trademark name: SOYLEIC®. Traditional soybean oil contains 10% palmitic acid, 4% stearic acid, 18% oleic acid, 55% linoleic acid and 13% linolenic acid (Clemente & Cahoon, 2009). With > 75% oleic acid and less than 3% linolenic acid, HOLL soybean oil has emerged as a valuable oil with potential health benefits as well as functional performances. Due to increased consumer awareness of the impact of dietary lipids on health, the introduction of HOLL soybean oil is expected to replace current commodity oils, especially those with high content of saturated fatty acids (Napolitano et al., 2018).

Ice cream and frozen desserts are food products that typically contain high saturated fat. Fat sources in these products are cream for dairy-based ice cream and palm or coconut oil for plant-based ice cream (Munk et al., 2018). These fats can crystallize and partially coalesce during the ice cream making process, which contributes not only to structural retention and melting resistance of ice cream, but also to smooth sensory perception (Akbari et al., 2019; Méndez-Velasco & Goff, 2012; Rolon et al., 2017). With its high oleic acid and low linolenic acid contents, HOLL soybean oil has the potential to produce a nutritionally improved ice cream.

Oil can be extracted from a variety of oilseeds in several ways including mechanical cold pressing. This oil extraction method is currently used for premium oil due to its low yield compared to the conventional solvent extraction process (Cakaloglu et al., 2018). Cold pressing of oil can be achieved by mechanically grinding or milling, bladder pressing, low resistance expeller pressing, modified atmospheric crushing and modified atmospheric packing (Centra Foods, 2017). Mechanical cold pressing provides several advantages.

Throughout the process, no solvent such as hexane is used, making the process more environmentally friendly (Al Juhaimi et al., 2018). Without the high heat treatment and refining process, cold pressed oils are obtained without any alterations in functionality, color, and flavor while bioactive phytochemicals can be preserved (Chew, 2020). For example, cold pressed soybean oil contains γ- and δ-tocopherols that are known antioxidants. A great variety of constituents including neutral lipids, polar lipids, fibers, phytosterols, phenolic compounds and other bioactive compounds are present in cold pressed oil (Ramadan, 2020).

High oleic oil normally has a lower melting temperature of 13.4 ºC than saturated oil due to their fatty acid profile and the higher degree of unsaturation (Marín-Suárez et al., 2016). Since high oleic oil contains some fractions of other polyunsaturated fatty acids, the melting temperature may be lower. High oleic sunflower oil melts at -7 ºC (Sung & Goff, 2010), whereas coconut oil melts at 40 ºC and palm kernel oil melts at 31 ºC (Marín-Suárez et al., 2016; Sung & Goff, 2010). The utilization of high oleic oil in ice cream for the replacement of milkfat, coconut oil or palm oil has been limited as high oleic oil does not exhibit an appropriate temperature-dependent melting profile and desirable partial coalescence formation of fat. Milkfat, a saturated fat, is an important ingredient in ice cream formulation with its ability to not only increase richness, but also provide desirable texture and meltdown property. The crystallization of milkfat during ageing which contributes to partial coalescence is critical to structure formation of ice cream. An oil with a low melting temperature is not able to crystallize at the temperature of 4 ºC and does not enable the partial coalesced fat structure to form during the freezing/whipping process. Meanwhile, fat globules can spread at the air interface, which leads to thinning of the lamella between air cells, resulting in destabilization and collapse of the air phase. Therefore, ice cream made with high oleic oil has lower overrun and poor meltdown properties (Marín-Suárez et al., 2016; Sung & Goff, 2010), making the use of high oleic oil to replace the saturated milk fat in ice cream a challenge.

To date, the investigations on the application of HOLL soybean oil in ice cream formulations are still limited. Although previous studies utilized high oleic oil in ice cream, the quality of ice cream was unsatisfactory in terms of lower overrun when compared to that made with coconut oil (Marín-Suárez et al., 2016). The objective of this chapter was to determine the effect of SOYLEIC™ soybean oil on the physical properties of ice cream.

3.2 Materials & Methods

3.2.1 Ice cream preparation

3.2.1.1 Materials

Soymilk, sugar, heavy cream, vegetable oil and distilled water were obtained from a local supermarket. HO soybean oil was provided by the Missouri Soybean Merchandising Council (Jefferson City, MO). The SOYLEIC® soybean cultivar SA17-8882 was grown and harvested at the Bradford Research Farm, University of Missouri (Columbia, MO). SOYLEIC™ soybean oil was extracted by pressing cracked soybeans using a tubular radial expeller (Scott Tech Model ERT60II, Scott Tech Equipment, Vinhedo, Brazil) at the USDA-ARS-NCAUR laboratory in Peoria, IL. The backpressure applied during pressing was maintained by adjusting the gap at the discharge end by retracting the screw 2.5 turns from the closed position. The process was controlled such that the temperature of the oil was maintained below 53 ºC. Proximate analysis, fatty acid profile, and phospholipid content of the oils were determined at the University of Missouri Agricultural Experiment Station, using analytical methods approved by AOAC International.

3.2.1.2 Formulation

Four ice cream samples were prepared based on using four different fats including commercial vegetable oil (COM-VO), commercial HO soybean oil (COM-HO), cold-pressed SOYLEIC™ soybean oil (SOYLEIC) or heavy cream (CREAM). All ice cream formulations consisted of 5% protein, 10% fat and 15% sugar. No additional stabilizer or emulsifier was added.

3.2.1.3 Processing

Ingredients were blended in a Thermomix® TM5 (Vorwerk & Co. KG, Wuppertal, Germany), heated to 75 ºC and held for 20 s for pasteurization. The mix was homogenized using a two-stage homogenizer at 500/2000 psi, immediately followed by cooling at refrigeration temperature (4 ºC). The ice cream was produced by freezing the mix while whipping in a Taylor Batch Ice Cream Freezer (Model 121-27, Taylor Inc., Rockton, IL) for 15 min. The ice cream was hardened in a -40 ºC freezer. Samples were transferred into a -20 ºC freezer one day before instrumental measurements.

3.2.2 Instrumental measurements

3.2.2.1 Fat globule size

Fat globule size distribution of ice cream mixes and thawed ice creams was determined by the laser diffraction technique using a Mastersizer 3000 equipped with Hydro EV sample dispersion unit (Malvern Panalytical, Worcestershire, UK). Drops of mix were added into the Hydro EV circulating at 3000 rpm until obscuration values of 12-15% were obtained. The refractive indices of soybean oil (1.47) and water (1.33) were set for particle and dispersant, respectively (Marín-Suárez et al., 2016). Hardened samples were tempered at 4 ºC for 24 hours before measurement. Each analysis was conducted in triplicate.

3.2.2.2 Rheological measurements

The rheological properties of ice cream mixes and thawed ice creams were measured using a Rheometer (Kinexus Pro, NETZSCH, Selb, Germany). The ice cream mix was loaded on the lower plate and the upper plate geometry (50 mm) was gently lowered to a gap of 0.15 mm. Flow behavior was determined under a shear rate ramp from 0.01/s to 100/s at 25 ºC and under a solvent trap setting to prevent evaporation. Hardened samples were tempered at 4 ºC for 24 hours before measurement. Rheological data were modelled according to the Power Law model. Each analysis was conducted in triplicate.

3.2.2.3 Overrun

A 6 oz. styrofoam container was filled with either ice cream mix or ice cream and the weight was recorded. The overrun of each ice cream sample was determined based on the weights of ice cream mix and ice cream using Eq. 1 (Lin, 2012):

$$\%\ Overrun = \frac{weight\ of\ mix - weight\ of\ ice\ cream}{weight\ of\ ice\ cream} * 100\% \qquad (1)$$

Each analysis was conducted in triplicate.

3.2.2.4 Texture analysis

The textural properties of ice cream samples were measured using a Texture Analyzer (TA.HD*plus*C, Texture Technologies Corp., South Hamilton, MA) equipped with a 100 kg load cell and a 1/2" diameter stainless steel ball probe (TA-18). The ice cream samples were tempered to -20 ºC for 24 h before analysis. The probe was immersed in ice cold water before each measurement to minimize the variation caused by the influence of temperature on the measurement of textural properties. The penetration speed of the probe was 1.0 mm/s to 10 mm. The pre-penetration and post-penetration speeds were 1.0 mm/s. Hardness was measured as the peak compression force during penetration and adhesiveness as the peak negative force during retraction (Guinard et al., 1997). Each analysis was conducted in triplicate.

3.2.2.5 Melting rate

The screen drip-through test developed by Goff & Hartel (2013) was used to determine the melting rate. Ice cream sample (120 g) was cut from the container and placed over a metal wire screen (36/cm^2). The screen was placed on a ring stand over a beaker on a scale. Weight of the ice cream drained into the beaker at ambient temperature was recorded every 10 min for 2 h. The ratio of the weight of drained ice cream to the original weight was plotted versus time as the melting profile. The slope of the linear portion of the profile was calculated as the melting rate. Each analysis was conducted in triplicate.

3.2.3 Statistical analysis

The data obtained from instrumental measurements were analyzed using one-way analysis of variance (ANOVA) to determine the significant differences ($P < 0.05$) among the samples prepared with different fats. Means were compared using the Tukey Honest Significant Difference test ($P < 0.05$). RStudio (Version 3.6.1, RStudio Inc, Boston, MA, USA) was used for data analyses.

3.3 Results & Discussion

3.3.1 Characterization of ice cream mixes

3.3.1.1 Fat droplet size distribution

Droplet size distribution represents homogenization efficiency in ice cream mixes. All ice cream mixes showed bimodal droplet size distributions (Figure 3-1A). The major peak with small droplet sizes had the mean diameters of 0.56 µm for COM-VO, COM-HO and SOYLEIC and 0.49 µm for CREAM. The minor peak with larger sizes had the mean diameter of 55.3 µm for all samples. This distribution pattern was in accordance with the findings by Chen et al. (2019): that the fat globule size distributions of soy-based ice cream mixes were bimodal, with the first peak having the mean diameters of 0.1-10 µm and the second peak having the mean diameters of 10-100 µm. The bimodal oil distributions of ice cream mixes indicated the formation of small oil droplets after homogenization as well as a small population of aggregated oil droplets. Higher saturated fat content in cream or difference in fatty acid and phospholipid compositions among vegetable oils (Table 3-1) did not affect fat droplet sizes.

A new peak was present at 3.34 µm for COM-VO and CREAM ice cream samples, which indicates fat destabilization happened, leading to flocculation or formation of small fat globule clusters. More degree of fat destabilization was observed in COM-VO compared to CREAM.

Table 3-1. Composition of soybean oils

	COM-VO	COM-HO	SOYLEIC™
Oleic acid (%)	21.0	74.3	75.4
Linoleic acid (%)	53.0	7.99	7.09
Linolenic acid (%)	6.79	1.51	2.20
Stearic acid (%)	4.06	3.41	3.48
Palmitic acid (%)	10.6	7.21	7.46
Phospholipid (mg/100g)	26.9	92.5	42.1

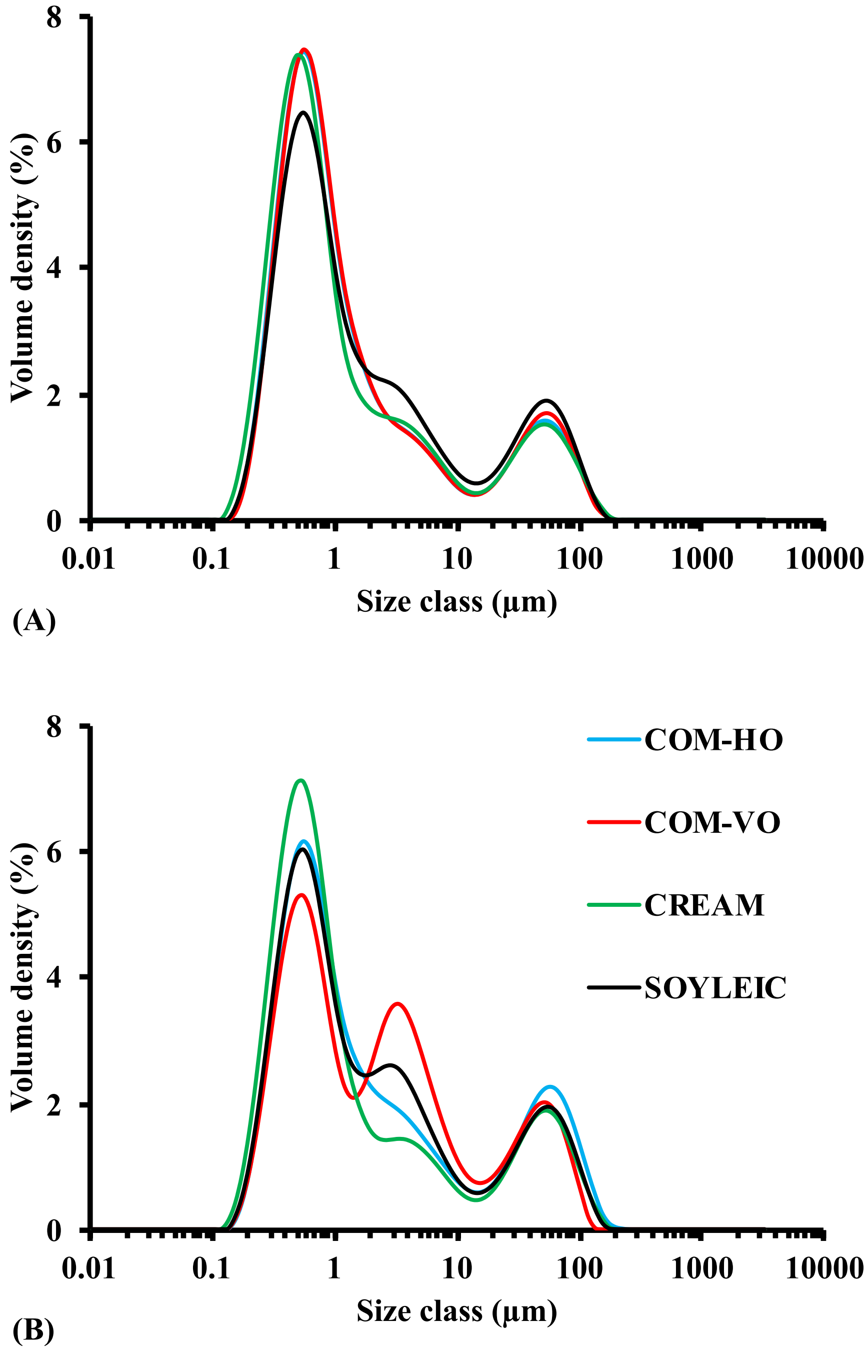

Figure 3-1. Particle size distributions of fresh ice cream mixes (A) and thawed ice cream (B)

3.3.1.2 Rheological properties

Rheological properties of ice cream mix could influence foamability and foam stability during whipping and freezing. Thus, in turn, foamability and foam stability both correlate with the overrun as well as the physical and sensory properties of ice cream (Goff & Hartel, 2013; Güven et al., 2018; Toker et al., 2013). The Power Law model has been commonly used to quantify the flow behavior of fluids. Consistency coefficient (K) provides a measure of the relative viscosity of a fluid and flow behavior index (n) shows the degree of shear-thinning behavior (Akalın et al., 2008). Viscosity flow curves for all ice cream mixes are shown in Figure 3-2. All ice cream mixes exhibited similar shear-thinning behavior between 0.01 to 100 s^{-1} shear rates with no significant difference in n or K values ($P > 0.05$). Im et al. (1994) found that the viscosity of dairy based ice cream mix decreased when milk fat was partially substituted with canola oil and soybean oil. The addition of an emulsifier and the ageing process led to crystallization of milkfat; thus, a mix with higher milkfat could have higher viscosity. In this study, no emulsifier was added, and the processing did not include ageing of the mix.

The consistency coefficient was significantly increased in COM-VO ice cream compared to its fresh ice cream emulsion. This increase could be correlated to more degree of fat destabilization found when comparing fat globule size distributions of fresh ice cream mixes and ice cream samples.

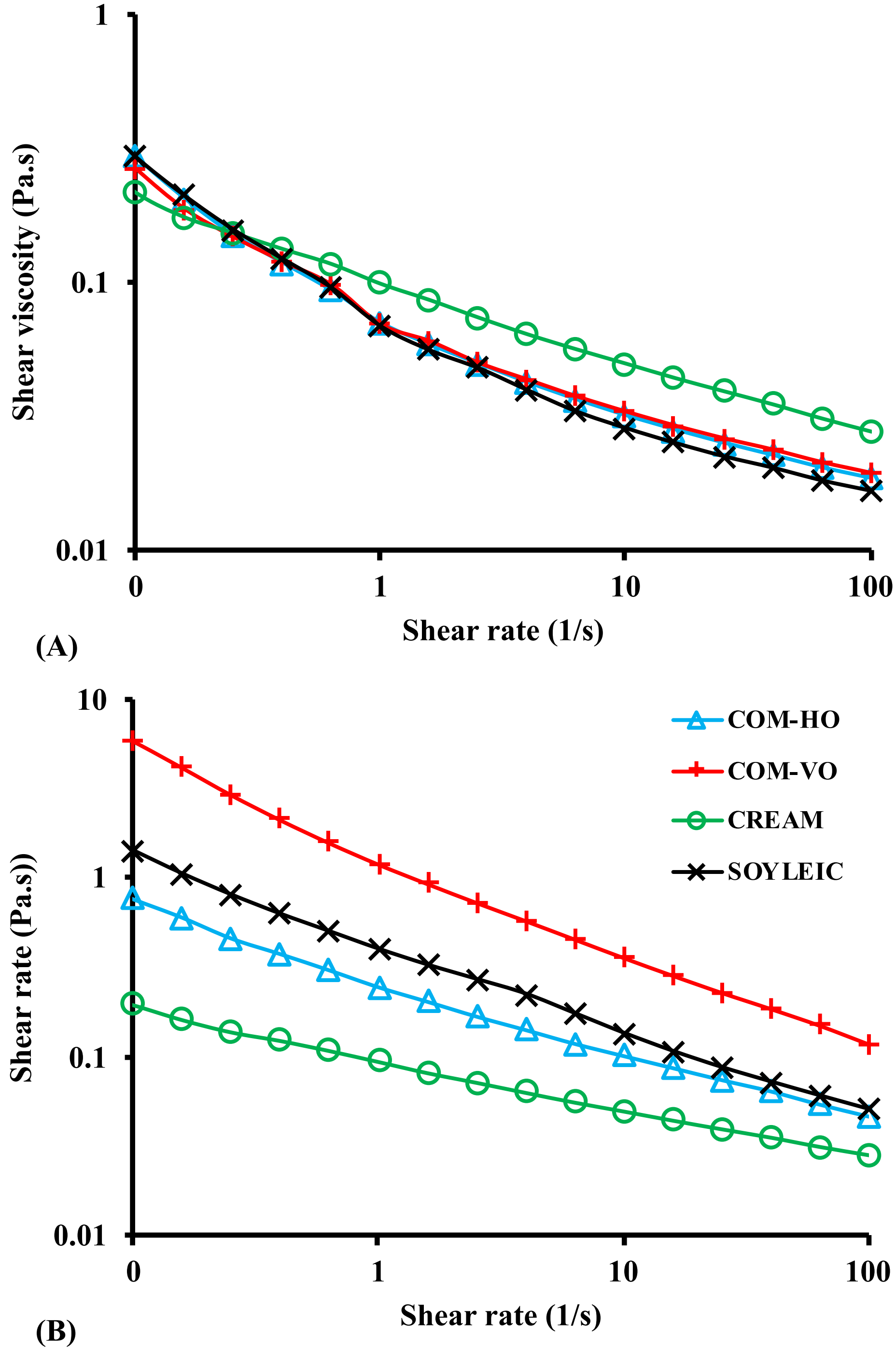

Figure 3-2. Flow behavior curves of fresh ice cream mixes (A) and thawed ice cream (B)

3.3.2 Characterization of ice cream

3.3.2.1 Overrun and texture analysis

Overrun measures the increase in the volume of ice cream caused by incorporating air during the freezing process. The amount and stability of air cells influence the physical and sensory properties of ice cream (Marshall, 1995). Overrun data is shown in Table 3-2. Ice cream made with CREAM had an overrun of 37 ± 1% when extruded from the batch freezer after 15 min of whipping. The low overrun is typical for ice cream processed by this type of ice cream freezer. The overruns of COM-HO (38 ± 5%) and SOYLEIC (39 ± 6%) were similar to CREAM ($P > 0.05$) and all were significantly higher ($P < 0.05$) than that of COM-VO (25 ± 1%). For vegetable oil, oil wetting or spreading could lead to low overrun and a weak structure of ice cream by thinning the lamella between bubbles and destabilizing the air phase (Marín-Suárez et al., 2016; Sung & Goff, 2010). Oil with higher unsaturated fatty acids was found to have lower foamability. COM-HO and SOYLEIC^{TM} oils contained higher oleic acid as well as lower linoleic and linolenic acids contents compared to COM-VO (Table 3-2). In addition, the higher phospholipid content in COM-HO and SOYLEIC^{TM} oils could contribute to higher foamability and foam stability of the mixes during whipping in the batch freezer which resulted in overrun comparable to that of CREAM and that was higher than COM-VO.

The texture of ice cream strongly depends on its composition (Skryplonek et al., 2019). Results of the texture analysis are shown in Table 3-2. With lower saturated fat content, COM-VO, COM-HO and SOYLEIC^{TM} oils have lower melting points than milk fat. In this study, there was no significant difference in hardness among samples formulated with different fats (*P > 0.05*) despite the lower overrun of COM-VO. The hardness of ice cream was reported to be linked to the rheological properties of the ice cream mix (Muse & Hartel, 2004). The rheological properties of mixes were found to be similar in this study, which could possibly explain the lack of difference in the hardness of ice cream. Adhesiveness represents the stickiness of ice cream, which could be correlated to the sensory characteristics of mouth coating and iciness (BahramParvar et al., 2013). Adhesiveness results indicated the ice cream made with SOYLEIC^{TM} soybean oil was less sticky than other samples.

3.3.2.2 Melting properties

Güven et al (2018) found that using vegetable oils, such as hazelnut and olive oils, increased the melting rate of ice cream. With higher unsaturated fat content, COM-VO, COM-HO and SOYLEIC should have lower melting temperature than CREAM; however, the melting rates among samples shown in Table 3-2 were not significantly influenced by the type of fat (*P > 0.05*). Interestingly, shape-retention behaviors during melting were different among samples. CREAM was able to maintain its original shape while ice cream made with vegetable oils collapsed and spread out over the mesh during the meltdown. Investigating the effect of blended fats on ice cream, Sung and Goff (2010) demonstrated the collapse of ice cream foam during melting as the solid fat content in blended fats decreased. It was proposed that coalescence was enhanced while the degree of partial coalescence was limited. As a result, the ice cream was not able to hold its shape during melting. In addition, melted ice crystals also ruptured the air cells which contributed to the structural collapse.

Table 3-2. Physical and textural properties of the ice cream samples made with different fat sources

Product	Overrun (%)*	Hardness (g)*	Adhesiveness (g)*	Melting rate (% melted per min after 40 min)*
COM-HO	38 ± 5[a]	45401 ± 4690[a]	-701 ± 55[b]	0.95 ± 0.20[a]
COM-VO	25 ± 1[b]	59364 ± 4588[a]	-623 ± 173[b]	0.89 ± 0.22[a]
CREAM	37 ± 1[a]	50610 ± 7656[a]	-868 ± 105[b]	1.14 ± 0.02[a]
SOYLEIC	39 ± 6[a]	47575 ± 4027[a]	-320 ± 60[a]	0.98 ± 0.22[a]

Note: *Data represents mean ± standard deviation. Different letters denote significant difference between samples within columns ($P < 0.05$).

3.4 Conclusion

SOYLEIC^{TM} soybean oil was extracted from HOLL soybean variety by cold press processing technique and successfully used to formulate plant-based ice cream. Fat globule distributions and rheological properties were characterized in ice cream mixes and thawed ice cream samples to determine any changes resulted from freezing/whipping process. Results showed fat sources did not change the physical properties of the ice cream mixes. A greater degree of fat destabilization during freezing/whipping process was found in the ice cream prepared with commercial vegetable oil.

Physical and textural properties of ice cream were evaluated. No significant differences in hardness were found among ice creams made with different fat sources despite the lower overrun of ice cream made with commercial vegetable oil. The ice cream prepared with SOYLEIC^{TM} soybean oil was less sticky. Although soybean oils should have lower melting temperatures than milk fat, melting rates were statistically similar among all ice creams. Different melting behaviors were observed between ice creams formulated using soybean oils and cream. Ice creams with soybean oils were susceptible to shape collapse.

Further sensory analysis is necessary to investigate the effect of SOYLEIC^{TM} soybean oil on sensory properties and consumer acceptance of ice cream.

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Chapter 4 SENSORY ANALYSIS OF SOY-BASED ICE CREAM FORMULATED WITH COLD-PRESSED HIGH OLEIC LOW LINOLENIC SOYBEAN OIL

4.1 Introduction

Plant-based ice creams were successfully formulated using cold pressed SOYLEIC^{TM} soybean oil containing high oleic acid but low linoleic acid. Results from the previous chapter have shown that differences in overrun, stickiness and melting behavior were found among soy-based ice creams made with either commercial vegetable oil (COM-VO), commercial HO soybean oil (COM-HO), cold-pressed SOYLEIC^{TM} soybean oil (SOYLEIC) or heavy cream (CREAM).

In addition to physical properties, the flavor aspect also plays an important in determining ice cream quality. Traditional soybean oil has low oxidative stability due to the relatively high percentage of the polyunsaturated fatty acids, linoleic acid and linolenic acid (Pham et al., 2012). The oxidative breakdown of these fatty acids produces beany and rancid odors in food products, whereas the oleic acid in HOLL soybean oil, is a monounsaturated fat that provides a relatively high resistance to oxidative degradation to HOLL soybean oil (Napolitano et al., 2018). From this aspect, using HOLL soybean oil may result in improved flavor acceptability of ice cream.

Sensory analysis is necessary for the perception evaluation of SOYLEIC in comparison with COM-VO, COM-HO and CREAM as satisfactory sensory results are an important criterion in new product development in the food industry. In this chapter, sensory evaluation including descriptive analysis and consumer acceptance testing, was conducted to investigate differences in appearance, textural and flavor characteristics of ice creams formulated with SOYLEIC™ soybean oil in comparison with other fats and the relationship between consumer preference and perception characteristics of these ice creams.

4.2 Materials & Methods

4.2.1 Preparation of ice cream for sensory analysis

Ice cream samples were prepared using the same formulation and processing as described in the previous chapter. Soymilk, sugar, heavy cream, vegetable oil and distilled water were obtained from the local supermarket. HO soybean oil was provided by the Missouri Soybean Merchandising Council (Jefferson City, MO). SOYLEIC™ soybean oil was extracted from the SOYLEIC® soybean cultivar SA17-8882 by cold pressing technique. All ice creams consisted of 5% protein, 10% fat and 15% sugar, formulated using either commercial vegetable oil (COM-VO), commercial HO soybean oil (COM-HO), cold-pressed SOYLEIC™ soybean oil (SOYLEIC) or heavy cream (CREAM). No additional stabilizer or emulsifier was added.

Ingredients were blended and pasteurized in a Thermomix® TM5 (Vorwerk & Co. KG, Wuppertal, Germany) at 75 ºC and held for 20 s. The hot mix was homogenized using a two-stage homogenizer at 500/2000 psi, immediately followed by cooling at refrigeration temperature (4 ºC). The ice cream was produced by freezing the mix while whipping in a Taylor Batch Ice Cream Freezer (Model 121-27, Taylor Inc., Rockton, IL) for 15 min.

After extruding from the ice cream freezer, ice creams were scooped into 2 oz. sugarcane bagasse portion cups, labeled with randomly generated 3-digit codes. Samples were stored in -40 ºC freezer until one day before being served.

4.2.2 Sensory evaluation

The study was reviewed and approved by the University of Missouri IRB (#2023044) and informed consent from each subject was obtained prior to participating in the study.

4.2.2.1 Descriptive sensory analysis

Descriptive sensory analysis (DA) was used to differentiate the perceived sensory characteristics of ice cream prepared with different fats. The twelve panelists were all students at the University of Missouri – Columbia. A list of pre-decided sensory attributes (Prindiville et al., 2000; Thompson et al., 2009; Warner, 1985; Yang et al., 2016) was provided by the panel leader in the lexicon development session. Panelists were provided with ice cream samples and asked to describe their differences. The sensory attributes were modified after the panelists' feedback. The descriptive terms included one appearance attribute: color; eight texture attributes: hardness, melting rate, iciness, creaminess, mouth coating, smoothness, gumminess and denseness; and eight flavor attributes: sweetness, raw soy, painty, malty, sweet aftertaste, raw soy aftertaste, painty aftertaste and malty aftertaste. Definitions and references for each attribute are listed in Tables 4-1 and 4-2. The panelists were trained in five training sessions using product and attribute references.

Upon completing the training sessions, panelists were asked to express the intensity of each attribute in the ice cream samples on a 15-cm unstructured line scale, anchored with low intensity = 0 and high intensity = 15. Four ice cream samples were randomly evaluated by twelve panelists in six sessions giving three replicate data sets. A 12x12 William Latin square design was used to balance the carry-over effect (Bower & Whitten, 2000). Ice cream samples were transferred from a -40 ºC freezer to a -20 ºC freezer one day before the actual tests. During the sensory testing, panelists followed this sequence: 1) observe the appearance (color) under white light and describe the color; 2) describe the scoopability as hardness; 3) put a spoonful of sample into the mouth and count the seconds to completely melt the sample; 4) describe the textural attributes; and 5) describe the flavor attributes. Panelists were asked to masticate a piece of unsalted cracker and then rinse their mouth with water before starting the test and in between samples to reset their palate and minimize carry-over effect. Panelists were also asked to put a vertical line in the appropriate place that best reflected their evaluation of the sample.

Table 4-1. Texture attributes for descriptive sensory panel

	Attributes	Definition	Reference
Appearance	Color	White to yellow color (canary yellow) under white light	Ref 3 = white; Ref 12 = yellow
Scoopability	Hardness	The resistance of ice cream to scooping	Ref 3 = store brand ice cream; Max – ice cream stored at -40 ºC (cannot be scooped)
Texture	Melting rate	The seconds to completely melt a spoonful of ice cream while rubbing if gently against the roof of the mouth with the tongue	
	Iciness	Perception of crystal-like particles in the sample	Ref 12 = iciness ice cream sample
	Creaminess	Degree of fat-like, full body liquids after melting in mouth	Ref 3 = 10% cream + 90 % whole milk; Ref 12 = 60% cream + 40% whole milk
	Mouth coating	Degree of fatty mouth or coated mouth after tasting	Ref 3 = 10% cream + 90 % whole milk; Ref 12 = 60% cream + 40% whole milk
	Smoothness	Smoothness/grittiness is perceived after compressing sample against the roof of the mouth	Ref 3 = Raymundo's homestyle classic caramel flan; Ref 12 = Jello vanilla pudding
	Gumminess	The perception of stickiness (like gum) between tongue and roof of the mouth when rubbing sample against the roof of the mouth	Ref 3 = Jello vanilla pudding; Ref 12 = store brand ice cream
	Denseness	The degree of compactness of the sample when pressed between the tongue and the roof of the mouth	Ref 3 = store brand ice cream; Ref 12 = refrozen store brand ice cream

Table 4-2. Flavor attributes for descriptive sensory panel

	Attributes	Definition	Reference
Flavor	Sweetness	The intensity of sweetness (sucrose-like)	Ref 3 = 5% sugar solution; Ref 12 = 15% sugar solution
	Raw soy (beany, grassy)	The intensity of flavor (beany and grassy) associated with raw soybeans	Ref 12 = soybeans soaked overnight then blended into a slurry of ratio 1:7 with distilled water
	Painty	The intensity of flavor (rancid and painty) associated with aged soybean oil	Ref 12 = soybean oil aged 8 d at 60 ºC Ref 7.5 = fresh soybean oil
	Malty	Sweet fermented aromatic associated with dried sprouted grains	Ref 12 = grape nuts cereal, 20 g in 500 mL water
Aftertaste	Sweetness aftertaste	The intensity of sweetness perceived after swallowing the sample	The same reference with sweetness.
	Raw soy (beany, grassy) aftertaste	Then intensity of flavor (beany and grassy) associated with raw soybeans perceived after swallowing the sample	The same reference with raw soy
	Painty aftertaste	The intensity of flavor (rancid and painty) associated with aged soybean oil perceived after swallowing the sample	The same reference with painty
	Malty aftertaste	Sweet fermented aromatic associated with dried sprouted grains perceived after swallowing the sample	The same reference with malty

4.2.2.2 Consumer acceptance testing

A 4x4 William Latin square design was used for the consumer test. A total of 82 volunteers who were students and staff members at the University of Missouri – Columbia participated in the consumer study. Ice creams were tempered at -20 ºC for 24 h and held at -20 ºC until served. Cups were labeled with random 3-digit numbers. Panelists were asked to masticate a piece of unsalted cracker and then rinse their mouth with water before starting the test and in between samples to reset their palate and minimize carry-over effects. Each participant tasted the four ice cream samples monadically in a randomized order and evaluated the degree of liking of the overall liking (DOL), flavor liking (DOF), texture liking (DOT) and appearance liking (DOA) on a 9-point hedonic scale where 1= dislike extremely to 9 = like extremely. At the end, panelists were asked to rank all four samples from the most favorite being number 1 to the least favorite being number 4. Participants were asked to fill out a questionnaire to collect their demographic information, shopping habits, frequency of consuming frozen desserts, types of frozen desserts consumed, factors influencing choice of frozen desserts and attitudes towards the healthfulness of plant-based foods and soy-based foods.

4.2.3 Statistical analysis

A descriptive analysis (DA) panel was conducted for six sessions (6 samples per session) giving three replicate data sets. There were very few (0.1%) missing values and missing values were replaced with grand means for that attribute. Mixed ANOVA models (lmerTest package) with the Tukey Honest Significant Difference test were applied to both descriptive data and hedonic data to determine significant differences ($P < 0.05$). Sources of variation include products, panelists, replications, and their two-way interactions in the ANOVAs of the descriptive data, and products and panelists in the ANOVAs of the hedonic data. Products were considered fixed effects, while the other parameters were considered random effects. Multivariate Analysis of Variance (MANOVA) with Wilk's Lambda (dplyr package) was used to test whether there were differences among products overall.

Principal component analysis (PCA) (FactoMineR and factoextra packages) was performed on the covariance matrix for descriptive data to determine relationships among attributes and differences among groups. Averaged data was used as the focus was on product differences. A Wilcoxon rank sum (Kruskal-Wallis) test was used to evaluate significant differences ($P < 0.05$) in the preference ranking of products. Partial Least Square (PLS) regression (pls package) was conducted to study the relationship between descriptive sensory attributes and consumer overall liking. Internal preference mapping was performed on the consumer hedonic data to analyze the preference of consumer for ice creams.

4.3 Results & Discussion

4.3.1 Mixed ANOVA and MANOVA for DA

Tables 4-3 and 4-4 present mean values of all sensory attributes across the four soy-based ice creams, and Figure 4-1 and 4-2 show visual spider plots. For the appearance, there was no significant difference in color among CREAM, COM-VO and COM-HO; however, SOYLEIC was significantly more yellow ($P < 0.05$). The ANOVA results show there were no significant differences in hardness, melting rate, iciness, smoothness, gumminess, or denseness among samples despite the difference in overrun. Although COM-VO had lower overrun, the panelists did not note the differences in denseness or hardness during consumption. Significant differences were found in creaminess with COM-HO and SOYLEIC showing lower creaminess than CREAM. SOYLEIC also had lower mouth coating than CREAM. No significant difference was found in creaminess and mouth coating between COM-VO and CREAM. This could possibly be due to the lower overrun and higher degree of fat destabilization of COM-VO, which contributed to the oil coating sensation. Studying the effect of vegetable oil on frozen desserts, Im et al. (Im et al., 1994) reported that replacing milkfat with vegetable oil did not affect the sensory properties of frozen dessert until at least two-thirds of the milkfat was replaced. The difference could be that our formulations did not contain emulsifier and no ageing process was included in the ice cream production. The change of fat compositions alone did not drastically affect the textural properties of the ice cream.

Ice cream made with SOYLEIC™ oil and CREAM had significantly lower painty and painty aftertaste intensities than COM-VO ($P < 0.05$). Painty and painty aftertaste intensities of COM-HO were higher than for CREAM and SOYLEIC, although they were not statistically significant. The use of CREAM resulted in the least raw soy, raw soy aftertaste, malty and malty aftertaste intensities. SOYLEIC had lower intensities of raw soy, raw soy aftertaste, malty and malty aftertaste than COM-VO and COM-HO, although they were not statistically significant. Sweetness and sweetness aftertaste were similar for all samples.

Multivariate analysis of variance (MANOVA) compares more than one dependent variable simultaneously, which can detect whether groups differ along a combination of attributes. MANOVA is commonly used when variables have somewhat correlation. Here, the result of MANOVA test (Wilk's lambda = 0.44528, df = 3, $P < 0.05$) has shown the four soy ice creams with different types of oil/fat are different.

Table 4-3. Mean values of texture attributes for ice creams for descriptive analysis

	Texture attributes*								
Product	Color	Hardness	Melting rate	Iciness	Creaminess	Mouth coating	Smoothness	Gumminess	Denseness
COM-HO	6.09^{b}	7.99^{a}	11.2^{a}	6.58^{a}	6.64^{b}	6.58^{b}	6.94^{a}	5.90^{a}	7.87^{a}
COM-VO	6.56^{b}	8.39^{a}	11.4^{a}	5.36^{a}	$7.67^{a,b}$	$7.27^{a,b}$	7.82^{a}	5.73^{a}	7.95^{a}
CREAM	6.54^{b}	8.47^{a}	10.9^{a}	5.10^{a}	8.49^{a}	8.08^{a}	8.08^{a}	5.89^{a}	8.22^{a}
SOYLEIC	8.50^{a}	8.42^{a}	11.5^{a}	5.54^{a}	6.78^{b}	6.68^{b}	7.36^{a}	5.67^{a}	7.98^{a}

Note: *Data represents mean. Different letters denote significant difference between samples within columns ($P < 0.05$).

Table 4-4. Mean values of flavor attributes for ice creams for descriptive analysis

	Flavor attributes*							
Product	Sweetness	Raw soy	Painty	Malty	Sweetness aftertaste	Raw soy aftertaste	Painty aftertaste	Malty aftertaste
COM-HO	6.97^{a}	7.56^{a}	4.78^{a,b}	5.81^{a}	6.01^{a}	6.50^{a}	3.89^{a,b}	5.08^{a}
COM-VO	6.79^{a}	6.87^{a}	5.91^{a}	5.90^{a}	5.93^{a}	6.42^{a}	5.38^{a}	5.63^{a}
CREAM	6.13^{a}	4.20^{b}	2.50^{b}	3.05^{b}	4.96^{a}	3.60^{b}	2.03^{b}	2.51^{b}
SOYLEIC	6.10^{a}	6.13^{a,b}	3.03^{b}	4.49^{a,b}	5.69^{a}	5.67^{a,b}	2.64^{b}	3.95^{a,b}

Note: *Data represents mean. Different letters denote significant difference between samples within columns ($P < 0.05$).

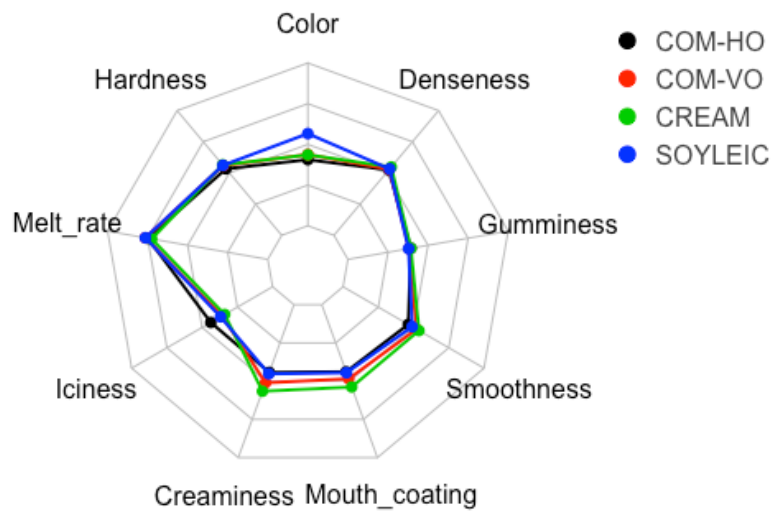

Figure 4-1. Spider plot of the mean sensory scores of texture attributes for ice cream samples

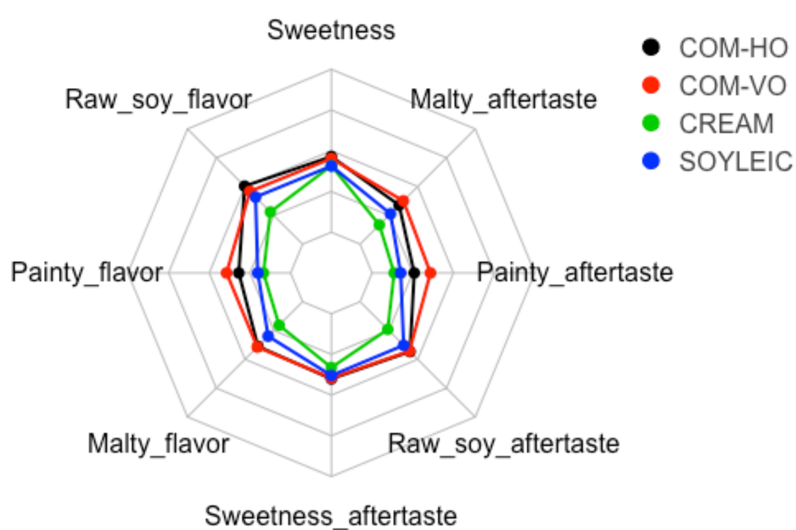

Figure 4-2. Spider plot of the mean sensory scores of flavor attributes for ice cream samples

4.3.2 Principal Component Analysis (PCA)

PCA is a multivariate technique that analyzes data containing inter-correlated quantitative dependent variables. It is used to provide new variables called principal components (PCs) that best explain the variation of the data and to display the pattern of similarity or difference of the products (Abdi & Williams, 2010; Bro & Smilde, 2014; Westad et al., 2003). Averaged data were used in PCA as our focus was on product differences. The first and second PCs explained 84.8% of the variance. The PC1, PC2 and PC3 account for 65.9%, 18.8% and 15.2% variation, respectively.

PCA (Figure 4-3) showed that the ice cream samples differed along a dimension (PC1), which described the contrast between all flavor attributes of sweetness, raw soy flavor, painty flavor, malty flavor, sweetness aftertaste, raw soy flavor aftertaste, painty aftertaste and malty aftertaste and two texture attributes of melting rate and iciness at one end; and the attributes of hardness, creaminess, mouth coating, smoothness and denseness at the other end. This suggests that all flavor attributes along with melting rate and iciness vary together while hardness, creaminess, mouth coating, smoothness and denseness vary oppositely. This component can be viewed as a measure of the quality of flavor related attributes. Furthermore, we see that the first PC correlates most strongly with raw soy flavor. In fact, we could state that based on the correlation of 0.9998, this PC is primarily a measure of the raw soy flavor. PC2 is driven by gumminess and color, in that it increases with increasing gumminess and decreasing color.

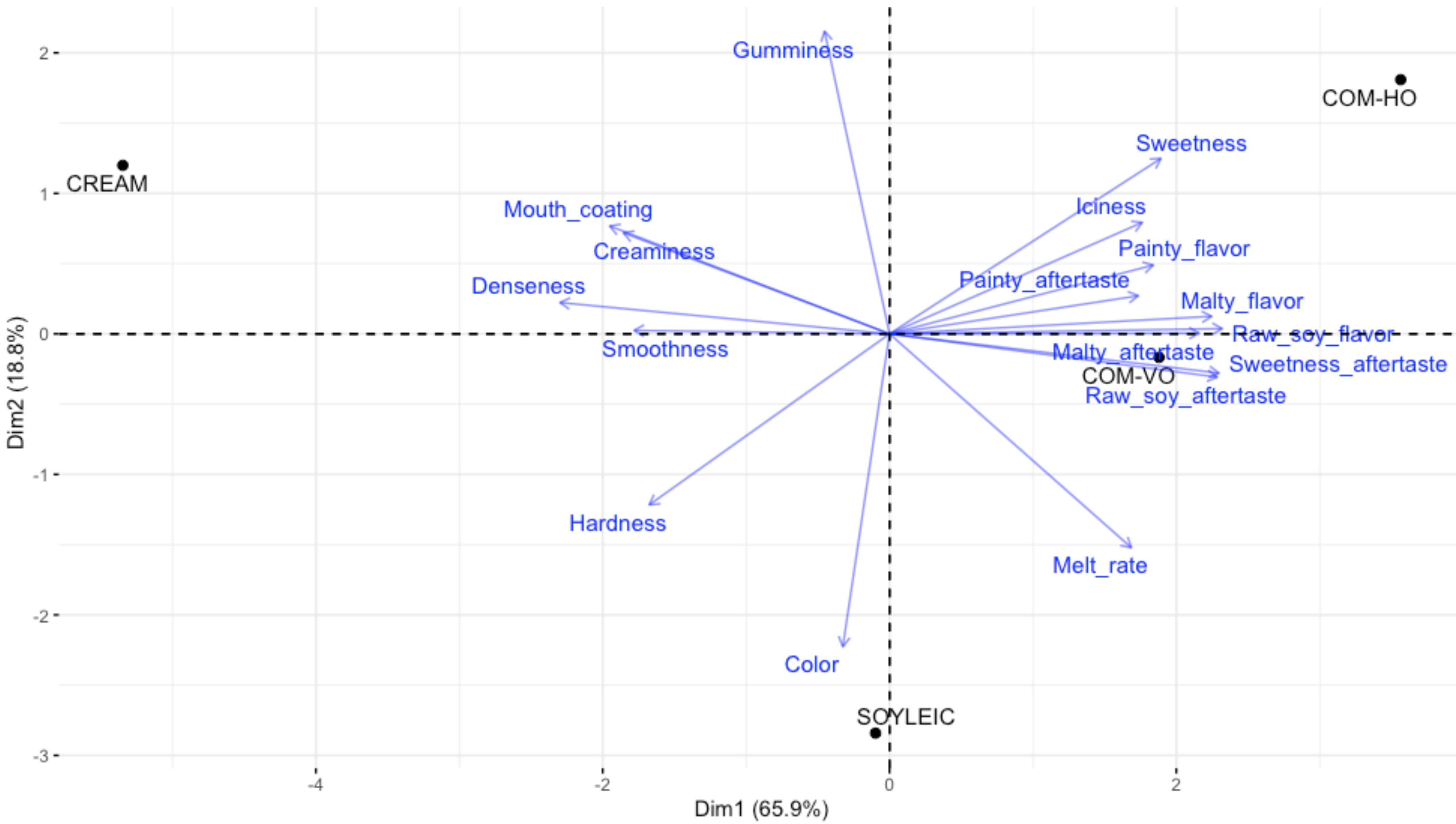

Figure 4-3. PCA biplot of the mean descriptive sensory data for four ice cream samples. Vectors in blue represented the descriptive sensory attributes and solid points represented the ice cream products.

4.3.3 Consumer acceptance test

Participants included 32 males and 50 females with the age ranging from 18 to 65 years old. More than half (51%) of our panelists consume frozen dessert 2-4 times a month while 21% consume it less than once a month and 28% consume it more than once a month. The top three types of frozen dessert that panelists would consume are premium ice cream and super-premium ice creams, as well as novelties, and frozen confections. Only 18% said they consume plant-based frozen dessert. Almost all panelists (99%) identified "flavor" as an important factor that influences their choice of a frozen dessert product, while 73% of the panelists would consider price and 54% of the panelists would consider texture of the frozen dessert product as important factors. Only 28% of the panelists would consider the healthfulness of the product when purchasing frozen desserts.

Consumer hedonic ratings are shown in Table 4-5. Consumer acceptance of the four soy-based ice creams ranged on average between "Like slightly" and "Like moderately." Significant differences were shown for DOL, DOF, DOT and DOA among products. Consumers rated ice creams made with SOYLEIC and CREAM significantly higher ($P < 0.05$) in DOL and DOF than COM-VO and COM-HO ice creams. This indicates that consumers preferred samples with SOYLEIC and CREAM in terms of flavor aspects. CREAM had a significantly higher appearance liking than the other three samples ($P < 0.05$). Texture liking was also significantly higher for SOYLEIC and CREAM ice creams compared to the COM-VO ($P < 0.05$) product; however, in the descriptive panel, the intensities of creaminess and mouth coating for SOYLEIC were statistically similar to COM-VO, but lower than CREAM.

CREAM was ranked as the most favorite sample followed by SOYLEIC. There was no significant difference in the preference between COM-VO and COM-HO ($P > 0.05$). Based on the Pearson coefficient results, DOL had the highest positive significant correlation (0.996, $P = 0.004$) with DOF, which indicates that flavor was the most important determinant in consumer acceptability. This result is consistent with the result from the survey answer that 99% panelists would rank "flavor" as the factor that influenced their choice of frozen dessert products.

Table 4-5. Hedonic ratings for ice creams by consumers

Product	Overall Liking (DOL)	Flavor Liking (DOF)	Appearance Liking (DOA)	Texture Liking (DOT)	Rank
COM-HO	5.4[b]	5.0[b]	6.7[b]	6.5[b,c]	3.2[a]
COM-VO	5.4[b]	5.1[b]	6.4[b]	6.1[c]	3.0[a]
CREAM	7.2[a]	7.0[a]	7.2[a]	7.1[a]	1.6[c]
SOYLEIC	6.6[a]	6.5[a]	6.7[b]	6.7[a,b]	2.2[b]

Note: *Data represents mean. Different letters denote the significance ($P < 0.05$) between ice cream products within columns.

4.3.4 Relationships among descriptive attributes and hedonic overall liking

To study the relationships between the independent descriptive attributes and the consumer liking, Partial Least Square (PLS) regression was conducted. From the PLS results (Figure 4-4), 92.2% and 7.7% of the consumer hedonic data were explained by component 1 and component 2, respectively. For descriptive data, component 1 explained 65.4% of variance and component 2 explained 17.4% of variance.

The correlation loading revealed that flavor attributes disliked by consumers were painty flavor, painty aftertaste, raw soy flavor, raw soy aftertaste, malty flavor, and malty aftertaste, while consumers liked attributes including denseness, smoothness, mouth coating, and creaminess. Interestingly, sweetness and sweetness aftertaste showed a negative correlation to DOL in this study. Sweetness is normally a desirable attribute for ice creams to a certain degree. However, within the four ice cream samples, sweetness and sweetness aftertaste varied together with all other flavor attributes that were negatively correlated to DOL. Furthermore, according to the PLS plot, SOYLEIC or CREAM were differentiated from the other two ice creams formulated with COM-VO and COM-HO. SOYLEIC and CREAM had a positive correlation while COM-VO and COM-HO were negatively correlated to overall liking. The COM-VO and COM-HO had high intensity in sweetness, sweetness aftertaste, painty flavor, painty aftertaste, raw soy flavor, raw soy aftertaste, malty flavor, and malty aftertaste compared to SOYLEIC and CREAM. Additionally, SOYLEIC showed more yellow color when compared with other samples. The correlation loadings of PLS showed all attributes except gumminess were significant attributes.

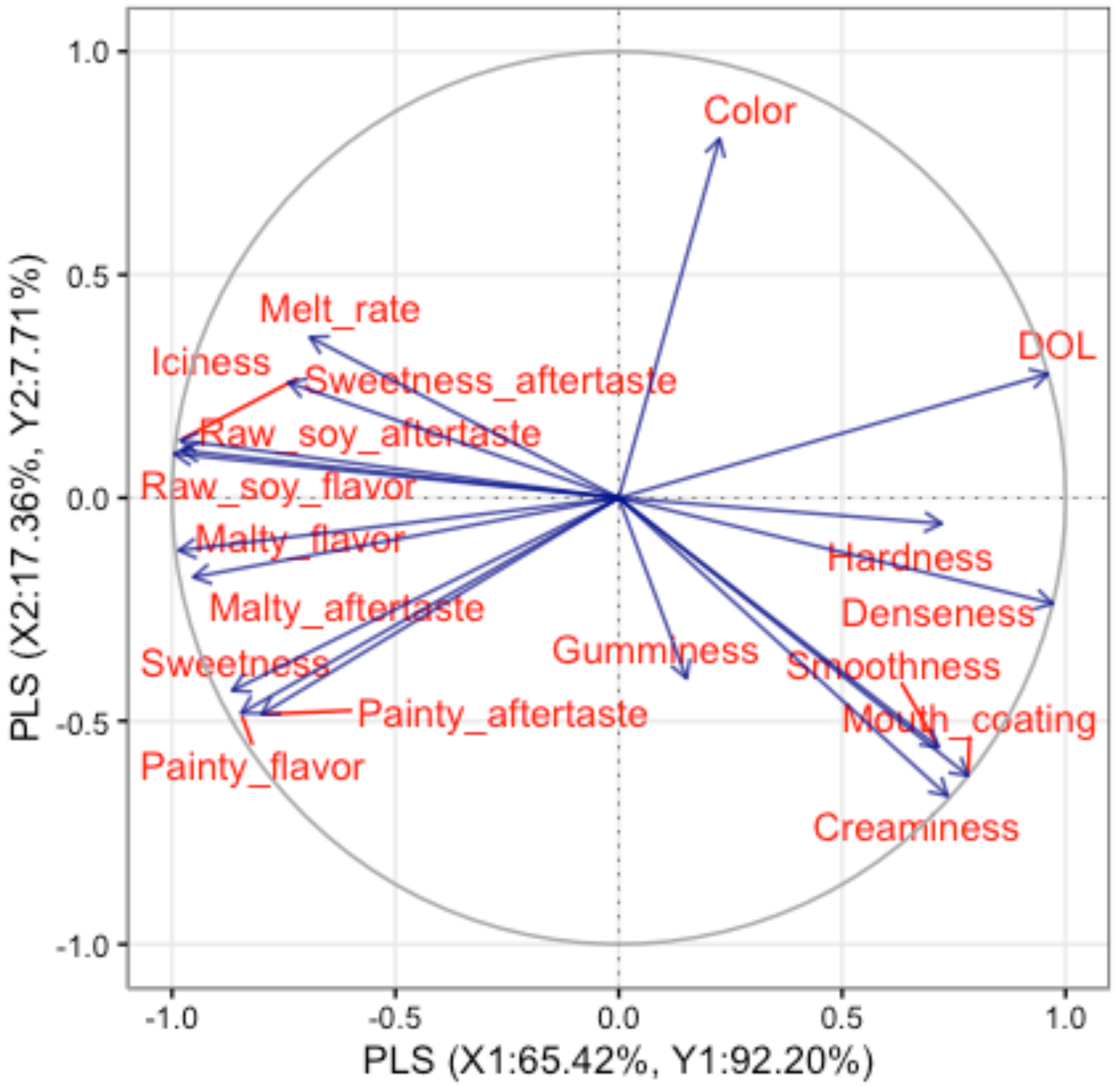

Figure 4-4. Correlation loadings of PLS regression of the integrated descriptive sensory and consumer hedonic data for the four ice cream samples

4.3.5 Preference Mapping

Preference mapping techniques have been used to provide the main directional preferences of consumers for a set of products based on each individual consumer included in the consumer acceptance sensory panel. Internal preference mapping is built from the PCA of the hedonic ratings by consumers (Guinard, 2002). Figure 4-5 shows the biplot of internal preference mapping, with solid points representing each consumer. According to the biplot, SOYLEIC and CREAM were more popular than COM-VO and COM-HO as more panelists were observed around SOYLEIC and CREAM products.

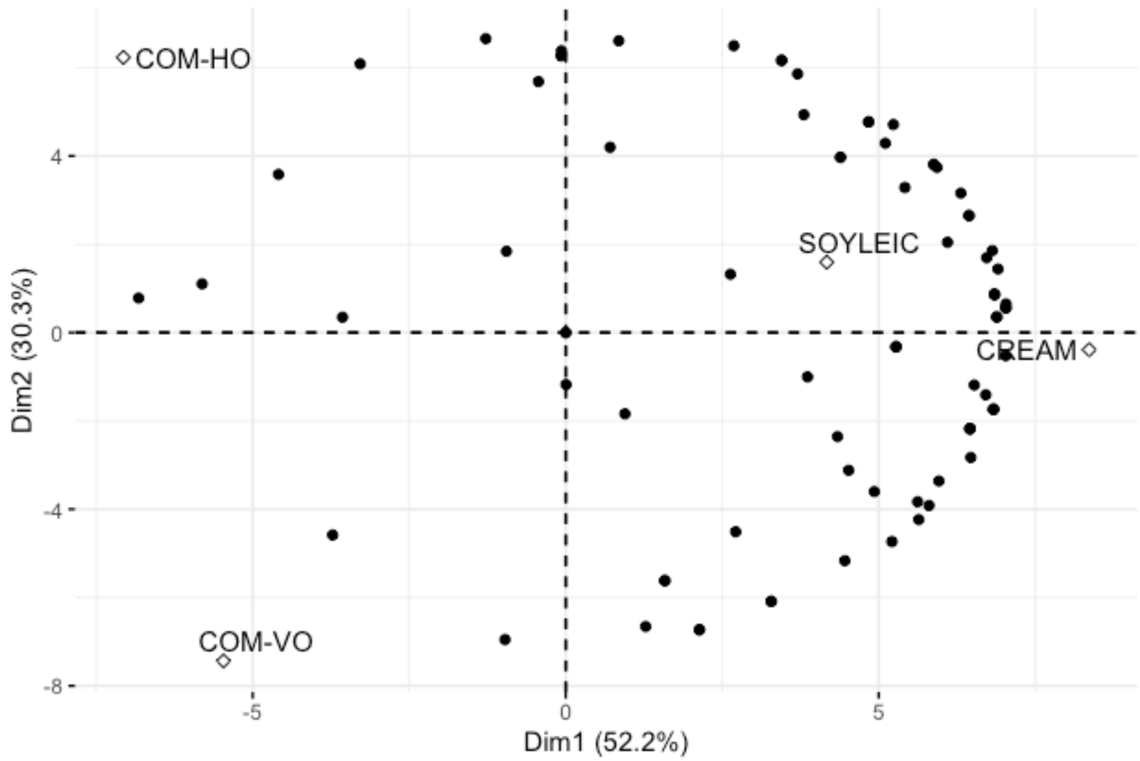

Figure 4-5. Internal preference mapping of the consumer overall degree of liking of ice creams. Solid points represented each individual consumer.

4.4 Conclusion

The results from descriptive sensory analysis showed that no significant differences were found in texture attributes except mouth coating which was higher for ice cream with cream than those with commercial HO soybean oil and SOYLEIC™ soybean oil. Ice cream made with SOYLEIC™ soybean oil had lower off-flavor of painty as well as painty aftertaste than that made with commercial vegetable oil.

The ratings provided by consumers showed that ice creams containing SOYLEIC™ soybean oil and dairy cream received higher preference compared to those made with commercial vegetable oil and commercial HO oil.

The overall liking of these soy-based ice creams was negatively correlated with flavor attributes of painty flavor, painty aftertaste, raw soy flavor, raw soy aftertaste, malty flavor, and malty aftertaste, but positively correlated with textural attributes including denseness, smoothness, mouth coating, and creaminess.

With better flavor and overall consumer liking, proprietary SOYLEIC™ soybean oil has potential to be used in formulating plant-based ice cream.

4.5 References

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Chapter 5 PRELIMINARY STUDY: EFFECTS OF SOY PROTEIN ISOLATE GEL PARTICLES STRUCTURED SOLID OILS ON FOAMING PROPERTIES OF MODEL ICE CREAM MIX

5.1 Introduction

Ice cream is an indulgent frozen dessert (Hartel et al., 2017), containing high fat levels. Traditional ice cream is made up of at least 10% milk fat. Milk fat is consisted of approximately 98% triglycerides (Lindmark Månsson, 2008), which have a wide melting range from 37 to –40 ºC (De Man, 1961). The combination of liquid and crystalline form of milk fat plays an important role in determining the texture and body of ice cream (Ohmes et al., 1998). During ice cream processing, milk fat is homogenized into milk fat globules of smaller diameters (< 2 μm) and evenly dispersed in the ice cream mix. Following homogenization, an aging step takes place, which allows the fat to cool down and crystallize as well as protein/emulsifier to rearrange. Then, the ice cream mix undergoes a dynamic freezing/whipping process, in which fat globules create a partially coalesced network surrounding the air bubbles, in turn, stabilize the foam structure of ice cream (Goff, 1997; Schmidt, 2004). Therefore, milk fat not only contributes to highly desirable attributes including creaminess and smoothness of ice cream but also good melting properties (Ohmes et al., 1998).

Currently, vegetable unsaturated liquid oils are used as fat components in ice cream formulation to substitute palm or coconut fats which are high in saturated fatty acids (Munk et al., 2018). However, the challenges for utilizing vegetable oils to formulate ice cream are from the lack of fat crystal at certain temperature, slow crystallization rate and lack of desired temperature-dependent melting profile (Nazaruddin et al., 2008). According to our previous work, the soy-based ice cream samples formulated with liquid vegetable oils were susceptible to shape collapse during melting test and showed less creaminess and mouth coating intensities than the ice cream made with heavy cream.

These technological limitations of vegetable oil could be overcome by texturing the liquid oil into solid form, such as oleogelation (Munk et al., 2018; Zulim Botega et al., 2013). The utilization of solid-like structured oil aims to mimic the behavior of milk fat by facilitating the creation of partially coalesced fat globule network that coats air cells and stabilizes the incorporated air bubbles in ice cream structure (Zulim Botega et al., 2013). Therefore, in order to stabilize the foam structure of ice cream, one approach is to transform liquid oil into solid-like texture which is hypothesized to play a similar role as crystalline milk fat in ice cream. Gel particles such as heated soy protein isolate (SPI) gel can be used to structure the liquid oil.

Another stabilization phenomenon that could possibly enhance foam stability by solid fat particles is the Pickering foam effect. Pickering foam is of great interest, in which solid particles form a close-packed particle barrier around air cells, serving as a mechanical barrier to inhibit bubble coalescence as well as providing the electrostatic force to keep the bubbles apart (Dickinson, 2010; Hunter et al., 2008). In addition, solid particles with appropriate sizes were found to have greater foam stability by structuring themselves and causing jamming within the foam lamellae and plateau borders to slow down the drainage (Amani et al., 2022; Chen et al., 2016; Denkov et al., 2020) as well as to increase the local viscosity to decrease the channel shrinking and film thinning (Denkov et al., 2020).

Hereby, in this preliminary study, structured oils with different oil fractions were fabricated using heated SPI gel and the effect of these solid-like structured oils on foamability and foam stability of stimulated ice cream mixes was evaluated. The objective of the preliminary experiment was to select the structured oil that could provide the best foam stability to formulate ice cream.

5.2 Materials & Methods

5.2.1 Materials

Soymilk, vegetable oil, sugar and distilled water were obtained from a local supermarket. SPI was purchased from Bob's Red Mill Natural Food (Milwaukie, OR).

5.2.2 Preparation of heated SPI gel structured oil with different oil fractions

5.2.2.1 Preparation of heated SPI gel

10% SPI dispersion was sonicated using a Sonics® VCX750 (Sonics & Materials Inc., Newtown, CT) at 80% amplitude for 15 minutes, stirred for 2 hours at room temperature, and heated in a water bath at 90 ºC for 30 min, followed by immediate cooling down in an ice bath. The resultant SPI gel was stored at refrigeration temperature overnight.

5.2.2.2 Preparation of structured oils containing different oil fractions

Heat-set SPI gel was first manually broken into small pieces using a spatula and then stirred on a magnetic stirring plate at 500 rpm. Oil was gradually added into the heat-set SPI gel while being sheared at 1,100 rpm to create structured oils with different oil fractions. The final oil fractions in structured oils were 50% (SO_50%), 67% (SO_67%) and 75% (SO_75%) for 1:1, 2:1 and 3:1 oil to SPI gel ratio, respectively.

5.2.3 Foaming properties of model ice cream mixes

Model ice cream mixes made with structured oil containing different oil fractions (SO_50%, SO_67% and SO_75%) or unstructured oil (CONTROL) were subjected to foaming test. The ice cream mixes were produced by homogenizing the blends of soy milk, sugar and fat using an IKA Disperser T 25 digital ULTRA-TURRAX (IKA Works, Inc., Wilmington, NC) at 12,000 rpm for 1 min. All simulated ice cream mixes were frothed in a volumetric cylinder using a milk frother (Aerolatte® Ltd., Hertfordshire, UK) for 2 min. Foam volume and drained liquid volume were recorded over a 30 min period. Overrun, foam stability and liquid drainage were calculated using Eq. 2 – Eq. 4 (Ingadottir & Kristinsson, 2003), respectively:

$$\%\,Overrun = \frac{V_0 - V_{L0}}{V_{L0}} * 100\% \qquad (2)$$

$$\%\,Foam\ stability = \frac{V_t}{V_0} * 100\% \qquad (3)$$

$$\% \, Liquid \, drainage = \left(1 - \frac{V_i - V_{Lt}}{V_i - V_{L0}}\right) * 100\% \qquad (4)$$

Where:

V_i: initial liquid volume

V_0: foam volume at 0 min

V_t: foam volume at t min

V_{L0}: volume of liquid at 0 min

V_{LT}: volume of liquid at t min

5.3 Results & Discussion

5.3.1 Formation of structured oils and effect of structured oil on foaming properties of model ice cream mix

Structure oils ranging from 50% to 75% oil fractions were fabricated successfully by gradually blending oil into heated SPI gel. The firmness of structured oil increased as the oil fraction increased.

Th foaming capacity was determined by the relative ratio of the volume of foam generated to initial liquid volume. The use of solid structure oil resulted in lower relative overruns from 200% for CONTROL to 150% for SO_75%, 140% for SO_67% and 140% for SO_50%. The presence of solid particles could possibly influence the rheological properties which in turn affect the foaming ability of the ice cream mix (Denkov et al., 2020).

There are three primary instability processes in foams, including drainage caused by gravity, bubble coalescence caused by thinning and breakage of foam lamellae and disproportionation caused by gas diffusion from smaller bubbles to larger bubbles (Wang et al., 2016). Figure 5-1 shows the foam stability of model ice cream mix in terms of percent foam stability and percent liquid drainage over a 30-min time period. Percent foam stability was described as the ratio of the volume of foam remained at time t compared to the initial foam volume while the percent liquid drainage was calculated as the ratio of the liquid drained from the foam at time t to the initial liquid drained before frothing (Ingadottir & Kristinsson, 2003). Results showed that gel-like structured oils could effectively slow down the liquid drainage of foam generated from model ice cream mix compared to unstructured oil. Furthermore, SO_75% showed higher percent foam stability than CONTROL while SO_67% and SO_50% had a very limited or no effect on the foam stability, which indicates structured oil with certain gel strength could inhibit the bubble coalescence in foam. The stabilization of foam could be contributed to the Pickering effect of solid fat particles as well as the physical accumulation of solid fat particles in the foam films. Changes in rheological properties of foam medium such as an increase in yield stress has also been reported as a stabilization effect in the solid particle-containing foams (Denkov et al., 2020).

Therefore, the heated SPI gel structured oil containing 75% oil content which resulted in a higher foam stability but a lower liquid drainage, was selected to formulate ice cream as a solid fat component for the next step.

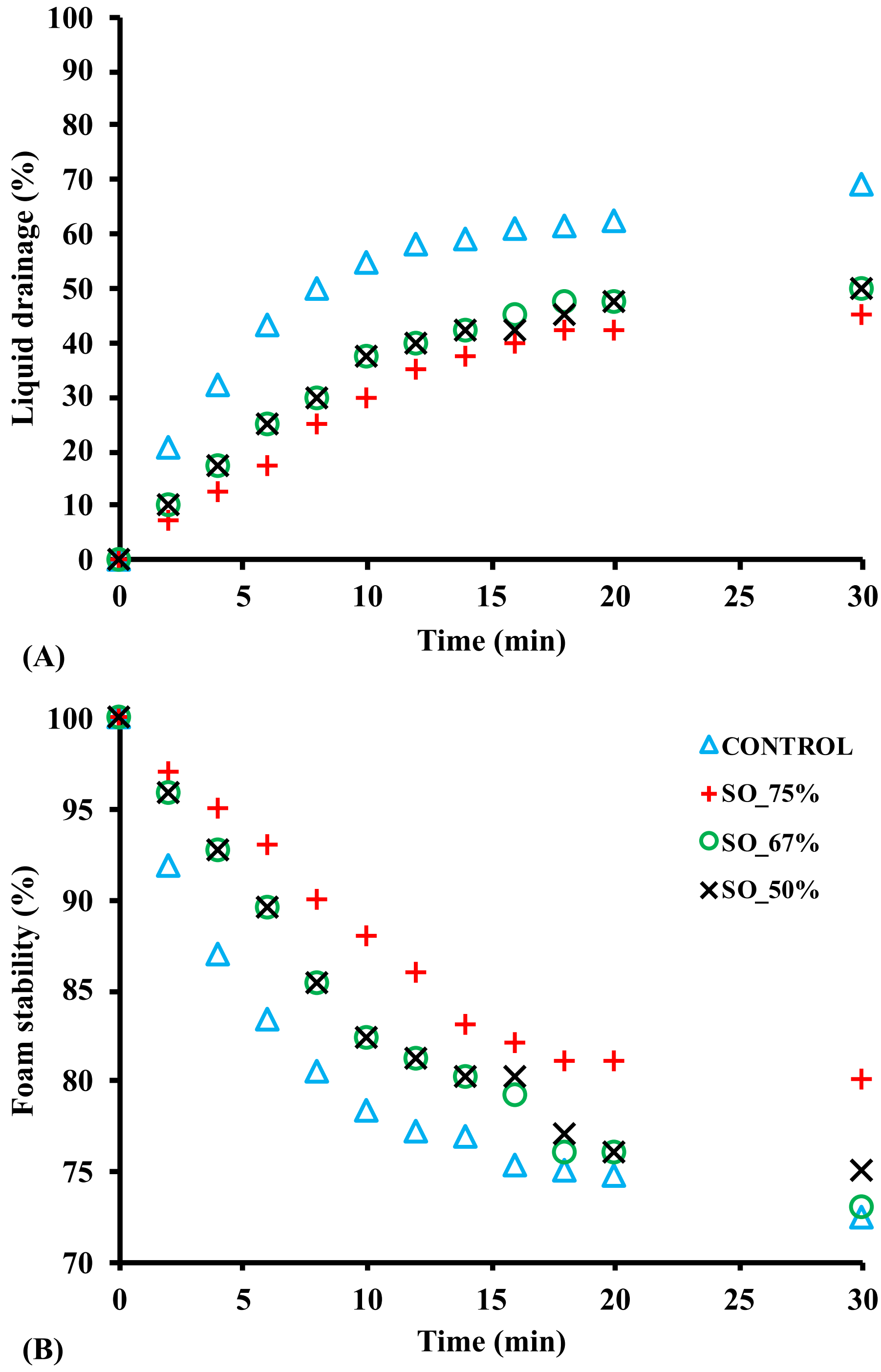

Figure 5-1. Percent liquid drainage (A) and percent foam stability (B) of model ice cream mixes containing heated SPI gel structured oil with different oil fractions

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Chapter 6 EFFECT OF HEAT-SET SOY PROTEIN ISOLATE GEL PARTICLES STRUCTURED HIGH INTERNAL PHASE EMULSION ON THE PHYSICAL AND SENSORY PROPERTIES OF SOY-BASED ICE CREAM

6.1 Introduction

Current growing attention to health conditions linked to consumption of *trans* and saturated fats (Dhaka et al., 2011; Elson & Alfin-Slater, 1992) has driven increasing demands for healthy alternatives to *trans* or saturated fats in foods. Vegetable oils consisting of high unsaturated fatty acid profile are believed to be healthier (Vieira et al., 2015). However, it can be challenging to use such vegetable oils in formulating foods without compromising desirable physical and sensory quality. Examples include ice cream and bakery goods which require functional properties from solid fats (Temkov & Mureșan, 2021; Vieira et al., 2015). Saturated fatty acids in milk fat play an important role in structural formation of ice cream. During freezing and whipping process, distinct milk fat globules destabilize to partially coalesce. The network formed by fat globule agglomerates is responsible for the shape retention of ice cream body and smooth perception property (Hartel et al., 2017; Muse & Hartel, 2004).

SOYLEIC™ soybean oil is an emerging high oleic low linolenic (HOLL) soybean oil, patented by the Missouri Soybean Merchandising Council (MSMC). From previous study, we found that soy milk-based ice cream formulated with SOYLEIC™ soybean oil received higher acceptance among consumer with significantly higher flavor liking, texture liking and overall liking scores than that with commercial vegetable oil. However, from the descriptive sensory panel, using oils containing high oleic fatty acid decreased mouth coating and creaminess for ice cream compared to using heavy cream.

The melting temperature of oleic acid is 13.4 ºC (Marín-Suárez et al., 2016). For high oleic oil still containing some fractions of other polyunsaturated fatty acids, the melting temperature may be lower. High oleic sunflower oil melts at –7 ºC (Sung & Goff, 2010) whereas coconut oil melts at 40 ºC and palm kernel oil melts at 31 ºC (Marín-Suárez et al., 2016; Sung & Goff, 2010). Crystallization of oil with high melting point favors oil movement and enhances coalescence, thus causing destabilization during aging of ice cream mix. Due to the relatively low melting temperature, high oleic oil is not able to crystalize at 4 ºC. Additionally, the slow crystallization rate of high oleic oil does not enable the partial coalesced fat structure to form during the freezing/whipping process of ice cream. Meanwhile, fat globules can spread at the air interface, which leads to the thinning of the lamella between air cells, resulting in destabilization and collapse of the air phase. Therefore, ice cream made with high oleic oil has lower overrun and poor meltdown properties (Marín-Suárez et al., 2016; Sung & Goff, 2010).

Using high oleic oil to replace the saturated milk fat, coconut oil or palm oil in ice cream is challenging as this sort of oil does not exhibit appropriate temperature-dependent melting profile and desirable partial coalescence formation of fat. This problem could be overcome by blending appropriate amount of solid-saturated fat with high oleic oil (Sung & Goff, 2010) or using oleogel (Zulim Botega et al., 2013a, 2013b). The creation of oleogel was aimed to texture liquid oil into solid form. This approach aims to facilitate the rate of fat destabilization of unsaturated fatty acid and in turn favor fat partial coalescence and ice cream network structuring. Zulim Botega et al. (2013b) reported that oleogel made from

90% high oleic sunflower oil and 10% rice bran wax was successfully incorporated into ice cream and the resulting ice cream possessed higher overrun compared to ice cream made from liquid oil. Oleogel droplets were observed to behave like crystallized fat droplets in ice cream and the level of fat destabilization in oleogel ice cream was similar to that of milkfat ice cream. However, the practical use of oleogel in food products is limited for a number of reasons. First, there are limited types of food grade oleogelator. Examples include beeswax, rice bran wax, candelilla wax, sunflower wax, hydroxypropyl methylcellulose, phytosterol and monoglycerides (Zhao et al., 2022). The concentration of oleogelator that is sufficient for structuring oil must meet food regulations. There is a knowledge gap about their interactions with other food components and their behaviors under different processing conditions. Additionally, cost and availability must be considered (Park & Maleky, 2020).

Like oleogel, high internal phase emulsions (HIPEs) also exhibit a solid like structure and contain high volume fraction of oil, which makes it another promising structured oil system for solid fat replacement. Food grade proteins and polysaccharides have been commonly used to form HIPEs (Bascuas et al., 2021). Current studies on HIPEs focus on its applications in dressings, mayonnaise, sauces, dips and spreads (Bai et al., 2021; Huang et al., 2022a, 2022b). The investigation of HIPEs as a milk fat replacement in ice cream is still lacking.

Ice cream is a frozen foam. There are three common foam destabilization mechanisms, coalescence, liquid drainage and disproportionation (Denkov et al., 2020). As the foam structure of ice cream is that air cells are dispersed in a solid continuous phase, disproportionation is usually not a concern for causing foam destabilization in ice cream

(Dickinson, 2010). Liquid drainage caused by gravity and air bubble coalescence caused by thinning and breakages of liquid films are the major destabilization phenomenon of foam here (Denkov et al., 2020; Wang et al., 2016). Pickering foam has gained increasing attention in various area including food technology, material science, oil recovery, cosmetic products and drug deliver (Ortiz et al., 2020). The basic mechanisms of Pickering stabilization have been established on the formation of compact-packed solid particle barrier at the gas-liquid interfaces (Bon, 2014; Dickinson, 2010). The steric force resulted from the solid particles layer at the interfaces of gas-liquid inhibits bubbles from coming in contact and coalescing (Du Sorbier et al., 2015; Wu & Ma, 2016).

In this study, we aimed to improve the physical and textural properties of soy-based ice cream by utilizing HIPE structured oil (ϕ=0.75). Our hypothesis was that gel-type HIPEs structured by protein gel particles could stabilize the foam structure in ice cream via Pickering effect. Preliminary experiments were conducted to determine the optimum formulation of HIPE by evaluating the effect of HIPE candidates on the foam ability and foam stability of model ice cream mixes. The effects of HIPE on the physical and textural properties as well as sensory properties of ice cream were determined.

6.2 Materials & Methods

6.2.1 Materials

Soymilk, sugar and distilled water were obtained from a local supermarket. SPI was purchased from Bob's Red Mill Natural Food (Milwaukie, OR). HO soybean oil was provided by the Missouri Soybean Merchandising Council (Jefferson City, MO).

6.2.2 Preparation of SPI particles

SPI was first dispersed in distilled water. The concentrations of SPI dispersions for heat-set SPI gel and heated SPI aggregates were 10% (w/w) and 9% (w/w), respectively. The 10% SPI dispersion was in paste form while 9% SPI dispersion was in liquid form. Thereby, sonication treatment was applied to the 10% SPI dispersion using a Sonics® VCX750 (Sonics & Materials Inc., Newtown, CT) at 80% amplitude for 15 min (Jambrak et al., 2009). Both SPI dispersions were magnetically stirred at room temperature for 2 hours to allow hydration and heated at 90 ºC for 30 min (Matsumiya & Murray, 2016) in a water bath. They were immediately cooled down in a cold ice bath and stored at refrigeration temperature (4 ºC).

6.2.3 Fabrication of heat-set SPI gel stabilized HIPE

Heat-set SPI gel was first manually broken into small pieces using a spatula and then gently stirred using a magnetic stirring plate at 500 rpm. Fabrication of SPI gel particles-stabilized

HIPE (ϕ=0.75) was achieved via a two-step method by gradually blending oil into heat-set SPI gel particles while being sheared at 1,100 rpm within 5 minutes. After mixing all oil, HIPE gel was additionally sheared for 5 minutes to obtain a homogeneous mixture.

6.2.4 Stability of HIPE

The stability against thermal treatment was tested according to the method described by Wang et al. (2022) with a minor modification. HIPEs were placed in glass scintillation vials sealed with caps and heated in a water bath at 90 ºC for 30 min and then subjected to cooling down in an ice bath. Heat stability was evaluated by visual observation of oil release.

6.2.5 Ice cream preparation

6.2.5.1 Ice cream formulation

Four ice cream samples were prepared with or without different SPI systems: control without any additional SPI (CONTROL), unmodified SPI powder (SPI), heated SPI aggregates (H-AGG) and heated SPI gel particles structured HIPE (HIPE). CONTROL contained 2.14% protein and others with SPI systems contained 2.43% protein. All ice cream formulations consisted of 10% fat and 15% sugar. No additional stabilizers or emulsifiers were added in this formulation. Detailed formulations are shown in Table 6-1.

6.2.5.2 Processing

Ingredients were blended in a Thermomix® TM5 (Vorwerk & Co. KG, Wuppertal, Germany), heated to 75 ºC and held for 20 s for pasteurization. The mix was homogenized using a two-stage homogenizer at 500/2000 psi, followed by cooling immediately at refrigeration temperature (4 ºC). The ice cream was produced by freezing the mix while whipping in a Taylor Batch Ice Cream Freezer (Model 121-27, Taylor Inc., Rockton, IL) for 15 min. The ice cream was hardened in a -40 ºC freezer. Samples were transferred into a -20 ºC freezer one day before sensory evaluation or instrumental measurements.

Table 6-1. Ice cream formulations

	CONTROL	SPI	H-AGG	HIPE
Total protein (%)	2.14	2.43	2.43	2.43
From soy milk (%)	2.14	2.14	2.14	2.14
From unmodified SPI (%)	0	0.29	0	0
From heated SPI aggregates (%)	0	0	0.29	0
From HIPE (%)	0	0	0	0.29
Total fat (%)	10	10	10	10
Total sugar (%)	15	15	5	15

6.2.6 Instrumental measurements

6.2.6.1 Fat globule size

Fat globule size distribution of ice cream mixes and melted ice creams was determined by the laser diffraction technique using a Mastersizer 3000 equipped with Hydro EV sample dispersion unit (Malvern Panalytical, Worcestershire, UK). Drops of mix were added into the Hydro EV circulating at 3000 rpm until obscuration values of 12-15% were obtained. The refractive indices of soybean oil (1.47) and water (1.33) were set for particle and dispersant, respectively (Marín-Suárez et al., 2016). Hardened samples were tempered at 4 ºC for 24 hours before measurement. Each analysis was conducted in triplicate.

6.2.6.2 Rheological measurements

The rheological properties of mixes and melted ice creams were measured using a Rheometer (Kinexus Pro, NETZSCH, Selb, Germany). The ice cream mix was loaded on the lower plate and the upper plate geometry (50 mm) was gently lowered to a gap of 0.15 mm. Flow behavior was determined under a shear rate ramp from 0.01/s to 100/s at 25 ºC and under a solvent trap setting to prevent evaporation. Hardened samples were tempered at 4 ºC for 24 hours before measurement. Rheological data were modelled according to the Power Law model. Each analysis was conducted in triplicate.

6.2.6.3 Overrun

A 6 oz. styrofoam container was filled with either ice cream mix or ice cream and the weight was recorded. The overrun of each ice cream sample was determined based on the weights of ice cream mix and ice cream using Eq. 1 (Lin, 2012):

$$\%\ Overrun = \frac{weight\ of\ mix - weight\ of ice\ cream}{weight\ of\ ice\ cream} * 100\% \qquad (1)$$

Each analysis was conducted in triplicate.

6.2.6.4 Texture analysis

The textural properties of ice cream samples were measured using a Texture Analyzer (TA.HD*plus*C, Texture Technologies Corp., South Hamilton, MA) equipped with a 100 kg load cell and a 1/2” diameter stainless steel ball probe (TA-18). The ice cream samples were tempered to -20 ºC for 24 h before analysis. The probe was immersed in ice cold water before each measurement to minimize the variation caused by the influence of temperature on the measurement of textural properties. The penetration speed of the probe was 1.0 mm/s to 10 mm. The pre-penetration and post-penetration speeds were 1.0 mm/s. Hardness was measured as the peak compression force during penetration and adhesiveness as the peak negative force during retraction (Guinard et al., 1997). Each analysis was conducted in triplicate.

6.2.6.5 Melting rate

The screen drip-through test developed by Goff & Hartel (2013) was used to determine the melting rate. Ice cream sample (about 120 g) was cut from the container and placed over a metal wire screen (36/cm^2). The screen was placed on a ring stand over a beaker on a scale. Weight of the ice cream drained into the beaker at ambient temperature was recorded every 10 min for 2 h. The ratio of the weight of drained ice cream to the original weight was plotted versus time as the melting profile. The slope of the linear portion of the profile was calculated as the melting rate. Each analysis was conducted in triplicate.

6.2.7 Sensory evaluation

The study was reviewed and approved by the University of Missouri IRB (#2023044) and informed consent from each subject was obtained prior to participating in the study.

Descriptive sensory analysis (DA) was used to differentiate the perceived sensory characteristics of ice cream prepared with different fats. The eleven panelists were all students at the University of Missouri – Columbia. A list of sensory attributes (Prindiville et al., 2000; Thompson et al., 2009; Warner, 1985; Yang et al., 2016) was provided by the panel leader. The sensory descriptive terms included eight texture attributes: hardness, melting rate, iciness, creaminess, mouth coating, smoothness, gumminess and denseness; and four flavor attributes: sweetness, raw soy, sweet aftertaste and raw soy aftertaste. Definitions and references for each attribute are listed in Tables 6-2 and 6-3. The panelists were trained in three training sessions in three consecutive days using product and attribute references.

Upon completing the training sessions, panelists were asked to express the intensity of each attribute in the ice cream samples on a 15-cm unstructured line scale, anchored with low intensity = 0 and high intensity = 15. Four ice cream samples were randomly evaluated by eleven panelists in six sessions giving three replicate data sets. A 12x12 William Latin square design was used to balance the carry-over effect (Bower & Whitten, 2000). Ice cream samples were transferred from a -40 ºC freezer to a -20 ºC freezer one day before the actual tests. During the sensory testing, panelists followed this sequence: 1) describe the scoopability as hardness; 2) put a spoonful of sample into the mouth and count the seconds to completely melt the sample; 3) describe the textural attributes; and 4) describe the flavor attributes. Panelists were asked to masticate a piece of unsalted cracker and then rinse their mouth with water before starting the test and in between samples to reset their palate and minimize carry-over effect. Panelists were also asked to put a vertical line in the appropriate place that best reflected their evaluation of the sample.

Table 6-2. Texture attributes for descriptive sensory panel

	Attributes	Definition	Reference
Scoopability	Hardness	The resistance of ice cream to scooping	Ref 3 = store brand ice cream; Max – ice cream stored at -40 ºC (cannot be scooped)
Texture	Melting rate	The seconds to completely melt a spoonful of ice cream while rubbing if gently against the roof of the mouth with the tongue	
	Iciness	Perception of crystal-like particles in the sample	Ref 12 = iciness ice cream sample
	Creaminess	Degree of fat-like, full body liquids after melting in mouth	Ref 3 = 10% cream + 90 % whole milk; Ref 12 = 60% cream + 40% whole milk
	Mouth coating	Degree of fatty mouth or coated mouth after tasting	Ref 3 = 10% cream + 90 % whole milk; Ref 12 = 60% cream + 40% whole milk
	Smoothness	Smoothness/grittiness is perceived after compressing sample against the roof of the mouth	Ref 3 = Raymundo's homestyle classic caramel flan; Ref 12 = Jello vanilla pudding
	Gumminess	The perception of stickiness (like gum) between tongue and roof of the mouth when rubbing sample against the roof of the mouth	Ref 3 = Jello vanilla pudding; Ref 12 = store brand ice cream
	Denseness	The degree of compactness of the sample when pressed between the tongue and the roof of the mouth	Ref 3 = store brand ice cream; Ref 12 = refrozen store brand ice cream

Table 6-3. Flavor attributes for descriptive sensory panel

	Attributes	Definition	Reference
Flavor	Sweetness	The intensity of sweetness (sucrose-like)	Ref 3 = 5% sugar solution; Ref 12 = 15% sugar solution
	Raw soy (beany, grassy)	The intensity of flavor (beany and grassy) associated with raw soybeans	Ref 12 = commercial soy milk
Aftertaste	Sweetness aftertaste	The intensity of sweetness perceived after swallowing the sample	The same reference with sweetness
	Raw soy (beany, grassy) aftertaste	Then intensity of flavor (beany and grassy) associated with raw soybeans perceived after swallowing the sample	The same reference with raw soy

6.2.8 Statistical analysis

6.2.8.1 Instrumental data

The data obtained from instrumental measurements were analyzed using one-way analysis of variance (ANOVA) to determine the significant differences ($P < 0.05$) among the samples prepared with different SPI systems. Means were compared using the Tukey Honest Significant Difference test ($P < 0.05$). RStudio (Version 3.6.1, RStudio Inc, Boston, MA, USA) was used for data analyses.

6.2.8.2 Sensory data

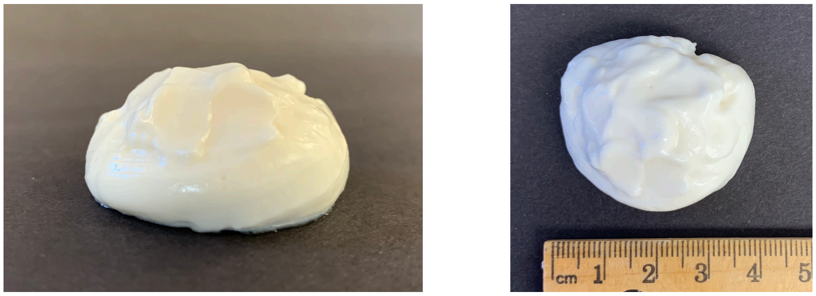
A descriptive analysis (DA) panel was conducted for six sessions (6 samples per session) giving three replicate data sets. Data from one panelist was excluded due to a relatively higher likelihood of missing evaluation. Descriptive data collected from other ten panelists had 0.1% missing values and missing values were replaced with variable grand mean. Mixed ANOVA models (lmerTest package) with the Tukey Honest Significant Difference test were applied to descriptive data to determine significant differences ($P < 0.05$). Sources of variation include products, panelists, replications, and their two-way interactions in the ANOVAs of the descriptive data. Products were considered fixed effects, while the other parameters were considered random effects. Principal component analysis (PCA) (FactoMineR and factoextra packages) was performed on the covariance matrix for descriptive data to determine relationships among attributes and differences among groups. Averaged data was used as the focus was on product differences (Næs et al., 2021).

6.3 Results & Discussion

6.3.1 Formation and characterization of HIPEs stabilized by SPI gel particles

Recently, successful formations of HIPEs have been reported by Huang and other researchers (Huang et al., 2022a, 2022b) using SPI-chitosan complexes or heated SPI particles at acidic pH of 3.0. In our study, we demonstrated the use of heated SPI gel particles at neutral pH to stabilize HIPE with a 0.75 internal fraction. The resultant HIPE shown in Figure 6-1 exhibited a form of self-supporting gels. The mechanisms for elastic gel network formation of HIPEs were believed to be the high density of inter-droplet hydrophobic interactions between adjacent droplets and high droplet Laplace pressure in the gel (Huang et al., 2022a, 2022b).

The thermal stability of the HIPE was evaluated, which could provide important information about the stability of the HIPE upon pasteurization heating condition. HIPE were stable against heating (at 90 ºC for 30 min). Xu et al. (2019) mentioned that heating could cause structural rearrangement of adsorbed proteins as well as increase the lateral interactions between adsorbed proteins, increasing the elasticity of HIPEs. Such thermal stability of HIPEs were also observed in the HIPEs. Such heat stability has also been seen in HIPEs stabilized by ovalbumin (Xu et al., 2018) and meat protein (Li et al., 2020). Additionally, no noticeable change in the appearance of HIPEs or oil leakage were visually observed upon heating.



(A) **(B)**

Figure 6-1. Appearance of heated SPI gel particles stabilized HIPE (f=0.75) with front view (A) and top view (B)

6.3.2 Fat droplet size distribution and fat destabilization

All four ice cream mixes exhibited similar bimodal size distributions as shown in Figure 6-2. The average droplet diameters were 0.56 µm for CONTROL and H-AGG, 0.63 µm for SPI and 0.49 µm for HIPE. The mean diameter sizes of the minor peak with larger droplets were similar among CONTROL, H-AGG and HIPE at 48.3 µm, 55.4 µm and 48.72 µm, respectively, and 29.3 µm for SPI. These results indicate that the semi-solid HIPE could efficiently emulsify and stabilize the fat droplets similar to the use of unstructured oil via typical two-stage homogenization process and form small fat globules between 0.1-10 µm along with a small portion of aggregated fat globules between 10-100 µm. The size distribution results were aligned with the bimodal fat droplet size distributions of soy-based ice cream found by Chen et al. (Chen et al., 2019). The emulsifying properties of SPI led to stable emulsions against oil flocculation and coalescence by the formation of a coating at the oil/water interface. Unmodified SPI particles should have smaller sizes than heated SPI aggregates. Therefore, aggregated oil droplets of smaller sizes were the results in the ice cream mixes of SPI. In addition, by comparing the percent area, the second peak accounted for a greater portion in the size distribution for H-AGG (6.1%) than for SPI (3.2%). Therefore, the second peak of H-AGG might be partially contributed from the particles of heated SPI aggregates.

In comparison with ice cream mixes, the fat globule sizes were not significantly changed in frozen ice cream. The presence of gellan gum in commercial soy milk used in ice cream formulation may provide viscosity of ice cream mixes, thereby aiding the formation of stable emulsions and decrease the degree of fat destabilization during freezing. Our ice cream formulation did not include any emulsifiers, which might limit the occurrence of fat destabilization.

Currently, as there is no publication about HIPE in ice cream available, studies on the application of oleogels in ice cream were used as parallel comparisons. Munk et al. (Munk et al., 2018) reported that ethylcellulose structured high oleic sunflower oil gel inhibited coalescence of oil droplets. The firm gel property of oleogels was attributable to the resistance against coalescence.

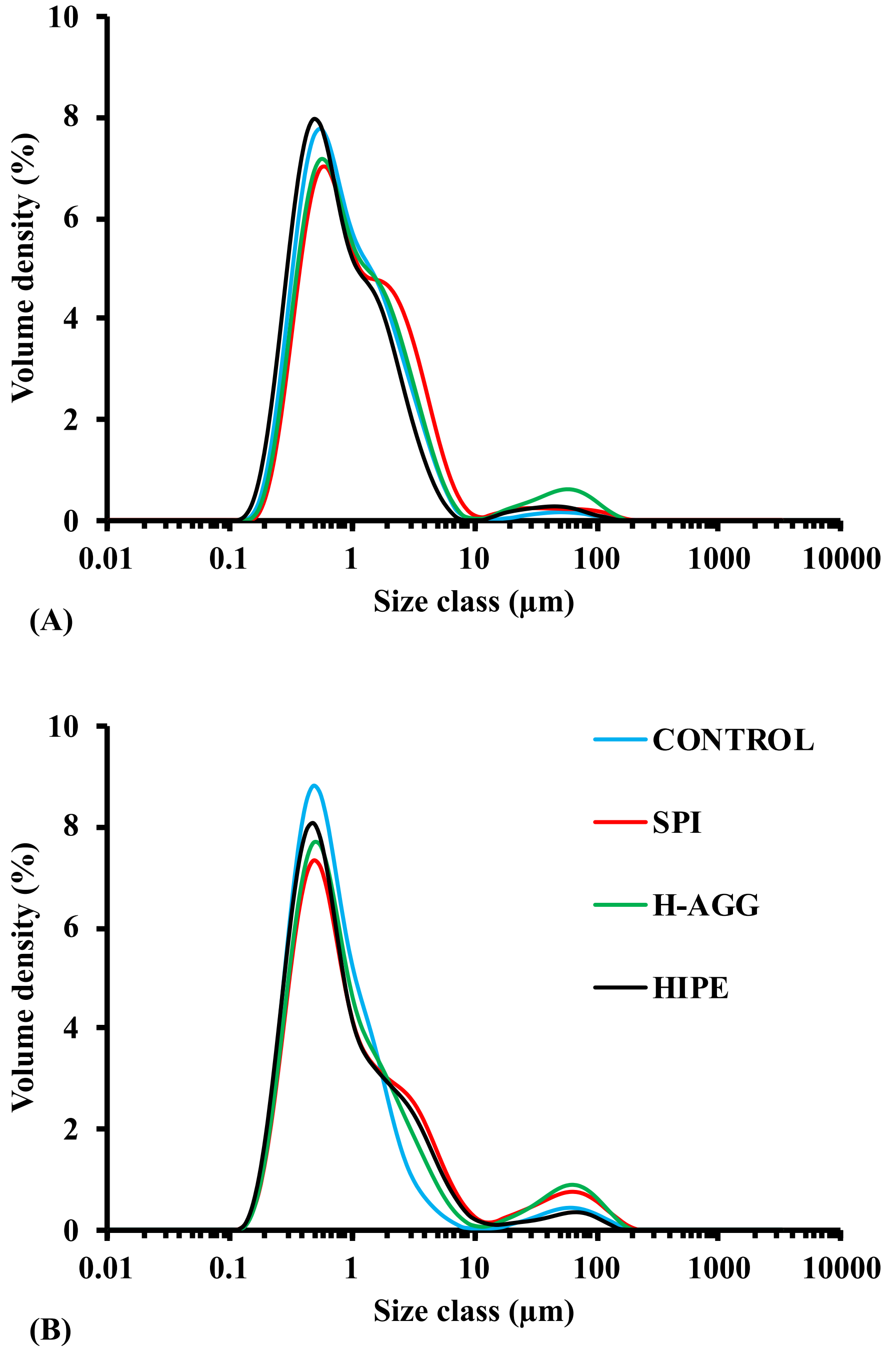


Figure 6-2. Particle size distributions of fresh ice cream mixes (A) and thawed ice cream (B)

6.3.3 Rheological properties

Rheological properties of ice cream mixes depend on their compositions, particle sizes, and interactions between individual constitutes (Chen et al., 2019). Viscosity flow curves shown in Figure 6-3A were fitted using the Power Law model. All mixes showed similar pseudoplastic behaviors with no significant difference ($P > 0.05$) in flow behavior index (n) across a range of 0.01 to 100 s^{-1} shear rates. However, mixes made with HIPE had higher viscosity than CONTROL. Despite similar fat globule sizes, the semi-solid form of HIPE led to a significant increase in consistency coefficient (K) compared to control ($P < 0.05$) while neither SPI powder nor heated SPI aggregates showed any effect ($P > 0.05$). Previous study reported higher viscosity in SPI-fortified ice cream mixes than the control without any substitution, which resulted from higher protein levels in the SPI-fortified mixes (Friedeck et al., 2003). In this study, the amount of additional protein in SPI and H-AGG was small, only accounting for 0.29% of total mixes. The viscosity provided from SPI and H-AGG could have limited impacts on the ice cream mix and might not be noticeable in the whole system.

Clusters of fat globules and larger fat globule sizes were found to correlate to an increase in viscosity of thawed ice cream (Sherwood & Smallfield, 1926). As shown in Figure 6-3B, thawed ice cream did not exhibit statistically significant increase in viscosities compared to fresh ice cream mixes. This result was in accordance with the limited fat destabilization occurring during freezing and whipping.

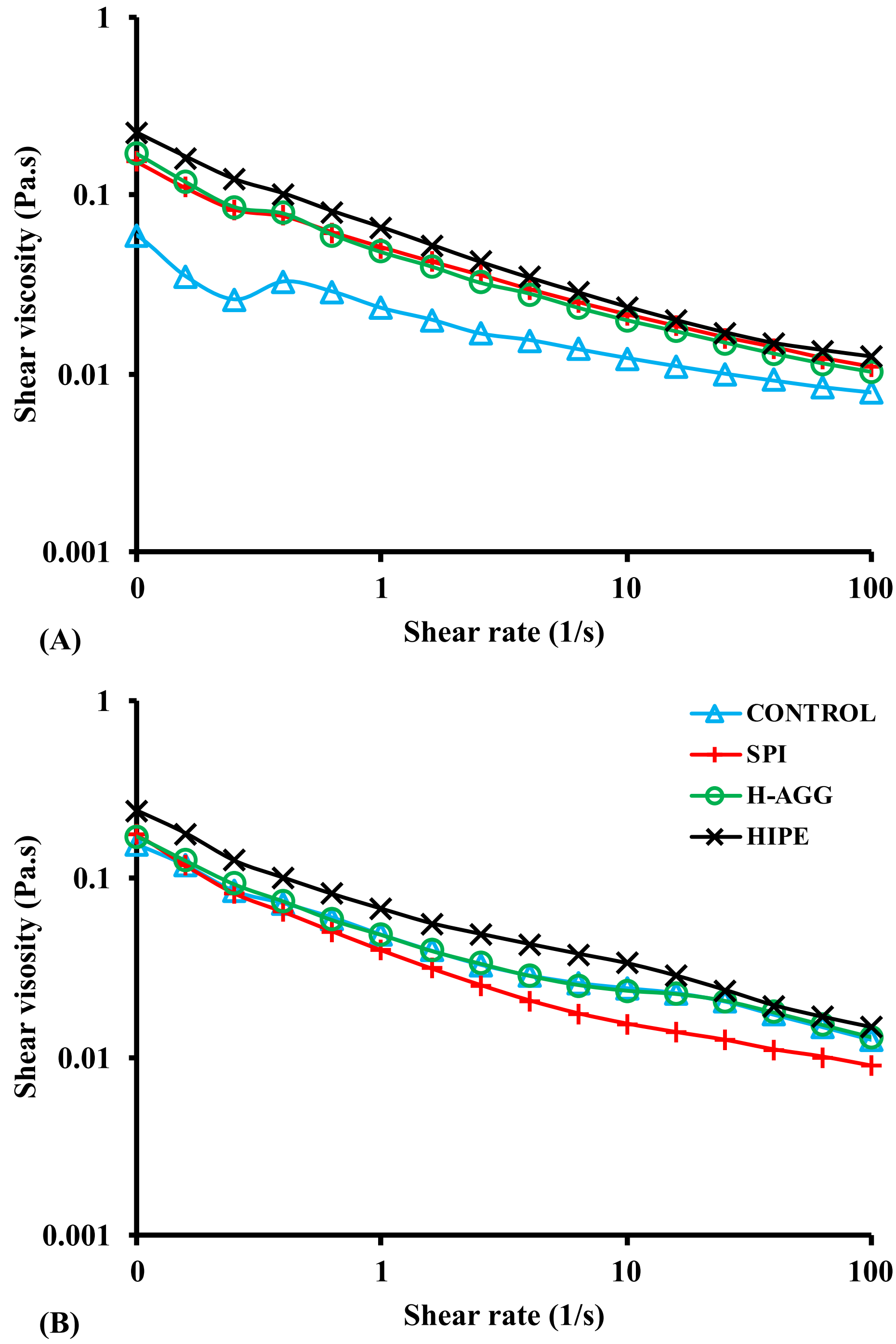


Figure 6-3. Flow behavior curves of fresh ice cream mixes (A) and thawed ice cream (B)

Table 6-4. Rheological properties of ice cream mixes and melted ice cream

Product	Ice cream mix		Melted ice cream	
	Consistency coefficient (K)	Flow behavior index (n)	Consistency coefficient (K)	Flow behavior index (n)
CONTROL	0.026 ± 0.005^{b}	0.70 ± 0.04^{a}	0.058 ± 0.003^{ab}	0.63 ± 0.04^{a}
SPI	0.055 ± 0.006^{ab}	0.62 ± 0.02^{a}	0.051 ± 0.005^{ab}	0.52 ± 0.09^{a}
H-AGG	0.057 ± 0.018^{ab}	0.57 ± 0.05^{a}	0.062 ± 0.006^{a}	0.60 ± 0.10^{a}
HIPE	0.069 ± 0.001^{a}	0.58 ± 0.00^{a}	0.083 ± 0.010^{a}	0.59 ± 0.02^{a}

Note: *Data represents mean ± standard deviation. Different letters denote significant difference between samples ($P < 0.05$).

6.3.4 Overrun and texture analysis of ice cream

Data of physical and textural properties are shown in Table 6-5. The overrun results were statistically similar among CONTROL and ice creams with different SPI particles, which could be due to similar rheological properties and fat globule sizes in ice cream mixes. Although SPI originally should have foaming ability, the small amount of SPI or heated SPI aggregates added might be insufficient to significantly increase the ability to foam. There was no difference in overrun between CONTROL and HIPE, although the ice cream mix for HIPE was more viscous than that for CONTROL.

Results of the effect of oleogel on the overrun of ice cream from literature were mixed. No difference in overrun was reported between ice cream made with ethylcellulose oleogel and high oleic sunflower oil (Munk et al., 2018). In another study, increased overrun in ice cream was observed for white beeswax structured oleogel by Jing et al. (Jing et al., 2022). Rice bran wax oleogel was also able to incorporate more amount of air in ice cream compared to liquid oil ice cream (Zulim Botega et al., 2013b).

Textural properties of ice cream were characterized using a puncture test. The hardness of H-AGG and HIPE were similar to CONTROL ($P > 0.05$) and all were significantly higher ($P < 0.05$) than that of SPI. Hardness is affected by the microstructure elements of ice cream, including overrun, size and amount of ice crystals, and fat globule agglomeration (Inoue et al., 2009). Larger fat globule agglomerates resulted from greater fat destabilization lead to higher hardness (Muse & Hartel, 2004). With similar overruns, the lower hardness of SPI might be attributed to its relatively smaller size fat globule agglomerates.

Adhesiveness represents the stickiness of ice cream. Many factors could influence the measurement of adhesiveness, such as iciness and viscosity. No statistical difference was found for adhesiveness, which could also be explained by the similar rheological properties found in melted ice cream.

6.3.5 Melting properties

Many factors can affect the melting properties of ice cream: overrun, ice crystal size and extent of fat agglomeration are considered the most significant factors (Muse & Hartel 2004). The melt-down resistance is shown in Figure 6-4. HIPE and H-AGG started to melt slightly behind CONTROL and SPI. HIPE exhibited better melt-down resistance among samples. After being placed at room temperature for 2 hours, the percent melted weight for HIPE (80.0 ± 2.6%) was significantly lower ($P < 0.05$) than that for CONTROL (91.7 ± 2.1%), SPI (88.0 ± 2.6%) and H-AGG (86.0 ± 1.0%). However, melting rates calculated as the slope of the linear region of the melt rate profile were not significantly different among all treatments.

Higher melt resistance of samples with oleogels made from rice bran wax and carnauba wax compared to milk fat were reported by Zulim Botega et al. (Zulim Botega et al., 2013a, 2013b) and Airoldi et al. (Airoldi et al., 2020), respectively. The high melting point of waxes used in structuring oleogel was believed to contribute to the increased melt resistance. Here, this heat-set SPI gel structured HIPE might have elevated melting point compared to liquid oil, which led to improved melt-down resistance.

Table 6-5. Physical and textural properties of the ice cream samples made with different fat sources

Product	Overrun (%)*	Hardness (g)*	Adhesiveness (g)*
CONTROL	32 ± 4[a]	25436 ± 2912[a]	-617 ± 90[a]
SPI	30 ± 3[a]	17389 ± 425[b]	-549 ± 114[a]
H-AGG	28 ± 1[a]	24411 ± 810[a]	-611 ± 180[a]
HIPE	31 ± 3[a]	22162 ± 1648[a]	-709 ± 91[a]

Note: *Data represents mean ± standard deviation. Different letters denote significant difference between samples within columns ($P < 0.05$).

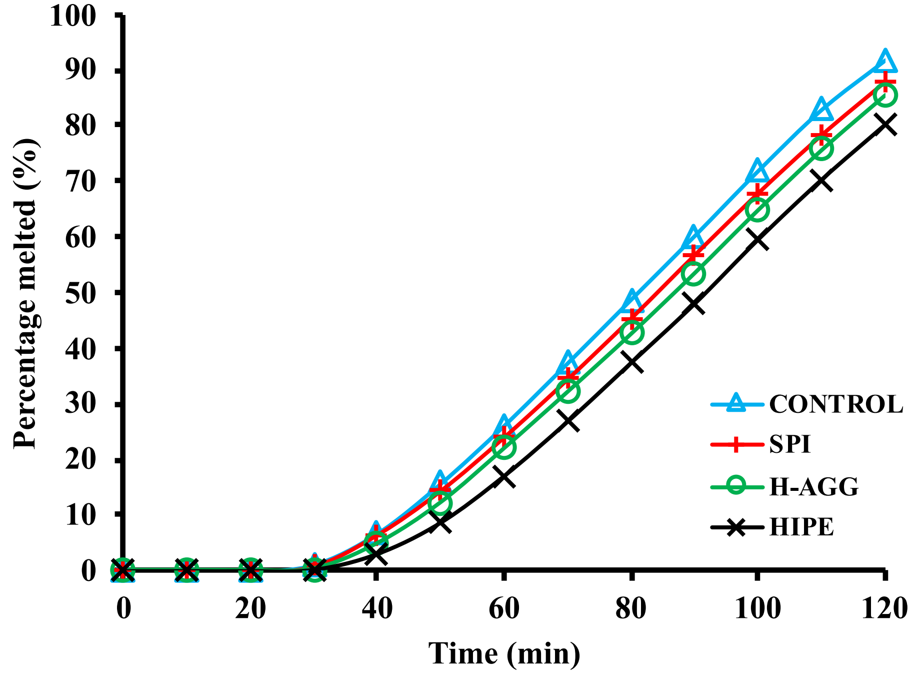


Figure 6-4. Melt rate profile for ice cream samples

Table 6-6. Melt percentage

Product	% weight melted at 60 min*	% weight melted at 120 min*	Melting rate (% melted per min after 40 min)*
CONTROL	26.0 ± 3.5^{a}	91.7 ± 2.1^{a}	1.09 ± 0.07^{a}
SPI	24.0 ± 2.0^{a}	88.0 ± 2.7^{a}	1.04 ± 0.02^{a}
H-AGG	21.0 ± 1.7^{a}	86.0 ± 1.0^{a}	1.04 ± 0.03^{a}
HIPE	17.0 ± 5.6^{a}	80.0 ± 2.7^{b}	$1..00 \pm 0.03^{a}$

Note: *Data represents mean ± standard deviation. Different letters denote significant difference between samples within columns ($P < 0.05$).

6.3.6 Descriptive sensory analysis

6.3.6.1 ANOVA

Descriptive analysis is a useful sensory evaluation tool to investigate differences in sensory characteristics that are important among a range of products (Yang & Lee, 2019). This information is critical when a new product or a new ingredient is developed. Successful products should have satisfactory sensory properties (Hwang, 2020). HIPEs is still a new material in ice cream formulation. It is important to understand how it impacts ice cream perception characteristics.

A mixed model was used to analyze if there was any significant difference among sensory attributes for the set of products. Tables 6-7 and 6-8 summarize the mean intensity of texture and flavor attributes for ice creams for descriptive analysis, respectively. No significant difference was found for any of the attributes. Results from instrumental analysis on physical and textural properties of ice cream only showed HIPE had higher melt-down resistance than CONTROL.

Although no significant difference was found, a general trend that could be observed from the descriptive panel is that compared to CONTROL, HIPE had higher creaminess and smoothness but lower iciness. Further modification in either HIPE formulation or ice cream making processing is needed to improve the application of HIPE in ice cream.

Table 6-7. Mean intensity of texture attributes for ice creams for descriptive analysis

	Texture attributes							
Product	Hardness	Melting rate	Iciness	Creaminess	Mouth coating	Smoothness	Gumminess	Denseness
CONTROL	7.09^{a}	8.29^{a}	7.02^{a}	5.73^{a}	5.61^{a}	6.45^{a}	6.20^{a}	6.49^{a}
SPI	7.13^{a}	8.56^{a}	7.02^{a}	5.72^{a}	5.82^{a}	6.17^{a}	6.14^{a}	6.34^{a}
H-AGG	7.64^{a}	8.62^{a}	6.76^{a}	5.56^{a}	5.62^{a}	6.46^{a}	6.07^{a}	6.31^{a}
HIPE	7.46^{a}	8.38^{a}	6.67^{a}	6.14^{a}	5.89^{a}	6.76^{a}	6.08^{a}	6.53^{a}

Note: *Data represents mean. Different letters denote significant difference between samples within columns ($P < 0.05$).

Table 6-8. Mean intensity of flavor attributes for ice creams for descriptive analysis

	Flavor attributes			
Product	Sweetness	Raw soy	Sweetness aftertaste	Raw soy aftertaste
CONTROL	5.79[a]	6.06[a]	5.58[a]	5.96[a]
SPI	5.90[a]	6.50[a]	5.33[a]	6.55[a]
H-AGG	5.78[a]	6.40[a]	5.23[a]	6.20[a]
HIPE	5.85[a]	6.57[a]	5.34[a]	6.51[a]

Note: *Data represents mean. Different letters denote significant difference between samples within columns ($P < 0.05$).

6.3.6.2 PCA

The PCA method analyzes the relationships among attributes. PCA result showed that the first two PCs explained the majority of the variation (75.7%) and the first three PCs explained all of the variation (100%). The PC1, PC2 and PC3 accounted for 45.4%, 30.3% and 24.3% of the variance, respectively. All the relationships of the sensory attributes could be explained by the first three principal dimensions.

PCA biplots of loadings of descriptive sensory attributes along with PC scores of four ice cream samples for the first three dimensions are shown in Figure 6-5 – Figure 6-7, respectively. The first PC was dominated by soy flavor, soy aftertaste, hardness, mouth coating in the one direction, and sweet after taste, iciness and gumminess in the opposite direction. Soy flavor was the most crucial attribute in PC1. Ice creams with added SPI showed more intensity of soy flavor. Texture attributes including creaminess, smoothness and denseness controlled the second PC together and melt rate controlled the second PC oppositely. The third PC was controlled by sweetness and hardness.

The score from HIPE was at the same space as the direction of texture attributes including creaminess, smoothness and mouth coating, but at the opposite space compared to the iciness direction. Meanwhile, CONTROL was near the vector of iciness. This suggests that HIPE was creamier, smoother, showed more mouth coating and was less icy than CONTROL. SPI and H-AGG were considered similar and were classified differently from either HIPE or CONTROL.

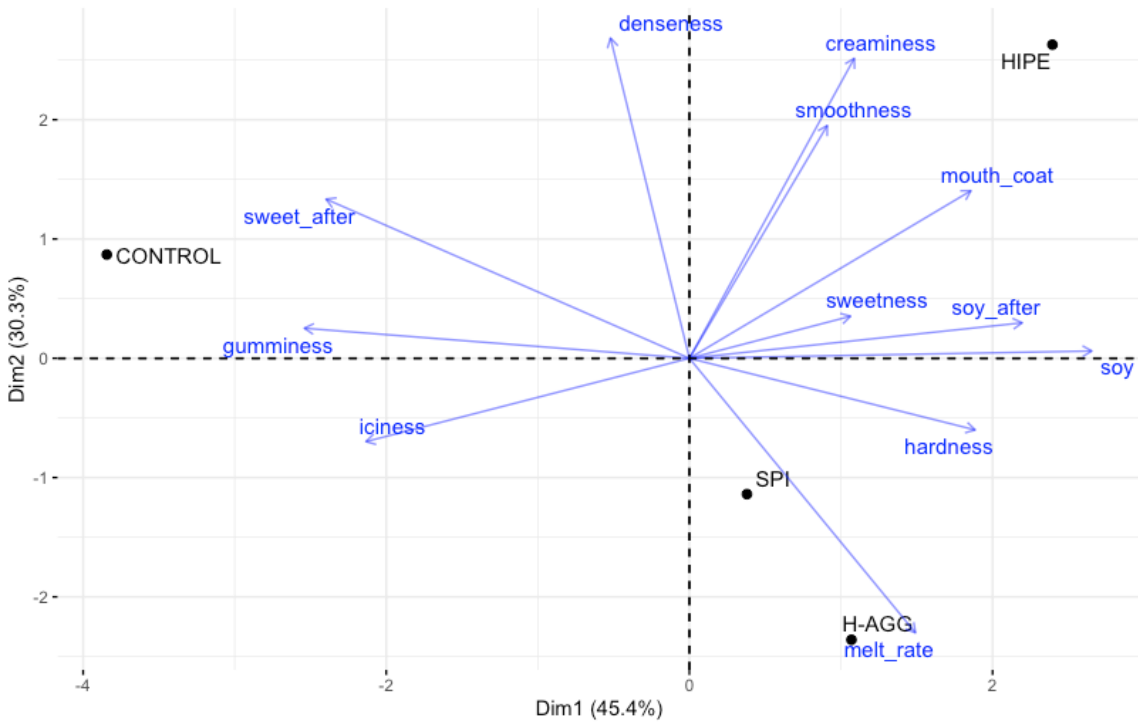


Figure 6-5. PCA biplot of the mean descriptive sensory data for four ice cream samples on PC 1 and PC 2. Vectors in blue represented the descriptive sensory attributes and solid points represented the ice cream products.

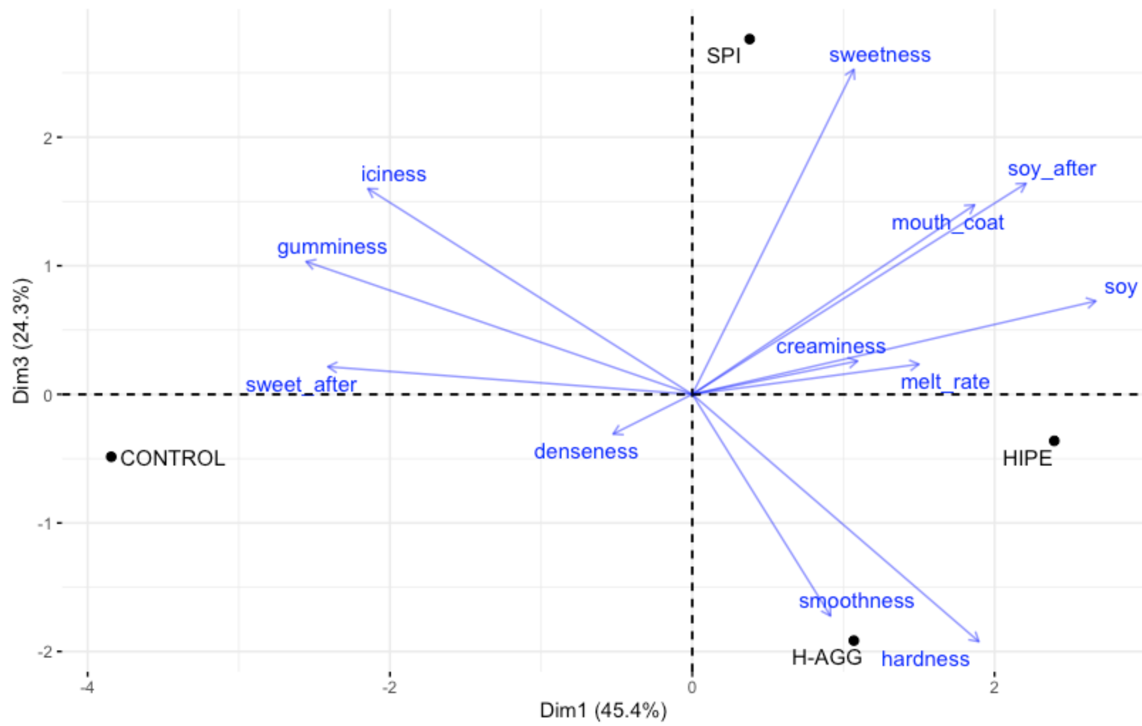


Figure 6-6. PCA biplot of the mean descriptive sensory data for four ice cream samples on PC 1 and PC 3. Vectors in blue represented the descriptive sensory attributes and solid points represented the ice cream products.

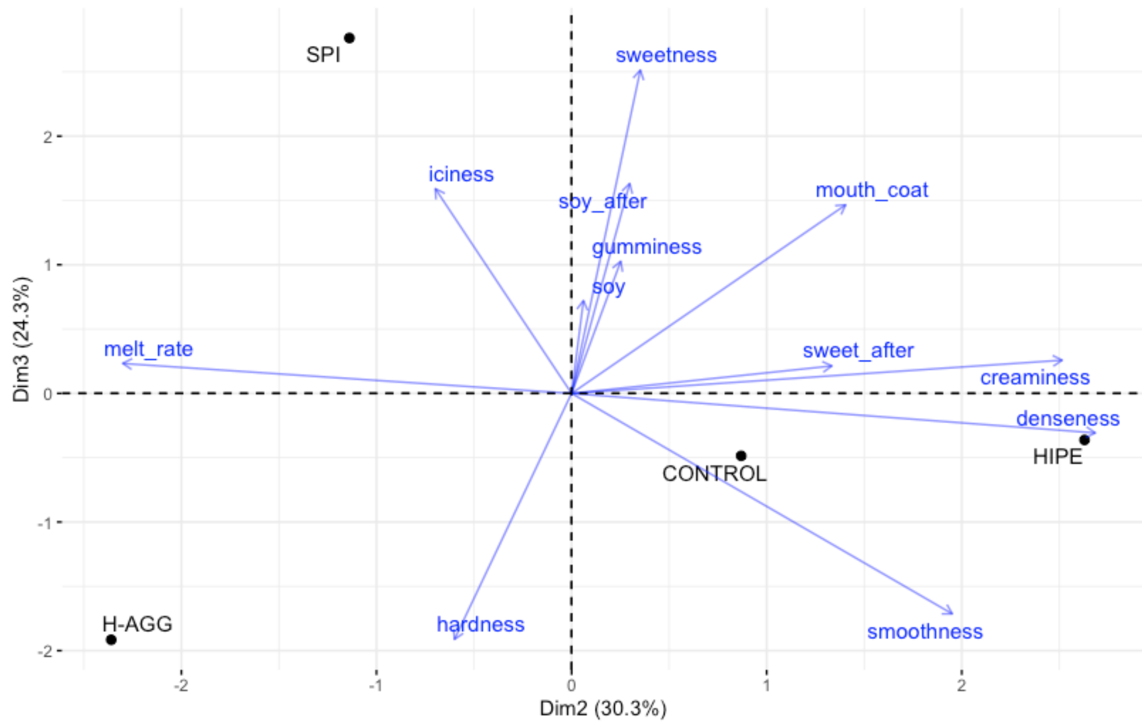


Figure 6-7. PCA biplot of the mean descriptive sensory data for four ice cream samples on PC 2 and PC 3. Vectors in blue represented the descriptive sensory attributes and solid points represented the ice cream products.

6.4 Conclusion

Heat-set SPI gel was used to structure HIPE (ϕ=0.75) which exhibited gel-like texture. This study was the first to incorporate HIPE in a soy milk-based ice cream formulation via typical ice cream making processes. The use of HIPE led to higher mix viscosity and improved melt resistance of ice cream. These slight improvements did not result in significant differences for any of the sensory attributes. However, the general trend has shown ice cream containing gelled HIPE tended to be creamier, smoother, showed more mouth coating and was less icy than that made from liquid oil. Further investigation could include the optimization of HIPE as well as modification of ice cream processing conditions.

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Chapter 7 CONCLUSION

The first objective of this study was to investigate the performance of cold pressed HOLL soybean oil (SOYLEIC^{TM}) in formulating plant-based ice cream. Results indicate that SOYLEIC^{TM} soybean oil showed technological advantage of improving the overrun of ice cream possibly due to the presence of phospholipids preserved by cold pressing technique. The high oleic fatty acid profile of SOYLEIC^{TM} soybean oil produced a clean flavor in ice cream with less painty off flavor and painty aftertaste. Consumer testing showed that consumer preference of ice cream products was positively correlated to textural attributes including denseness, smoothness, mouth coating, and creaminess while flavor attributes of painty flavor, painty aftertaste, raw soy flavor, raw soy aftertaste, malty flavor, and malty aftertaste were disliked by consumers.

In the second part of this study, structured oil as a solid fat substitute was developed and incorporated into soy-based ice cream with the goal to improve the textural properties. Heated SPI gel particles were used to form and stabilize HIPEs. The optimum formulation produced a gel-like HIPE that was stable against heating. Ice cream made with HIPE was resistant to melt down which could be due to the presence of solid particles. Although descriptive sensory results showed no significant differences in all sensory attributes, ice cream made with HIPE were less icy, more smooth, creamier and more mouth coating compared to liquid oil in general direction.

Overall, proprietary SOYLEIC^{TM} soybean oil has potential to be used in formulating plant-based ice cream with improved taste and nutrition. Modification of liquid oil into gel-like

HIPE could further improve the texture of ice cream without the use of emulsifier and stabilizer. Future investigation could focus on continued developing and optimizing HIPEs for its application in clean label plant-based ice cream.

APPENDIX

Appendix 1. Consent form

STUDY CONSENT FORM TO PARTICIPATE IN A RESEARCH STUDY

Study Title: Evaluating and optimizing the use of high oleic low linolenic (HOLL) soybean oil in ice cream

Introduction: This research project involves gathering data on ice cream made from different types of fat. The data will be collected for analysis and may be published. You must be at least 18 years of age to participate.

Purpose: The purpose of this study is to evaluate the use of high oleic acid low linolenic (HOLL) soybean oil in ice cream. The HOLL soybean line was developed at the University of Missouri.

Voluntary: The survey is entirely voluntary. You may refuse to answer any question or choose to withdraw from participation at any time without any penalty or loss of benefits to which you are otherwise entitled.

Study procedures: You will come to the lobby of Eckles Hall for an in-person sensory evaluation (roughly lasting 30 minutes to 1 hr) consisting of:

- Consuming 4 research product samples.
- Completing a 9-point hedonic scale based on flavor, texture, and appearance.
- Completing a demographic and purchase survey.

Risks: There is no substantial risk related to the study. The risk will involve having the allergic reaction or intolerance to the ingredients used in the study (listed below).

Screening:
If you are allergic to any type of dairy, soybean product or ingredients listed below do not participate in this sensory study. The ice cream samples may contain one or more of the following ingredients:

Milk	Soybean oil	Cream	Soybean fiber
Soy protein	Coconut oil	vanilla extract	lactose

Compensation: You will receive a coupon for a one-scoop of Buck's ice cream.

Benefits: Your participation, however, will enrich the knowledge on ice cream science and may contribute to the development of novel ice cream products using Missouri soybean.

Confidentiality: Your confidentiality will be maintained in that your name will not appear on the ballot or in the published study itself. The data will only be reported in aggregate form.

Thank you for your assistance in developing new ice cream products. Although great strides have been made in the instrumental analysis of foods, the development of new foods still requires the human sensory response and feedback. Your efforts are greatly appreciated.

If you have any questions regarding the study, please contact Dr. Bongkosh Vardhanabhuti (573-8821437 or vardhanabhutib@missouri.edu). If you have questions regarding your rights as a participant in research, please feel free to contact the University of Missouri Institutional Review Board at (573) 882-3181 or irb@missouri.edu.

If you want to talk privately about your rights or any issues related to your participation in this study, you can contact University of Missouri Research Participant Advocacy by calling 888-280-5002 (a free call), or emailing MUResearchRPA@missouri.edu

Please keep one copy of this consent form for your records!

Appendix 2. Ballot scale for descriptive sensory panel of study 1

Sample number __________

Appearance:
Color

Low High

Scoopability:
Hardness

Low High

Texture:
Rate of melt __________ sec.

Iciness

Low High

Creaminess

Low High

Mouth coating

Low High

Smoothness

Low High

Gumminess

Low High

Denseness

Low High

Flavor:
Sweetness

Low High

Raw soy (beany and grassy) flavor

Low High

Painty flavor

Low High

Malty flavor

Low High

Aftertaste:
Sweetness aftertaste

Low High

Raw soy (beany and grassy) flavor aftertaste

Low High

Painty flavor aftertaste

Low High

Malty flavor aftertaste

Low High

Appendix 3: Questionnaire and hedonic scale for consumer acceptance

Study of Consumer Acceptance of Soy Frozen Dessert

Instructions:
This sensory test is about soy frozen dessert with different types of oil/fat. There are three portions to this test. You will fill out our questionnaire. Then you will be tasting four soy frozen dessert samples and evaluating them one after another. Finally, please rank the four soy frozen dessert samples together.

First Part: Please complete the following questionnaire:

1. Gender: Male ☐ Female ☐ Prefer not to answer ☐

2. What is your age group?

<18 ☐ 18-25 ☐ 26-35 ☐ 36-45 ☐ 46-55 ☐ 56-65 ☐ 65< ☐

3. Do you shop for your household, even if it is you alone? Yes ☐ No ☐

Please answer the following questions. There are no right or wrong answer. We want to know about you and what you think. Please ask if you have any questions!

4. How often do you consume frozen desserts (ice cream, frozen yogurt)? (check one)

☐ Never
☐ Less than once a month
☐ 2-4 times a month
☐ More than once a week

5. What types of frozen desserts do you consume? (Check all that apply)

☐ Premium, super-premium ice cream (e.g., Eddy's, Breyers, Ben & Jerry's, etc.)
☐ Low-fat/fat-free ice cream (e.g., Ben & Jerry's, Haagen Dazs Low Fat, etc.)
☐ Novelties (e.g., Ice cream sandwiches, Klondike bars, etc.)
☐ Sherbet
☐ Frozen Confections (popsicles, fruit juice bars)
☐ Plant-based frozen dessert (e.g., soymilk frozen dessert, almondmilk frozen dessert, etc.)

6. What factors influence your choice of frozen dessert products? (check all that apply):

Price ☐ Texture ☐ Flavor ☐ Health ☐ Availability ☐

7. Check one that best indicates how you feel about the following statement:
"Plant based foods are healthy foods"

☐ Strongly agree
☐ Agree
☐ Neither disagree nor agree (don't know)
☐ Disagree
☐ Strongly disagree

8. Check one that best indicates how you feel about the following statement:
"Soy foods are healthy foods"

☐ Strongly agree
☐ Agree
☐ Neither disagree nor agree (don't know)
☐ Disagree
☐ Strongly disagree

9. If the price per container were the same and the flavor/texture were the same or better, would you purchase a plant-based frozen dessert made with high oleic soybean oil? (check one)

☐ Definitely would buy
☐ Probably would buy
☐ Maybe would buy
☐ Would not buy

Thank you very much!

Second Part: Please choose one of the point in the forms below for each sample, according to your experience.

Instructions:

1. Please masticate (eat) a piece of cracker then rinse your mouth with water before you start the test

2. Write the three-digit code of the ice cream sample, shown on the sample cup, on the line provided on each ballot on next page.

3. Place a spoonful of sample in your mouth and then rate how much you like or dislike the sample by placing a mark on the scale that best describes your opinion.

4. Consume another piece of cracker than rinse your mouth with water before you start the next sample

5. When you are done with your evaluation, please flip the light switch letting a lab assistant know you are ready for your next sample

6. Remember, do not re-taste the samples during the test.

7. Thank you for participating in the study!

If at any time you have a question about the test or directions, please ask the lab assistant.

Consumer Ballot for Frozen Dessert

Three-digit Sample Code: _______________

How would you rate the **"OVERALL LIKING"** of this product?

Extremely dislike	Dislike very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How would you rate the **"OVERALL FLAVOR"** of this product?

Extremely dislike	Dislike very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How would you rate the **"OVERALL APPEARANCE"** of this product?

Extremely dislike	Dislike very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

How would you rate the **"OVERALL TEXTURE"** of this product?

Extremely dislike	Dislike very much	Dislike Moderately	Dislike Slightly	Neither like nor dislike	Like slightly	Like moderately	Like very much	Like extremely

Third Part: Please rank the sample in an order from BEST sample to worst sample:

Sample # ________ > Sample # ________ > Sample # ________ > Sample # ________

Thank you again for participating in the study!

Appendix 4: Williams Design for quantitative descriptive test of study 1

Sample	Product code
COM-HO	256
COM-VO	132
CREAM	848
SOYLEIC	652

(Replication 1)

Panelist	Session 1					
	1	2	3	4	5	6
J1	132	652	256	848	132	132
J2	256	132	132	652	652	848
J3	652	132	848	256	256	132
J4	848	132	652	256	132	848
J5	132	256	652	132	848	652
J6	256	848	652	652	848	132
J7	652	848	132	132	256	256
J8	848	652	256	132	652	256
J9	132	256	848	848	652	652
J10	256	848	132	652	848	256
J11	652	256	848	848	256	652
J12	848	652	256	256	132	848

Panelist	Session 2					
	1	2	3	4	5	6
J1	652	256	848	848	256	652
J2	848	132	256	256	652	848
J3	652	652	848	848	256	132
J4	256	652	132	256	652	848
J5	256	848	652	132	848	256
J6	256	256	132	132	848	652
J7	132	848	652	652	848	256
J8	848	132	256	652	132	848
J9	132	256	256	848	132	652
J10	652	848	132	652	256	132
J11	132	132	848	256	652	132
J12	848	652	652	132	132	256

(Replication 2)

Panelist	Session 3					
	1	2	3	4	5	6
J1	132	848	848	652	132	256
J2	256	652	652	256	132	848
J3	652	256	132	652	256	256
J4	848	132	256	848	652	132
J5	132	652	256	256	652	652
J6	256	132	652	652	848	256
J7	652	256	256	848	652	132
J8	848	652	132	256	848	132
J9	132	848	848	132	256	848
J10	256	848	652	132	256	848
J11	652	256	848	132	132	652
J12	848	132	132	848	848	652

Panelist	Session 4					
	1	2	3	4	5	6
J1	848	132	256	652	652	256
J2	256	132	652	848	848	132
J3	652	848	848	132	132	848
J4	256	848	652	652	132	256
J5	848	256	132	848	848	132
J6	132	652	848	256	132	848
J7	132	848	256	132	652	848
J8	132	652	848	256	256	652
J9	652	652	256	256	652	132
J10	652	132	132	848	256	652
J11	848	256	132	652	848	256
J12	256	256	652	132	256	652

(Replication 3)

Panelist	Session 5					
	1	2	3	4	5	6
J1	132	652	848	848	256	132
J2	256	848	652	652	132	256
J3	652	256	132	848	848	652
J4	848	652	256	256	652	132
J5	132	256	256	848	652	132
J6	256	848	132	132	256	652
J7	652	256	848	132	256	256
J8	848	132	652	652	132	256
J9	132	652	848	256	652	848
J10	256	132	652	256	848	848
J11	652	848	132	132	848	652
J12	848	132	256	652	132	848

Panelist	Session 6					
	1	2	3	4	5	6
J1	132	652	256	256	652	848
J2	848	132	652	256	132	848
J3	652	256	132	132	848	256
J4	132	256	848	848	652	132
J5	848	652	256	848	652	132
J6	652	848	848	132	256	652
J7	652	848	132	132	848	652
J8	848	848	256	652	132	256
J9	132	652	848	256	256	132
J10	256	132	652	652	132	848
J11	256	256	132	848	256	652
J12	256	132	652	652	848	256

Appendix 5. Ballot scale for descriptive sensory panel of study 2

Sample number __________

Scoopability:
Hardness

Low High

Texture:
Rate of melt __________ sec.

Iciness

Low High

Creaminess

Low High

Mouth coating

Low High

Smoothness

Low High

Gumminess

Low High

Denseness

Low High

Flavor:
Sweetness

Low High

Raw soy (beany and grassy) flavor

Low High

Malty flavor

Low High

Aftertaste:
Sweetness aftertaste

Low High

Raw soy (beany and grassy) flavor aftertaste

Low High

Appendix 6: Williams Design for quantitative descriptive test of study 2

Sample	Product code
CONTROL	230
SPI	415
H-AGG	510
HIPE	898

(Replication 1)

Panelist	Session 1					
	1	2	3	4	5	6
J1	230	510	415	230	510	415
J2	898	510	898	415	230	230
J3	415	230	510	510	898	230
J4	510	898	415	415	230	230
J5	230	898	415	898	898	510
J6	898	898	230	510	415	415
J7	415	230	898	898	510	898
J8	510	230	230	415	415	510
J9	230	415	510	510	230	898
J10	898	415	510	230	415	898
J11	415	510	230	898	510	415

Panelist	Session 2					
	1	2	3	4	5	6
J1	898	510	898	898	230	415
J2	415	510	898	230	510	415
J3	898	415	230	510	415	898
J4	510	898	230	898	415	510
J5	510	415	415	230	230	510
J6	898	230	510	510	415	230
J7	415	510	230	415	510	230
J8	510	898	898	415	898	230
J9	415	415	510	230	898	898
J10	230	898	510	510	230	415
J11	230	230	415	898	510	898

(Replication 2)

Panelist	Session 3					
	1	2	3	4	5	6
J1	230	415	415	415	898	230
J2	898	230	510	898	510	510
J3	415	230	898	415	510	415
J4	510	510	230	898	415	230
J5	230	510	415	510	415	898
J6	898	510	230	898	898	415
J7	415	230	415	510	230	510
J8	510	898	510	230	230	898
J9	230	898	898	510	510	898
J10	898	415	510	230	898	415
J11	415	415	230	230	415	510

Panelist	Session 4					
	1	2	3	4	5	6
J1	510	510	898	510	230	898
J2	230	898	415	415	415	230
J3	898	230	230	510	898	510
J4	415	898	230	510	415	898
J5	230	230	415	898	898	510
J6	510	230	510	415	230	415
J7	415	898	898	230	510	898
J8	415	510	415	898	230	415
J9	510	415	230	230	415	415
J10	230	415	230	898	510	510
J11	898	510	510	898	898	230

(Replication 3)

Panelist	Session 5					
	1	2	3	4	5	6
J1	230	415	510	898	415	230
J2	898	230	415	230	230	510
J3	415	510	898	230	415	415
J4	510	510	230	898	230	415
J5	230	230	898	510	415	510
J6	898	415	415	510	898	230
J7	415	898	230	230	510	230
J8	510	230	415	415	898	898
J9	230	510	230	510	898	898
J10	898	415	510	898	510	415
J11	415	898	898	415	510	510

Panelist	Session 6					
	1	2	3	4	5	6
J1	898	230	415	510	898	510
J2	510	510	415	898	898	415
J3	898	898	510	230	510	230
J4	898	898	415	415	230	510
J5	230	898	510	415	415	898
J6	510	415	510	898	230	230
J7	415	510	898	510	415	898
J8	415	230	898	230	510	510
J9	415	415	230	898	510	415
J10	230	510	230	230	898	415
J11	510	230	230	415	230	898

Appendix 7: R code

```
library(readxl)
library(tidyverse)
library(dplyr)
library(car)
library(FactoMineR)
library(lmerTest)
library(lsmeans)
library(fmsb)
library(SensoMineR)
library(ggplot2)
library(scales)
library(grid)
library(plyr)
library(gridExtra)
library(pls)
library(mdatools)
library(ggrepel)
library(corrplot)
```

Descriptive panel data for SOYLEIC^{TM} soybean oil formulated ice cream:

```
df <- read_excel("Descriptive Panel Data.xlsx")
## set panelist, session, replication and treatment as factors
df$Panelist = as.factor(df$Panelist)
df$Session = as.factor(df$Session)
df$Replication = as.factor(df$Replication)
df$Product = as.factor(df$Product)

df_summary_all = df[ , -c(1:3)] %>%
  group_by(Product) %>%
  summarise_all(mean)
print.data.frame(df_summary_all)
```

<u>## PCA</u>

```
X = df_summary_all[, -1]
rownames(X) = c("COM-HO", "COM-VO", "CREAM", "SOYLEIC")
DA.pca = PCA(X, graph = FALSE)
fviz_pca_biplot(DA.pca, col.var = "blue", col.ind = "black", repel = TRUE)

## MANOVA
DA.manova = manova(cbind(Color, Hardness, Melt_rate, Iciness, Creaminess,
Mouth_coating, Smoothness, Gumminess,
                 Denseness, Sweetness, Raw_soy_flavor, Painty_flavor, Malty_flavor,
Sweetness_aftertaste,
```

```
               Raw_soy_aftertaste, Painty_aftertaste, Malty_aftertaste) ~ Product,
data=df)
summary(DA.manova, test="Wilks")
```

<u>## Mixed model ANOVA</u>

```
DA.mixed.color = lmer(Color ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.color)
rand(DA.mixed.color)
emmeans(DA.mixed.color, list(pairwise ~ Product))

DA.mixed.hardness = lmer(Hardness ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.hardness)
rand(DA.mixed.hardness)
emmeans(DA.mixed.hardness, list(pairwise ~ Product))

DA.mixed.melt = lmer(Melt_rate ~ Product + (1|Panelist) + (1|Session) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.melt)
rand(DA.mixed.melt)
emmeans(DA.mixed.melt, list(pairwise ~ Product))

DA.mixed.iciness = lmer(Iciness ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.iciness)
rand(DA.mixed.iciness)
emmeans(DA.mixed.iciness, list(pairwise ~ Product))

DA.mixed.creaminess = lmer(Creaminess ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.creaminess)
rand(DA.mixed.creaminess)
emmeans(DA.mixed.creaminess, list(pairwise ~ Product))

DA.mixed.mouthcoating = lmer(Mouth_coating ~ Product + (1|Panelist) + (1|Replication)
+ (1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.mouthcoating)
rand(DA.mixed.mouthcoating)
emmeans(DA.mixed.mouthcoating, list(pairwise ~ Product))
```

```
DA.mixed.smoothness = lmer(Smoothness ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.smoothness)
rand(DA.mixed.smoothness)
emmeans(DA.mixed.smoothness, list(pairwise ~ Product))

DA.mixed.gumminess = lmer(Gumminess ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.gumminess)
rand(DA.mixed.gumminess)
emmeans(DA.mixed.gumminess, list(pairwise ~ Product))

DA.mixed.denseness = lmer(Denseness ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.denseness)
rand(DA.mixed.denseness)
emmeans(DA.mixed.denseness, list(pairwise ~ Product))

DA.mixed.sweet = lmer(Sweetness ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.sweet)
rand(DA.mixed.sweet)
emmeans(DA.mixed.sweet, list(pairwise ~ Product))

DA.mixed.rawsoy = lmer(Raw_soy_flavor ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.rawsoy)
rand(DA.mixed.rawsoy)
emmeans(DA.mixed.rawsoy, list(pairwise ~ Product))

DA.mixed.painty = lmer(Painty_flavor ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
anova(DA.mixed.painty)
rand(DA.mixed.painty)
emmeans(DA.mixed.painty, list(pairwise ~ Product))

DA.mixed.malty = lmer(Malty_flavor ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
            data=df, REML = TRUE)
```

```
anova(DA.mixed.malty)
rand(DA.mixed.malty)
emmeans(DA.mixed.malty, list(pairwise ~ Product))

DA.mixed.sweetaft = lmer(Sweetness_aftertaste ~ Product + (1|Panelist) + (1|Replication)
+ (1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
          data=df, REML = TRUE)
anova(DA.mixed.sweetaft)
rand(DA.mixed.sweetaft)
emmeans(DA.mixed.sweetaft, list(pairwise ~ Product))

DA.mixed.rawsoyaft = lmer(Raw_soy_aftertaste ~ Product + (1|Panelist) + (1|Replication)
+ (1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
          data=df, REML = TRUE)
anova(DA.mixed.rawsoyaft)
rand(DA.mixed.rawsoyaft)
emmeans(DA.mixed.rawsoyaft, list(pairwise ~ Product))

DA.mixed.paintyaft = lmer(Painty_aftertaste ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
          data=df, REML = TRUE)
anova(DA.mixed.paintyaft)
rand(DA.mixed.paintyaft)
emmeans(DA.mixed.paintyaft, list(pairwise ~ Product))

DA.mixed.maltyaft = lmer(Malty_aftertaste ~ Product + (1|Panelist) + (1|Replication) +
(1|Product:Panelist) + (1|Product:Replication) + (1|Panelist:Replication),
          data=df, REML = TRUE)
anova(DA.mixed.maltyaft)
rand(DA.mixed.maltyaft)
emmeans(DA.mixed.maltyaft, list(pairwise ~ Product))
```

Consumer acceptance data for SOYLEIC™ soybean oil formulated ice cream:

Questionnaire

```
demographic = read_excel("Consumer sensory.xlsx", sheet =
"demograhpic_questionnaire")
**Gender**
demographic$Q1_gender[demographic$Q1_gender==1] = "Male"
demographic$Q1_gender[demographic$Q1_gender==2] = "Female"
gender_tbl = demographic %>%
  group_by(demographic$Q1_gender) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
gender_tbl
```

```
**Age**
demographic$Q2_age[demographic$Q2_age==1] = "<18"
demographic$Q2_age[demographic$Q2_age==2] = "18-25"
demographic$Q2_age[demographic$Q2_age==3] = "26-35"
demographic$Q2_age[demographic$Q2_age==4] = "36-45"
demographic$Q2_age[demographic$Q2_age==5] = "46-55"
demographic$Q2_age[demographic$Q2_age==6] = "56-65"
demographic$Q2_age[demographic$Q2_age==7] = ">65"
age_tbl = demographic %>%
  group_by(demographic$Q2_age) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
print(age_tbl)

**Whether shop for household or not**
demographic$Q3_household_shop[demographic$Q3_household_shop==1] = "YES"
demographic$Q3_household_shop[demographic$Q3_household_shop==2] = "NO"
demographic$Q3_household_shop[demographic$Q3_household_shop==3] = "NA"
household_shop_tbl = demographic %>%
  group_by(demographic$Q3_household_shop) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
household_shop_tbl

**Frequency consume frozen desserts**
demographic$Q4_freq_eat[demographic$Q4_freq_eat==1] = "1_Never"
demographic$Q4_freq_eat[demographic$Q4_freq_eat==2] = "2_Less than once a month"
demographic$Q4_freq_eat[demographic$Q4_freq_eat==3] = "3_2-4 times a month"
demographic$Q4_freq_eat[demographic$Q4_freq_eat==4] = "4_More than once a week"
freq_consume_tbl = demographic %>%
  group_by(demographic$Q4_freq_eat) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
freq_consume_tbl

**What types of frozen desserts do you consume?**
frozen_dessert_type = c("Premium, super-premium ice cream", "Low-fat, fat-free ice
cream", "Novelties", "Sherbet", "Frozen Confections", "Plant-based frozen dessert")
typ_n = c(sum(demographic$Q5_type_eat_1), sum(demographic$Q5_type_eat_2),
sum(demographic$Q5_type_eat_3), sum(demographic$Q5_type_eat_4),
sum(demographic$Q5_type_eat_5), sum(demographic$Q5_type_eat_6))
typ_pct = typ_n/82
fd_type_tbl = data.frame(cbind(frozen_dessert_type, typ_n, typ_pct))
print(fd_type_tbl)
**What factors influence your choice of frozen dessert prodcuts?**
influence_factor = c("Price", "Texture", "Flavor", "Health", "Availability")
```

```
fac_n = c(sum(demographic$Q6_factor_1), sum(demographic$Q6_factor_2),
sum(demographic$Q6_factor_3), sum(demographic$Q6_factor_4),
sum(demographic$Q6_factor_5))
fac_pct = fac_n/82
inf_fac_tbl = data.frame(cbind(influence_factor, fac_n, fac_pct))
print(inf_fac_tbl)

**Plant based foods are healthy foods**
demographic$Q7_plantfood_healthy[demographic$Q7_plantfood_healthy==1] =
"1_Stongly agree"
demographic$Q7_plantfood_healthy[demographic$Q7_plantfood_healthy==2] =
"2_Agree"
demographic$Q7_plantfood_healthy[demographic$Q7_plantfood_healthy==3] =
"3_Neither disagree nor agree"
demographic$Q7_plantfood_healthy[demographic$Q7_plantfood_healthy==4] =
"4_Disagree"
demographic$Q7_plantfood_healthy[demographic$Q7_plantfood_healthy==5] =
"5_Strongly disagree"
plantfoods_tbl = demographic %>%
  group_by(demographic$Q7_plantfood_healthy) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
plantfoods_tbl

**Soy foods are healthy foods**
demographic$Q8_soyfood_healthy[demographic$Q8_soyfood_healthy==1] =
"1_Strongly agree"
demographic$Q8_soyfood_healthy[demographic$Q8_soyfood_healthy==2] = "2_Agree"
demographic$Q8_soyfood_healthy[demographic$Q8_soyfood_healthy==3] = "3_Neither
disagree nor agree"
demographic$Q8_soyfood_healthy[demographic$Q8_soyfood_healthy==4] =
"4_Disagree"
demographic$Q8_soyfood_healthy[demographic$Q8_soyfood_healthy==5] =
"5_Strongly disagree"
soyfoods_tbl = demographic %>%
  group_by(demographic$Q8_soyfood_healthy) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
soyfoods_tbl

**Whether buy a plant-based frozen dessert with high oleic soybean oil**
demographic$Q9_if_buy[demographic$Q9_if_buy==1] = "1_Definitely would buy"
demographic$Q9_if_buy[demographic$Q9_if_buy==2] = "2_Probably would buy"
demographic$Q9_if_buy[demographic$Q9_if_buy==3] = "3_Maybe would buy"
demographic$Q9_if_buy[demographic$Q9_if_buy==4] = "4_Would not buy"
ifbuy_tbl = demographic %>%
```

```
  group_by(demographic$Q9_if_buy) %>%
  summarise(n=n()) %>%
  mutate(freq = prop.table(n))
ifbuy_tbl
```

Mixed model ANOVA

```
liking = read_excel("Consumer sensory.xlsx", sheet = "sensory_evaluation")
liking$Product = as.factor(liking$Product)
colnames(liking)[3:6] = c("DOL", "DOF", "DOA", "DOT")

DOL = lmer(Overall_liking ~ Product + (1|Panelist), data=liking)
anova(DOL)
rand(DOL)
emmeans(DOL, list(pairwise ~ Product))

DOF = lmer(Flavor_liking ~ Product + (1|Panelist), data=liking)
anova(DOF)
rand(DOF)
emmeans(DOF, list(pairwise ~ Product))

DOA = lmer(Appearance_liking ~ Product + (1|Panelist), data=liking)
anova(DOA)
rand(DOA)
emmeans(DOA, list(pairwise ~ Product))

DOT = lmer(Texture_liking ~ Product + (1|Panelist), data=liking)
anova(DOT)
rand(DOT)
emmeans(DOT, list(pairwise ~ Product))

## Wilcoxon rank sum (Kruskal-Wallis) test
kruskal.test(Rank ~ Product, data=liking)
pairwise.wilcox.test(liking$Rank, liking$Product)
```

PLS

```
liking_mean = liking[ , -1] %>%
  group_by(Product) %>%
  summarise_all(mean)
descriptive_mean = descriptive[ , -c(1:3)] %>%
  group_by(Product) %>%
  summarise_all(mean)

df = merge(liking_mean, descriptive_mean, by="Product")
dol_df = df[ , -c(1, 3:6)]
rownames(dol_df) = c("COM-HO", "COM-VO", "CREAM", "Soyleic")
```

```
dol_pls = plsr(DOL ~ ., data = dol_df, scale = TRUE)
summary(dol_pls)
validationplot(dol_pls)
coef(dol_pls)
biplot(dol_pls, which = c("x", "y", "scores", "loadings"), var.axes = TRUE)
plot(dol_pls, plottype = "correlation")

### PLS Correlation Plot ###
## begin
S_plsr = scores(dol_pls)[, comps = c(1,2), drop=FALSE]
c1_plsr = cor(model.matrix(dol_pls), S_plsr)

df_cor = as.data.frame(c1_plsr)

df_depend_cor <- as.data.frame(cor(dol_df[,1], S_plsr))
rownames(df_depend_cor) = "DOL"

plot_loading_correlation  <-  rbind(df_cor, df_depend_cor)

plot_loading_correlation1 <- setNames(plot_loading_correlation, c("comp1", "comp2"))

#Function to draw circle
circleFun <- function(center = c(0,0),diameter = 1, npoints = 100){
  r = diameter / 2
  tt <- seq(0,2*pi,length.out = npoints)
  xx <- center[1] + r * cos(tt)
  yy <- center[2] + r * sin(tt)
  return(data.frame(x = xx, y = yy))
}

dat_plsr <- circleFun(c(0,0),2,npoints = 100)

ggplot(data=plot_loading_correlation1 , aes(comp1, comp2))+
  ylab("")+xlab("")+ggtitle(" ")+
  theme_bw() +
  geom_hline(aes(yintercept = 0), size=.2, linetype = 3)+
  geom_vline(aes(xintercept = 0), size=.2, linetype = 3)+
  geom_text_repel(aes(label=rownames(plot_loading_correlation1),
                  colour=ifelse(rownames(plot_loading_correlation1)!='dependent',
'red','darkblue')))+
  scale_color_manual(values=c("red","darkblue"))+
  scale_x_continuous(breaks = c(-1,-0.5,0,0.5,1))+
  scale_y_continuous(breaks = c(-1,-0.5,0,0.5,1))+
  coord_fixed(ylim=c(-1, 1),xlim=c(-1, 1))+xlab("PLS (X1:65.42%, Y1:92.20%)")+
  ylab("PLS (X2:17.36%, Y2:7.71%)")+ theme(axis.line.x =
element_line(color="darkgrey"),
```

```
                    axis.line.y = element_line(color="darkgrey"))+
  geom_path(data=dat_plsr ,
          aes(x,y), colour = "darkgrey")+
  theme(legend.title=element_blank())+
  theme(axis.ticks = element_line(colour = "black"))+
  theme(axis.title = element_text(colour = "black"))+
  theme(axis.text = element_text(color="black"))+
  theme(legend.position='none')+
  theme(panel.grid.minor = element_blank()) +
  geom_segment(data=plot_loading_correlation1, aes(x=0, y=0, xend=comp1,
yend=comp2),
            arrow=arrow(length=unit(0.2,"cm")), alpha=0.75,
            colour=ifelse(rownames(plot_loading_correlation1)=='dependent',
'red','darkblue'))
## end

par(mfrow = c(2,2))
biplot(dol_pls, which = "x", var.axes = TRUE)
biplot(dol_pls, which = "y", var.axes = TRUE)
biplot(dol_pls, which = "scores", var.axes = TRUE)
biplot(dol_pls, which = "loadings", var.axes = TRUE)
```

Preference mapping

```
DOL_df = liking[, 1:3]
DOL_df$DOL = as.integer(DOL_df$DOL)
DOL_transpose = spread(DOL_df, Panelist, DOL)

preference_df = merge(descriptive_mean, DOL_transpose, by="Product")
rownames(preference_df) = c("COM-HO", "COM-VO", "CREAM", "SOYLEIC")

## Internal Preference Mapping
DOL_in = DOL_transpose[,-1]
rownames(DOL_in) = c("COM-HO", "COM-VO", "CREAM", "SOYLEIC")

in_pref = PCA(DOL_in, graph = FALSE)
fviz_pca_biplot(in_pref, axes = c(1,2), geom.ind = "text", geom.var = "point",
            col.var = "black", col.ind = "black", repel = TRUE) + geom_point(shape = 5)

## External Preference Mapping
senso.data = preference_df[, 2:18]
hedo.data = preference_df[, 19:100]
res.pca = PCA(senso.data, graph = FALSE)
carto(res.pca$ind$coord[,1:2], hedo.data, regmod = 3)
```

Descriptive sensory data for HIPE formulated ice cream

```
SFD_DS = read_excel("SFD2_descriptive.xlsx")
SFD_DS[, 'replication'] = NA
SFD_DS$replication[SFD_DS$session==1] = 1
SFD_DS$replication[SFD_DS$session==2] = 1
SFD_DS$replication[SFD_DS$session==3] = 2
SFD_DS$replication[SFD_DS$session==4] = 2
SFD_DS$replication[SFD_DS$session==5] = 3
SFD_DS$replication[SFD_DS$session==6] = 3
SFD_DS = subset(SFD_DS, panelist!=2)
SFD_DS$panelist=as.factor(SFD_DS$panelist)
SFD_DS$session=as.factor(SFD_DS$session)
SFD_DS$order=as.factor(SFD_DS$order)
SFD_DS$product=as.factor(SFD_DS$product)
SFD_DS$replication=as.factor(SFD_DS$replication)

ds_summary_all = SFD_DS[ , -c(1:3, 17)] %>%
  group_by(product) %>%
  summarise_all(mean)
print.data.frame(ds_summary_all)
```

PCA

```
X = ds_summary_all[, -1]
rownames(X) = c("CONTROL", "SPI", "H-AGG", "HIPE")
DA.pca = PCA(X, graph = FALSE)
fviz_pca_biplot(DA.pca, col.var = "blue", col.ind = "black", repel = TRUE)
fviz_pca_biplot(DA.pca, axes = c(2,3), col.var = "blue", col.ind = "black", repel = TRUE)
fviz_pca_biplot(DA.pca, axes = c(1,3), col.var = "blue", col.ind = "black", repel = TRUE)
```

Mixed model ANOVA

```
DA.mixed.hardness = lmer(hardness ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                    data=SFD_DS, REML = TRUE)
anova(DA.mixed.hardness)
rand(DA.mixed.hardness)

DA.mixed.melt = lmer(melt_rate ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                 data=SFD_DS, REML = TRUE)
anova(DA.mixed.melt)
rand(DA.mixed.melt)

DA.mixed.iciness = lmer(iciness ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                    data=SFD_DS, REML = TRUE)
anova(DA.mixed.iciness)
```

```
rand(DA.mixed.iciness)

DA.mixed.cream = lmer(creaminess ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                 data=SFD_DS, REML = TRUE)
anova(DA.mixed.cream)
rand(DA.mixed.cream)

DA.mixed.mouth = lmer(mouth_coat ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                 data=SFD_DS, REML = TRUE)
anova(DA.mixed.mouth)
rand(DA.mixed.mouth)

DA.mixed.smooth = lmer(smoothness ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                  data=SFD_DS, REML = TRUE)
anova(DA.mixed.smooth)
rand(DA.mixed.smooth)

DA.mixed.gum = lmer(gumminess ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                data=SFD_DS, REML = TRUE)
anova(DA.mixed.gum)
rand(DA.mixed.gum)

DA.mixed.dense = lmer(denseness ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                 data=SFD_DS, REML = TRUE)
anova(DA.mixed.dense)
rand(DA.mixed.dense)

DA.mixed.sweetness = lmer(sweetness ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                 data=SFD_DS, REML = TRUE)
anova(DA.mixed.sweetness)
rand(DA.mixed.sweetness)

DA.mixed.soy = lmer(soy ~ product + (1|panelist) + (1|replication) + (1|product:panelist)
+ (1|product:replication) + (1|panelist:replication),
                    data=SFD_DS, REML = TRUE)
anova(DA.mixed.soy)
rand(DA.mixed.soy)

DA.mixed.sweet_after = lmer(sweet_after ~ product + (1|panelist) + (1|replication) +
(1|product:panelist) + (1|product:replication) + (1|panelist:replication),
```

```
                       data=SFD_DS, REML = TRUE)
anova(DA.mixed.sweet_after)
rand(DA.mixed.sweet_after)

DA.mixed.soy_after = lmer(soy_after ~ product + (1|panelist) + (1|replication) + (1|product:panelist) + (1|product:replication) + (1|panelist:replication),
                         data=SFD_DS, REML = TRUE)
anova(DA.mixed.soy_after)
rand(DA.mixed.soy_after)
```

VITA

Yun Wang was born in Shanghai, China. She earned her bachelor's degree in Bioengineering from East China University of Science and Technology in Shanghai, China and master's degree in Bioengineering with Dr. Fu-hung Hsieh from University of Missouri, Columbia, Missouri. After receiving her master's degree, she worked as a Research/Lab Technician for Dr. Bongkosh Vardhanabhuti in the Food Science Department at University of Missouri, Columbia, Missouri. She decided to pursue a higher degree in Food Science area. In 2018, she entered the Food Science doctoral program from University of Missouri, Columbia, Missouri under the direction of Dr. Bongkosh Vardhanahbuti. While studying Food Science major, she obtained a minor in Statistics. She completed her internship at Tate & Lyle, Hoffman Estates, IL in the summer of 2022.