USAGE OF MULTIPLE EMULSIONS TO DESIGN LOW-FAT ICE CREAM

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ABSTRACT

USAGE OF MULTIPLE EMULSIONS TO DESIGN LOW FAT ICE CREAM

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The primary objective of this thesis was to design similarly perceived low fat ice cream with regular fat ice cream by using multiple emulsions. Thus, it was aimed to prepare multiple emulsion formulations by using food grade stabilizers and emulsifiers and to reduce the amount of fat in ice cream considerably. With the use of polyglyserol poliricinoleate (PGPR) and PGPR-lecithin blend as emulsifier in the primary phase and different gums such as guar gum (GG), locust bean gum (LBG) and gum tragacanth (GT) and their blends as stabilizer in the secondary phase, the most stable multiple emulsions were formed.

There was no difference between multiple emulsions prepared with PGPR-lecithin blend and only PGPR in terms of particle size, rheological properties and stability. Different gum formulations affected the emulsion characteristics in different way. Guar gum (1%) and GG-GT (0.75%-0.25%) were chosen since they provided good emulsions with acceptable particle size, apparent viscosities and stability values. Ice cream samples prepared accordingly and PGPR-lecithin and GG-GT blends had higher overrun values, better meltdown resistance and higher overall acceptability in sensory analysis.

Reduced fat and regular fat containing ice cream prepared with conventional method were compared with ice cream prepared with multiple emulsion method in terms of rheological properties, overrun, melt-down resistance and sensory properties. Reduction of fat to 2.82% by means of double emulsion was possible without affecting ice cream quality adversely.

Keywords: Multiple emulsions, reduced fat, ice cream

ÖZ

ÇOKLU EMÜLSİYONLAR KULLANILARAK AZ YAĞLI DONDURMA TASARIMI

Tekin, Ezgi
Yüksek Lisans, Gıda Mühendisliği Bölümü
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Bu tezin ana amacı, çoklu emülsiyon yöntemiyle normal yağlı dondurma ile duyusal olarak benzer algılanan az yağlı dondurma tasarlamaktır. Böylece, gıda bazlı stabilizatör ve emülgatör kullanılarak çoklu emülsiyon formülasyonları hazırlamak ve dondurmada yağ miktarını önemli ölçüde azaltmak hedeflenmiştir. İlk fazda poligliserol polirisinolat esterleri (PGPR) ve PGPR-lesitin karışımı kullanılarak ve ikinci fazda ise guar zamkı, keçiboynuzu zamkı ve kitre zamkı ve bunların karışımları kullanılarak en stabil çoklu emülsiyon hazırlanmıştır.

PGPR-lesitin karışımı kullanılarak hazırlanan çoklu emülsiyonların parçacık boyutu, reolojik özellikleri ve stabilite değerleri açısından sadece PGPR kullanılarak hazırlanan emülsiyonlar arasında bir farklılık gözlemlenmemiştir. Farklı gum formulasyonları emülsiyon özelliklerini farklı şekilde etkilemiştir. Guar zamkı (1%) ve GG-GT (0.75%-0.25%) karışımı kabul edilebilir parçacık boyutu, viskozite ve stabilite değerleri ile iyi emülsiyon oluşturdukları için seçilmiştir. Hazırlanan dondurma örneklerinden PL-GGGT, daha yüksek hava içeriği, erime direnci ve yüksek kabul edilebilirlik değerlerine sahiptir.

Geleneksel yöntemle hazırlanmış yağı azaltılmış dondurma ve normal yağlı dondurma, çoklu emulsiyon yöntemi ile yağı azaltılmış dondurma ile reolojik özellikler, hava içeriği, erime direnci özelliği ve duyusal analizler açısından karşılaştırılmıştır. Çoklu emülsiyon sayesinde dondurma kalitesini olumsuz yönde etkilemeden yağ oranının %2.82'ye düşürülmesi mümkün olmuştur.

Anahtar kelimeler: Çoklu emülsiyon, yağı azaltılmış, dondurma

To all new challenges...

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INTRODUCTION

1.1 Multiple Emulsions

The multiple emulsions are named as ‘emulsions of emulsions’ where the coexisting of both phases of single emulsions namely water-in-oil and oil-in-water takes place (Garti, 1997a). Such complex polydispersed system was firstly described by William Seifriz (1925). Since then, the formulations, stability, characterization and extension of application areas have been studied in detail. Nevertheless, there is still need to study multiple emulsion concept and possible applications due to inadequate number of researches.

The concept of multiple emulsions is based on dispersing a single emulsion into another phase. There are two main types of multiple emulsions which are water-in-oil-in-water (W/O/W) and oil-in-water-in-oil (O/W/O) (Figure 1.1). There is a dispersed phase as well as continuous phase for each type of multiple emulsions. For the case W/O/W, oil droplets containing smaller water droplets are dispersed in continuous water phase. Likewise, small oil globules are entrapped in the first water phase and these oil-water globules are further dispersed in continuous oil phase to form O/W/O type of multiple emulsions (Benichou et al., 2004).

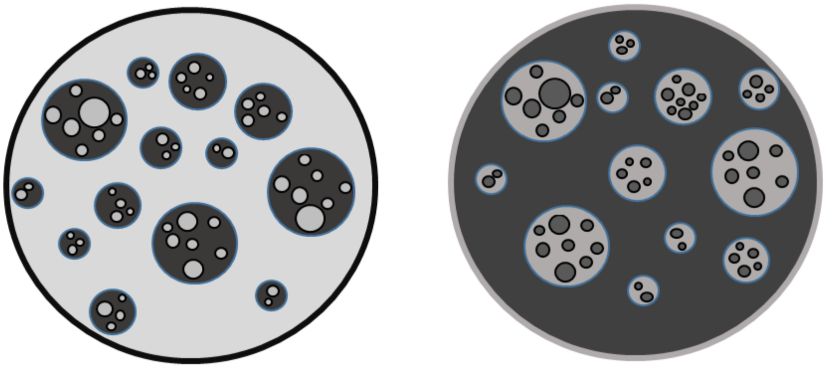


Figure 1.1 Schematic illustration of multiple emulsions: W/O/W and O/W/O

As already known for single emulsions, multiple emulsions and so called double emulsions are thermodynamically unstable systems. It is the result of cohesive forces being greater than the adhesive forces between the liquids that are accumulated as excess free energy at the surface of the droplet (Aserin, 2007). This can be explained from Equation 1.1.

$$G = \gamma \times \Delta A \tag{1.1}$$

where G, γ, A represent the interfacial free energy (kj), the interfacial tension (kj/m^2) and the total area of dispersed phase (m^2), respectively. If the interfacial area increases, system gains tendency to turn back to two separate emulsions to reduce the area of the interface. In other words, the droplets merge and destruct the emulsion system.

1.2 Application Areas of Multiple Emulsions

Throughout the investigation of multiple emulsions, application areas have been extended by many authors due to their achievements in the field of pharmacy and cosmetics. Their applicability in the food products was shown to be promising by some authors (Garti, 1997b; Muschiolik, 2007; Sapei et al., 2012). Double emulsions are generally used for the controlled release of sensitive and active ingredients from the inner to the outer phase (Benichou et al., 2004). Encapsulation of compounds and microcapsule production are the major applications of the multiple emulsions (Vladisavljević & Williams, 2005; Vladisavljević, 2015).

Besides, Dickinson stated two areas for the application of double emulsion in the food systems. One is encapsulation of sensitive and valuable compounds and the other is reduction of fat content to propose healthier food products to consumer (Dickinson, 2011). In food applications, encapsulation of vitamins (Benichou et al., 2006; O'Regan & Mulvihill, 2009), minerals (Marquez & Wagner, 2010), and probiotics (Zhang et al., 2015) have been studied. However, there have been only few attempts to reduce the fat content of the food products by using multiple emulsions. Lobato-Calleros and colleagues (2008) studied the structural and textural properties of reduced fat cheese, while Marquez & Wagner (2010) formulated whipped cream with low fat content, and Cofrades and colleagues (2013) designed reduced fat pork meat by using multiple emulsions.

Overall, multiple emulsions have been utilized to encapsulate minerals, vitamins, unwanted flavors, sensitive or valuable compounds as well as to reduce fat, sugar, and salt content of the products throughout the investigation in the field of food. However, there have been some challenges about the fat reduction concept due to limited stability and the sensorial inadequacy in the final products. Hence, there should be new approaches to solve the existing problems and to propose new strategies for the progress of the multiple emulsions formulations.

1.3 Preparation Techniques of Multiple Emulsions

There are various techniques used for the preparation of the multiple emulsions depending on the type of apparatus utilized as well as the approach used.

1.3.1 Solvent Evaporation Method

Solvent evaporation technique (Figure 1.2) is one of the main preparation methods widely applied in the pharmacy, especially in drug delivery systems (Garti & Bisperink, 1998). In this particular method, the internal phase of the emulsion is evaporated. There is no elevation in temperatures and no need to separate the phases. In the stepwise approach, first biodegradable polymers are dissolved in organic solvent and then the drug is introduced into the solution of the organic polymer. After that, the organic phase is emulsified in the second continuous phase. This step determines the size of the droplet and affects the effectiveness of the encapsulation, release matter and the yield greatly. In order to strengthen the particle or the droplet, organic solvent is evaporated. The evaporation is facilitated by means of pressure reduction, temperature elevation and mixing conditions. Further, the recovery and drying are performed to finalize the procedure. This can be achieved by centrifugation, simple filtration or ultrafiltration depending on the purpose (Naik et al., 2012).

1.3.2 Phase Inversion Method

It is known as one step emulsification. In phase inversion method, there is a ‘mesophase’ between O/W (oil-in-water) and W/O (water-in-oil) which can be manipulated in the presence of non-ionic surfactant. Phase inversion can be categorized as transitional and catastrophic. In the transitional phase inversion, there is a reversal of affinity of the emulsifier that is triggered by temperature change, amount of salt in the aqueous phase and also polarity of organic phase. The phase inversion temperature (PIT) is of concern at which the HLB (hydrophilic-lipophilic balance) values are equilibrated at the interface and inversion takes place as well. Catastrophic phase inversion happens when the volume fractions of dispersed and continuous phase change. In other words, the increase in the volume fraction of dispersed phase will result in a sudden phase inversion (Morais et al., 2008). These inversion types are illustrated in Figure 1.3.

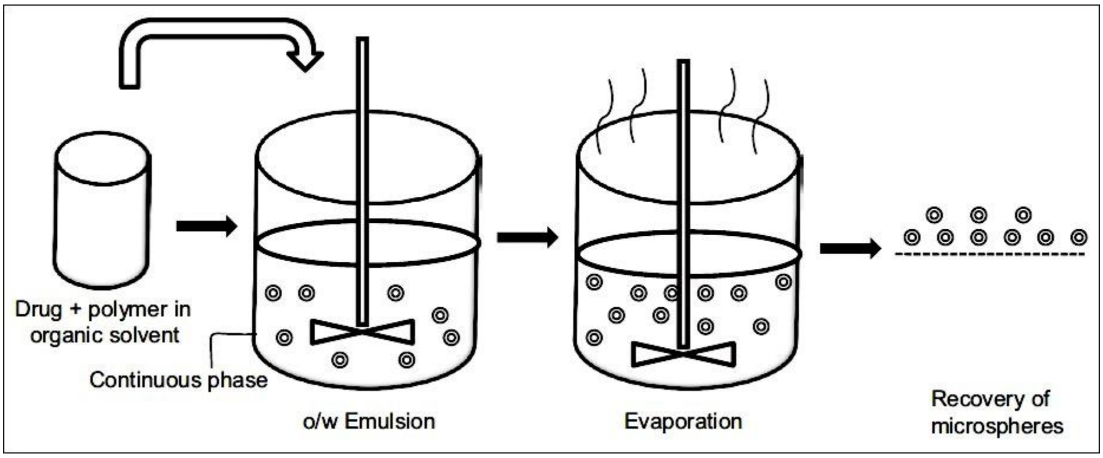


Figure 1.2 Solvent evaporation method to obtain microcapsules (Suresh et al. , 2014)

Upon dilution with water, Fernandez et al. (2004) observed that phase inversion took place. In addition to that, Forgiarini et al. (2001) detected the phase inversion by looking at the conductivity values. With the rise of the temperature, the conductivity increased slightly and instantly and then it decreased at a point where phase was inverted from O/W to W/O. The control of the phase inversion is so difficult that this can be considered as a disadvantage of this technique for double or single emulsions formation (Garti, 1997b).

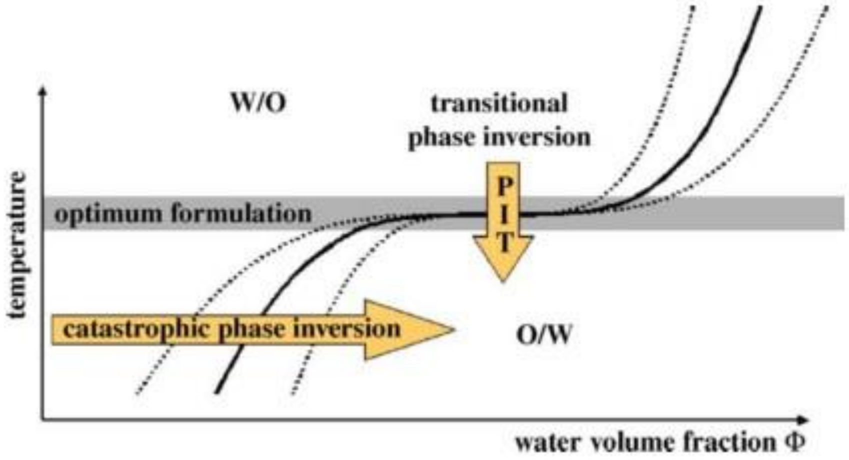


Figure 1.3 Catastrophic and transitional phase inversion illustration (Fernandez et al. 2004).

1.3.3 Two Step Emulsification

Another emulsification method well worth to mention is the two step emulsification (Figure 1.4) which requires two sets of surfactants. Although droplets formed by this method result in larger globule size, it provides better controlled preparations of double emulsions. As a first step of preparation of the W/O/W type of double emulsion, primary emulsion (W/O) is formed by adding aqueous phase into oil with lipophilic emulsifier under high shear conditions in order to achieve stable small droplet formation. After homogenization of the primary phase, it is gently mixed with the secondary water phase including hydrophilic surfactant under milder conditions.

This technique is widely used when forming double emulsions because this technique is easy to follow, applicable, controllable and straightforward. By two-step emulsification, Leal-Calderon (2012) studied the feasibility to obtain low oil content with high internal droplet fraction in multiple emulsions, Weiss et al.(2005), investigated the release of encapsulated hydrophilic compounds with polysaccharide gel. Pays et al.(2002), pointed out the NaCl release mechanism by setting equal osmotic pressure for both phases.

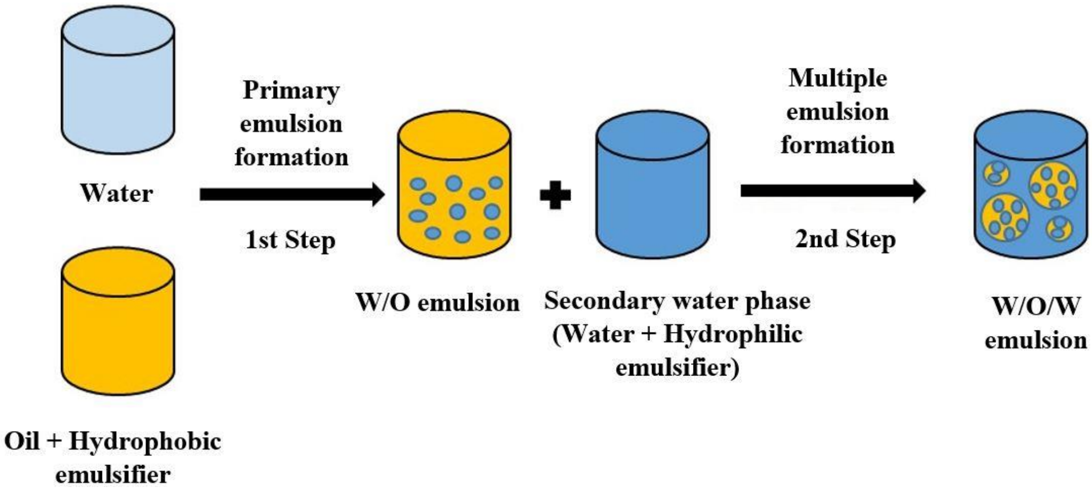


Figure 1.4 Schematic view of two step emulsification method to form W/O/W type of multiple emulsions.

1.3.4 Membrane Emulsification

Among different emulsification methods, membrane emulsification (Figure 1.5) is relatively a recent method. It has a great potential if monodisperse nature and narrow size of the droplets are maintained so that the productivity is increased. Basically, membrane emulsification can be performed either by premix or cross-flow. In premix emulsification, a coarse emulsion which is previously prepared is subjected to a membrane in order to miniaturize the droplets. The passage through the membrane is repeated for the need of the specific droplet size range. In other words, premix emulsification breaks up droplets into smaller droplets. This method is used when the dispersed phase is higher in concentration. Although it is applied many times to the formed emulsions, it is a single stage operation. For the case of cross-flow emulsification, dispersion of one phase into another phase is achieved with the help of a membrane. Upon the formation of a droplet over the membrane, it is flowed away. Unlike premix emulsification, cross-flow emulsification does not require a formed double emulsion. It just needs separated phases like secondary water phase and a primary (water-in-oil) phase or secondary oil phase with a primary (oil-in-water) phase.

The methodology of the membrane emulsification is based on the membrane type, mean pore size and porosity, crossflow velocity, transmembrane pressure and lastly emulsifier type. Also, interfacial forces and shear stress due to wall have also great importance when considering membrane emulsification. Moreover, the droplet formation regime is dependent on the wetting properties of the membrane surfaces (Lambrich & Schubert, 2005).

The advantages of the membrane emulsification are the monodisperse nature of the formed emulsion droplets, the lower energy consumption during emulsification, and narrower droplet size distributions. On the other hand, lower productivity for larger scale applications restricts the usage of membrane emulsification (Mohanty & Purkait, 2011).

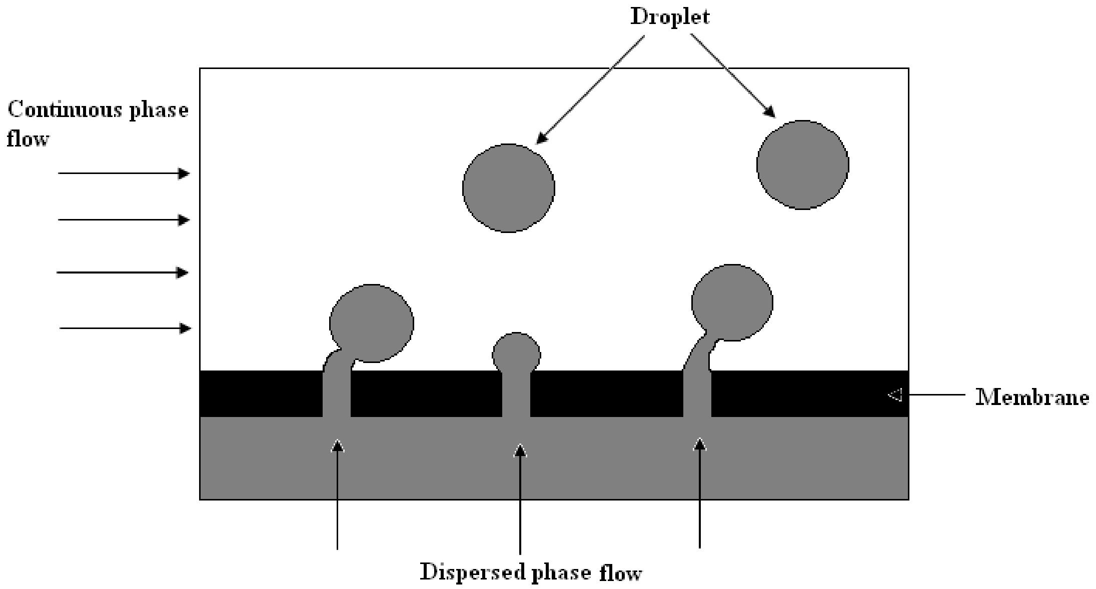


Figure 1.5 Membrane emulsification crossflow type (Sajjadi, 2006).

1.3.5 Microchannel Emulsification

One of the most commonly used devices for the fabrication of the multiple emulsions is microchannel device (Figure 1.6) which results in monodisperse droplets in the resultant emulsions with a controlled droplet size. It is also known as micro fabricated channel array. It is generally used as a secondary stage for the emulsification process where the premix is introduced to the secondary phase of the multiple emulsions. As in the case of membrane emulsification, the rate of production is very low for microchannel emulsification. Although this method produces lower rate of droplet formation compared with other emulsification methods except membrane emulsification, the usage of this method has been increasing due to less energy loss during production and narrow droplet size distribution at the end (McClements, 2005). Sugiura et al. (2002) found that the longer and narrower channel led to pressure drop during detachment of the droplets which shortened time of detachment and further resulted in the better emulsion fabrication rates.

T-junction is one particular type of microchannel emulsification method to produce desirable double or single emulsion. This device is designed as two different channels which are perpendicular to each other, so it is recognized like a T-shape. In each of the channels, the dispersed and continuous phases are introduced separately for the formation of the emulsion. The emulsion can either be single or multiple as in the case for the other methods. The resulting droplets are determined by the flow regime, detachment time and channel dimensions (Steegmans et al., 2009). Moreover, there are several T-junction models proposed by different researchers for different flow regimes of the phases.

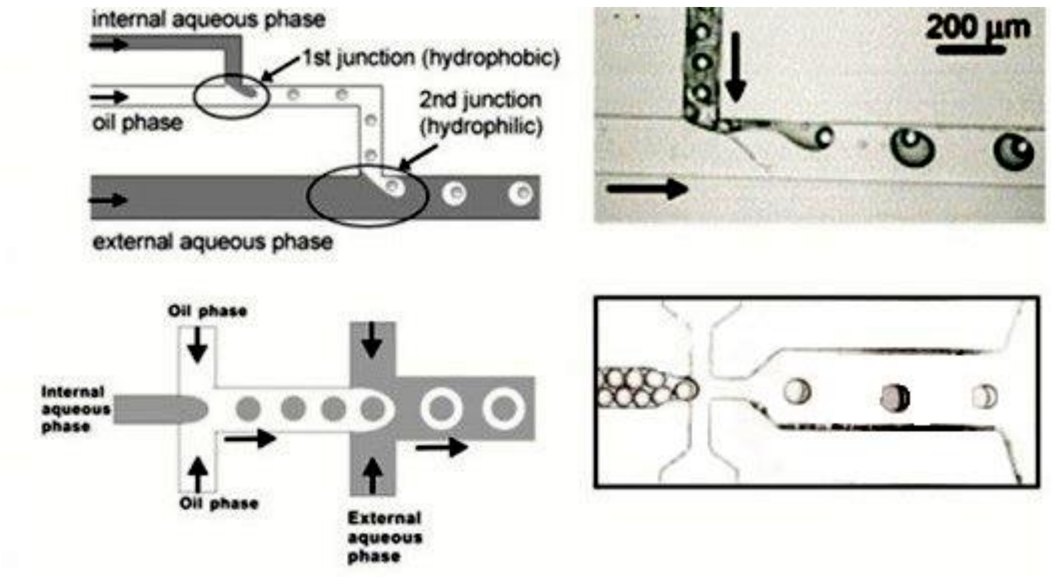


Figure 1.6 Illustration of emulsion formation by microchannel devices.

1.3.6 Emulsification via microcapillary devices

Coaxial assemblies of glass capillaries on glass slides generate a microcapillary device (Figure 1.7) so that their wettability can be controlled via chemical used either hydrophobic or hydrophilic. Two capillaries are aligned coaxially such that one fluid is passing through the capillary and the other is flowing inside the square capillary with the same direction. The droplet size distribution changes according to the flow rate of the fluids (Shah et al., 2008). Kim et al. (2013) stated the possibility to enhance the capacity of the droplet formation of the capillary device at a given time by proposing the parallelized devices. In this study, they ended up with the acceptable monodispersed particles with high encapsulation efficiency and stable double emulsion drops. Furthermore, they provided that size distribution can be decreased via employing similar size of all orifices and shortening the distance between them.

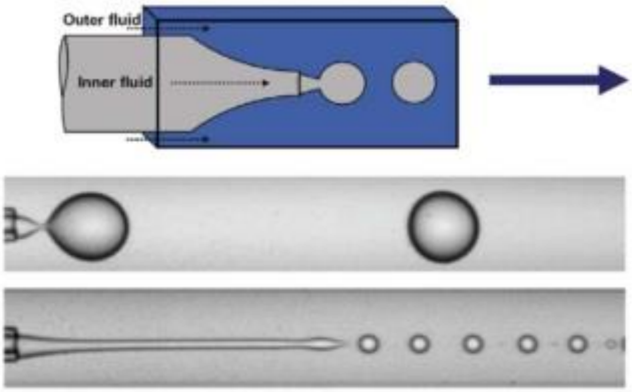


Figure 1.7 Schematic representation of microcapillary device and droplet formation illustration.

1.4 Homogenization Methods

There are several different kinds of homogenization devices used in the field of chemical, pharmaceutical and food industry. The most widely used devices are high speed mixers, high pressure homogenizers, rotor stator systems and ultrasound homogenizers.

1.4.1 High speed mixers

Generally the term high speed mixer covers high shear/speed homogenizers having highly centralized energy dissipation close to the mixing head (Zhang et al., 2012). Despite its wide use and easy control, the high speed mixers, shown in Figure 1.8, or high shear homogenizers does not reduce particle size much but reduce the polydispersity of the emulsion (Pinnamaneni et al., 2003). According to the study on the liquid-liquid emulsion, the droplet size was reduced by the intensity of the homogenization and thereby reached to the steady value (Maa & Hsu, 1996).

Typical usage of high shear homogenizer can be visualized in cosmetics, paint, food, pharmacy, and chemical industry as in line or batch wise. The narrow space is present as in the rotor stator systems to aid the generation of the shear and mechanical forces (Zhang et al., 2012). It is based on the mechanical agitation at high speed to homogenize the emulsions. Furthermore, it is generally used in the first step of the double emulsion formation.

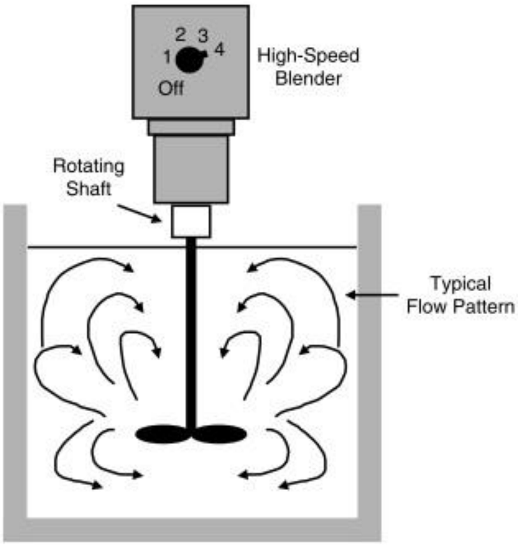


Figure 1.8 High speed blender/mixers (McClements, 2005)

1.4.2 High pressure homogenizers

It is also called microfluidizer which is composed of an inlet fluid, pump and two channels basically (McClements, 2005). The theory of the formation of extremely small droplets is built on the disruptive forces generated under high energy consumption (Qian & McClements, 2011). The schematic representation of the high pressure homogenizer is given in Figure 1.9.

Basically, a narrow gap and a high pressure pump exist in a microfluidizer. In microfluidizer; the fluid is passed through the homogenizers and velocity of the fluid is increased with the help of pump and then collision takes place at which droplets are disturbed. The study conducted by Qian & McClements (2011) suggested that small molecule emulsifiers tended to produce smaller droplets than biopolymers. Also, they stated that high viscous and water miscible co-solvent addition aided reduction of the droplets size.

The droplet formation and fragmentation were deeply studied in order to gain insight for understanding the emulsification process (Innings & Trägårdh, 2005; Hakanssons et al., 2011). They stated that the mechanism of action and the process parameters affected the final product properties.

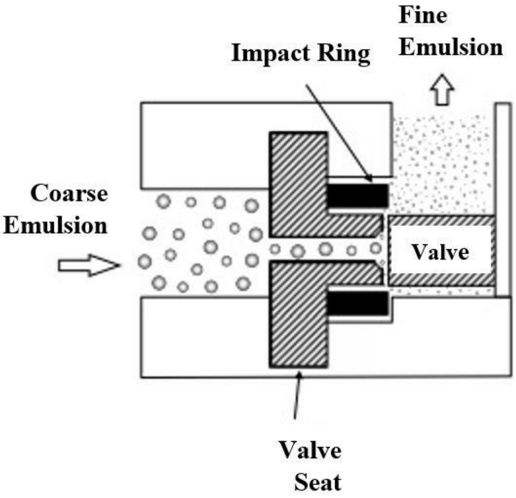


Figure 1.9 High pressure valve homogenizer (McClements, 2005).

The effect of the high pressure homogenization on the final emulsion quality was intensively studied. For instance, McCrae (1994) found out that the homogenization of milk by high pressure homogenizer resulted in smaller particles and high protein coverage on the fat particles which restricted the formation of fat clusters after microfluidization. The results were similar to the study of Dalgleish et al. (1996). Microfluidization technique has been widely used in dairy industry including ice cream (Olson et al., 2003; Cavender & Kerr, 2013), cheddar cheese (Lemay et al., 1994), mozzarella cheese (Hekken et al., 2007), fish oil enriched milk (McCrae, 1994; Let et al., 2007), and yogurt (Ciron et al., 2010).

1.4.3 Rotor stator systems

The mostly known systems are colloid mills as shown in Figure 1.10. Colloid mill contains two discs; one is rotating and the other one is static. An emulsion is fed into the center of the homogenizer, and high speed rotation produces the high shear force and mechanical collision that break up the droplets to reduce the size of the individual droplets. During processing, the centrifugal forces are generated because of the high rotational speed of the disc that pushes fluid from the center to the edge of the mill such that there is no need for extra pressure input. Moreover, the assembly of the two discs provides droplet disruption in between droplets are broken down into smaller particles.

The size of the gap between the rotor and stator, the surface of the discs, rotation speed can be adjusted to change the shear forces generated during processing. At high speed of rotations, the flow regime becomes turbulent where the power, and residence time is of concern for resulting droplet size (Maa & Hsu, 1996).

This technique usually used as secondary step for the emulsification because it is very effective in reducing the droplet size of previously formed emulsions called coarse emulsions. Additionally, this systems are widely used in the food industry for homogenization of the medium to high viscous fluids (McClements, 2005). However, it is limited in scale up because the assembly of the rotor and a stator make the internal circulation (Maa & Hsu, 1996).

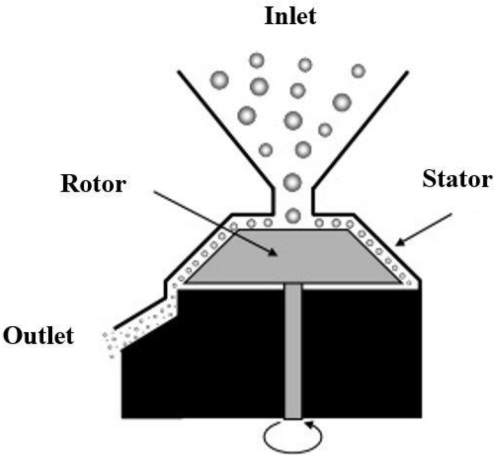


Figure 1.10 Colloid mill schematic representation (McClements, 2005).

1.4.4 Ultrasound homogenizers

Ultrasound homogenizers (Figure 1.11) have been readily utilized for the emulsion formation purposes (Wood & Loomis, 1927). Thereafter, it is used by food (Wu et al., 2000; Nassar et al., 2004; Ghosh et al., 2013) and pharmacy industry (Wyber et al., 1997; Tang et al., 2003).

It basically includes a generator, a converter and a probe to perform sonication. A generator is used to convert electrical current to the desired sonication level while the converter works as a transformer of energy to mechanical vibrations (Jafari et al., 2007).

The ultrasonic waves generate intense shear and pressure gradients. If it is applied to the water and oil mix, it helps liquids to intermingle and globules to be broken down into smaller ones. There are two types of ultrasonic homogenizers found in food usage; piezoelectric transducers and liquid jets. Piezoelectric transducers are generally used for small scale while the latter one is used in industries for larger volumes. There are many advantages for liquid jet homogenizer such as energy efficient, capable of producing small droplets and suitable for continuous production (Akoh & Min, 2008).

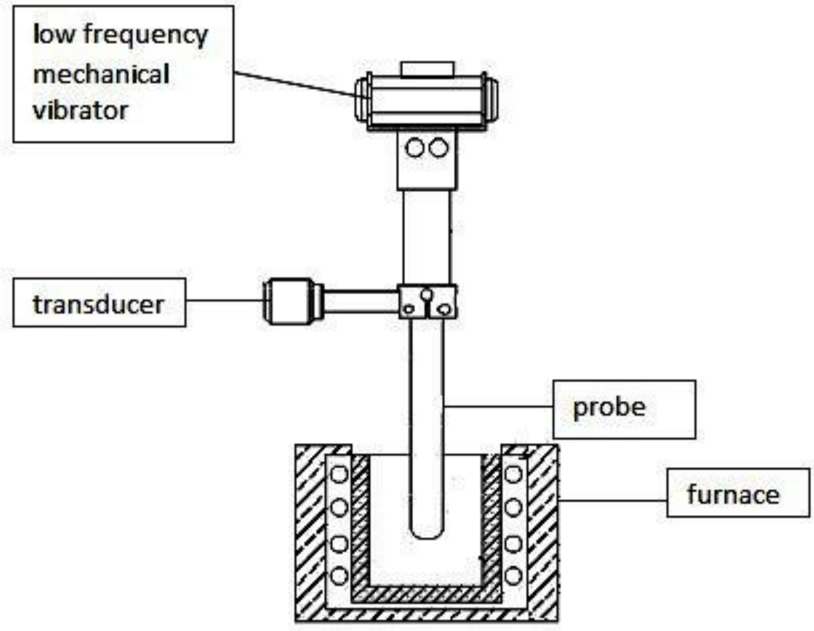


Figure 1.11 Ultrasound homogenizer ("Continuous and Static Casting with Ultrasound" , 2013).

1.5 Instability Mechanisms

Emulsions are broken down in various ways including creaming/sedimentation, flocculation, phase inversion, coalescence and Ostwald ripening. These mechanisms are the results of thermodynamically instable nature of the double emulsions. Also, continuous movement of particles due to Brownian motion increases the possibility of destabilization (Frasch-Melnik, 2011). The process of creaming/sedimentation starts with the motion of particles upward/downward due to density differences in particles and continuous phase. Flocculation of the droplets is caused by attractive forces existing between particles as well as collision of particles those results in aggregation. Phase inversion takes place when the continuous phase becomes dispersed phase and vice versa. Coalescence is the process of film disruption between droplets. Lastly, the Ostwald ripening, also known as disproportionation is the process in which small particles gets smaller while larger droplets becomes larger due to diffusion (Tadros, 2013). All that destabilization mechanisms further causes phase separation as shown in the Figure 1.12.

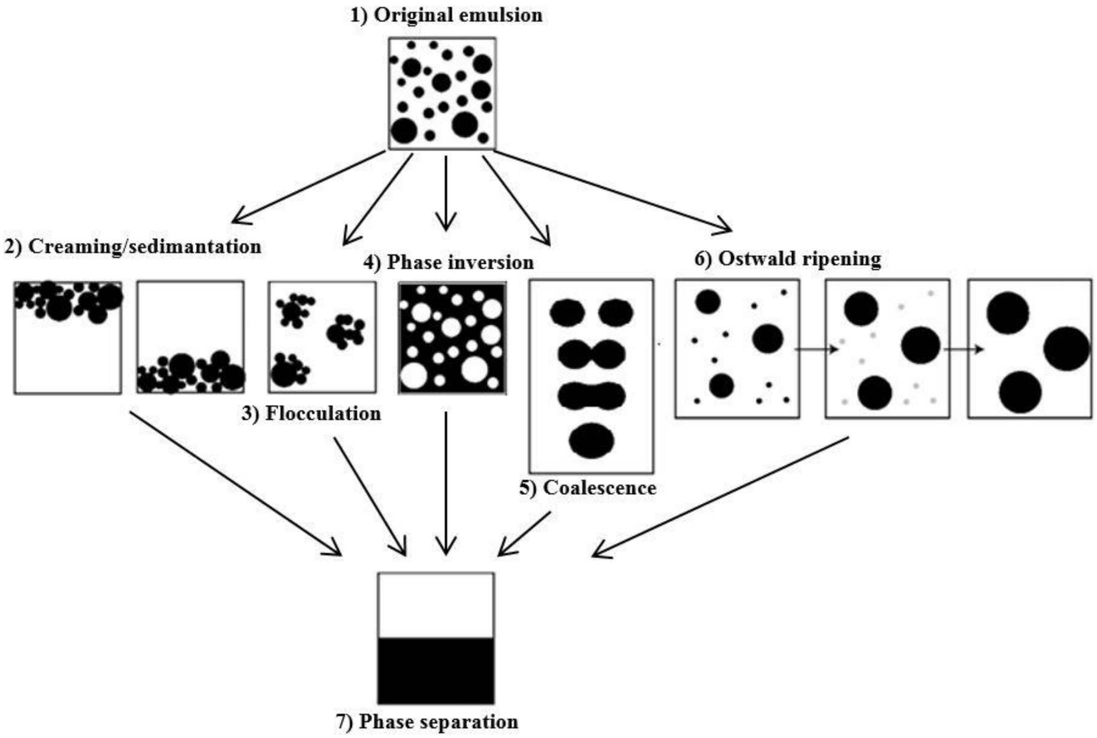


Figure 1.12 Mechanisms of instability of emulsions.

1.6 Emulsifiers & Surfactants

Emulsifiers are known to be the key elements in the formation of emulsions. Basically, they are composed of a hydrophobic tail and a hydrophilic head as shown in Figure 1.13. They are amphiphilic molecules that are located at the interfaces since they are not fully soluble in both oil and water phases (Robins, 2007). Their existence facilitates the formulations and stabilizations of emulsions, especially food emulsions. These molecules are also called as surfactants which contain both polar and non-polar parts that make them categorized as ionic, cationic, non-ionic, or amphoteric depending on the carried charges. Aionic surfactants have negative charges on the polar molecules whereas cationic surfactants have positive charges. Amphoteric surfactants composed of both the positive and negative charges appeared on the same molecule. Non-ionic surfactants unlike the amphoteric ones have no charges, but the dipole appears due to polar part of the molecule (Hasenhuettl & Hartel, 2008).

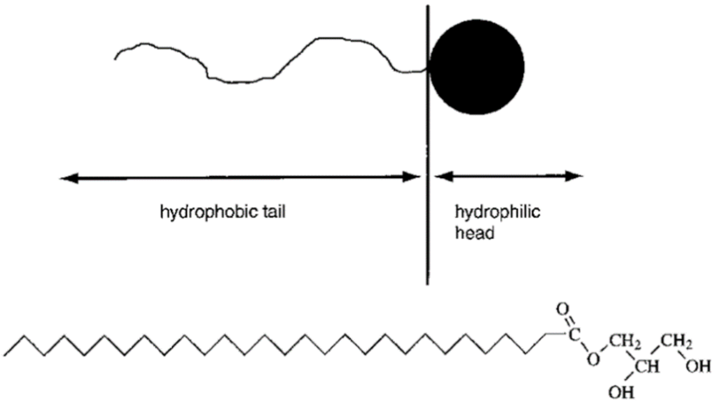


Figure 1.13 Basic structure of emulsifier (McClements & Weiss, 2005).

The functions of emulsifiers have long be investigated by many authors (Krog, 1977; Das & Kinsella, 1990; Dickinson, 1993; Wilde et.al., 2004). Emulsifiers act as surface active agents to reduce the interfacial tension between the phases. They have the ability to lower the tension. They interact with proteins (Dickinson, 2009), and/or carbohydrates (Rosel et.al., 2001) to enhance their physicochemical properties. Major functions of emulsifiers in the food systems are reducing surface tension, controlling emulsification and oil separation, reducing lubrication and stickiness, controlling viscosity of the medium, acting as a plasticizer, hydrating agent, crystal modifier, bloom inhibitor, release agent and foaming agent. In addition, they improve stability and sensory attributes, increase shelf life of products, shorten mixing time and reduce mixing tolerance of the emulsion systems (Hasenhuettl & Hartel, 2008).

In order to classify the type of emulsifiers hydrophile-lipophile balance (HLB) concept is uesd. This concept shows the affinity of the emulsifier for either oil or water phases (McClements, 2005). It determines whether the molecule is water dispersible or oil dispersible meaning that high HLB values are linked to water soluble while low HLB values are linked to oil soluble surfactants (Hasenhuettl & Hartel, 2008).

Lecithin, diacetyltartaric esters of monoglycerides (DATEM), mono and diglycerides, monosodium phosphate derivatives of mono and diglycerides are the common examples of emulsifiers used in food industry. They are confirmed as generally recognized as safe (GRAS) by Food and Drug Administration (FDA). Others like polysorbate 60, sorbitan monolaurate, sucrose esters of fatty acids and calcium stearoyl lactylate are categorized as direct food additives (Hasenhuettl & Hartel, 2008).

Lecithin is naturally occurring emulsifier found in egg yolk, and soybean oil with varying HLB value. Commercial lecithin contains varying phospghatidylcholin, phosphatidylinositol and phosphatidylethanolamine content which changes their behavior in the system (Frieberg et al., 2004).

As a hydrophobic emulsifier polyglycerol ester of polyricinoleic acid (PGPR) is commonly used in food industry very effectively (Wilson et al., 1998). It is used as viscosity modifier as well as moisture scavenger in chocolate industry (Hasenhuettl & Hartel, 2008).

In order to form double emulsions (W/O/W type); there is a need for secondary hydrophilic emulsifier/stabilizer or their blends. Proteins and hydrocolloids have been used to formulate stable emulsions in food industry (Koberstein-Hajda & Dickinson, 1996); Benichou et al., 2004) . Gums and gum exudates are also used as emulsifiers and stabilizer due to their ability to hold water and thicken the aqueous phase (Dickinson, 2003). In addition, some researchers showed that these gums enhanced product quality by controlling rheological and textural properties (Funami et al., 2005).

Guar gum is obtained from the seeds of the plant called *Cyamopsis tetragonoloba* which is very tolerant wild African species. It is a substitute of locust bean gum because it resembles in many aspects. They are both complex carbohydrate polymer of galactose and mannose but with a different ratios. Being a non-ionic stabilizer, guar gum is not pH dependent and also it has freeze-thaw stability. The usage of guar gum started in paper industry and has been spread to food industry. Ice cream, sauces, cake mixes cheese, fruit drinks and dressing are some of the examples of guar gum usage in food industry (Morris, 2010; Mudgil et al., 2014).

Locust bean gum, also known as carob gum, is widely used as stabilizer in many fields such as paper, pharmacy, cosmetics, textile and food. It is derived from carob tree or *Ceratonia silique* that cultivated in Mediterranean region. Gum is extracted from the yellow embryo or outer husk. It is shorter than guar gum in case of structure. It has been already used in ice cream, cheese, yogurt and meat products. It forms very good film such that it is used in painting in textile printing and paper industry (Prajapati et al., 2013; Barak & Mudgil, 2014).

Gum tragacanth is an exudate of *Astragalus gummifier* and it is a mixture of polysaccharides with calcium, magnesium and potassium. Gum tragacanth is a complex, highly branched, heterogeneous hydrophilic polysaccharide and anionic stabilizer. In addition, it is a natural gum and is in GRAS list. It is used in sauces, dressings, frozen desserts, bakery fillings and confectionary. It has been used as thickener, stabilizer and emulsifier in food systems. It consists of two separate fractions like water soluble (tragacanthic acid) and water swellable (bassorin). It was reported as gum tragacanth composed of fucose, xylose, galacturonic acid and methoxyl groups, and low proportions of arabinose and methoxyl/galacturonic acid. These components with high proportions result in higher viscosity (Verbeken et al., 2003; Mohammadifar et al., 2006; Cash & Caputo, 2010).

1.7 Ice Cream Manufacture

Ice cream is globally consumed frozen dairy dessert. Its history dates back to the first century when snow was used to cool food and beverages. Then, it had progressively evolved to become ice cream through the centuries.

Basically, ice cream mix iscomposed of seven compounds before it is frozen, namely fat, milk solid non-fat (MSNF), sweeteners, stabilizers, emulsifiers, water and flavors. After freezing air becomes the most important quality parameter. Together with the air, all ingredients make up the ice cream and its structure.

Dairy fat (cream or milk) or non-dairy fat has a vital role in ice cream structure and texture. Fat also contributes to sense of lubrication and richness in flavor. MSNF, derived from lactose, casein, whey proteins, minerals, vitamins, and other components comes from milk. Proteins are major determinants of the structure ice cream in terms of emulsification, whipping and water binding in the system. Initially, they aid air bubble formation in mix and due to its capacity to hold water; it increases the viscosity of the mix and retards meltdown time. Addition of sweeteners not only contributes its flavor but also improves texture and provides controlled hardness by depressing the freezing point of the mix. Stabilizers and emulsifiers have the greatest significance in the mixture due to their functions in the system. They built up body and texture; regulate ice crystal growth, form good stand up and melting resistance properties (Goff & Hartel, 2013).

Typical ice cream microstructure is comprised of ice crystals and air bubbles ranging from 20 μm to 100 μm, and fat globule size of 1μm to 0.1mm. Other ingredients sugar, polysaccharides, and milk proteins are called as ‘matrix’(Crilly et al., 2008).

Ice cream manufacture starts with the preparation of dry and liquid ingredients separately and blended in the tank to further pasteurize batch wise or continuously as illustrated in Figure 1.14. After pasteurization, mix is homogenized and cooled to refrigeration temperature for cool aging process which ranges from 2 hours to 24 hours. After aging, flavor or coloring agent addition is done prior to freezing to draw temperature (-5°C). During dynamic freezing, air is incorporated into the system to modify the structure of ice cream. The process is followed by the hardening of ice cream to finalize the structure as well as minimizing the changes occurring in structure during storage.

It is traditionally accepted that smooth texture with regular fat content ice cream is desirable in the market. However, trend towards the consumption of low fat ice cream as in the case of all types of foods is increasing. This is the result of more people giving attention to their diets for reducing the risk of health problems in today’s world. Hence, providing low fat food products having the same properties of regular fat is a challenging task.

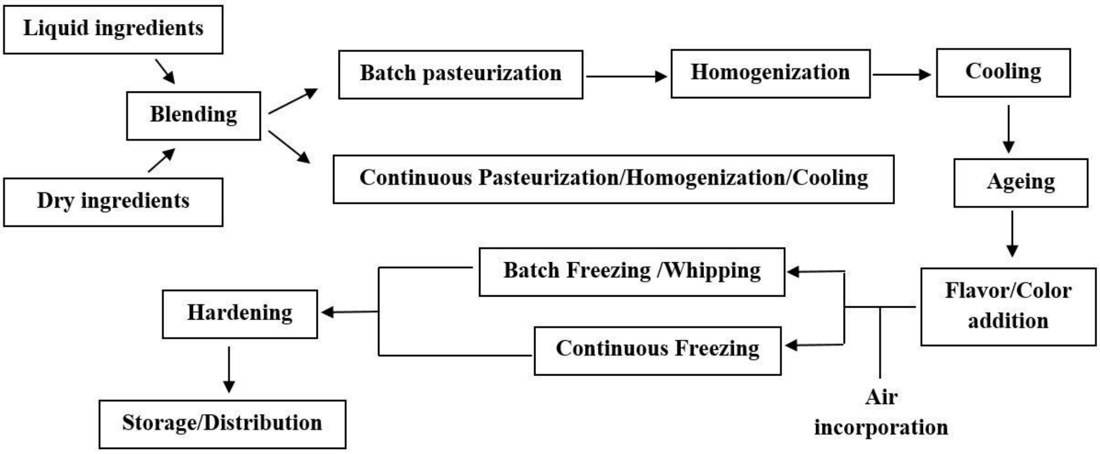


Figure 1.14 Ice cream production flow chart.

1.8 Aim of the Study

In the last years, the significant rise in the number of health problems throughout the world increased the tendency of consumption of low fat food products in diets. Beyond that, there are considerable beliefs and proofs about the cause that is related to the obesity of today's chronic diseases such as cardiovascular disease, diabetics, hypertension, and musculoskeletal system disorders. Since obesity is designated as the main reason of many diseases and it can be suppressed by reducing the usage of fats in diets, designing low calorie food products has a significant importance for the food industry.

By this time, there have been many attempts for fat reduction in some food systems by providing fat replacers or components of imitating fat. However, the limiting factor of the fat reduction in food systems is the sensory differences between the product with regular fat and reduced fat. Therefore, the majority of the concern is acquiring the equivalent properties of the targeted food system. Usage of multiple emulsions with food grade emulsifiers is proposed to be an alternative way to produce low calorie food products. However, multiple emulsions are instable and need to be stabilized by using surfactants having quotas in food systems that vary according to the specific food product. Due to limitations of surfactants, the studies with the use of multiple emulsions for fat reduction in food systems cannot go beyond other than meat, fresh cheese and whipped cream. In literature, there are limited studies on usage of multiple emulsions for reduction of fat in food systems. On the other hand, there is no study in literature about reducing the amount of fat content of ice cream by using multiple emulsions. The reason of choosing ice cream as a target product in this particular study is not only its being an extensively consumed product among the frozen dairy desserts, but also its complex structure to be studied more broadly. One of the most commonly used categorization of ice cream is based on its varying fat content. The regular fat ice cream usually contains 10-12% fat, higher fat ice cream (i.e. premium type) with a 12-16% fat, low-fat 5% or non-fat ice cream with a fat content of 2% are the major types of the industrially produced ice cream. In principle, ice cream is a complex network of fat, ice crystals, air bubbles and matrix that are the major parts constituting the oil-in-water emulsion. All these provide a basis of the application of the multiple emulsions for the purpose of the fat reduction in ice cream.

The primary objective of this study was to design similarly perceived low fat ice cream as regular fat ice cream by using multiple emulsions. In this manner, it was aimed to prepare multiple emulsion formulations by using food grade stabilizers and emulsifiers and to reduce remarkable amount of fat in ice cream. With the use of polyglycerol poliricinoleate (PGPR) and lecithin in the primary phase and different gums and their blends as stabilizers in the secondary phase, the most stable multiple emulsion was formed. In addition, the most appropriate phase ratios were designated by the preliminary experiments. Entirely, the best formulation with the longest stability, the smallest particle size, and the best rheological properties were addressed to suggest formulation of double emulsified ice cream product.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

Sunflower oil was purchased from Yudum Gıda San. ve Tic. A.Ş. (Ataşehir, İstanbul). Polyglycerol polyricinoleate (PGPR) was obtained from ETİ Gıda San. ve Tic. A.Ş. (Eskişehir, Turkey). Soy lecithin was supplied from LIPOID GmbH (Ludwigshafen, Germany). Calcium chloride, guar gum, gum tragacanth and locust bean gum (from *Ceratonia siliqua* seeds) were purchased from Sigma Aldrich Chemical Co. (St Louis, MO, USA). Non-fat milk powder was purchased from Makeks Makina Gıda San. Dis. Tic. Ltd. Şti. (Üsküdar, İstanbul, Turkey). It was used to formulate ice cream to get proper milk solids as in the standard ice cream formulations. Table sugar was taken from Türkiye Şeker Fabrikaları A.Ş. (Turkey) to provide sweetness in the ice cream. Vanilla flavor was supplied from Dr. Oetker Gıda San ve Tic. A.Ş. (İzmir, Turkey) and it was used for flavoring in the ice cream formulations.

2.2 Methods

2.2.1 Double Emulsion Preparation

Two step emulsification method was used to form double emulsions. Calcium chloride ($CaCl_2$) was mixed with distilled water using magnetic stirrer (Heidolph MR 3001 K, Heidolph Instruments GmbH Co, Schwabach, Germany) at a rate of 700 rpm for 1 hour at room temperature to obtain stock solution at a concentration of 1%

(w/w) to be used as (W1) phase. Oil (O) phase, which was the exterior part of the single emulsion (W1/O), was prepared by mixing lipophilic emulsifier with sunflower oil. As emulsifier, 1.5% (w/w) PGPR + 0.5% (w/w) lecithin or 2% (w/w) PGPR was used. The solution was pre-homogenized with high speed homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia) for 15 s at 5000 rpm prior to putting into the water bath at 50°C with shaking at 70 rpm for 15 min. For the second water (W2) phase, three different gums including guar gum (GG), locust bean gum (LBG) and gum tragacanth (GT) with different combinations were used. Total gum concentration in W_2 phase was 1% except 0.75% GG + 0.50% GT. The concentrations of each gum used in different formulations were given in Table 2.1. The gum solutions were prepared by dissolving the appropriate amounts of gums in distilled water with the help of magnetic stirrer (Are Heating Magnetic Stirrer, Velp Scientifica, Usmate (MB), Italy) used at a level of 8 for 50 min.

Then, in order to form primary (W1/O) phase, immediately after taking oil containing lipophilic emulsifier from water bath, the salt solution (1% $CaCl_2$) was mixed gently via high speed homogenizer (IKA T25 Digital Ultra-Turrax, Selangor, Malaysia) at harsh conditions; 16000 rpm for 10 min. Water to oil ratio of the primary emulsion was 6:4 by weight, in which 60 gram of salt solution (1% $CaCl_2$) was introduced into 40 gram of oil containing lipophilic emulsifier. The conditions of the preparations and ratios were determined by preliminary experiments.

In the second step of the emulsification, the double emulsion is formed by using food processor (Arçelik K-1190 Robolio, 700W, Arçelik Inc. Istanbul, Turkey). In this stage, ratio of primary emulsion (W1/O) to secondary water phase (W2) containing gum was 1:9 and it was formed by mixing 20 g of primary phase (W1/O) and 180 gram of previously prepared gum solution (W2). The mixture was homogenized using food processor at a level of 3 for 5 min. In all experiments, amount of double emulsion was fixed to 200 g. Rotary level and mixing time combination was determined by preliminary experiments.

Table 2.1 Formulations and gum blends used in the secondary water phase.

Formulation #	%Guar Gum (GG) in W2	% Gum Tragacanth (GT) in W2	% Locust Bean Gum (LBG) in W2
1	1.00	-	-
2	-	1.00	-
3	-	-	1.00
4	0.50	0.50	-
5	0.50	-	0.50
6	-	0.50	0.50
7	0.75	0.25	-
8	0.75	0.50	-

For further analysis, samples taken from each double emulsion were placed in the refrigerator at 4°C and in freezer at -18°C. Every experiment was duplicated for the accuracy of the results.

2.2.2 Ice Cream Preparation

2.2.2.1 Ice Cream Mix Preparation with Conventional Method

Compositions of low fat and regular fat ice cream mixes were given in Table 2.2. The ingredients were weighed and directly mixed at 50°C to prepare ice cream mix. Thereafter, the mix was pasteurized at 80°C for 25 s to ensure no pathogenic bacteria present (Cogne et al., 2003). Then, the ice cream mix was cooled down to a temperature of 4°C and aged at 4°C for 24 h.

Table 2.2 Compositions and ingredients of ice cream samples

Ingredients	Low Fat Ice Cream Composition (%)	Regular Fat Ice Cream Composition (%)
Fat	2.82	12
Sugar	18	18
Non-fat milk powder	11	11
Stabilizer/Emulsifier	0.69	0.69
Water	67.49	58.31

2.2.2.2 Ice Cream Mix Preparation with Double Emulsion

All the compositions and the mixing conditions were the same with ice cream mix preparation with conventional method. Double emulsion procedure was followed as explained in the double emulsion preparation section. Then, required amount of double emulsion was weighed and mixed thoroughly with sugar (18%) and non-fat milk powder (11%) at 50°C. Ice cream mix was then subjected to pasteurization at 80°C for 25s. After pasteurization, ice cream mix was immediately cooled down to 4°C in ice bath and ripened at 4°C for 24h.

2.2.2.3 Ice Cream Mix Freezing

After overnight refrigeration at 4°C, samples were loaded into freezer container where 0.1% vanilla flavor was added prior to freezing. Batch freezing of 0.5 kg ice cream mix was performed using De’Longhi II Gelatiao ICK5000 (De’Longhi Appliances S.r.l. - Via L. Seitz, 47, 31100 Treviso, Italy) for 40 min processing time. Ice cream was frozen to draw temperature of -5°C. Frozen ice cream was loaded into cups for storage at -18°C for further analysis.

2.2.3 Analysis of the Emulsions

2.2.3.1 Particle Size Measurements

Particle size distribution of the double emulsions was determined by using laser diffraction particle size analyzer (Malvern 3000, Malvern Instruments Ltd., Worcestershire, UK). The measurements were performed based on Mie Scattering Theory. The variables of the software were determined thoroughly. Dispersed medium was water and the particle refractive index was taken as 1.33. Likely, refractive index and absorbance of oil were 1.47 and 0.01, respectively. Obscuration range was chosen as 1% to 20%. Measurements were undertaken when the medium was stirred at 1600 rpm to avoid disruption of the particles. The analyses were done in duplicate for the accuracy of the results. Sauter mean diameter was calculated with Equation 2.1 (McClements, 2005):

$$D_{32} = \frac{\sum n_i {d_i}^3}{\sum n_i {d_i}^2} \tag{2.1}$$

where, d_i and n_i are the diameter of the particles and number of related particles per unit volume of all particles.

Span values were also determined to address the particle size distribution range as given in Equation 2.2.

$$Span = \frac{d_{v0.9} - d_{v0.1}}{d_{v0.5}} \tag{2.2}$$

where $d_{v0.9}$ defined as the diameter 90% of the distribution lie below this value, $d_{v0.1}$ is 10% of the distribution lie below $d_{v0.1}$, and $d_{v0.5}$, the median, is defined as the diameter where half of the population lies below this value.

2.2.3.2 Rheological Measurements

Rheological measurements of double emulsion samples were performed via rheometer with a cone and plate geometry (Kinexus, Malvern Instruments, Ltd, Worcestershire, UK) immediately after preparation by loading 1.19 ml of sample on the specified area of the equipment. Measurements were performed at 25°C. Cone angle, diameter and gap were 4°, 40mm and 0.001mm, respectively. Apparent viscosities were recorded under applied shear rate ranging from 0.1 to 100 s^{-1}. All measurements were duplicated for the accuracy of the results.

2.2.3.3 Stability Measurements

Stability measurements of the double emulsion samples were carried out three different ways. As the first way, the measurements were done by centrifugation method. Freshly produced double emulsions of 8 g were loaded into 10 ml centrifuge tubes sealed with caps. Then they were subjected to 5000 rpm for 5 min at 20°C in the centrifuge (Hettich Mikro200/200R, Sigma Laborzentrifugen GmbH, Germany). The height of the emulsion before centrifugation (H_E) and the height of the supernatant after centrifugation (H_s) were measured and emulsion stability values were calculated as described in the Equation 2.3. Experiments were replicated twice.

$$Emulsion\ Stability(\%) = \frac{H_S}{H_E} \times 100 \tag{2.3}$$

The two other ways of measuring the emulsion stability were storing 10 g of the emulsion samples after loading into glass tubes in the refrigerator at 4°C and -18°C to observe their gravitational separation after 7 days of storage. After 7 days, the separations of the phases were measured and the emulsion stabilities were calculated as described in the Equation 2.3.

2.2.3.4 Optical Imaging

The morphology of the double emulsions was monitored under inverted light microscope (PrimoVert, Zeiss, Jena, Germany) equipped with microscopic camera (Sony CCD Color Digital Video C-Mount Microscope Camera, Tokyo, Japan) connected to the computer. The prepared emulsions of very thin layer was formed on microscope slide and placed on the microscope. The software TopView was used by adjusting hue, saturation, brightness, contrast and gamma values of (41, 167, 25, 5 and 131), respectively. The images of double emulsions were taken at 40× magnification level.

2.2.4 Analysis of the Ice Cream

2.2.4.1 Overrun Measurement

Unit volume of ice cream mix (just before freezing) and frozen ice cream samples (immediately after freezing) were weighed with a precision of 0.1 g (A&D EK-3000i, Precision Weighing Balances, Haverhill, MA, USA) and values were recorded.

Overrun values of the ice cream samples were determined according to the formula described by Equation 2.4;

$$Overrun\ (\%) = \frac{W_{ICM} - W_{ICE}}{W_{ICE}} \times 100 \tag{2.4}$$

where W_{ICM}, and W_{ICE} represent weight of unit volume of ice cream mix and weight of unit volume of ice cream after freezing, respectively.

2.2.4.2 Meltdown Rate

All ice cream samples were weighed and loaded into cups of 125 g and then stored at -18°C for 24 hour before carrying out melting test. The ice cream samples placed on mesh grid (mesh size 2×2 mm) and allowed to stand at 29°C. The weight of the material passing through the mesh recorded for every 10 min for an hour after first drop appeared on the container. Also, time of the first drip was recorded for each sample for comparison. Then, with the collected data melting rate was calculated. The experimental setup was done according to literature (Granger et al., 2005).

2.2.4.3 Rheological Measurements

To assess the dynamic rheological behavior of ice cream products, oscillatory-thermo rheometer was used (AR 2000ex, TA Instruments, New Castle, US) equipped with temperature control unit. Samples were formed into cylindrical tablets with 5mm thickness and 40 mm diameter and they were stored at -18°C for one day prior to measurements. Plate-plate geometry was used for the rheological analysis of ice cream samples. Movable hood covering was used to minimize heat exchange with the surroundings.

In order not to exceed the linear viscoelastic regime (LVR) of ice cream, 1.59 Hz (angular frequency of 10 s^{-1}) was selected. Then, frequency sweep test was performed at -5°C. Also, 0.0002 constant amplitude was chosen for the oscillatory measurements at which linear behavior was seen for both storage (G') and loss modulus (G''). The gap width was chosen as 2 mm for all measurements.

For the oscillatory thermo rheometer, temperature was increased from -5°C to 15°C, which is called temperature sweep test, with a heating rate of 1°C/min. The measurements were carried out within the LVR of ice cream at constant deformation amplitude of 0.0002 and frequency of 1.59 Hz. The gap width was 2mm as in the frequency sweep test. At least two measurements were performed for each ice cream formulation. The storage and loss moduli were analyzed for the characterization of ice cream behaviors.

2.2.4.4 Sensory Analysis

Three different ice cream formulations which contain regular fat, low fat and double emulsion with reduced fat were used for the sensory analysis.

The ballot development was done as described by Aime et al. (2001). Sensory analysis was performed with 8 trained panelists at Unilever Algida Konya factory. The panelists had at least 2 years of experience who passed the sensory test conducted by the Quality Department of Algida, Unilever.

In order to control temperature of the samples during transportation, insulated cabinet filled with dry ice tablets were used. Serving temperature of the ice cream samples for testing was between -12°C and -15°C. Specter and Setser (1994) performed sensory analysis of the samples at -12±1°C and Aime et al. (2001) analyzed their samples between temperatures -13°C and -11°C.

Ice cream samples were randomly coded with three digit numbers and randomly distributed to the panelists. The 6-point scale was used for the specified sensory attributes summarized as in the Table 2.3.

2.2.1 Statistical Analysis

In order to monitor whether there is a significant difference between the results or not, analysis of variance (ANOVA) was used ($p \leq 0.05$). If significant difference was found, Tukey’s Test was applied ($p \leq 0.05$) on Minitab (Version 16.2.0.0, Minitab Inc., Coventry, United Kingdom). Pearson comparison test was used for correlation.

Table 2.3 Sensory Analysis Card

	Least					Most
Sensory Attributes	1	2	3	4	5	6
Viscosity						
Iciness						
Intensity of coldness						
Mouth-coating						
Overall Acceptability						

CHAPTER 3

RESULTS AND DISCUSSION

3.1 Double Emulsion

Different formulations were used in the preparation of primary and secondary emulsions and their effects were monitored by means of particle size, rheological properties, stability and morphological properties. Additionally, all the parameters were statistically analyzed and tabulated in Appendix A.

3.1.1 Particle Size of Double Emulsion

Emulsion characterization and stabilization have been extensively studied by means of particle size due to the fact that particle size affects surface properties, stability, and structure of the emulsions (Hunter et al., 2008). Hence, the particle size and particle size distribution of the multiple emulsions were investigated in this particular study.

Both monomodal and bimodal distributions were observed in the double emulsion formulations used in this study (Figure 3.1 and Figure 3.2). Although particle size distribution analyses have been performed by many authors, there is no common trend stated. Similarly, findings about this particular study did not show a general trend for different double emulsion formulations. Formulations of PGPR as a primary emulsifier and GG-GT and LBG-GT blends as stabilizers had monomodal size distribution while GG, GT, LBG, and GG-LBG blends had bimodal distributions (Figure 3.1 and Figure 3.2). On the other hand, double emulsions with PGPR-lecithin blend as a hydrophobic emulsifier had monomodal size distribution when stabilizers were GT, GG-GT (0.5%-0.5%) and LBG-GT blends. In addition, GG, LBG, GG-LBG and GG-GT (0.75%-0.25% & 0.75%-0.50%) blends had bimodal size distribution for the formulations prepared with PGPR-lecithin as a primary phase emulsifier. The average span values were given in Table 3.1. Span values, lower than 1.865, corresponded to monomodal size distribution while higher values showed polydispersed particle size distribution in double emulsion formulations.

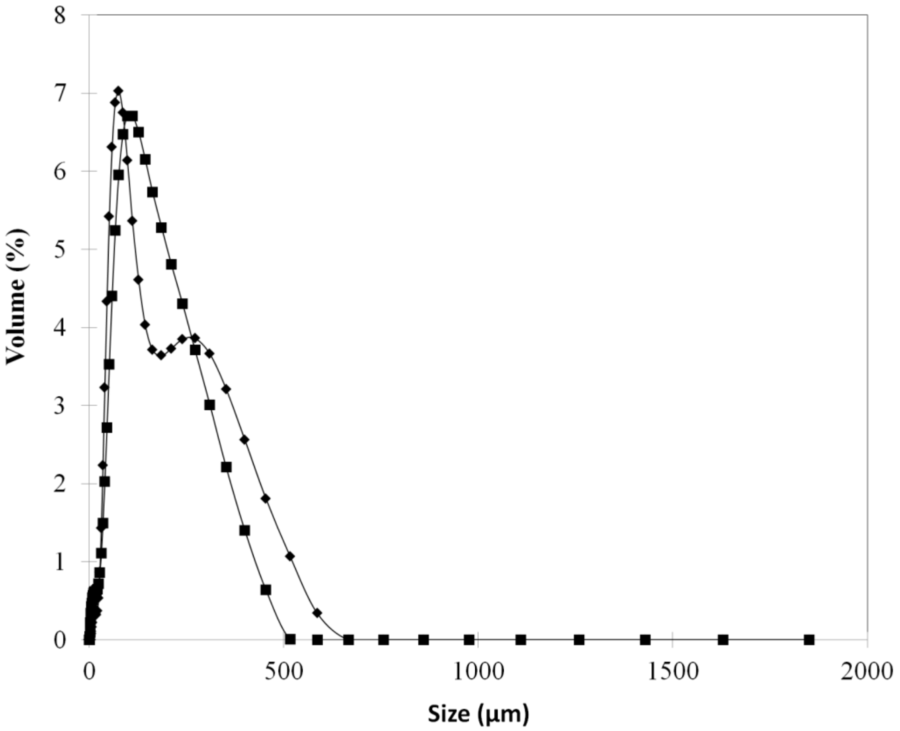


Figure 3.1 Particle size distributions of double emulsions formed with 2% PGPR (square) and 1.5% PGPR-0.5% lecithin blend (diamond) as a primary emulsifier and GG (1%) as stabilizers.

It was stated in the literature that particle size distribution was affected from the conditions of homogenization, composition and nature of emulsion (Huang et al., 2001; Jafari et al., 2008; Bou et al., 2014). The change in size distributions may be due to varying conformations between the emulsifiers and stabilizers present in the emulsions.

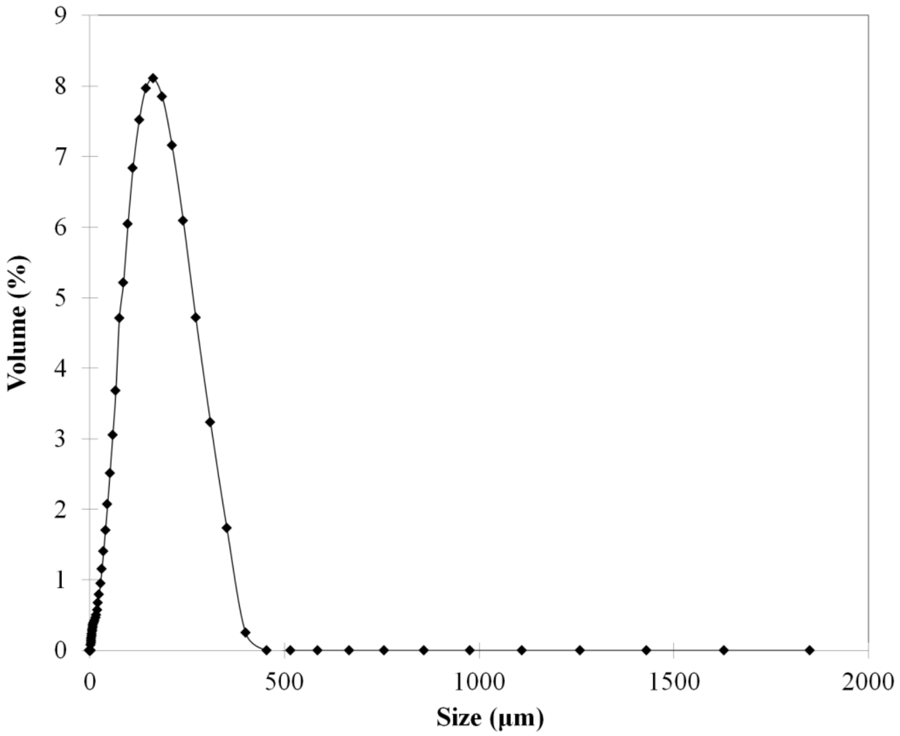


Figure 3.2 Particle size distributions of double emulsions formed with 2% PGPR as a primary emulsifier and GG-GT (0.75%-0.25%) blend as stabilizer.

Table 3.1 Span values of different double emulsion formulations indicating polydispersity.

Gum Type /Conc.	Emulsifier Type	Span	Polydispersity
1% GG	PGPR	1.909	Bimodal
	PGPR+ Lecithin	3.708	Bimodal
1% GT	PGPR	2.018	Bimodal
	PGPR+ Lecithin	1.482	Monomodal
1% LBG	PGPR	2.228	Bimodal
	PGPR+ Lecithin	3.176	Bimodal
0.5% GG+ 0.5% GT	PGPR	1.838	Monomodal
	PGPR+ Lecithin	1.865	Monomodal
0.5% GG+ 0.5% LBG	PGPR	2.723	Bimodal
	PGPR+ Lecithin	3.962	Bimodal
0.5% LBG+ 0.5% GT	PGPR	1.760	Monomodal
	PGPR+ Lecithin	1.699	Monomodal
0.75% GG+ 0.25% GT	PGPR	1.745	Monomodal
	PGPR+ Lecithin	2.065	Bimodal
0.75% GG+ 0.50% GT	PGPR	1.742	Monomodal
	PGPR+ Lecithin	2.007	Bimodal

Sauter mean diameters (D_{32}) of each formulation were designated and statistically analyzed for the determination of multiple emulsions both on the 1st day and the 7th days of storage, respectively (Figure 3.3 & Figure 3.4).

Based on the statistical analysis of 1st day measurements, types of hydrophobic emulsifier used in the primary phase did not affect the particle size of double emulsions (Table A.1). Further, Sauter mean diameters of each PGPR and PGPR-lecithin blend containing formulations were analyzed individually to show statistical differences between stabilizer formulations (Table A.2 & Table A.3).

As can be seen from Figure 3.3 and Figure 3.4, Sauter mean diameters of the emulsions prepared with PGPR as a hydrophobic emulsifier ranged between 30 µm to 75 µm, while those prepared with PGPR-lecithin blend changed between 25 µm to 85 µm for based on 1st day measurements. Stabilizers used in double emulsions significantly affected the particle size. On the 1st day, the smallest particle size was obtained when GG, LBG, GT and LBG-GG blend were used as stabilizer in secondary water phase (W_2) for the emulsions containing PGPR as a hydrophobic emulsifier (Figure 3.3). Also, it can be interpreted that there was no significant difference between the formulations of LBG-GT (0.5%-0.5%) and GG-GT (0.75%-0.25% and 0.75%-0.50%) in terms of D_{32} values when PGPR was used.

In the case of PGPR-lecithin blend, the lowest average particle size was obtained when emulsions were prepared with LBG alone, or GG-LBG (0.5%-0.5%) blend. The highest average particle size was observed in double emulsions containing GG alone and GG-GT (0.75%-0.50%) blend based on 1st day measurements.

Sauter mean diameters of all samples were also analyzed at the end of seven days storage at 4°C (Figure 3.3 & Figure 3.4). Particle sizes of double emulsions prepared with only PGPR and PGPR-lecithin blend were significantly different (Table A.4). The results obtained based on 7th day measurements also showed that the largest particle size was observed for the formulations containing GG-GT (0.75%-0.25%) blend as a stabilizer when PGPR was used as an emulsifier. For the samples prepared with PGPR-lecithin blend, the largest particle size was obtained when stabilizers were GG and GT.

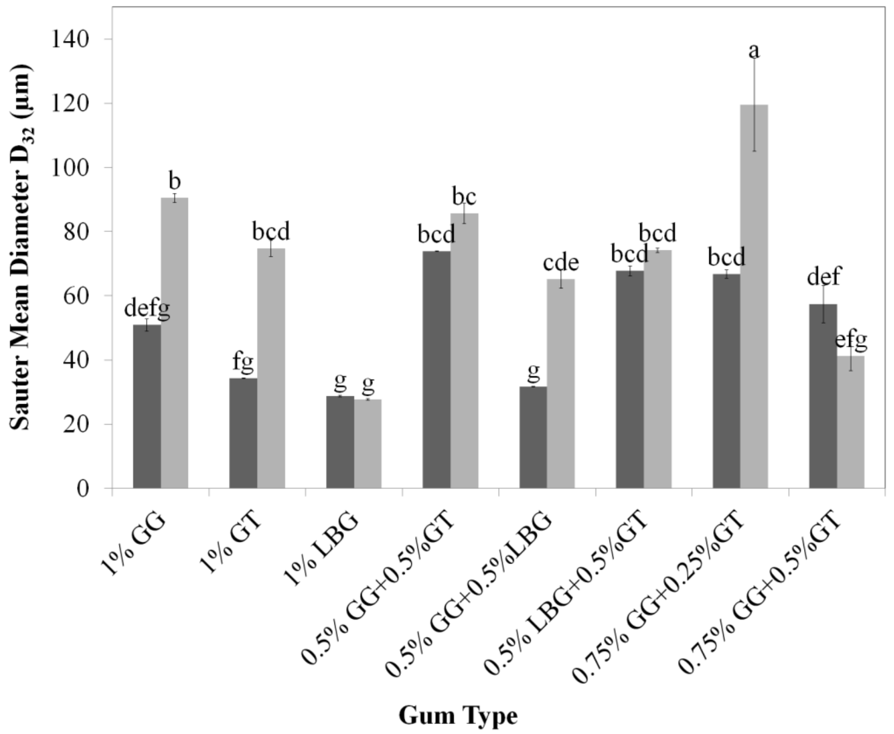


Figure 3.3 Sauter mean diameters of different double emulsion formulations containing PGPR in the oil (O) phase based on 1st and 7th day of measurements. Black columns represent 1st day measurements and grey columns represent 7th day measurements. Bars having different letters indicate significant difference ($p \leq 0.05$).

Particle size of double emulsions changed significantly during storage (Table A.5 & Table A.6). Generally an increase in particle size was observed during storage for the emulsions prepared with PGPR. However, for emulsions containing LBG (1%), GG-GT (0.5%-0.5% & 0.75%-0.50%) and LBG-GT (0.5%-0.5%) blends, D_{32} remained constant. Increase in average droplet size can be ascribed to swelling of particles (Leal-Calderon et al., 2012) and Ostwald ripening in which larger droplets might get larger and smaller ones become smaller. In general, Sauter mean diameters showed no significant difference during storage of emulsions containing PGPR-lecithin blends. This indicated that PGPR-lecithin containing emulsions were stable.

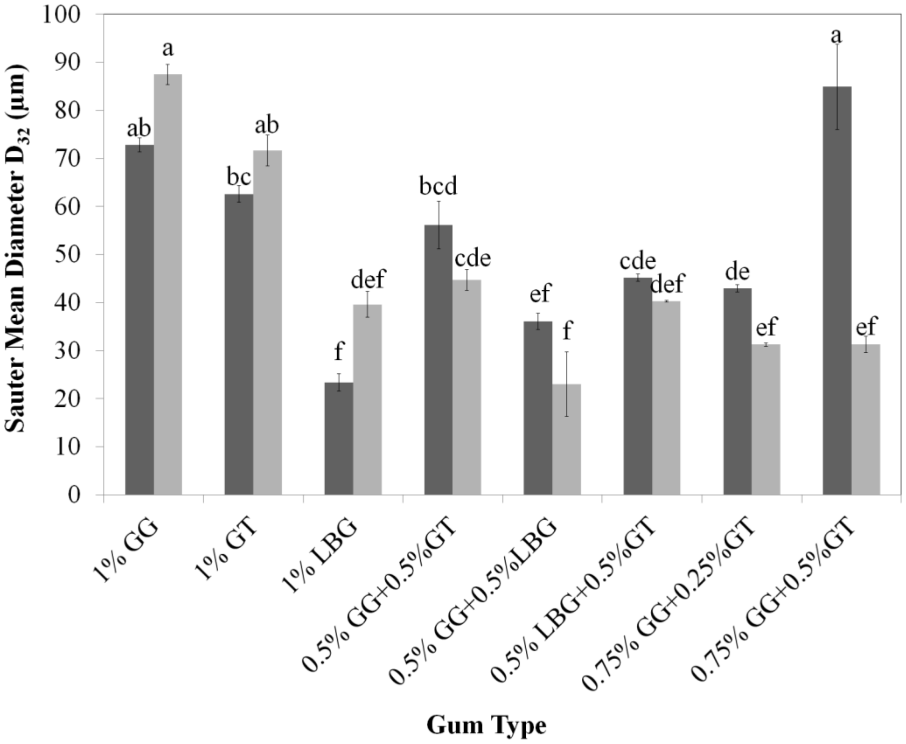


Figure 3.4 Sauter mean diameters of different double emulsion formulations containing PGPR-lecithin blend in the oil (O) phase based on 1st and 7th day of measurements. Black columns represent 1st day measurements and grey columns represent 7th day measurements. Bars having different letters indicate significant difference ($p \leq 0.05$).

3.1.2 Rheological Properties of Double Emulsions

Rheological properties of emulsions have been investigated by many authors since it indicates the physical state of the emulsion (Vasiljevic et al., 2006). Rheological properties of double emulsion formulations were assessed by considering the effects of emulsifiers in the first phase, stabilizers and their blends in secondary phase. For all emulsion formulations, shear thinning behavior was observed (Figure 3.5 and Figure 3.6). Obtained results were analogous to the results in literature (Carrillo et al., 2015; Pal, 2011; Garti & Bisperink, 1998). As shear rate was increased, apparent viscosities of emulsion decreased due to the deformation of primary phase droplets (Carrillo et al., 2015).

As can be seen from the Figure 3.5 & Figure 3.6, formulation with GG-GT (0.75%-0.50%) blend had the highest while formulation with LBG-GT blend had the lowest apparent viscosity values initially. Double emulsions prepared with PGPR or PGPR-lecithin blend showed similar trend under the applied stress.

Rheological properties are characterized using models to describe structural viscosity of the emulsions (Carrillo-Navas et al., 2012). For the flow properties of double emulsions, there are different models used, but in this particular study power law was well fitted to the experimental data ($r^2 \geq 0.98$). Power law is explained in Equation 3.1 (Sahin & Sumnu, 2006);

$$\eta = K\,\dot{\gamma}^{n} \tag{3.1}$$

where, n represents dimensionless flow behavior index, K represents consistency index (Pa s^n), $\dot{\gamma}$ is shear rate (s^{-1}) and η is apparent viscosity (Pa.s).

Power law model constants for different double emulsion formulations were given in Table 3.2.

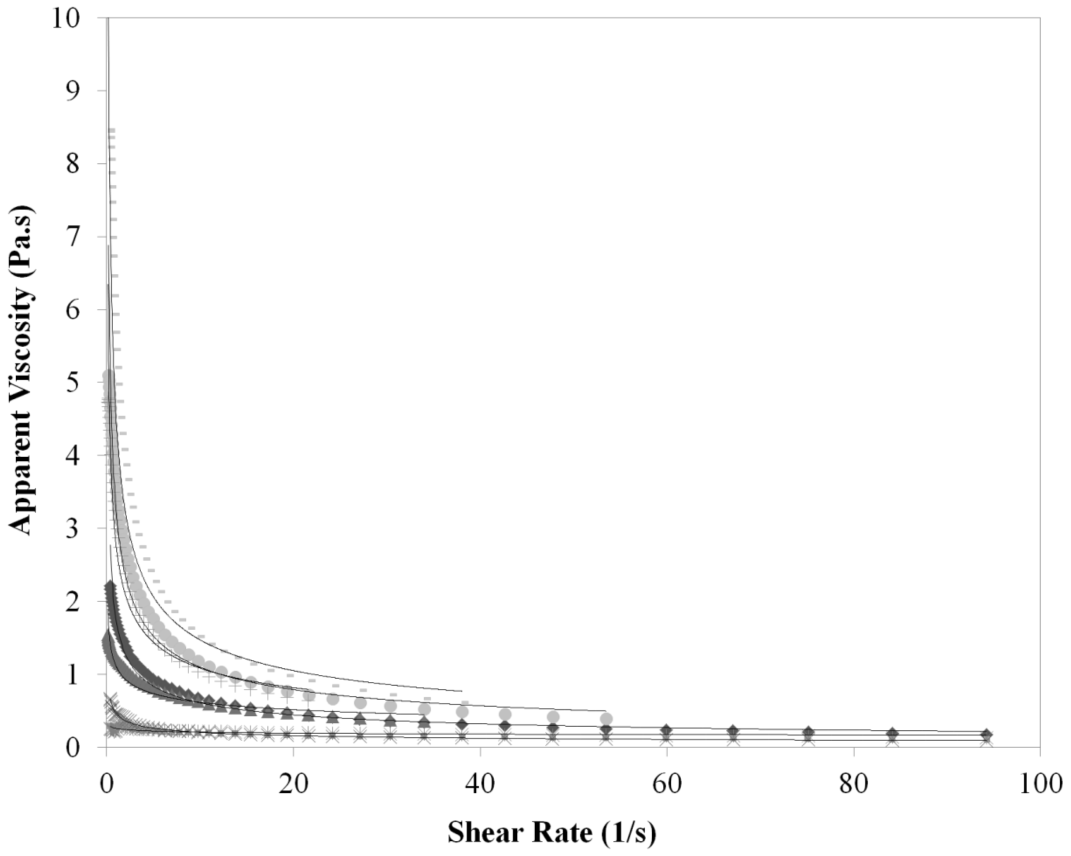


Figure 3.5 Apparent viscosity of double emulsions containing PGPR-lecithin blend in the oil phase and different gum formulations in the secondary aqueous phase; 1% GG (●), 1% GT(×), 1% LBG(✳), 0.5% GG + 0.5% GT (♦), 0.5% GG + 0.5% LBG (▲), 0.5% LBG + 0.5% GT (■), 0.75% GG + 0.25% GT (+), 0.75% GG + 0.5% GT (-). Markers represent data points, lines represent power law model.

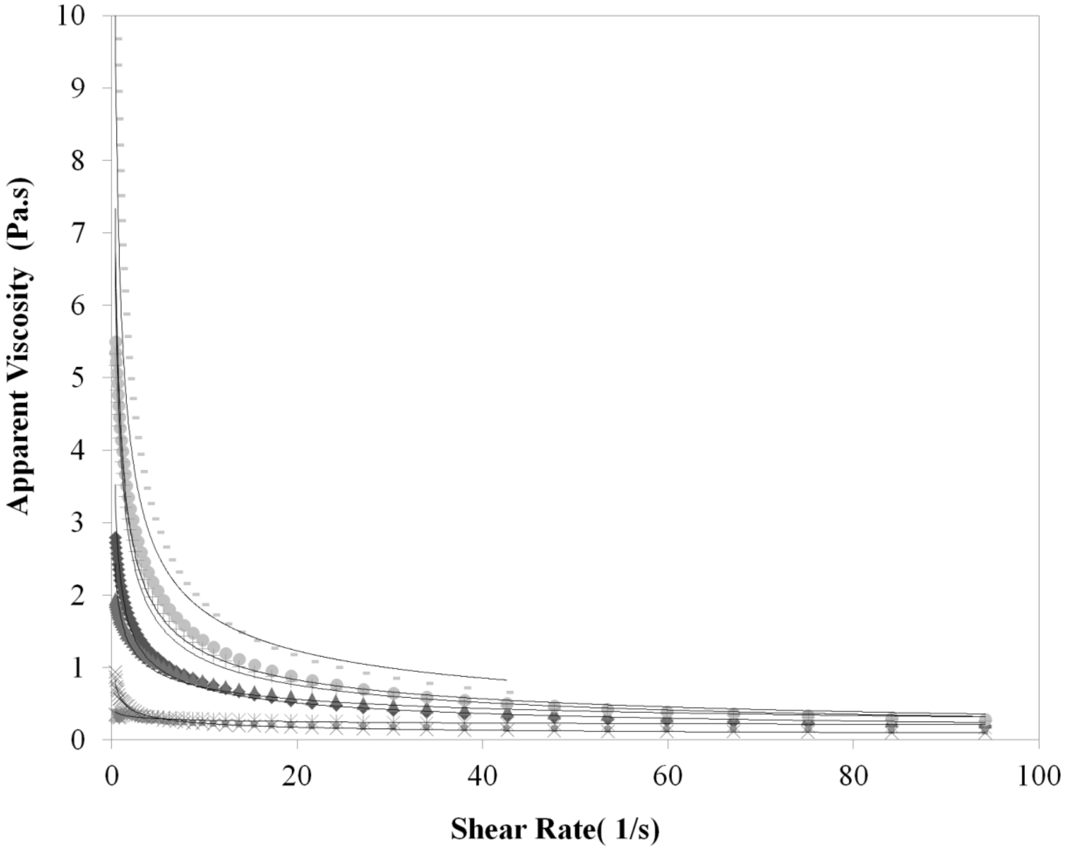


Figure 3.6 Apparent viscosity of double emulsions containing PGPR in the oil phase and different gum formulations in the secondary aqueous phase; 1% GG (●), 1% GT(×), 1% LBG(✳), 0.5% GG + 0.5% GT (♦), 0.5% GG + 0.5% LBG (▲), 0.5% LBG + 0.5% GT (■), 0.75% GG + 0.25% GT (+), 0.75% GG + 0.5% GT (-). Markers represent data points, lines represent power law model.

Apparent viscosities of different formulations at constant shear stress (5.5 Pa) were also compared (Figure 3.7). The highest apparent viscosities were obtained when GG (1%) or GG-GT (0.75%-0.50%) blend was used in the case of both PGPR or PGPR-lecithin blend were used as emulsifier (Table A.7 and Figure 3.7).

Table 3.2 Power law model fit constants for different formulations of double emulsions. Columns with different letters indicate statistical differences ($p \leq 0.05$).

Gum Type	Emulsifier Type	K (Pa s^n)	n	R^2
1% GG	PGPR	6.76^{ab}	0.36^{h}	0.98
1% GT	PGPR	0.53^{g}	0.63^{cd}	0.99
1% LBG	PGPR	0.42^{g}	0.82^{a}	0.99
0.5% GG+0.5% GT	PGPR	2.50^{defg}	0.47^{ef}	0.99
0.5% GG+0.5% LBG	PGPR	1.87^{efg}	0.58^{d}	0.99
0.5% LBG+0.5% GT	PGPR	0.40^{g}	0.74^{b}	0.99
0.75% GG+0.25% GT	PGPR	4.57^{bcde}	0.39^{gh}	0.99
0.75% GG+0.50% GT	PGPR	7.60^{a}	0.35^{h}	0.99
1% GG	PGPR-Lecithin	5.01^{abcd}	0.40^{gh}	0.99
1% GT	PGPR-Lecithin	0.46^{g}	0.64^{c}	0.99
1% LBG	PGPR-Lecithin	0.33^{g}	0.82^{a}	0.99
0.5% GG+0.5% GT	PGPR-Lecithin	2.01^{efg}	0.49^{e}	0.99
0.5% GG+0.5% LBG	PGPR-Lecithin	1.43^{fg}	0.60^{cd}	0.99
0.5% LBG+0.5% GT	PGPR-Lecithin	0.33^{g}	0.75^{b}	0.99
0.75% GG+0.25% GT	PGPR-Lecithin	3.43^{cdef}	0.43^{fg}	0.99
0.75% GG+0.50% GT	PGPR-Lecithin	5.69^{abc}	0.39^{gh}	0.99

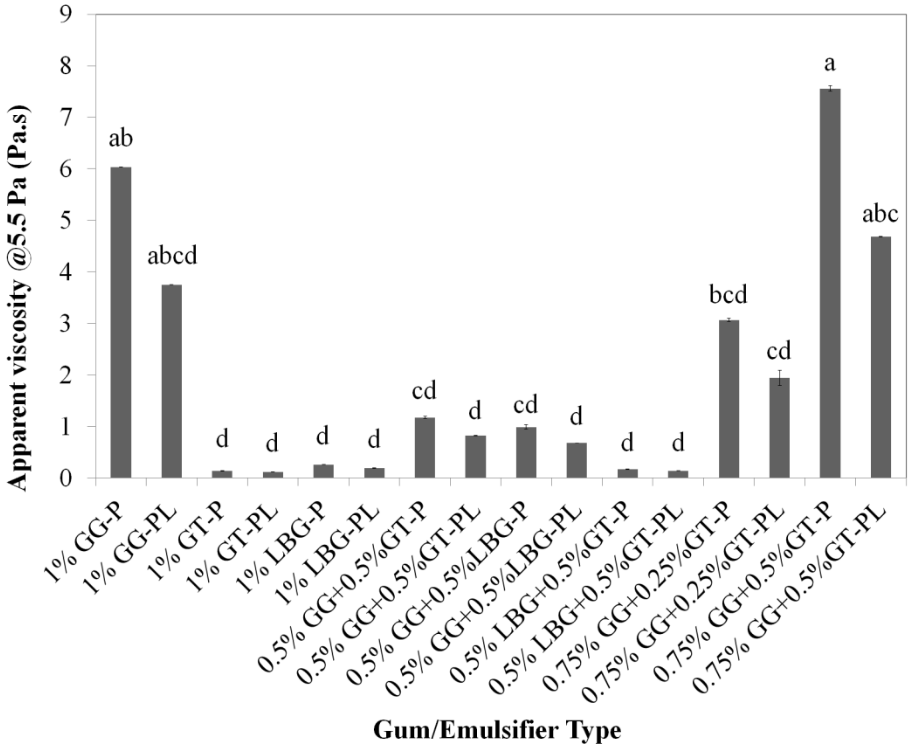


Figure 3.7 Apparent viscosities of different double emulsion formulations at 5.5 Pa. Bars having different letters indicate significant difference ($p \leq 0.05$).

In the emulsions prepared with PGPR alone, GG-GT (0.75%-0.50%) blend and GG (1%) provided the highest consistency coefficient (Table 3.2). Increasing GT concentration caused an increase in consistency index (Table 3.2). The table also indicated that increasing guar gum concentration of the medium resulted in improvement in the consistency coefficient. The reason of such increase might be the thickening ability of the guar gum as well as its aggregation in the emulsion. In literature, increasing guar gum concentration in the emulsion resulted in rise in both consistency coefficient and viscosity of the emulsion (Erçelebi & Ibanoğlu, 2009). Also, addition of guar gum to the formulation of cake batter improved viscosity of the samples due to its higher molecular weight compared to locust bean gum (Turabi et al., 2008). In the case of PGPR-lecithin blend in primary phase, similar results were observed with those prepared with PGPR alone.

In the literature, it was stated that emulsions containing smaller droplet size tended to be more resistant to the shear stress which contradicts with the findings of this study (Pal, 2011). As can be seen from the Figure 3.4, in the case of PGPR-lecithin blend, the smallest particle size was obtained when locust bean gum was incorporated in the external aqueous phase. However, locust bean gum and its blends had low consistency coefficients (Table 3.2) and low apparent viscosity values (Figure 3.7) regardless of primary hydrophobic emulsifier used. This might be related to the instability of the droplets. Visual phase separation was observed very quickly after preparation of double emulsions containing locust bean gum. Bigger sized droplets formed a cream layer on the top. In this situation, smaller mean particle size did not show higher stability since particle size analysis was performed in the lower layer. Another study revealed that addition of locust bean gum reduced the protein adsorption in the emulsion (Makri & Doxastakis, 2006). Weak adsorption at the interface results in weak film strength which further causes film rupture. This phenomenon seems reasonable when compared to the results of this experiment.

Moreover, the flow behavior index (n) is related to shear sensitivity or deformability of the samples. In shear thinning fluids, smaller values of flow behavior index were associated with higher shear sensitivity (Saiki & Prestidge, 2005). Therefore, n values were also analyzed statistically to describe their shear deformability (Table A.9). The n values of LBG containing samples (Table 3.2) were the highest and significantly different from other emulsion formulations, meaning that, LBG containing emulsions were less shear-dependent and they were slightly deformed by the applied shear. On the other hand, considering primary emulsifiers individually, emulsions formulated with GG (1%) and GG-GT (0.75%-0.25% and 0.75%-0.50%) blends had the lowest n values indicating high deformability of these samples.

3.1.3 Stability of Double Emulsion

Double emulsion stability is very important issue and it has been studied in order to extend the applicability of the double emulsions. There have been different methods to measure the stability of the emulsions. To address the stability of the double emulsions, centrifugation assay (Huang et al., 2001; Turabi et al., 2008), and visual phase separation (Osano et al., 2014) under varying conditions like storage at different temperatures or storage after heat shock were used (Oppermann et al., 2015).

In this study, phase separation after centrifugation and phase separation under gravity when stored at +4°C and -18°C for 7 days were used to quantify the emulsion stability.

Average stability values of all formulations determined using centrifugation assay were demonstrated in the Figure 3.8. For emulsions containing PGPR as a hydrophobic emulsifier, stabilizers affected emulsion stability significantly (Table A.10). Stability of LBG (1%) containing emulsion was different from emulsion containing GT (1%) and GG-GT (0.75%-0.25%) blend, and emulsions containing GT (1%) was different from GG-LBG (0.5%-0.5%) blend containing emulsion in terms of stability. On the other hand, when the emulsions were prepared with PGPR-lecithin blend as a hydrophobic emulsifier, the lowest stability was observed for the emulsions containing LBG (1%), GG (1%), and GG-LBG (0.5%-0.5%) blend. The highest stability values were obtained when LBG-GT (0.5%-0.5%) and GG-GT (0.75%-0.50%) blends were used. These ES (%) values might be correlated with n values given in Table 3.2. Emulsions prepared with PGPR-lecithin blend and GG (1%) or GG-GT (0.75%-0.25%) or GG-GT (0.75%-0.50%) had the lowest n-value, indicating the highest shear dependency as compared to other formulations. This resulted in lowest stability after centrifugation. During centrifugation, shear forces acting on the samples affected the behavior of the emulsions in such a way that more shear dependent samples showed more phase separation. Moreover, as can be followed from the Figure 3.5 & Figure 3.6, the apparent viscosity change was sharper for the formulations of GG-GT (0.75%-0.50%) blend while apparent viscosity values were slightly affected for the emulsions containing LBG-GT (0.5%-0.5%) blends in which PGPR or PGPR-lecithin blend were used in primary phase. This explained why emulsion containing LBG-GT (0.5%-0.5%) had higher stability than emulsion containing GG-GT (0.75%-0.50%) blend. On the other hand, emulsions containing GT (1%) and PGPR or PGPR-lecithin blend showed small change in the viscosity with respect to shear and had smoother curve as compared to emulsions containing GG-GT (0.75%-0.50%) blend.

There are various mechanisms to characterize the emulsion stability like sedimentation, creaming, flocculation, coalescence, phase inversion, Ostwald ripening, coagulation, and aggregation (Kulmyrzaev et al., 2000; Tadros, 2013). The emulsion stability results can be explained by creaming based on the observations at the end of the seven days of storage. During storage, the larger particles get larger while smaller ones might get smaller which is known as Ostwald ripening. Larger droplets formed a cream layer at the top of the emulsions. At the end of seven days of storage at refrigerator (+4°C), creaming layer were quantified and described as ES (%) as shown in Figure 3.9. In other words, emulsions having higher viscosity values exhibited higher ES (%) values. The highest stability obtained by the formulations containing PGPR; were GG (1%), GG-GT (0.75%-0.25% and 0.75%-0.50%) blends which were statistically not different from emulsions prepared with PGPR-lecithin blends (Table A.11).

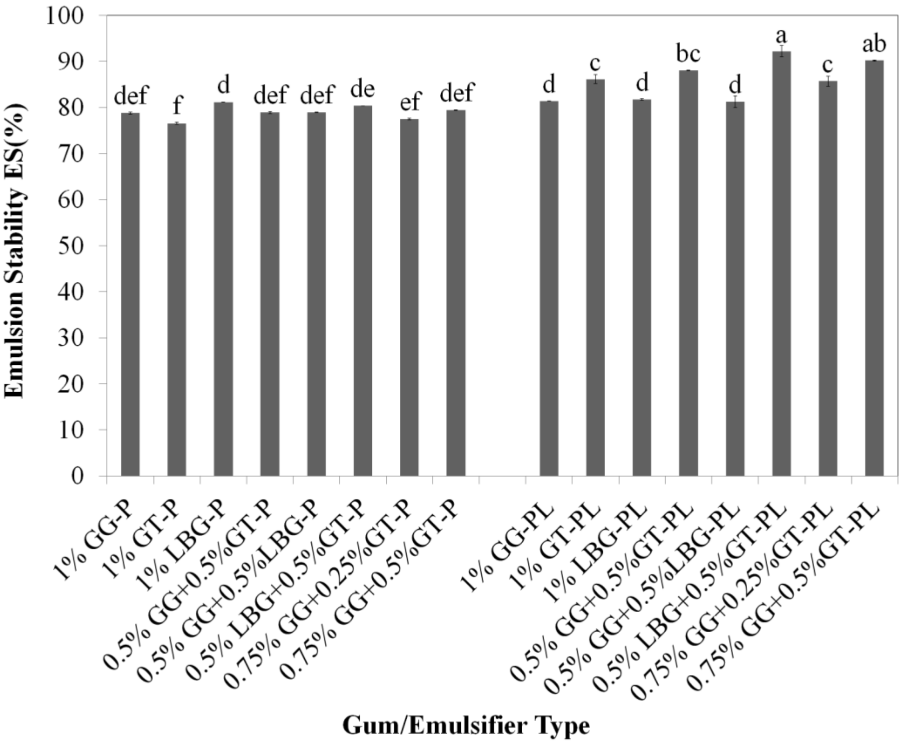


Figure 3.8 Stability values of different double emulsion formulations in accordance with centrifugation.

Partial replacement of PGPR by lecithin improved stability of some of the emulsion formulations like GT containing ones. It might be due to synergistic effect of PGPR and lecithin when incorporated in the primary phase.

Furthermore, in literature, protein-polysaccharide complexes have been used to stabilize the double emulsions to produce more stable structure based on control of the rheology of aqueous phase (Leal-Calderon et al., 2007). Also, it was reported that protein-polysaccharide complexes provides very good coverage and higher encapsulation yields (Jiménez-Alvarado et al., 2009). This concludes the relationship between the viscosity and stability of the emulsion that higher viscosity values provided better stability and lower phase separation which was similar to the findings of this particular study in accordance with storage stability.

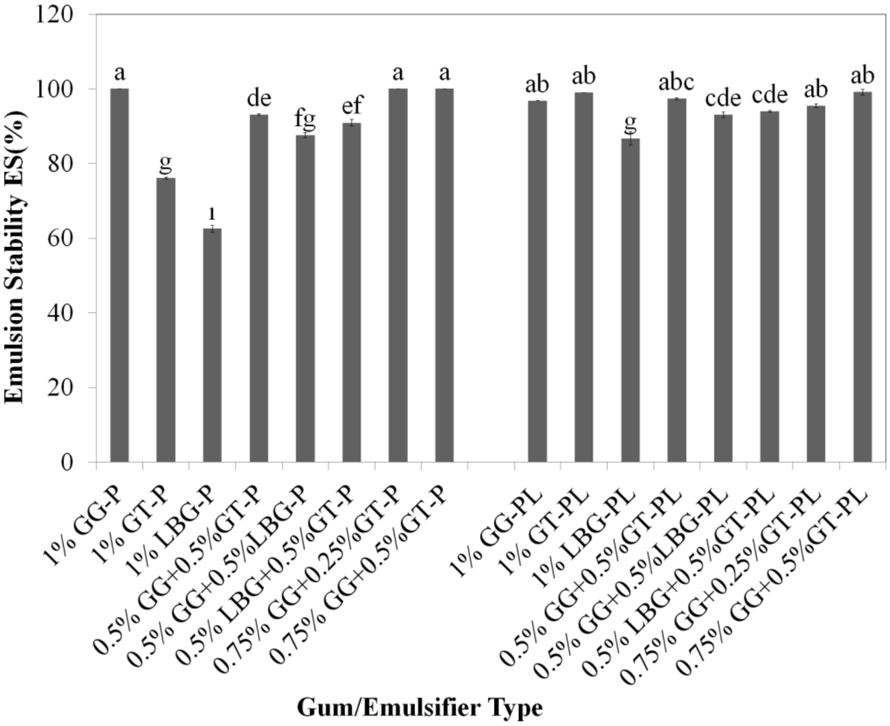


Figure 3.9 Stability values of different double emulsion formulations after 7 days of storage at +4°C.

In order to analyze the freeze-thaw stability, emulsions were stored at -18°C for seven days. At the end of the storage period, samples were taken out and waited for being thawed at room temperature. All samples kept their stability except GT (1%) and LBG (1%) containing PGPR as a primary emulsifier which formed a small creaming layer during thawing (Data is not shown). This reduces its emulsion stability to 90%. It is possible that emulsions can form cream layer before they totally get frozen.

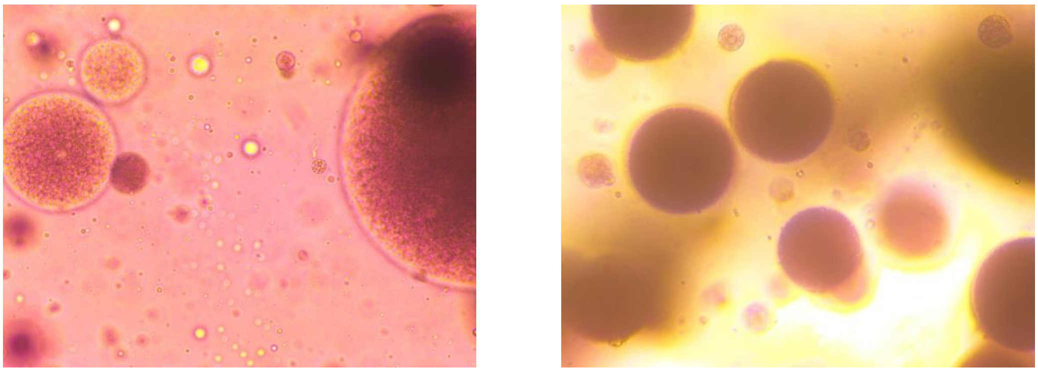
3.1.4 Morphological Properties of Double Emulsion

In order to observe the morphology of the double emulsion formulations, inverted microscope was used. The optical images were taken to differentiate the formulations.

Optical images of double emulsions with different formulations can be seen in (Figure 3.10 to Figure 3.17).

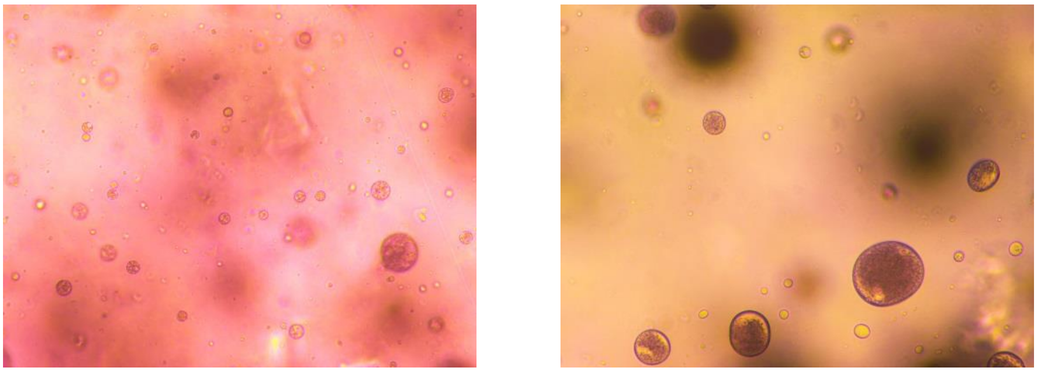
Double emulsion prepared with LBG (1%) (Figure 3.11) in the outer water phase had lower particle size when compared to GG (1%) containing one (Figure 3.10). The largest particle size was obtained when GG-GT (0.75%-0.50%) blend was used as stabilizer in PGPR-lecithin containing double emulsions (Figure 3.17). The reason of having the largest particle size can be explained by incorporation of higher amount of stabilizer when compared to other emulsions.

Furthermore, it can be interpreted from the optical images that samples of 1% GG, 1% LBG, and GG-LBG (0.5%-0.5%) (lipophilic emulsifier: PGPR-lecithin blend), 1% GT, 1% LBG and 0.5% GG+ 0.5% LBG (lipophilic emulsifier: PGPR) showed bimodal distributions while others showed monomodal size distribution (Figure 3.10 to Figure 3.17).



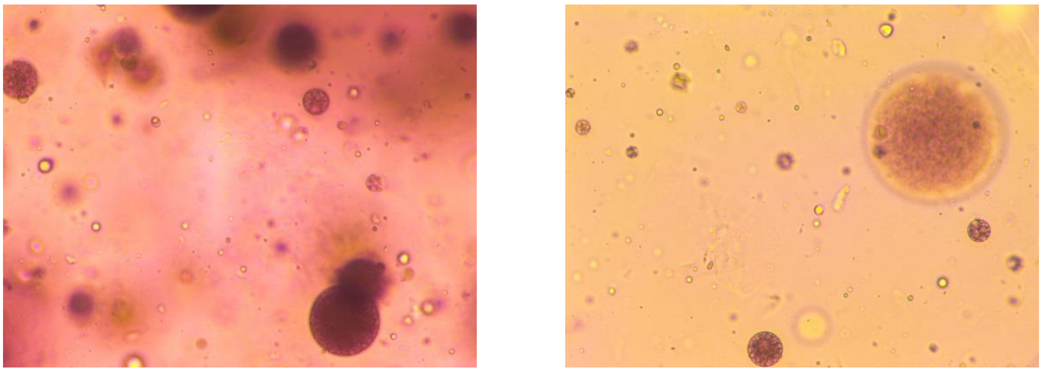
A B

Figure 3.10 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 1% GG in secondary outer phase.



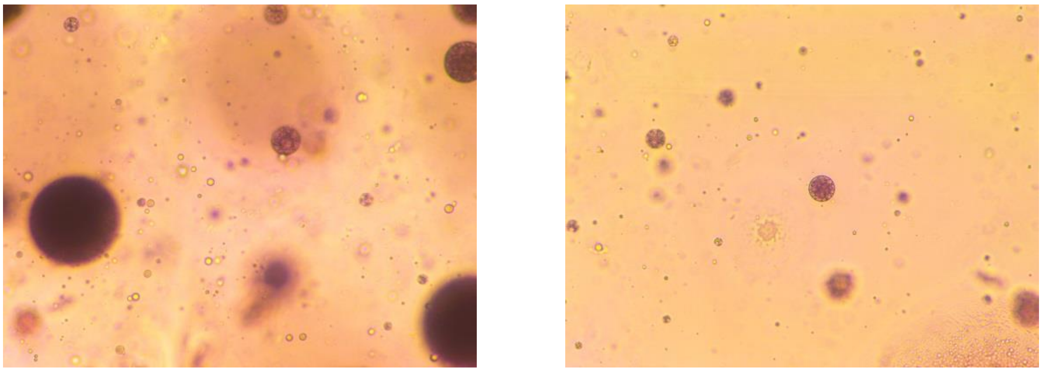
A B

Figure 3.11 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 1% LBG in secondary outer phase.



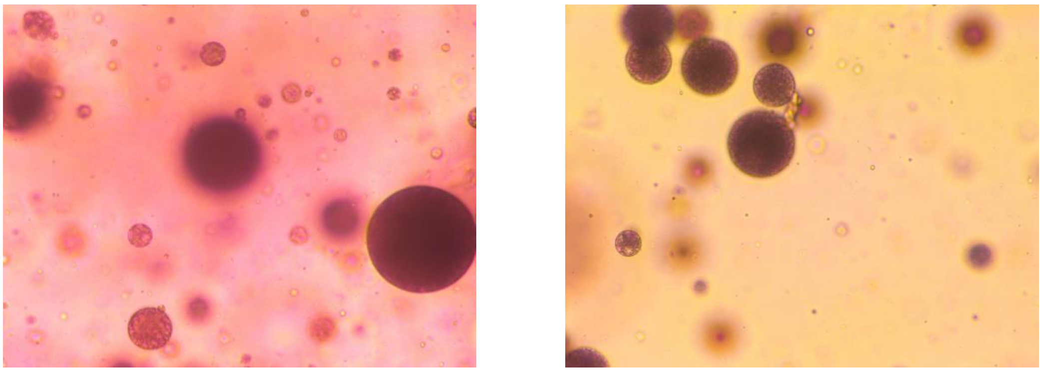
A B

Figure 3.12 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 1% GT in secondary outer phase.



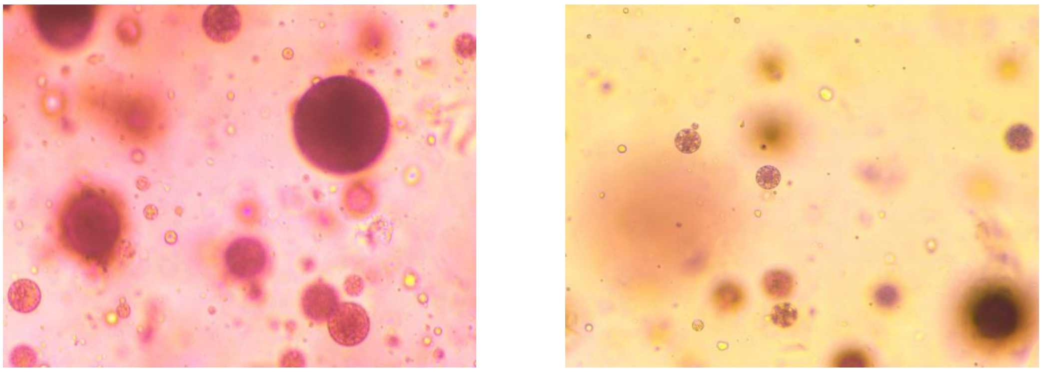
A B

Figure 3.13 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 0.5% GG + 0.5% GT in secondary outer phase.



A B

Figure 3.14 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 0.5% GG + 0.5% LBG in secondary outer phase.



A B

Figure 3.15 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 0.5% LBG + 0.5% GT in secondary outer phase.

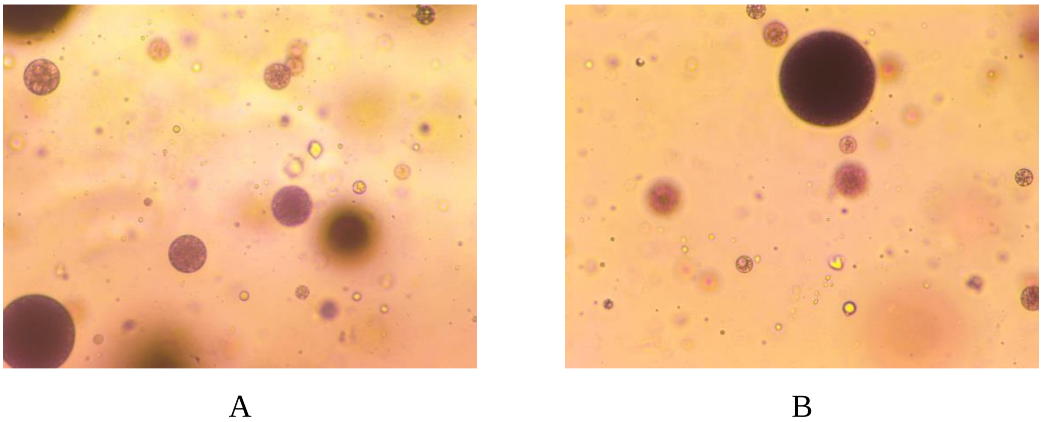


Figure 3.16 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 0.75% GG + 0.25% GT in secondary outer phase.

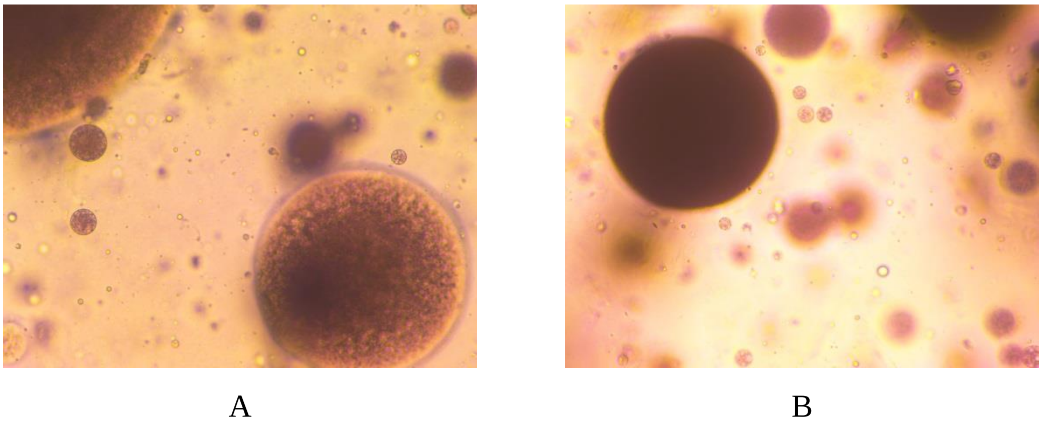


Figure 3.17 Optical images of double emulsions containing 1.5% PGPR and 0.5% lecithin (A) or 2% PGPR (B) in oil phase and 0.75% GG + 0.50% GT in secondary outer phase.

3.2 Ice Cream Manufacture with Double Emulsion

Ice cream is a multiphase system such that ice crystals, air bubbles and fat globules are incorporated in a highly viscous frozen matrix phase (Eisner et al., 2005). The typical ice cream contains 10 % fat up to 16 % (Goff, 1997).

The most suitable emulsifier and gum type were chosen based on the results obtained from the double emulsion experiments for ice cream production. As a primary phase emulsifier, PGPR-lecithin combination showed similar results with the PGPR containing emulsions in terms of particle size and rheological properties. Stability of the double emulsion formulations based on the gravitational separation results at +4°C were considered since more consistent results were observed. The double emulsions containing PGPR and PGPR-lecithin blends had similar stability values. Since, it is better to reduce the usage of PGPR by replacing food grade lecithin, PGPR-lecithin combination was chosen as emulsifier. It is important to note that although lower particle size was observed for the emulsions containing LBG or GT, they had low viscosity with low consistency coefficients and they were not stable during storage. GG (1%) and blend of GG-GT (0.75%-0.25%) were chosen as secondary phase stabilizers regarding particle size, rheology and stability as well as freeze-thaw stability.

After formulating the ice cream recipes which were produced with double emulsion, the control samples were prepared accordingly. In this study, ice cream samples, containing the same concentration of fat as in ice cream prepared with double emulsion and ice cream with regular fat content were also prepared with conventional method to assess the difference between them.

3.2.1 Overrun of Ice Cream Formulations

Overrun is an important physical characteristic of the ice cream since it affects most of the quality attributes in the ice cream product. Also, it is a good indicator of aeration process and network formation in the ice cream. Besides, it is correlated with the flavor, taste and thermal properties of ice cream (Soukoulis et al., 2010). Increasing overrun values offered smaller air cell bubbles and ice crystals such that it resulted in softer ice cream at the end (Sofjan & Hartel, 2004).

Overrun values of 6 different ice cream formulations were tabulated in Table 3.3. The amount of fat varied from 2.82% (double emulsion and low fat) to 12% (regular fat). The reason for the lower overrun values obtained for regular fat formulations might be weak network formation in the ice cream. The weakness of the network might be due to lower amount of emulsifier/stabilizer corresponding to the amount of fat. In other words, the same amount of emulsifier/stabilizer was used for all formulations, but this amount of emulsifier/stabilizer may not be sufficient to obtain strong network in regular fat containing ice cream.

Ice cream produced by using double emulsion had higher overrun values than regular fat containing formulations prepared conventionally. This showed that double emulsion improved air content and network formation in the ice cream formulations when compared to regular fat ice cream (Table A.12 and Table A.13). The analysis of overrun indicated that replacing small amount of GG with GT resulted in improvement of aeration in the ice cream to some extent. A study conducted to analyze the effect of GT on textural properties of cheese concluded that samples prepared with high concentration of GT were softer as the concentration of gum increased from 0.25 g/kg of product to 1 g/kg of product (Rahimi et al., 2007). GT and whey protein concentrate were used in another research to replace the amount of fat in yogurt product in which addition of GT provided more open structure to the product (Aziznia et al., 2008). These proved that GT aided the formation of network and more open structure in the ice cream formulations.

Table 3.3 Overrun values (%) of different ice cream formulations. Rows containing different letters are significantly different ($p \leq 0.05$).

Ice Cream Sample	Double Emulsion	Low Fat	Regular Fat
PL-GGGT*	90^{a}	83^{ab}	77^{b}
PL-GG**	85^{a}	75^{ab}	70^{b}

* PL-GGGT: PGPR-lecithin blend as hydrophobic emulsifier and guar gum-gum tragacanth as hydrophilic gum.

** PL-GG: PGPR-lecithin blend as hydrophobic emulsifiers and guar gum as a hydrophilic gum.

3.2.2 Meltdown Properties of Ice Cream Formulations

Ice cream structure can also be explained by analyzing the rate of melting under controlled conditions. Meltdown properties of ice cream give essential information about fat aggregation which is very crucial to characterize sensorial attributes of ice cream (Bolliger et al., 2000).

In Figure 3.18 & Figure 3.19, melting rates of six different ice cream formulations were shown. In Figure 3.18, melting curves of ice cream containing GG-GT were drawn and their rates were determined by linear regression ($r^{2}>0.95$) analysis (Table 3.4). Figure 3.19 shows the melting rates of ice cream formulations containing guar gum as stabilizer.

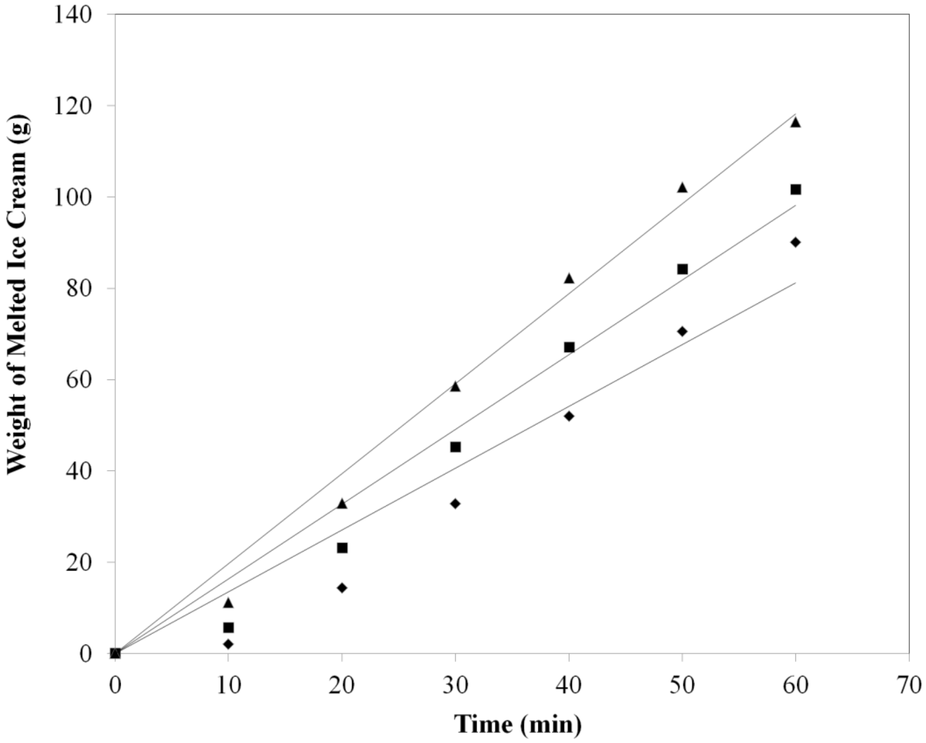


Figure 3.18 Melting curve of ice cream formulations containing PGPR-lecithin as hydrophilic emulsifiers and GG-GT as hydrophilic gums with a varying fat concentration; regular fat (triangle), low fat (square) and double (diamond). Markers represent data points, lines represent linear model.

The rate of melting was the lowest in the case of ice cream produced with double emulsion when GG or GG-GT was used as stabilizers (Table 3.4). Regular fat containing formulations melted more within the same time interval and found to be significantly different from the others. However, time of first drip of the samples did not show any significant difference (Table A.14 and Table A.15). In literature, it was stated that some of the proteins including casein micelles might not be adsorbed due to the effect of low molecular weight lipid emulsifiers in the frozen phase. However, they still continue to contribute the structure of the final product. As a consequence, it is very likely that there may not be any difference observed in time of first drip of the samples although there is significant difference between the melting rates once the structure starts to deform (Goff, 2002) (Table A.16 and Table A.17). This was also valid for ice cream formulations containing GG due to similar observations as presented in Table 3.4 and Figure 3.19. It is worth noting that independent of the stabilizer used in the ice cream mixes, the order of melting rates were the same; double emulsion < low fat < regular fat.

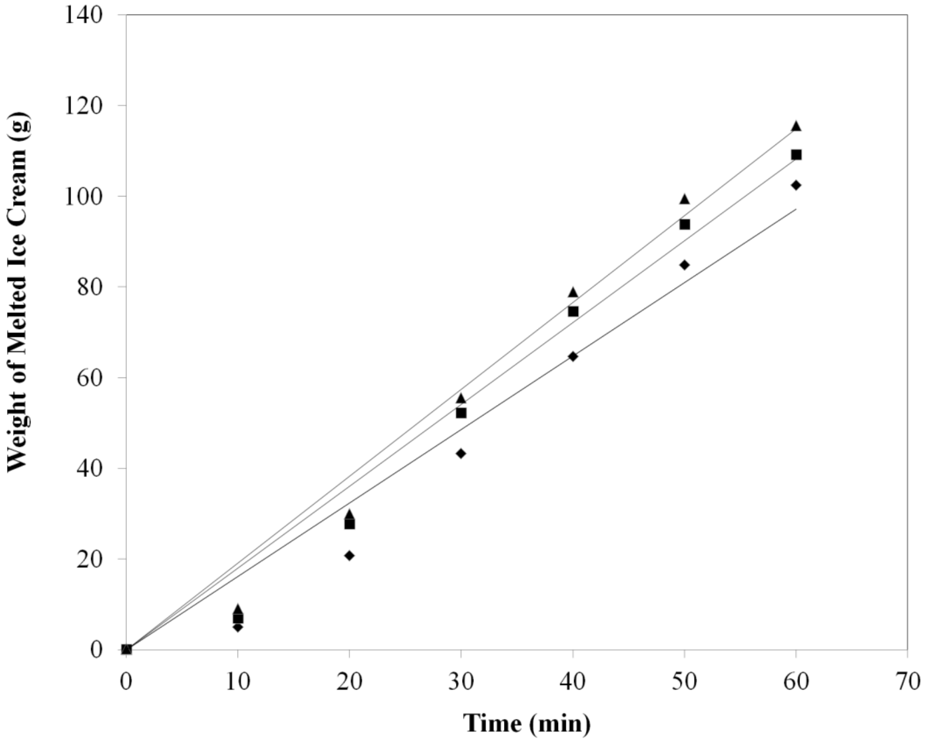


Figure 3.19 Melting curve of ice cream containing PGPR-lecithin (PL) as hydrophilic emulsifiers and GG as hydrophilic gum with a varying fat concentration; regular fat (triangle), low fat (square) and double (diamond). Markers represent data points, lines represent linear model.

The melting rate was inversely correlated with the overrun values tabulated in Table 3.2 and Table 3.3 (with a correlation coefficient of -0.873 and a p-value of 0.000). Similar results were obtained in literature (Segall & Goff, 2002). This can be explained by air cells which slow down the melting rate (Goff, 1997). Melting properties of ice cream could be improved via addition of dietary fiber (Soukoulis et al., 2009), emulsifier/stabilizer, fat components, sweeteners, and mineral salts (Goff & Hartel, 2013).

Table 3.4 Melting rate and time of first drip of ice cream formulations; PL-GGGT: PGPR-lecithin blend as hydrophobic emulsifiers and guar gum-gum tragacanth as hydrophilic gum; and PL-GG: PGPR-lecithin blend as hydrophobic emulsifiers and guar gum as a hydrophilic gum. Having capital or small letters stands for individual statistical analysis.

Formulation Type	Fat Content	Rate of Melting (g/min)	Time of First Drip (min)
PL-GGGT	Regular fat (12%)	1.970^{a}	12.5^{a}
	Low fat (2.82%)	1.635^{b}	13.0^{a}
	Double (2.82%)	1.354^{c}	14.0^{a}
PL-GG	Regular fat (12%)	1.917^{A}	10.0^{A}
	Low fat (2.82%)	1.802^{B}	12.0^{A}
	Double (2.82%)	1.621^{C}	14.0^{A}

3.2.3 Rheological Properties of Ice Cream Formulations

Rheology is another significant characteristic that has been investigated extensively for the correlation of microstructure of the ice cream with quality parameters (Wildmoser et al., 2004). For the determination of ice cream rheology, oscillation thermo-rheometer has been used because it provides useful information about the structural networks regarding both thermal and mechanical properties (Granger et al., 2005).

In this study, by performing oscillatory thermo-rheometer, storage (G′) and loss modulus (G″) of six different ice cream formulations were assessed; PL-GGGT (double emulsion, low fat and regular fat) and PL-GG (double emulsion, low fat and regular fat).

The G′ values explain the solid body like behavior of material in terms of elastically stored deformation energy while G″ values represent viscous fluid behavior corresponding the lost deformation energy (Wildmoser et al., 2004).

Figure 3.20 presents temperature sweep test results of ice cream prepared with double emulsion and PL-GGGT. As can be observed from Figure 3.20, both storage (G′) and loss modulus (G″) decreased as temperature increased. The steep decrease of G′ and G″ values was observed between the temperatures -5°C to -2°C where the ice fraction decreased as mentioned in the study conducted by Granger and the colleagues (2005). This change was attributed to the decrease in ice crystals. A more pronounced decrease was observed in the samples of ice cream prepared by double emulsion in the temperatures between -5°C and 0°C. This might be explained by the presence of ice crystals mainly in the outer phase of the double emulsion. In the outer phase of the double emulsions, there were milk solids and gum blends that control the structure of ice cream by providing a network while in the conventional ice cream formulations; both regular fat and low fat containing ones, all ingredients were present in the simple emulsion. In other words, in conventional ice cream formulations (regular fat and low fat), there exist emulsifiers (PGPR-lecithin blend) as well as milk solids and gum blends in the simple emulsion that may probably form more complex matrix in the system by the interaction of emulsifiers and stabilizers with fat. As a result, less decrease in G′ and G″ values of ice cream formulations containing low fat and regular fat can be clearly noticed. In the temperature range between 0°C to 15°C in which all ice crystals melted, only fat phase and air bubbles determine the rheological behavior as well as quality characteristics (Wildmoser et al., 2004). The G″ values, describing the flowability of ice cream, were higher in the regular fat formulations followed by low fat and double emulsion for the ice cream prepared with PL-GGGT.

In the case of ice cream formulations prepared with PL-GG, the values of G′ and G″ can be differentiated (Figure 3.21). As compared to PL-GGGT ice cream samples, the change in the G′ and G″ values were smoother in the temperature range -5°C to 0°C. The reason may be the presence of less air and as a consequence lower overrun values of ice cream formulations containing PL-GG as given in Table 3.3 that corresponded to less integrity of icy microstructure and less sensorial impression of coldness (Wildmoser et al., 2004). The G′ and G″ values decreased over temperature ranges as observed in samples of PL-GGGT. The samples with low fat, regular fat and double emulsion showed similar behavior.

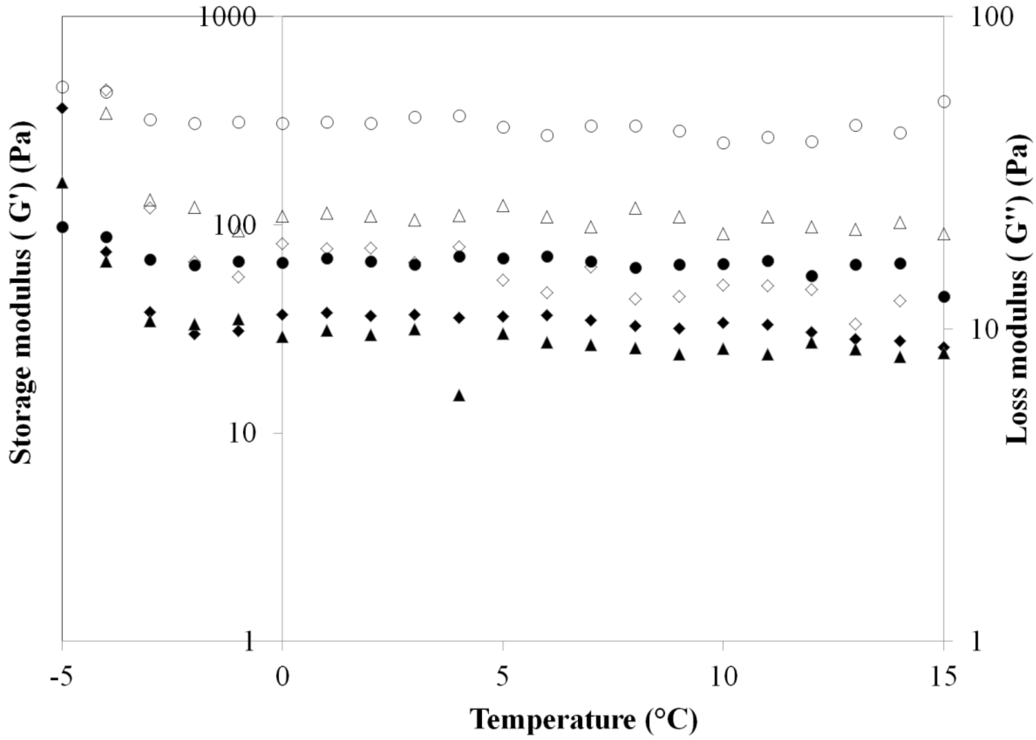


Figure 3.20 Change in storage (G′) and loss modulus (G″) as a function of temperature obtained by temperature sweep test of ice cream prepared by PL-GGGT with double emulsion G′(diamond), low fat G′(triangle) , regular fat G′(circle). Filled markers represent storage, empty markers represent loss modulus.

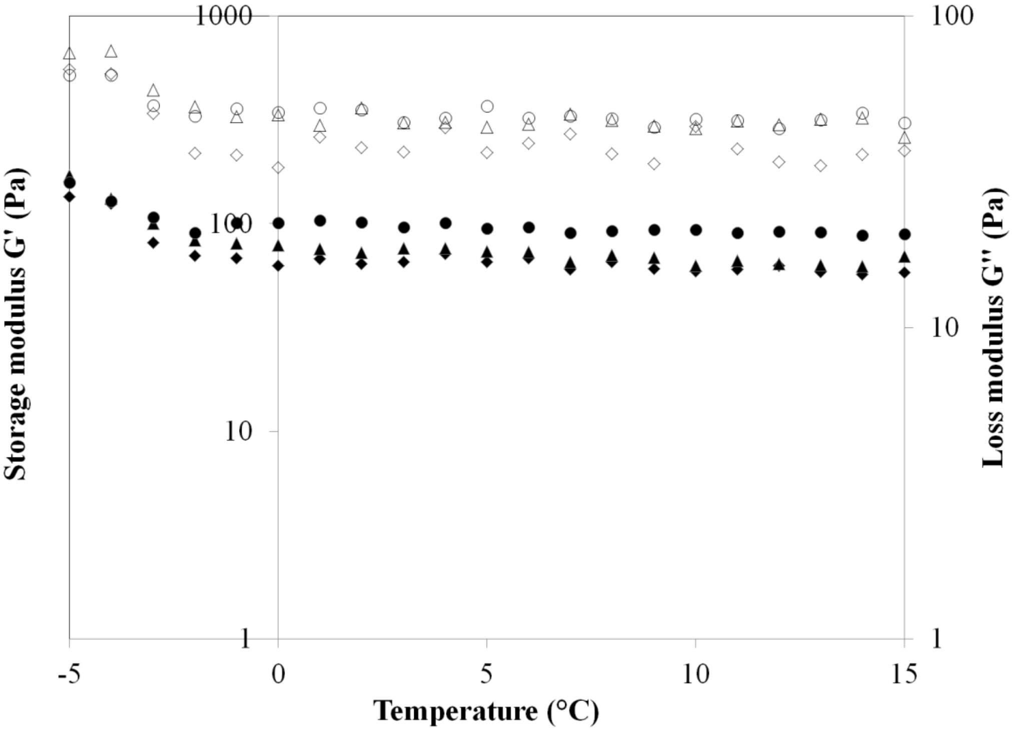


Figure 3.21 Change in storage (G′) and loss modulus (G″) as a function of temperature obtained by temperature sweep test of ice cream prepared by PL-GG with double emulsion G′(diamond), low fat G′(triangle) , regular fat G′(circle). Filled markers represent storage, empty markers represent loss modulus.

In the range of temperatures between 0°C to 15°C, the values of G″ reached a plateu for all formulations. As shown in Figure 3.20 & Figure 3.21, there was no clear change in the values of G′ and G″ to pronounce the effects of stabilizers in the ice cream formulations for this study.

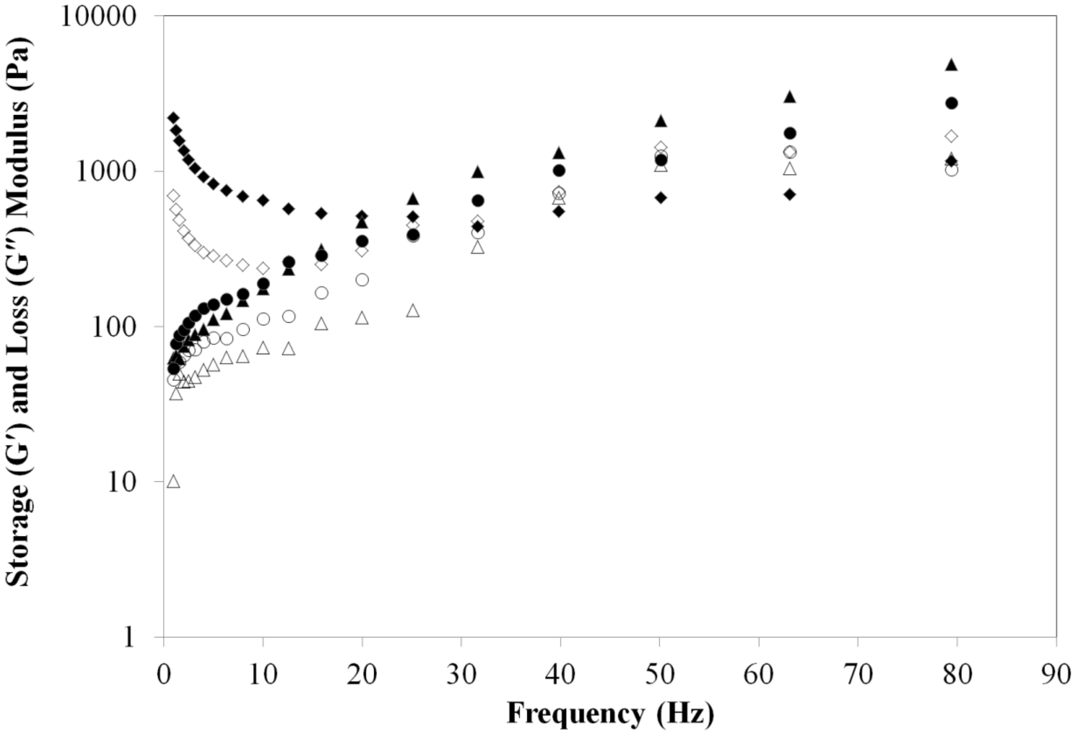


Figure 3.22 Change in storage (G′) and loss modulus (G″) as a function of frequency obtained by frequency sweep test of ice cream prepared by PL-GGGT with double emulsion at -5°C; G′(diamond), low fat G′(triangle) , regular fat G′(circle). Filled markers represent storage, empty markers represent loss modulus.

Frequency sweep test was also performed at -5°C in order to assess the frequency dependency of the ice cream samples as well as viscoelastic behaviors (Figure 3.22 & Figure 3.23). In Figure 3.22, only double emulsion formulated ice cream with PL-GGGT sample showed decrease in the values of both G′ and G″ values up to 30 Hz. However, after 30 Hz, crossover point occurred which means transition from more elastic behavior (G′> G″) to more viscous behavior (G′ < G″) occurred (Adapa et al., 2000). For the low fat and regular fat formulations, no crossover point observed. Moreover, the trend of low fat and regular fat formulations was different; the values of G′ and G″ increased with increasing frequency. In literature, rheological behavior of ice cream samples were similar to our findings for low fat and regular fat formulations (Wildmoser et al., 2004). The differences between the samples may be due to the microstructure differences. PL-GGGT containing ice cream formulation behaved differently in the frequency sweep test. Two different gums namely guar gum and gum tragacanth may form different structure in the ice cream interacting with ingredients.

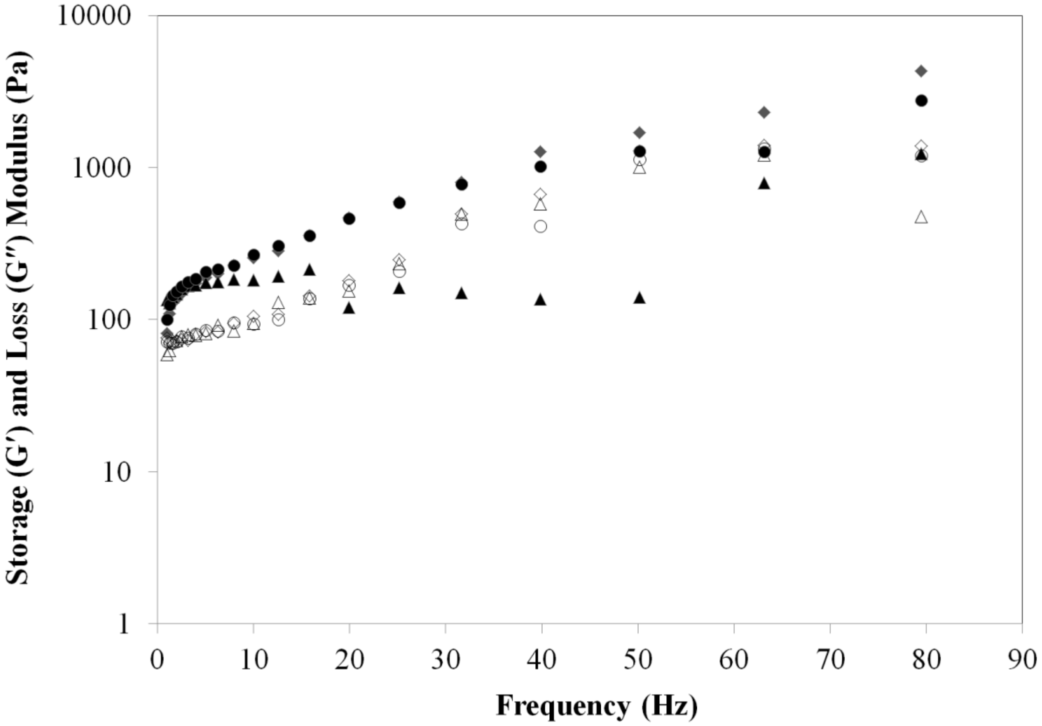


Figure 3.23 Change in storage (G′) and loss modulus (G″) as a function of frequency obtained by frequency sweep test of ice cream prepared by PL-GG with double emulsion at -5°C; G′(diamond), low fat G′(triangle), regular fat G′(circle). Filled markers represent storage, empty markers represent loss modulus.

The frequency sweep test applied at -5°C for the formulations containing PL-GG is shown in Figure 3.23. The same trend was observed for all formulations except low fat sample in terms of G′. The G′ and G″ increased with increasing frequency levels. The decrease in the G′ values of low fat formulation may be due to loss of structure in the network.

3.2.4 Sensory Analysis of Ice Cream Formulations

Ice cream is an extensively studied product due to its massive consumption. Therefore, sensory attributes of ice cream has a great importance. The attributes mostly examined are viscosity, iciness, intensity of coldness, mouth-coating, and overall acceptability (Aime et al., 2001; Wildmoser et al., 2004; Thompson, 2007).

Sensory analysis was performed for six different ice cream samples, PL-GGGT containing ones (double emulsion, low fat, regular fat) and PL-GG containing ones (double emulsion, low fat, regular fat). Ice cream prepared with double emulsion and conventional low fat containing ice cream had 2.82% fat while regular fat containing ice cream had 12% fat. Sensory analysis results were given in Table 3.5.

In the light of sensory analysis, in the case of ice cream samples prepared with PL-GG, viscosity values of the samples did not vary significantly. Viscosity of regular fat containing formulation had significantly lower viscosity than ice cream with low fat and double emulsion when GG-GT blend was used as stabilizer.

Iciness is another important parameter of concern that affects perception of coldness of ice cream while consuming. The higher the water in the ice cream, the larger the ice crystals that causes higher perceived intensity of coldness (Aime et al., 2001). Iciness of regular fat containing ice cream sample formulated with GG-GT blend was significantly lower than low fat containing formulation, but not different from ice cream prepared with double emulsion (Table 3.5).

Table 3.5 Comparison of sensory attributes of ice cream different samples. Columns with different letters indicate significant difference ($p \leq 0.05$).

Sample	Sample code	Viscosity	Iciness	Intensity of coldness	Mouth-coating	Overall Acceptability
Double PL-GG	148	3.75^{ab}	2.00^{abc}	3.62^{ab}	3.625^{b}	3.12^{ab}
Low Fat PL-GG	256	3.62^{ab}	2.87^{ab}	4.62^{a}	2.00^{c}	2.2^{b}
Regular Fat PL-GG	328	3^{ab}	1.50^{bc}	3.00^{bc}	5.00^{a}	2.62^{b}
Double PL-GGGT	734	4.25^{a}	2.50^{abc}	3.62^{ab}	3.12^{b}	3.75^{a}
Low Fat PL-GGGT	617	3.75^{a}	3.50^{a}	4.25^{ab}	2.00^{c}	2.12^{b}
Regular Fat PL-GGGT	536	2.37^{b}	1.25^{c}	2.00^{c}	5.50^{a}	3.00^{ab}

Intensity of coldness of low fat containing ice cream was similar to the ice cream prepared with double emulsion. Only, regular fat containing formulation had similar values with double emulsion formulation when ice cream samples were prepared with PL-GG.

In the case of mouth-coating, the highest scores were given to regular fat containing ice cream. Good mouth-coating is generally attributed to high amount of fat in ice cream (Koeferli et al., 1996; Aime et al., 2001). Usage of double emulsion in ice cream improved mouth-coating significantly as compared to low fat formulations prepared conventionally.

Overall acceptability of the samples was also evaluated by the panelists. Ice cream formulated by double emulsion containing PL-GGGT had comparable score with the regular fat ice cream with PL-GGGT. When PL-GG was used, overall acceptability of ice cream with double emulsion was similar with that of low fat and regular fat containing ones.

The correlation between sensory attributes and fat levels were also determined (Table A.18). There was a strong positive correlation between the fat level and mouth-coating (Table 3.6). Increasing fat content of the ice cream improved the mouth-coating ability of ice cream as also stated in the literature (Koeferli et al., 1996). Amount of fat in ice cream alters viscosity which is correlated with the mouth-coating (Aime et al., 2001). There were negative correlations between fat levels and viscosity, iciness and intensity of coldness, meaning that when fat content of ice cream formulations was decreased, the values regarding these attributes increased. This is reasonable because fat has a crucial role in developing the structure (Goff et al., 1999).

In conclusion, ice cream prepared with double emulsion method showed improvement in sensory attributes and resulted in better scores than low fat formulations in terms of mouth-coating and overall acceptability when GG-GT blend was used. In addition, overall acceptability of this product was comparable with regular fat ice cream.

Table 3.6 The correlation coefficients of sensorial attributes with fat level of ice cream formulations ($p \leq 0.05$)

		Viscosity	Iciness	Intensity of coldness	Mouth-coating	Overall Acceptability
Fat Level	r	-0.417	-0.499	-0.566	0.837	0
	p-value	0.003	0	0	0	1

CHAPTER 4

CONCLUSIONS AND RECOMMENDATIONS

For the determination of the best double emulsion formulation particle size analysis, rheological measurements, stability analyses and optical imaging were performed. No significant differences was found between the PGPR and PGPR-lecithin blend containing double emulsions in terms of particle size, stability and viscosity. This showed that 0.5% PGPR can be replaced with lecithin in double emulsions. It was concluded that double emulsions prepared with PGPR-lecithin blend with GG (1%) and GG-GT blend (0.75%-0.25%) had longer stability values during storage as compared to other stabilizers. These samples had also similar values in terms of apparent viscosities and consistency coefficients. Thus, they could be used in low fat ice cream preparation.

Ice cream samples were prepared by incorporating double emulsions formulated with PGPR-lecithin blend as emulsifier and GG (1%) or GG-GT (0.75%-0.25%) blend as stabilizer and their effects were observed in terms of overrun, meltdown resistance, rheology and sensory analysis. The results were compared with the properties of regular fat ice cream and low fat ice cream prepared conventionally. Ice cream prepared by double emulsions had higher overrun values than regular fat containing one. Higher the overrun values, lower the melting rate of the ice cream was. The lowest melting rate was obtained in the case of ice cream prepared with double emulsion.

Usage of double emulsion in ice cream preparation improved mouth-coating significantly, but this sensory attribute was not as good as regular fat ice cream formulation. When PL-GGGT was used, ice cream samples prepared with double emulsion formulation of had similar acceptability values with regular fat containing formulation. In addition, it had higher overall acceptability value as compared to low fat ice cream. Negative correlations were found between fat levels and viscosity, intensity of coldness, or iciness. Levels of fat were positively correlated with the mouth-coating while no correlation was found for the overall acceptability.

By implementing double emulsions into ice cream, fat reduction to 2.82% could be achieved without affecting quality of ice cream adversely. Also, improvement was observed in terms of overrun and meltdown resistance of the ice cream when double emulsion was incorporated. In addition, better results were obtained in terms of mouth-coating and overall acceptability by the help of double emulsion as compared to low fat ice cream formulations. As a conclusion, the usage of multiple emulsions can be recommended to reduce the fat content of ice cream.

For further studies, incorporating double emulsions into different food products like chocolate and cake batter can be studied to reduce the amount of fat or sugar. Different fat types can also be used and their effects can be monitored under the usage of multiple emulsions. Moreover, it may be recommended that outer phase composition of multiple emulsions could be improved by the addition of electrolytes to enhance stability.

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APPENDIX A

STATISTICAL ANALYSIS

Table A.1 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared with different formulations and blends of hydrophobic emulsifiers of PGPR and lecithin and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) based on 1st day measurements.

General Linear Model: D[3,2] 1st versus Gum Type/Conc; Emulsifier Type/

```
Factor               Type   Levels  Values
Gum Type/Conc        fixed       8  0,5% GG+0,5%GT; 0,5% GG+0,5%LBG; 0,5%
                                    LBG+0,5%GT; 0,75% GG+0,25%GT; 0,75%
                                    GG+0,5%GT; 1% GG; 1% GT; 1% LBG
Emulsifier Type/Conc fixed       2  1,5%PGPR+0,5%Lecithin; 2%PGPR


Analysis of Variance for D[3,2] 1st, using Adjusted SS for Tests

Source                             DF    Seq SS   Adj SS  Adj MS      F
P
Gum Type/Conc                       7   6699,42  6699,42  957,06  48,79
0,000
Emulsifier Type/Conc                1     20,32    20,32   20,32   1,04
0,324
Gum Type/Conc*Emulsifier Type/Conc  7   3464,55  3464,55  494,94  25,23
0,000
Error                              16    313,85   313,85   19,62
Total                              31  10498,15


S = 4,42899   R-Sq = 97,01%   R-Sq(adj) = 94,21%


Unusual Observations for D[3,2] 1st

Obs  D[3,2] 1st      Fit  SE Fit  Residual  St Resid
 31     76,0000  84,9000  3,1318   -8,9000     -2,84 R
 32     93,8000  84,9000  3,1318    8,9000      2,84 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence
```

```
Gum Type/Conc      N  Mean  Grouping
0,75% GG+0,5%GT    4  71,1  A
0,5% GG+0,5%GT     4  65,0  A B
1% GG              4  61,9  A B
0,5% LBG+0,5%GT    4  56,5    B C
0,75% GG+0,25%GT   4  54,9    B C
1% GT              4  48,4      C
0,5% GG+0,5%LBG    4  33,9        D
1% LBG             4  26,0        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier Type/Conc    N  Mean  Grouping
1,5%PGPR+0,5%Lecithin  16  53,0  A
2%PGPR                 16  51,4  A
```

Table A.2 One-way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared with different formulations; hydrophobic emulsifier of PGPR and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) for comparison of 1st and 7th day measurements

One-way ANOVA: D_{32} versus Gum Type/Conc

```
Source          DF       SS      MS      F      P
Gum Type/Conc   15  19489,5  1299,3  34,83  0,000
Error           16    596,9    37,3
Total           31  20086,4

S = 6,108   R-Sq = 97,03%   R-Sq(adj) = 94,24%



Level                  N    Mean  StDev
0,5% GG+0,5%GT-1st     2   73,90   0,14
0,5% GG+0,5%GT-7th     2   85,65   4,45
0,5% GG+0,5%LBG-1st    2   31,65   0,07
0,5% GG+0,5%LBG-7th    2   65,20   3,96
0,5% LBG+0,5%GT-1st    2   67,75   2,19
0,5% LBG+0,5%GT-7th    2   74,20   0,99
0,75% GG+0,25%GT-1st   2   66,80   1,84
0,75% GG+0,25%GT-7th   2  119,50  20,51
0,75% GG+0,5%GT-1st    2   57,35   8,13
0,75% GG+0,5%GT-7th    2   41,25   6,43
1% GG-1st              2   50,90   2,69
1% GG-7th              2   90,45   2,05
1% GT-1st              2   34,25   0,07
1% GT-7th              2   74,70   3,54
1% LBG-1st             2   28,70   0,28
1% LBG-7th             2   27,70   0,42
```

```
                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level                 ----+---------+---------+---------+-----
0,5% GG+0,5%GT-1st                    (--*--)
0,5% GG+0,5%GT-7th                       (---*--)
0,5% GG+0,5%LBG-1st    (---*--)
0,5% GG+0,5%LBG-7th                (--*--)
0,5% LBG+0,5%GT-1st                 (--*--)
0,5% LBG+0,5%GT-7th                   (--*--)
0,75% GG+0,25%GT-1st               (--*--)
0,75% GG+0,25%GT-7th                                 (--*--)
0,75% GG+0,5%GT-1st             (--*--)
0,75% GG+0,5%GT-7th        (--*--)
1% GG-1st                     (--*--)
1% GG-7th                                  (--*--)
1% GT-1st               (--*--)
1% GT-7th                             (--*--)
1% LBG-1st             (--*--)
1% LBG-7th            (--*--)
                      ----+---------+---------+---------+-----
                         30        60        90       120

Pooled StDev = 6,11


Grouping Information Using Tukey Method

Gum Type/Conc         N    Mean  Grouping
0,75% GG+0,25%GT-7th  2  119,50  A
1% GG-7th             2   90,45    B
0,5% GG+0,5%GT-7th    2   85,65    B C
1% GT-7th             2   74,70    B C D
0,5% LBG+0,5%GT-7th   2   74,20    B C D
0,5% GG+0,5%GT-1st    2   73,90    B C D
0,5% LBG+0,5%GT-1st   2   67,75    B C D
0,75% GG+0,25%GT-1st  2   66,80    B C D
0,5% GG+0,5%LBG-7th   2   65,20      C D E
0,75% GG+0,5%GT-1st   2   57,35        D E F
1% GG-1st             2   50,90        D E F G
0,75% GG+0,5%GT-7th   2   41,25          E F G
1% GT-1st             2   34,25            F G
0,5% GG+0,5%LBG-1st   2   31,65              G
1% LBG-1st            2   28,70              G
1% LBG-7th            2   27,70              G

Means that do not share a letter are significantly different.
```

Table A.3 One-way ANOVA and Tukey’s Comparison Test for particle size of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) for comparison of 1st and 7th day measurements.

One-way ANOVA: D_{32} versus Gum Type/Conc

```
Source         DF       SS     MS      F      P
Gum Type/Conc  15  12878,9  858,6  36,04  0,000
Error          16    381,2   23,8
Total          31  13260,1

S = 4,881   R-Sq = 97,13%   R-Sq(adj) = 94,43%
```

```
Level                 N    Mean   StDev
0,5% GG+0,5%GT-1st    2  56,150   7,000
0,5% GG+0,5%GT-7th    2  44,750   3,041
0,5% GG+0,5%LBG-1st   2  36,100   2,404
0,5% GG+0,5%LBG-7th   2  23,000   9,475
0,5% LBG+0,5%GT-1st   2  45,150   1,061
0,5% LBG+0,5%GT-7th   2  40,250   0,212
0,75% GG+0,25%GT-1st  2  42,950   1,061
0,75% GG+0,25%GT-7th  2  31,250   0,495
0,75% GG+0,5%GT-1st   2  84,900  12,587
0,75% GG+0,5%GT-7th   2  31,300   2,404
1% GG-1st             2  72,800   2,121
1% GG-7th             2  87,500   2,970
1% GT-1st             2  62,600   2,404
1% GT-7th             2  71,600   4,525
1% LBG-1st            2  23,400   2,546
1% LBG-7th            2  39,600   3,818

                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level                 --+---------+---------+---------+-------
0,5% GG+0,5%GT-1st                    (---*---)
0,5% GG+0,5%GT-7th               (--*---)
0,5% GG+0,5%LBG-1st         (---*---)
0,5% GG+0,5%LBG-7th   (---*--)
0,5% LBG+0,5%GT-1st              (---*--)
0,5% LBG+0,5%GT-7th           (---*---)
0,75% GG+0,25%GT-1st            (--*---)
0,75% GG+0,25%GT-7th      (---*--)
0,75% GG+0,5%GT-1st                                  (--*---)
0,75% GG+0,5%GT-7th       (---*--)
1% GG-1st                                      (--*---)
1% GG-7th                                             (---*--)
1% GT-1st                                 (--*---)
1% GT-7th                                     (---*--)
1% LBG-1st            (---*--)
1% LBG-7th                    (---*--)
```

```
                      --+---------+---------+---------+-------
                       20        40        60        80

Pooled StDev = 4,881


Grouping Information Using Tukey Method

Gum Type/Conc          N    Mean  Grouping
1% GG-7th              2  87,500  A
0,75% GG+0,5%GT-1st    2  84,900  A
1% GG-1st              2  72,800  A B
1% GT-7th              2  71,600  A B
1% GT-1st              2  62,600    B C
0,5% GG+0,5%GT-1st     2  56,150    B C D
0,5% LBG+0,5%GT-1st    2  45,150      C D E
0,5% GG+0,5%GT-7th     2  44,750      C D E
0,75% GG+0,25%GT-1st   2  42,950        D E
0,5% LBG+0,5%GT-7th    2  40,250        D E F
1% LBG-7th             2  39,600        D E F
0,5% GG+0,5%LBG-1st    2  36,100          E F
0,75% GG+0,5%GT-7th    2  31,300          E F
0,75% GG+0,25%GT-7th   2  31,250          E F
1% LBG-1st             2  23,400            F
0,5% GG+0,5%LBG-7th    2  23,000            F

Means that do not share a letter are significantly different.
```

Table A.4 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared with different formulations and blends of hydrophobic emulsifiers of PGPR and PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) after 7 days of storage at +4°C.

General Linear Model: D_{32} 7th versus Gum Type/Conc; Emulsifier Type/

```
Factor                Type   Levels  Values
Gum Type/Conc         fixed       8  0,5% GG+0,5%GT; 0,5% GG+0,5%LBG; 0,5%
                                     LBG+0,5%GT; 0,75% GG+0,25%GT; 0,75%
                                     GG+0,5%GT; 1% GG; 1% GT; 1% LBG
Emulsifier Type/Conc  fixed       2  1,5%PGPR+0,5%Lecithin; 2%PGPR


Analysis of Variance for D[3,2] 7th, using Adjusted SS for Tests

Source                              DF   Seq SS   Adj SS  Adj MS       F
P
Gum Type/Conc                        7  11156,1  11156,1  1593,7   38,39
0,000
Emulsifier Type/Conc                 1   5481,0   5481,0  5481,0  132,02
0,000
Gum Type/Conc*Emulsifier Type/Conc   7   7172,2   7172,2  1024,6   24,68
0,000
Error                               16    664,3    664,3    41,5
```

```
Total                               31  24473,7


S = 6,44336   R-Sq = 97,29%   R-Sq(adj) = 94,74%


Unusual Observations for D[3,2] 7th

Obs  D[3,2] 7th      Fit  SE Fit  Residual  St Resid
 25     134,000  119,500   4,556    14,500      3,18 R
 26     105,000  119,500   4,556   -14,500     -3,18 R

R denotes an observation with a large standardized residual.




Grouping Information Using Tukey Method and 95,0% Confidence

Gum Type/Conc      N  Mean  Grouping
1% GG              4  89,0  A
0,75% GG+0,25%GT   4  75,4  A B
1% GT              4  73,2    B
0,5% GG+0,5%GT     4  65,2    B C
0,5% LBG+0,5%GT    4  57,2      C D
0,5% GG+0,5%LBG    4  44,1        D E
0,75% GG+0,5%GT    4  36,3          E
1% LBG             4  33,6          E

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Emulsifier Type/Conc    N  Mean  Grouping
2%PGPR                 16  72,3  A
1,5%PGPR+0,5%Lecithin  16  46,2    B

Means that do not share a letter are significantly different.
```

Table A.5 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared with different formulations; hydrophobic emulsifier of PGPR and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) for comparison of 1st and 7th day measurements.

General Linear Model: D_{32} versus Gum Type/Conc; Day

```
Factor          Type   Levels  Values
Gum Type/Conc   fixed       8  0,5% GG+0,5%GT; 0,5% GG+0,5%LBG; 0,5%
LBG+0,5%GT;
                               0,75% GG+0,25%GT; 0,75% GG+0,5%GT; 1% GG; 1%
GT;
                               1% LBG
Day             fixed       2  1st; 7th
```

```
Analysis of Variance for D[3,2], using Adjusted SS for Tests

Source             DF   Seq SS   Adj SS  Adj MS      F      P
Gum Type/Conc       7  11946,3  11946,3  1706,6  45,74  0,000
Day                 1   3500,8   3500,8  3500,8  93,83  0,000
Gum Type/Conc*Day   7   4042,4   4042,4   577,5  15,48  0,000
Error              16    596,9    596,9    37,3
Total              31  20086,4


S = 6,10806   R-Sq = 97,03%   R-Sq(adj) = 94,24%


Unusual Observations for D[3,2]

Obs   D[3,2]      Fit  SE Fit  Residual  St Resid
 29  134,000  119,500   4,319    14,500      3,36 R
 30  105,000  119,500   4,319   -14,500     -3,36 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence

Gum Type/Conc      N  Mean  Grouping
0,75% GG+0,25%GT  4  93,2  A
0,5% GG+0,5%GT    4  79,8  A B
0,5% LBG+0,5%GT   4  71,0    B
1% GG             4  70,7    B
1% GT             4  54,5      C
0,75% GG+0,5%GT   4  49,3      C
0,5% GG+0,5%LBG   4  48,4      C
1% LBG            4  28,2        D

Means that do not share a letter are significantly different.


Grouping Information Using Tukey Method and 95,0% Confidence

Day   N  Mean  Grouping
7th  16  72,3  A
1st  16  51,4    B

Means that do not share a letter are significantly different.
```

Table A.6 Two way ANOVA and Tukey's Comparison Test for particle size of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) for comparison of 1st and 7th day measurements.

General Linear Model: D_{32} versus Gum Type/Conc; Day

```
Factor          Type   Levels  Values
Gum Type/Conc   fixed       8  0,5% GG+0,5%GT; 0,5% GG+0,5%LBG; 0,5%
LBG+0,5%GT;
                               0,75% GG+0,25%GT; 0,75% GG+0,5%GT; 1% GG; 1%
GT;
                               1% LBG
Day             fixed       2  1st; 7th


Analysis of Variance for D[3,2], using Adjusted SS for Tests

Source              DF    Seq SS   Adj SS   Adj MS      F      P
Gum Type/Conc        7   8983,98  8983,98  1283,43  53,87  0,000
Day                  1    375,38   375,38   375,38  15,76  0,001
Gum Type/Conc*Day    7   3519,58  3519,58   502,80  21,10  0,000
Error               16    381,19   381,19    23,82
Total               31  13260,13


S = 4,88102   R-Sq = 97,13%   R-Sq(adj) = 94,43%


Unusual Observations for D[3,2]

Obs   D[3,2]      Fit  SE Fit  Residual  St Resid
 15  76,0000  84,9000  3,4514   -8,9000     -2,58 R
 16  93,8000  84,9000  3,4514    8,9000      2,58 R

R denotes an observation with a large standardized residual.


Grouping Information Using Tukey Method and 95,0% Confidence


Gum Type/Conc     N  Mean  Grouping
1% GG             4  80,1  A
1% GT             4  67,1    B
0,75% GG+0,5%GT   4  58,1    B C
0,5% GG+0,5%GT    4  50,5      C D
0,5% LBG+0,5%GT   4  42,7        D E
0,75% GG+0,25%GT  4  37,1          E F
1% LBG            4  31,5          E F
0,5% GG+0,5%LBG   4  29,5            F

Means that do not share a letter are significantly different.
Grouping Information Using Tukey Method and 95,0% Confidence
Day   N  Mean  Grouping
1st  16  53,0  A
7th  16  46,2    B
```

Table A.7 One-way ANOVA and Tukey’s Comparison Test for viscosity at 5.5 Pa of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR and PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG).

One-way ANOVA: Apparent Viscosity versus Gum/Emulisfier Types

```
Source                DF       SS      MS      F      P
Gum/Emulisfier Types  15  166,984  11,132  12,27  0,000
Error                 16   14,511   0,907
Total                 31  181,496

S = 0,9523   R-Sq = 92,00%   R-Sq(adj) = 84,51%


Level                N    Mean   StDev
0,5% GG+0,5%GT-p     2  1,1750  0,0354
0,5% GG+0,5%GT-pl    2  0,8250  0,0071
0,5% GG+0,5%LBG-p    2  0,9900  0,0566
0,5% GG+0,5%LBG-pl   2  0,6800  0,0000
0,5% LBG+0,5%GT-p    2  0,1650  0,0071
0,5% LBG+0,5%GT-pl   2  0,1400  0,0000
0,75% GG+0,25%GT-p   2  3,0650  0,0495
0,75% GG+0,25%GT-pl  2  1,9450  0,2051
0,75% GG+0,5%GT-p    2  7,5550  0,0778
0,75% GG+0,5%GT-pl   2  4,6850  0,0071
1% GG-p              2  6,0350  0,0014
1% GG-pl             2  3,7500  0,0015
1% GT-p              2  0,1350  0,0071
1% GT-pl             2  0,1200  0,0000
1% LBG-p             2  0,2600  0,0000
1% LBG-pl            2  0,1950  0,0071

                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level                ----+---------+---------+---------+-----
0,5% GG+0,5%GT-p        (----*----)
0,5% GG+0,5%GT-pl      (----*----)
0,5% GG+0,5%LBG-p       (---*----)
0,5% GG+0,5%LBG-pl     (---*----)
0,5% LBG+0,5%GT-p    (----*---)
0,5% LBG+0,5%GT-pl   (---*----)
0,75% GG+0,25%GT-p            (----*----)
0,75% GG+0,25%GT-pl        (---*----)
0,75% GG+0,5%GT-p                            (----*----)
0,75% GG+0,5%GT-pl                  (----*---)
1% GG-p                                 (---*---)
1% GG-pl                         (---*---)
1% GT-p              (---*----)
1% GT-pl             (---*----)
1% LBG-p             (----*----)
1% LBG-pl            (----*---)
                     ----+---------+---------+---------+-----
                       0,0       3,0       6,0       9,0

Pooled StDev = 0,9523
```

```
Grouping Information Using Tukey Method

Gum/Emulisfier Types  N   Mean  Grouping
0,75% GG+0,5%GT-p     2  7,555  A
1% GG-p               2  6,035  A B
0,75% GG+0,5%GT-pl    2  4,685  A B C
1% GG-pl              2  3,750  A B C D
0,75% GG+0,25%GT-p    2  3,065    B C D
0,75% GG+0,25%GT-pl   2  1,945      C D
0,5% GG+0,5%GT-p      2  1,175      C D
0,5% GG+0,5%LBG-p     2  0,990      C D
0,5% GG+0,5%GT-pl     2  0,825        D
0,5% GG+0,5%LBG-pl    2  0,680        D
1% LBG-p              2  0,260        D
1% LBG-pl             2  0,195        D
0,5% LBG+0,5%GT-p     2  0,165        D
0,5% LBG+0,5%GT-pl    2  0,140        D
1% GT-p               2  0,135        D
1% GT-pl              2  0,120        D

Means that do not share a letter are significantly different.
```

Table A.8 One-way ANOVA and Tukey's Comparison Test for consistency coefficients (K) of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR and PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG).

One-way ANOVA: K versus Gum/Emulisfier Types

```
Source                DF       SS      MS      F      P
Gum/Emulisfier Types  15  185,873  12,392  25,81  0,000
Error                 16    7,681   0,480
Total                 31  193,554

S = 0,6928   R-Sq = 96,03%   R-Sq(adj) = 92,31%



Level                  N    Mean   StDev
0,5% GG+0.5% GT-p      2  2,5000  0,0283
0,5% GG+0.5% GT-pl     2  2,0150  0,0212
0,5% GG+0.5% LBG-p     2  1,8715  0,0841
0,5% GG+0.5% LBG-pl    2  1,4365  0,0106
0,5% LBG+0.5% GT-p     2  0,4015  0,0021
0,5% LBG+0.5% GT-pl    2  0,3395  0,0021
0,75% GG+0.25% GT-p    2  4,5500  0,0141
0,75% GG+0.25% GT-pl   2  3,4300  0,2263
0,75% GG+0.50% GT-p    2  7,6050  0,0354
0,75% GG+0.50% GT-pl   2  5,6950  0,0354
1% GG-p                2  6,7600  0,4890
```

```
1% GG-pl              2  5,0145  0,1929
1% GT-p               2  0,5305  0,0021
1% GT-pl              2  0,4655  0,0035
1% LBG-p              2  0,4255  0,0035
1% LBG-pl             2  0,3350  0,0085

                      Individual 95% CIs For Mean Based on
                      Pooled StDev
Level                 ---+---------+---------+---------+------
0,5% GG+0.5% GT-p              (---*---)
0,5% GG+0.5% GT-pl           (---*---)
0,5% GG+0.5% LBG-p          (---*----)
0,5% GG+0.5% LBG-pl        (---*---)
0,5% LBG+0.5% GT-p    (----*---)
0,5% LBG+0.5% GT-pl   (---*----)
0,75% GG+0.25% GT-p                    (---*---)
0,75% GG+0.25% GT-pl               (---*---)
0,75% GG+0.50% GT-p                                (---*----)
0,75% GG+0.50% GT-pl                        (---*---)
1% GG-p                                         (---*---)
1% GG-pl                                 (---*---)
1% GT-p                (---*---)
1% GT-pl               (---*---)
1% LBG-p               (---*---)
1% LBG-pl             (---*---)
                      ---+---------+---------+---------+------
                       0,0       2,5       5,0       7,5

Pooled StDev = 0,6928


Grouping Information Using Tukey Method

Gum/Emulisfier Types  N   Mean  Grouping
0,75% GG+0.50% GT-p   2  7,605  A
1% GG-p               2  6,760  A B
0,75% GG+0.50% GT-pl  2  5,695  A B C
1% GG-pl              2  5,015  A B C D
0,75% GG+0.25% GT-p   2  4,550    B C D E
0,75% GG+0.25% GT-pl  2  3,430      C D E F
0,5% GG+0.5% GT-p     2  2,500        D E F G
0,5% GG+0.5% GT-pl    2  2,015          E F G
0,5% GG+0.5% LBG-p    2  1,872          E F G
0,5% GG+0.5% LBG-pl   2  1,437            F G
1% GT-p               2  0,530              G
1% GT-pl              2  0,466              G
1% LBG-p              2  0,425              G
0,5% LBG+0.5% GT-p    2  0,402              G
0,5% LBG+0.5% GT-pl   2  0,340              G
1% LBG-pl             2  0,335              G

Means that do not share a letter are significantly different.
```

Table A.9 One-way ANOVA and Tukey's Comparison Test for flow behavior index (n) of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR and PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG).

One-way ANOVA: n versus Gum/Emulisfier Types

```
Source                DF        SS        MS        F      P
Gum/Emulisfier Types  15  0,837272  0,055818  337,02  0,000
Error                 16  0,002650  0,000166
Total                 31  0,839922

S = 0,01287   R-Sq = 99,68%   R-Sq(adj) = 99,39%



Level                N     Mean    StDev
0,5% GG+0,5%GT-p     2  0,46500  0,00707
0,5% GG+0,5%GT-pl    2  0,49000  0,00000
0,5% GG+0,5%LBG-p    2  0,58500  0,00707
0,5% GG+0,5%LBG-pl   2  0,60000  0,00000
0,5% LBG+0,5%GT-p    2  0,74000  0,00000
0,5% LBG+0,5%GT-pl   2  0,75000  0,00000
0,75% GG+0,25%GT-p   2  0,39000  0,00000
0,75% GG+0,25%GT-pl  2  0,42500  0,00707
0,75% GG+0,5%GT-p    2  0,35000  0,00000
0,75% GG+0,5%GT-pl   2  0,39000  0,00000
1% GG-p              2  0,36000  0,04243
1% GG-pl             2  0,39500  0,02121
1% GT-p              2  0,63000  0,01414
1% GT-pl             2  0,64500  0,00707
1% LBG-p             2  0,82000  0,00000
1% LBG-pl            2  0,82000  0,00000

                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level                --------+---------+---------+---------+-
0,5% GG+0,5%GT-p             (*)
0,5% GG+0,5%GT-pl             (-*)
0,5% GG+0,5%LBG-p                    (*)
0,5% GG+0,5%LBG-pl                    (*)
0,5% LBG+0,5%GT-p                              (*-)
0,5% LBG+0,5%GT-pl                              (*)
0,75% GG+0,25%GT-p      (*)
0,75% GG+0,25%GT-pl       (*-)
0,75% GG+0,5%GT-p    (*-)
0,75% GG+0,5%GT-pl      (*)
1% GG-p               (*)
1% GG-pl                (*-)
1% GT-p                                 (*)
1% GT-pl                                 (*)
1% LBG-p                                            (-*)
1% LBG-pl                                           (-*)
                     --------+---------+---------+---------+-
                           0,45      0,60      0,75      0,90
```

```
Pooled StDev = 0,01287


Grouping Information Using Tukey Method

Gum/Emulisfier Types  N     Mean  Grouping
1% LBG-pl             2  0,82000  A
1% LBG-p              2  0,82000  A
0,5% LBG+0,5%GT-pl    2  0,75000    B
0,5% LBG+0,5%GT-p     2  0,74000    B
1% GT-pl              2  0,64500      C
1% GT-p               2  0,63000      C D
0,5% GG+0,5%LBG-pl    2  0,60000      C D
0,5% GG+0,5%LBG-p     2  0,58500        D
0,5% GG+0,5%GT-pl     2  0,49000          E
0,5% GG+0,5%GT-p      2  0,46500          E F
0,75% GG+0,25%GT-pl   2  0,42500            F G
1% GG-pl              2  0,39500              G H
0,75% GG+0,5%GT-pl    2  0,39000              G H
0,75% GG+0,25%GT-p    2  0,39000              G H
1% GG-p               2  0,36000                H
0,75% GG+0,5%GT-p     2  0,35000                H

Means that do not share a letter are significantly different.
```

Table A.10 One-way ANOVA and Tukey's Comparison Test for stability of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR and PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) based on centrifugation assay.

One-way ANOVA: ES(%) Centrifuge versus Gum/Emulsifier Type

```
Source               DF       SS      MS      F      P
Gum/Emulsifier Type  15  650,572  43,371  65,06  0,000
Error                16   10,665   0,667
Total                31  661,238

S = 0,8165   R-Sq = 98,39%   R-Sq(adj) = 96,87%


                                       Individual 95% CIs For Mean Based on
                                       Pooled StDev
Level                N    Mean  StDev  ---------+---------+---------+------
---+
0,5% GG+0,5%GT-p     2  78,910  0,354      (--*-)
0,5% GG+0,5%GT-pl    2  88,010  0,057                         (-*-)
0,5% GG+0,5%LBG-p    2  78,930  0,127      (--*-)
0,5% GG+0,5%LBG-pl   2  81,250  1,754           (--*-)
0,5% LBG+0,5%GT-p    2  80,345  0,021         (--*-)
0,5% LBG+0,5%GT-pl   2  92,200  1,697                                 (-*--
```

```
)
0,75% GG+0,25%GT-p   2  77,485  0,304    (-*-)
0,75% GG+0,25%GT-pl  2  85,695  1,506                    (-*--)
0,75% GG+0,5%GT-p    2  79,430  0,028       (--*-)
0,75% GG+0,5%GT-pl   2  90,180  0,226                             (-*--)
1% GG-p              2  78,800  0,354      (--*-)
1% GG-pl             2  81,410  0,014           (--*-)
1% GT-p              2  76,575  0,332  (-*--)
1% GT-pl             2  86,120  1,372                     (-*--)
1% LBG-p             2  81,175  0,035           (-*--)
1% LBG-pl            2  81,740  0,184            (-*--)
                                       ---------+---------+---------+------
---+
                                             80,0      85,0      90,0
95,0

Pooled StDev = 0,816


Grouping Information Using Tukey Method

Gum/Emulsifier Type  N    Mean  Grouping
0,5% LBG+0,5%GT-pl   2  92,200  A
0,75% GG+0,5%GT-pl   2  90,180  A B
0,5% GG+0,5%GT-pl    2  88,010    B C
1% GT-pl             2  86,120      C
0,75% GG+0,25%GT-pl  2  85,695      C
1% LBG-pl            2  81,740        D
1% GG-pl             2  81,410        D
0,5% GG+0,5%LBG-pl   2  81,250        D
1% LBG-p             2  81,175        D
0,5% LBG+0,5%GT-p    2  80,345        D E
0,75% GG+0,5%GT-p    2  79,430        D E F
0,5% GG+0,5%LBG-p    2  78,930        D E F
0,5% GG+0,5%GT-p     2  78,910        D E F
1% GG-p              2  78,800        D E F
0,75% GG+0,25%GT-p   2  77,485          E F
1% GT-p              2  76,575            F

Means that do not share a letter are significantly different.
```

Table A.11 One-way ANOVA and Tukey's Comparison Test for stability of double emulsions prepared with different formulations; hydrophobic emulsifiers of PGPR and PGPR-lecithin blend and stabilizers of guar gum (GG), gum tragacanth (GT) and locust bean gum (LBG) based on 7 days of storage at +4°C.

One-way ANOVA: ES(%) (+4°C) versus Gum Type/Conc

```
Source          DF        SS       MS       F      P
Gum Type/Conc   15  3062,688  204,179  241,11  0,000
Error           16    13,549    0,847
Total           31  3076,237
```

```
S = 0,9202   R-Sq = 99,56%   R-Sq(adj) = 99,15%



Level                N     Mean  StDev
0,5% GG+0,5%GT-p     2   93,065  0,290
0,5% GG+0,5%GT-pl    2   97,340  0,354
0,5% GG+0,5%LBG-p    2   87,535  0,997
0,5% GG+0,5%LBG-pl   2   93,025  1,025
0,5% LBG+0,5%GT-p    2   90,915  1,294
0,5% LBG+0,5%GT-pl   2   93,990  0,240
0,75% GG+0,25%GT-p   2  100,000  0,000
0,75% GG+0,25%GT-pl  2   95,500  0,707
0,75% GG+0,5%GT-p    2  100,000  0,000
0,75% GG+0,5%GT-pl   2   99,130  1,103
1% GG-p              2  100,000  0,000
1% GG-pl             2   96,835  0,035
1% GT-p              2   76,130  0,368
1% GT-pl             2   99,000  0,000
1% LBG-p             2   62,530  1,259
1% LBG-pl            2   86,730  2,475

                     Individual 95% CIs For Mean Based on
                     Pooled StDev
Level                ---------+---------+---------+---------+
0,5% GG+0,5%GT-p                              (-*)
0,5% GG+0,5%GT-pl                                 (*)
0,5% GG+0,5%LBG-p                         (*)
0,5% GG+0,5%LBG-pl                            (-*)
0,5% LBG+0,5%GT-p                            (*)
0,5% LBG+0,5%GT-pl                             (*)
0,75% GG+0,25%GT-p                                  (*)
0,75% GG+0,25%GT-pl                             (-*)
0,75% GG+0,5%GT-p                                   (*)
0,75% GG+0,5%GT-pl                                 (-*)
1% GG-p                                             (*)
1% GG-pl                                          (*)
1% GT-p                          (*-)
1% GT-pl                                           (-*)
1% LBG-p             (*)
1% LBG-pl                                (*)
                     ---------+---------+---------+---------+
                             72        84        96       108

Pooled StDev = 0,920


Grouping Information Using Tukey Method

Gum Type/Conc        N     Mean  Grouping
1% GG-p              2  100,000  A
0,75% GG+0,5%GT-p    2  100,000  A
0,75% GG+0,25%GT-p   2  100,000  A
0,75% GG+0,5%GT-pl   2   99,130  A B
1% GT-pl             2   99,000  A B
0,5% GG+0,5%GT-pl    2   97,340  A B C
1% GG-pl             2   96,835  A B C
0,75% GG+0,25%GT-pl  2   95,500    B C D
0,5% LBG+0,5%GT-pl   2   93,990      C D E
```

```
0,5% GG+0,5%GT-p     2   93,065        D E
0,5% GG+0,5%LBG-pl   2   93,025        D E
0,5% LBG+0,5%GT-p    2   90,915          E F
0,5% GG+0,5%LBG-p    2   87,535            F G
1% LBG-pl            2   86,730              G
1% GT-p              2   76,130                H
1% LBG-p             2   62,530                  I

Means that do not share a letter are significantly different.
```

Table A.12 One way ANOVA and Tukey's Comparison Test for overrun values of ice cream samples; double, low fat and regular fat of PL-GGGT.

One-way ANOVA: Overrun versus Sample

```
Source  DF      SS     MS      F      P
Sample   2  169,33  84,67  10,58  0,044
Error    3   24,00   8,00
Total    5  193,33

S = 2,828   R-Sq = 87,59%   R-Sq(adj) = 79,31%


                               Individual 95% CIs For Mean Based on
                               Pooled StDev
Level        N    Mean  StDev  ---------+---------+---------+---------+
Double-GGGT  2  90,000  2,828                   (---------*--------)
RF-GGGT      2  77,000  2,828  (--------*--------)
LF-GGGT      2  83,000  2,828          (---------*--------)
                               ---------+---------+---------+---------+
                                     77,0      84,0      91,0      98,0

Pooled StDev = 2,828


Grouping Information Using Tukey Method

Sample       N    Mean  Grouping
Double-GGGT  2  90,000  A
LF-GGGT      2  83,000  A B
RF-GGGT      2  77,000    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample

Individual confidence level = 97,50%


Sample = Double-GGGT subtracted from:

Sample     Lower   Center   Upper   -+---------+---------+--------+-------
-
```

```
RF-GGGT  -24,820  -13,000  -1,180   (---------*---------)
LF-GGGT  -18,820   -7,000   4,820        (---------*---------)
                                    -+---------+---------+---------+-------
-
                                   -24       -12         0        12


Sample = RF-GGGT subtracted from:

Sample    Lower  Center   Upper   -+---------+---------+---------+--------
LF-GGGT  -5,820   6,000  17,820                   (---------*---------)
                                  -+---------+---------+---------+--------
                                 -24       -12         0        12
```

Table A.13 One way ANOVA and Tukey's Comparison Test for overrun values of ice cream samples; double, low fat and regular fat of PL-GG.

One-way ANOVA: Overrun versus Sample

```
Source    DF      SS      MS      F      P
Sample     2  233,33  116,67  19,44  0,019
Error      3   18,00    6,00
Total      5  251,33

S = 2,449   R-Sq = 92,84%   R-Sq(adj) = 88,06%


                             Individual 95% CIs For Mean Based on
                             Pooled StDev
Level      N    Mean  StDev  --------+---------+---------+---------+-
Double-GG  2  85,000  2,828                       (------*-------)
RF-GG      2  70,000  2,828  (-------*-------)
LF-GG      2  75,000  1,414        (-------*-------)
                             --------+---------+---------+---------+-
                                  70,0      77,0      84,0      91,0

Pooled StDev = 2,449

Grouping Information Using Tukey Method

Sample     N    Mean  Grouping
Double-GG  2  85,000  A
LF-GG      2  75,000  A B
RF-GG      2  70,000    B

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample_1

Individual confidence level = 97,50%


Sample   = Double-GG subtracted from:
```

```
Sample      Lower   Center   Upper   -+---------+---------+---------+------
--
RF-GG     -25,236  -15,000  -4,764   (--------*-------)
LF-GG     -20,236  -10,000   0,236       (--------*-------)
                                     -+---------+---------+---------+------
--
                                    -24       -12         0        12

Sample   = RF-GG subtracted from:

Sample     Lower  Center   Upper   -+---------+---------+---------+--------
LF-GG     -5,236   5,000  15,236                    (-------*--------)
                                   -+---------+---------+---------+--------
                                  -24       -12         0        12
```

Table A.14 One way ANOVA and Tukey's Comparison Test for melting rates of ice cream samples; double, low fat and regular fat of PL-GGGT.

One-way ANOVA: Melting Rate versus Sample

```
Source  DF         SS         MS          F      P
Sample   2  0,3803923  0,1901962  126797,44  0,000
Error    3  0,0000045  0,0000015
Total    5  0,3803968

S = 0,001225   R-Sq = 100,00%   R-Sq(adj) = 100,00%

                                  Individual 95% CIs For Mean Based on
                                  Pooled StDev
Level        N     Mean    StDev  ------+---------+---------+---------+---
Double-GGGT  2  1,35400  0,00141  (*
RF-GGGT      2  1,97000  0,00141                                       *
LF-GGGT      2  1,63550  0,00071                  *
                                  ------+---------+---------+---------+---
                                      1,44      1,60      1,76      1,92

Pooled StDev = 0,00122

Grouping Information Using Tukey Method

Sample       N     Mean  Grouping
RF-GGGT      2  1,97000  A
LF-GGGT      2  1,63550    B
Double-GGGT  2  1,35400      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample

Individual confidence level = 97,50%


Sample = Double-GGGT subtracted from:
```

```
Sample     Lower   Center    Upper  ----+---------+---------+---------+----
-
RF-GGGT  0,61088  0,61600  0,62112
(*
LF-GGGT  0,27638  0,28150  0,28662                          *
                                    ----+---------+---------+---------+----
-
                                     -0,25      0,00      0,25      0,50

Sample = RF-GGGT subtracted from:

Sample      Lower    Center     Upper  ----+---------+---------+---------+-
----
LF-GGGT  -0,33962  -0,33450  -0,32938  (*
                                       ----+---------+---------+---------+-
----
                                        -0,25      0,00      0,25      0,50
```

Table A.15 One way ANOVA and Tukey's Comparison Test for melting rates of ice cream samples; double, low fat and regular fat of PL-GG.

One-way ANOVA: Melting Rate versus Sample

```
Source    DF         SS         MS        F      P
Sample     2  0,0890680  0,0445340  3929,47  0,000
Error      3  0,0000340  0,0000113
Total      5  0,0891020

S = 0,003367   R-Sq = 99,96%   R-Sq(adj) = 99,94%


                                Individual 95% CIs For Mean Based on
                                Pooled StDev
Level      N     Mean    StDev  --------+---------+---------+---------+-
Double-GG  2  1,62100  0,00283  (*)
RF-GG      2  1,91700  0,00424                                     (*)
LF-GG      2  1,80200  0,00283                      (*)
                                --------+---------+---------+---------+-
                                      1,680     1,760     1,840     1,920

Pooled StDev = 0,00337

Grouping Information Using Tukey Method

Sample     N     Mean  Grouping
RF-GG      2  1,91700  A
LF-GG      2  1,80200    B
Double-GG  2  1,62100      C

Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample_1
```

```
Individual confidence level = 97,50%

Sample   = Double-GG subtracted from:

Sample      Lower   Center    Upper    -+---------+---------+---------+----
----
RF-GG     0,28193  0,29600  0,31007                                     (-
*)
LF-GG     0,16693  0,18100  0,19507                            (*)
                                       -+---------+---------+---------+----
----
                                     -0,12      0,00      0,12      0,24

Sample   = RF-GG subtracted from:

Sample       Lower    Center     Upper
LF-GG     -0,12907  -0,11500  -0,10093

Sample      -+---------+---------+---------+--------
LF-GG        (*-)
            -+---------+---------+---------+--------
          -0,12      0,00      0,12      0,24
```

Table A.16 One way ANOVA and Tukey's Comparison Test for time of first drip of ice cream samples; double, low fat and regular fat of PL-GGGT.

One-way ANOVA: Time of first drip versus Sample

```
Source  DF     SS     MS     F      P
Sample   2  2,333  1,167  1,40  0,372
Error    3  2,500  0,833
Total    5  4,833

S = 0,9129   R-Sq = 48,28%   R-Sq(adj) = 13,79%
Level        N    Mean  StDev
Double-GGGT  2  14,000  1,414
RF-GGGT      2  12,500  0,707
LF-GGGT      2  13,000  0,000

             Individual 95% CIs For Mean Based on Pooled StDev
Level           +---------+---------+---------+---------
Double-GGGT               (------------*-------------)
RF-GGGT         (------------*-------------)
LF-GGGT            (-------------*------------)
                +---------+---------+---------+---------
             10,5      12,0      13,5      15,0

Pooled StDev = 0,913

Grouping Information Using Tukey Method

Sample       N     Mean  Grouping
Double-GGGT  2  14,0000  A
LF-GGGT      2  13,0000  A
RF-GGGT      2  12,5000  A
```

```
Means that do not share a letter are significantly different.

Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample

Individual confidence level = 97,50%

Sample = Double-GGGT subtracted from:

Sample     Lower   Center   Upper    -+---------+---------+---------+------
--
RF-GGGT  -5,3149  -1,5000  2,3149    (--------------*--------------)
LF-GGGT  -4,8149  -1,0000  2,8149      (--------------*--------------)
                                     -+---------+---------+---------+------
--
                                   -5,0      -2,5       0,0       2,5

Sample = RF-GGGT subtracted from:

Sample     Lower  Center   Upper    -+---------+---------+---------+-------
-
LF-GGGT  -3,3149  0,5000  4,3149            (--------------*--------------)
                                    -+---------+---------+---------+-------
-
                                  -5,0      -2,5       0,0       2,5
```

Table A.17 One way ANOVA and Tukey's Comparison Test for time of first drip of ice cream samples; double, low fat and regular fat of PL-GG.

One-way ANOVA: Time of first drip versus Sample

```
Source    DF      SS    MS      F      P
Sample     2  16,00  8,00  6,00  0,089
Error      3   4,00  1,33
Total      5  20,00

S = 1,155   R-Sq = 80,00%   R-Sq(adj) = 66,67%


                             Individual 95% CIs For Mean Based on Pooled
StDev
Level       N    Mean  StDev    +---------+---------+---------+---------
Double-GG  2  14,000  1,414                    (---------*---------)
RF-GG      2  10,000  0,000    (---------*---------)
LF-GG      2  12,000  1,414            (---------*---------)
                               +---------+---------+---------+---------
                             7,5      10,0      12,5      15,0

Pooled StDev = 1,155


Grouping Information Using Tukey Method

Sample     N    Mean  Grouping
```

```
Double-GG  2  14,000  A
LF-GG      2  12,000  A
RF-GG      2  10,000  A

Means that do not share a letter are significantly different.


Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of Sample_1

Individual confidence level = 97,50%


Sample   = Double-GG subtracted from:

Sample     Lower  Center  Upper   --+---------+---------+---------+-------
RF-GG     -8,825  -4,000  0,825   (-----------*-----------)
LF-GG     -6,825  -2,000  2,825        (-----------*-----------)
                                  --+---------+---------+---------+-------
                                 -8,0      -4,0       0,0       4,0

Sample   = RF-GG subtracted from:

Sample     Lower  Center  Upper   --+---------+---------+---------+-------
LF-GG     -2,825   2,000  6,825               (-----------*-----------)
                                  --+---------+---------+---------+-------
                                 -8,0      -4,0       0,0       4,0
```

Table A.18 Pearson Correlations of fat levels with sensory parameters ($p \leq 0.05$).

Correlations: Fat Level; Intensity of coldness

```
Pearson correlation of Fat Level and Intensity of coldness = -0,566
P-Value = 0,000
```

Correlations: Fat Level; Iciness

```
Pearson correlation of Fat Level and Iciness = -0,499
P-Value = 0,000
```

Correlations: Fat Level; Mouth-coating

```
Pearson correlation of Fat Level and Mouth-coating = 0,837
P-Value = 0,000
```

Correlations: Fat Level; Overall Acceptability

```
Pearson correlation of Fat Level and Overall Acceptability = -0,000
P-Value = 1,000
```

Correlations: Fat Level; Viscosity

```
Pearson correlation of Fat Level and Viscosity = -0,417
P-Value = 0,003
```

Correlations: Intensity of coldness; Iciness

```
Pearson correlation of Intensity of coldness and Iciness = 0,440
P-Value = 0,002
```