

EFFECTS OF DIFFERENT TYPE OF WAXES ON COCOA CREAM

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ABSTRACT

EFFECTS OF DIFFERENT TYPE OF WAXES ON COCOA CREAM

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Recently, the negative health effects of saturated and trans fatty acids are the hot topics for human health. It was found that, excessive consumption of saturated fatty acid correlated with the health problems. The most used fat, containing high saturated fatty acid, is the palm oil in the fast moving consumer goods.

In this study, oleogelation technique was tried to be used to decrease the saturated fat content of the cocoa cream containing palm oil. Two types of oleogels were developed by using carnauba wax (CW) and beeswax (BW) at different concentrations (5% and 8%) and high oleic sunflower oil (HOSO) was chosen as liquid phase. Two types of oleogels at different concentrations were replaced with the palm oil in cocoa cream with different ratios (50% and 100%). Crystal formation time, solid fat content (SFC), fatty acid composition, oxidative stability, textural and rheological behavior were analyzed. The results showed that, 50% and 100% replacement with palm oil decreased the saturated fat content in cocoa cream from 57% to 33-34% and 9-10% respectively. SFC ratio of the oleogel cocoa creams were not significantly different from palm oil at 35 °C (~ body temperature) ($p>0.05$). Peroxide and acidity of the oleogel cocoa creams did not exceed the acceptable limit during 3 months storage. However, BW oleogels were not as good as the CW oleogels. Wax type, wax

concentration significantly affected the hardness of the oleogels ($p < 0.05$). Because $G' > G''$ in oleogel cocoa creams, they were regarded as elastic gels.

Keywords: Oleogel, Saturated Fat, Beeswax, Carnauba Wax, Palm Oil,

ÖZ

FARKLI WAX TÜRLERİNİN KAKAO KREMASI ÜZERİNDEKİ ETKİSİ

Çiftçi, Selcan
Yüksek Lisans, Gıda Mühendisliği
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Son zamanlarda, doymuş ve trans yağ asitlerinin sağlığa olan olumsuz etkileri gündeme alınmıştır. Doymuş yağ asitlerinin aşırı tüketimi sağlık problemleriyle ilişkilendirilmiştir. Hızlı tüketim malları içinde en çok kullanılan ve doymuşluğu yüksek olan yağ palm yağıdır.

Bu çalışmada, oleojel tekniği kullanılarak palm yağı içeren kakao kremasının doymuş yağ oranı azaltılmaya çalışılmıştır. Karnaubu ve balmumu waxı farklı konsantrasyonlarda (%5 ve %8 yüksek oleic asitli ayçiçek yağı ile karıştırılarak oleojel elde edilmiştir. İki farklı oleojel, kakao kremasının içindeki palm yağı ile farklı oranlarda (%50 ve %100) yer değiştirilmiştir. Oleojel ve kakao kremalarının kristal oluşturma süresi, katı yağ içeriği, yağ asidi kompozisyon, oksidatif dayanıklılığı tekstürel ve reolojik davranışları analiz edilmiştir. Analiz sonuçlarına göre % 50 ve % 100 palm yağı ile yer değişimi kakao kremasındaki doymuş yağ miktarını % 57 'den sırasıyla % 33-34 ve % 9-10' a düşürmüştür. 35 °C'deki ölçüme göre palm yağlı ve oleojelli kakao kremaları arasında bir fark görülmemiştir. ($p>0.05$). Oleojel kakao kremaları 3 aylık depolama süresi boyunca kabul görülen maksimum peroksit ve asitlik değerini aşmamıştır. Bununla birlikte balmumu wax sonuçları carnauba wax sonuçları kadar iyi bulunmamıştır. Wax türü ve konsantrasyonu sertliği önemli ölçüde

etkilemiştir ($p < 0.05$). $G' > G''$ olduđu için bütn oleojel kakao kremaları elastik jel olarak kabul edilmiştir.

Anahtar Kelimeler: Oleojel, Doymuş Yađ, Karnauba Wax, Balmumu Waxı, Palm Yađı

To my family

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TABLE OF CONTENTS

ABSTRACT	v
ÖZ	vii
ACKNOWLEDGEMENTS	x
TABLE OF CONTENTS	xi
LIST OF TABLES	xiv
LIST OF FIGURES	xv
CHAPTERS	
1. INTRODUCTION	1
1.1. Health Problems Related to Trans Fatty Acids and Saturated Fats.....	1
1.2. Oleogels	2
1.2.1. Low Molecular Oleogels (LMOGs).....	4
1.2.1.1. Waxes.....	5
1.2.2. High Molecular Oleogelators (HMOGs)	8
1.3. Oil Structuring Approaches	9
1.3.1. Direct Dispersion of Structuring Material to the Oil Phase	9
1.3.2. Indirect Method Using Water Continuous Emulsions	10
1.4. Oleogel Applications in Food Industry.....	11
1.5. Characterization of Oleogels.....	12
1.5.1. Determination of Crystal Formation Time	12
1.5.2. Determination of Solid Fat Content	12
1.5.3. Determination of Fatty Acid Composition	14
1.5.4. Determination of Oxidative Stability	15

1.5.5. Determination of Texture Analysis	17
1.5.6. Determination of Rheological Properties	20
1.5.7. Objectives of the Study	22
2. MATERIAL AND METHODS	23
2.1. Materials	23
2.2. Oleogel Preparation	24
2.3. Cocoa Cream Preparation	24
2.4. Analysis of Oleogels and Cocoa Creams.....	26
2.4.1. Crystal Formation Time	26
2.4.2. Solid Fat Content	26
2.4.3. Fatty Acid Composition	26
2.4.4. Oxidative Stability	27
2.4.4.1. Peroxide Value	27
2.4.4.2. Acidity Value	27
2.4.5. Texture Analysis	28
2.4.6. Rheological Properties	29
3. RESULTS AND DISCUSSION.....	31
3.1. Crystal Formation Time.....	31
3.2. Solid Fat Content.....	32
3.3. Fatty Acid Composition.....	35
3.4. Oxidative Stability.....	39
3.5. Texture Analysis.....	46
3.6. Rheological Properties.....	51
4. CONCLUSION AND RECOMMENDATIONS	55

REFERENCES	57
APPENDICES.....	69
TABLES.....	69
STATISTICAL ANALYSIS.....	72

LIST OF TABLES

TABLES

Table 1.1. Textural Properties of Popular Terms	18
Table 2.1. Cocoa Cream Formulations	25
Table 3.1. CFT of Oleogels	31
Table 3.2. Fatty Acid Composition of Oils and 50% Oleogel Cocoa Creams	37
Table 3.3. Fatty Acid Composition of 100% Oleogel Cocoa Creams	38
Table 3.4. Texture Analysis of Oils and Oleogels	47
Table 3.5. Texture Analysis of Cocoa Creams	48

LIST OF FIGURES

FIGURES

Figure 1.1. Global Health Estimates in 2016; Death Causes	2
Figure 1.2. Molecular Characteristic of of oleogels (Davidovich-Pinhas, 2018).....	4
Figure 1.3. Heating and cooling cycle of thermo reversible supramolecular gels (Schön, 2014)	5
Figure 1.4. Chemical Structure of Carnauba Wax.....	6
Figure 1.5. Chemical Structure of Beeswax.....	7
Figure 1.6. Edible oil structuring: an overview and recent updates. Food & Function, 2016, 7, 20-29. Published by Thee Royal Society of Chemistry.	9
Figure 1.7. Gas Chromatograpy	15
Figure 1.8. Measurement Curve of the Texture Profile	19
Figure 1.9. Frequency Sweep	21
Figure 2.1. Texture Analyzer	28
Figure 3.1. (a) only PO cocoa cream, (b) only HOSO cocoa cream, (c) 50% oleogel cocoa cream BWO5, (d) 50% oleogel cocoa cream CWO5, (e) 50% oleogel cocoa cream BWO8, (f) 50% oleogel cocoa cream CWO8, (g) 100% oleogel cocoa cream BWO5, 100% oleogel cocoa cream CWO5, (i) 100% oleogel cocoa cream BWO8, (j) 100% oleogel cocoa cream CWO8.....	33
Figure 3.2. SFC Ratio of Oils and Oleogels.....	34
Figure 3.3. SFC Ratio of the Palm Oil and 50% Oleogel Cocoa Creams.....	35
Figure 3.4. PV of oils & oleogels during three months	40
Figure 3.5. PV of 50% oleogel cocoa creams during three months	41
Figure 3.6. PV of 100% oleogel cocoa creams during three months.....	42
Figure 3.7. Acidity of Oleogels & Oils during three months.....	43
Figure 3.8. Acidity of 50% Oleogel & Oil Cocoa Creams during three months	44
Figure 3.9. Acidity of 100% Oleogel & Oil Cocoa Creams during three months ...	46

Figure 3.10. Hardness Graph (a) BWO5 (b) BWO8 (c)50% Oleogel Cocoa Cream BWO5 (d)50% Oleogel Cocoa Cream BWO8 (e) 100% oleogel Cocoa Cream BWO5 (f) 100% Oleogel Cocoa Cream BWO8 50

Figure 3.11. Storage and Loss Modulus of the Palm Oil & Oleogels 52

Figure 3.12. Storage and Loss Modulus of the Oils & 50% oleogel Cocoa Creams 53

Figure 3.13. Storage and Loss Modulus of the Oils & 100% oleogel Cocoa Creams 53

CHAPTER 1

INTRODUCTION

1.1. Health Problems Related to Trans Fatty Acids and Saturated Fats

Recently, the negative health effects of saturated fatty acids and trans fatty acids (TFAs) are the hot topics for human health. Several studies have shown that excessive consumption of these types of components, especially TFAs, can increase the cardiovascular diseases. In addition, excessive intake of saturated fatty acids has also correlated with the coronary heart disease, Type 2 diabetes, obesity and other related diseases (Blake & Marangoni, 2014). According to 2016 World Health Organization (WHO), the most common death causes are the cardiovascular diseases (CVD) (WHO, 2018).

The negative health effects of both TFAs and saturated fatty acids have tried to be decreased by the FDA (Food and Drug Administration) and EFSA (European Food Safety Authority). Since 2006, FDA has postulated to show on the label, if the product includes trans fatty acids. Besides, EFSA warns if the consumption exceeds the 2% of the total energy of TFAs; it increases risk of cardiovascular diseases (Chaves et al., 2017). Negative health effects associated with the consuming of TFAs and saturated fat may be decreased by replacing with the unsaturated fats. Replacement of 5% daily energy intake from the saturated fats with carbohydrates would reduce the risk in the range of 22-37% related to CVD (Roche, 2005). In addition, FDA will not allow the addition of partially hydrogenated oils (PHOs) to products after January 1, 2020 due to including of TFAs (FDA, 2018).

Palm oil also used widely in food products for example; ice creams, biscuits, creams, shortenings, spreads. However, palm oil has been defined as potential harmful due to containing saturated fats and when they refined at high temperatures; they form

hazardous contaminants such as 3-Monochloropropane diol (3-MCPD) and Glycidyl esters (GE) (EFSA, 2018). Recently, because people have become more conscious about what they consume and healthy diets have become popular, the concept of structuring oils that could mimic the saturated fats has been developed (Rogers, 2011). However, due to saturated fat’s textural properties and mouth feel, replacing saturated fats for example in chocolates, ice creams or other food products with unsaturated oils is a technical challenge (Moriano & Alamprese, 2017). Novel techniques have become used by giving structure to the liquid oil like solid crystalline fat without its chemical structure has not been changed significantly.

Top 10 global causes of deaths, 2016

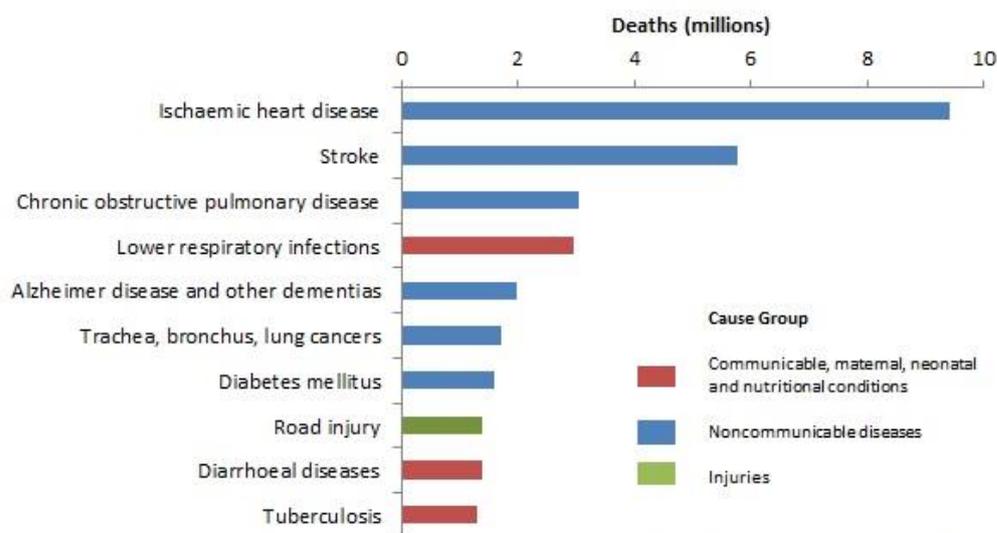


Figure 1.1. Global Health Estimates in 2016; Death Causes

1.2. Oleogels

Gel or jelly-like material can be defined as the material is between the liquid and solid state but does not flow like liquid (Schön, 2014). In other words, gels are the three-dimensional structures that immobilize the liquid phase. Gels are composed of two

parts; liquid phase and gelling agent (Kaushik et al., 2017). Generally, liquid phase forms the major part of the structure and gelator immobilizes up to 10^5 liquid (solvent) molecule per gelator molecule (Schön, 2014) According to polarity of the liquid part, gels can be categorized as emulgels, hydrogels and oleogels/organogels. When an emulsion is gelled, this two-phase formulation is called as emulgel; if the liquid part is water, then it is called hydrogel (Dickinson, 2012). These types of gels have been researched both food and cosmetic sectors and recently structuring of proteins and polysaccharides and their combinations have been shown (Dickinson, 2012; Juszczak et al., 2013). When the dispersed part is organic solvent such as vegetable oil and gelled with organogelator, then the gel is called organogel (Balasubramanian et al., 2014; Murdan, 2005).

Organogel can be defined as self-standing, anhydrous, thermo reversible and three-dimensional gel networks. Formation of the gel network, physical inter-chain interactions are needed such as van der waals, hydrogen bonding between the small organogelators (Yılmaz & Öğütçü, 2015). There are different methods for characterization of oleogels. Depending on the molecular type, oleogels can be categorized into two groups; low molecular weight oleogelators (LMOGs) and high molecular weight oleogelators (HMOGs) as shown in Figure 1.2.

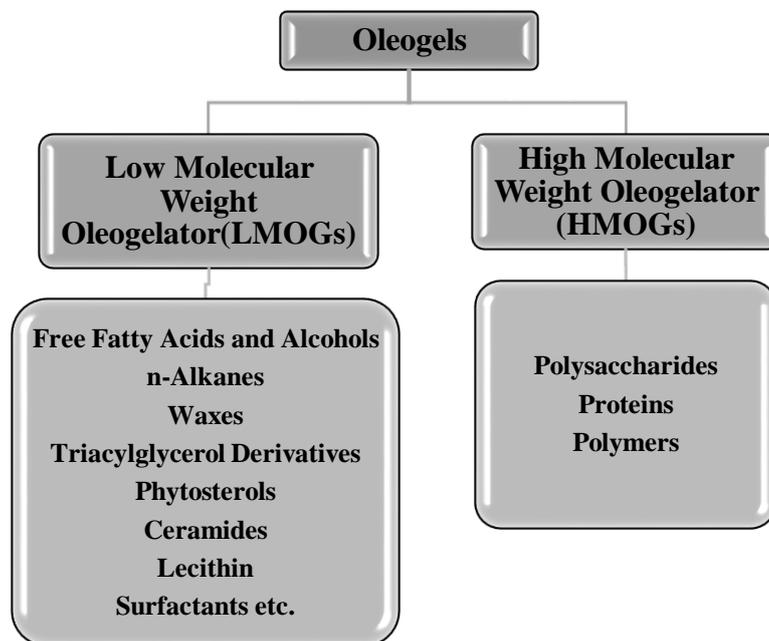


Figure 1.2. Molecular Characteristic of of oleogels (Davidovich-Pinhas, 2018)

1.2.1. Low Molecular Oleogels (LMOGs)

Low molecular weight oleogels could mimic fats and they have gelling ability of oils as low as 3% mass percentages. However, most food fats need minimum 20% solid fat content to display the solid properties (Co & Marangoni, 2018). LMOGs could form organized fat structure by imitating the natural ability of the triacylglycerides (TAGs). In a suitable solvent and appropriate gelation composition, supramolecular structures are formed by non-covalent bonds such as van der waals forces, hydrogen bondings, π - π stacking, dipole forces (Marangoni & Acevedo, 2012). The gelation mechanism depends on the aggregation which is initiated by the external forces such as temperature or shear forces (Vintiloiu & Leroux, 2008). Phase changing could occur when the structure heated up to sol-to gel transition temperature by breaking the 3D structure, but it could solidify and reforms 3D structure upon cooling (De Loos et al., 2005; Sangeetha & Maitra, 2005). Figure 1.3. shows the relation between the gel

and temperature. Variety of LMOGs exist in the literature on the basis of FFA and alcohols, *n*-alkanes, waxes, triacylglycerol derivatives, phytosterols, ceramides, lecithin, surfactants etc. that form 3D crystalline structure which entrap the oil within (Co & Marangoni, 2018; Marangoni & Garti, 2018).

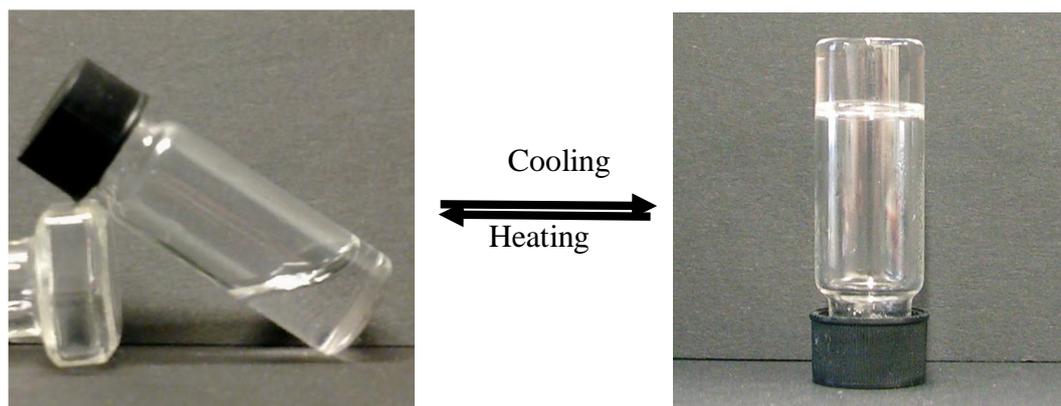


Figure 1.3. Heating and cooling cycle of thermo reversible supramolecular gels (Schön, 2014)

1.2.1.1. Waxes

Wax is defined as fatty substance containing long chain esters with or without functional group derived from the fatty acids or alcohols (Dassanayake et al., 2011; Marangoni & Garti, 2018). Besides containing high ester amount characteristically, waxes contain several other components such as, fatty acids, fatty alcohols, *n*-alkanes and sterol esters. Therefore, they exhibit hydrophobic characteristics and have high melting temperature points, so they are solid at room temperature (Jana & Martini, 2016). Among the several structuring agents, waxes are by the most promising materials due to their great oil binding capacities (Blake et al., 2014), as well as their economic value (excellent gelling capability at low concentrations, minimum 0.5% wt) (Hwang et al., 2012). Also, number of approved waxes exists for use in foods

(Patel, 2015). In addition to that, due to thermo reversible characteristics of gels which is formed by the waxes and stabilization of water in oil emulsions, tendency to use of waxes increases in food products (Patel et al., 2014, Patel et al., 2013). Waxes are generally used in food products, for example rice bran wax, candellila wax, carnauba wax, beeswax, sunflower wax, shellac wax and sugarcane wax.

Carnauba wax (CW) is one of the plants based natural wax obtained from the Brazilian Palm *Copernicia cerifera*, called also as “queen of waxes” (Chaves et al., 2017). Carnauba wax is one of the hardest natural waxes and it has very high melting temperature (81-86°C). Because it has higher price when it is compared with other plant-based waxes, carnauba wax is generally used in high-value applications. Automobile and surfboard wax, furniture polishes and shoe polish are the application area of the carnauba wax. Due to its GRAS status, it is used in food applications such as fruit and vegetable coating, confectionery coating. Composition of the carnauba wax resembles to that of rice bran wax. Contrary to candelilla wax, it contains nearly 1% wt hydrocarbons and 40% aliphatic esters as well as esters of hydroxylated cinnamic (30%), unesterified alcohols (12%) and x-hydroxy esters (14%) (Dassanayake et al., 2011; Edmund & Marangoni, 2012). Carnauba wax chemical structure is also seen in Figure 1.4. as $\text{CH}_3(\text{CH}_2)_{30}\text{CO}_2-(\text{CH}_2)_{33}\text{CH}_3$ (Libretex, 2019).

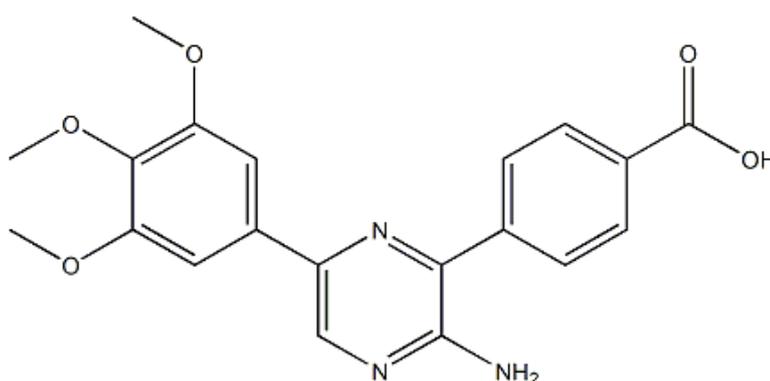


Figure 1.4. Chemical Structure of Carnauba Wax

Beeswax is an organic animal wax which is produced by the bees from the *Apis mellifera* L genus (Yılmaz & Öğütçü, 2014). Beeswax is accepted as a food additive by the worldwide and recognized as E901. Also, it is approved by Food & Drug Administration (FDA under Regulation 21 CFR 184.1973) (EU, 1995). Beeswax is used in various fields of the food industry as stabilizer, carrier for colors and flavors, glazing and coating agent on confectionary (except chocolate) and particular fruits (apples, melons, peaches, fresh citrus fruits, pears and pineapples), chewing gum base material for texturizing (EFSA, 2007). Yılmaz and Öğütçü (2014) state that beeswax is composed of approximately 70-71% total esters, 12-15% hydrocarbons, 9-11% free acids and 1-1,5% free alcohols. Its chemical structure $\text{CH}_3(\text{CH}_2)_{24}\text{CO}_2-(\text{CH}_2)_{29}\text{CH}_3$ as seen below the Figure 1.5. (Libretexts, 2019).

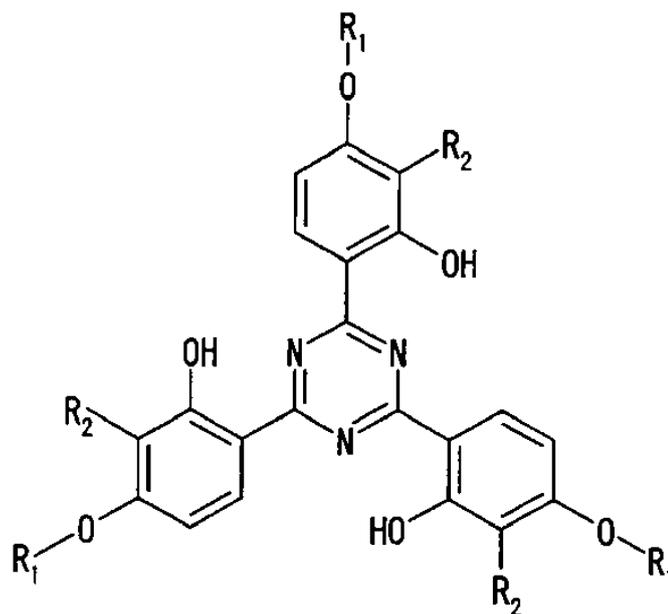


Figure 1.5. Chemical Structure of Beeswax

1.2.2. High Molecular Oleogelators (HMOGs)

High molecular oleogelators such as water-soluble polysaccharides and proteins, ethyl cellulose (EC), which do not form crystalline structure while forming gel network, are rarely used for the oleogelation process (Davidovich-Pinhas, 2018). Few polymeric gelators are used because of their poor solubility in oils except EC. EC is a linear polysaccharide obtained from the cellulose and when the substitution degree attains 2.3-2.6 it becomes soluble in organic oils. EC could form a solid elastic gel in edible oil. Its gelation principle is based on the heating above its glass transition temperature nearly 140 °C in the vegetable oil and then cooling. When EC polymers are cooled, they interact with each other and make gel with oil. EC's potential application is researched in food products such as heat-resistant chocolate, reduction of saturated fat content in frankfurter (Rogers et al., 2014, Zetzl et al., 2012).

The use of other HMOGs named protein based gelators is a challenge because of its chemical structure, i.e., amino acids, which do not dissolve in oil phase generally. However, recently, oleogel network has been developed by using whey protein isolate (Davidovich-Pinhas, 2018).

The use of polysaccharide is less common method in oil structuring applications. Strategy to form water soluble oleogel system is achieved by using oil-in-water emulsion stabilized by polysaccharides, for instance, methylcellulose, hydroxyl methyl cellulose (HMPC) or xanthan gum. This approach involves the 3 steps; polysaccharide is dissolved in the water then by oil-in-water emulsion and ultimately water dehydration is done to form the oleogel (Patel et al., 2014).

1.3. Oil Structuring Approaches

There are several characterization methods for the oleogelation and oil structuring. Patel and Dewettinck (2016) categorized oil structuring approaches into four main titles in their reviews. These are direct dispersion method, indirect method, oil sorption and structured biphasic systems (Figure 1.6).

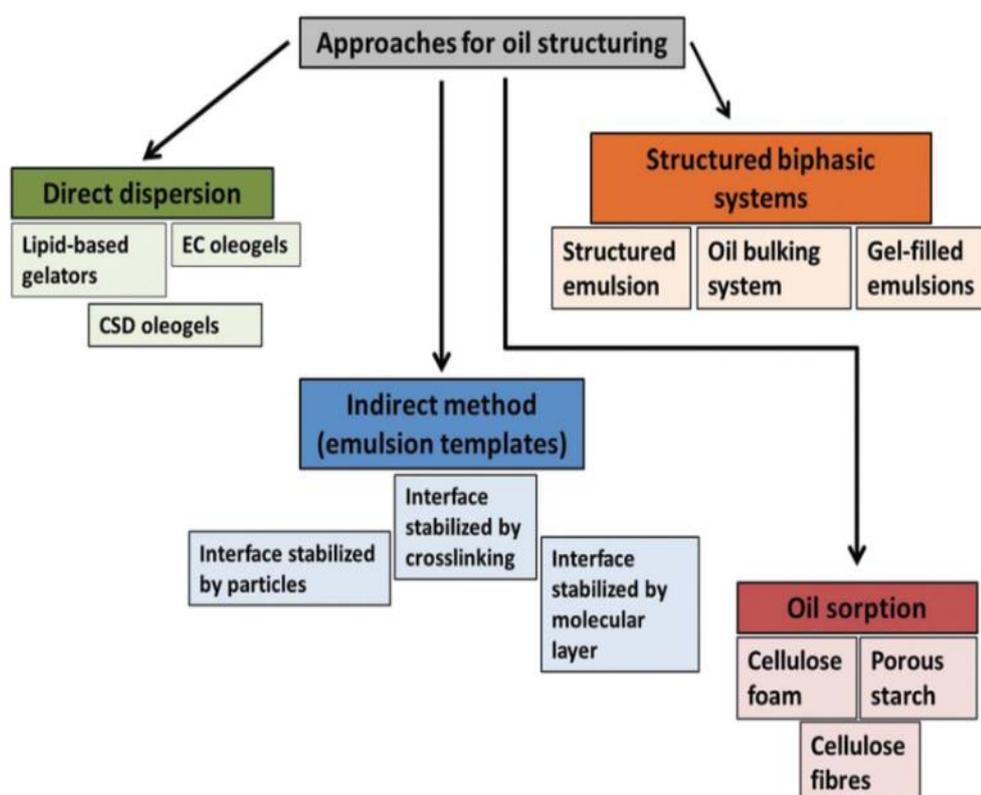


Figure 1.6. Edible oil structuring: an overview and recent updates. *Food & Function*, 2016, 7, 20-29. Published by The Royal Society of Chemistry.

1.3.1. Direct Dispersion of Structuring Material to the Oil Phase

When the gelling agent is lipid-based gelators such as fatty acids or fatty alcohols, mono glycerides (MAGs), waxes, etc., direct dispersion method is usually used by directly dispersing the agent into the liquid part (oil phase) at temperatures above their

melting point. Mixture is then cooled to lower temperatures under force or static conditions. This process is widely used for the oleogelation and it is a serial of conventional structuring process including solid fats. Nucleation, crystal growth, aggregation and network formation phases are both seen in solid fats and oil structuring using lipid-based gelators. On the other hand, their crystal morphologies, unidirectional growth tendencies and lower aggregation properties among the crystals which are formed differ from the solid fat crystals as such; network formation is formed at lower mass fraction of crystalline phase (Patel & Dewettinck, 2016).

Ethyl cellulose (EC) and colloidal silicon dioxide (CSD) (inorganic molecule) are the non-lipid direct dispersion agents. For EC, certain conditions should be supplied for the gelation mechanism which was mentioned above. Under these conditions, while cooling, gelation occurs with the association of polymer chains by hydrogen bonding (Davidovich-Pinhas et al., 2015). CSD gels are performed under high shear (>10 000 rpm) at room temperature so that aggregates of primary particles are de-agglomerated. After removal of shear, uniformly de-agglomerated particles re-agglomerate resulting very hard gel network (Patel et al., 2015).

1.3.2. Indirect Method Using Water Continuous Emulsions

The use of polymers as gelling agents for oil structuring is widely known concept. The use of polymers is very suitable for oil structuring because there are many polymers have been approved to be used in food products. However, since many of them are hydrophilic, i.e., low solubility in oil phase makes it difficult to use in oil structuring. For this reason, to use hydrophilic polymer, first of all it is needed to pre-hydrate in aqueous phase and then catch these forms in dehydrated forms to be used in physical entrapment in oil phase (Patel & Dewettinck, 2016).

1.4. Oleogel Applications in Food Industry

Historical development of oleogel came out from the term of gel which is described by Thomas Graham in 1861. Then with the Jordan Lloyd, which expressed the colloid structure or gel after 65 years, the term has been used widely (Pehlivanoğlu et al., 2018).

After increasing the importance of the health issues related to solid fats in food industry, researchers tried to seek alternative methods to form solid phase from liquid oils. Then oleogelation method has become an important place within the approaches. Interest of oleogelation has increased since 2006 (Pehlivanoğlu et al., 2018).

Because oleogels have been used to decrease the saturated fat and trans fat in the foods, they have been tried to be used in foods which have high in saturated fats or trans fats. γ -orzanol/ β -sitosterol oleogel emulsion was tried to be used in frankfurters by replacing the pork back fat partially (Panagiotopoulou et al., 2016). All quality parameters such as sensory, pH and oxidation level, chewiness, gumminess were in accepted intervals.

Oleogel applications were tried to solve some problems in food industry. For instance, EC was tried to be used in chocolate to produce heat resistant chocolate for warm climate (Stortz and Marangoni, 2013). Ethanol (EtOH) was used to solve the EC in melted chocolate and then it was evaporated so that EC could form oleogel in the fat phase of the chocolate. At 40 °C, while the hardness of the control chocolate could not be measured, EC chocolates could be measured (Stortz and Marangoni, 2013).

To date, most studied waxes for the oleogel application are sunflower wax (SFX), rice bran wax (RBW), beeswax (BW), carnauba wax (CW) and candelilla wax (CDW). Zulim Botega et al., investigated the potential application of RBW oleogels with HOSO in ice cream to enhance the unsaturated fat content. Although RBW oleogel was better than the HOSO ice cream, it was not as good as milk fat in terms of structure. However further optimization and mix composition could be investigated (2013).

Beside this, waxes were tried to be used in cookies or baked products as a shortening replacer. Mert and Demirkesen (2016) investigated the potential application of CDW oleogel with canola oil at different concentrations (3% and 6% w/w) in cookie. While applying, oleogel was replaced with shortening at different concentrations. Then, it was concluded that, to reduce the saturated fat content in baked products, step by step replacement could be a convenient approach.

Another study about the waxes, which was performed by Doan et al. (2016), was potential application of BW oleogel (1.5, 2, 2.5, 3, and 3.5%) with rice bran oil in the hazelnut filling by replacing the palm oil at different concentrations (17, 33, 50%). Rheological and thermal properties, oil binding capacity and microstructures were measured and compared with each other. The most suitable replacement ratio was determined as 17% according to gel strength. There was not found significant difference among the BW concentrations.

1.5. Characterization of Oleogels

1.5.1. Determination of Crystal Formation Time

Crystal formation time is important parameter for the product quality. For oleogels, the faster it forms gel, it approaches to palm oil structure and it can be more usable in food industry.

1.5.2. Determination of Solid Fat Content

Solid fat content (SFC) is known as the stability of fat-oil mixtures such as margarines i.e. solid/liquid ratio of lipid at different temperature ranges (Marcone et al., 2013). Solid fat content is one of the important parameters for the quality of the food products. Due to this reason, in oil industry there is a great interest for the SFC and instrumental methods are tried to be enhanced. These methods are; differential thermal scanning (DSC), dilatometry, continuous-line nuclear magnetic resonance (NMR),

and pulsed NMR (McClements & Povey, 1987). One of the SFC measurement methods, which is NMR, can be applied in various type of liquid and solid samples without changing the sample structure. Although sensitivity and detection limitations of the NMR should be developed, it is more advantageous than other common equipment such as high-pressure liquid chromatography (HPLC), mass spectrometry (MS) and gas chromatography (GC).

NMR was firstly used in the last of 1940s to explain the structure of the molecules in chemistry. Due to high cost of equipment, low scientific information, its development and wide range of usage were delayed to 1980s. After development of NMR and enhanced data collecting programs, NMR has been used widely in food related researches even in food microbiology (Marcone et al., 2013).

In addition, NMR could supply noninvasive, nondestructive and sensitive measurement and it could be used to measure and gathering information about the mobile protons, water and oil distribution and SFC content in food products (Oztop et al., 2014).

NMR's working principle is based on the intrinsic dipole of the atomic nucleus. This dipole moment is formed by the spin because of the finite angular momentum of atomic nucleus which is in ground state (Hoffman, 2012).

In solid fat content determination, the principle is based on the measurement of the signals. Measurement gives the short and long components. While short component defines the solid and liquid fat content of the sample, long component defines the only solid fat content. SFC ratio is calculated as

$$\text{SFC ratio} = \frac{\text{Short Component} - \text{Long Component}}{\text{Short Component}} \quad (\text{Equation 1})$$

1.5.3. Determination of Fatty Acid Composition

Fatty acid composition is another important parameter for the health issues. Fatty acids are long chain hydrocarbons having methyl group at the one end and the acid group at the other end. Fatty acids are divided into 3 categories which are saturated, mono unsaturated (MUFAs) and poly unsaturated (PUFAs). Saturated fatty acids do not contain any double bonds. Unsaturated fatty acids contain at least one carbon-carbon double bond; MUFAs contain one double bond; PUFAs contain more than one double bond. Fats which contain mostly PUFAs are liquid at room temperature and they are called as oils. Generally, vegetable oils contain PUFA having two and three double bonds. Fats play an important role in people's diet because they are source of energy.

The working principle of the gas chromatograph is by the help of the volatility difference between the compounds, complex mixtures are analyzed and separated.

There are four important and indispensable parts in the gas chromatography, which are carrier gas, injection system, temperature-controlled column and detector. Figure 1.7. shows the part of the GC. Generally, helium is used as a carrier gas because it is an inert gas. For injection system, split-splitless injector is commonly used. This injector can be used any kind of products with the good results independent from the solvent volatility or initial column temperature. However, its disadvantage is, when the splitter is not linear, the amount of sample which is reached to column is analyzed only (Jennings 2012). Separation column is very important part of the GC. Temperature is controlled by the controllable ovens. It is capable of controlling the 0,5°C intervals. Nowadays, fused silica capillary columns have been used mostly. While diameter of the column is between 0,25-0,5 mm, length of the column is between the 5-100 m (Şenlik, 2014).

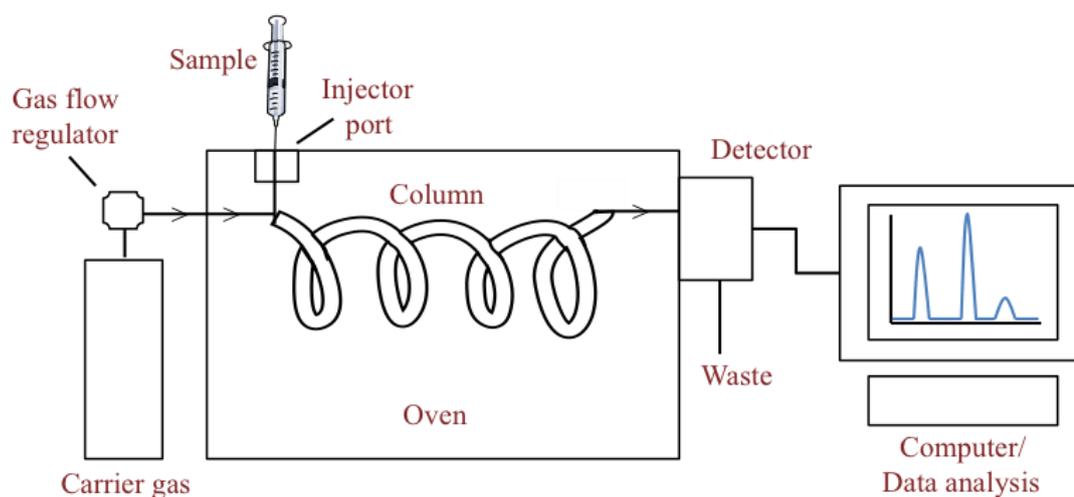
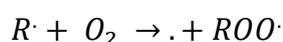
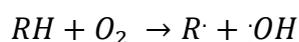


Figure 1.7. Gas Chromatography

1.5.4. Determination of Oxidative Stability

Rancidity is an important quality problem in the food industry. Oxidation produces rancid and off flavor as well as decreases the nutritional value of the food products. As a result, shelf life of the product depends on its oxidative stability. Degree of unsaturation also affects the oxidation stability. The more unsaturation level causes the more susceptibility to oxidation (Gray, 1978). Mechanism of oxidation is composed of three parts, which are initiation, propagation and termination (Frankel, 2014). Initiation involves the formation of free radicals in the fatty acids, which is started by the one of most significant initiator, O_2 ,

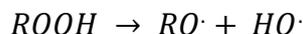
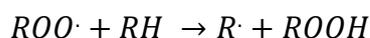


Where RH is the unsaturated fatty acids,

$R\cdot$ is a free radical which is formed by the removal of the unstable hydrogen which is adjoined to carbon atom with a double bond.

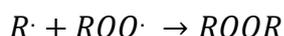
Fe^{+3} , Ca^{+2} or enzymes can initiate the formation of free radicals.

Propagation involves the reaction of radical and non-radical to form a new radical.



Where ROOH is the hydro peroxide which is the major initial oxidation product causes off-flavors by decomposing to form these compounds.

Termination is last stages of the autoxidation. In this stage, free radicals react with each other to form non-radical products. It is a long chain reaction stage since it continues until the enough radical concentration is achieved.



Acidity and peroxide value was measured to observe the oxidative stability of fatty acids at 25 °C. Iodometric method is widely used to measure the peroxide value of the food products. Measurement is based on the iodine produced from the potassium iodide (KI), by the peroxide which is found in the oils (Gray, 1978).

For the quality assessment, acidity is measured to determine the free fatty acids (FFA) in terms of %oleic acid. In accordance with European Pharmacopoeia, this analytical method is used for the waxes, fatty oils or pharmaceutical aids. In fatty oils, formation of free fatty acids results from the hydrolysis of the triacylglycerides during production or storage. Because waxes contain individual fatty acids, acidity could be used as criteria to determine the identity (Skiera et al., 2014).

One of the acidity measurement methods is the titrimetric method. It is based on the neutralization reaction of free fatty acids in the oils with the KOH or NaOH. Color change is observed until the pink color was seen permanently.

1.5.5. Determination of Texture Analysis

Texture is principally the response of tactual senses to physical actions that related to part of the body such as hand and the food. Tactual sense, which is touching, feeling the texture but sound for example crispiness, crunchiness also could be used as an alternative to determine the texture (Bourne, 2002). Some of the textural terms were given in the Table 1.1.

For the overall acceptability of foods, textural properties are very important because it could be changed according to product. Importance of texture could be classified into three parts; Critical, important and minor. Critical one is the dominant and critical quality characteristics such as potato chips, crackers, corn flakes. Important one is not much more significant as critical as but affects the overall quality. Cheeses, bread could be given as examples. Minor one has the negligible effect on the overall quality. Beverages and soups could be given as examples for this classification. The value of texture on food was indirectly expressed by the Schiffman (1977). By having people try 29 different foods, he asked them to define the foods according to their flavor. All samples such as apple, banana, fish, carrot were pureed to eliminate the effect of texture and then it was found that when the texture of the food was disrupted, flavor of the food was poorly perceived (Bourne, 2002).

Rather than other quality factors, texture was mostly affected by the culture. Afro-Americans of southern rural people thought that texture is the most important food pattern flavor's affect is lower than it (Jerome, 1975). Because texture consists of many different physical senses, it could be said as textural properties.

Since there are varied type of foods having different type of texture, different measurement techniques were developed.

Force measurement is the most commonly used technique to measure the texture. Force has three dimensions which are mass x length x time⁻² and its general unit is Newton (N). It is divided into eight subclasses that are; puncture test, compression-

extrusion test, cutting-shear test, compression test, tensile test, torsion test, blending and snapping test and deformation test (Bourne, 2002).

Puncture test method measures the force needed to push to probe into the food. These tests are the maximum-force tests. They can be divided into single-probe instruments and multiple probe instruments. Puncture tests are generally used instrumentation method for the solid particles.

Compression-extrusion test is based on the force, which is applied until the food become disrupted and flows. This test method is generally used for the gels, viscous fluids, fats.

Table 1.1. *Textural Properties of Popular Terms*

Mechanical Properties		
Primary parameters	Secondary Parameters	Popular Terms
Hardness		Soft-Firm-Hard
Cohesiveness	Brittleness Chewiness Gumminess	Crumbly-Crunchy- Brittle Tender-Chewy- Tough Short-Mealy-Pasty- Gummy
Viscosity		Thin-Viscous
Elasticity		Plastic-Elastic
Adhesiveness		Sticky-Tacky-Gooey
Geometrical Properties		
Particle Size and Shape		Gritty-Grainy-Coarse, etc.
Particle Shape and Orientation		Fibrous-Cellular- Crystalline etc.
Other Characteristics		
Moisture Content		Dry-moist-wet-watery
Fat Content	Oiliness Greasiness	Oily Greasy

Cutting shear test is not widely used test method to measure the texture. The Pea Tenderometer that was found in 1937, works with the cutting shear method. It composed of two grids of blades with the rotating constant speed one to other (Sahin & Sumnu, 2006).

Compression (deformation) test is used to measure the distance to compress the food under the standard force or to measure the force needed to compress a food in a standard distance (Sahin & Sumnu, 2006). There are two types of compression tests that uniaxial compression and bulk compression. While in uniaxial, compression was performed in one direction and other dimensions are unrestrained, in bulk, compression was performed in three dimensions with the help of hydraulic pressure. Uniaxial test is generally used for the solid products. Bulk compression also rarely used due to its slowness (Bourne, 2002).

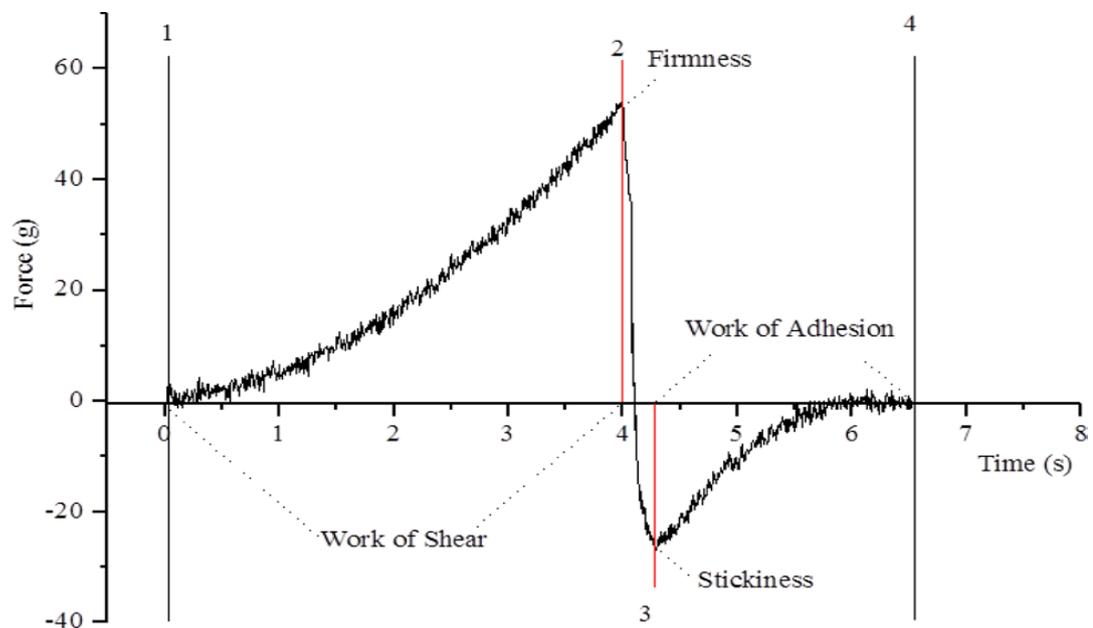


Figure 1.8. Measurement Curve of the Texture Profile

Tensile test is also rarely used method since the process is chewing involving compression not tension. The earliest tensile test was performed by the Howe and Bull (1927) on meat sample.

Torsion test is a method that force is applied the food by rotating one part of the sample around one axis according to other parts. Farinograph and mixograph are the example of torque measurement.

Bending and snapping method is mostly used for the brittle foods having sheet or bar shape. Strength and dimension of the food changed the force which is needed to snap (Sahin & Sumnu, 2006).

1.5.6. Determination of Rheological Properties

Rheology can be defined as the science of deformation and flow. The attitude of the real material is on the basis of viscous and elasticity parts. There are some terms to explain the rheological behavior of the materials.

Shear stress is the force applied to specific area tangentially between the upper plate and beneath the liquid part guide to flow in the liquid layer.

$$\tau = \frac{F}{A} = \frac{\text{Newton}}{m^2} = Pa \text{ (Pascal)} \quad (\text{Equation 2})$$

Shear rate is defined as the velocity difference dv between the two neighboring flowing layers having thickness of the particular layer dy (Schramm, 1994).

$$\dot{\gamma} = \frac{dv}{dy} \quad (\text{Equation 3})$$

All materials can be categorized as three main topics in terms of rheological behavior; viscous material, elastic material and viscoelastic material. Viscosity is defined as resistance to flow. Elasticity is defined as the ability of material to stock deformational energy to recover its shape after deformation. Viscoelasticity exhibits both elastic and viscous behavior and it changes with time and temperature. While viscous material is

a purely viscous that all added energy is dissipated into heat, elastic material is a purely elastic that all added energy is stored in material. Viscoelastic material shows both viscous and elastic material properties.

Materials's rheological behavior is measured with different instruments such as viscometers, rheometers, capillary viscometer and dynamic mechanical analyzer. In addition, there are three test modes which are, steady (viscometry), dynamic (oscillation) and transient (creep and recovery). While in steady test modes constant deformation is performed in a constant time, in dynamic test mode, deformation is performed as a sinusoidal function. In dynamic oscillation, the sample is oscillated in a set particular frequency.

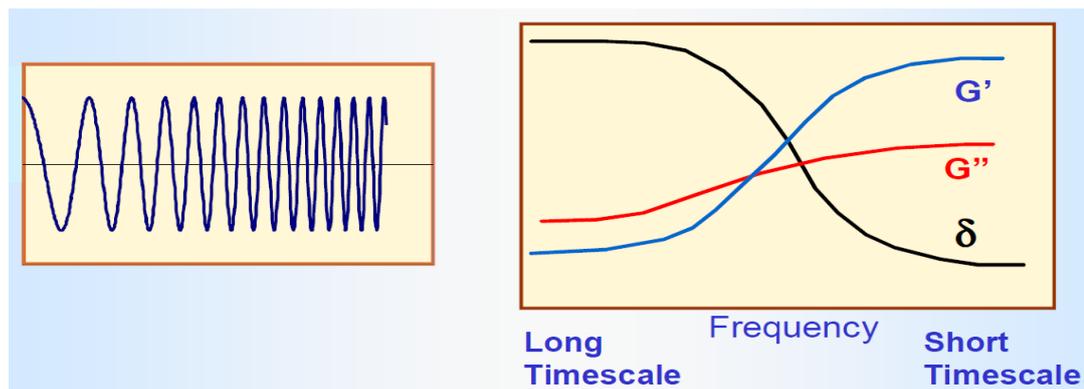


Figure 1.9. Frequency Sweep

Storage (elastic) modulus G' is the ability of the material to regain the energy and it is linked to the stiffness of material. That means, it measures the stored energy. Loss modulus G'' shows the ability of energy dissipation of material as heat. The ratio between the G' and G'' , which is $\tan\delta$, is important for viscoelastic materials regardless of shape and dimension. Frequency sweep, which is one of the dynamic test modes, is measured with the constant oscillation amplitude and temperature values (Figure 1.9.).

Strain sweep, the another dynamic test modes, while temperature and frequency is constant, it measures the increase of strain. While measurement is performed, linear viscoelastic region is selected. This dynamic test should be conducted for the unknown materials.

1.5.7. Objectives of the Study

With the increasing population, heavy workload, limited time, people have tended to consume unhealthy food products such as fast foods junk foods having palm oil etc. This eating trend has increased the cardiovascular diseases, obesity diabetes and related disorders. Alternative solutions could be developed to make the healthier products.

In this thesis, the main objective is to replace the palm oil with the wax oleogel and developing the cocoa cream filling. Replacing palm oil with liquid oil directly could cause technical challenge such as structure, mouth feel or oxidation problems (Stortz et al., 2012). Because of this reason, oleogel was tried to be developed with the waxes. Carnauba wax and beeswax are the food grade waxes which could make 3D crystalline structure with the liquid oil. HOSO, which is high oleic sunflower oil having 74,3% unsaturation level, could decrease the LDL cholesterol and increases the HDL (Cater et al., 1997). Thus in this study, cocoa cream filling having different percentage and type of oleogel, were tried to be measured in terms of crystal formation time, solid fat content, fatty acid composition, oxidative stability, textural and rheological properties and compared with the palm oil and HOSO cocoa cream filling.

CHAPTER 2

MATERIAL AND METHODS

2.1. Materials

Carnauba wax (CW), having flake structure and 80-86°C melting range, was received as sample from Foncepi Commercial Exportadora LTDA (Brazil).

Beeswax(BW) having white, waxy pellet structure, having a melting range of 61-66 °C and saponification value of 87-104 mg KOH/g was received as sample from Kahlwax GmbH & Co. KG (Trittau, Germany).

Palm oil, which is the palm olein, has 99.9% fat content and 38-42°C melting range, fat reduced alkalised cocoa powder having 11% cocoa butter content and 2% moisture content were purchased from Cargill Agriculture and Food Industry (Balıkesir, Turkey).

HOSO, having 9-9.5% saturated fat content was purchased from Trakya Birlik (Edirne, Turkey).

Whey powder having 1% fat content, 2.3% moisture content, skimmed milk powder having 2.1% moisture content were supplied from Ak Gıda (Karaman, Turkey),

Corn starch having 10% moisture content was purchased from Ak Nişasta (Kırklareli, Turkey).

Sunflower lecithin having brown oily free from solid particles supplied from Sime Darby Plantation Company (Netherlands).

2.2. Oleogel Preparation

First of all, HOSO was heated up to 90°C, then CW and BW were added to oil slowly with two different concentrations (5% and 8% w/w) separately. Mixtures were heated under gentle agitation for 20 minutes until the clear mixtures were obtained. Then the mixtures were hold in cold water bath for 2 hours without stirring to allow oleogel formation. Then the mixtures were kept at ambient temperature overnight.

2.3. Cocoa Cream Preparation

Prepared CW and BW oleogels were used for cocoa cream production. Cocoa creams were prepared with only palm oil, only HOSO, palm oil + oleogels with different ratio (50:50) and only oleogels. In total, 100 gr palm oil cocoa cream basis was composed of 52.9 % powdered sugar, 36.5% palm oil, 3.6% skimmed milk powder, 2.37% whey powder, 2.37% corn starch, 2.07% alkalized cocoa powder, 0.2% sunflower lecithin. Raw materials were mixed using mixer (Hobart, Germany) for 15 minutes at 124 rpm until the all materials were mixed homogeneously. HOSO cocoa cream was prepared with the same recipe but HOSO was used instead of palm oil. 50% oleogel cocoa creams were prepared by replacing the 50% of the palm oil with oleogel. 100% oleogel cocoa creams were also prepared by replacing 100% of the palm oil with the oleogel in recipe. In the final step, 10 cocoa cream samples were obtained; four of them is 50% oleogel cocoa cream, four of them is 100% oleogel cocoa cream and one of them is only HOSO cocoa cream, and the final one is only palm oil cocoa cream. Then they were deposited in classic biscuits and sandwiched. Table 2.1. shows cocoa cream formulations.

Table 2.1. Cocoa Cream Formulations

RAW MATERIALS	COCOA CREAM		50% OLEOGEL COCOA CREAM			100% OLEOGEL COCOA CREAM				
	PO	HOSO	5% CWO	8% CWO	5% BWO	8% BWO	5% CWO	8% CWO	5% BWO	8% BWO
Powdered Sugar	52.91	52.91	52.91	52.91	52.91	52.91	52.91	52.91	52.91	52.91
Sunflower Lecithin	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
HOSO	0.00	36.49	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
PO	36.49	0.00	18.25	18.25	18.25	18.25	0.00	0.00	0.00	0.00
5% CW	0.00	0.00	18.25	0.00	0.00	0.00	36.49	0.00	0.00	0.00
8% CW	0.00	0.00	0.00	18.25	0.00	0.00	0.00	36.49	0.00	0.00
5% BW	0.00	0.00	0.00	0.00	18.25	0.00	0.00	0.00	36.49	0.00
8% BW	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.49
Alkalized Coca	2.07	2.07	2.07	2.07	2.07	2.07	2.07	2.07	2.07	2.07
Whey Powder	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37
Starch (Corn)	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37	2.37
Skimmed Milk Powder	3.59	3.59	3.59	3.59	3.59	3.59	3.59	3.59	3.59	3.59
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00

2.4. Analysis of Oleogels and Cocoa Creams

2.4.1. Crystal Formation Time

Crystal formation time was determined by the method proposed by Ögütçü & Yılmaz, (2015). Previously formed oleogels were heated again to 90 °C then put in the tubes then turned to 90° in room temperatures. At the same time chronometer was started. Samples were observed until the no flowing occurs.

2.4.2. Solid Fat Content

Solid fat content of the samples was conducted with the nuclear magnetic resonance (NMR) analyzer (Spin Track SB4, Mary El, Russia) at three different temperatures, which are 4 °C, 25 °C and 35 °C. Samples were prepared accordant to method presented by Yılmaz and Ögütçü (2015). Firstly, approximately 3-4 ml of samples were weighed and put into NMR test tubes and melted completely in 90 °C water bath. Then the tubes were conditioned in 0 °C water bath for 1 hour. After that, samples and their parallels were conditioned at 4 °C, 25 °C and 35 °C for 30 minutes before measurement.

2.4.3. Fatty Acid Composition

For the fatty acid analysis oil was extracted from the cocoa creams. After that, nearly 100 mg of each sample was weighed and put into 12 ml vials. Then 100 mg of methyl nonadecanoate (C19:0) was added to same vial followed by the recording the vials. Each sample and methyl nonadecanoate were dissolved in 10 ml of toluene. After preparation of the samples, Agilent 7890A gas chromatograph (California, ABD) was used to analyze the fatty acid composition of the samples with the SPTM-2560 capillary column (100 m × 0.25 mm, 0.2 µm thickness). The oven temperature was set to 140 °C for 5 minutes and then it was increased to 210 °C (4 °C / min) and held for 10 minutes. After then, it was increased to 240 °C (5 °C /min) that was kept for 5 min

and ultimately, it was increased to 250°C (4°C / min) that was kept for 5 minutes. Helium gas was used as a carrier (Jang et al., 2015).

2.4.4. Oxidative Stability

2.4.4.1. Peroxide Value

To observe the oxidative stability of the creams, acidity and peroxide values were measured for three months. Peroxide value (PV) measurements were conducted by extracting oil from the 100 gr cocoa creams using hexane. Then oil and hexane mixtures were filtered to 125 ml volumetric flask to get rid of undesirable impurities by using filter paper having pore size 1,2 µm and 125 mm diameter (GVS Filter Technology, UK). Then the volumetric flask was put in rotary evaporator (Buchi, Switzerland) to evaporate the hexane. 5 grams of oils were weighed and color change was observed by titrimetric method. Experiment was done two times for all samples and averages were taken. PVs were expressed as meq/kg and calculated with following equation:

$$\text{Peroxide value} = [(V_1 - V_0) * C_{\text{tityo}} * 1000] / m \quad (\text{Equation 4})$$

Where V_1 is the consumption of 0.01 mol/l or 0.1 mol/l sodium thiosulfate solution in the main test, V_0 is the consumption of 0.01 mol/l or 0.1 mol/l sodium thiosulfate solution in the blank test, C_{tityo} is the molar concentration (molarity) of the sodium thiosulfate solution and m is the weighed portion of substance in grams (IFRA, 2011).

2.4.4.2. Acidity Value

Acidity of the oils was conducted with 1 gr of oil was measured and color change was observed by titrimetric method. Then acidity of the oil was calculated in terms of oleic acid and following equation:

$$\text{Acidity (in terms of oleic acid, \%)} = [f * N . \text{meg} * (V_1 - V_0) * 100] / m \quad (\text{Equation 5})$$

where N is the normality of Potassium Hydroxide mol/lit (0,05). While f is the conversion factor (it is taken as 1), meq is the equivalent grams of oleic acid (0,282), V_1 is the consumption of potassium hydroxide in the main test in terms of ml, V_0 is the consumption of potassium hydroxide in the blank test in terms of ml and m is the weighed portion of substance in grams (Dileesh et al., 2013).

2.4.5. Texture Analysis

Textural properties of the oleogels, cocoa creams and oils were measured with CT3 Texture Analyzer (Brookfield Ametek, Massachusetts, USA). For this purpose, 30 mm diameter and 45° conic acrylic probe was used to measure the hardness and stickiness with three replications. All samples were heated to 45 °C to melt all fat crystals and then they were cooled to room temperature by stirring to form homogeneous fat crystals. Samples were kept overnight before the measurement.



Figure 2.1. Texture Analyzer

2.4.6. Rheological Properties

Rheological properties were measured Kinexus Rheometer (Malvern Panalytical Ltd, Malvern, UK). Frequency sweep experiments were conducted to determine the elastic (G') modulus and viscous modulus (G'') between the 0.1-10 Hz with parallel plate geometry having 20 mm diameter and 2 mm gap. Before the frequency sweep the most suitable strain amplitude was determined (0.1) with the series of trials for the linear regime viscoelastic measurement. For this purpose, frequency was set 25 °C and 0.1 Hz respectively.

CHAPTER 3

RESULTS AND DISCUSSION

3.1. Crystal Formation Time

Crystal formation time of the wax oleogels were measured at 25°C and found that according to concentration of waxes CFT was changed. There was a significant difference between the CW at 5% and 8%. Also, there was a significant difference between the Beeswax at 5% and 8% concentrations ($p < 0.05$). The reason could be explained the fact that increasing the concentration decrease the CFT of the oleogels. Also as it was understood from the Table 3.1. increasing the concentration with the same amount but in different wax types led to significant differences on CFT. It means that, while CWO5 and BWO5 had the same CFT, CFT of CWO8 and BWO8 were significantly different. Increasing the concentration decrease the CFT of the BWO more than the CWO. In general, increasing the concentration decreased the CFT value (Öğütçü & Yılmaz, 2015).

Table 3.1. *CFT of Oleogels*

Wax Type	Oleogel Concentration	Temperature (°C)	CFT (min)
Carnauba Wax	5%	25	12.50 ± 0.62 ^a
	8%	25	10.03 ± 0.61 ^b
Beeswax	5%	25	13.03 ± 0.51 ^a
	8%	25	7.53 ± 0.35 ^c

*CFT denotes *Crystal Formation Time*

** Different letters on the bars show the significant difference ($p < 0.05$)

3.2. Solid Fat Content

SFC value of the edible oils determines the characteristic of the oil because it identifies the percentage of the solid particles of the fat at a specific temperature. SFC value of the oil express the behavior of the oil in terms of consistency or plasticity in food products at different temperature ranges (Yılmaz & Ögütçü, 2014).

SFC of the oleogels and cocoa creams was measured with the NMR spectrometry at three different temperatures which were 4 °C, 25 °C and 35 °C. Also, all cocoa creams were deposited into the biscuit pieces at 40 °C to show its capability of setting initially. Measurement of SFC value of the margarines and spread products could be a prediction method for the hardness, mouthfeel, heat resistance and flavor of the products (Hwang et al., 2013).

Figure 3.1. shows the cocoa cream sandwiches which was deposited at 40 °C. In Fig. 1a., which was the only palm oil cocoa cream, set initially, cocoa cream having only HOSO was spread due to its low SFC content (Figure 3.1b). 50% oleogel cocoa creams were similar each other. They behaved like an only PO cocoa cream. However, in 100% oleogel creams were not as successful as 50% oleogel creams. Cocoa cream having 100% oleogel CWO5 cream was softer than the 50% oleogel cocoa creams but it was not spread like the HOSO (Figure 3.1h). Nevertheless, the least acceptable one was 100% oleogel BWO5 cream because it slopped over the biscuit (Figure 3.1g). In the same way, cocoa cream having 100% oleogel BWO8 cream was spread small amount (Figure 3.1i). There were not big problems in both 100% oleogel CWO5 and CWO8 creams in terms of spreadability but they were not as successful as 50% oleogel cocoa creams.

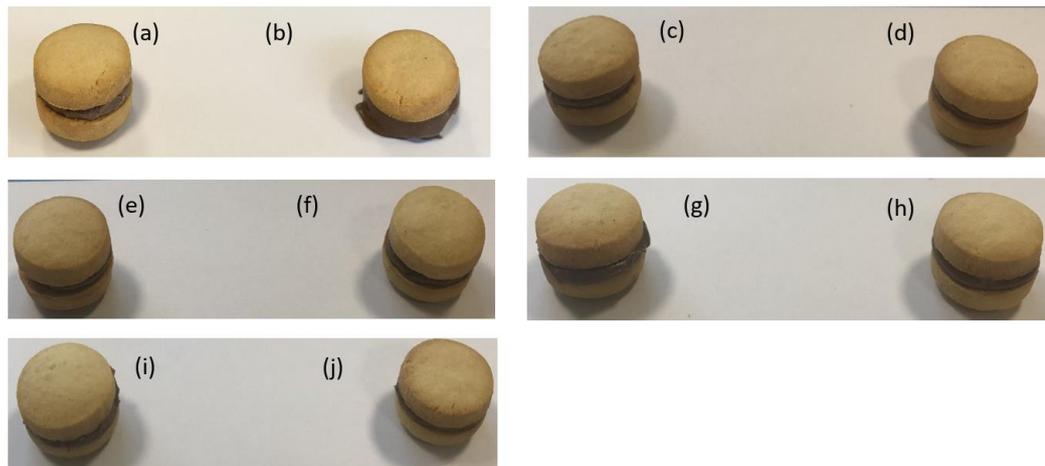


Figure 3.1. (a) only PO cocoa cream, (b) only HOSO cocoa cream, (c) 50% oleogel cocoa cream BWO5, (d) 50% oleogel cocoa cream CWO5, (e) 50% oleogel cocoa cream BWO8, (f) 50% oleogel cocoa cream CWO8, (g) 100% oleogel cocoa cream BWO5, (h) 100% oleogel cocoa cream CWO5, (i) 100% oleogel cocoa cream BWO8, (j) 100% oleogel cocoa cream CWO8

When oils and oleogels at 4 °C were compared, apart from the palm oil, there were not significant difference between the HOSO and oleogels ($p < 0.05$). While highest solid fat content rate for all temperature ranges was found as the palm oil, lowest amount was found as HOSO (Figure 3.2.). There is a correlation between the hardness and the SFC which means that harder fats have higher SFC value and the strong texture (Mert & Demirkesen, 2016). This measurement could explain the why palm oil had the highest hardness and texture of the HOSO could not be measured due to its structure (Table 3.4.).

For oleogels, increasing concentration led to slight increase in SFC but none of them could reach to SFC of the palm oil. While SFC ratio of palm oil decreased sharply after 25 °C, oleogels were slightly decreased.

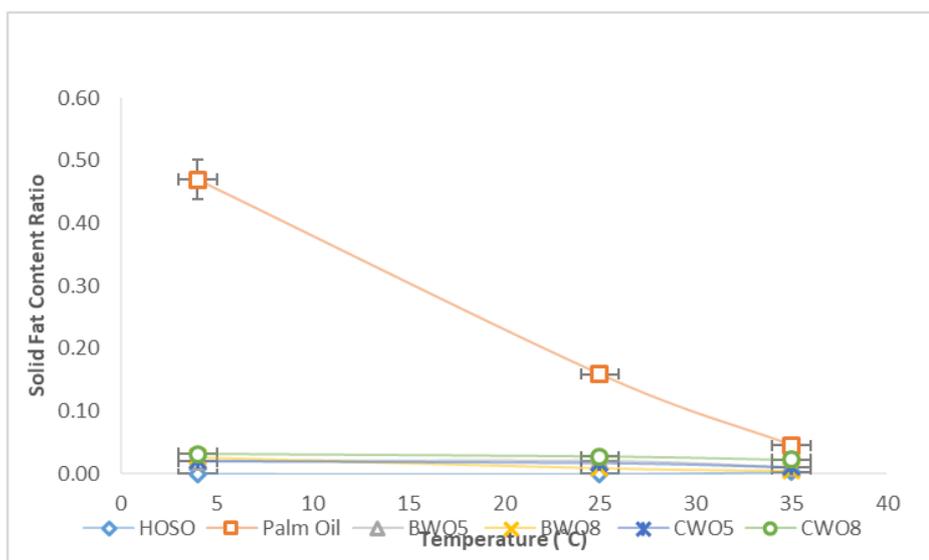


Figure 3.2. SFC Ratio of Oils and Oleogels

When palm oil cocoa cream and 50% oleogel cocoa creams were compared, behavior of the cocoa creams was similar to each other. It means that all cocoa creams were decreased sharply after 25 °C as it was expected because all cocoa creams contained palm oil. However, like in the Figure 3.2. and in the Figure 3.3, SFC ratio of the palm oil cocoa cream was the highest among the samples. Although fatty acid compositions the palm oil cocoa cream and the oleogel cocoa creams were different, at 35 °C, palm oil 50% BWO8 and 50% CWO8 cocoa creams had the same SFC ratio with the 0.26. Also, 50% BWO5 and 50% CWO5 were the same SFC ratio with the 0.25. This condition may enable advantage to oleogels for acting like a palm oil.

Doan et al., (2016) stated that in their study, with the same replacement ratio, increasing of beeswax concentration lead to almost no change in the SFC value like it was seen in this study.

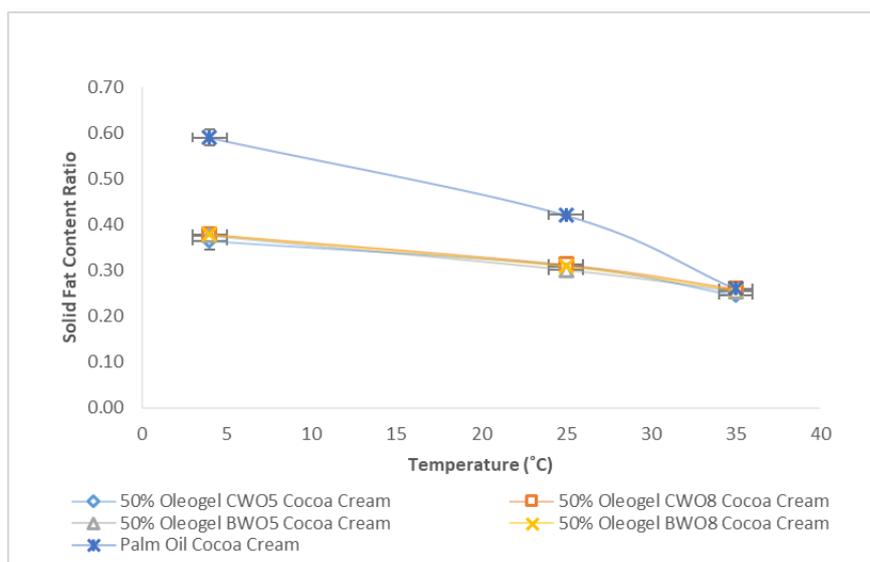


Figure 3.3. SFC Ratio of the Palm Oil and 50% Oleogel Cocoa Creams

3.3. Fatty Acid Composition

Fatty Acid compositions were measured for all cocoa creams. The mean of the results was seen in Table 3.2. and Table 3.3. The highest saturated fat amount was found in palm oil cocoa cream as expected because of palm oil's typical formulation. The first three remarkable fatty acids in palm oil was the C16:0 (palmitic acid), C18:1 (oleic acid) followed by the C18:2 (linoleic acid). Containing 41.82% C16:0, causes the highest saturated fat content to palm oil. C8:0 (Caprylic acid), C10:0 (Capric acid) and C12:0 (Lauric acid), which were the medium chain fatty acids, were found only in samples containing palm oil. Also, C14:0 (Mystric acid), a long chain fatty acid, was specific to samples containing palm oil.

HOSO cocoa cream had the highest oleic acid (C18:1) content with the 77.69 percent that was expected because the oil's name. The other remarkable fatty acids in HOSO was C18:2 (linoleic acid) and C16:0 (palmitic acid).

Replacing 50% of the palm oil with the oleogels could be a healthier alternative in terms of SFA, MUFA, PUFA and trans fatty acids. While in palm oil cocoa cream,

SFA was 57.22%, 50% oleogel cocoa creams had between the 33.82-34.71% SFA value. Also, cocoa creams with prepared 50% oleogel contained higher MUFA and PUFA and lower trans fatty acids (Table 3.3.).

Replacing 100% of the palm oil with the oleogel, changes remarkably SFA, MUFA, PUFA and trans fatty acids. While SFA, which was considered as deleterious effect on the human body, in palm oil cocoa cream was 57.22 ± 0.16 , 100% oleogel cocoa creams had 9.11-9.65% SFA interval. Specially contribution of C18:1 (oleic acid) on MUFA amount was undeniable.

In addition, replacing 100% of the palm oil with the oleogel decreased the SFA from 57.22 ± 0.16 , to below the 10 percent. Trans fatty acid amount also result in the significant decrease when compared with palm oil cocoa cream.

However, when 100% oleogel cocoa creams were compared with the HOSO cocoa cream, increase of SFA amount was observed in 100% oleogel cocoa cream. Because waxes contain long chain hydrocarbons, sterol esters, acyl-glycerols, they may cause increase of SFA value slightly (Mert & Demirkesen, 2016). Also increase in the trans fatty acids in 100% oleogel comparing the HOSO, could also explained with the same reason.

It was reported that replacement of nearly 5% of energy from saturated fatty acids by oleic acid reduced coronary heart disease risk between 20–40%, primarily with low-density lipoprotein (LDL) cholesterol reduction (Kris-Etherton, 1999). It was general acceptance that higher consumption of oleic acid and low intake of saturated fats were healthy way and may assist to protect body from cardiovascular diseases. Because of these reasons, replacement of palm oil with the oleogels may be a healthier alternative.

Table 3.2. Fatty Acid Composition of Oils and 50% Oleogel Cocoa Creams

Fatty Acid Comp.	Palm Oil	HOSO	50% Oleogel BW05	50% Oleogel BW08	50% Oleogel CW05	50% Oleogel CW08
C8:0	0.52±0.00	-	0.25±0.01	0.28±0.00	0.23±0.00	0.24±0.02
C10:0	0.42±0.00	-	0.19±0.01	0.21±0.00	0.17±0.00	0.18±0.00
C12:0	4.34±0.01	-	2.21±0.01	2.24±0.01	2.26±0.01	2.33±0.01
C14:0	2.33±0.02	-	1.19±0.00	1.20±0.01	1.19±0.00	1.21±0.00
C16:0	41.82±0.14	5.11±0.01	24.04±0.03	24.56±0.06	24.14±0.01	24.65±0.01
C16:1	0.09±0.00	-	0.13±0.00	0.19±0.00	0.12±0.01	0.13±0.01
C17:0	-	-	0.14±0.00	0.17±0.01	-	-
C18:0	7.38±0.02	3.17±0.02	5.22±0.01	5.30±0.00	5.18±0.01	5.23±0.01
C18:1 cis	33.66±0.22	77.69±0.01	54.30±0.00	53.44±0.02	54.34±0.01	53.57±0.02
C18:2 trans	0.69±0.00	0.50±0.02	0.64±0.01	0.67±0.02	0.60±0.01	0.61±0.00
C18:2 cis	8.10±0.05	12.21±0.01	10.32±0.00	10.28±0.01	10.35±0.05	10.33±0.03
C20:0	0.41±0.04	0.33±0.01	0.33±0.00	0.36±0.01	0.33±0.00	0.34±0.00
C18:3 n3	0.14±0.01	0.09±0.01	0.11±0.00	0.11±0.01	0.10±0.01	0.11±0.02
C20:1	0.10±0.00	0.22±0.00	0.27±0.00	0.29±0.00	0.27±0.02	0.28±0.02
C20:3 n6	-	0.69±0.00	0.31±0.01	0.29±0.01	0.29±0.01	0.26±0.01
C20:4	-	-	0.11±0.01	0.13±0.01	-	-
C21:0	-	-	0.12±0.01	0.15±0.00	-	-
C22:0	-	-	-	-	0.26±0.04	0.32±0.03
C24:0	-	-	0.13±0.00	0.15±0.01	0.18±0.00	0.20±0.00
SFA	57.22±0.16	8.61±0.01	33.82±0.00	34.61±0.04	33.94±0.05	34.71 ±0.02
MUFA	33.85±0.22	78.60±0.01	55.01±0.01	54.21±0.03	55.02±0.00	54.24±0.01
PUFA	8.24±0.06	12.30±0.00	10.53±0.01	10.52±0.03	10.45±0.04	10.44±0.02
TRANS	0.69±0.00	0.50±0.02	0.64±0.01	0.67±0.02	0.60±0.01	0.61±0.00

Table 3.3. Fatty Acid Composition of 100% Oleogel Cocoa Creams

<i>Fatty Acid Composition</i>	<i>100% Oleogel BW05</i>	<i>100% Oleogel BW08</i>	<i>100% Oleogel CW05</i>	<i>100% Oleogel CW08</i>
C8:0	-	-	-	-
C10:0	-	-	-	-
C12:0	-	-	-	-
C14:0	-	-	-	-
C16:0	5.10±0.01	5.12±0.02	5.08±0.03	5.14±0.04
C16:1	0.13±0.01	0.18±0.00	0.13±0.00	0.14±0.01
C17:0	0.21±0.01	0.30±0.00	-	-
C18:0	3.10±0.01	3.21±0.01	3.25±0.01	3.31±0.08
C18:1 cis	76.73±0.03	76.41±0.01	76.81±0.01	76.64±0.04
C18:2 trans	0.62±0.00	0.64±0.00	0.54±0.01	0.55±0.00
C18:2 cis	12.11±0.01	12.02±0.01	12.07±0.01	11.94±0.02
C20:0	0.24±0.00	0.28±0.01	0.23±0.00	0.26±0.01
C18:3 n3	0.09±0.00	0.08±0.01	0.08±0.01	0.07±0.01
C20:1	0.34±0.02	0.35±0.03	0.34±0.02	0.35±0.03
C20:3 n6	0.69±0.00	0.67±0.00	0.67±0.00	0.67±0.00
C20:4	0.18±0.00	0.24±0.01	-	-
C21:0	0.23±0.02	0.25±0.00	-	-
C22:0	-	-	0.44±0.03	0.53±0.11
C24:0	0.23±0.01	0.26±0.01	0.37±0.05	0.41±0.00
SFA	9.11±0.01	9.41±0.02	9.36±0.02	9.64±0.01
MUFA	77.89±0.00	77.60±0.02	77.95±0.03	77.80±0.00
PUFA	12.37±0.02	12.34±0.00	12.15±0.01	12.01±0.02
TRANS	0.62±0.00	0.64±0.00	0.54±0.01	0.55±0.00

3.4. Oxidative Stability

For the oxidation determination, peroxide value (PV) is generally used to determine the oxidative status of the oil. In the fresh oils, PV value is less than 10 meq/kg. In addition, rancidity could be perceived when the PV value is between the 20-30 meq/kg. Because as PV of oil exceeds the 10 meq/kg, oil is regarded as at high oxidation level, upper limit of vegetable oil for the acceptance is between the 15-20 meq/kg (Patel et al., 2014).

PV of both oleogels, oils, 50% oleogel and 100% oleogel cocoa creams were measured at 25 °C during three months. In this study, as it was seen from the Figure 3.4., Figure 3.5. Figure 3.6. all samples were below the upper acceptance limits. However, none of the oleogels and oleogel cocoa creams was not as good as palm oil and palm oil cocoa cream in terms of peroxide value at the end of the 90 days.

Before conducting the storage study, peroxide value of the all oleogels and oleogel cocoa creams were higher than the HOSO and palm oil (Figure 3.4., Figure 3.5. & Figure 3.6.). The reason could be explained that oils in the oleogels were subjected to high temperatures (90 °C) while preparation of the oleogels (Lim et al., 2017).

HOSO had the highest peroxide value among the samples after three months storage. It was not exceeding the upper limit but it was seen from the Figure 3.4. the most susceptible sample to oxidation was the HOSO. Because vegetable oils having triglyceride-based structure contain unsaturated fatty acids, they are more prone to lipid oxidation. The more unsaturation level, that is, the more double bonds, causes the more oxidation (Fox, & Stachowiak, 2007). Lim et al., (2017) stated in their study, peroxide value of canola oil had significantly increased faster than the candelilla wax oleogel, carnauba wax oleogel and beeswax oleogel under the accelerated storage conditions (60°C). In this study, peroxide value of HOSO was also increased similar to canola oil.

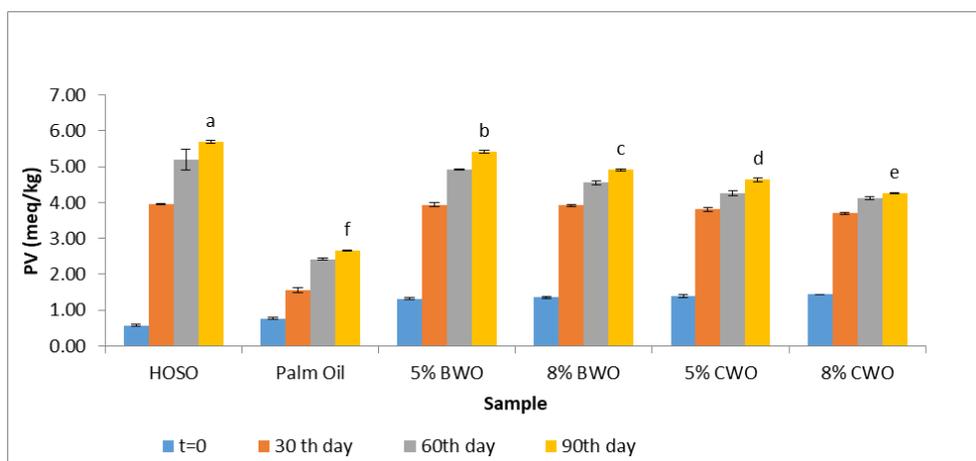


Figure 3.4. PV of oils & oleogels during three months

**Different letters on the bars show the significant difference ($p < 0.05$)

While vegetable oils, which contain high amount of monounsaturated fatty acids, generally autoxidized at higher temperatures, vegetable oils containing more double bonds especially linoleic (18:2) is easily oxidized at room temperature (Fox, & Stachowiak, 2007). As it was seen from Table 3.3. because cocoa creams containing HOSO, have higher linoleic acid (18:2) content, their oxidation stability were lower than the cocoa creams having PO. In addition, fatty acids, which contain 8-16 C, are classified as the saturated fatty acids. Because PO has nearly 41.78 % palmitic acid (16:0), its oxidation stability will be expected to higher than the HOSO (Chowdhury et al., 2007, Kano et al., 2012).

When the oleogels were compared in terms of peroxide value, wax type and wax concentration affected the oxidative stability significantly ($p < 0.05$). As it was seen from the Fig 3.6. the lowest peroxide value had the 8% CWO followed by the 5% CWO, 8% BWO and 5% BWO. Lim et al., (2017) reported that peroxide value of CWO with canola oil was lower than the BWO with canola oil although the results were not the same with this study because the study was conducted under the accelerated storage conditions (60 °C). Besides, in Table 3.2. & Table 3.3. PUFA in BW oleogels were higher than the CW oleogels they were more liable to oxidation.

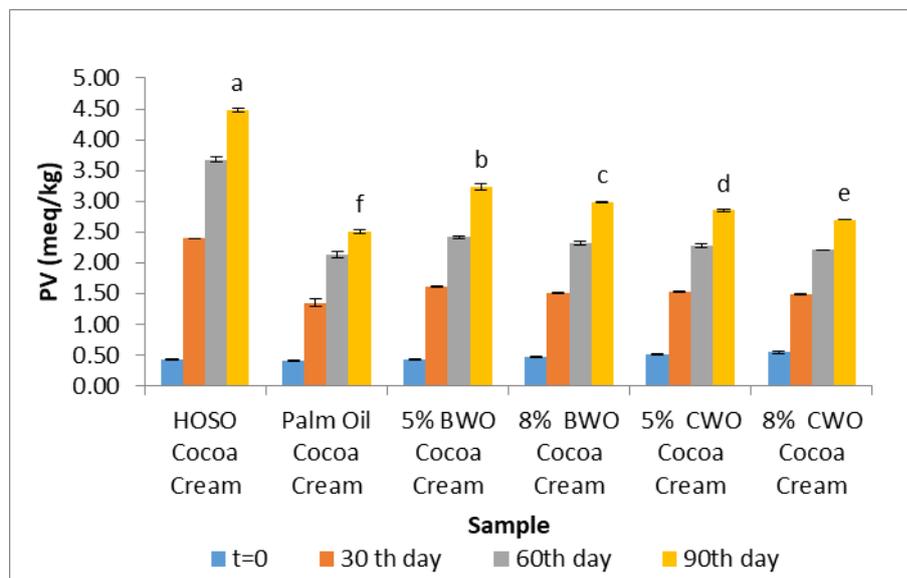


Figure 3.5. PV of 50% oleogel cocoa creams during three months

**Different letters on the bars show the significant difference ($p < 0.05$)

Also, peroxide value of 50% oleogel %8 BWO cocoa cream was lower than 50% oleogel 5% BWO one. Increasing the concentration may strengthen the network. Martins et al., (2017) also supported with the study that among the different BWO concentrations (2%, 4%, 6%, 8%), 2% BWO was the highest peroxide value due to its weaker network supported by the oleogelator.

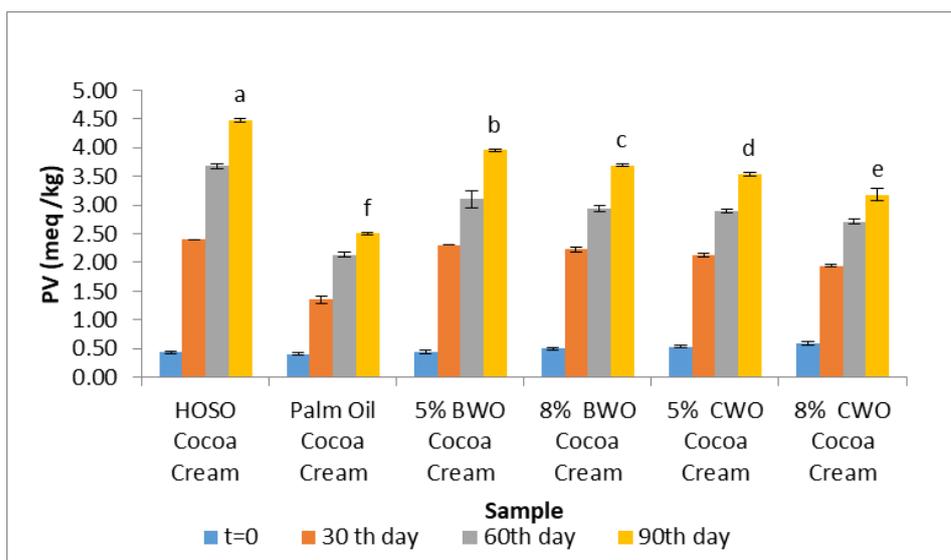


Figure 3.6. PV of 100% oleogel cocoa creams during three months

**Different letters on the bars show the significant difference ($p < 0.05$)

When the 50% and 100% oleogel cocoa creams were compared, PV value of 50% oleogel cocoa creams were lower than the 100% oleogel cocoa creams. This difference was the usage of palm oil in the 50% oleogel cocoa cream. As it was mentioned above, PO's saturated fat content could delay the oxidation.

Another oxidative stability is acidity. Although acidity value is very important parameter for the quality of the sample such as flavor, there was not enough studies about this subject. Figure 3.7. shows the acidity of the oils and the oleogels in terms of % oleic acid. According to Codex Alimentarius maximum limit of acid value of the refined oils should be less than 0,6 mg KOH/gr oil (1999). As it was seen from the figure, highest acidity value was the HOSO at the end of the three months. Under light of these informations, at the end of the three months, acid value of the HOSO and BWO5 were exceed the maximum limit with the 0.37% FFA and 0.34% FFA which is equivalent to 0.74% mg KOH/g and 0.68% mg KOH/g respectively (Berrios et al., 2007).

In addition, it was observed that there was no significant difference among the HOSO, 5% BWO and 8% BWO samples. Also it was found as palm oil has the lowest acidity. Acidity of oleogels before the starting of the storage experiment were higher than both HOSO and Palm oil. The reason could be explained the fact that, during oleogelation process because oelogels exposed to high temperature, their acidity could be increased.

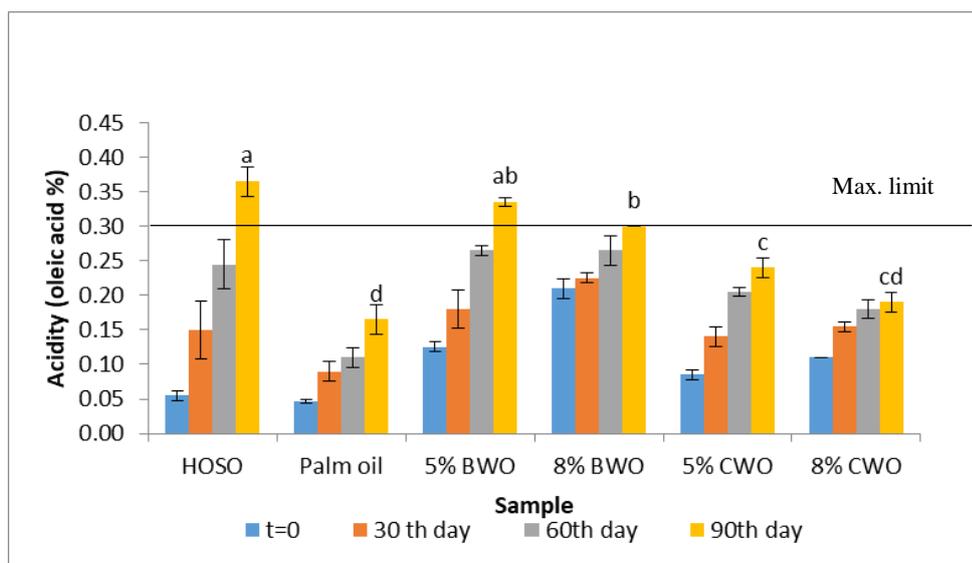


Figure 3.7. Acidity of Oleogels & Oils during three months

**Different letters on the bars show the significant difference ($p < 0.05$)

Also, Doan et al., (2015) measured acidity of the both BW and CW before conducting the experiment and found that acid value of BW (19.10 ± 0.06) was remarkably higher than the CW (5.88 ± 0.01). It was concluded that, intrinsic factor of the waxes affected the acidity. In another study stated that because carnauba was contained lower amount of free fatty acids and hydroxyl groups (Acid value; 2-7) than beeswax contained (Acid value ;17-24), degradation of beeswax structure was higher than the carnauba wax (Kheradmandnia et al., 2010). In this study, acidity of the samples was not measured as high as above studies because these studies directly measured the acid value of the waxes not the oleogels. Also, in this study acidity was measured in terms

of % oleic acid not in terms of mg KOH/g. In addition, wax characteristics may differ according to brand or type so it changes the acidity.

However, although acidity of 8% BWO was higher than the 5% BWO before starting the storage experiment, it formed a stronger three dimensional network and structure than the 5% BWO did. Despite the fact that, before starting the experiment BWO5 and BWO8 were significantly different to each other, at the end of the experiment there was no significant difference was observed ($p < 0.05$).

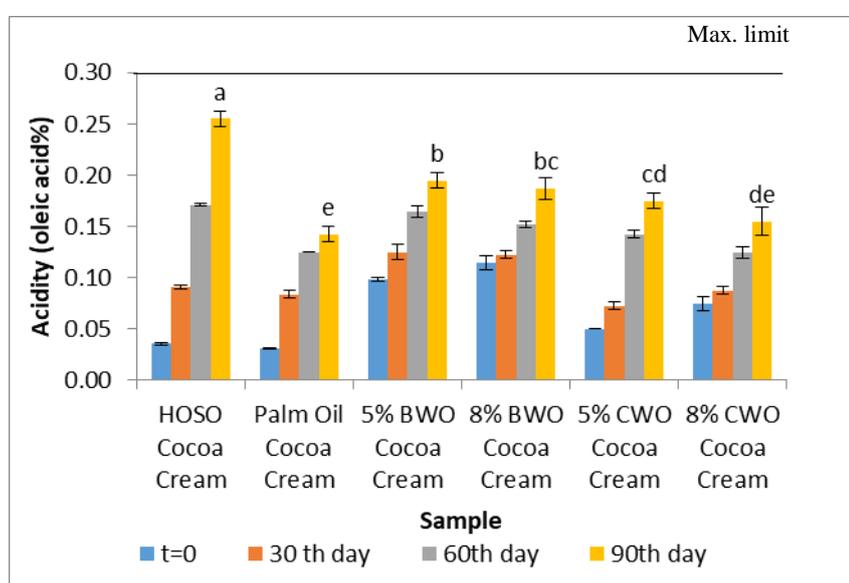


Figure 3.8. Acidity of 50% Oleogel & Oil Cocoa Creams during three months

**Different letters on the bars show the significant difference ($p < 0.05$)

When acidity of 50% oleogel cocoa creams were compared, acidity of HOSO cocoa cream was found as the highest among the samples as it was found in the oils and oleogel sample measurements (Figure 3.8.). The reason could be explained the fact that, because HOSO cocoa cream has oleic acid (18:1) and higher linoleic acid (18:2) than others, it was more susceptible to oxidation. Although oleic acid, which forms

the mainly part of the HOSO, is regarded as more stable than linoleic acid, it is a monounsaturated fatty acid, so it is not more stable than the saturated fatty acids.

The effects of fatty acid composition on oxidation stability is related to degree of unsaturation level of the oils (Kamal-Eldin, 2006). It was found from the Table 3.3. the more unsaturation level cocoa cream has, the more susceptible to oxidation it is.

Besides, since palm oil cocoa cream has 57.22 ± 0.16 % saturated fat content, its acidity was found as the lowest among the cocoa creams. However, there was no significant difference between the 50% oleogel BWO5 and BWO8 cocoa creams. It was concluded that concentration change did not affect the acidity of the oleogel cocoa creams. The same phenomena were seen also in the 50% oleogel CWO5 and 50% oleogel CWO8 oleogel cocoa creams. Apart from these comparisons, interestingly 50% oleogel CWO5 oleogel cocoa cream was not significantly different from the 50% oleogel BWO8 cocoa cream. The reason could be explained the fact that, BWO8 may form a strong three dimensional network than the CWO5 also concentration is higher than the CWO5, it could retard the acidity to a certain extend.

When 100% oleogel cocoa creams were compared, acidity of palm oil was significantly different from the other cocoa creams ($p < 0.05$). It was understood from the Figure 3.10., because HOSO and BWO cocoa creams were not significantly different, BWO could not retard the acidity development during storage. Also, increasing the beeswax concentration did not make a significant change. In the same way, CWO cocoa creams were not significantly different that increasing of concentration could not retard acidity.

Interestingly, acidity of 100% oleogel BWO cocoa creams and 100% oleogel CWO5 also close to each other.

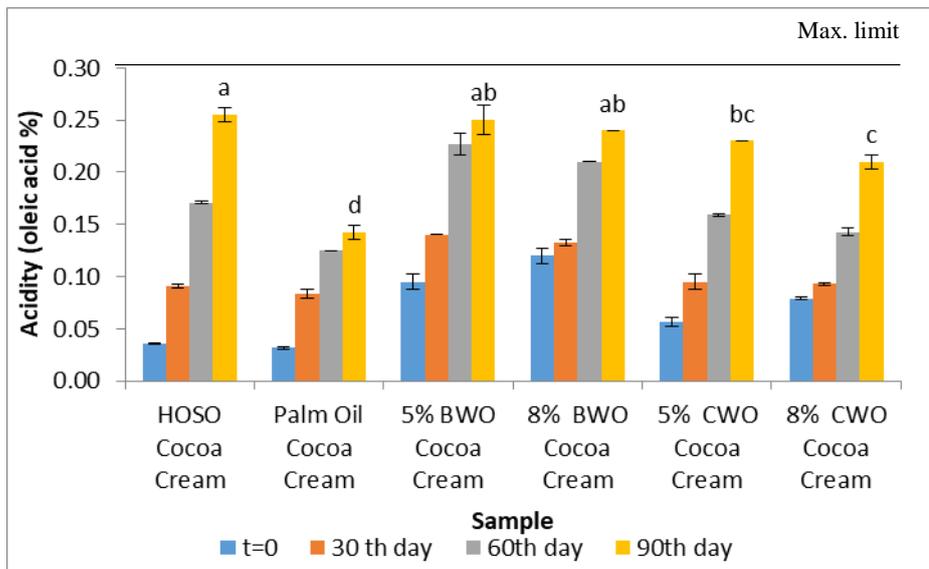


Figure 3.9. Acidity of 100% Oleogel & Oil Cocoa Creams during three months

**Different letters on the bars show the significant difference ($p < 0.05$)

3.5. Texture Analysis

In the texture analysis, which was applied one-cycle compression to measure the peak force, palm oil cocoa cream was the hardest one with the 49.88 ± 2.61 N, among the samples according to one-way ANOVA ($p < 0.05$). This result was expected because most part of the PO is crystallized form at the room temperature, it gave a structure to the cocoa cream. Hardness of HOSO and its cocoa cream could not be measured, because the probe of the texture analyzer had already penetrated the cocoa cream before the measurement. The reason could be SFC value of HOSO was very low at room temperature.

Effect of wax concentration on hardness was seen from the Table 3.4. Wax concentration affects the hardness significantly. Ögütçü (2014) stated in their study, beeswax concentration increased, the hardness value was also increased. It could be explained as, more wax concentration may cause strong three dimensional network and strong interactions. Also, in another study of Ögütçü & Yilmaz (2014), 3%, 7%

and 10% CWO with virgin olive oil were compared and found that there was a significant difference between the hardness of the oleogels.

Table 3.4. *Texture Analysis of Oils and Oleogels*

Sample	Hardness (N)
Palm Oil	9.6±0.49 ^a
HOSO	Not measured
5% BWO	1.4±0.13 ^d
8% BWO	3.3±0.10 ^c
5% CWO	2.6±0.04 ^c
8% CWO	4.9±0.25 ^b

***Different letters represent significant difference ($p < 0.05$)*

Lim et al., (2017) found a correlation between the hardness of the oleogels and the peroxide value between the carnauba wax oleogel and beeswax oleogel. The harder oleogel caused the lower peroxide value. This phenomena could be explained the fact that oil mobility was restricted so that oxidation will be retarded during storage. In this study, there was also a correlation between the hardness and peroxide value. The hardest oleogel was found as 8% CWO and the lowest peroxide value among the oleogels was found as 8% CWO (Figure 3.5.).

50% oleogel cocoa creams were significantly different from the 100% oleogels ($p < 0.05$). Ali et al., (2001) stated that increase of liquid phase in the chocolate caused softening. The difference among the oleogel cocoa creams may associate with this hypothesis.

However, while increase of concentration could cause a significant difference between the BWO cocoa cream samples, it did not create a big difference between the CWO cocoa cream samples among the 50% oleogel cocoa creams (Table 3.5.). It could be

explained the fact; beeswax could go into an interaction presence of the palm oil with the increasing concentration.

Besides the effects of oil or wax type on texture, non-fat solids also affect the hardness. Due to this reason, every cocoa cream was harder than its oleogel or oil. Increase in the hardness is related to increased solid amount. Delbaere et al., (2016) stated that in chocolate or fat continuous fillings, particle interactions such as van der Waals forces or hydrogen bonding between the polar particles could lead to formation of three dimensional network. Beside the particle-particle interactions particle-fat interaction could assist the three dimensional network. In this study, sugar, cocoa powder and other related ingredients were used to form cocoa cream. By strengthen the three dimensional network, these particles could make harder cocoa creams than their oleogels.

Among the 100% oleogel cocoa creams, 8% CWO cocoa cream was significantly different from the 5% BWO ($p < 0.05$). However, 8% BWO cocoa cream was not significantly different from the 5% CWO cocoa cream.

Table 3.5. *Texture Analysis of Cocoa Creams*

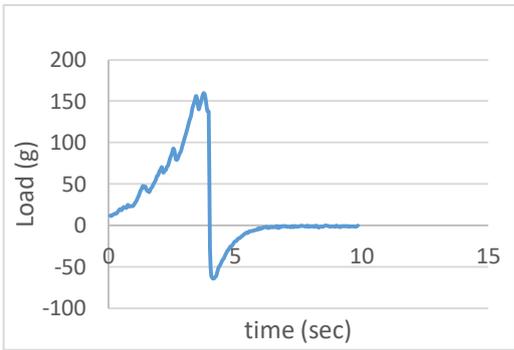
Sample	Hardness (N)
Palm Oil Cocoa Cream	49.88 ±2.61 ^a
HOSO Cocoa Cream	Not measured
50 % Oleogel 5% BWO Cocoa Cream	13.94±1.04 ^c
50 % Oleogel 8% BWO Cocoa Cream	18.59±2.16 ^b
50 % Oleogel 5% CWO Cocoa Cream	15.23±0.62 ^{bc}
50 % Oleogel 8% CWO Cocoa Cream	18.85±1.21 ^b
100 % Oleogel 5% BWO Cocoa Cream	3.40±0.59 ^e
100 % Oleogel 8% BWO Cocoa Cream	6.60±0.59 ^{de}
100 % Oleogel 5% CWO Cocoa Cream	5.15±0.69 ^{de}
100 % Oleogel 8% CWO Cocoa Cream	7.36±0.12 ^d

***Different letters represent significant difference ($p < 0.05$)*

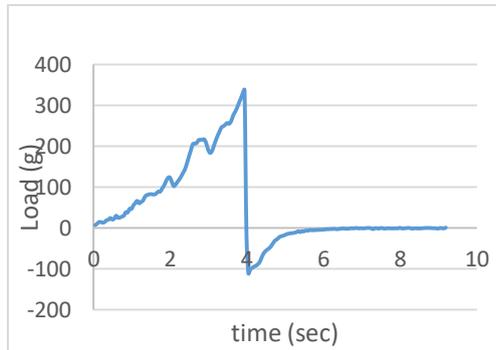
However, when measurement curves of the beeswax oleogels and their cocoa creams were analysed, fractures were seen in the both two replicates of the BWO oleogels. Walstra stated that gel fracture could occur with the breakage of the network chains between the crosslinks or the junction zones. (2003). Although BW oleogels were found in the frequency sweep test as a strong gel due to superiority of G' over G'' , it could not resist the force which was applied by the texture analyzer.

Talens & Krochta reported that while CW is a hard-elastic material and has a little viscous behavior, BW has less elastic but more viscous structure besides more easily deformed material. This hypothesis could be attributed to presence of significant amount of free fatty acids in the BW may develop the viscous qualities (2015).

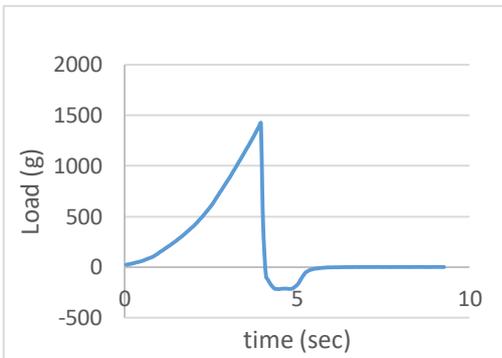
Interestingly, while these fractures were observed in the BW oleogels, any problem was observed in their cocoa creams. Adding of solid particles into the oleogel may harden it and they may build strong interaction with each other so that it resisted to force more than the oleogel phase. Also, presence of lecithin in the cocoa cream formulation may develop the elasticity of the BW oleogel so that they did not fracture. Aguilar-Zárate et al., (2019) stated in their study, constituting of oleogel by using lecithin mixture developed the elastic recovery properties and it supplied the high storage modulus (G').



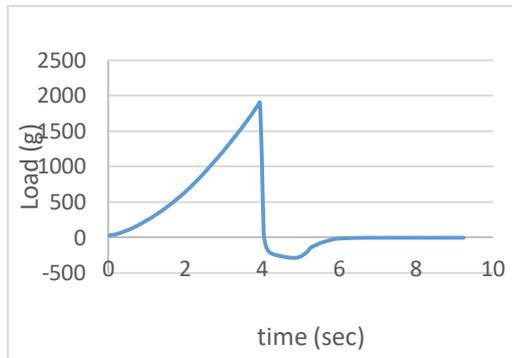
(a)



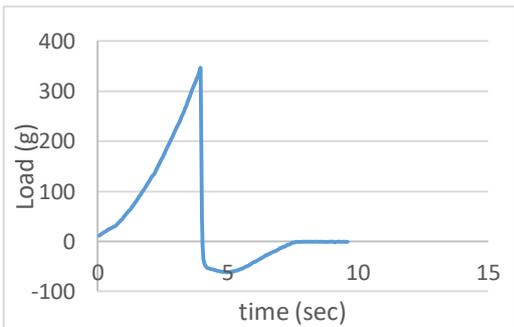
(b)



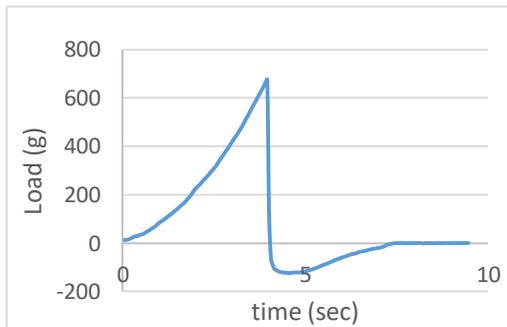
(c)



(d)



(e)



(f)

Figure 3.10. Hardness Graph (a) BWO5 (b) BWO8 (c)50% Oleogel Cocoa Cream BWO5 (d)50% Oleogel Cocoa Cream BWO8 (e) 100%oleogel Cocoa Cream BWO5 (f) 100% Oleogel Cocoa Cream BWO8

3.6. Rheological Properties

To measure the rheological properties of the oleogels and cocoa creams, non-destructive oscillation test was used. The viscoelastic behavior combines the structural changes of the gel formulations. It involves two parts which are storage or elastic modulus G' and loss modulus or viscous modulus G'' . While G' represents the solid-like properties, G'' represents the liquid like properties.

Frequency sweep of the oleogels and cocoa creams were measured in the linear viscoelastic region. Linear elastic means that the materials where the strain is directly proportional to the stress and strain turn back to zero when the stress is removed (Bourne, 2002). First of all, to find the linear viscoelastic region under the constant frequency the most suitable strain amplitude was determined as 0.1. Then frequency sweep analysis was conducted between the 0.1 and 10 Hz. HOSO could not be measured due to its liquid structure.

Among the all samples, palm oil has the highest storage (G') and loss modulus (G'') both in oleogels and cocoa creams that it was ascribed by the harder texture of the palm oil. All oleogels, palm oil and cocoa creams except HOSO cocoa creams showed higher storage (G') modulus than the loss (G'') modulus. It shows that the all cocoa creams except HOSO cocoa cream had the elastic characteristics (solid-like material) which means they had strong gel property. In general, purely elastic materials are not dependent to frequency, Nevertheless, food gels have small amount of part (G'') so, storage (G') modulus depends on the frequency (Ikeda & Foegeding, 1999). Also, Jang et al., (2015) stated that increasing storage (G') and loss modulus (G'') with the increasing frequency means that they were frequency dependent. As it was seen the Figure 3.11., 3.12. and 3.13. both storage and loss modulus increased with the increasing frequency.

It was found that G' and G'' increased with the increasing wax concentration (Figure 3.11.). Blake & Marangoni (2014) reported that in their study, between the 5% CWO and 8% CWO 20-fold difference was observed. However, in this study approximately

10-fold difference was observed. This difference could be arisen from the wax brand or type of carnauba wax.

Also type of wax affected the G' and G'' . In all formulations, G' and G'' of the CWO8 and CWO5 were found higher than the BWO8 and BWO5 respectively. It could be concluded that CW oleogels may form stronger gel structure than the BW oleogels.

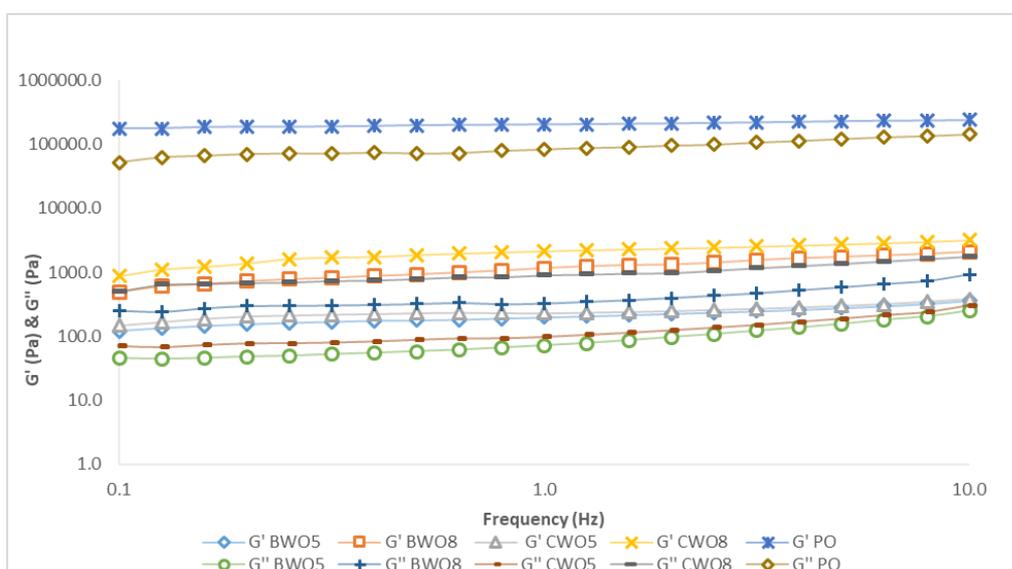


Figure 3.11. Storage and Loss Modulus of the Palm Oil & Oleogels

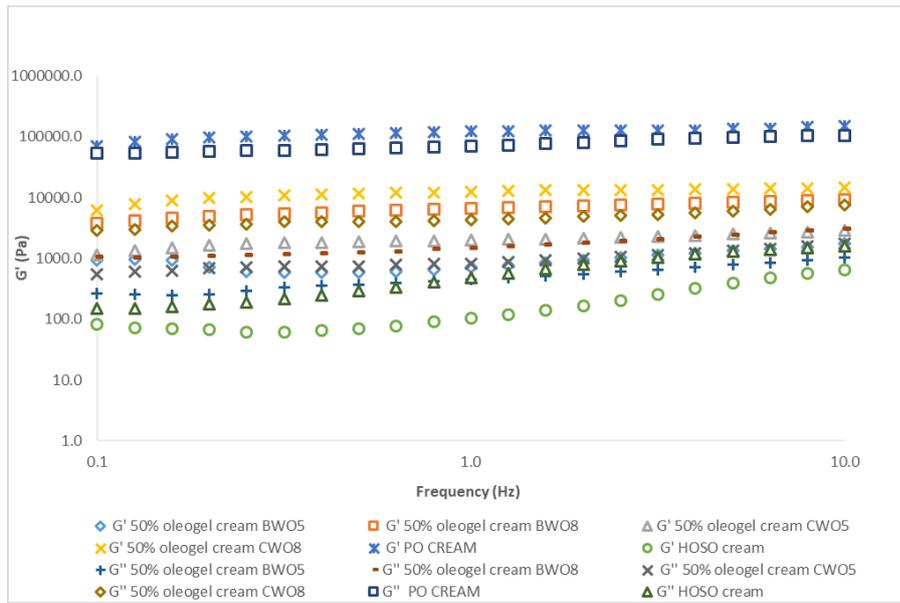


Figure 3.12. Storage and Loss Modulus of the Oils & 50% oleogel Cocoa Creams

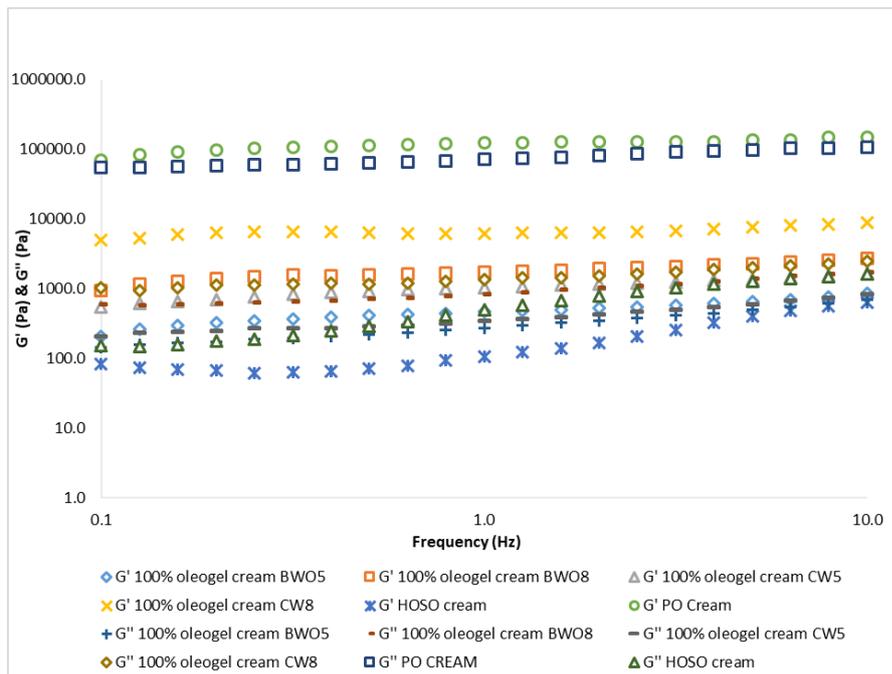


Figure 3.13. Storage and Loss Modulus of the Oils & 100% oleogel Cocoa Creams

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

In this study, the aim of oleogel usage as a fat replacer in the food products is to decrease the saturated fat content and trans fatty acids by satisfying the palm oil properties. In general, 50% oleogel cocoa creams were found as more successful than the 100% oleogel cocoa creams to imitate the palm oil cocoa cream properties in terms of SFC ratio, structure and oxidative stability. However, none of the oleogel could reach the palm oil physical properties.

In fatty acid compositions, by replacing the 50% of the palm oil with the oleogel cocoa creams, saturated fat content decreased from 57% to 33-34 % intervals. Also, the most successful sample was the CWO5 among the 50% oleogel cocoa creams in terms of trans fatty acids. (0.60 %). When 100% oleogel cocoa creams were compared, saturated fat content and trans fatty acid decreased below the 10% and 0.65% respectively.

SFC ratios of the oleogels, oils and cocoa creams were measured at three different temperatures which are 4 °C, 25 °C, 35 °C. While there was a significant difference between the samples containing only palm oil and others at 4 °C and 25 °C ($p < 0.05$), at 35 °C (nearly body temperature) all cocoa creams were not the significantly different ($p > 0.05$). This could be an advantage for the oleogels so that waxy mouthfeel could not be perceived.

At the end of the three months storage, HOSO had the highest peroxide value, but none of the oleogel and their cocoa creams did not exceed the maximum limit which was accepted. 50% oleogel CWO8 cocoa creams is the closest one to the palm oil

cocoa cream. Results of the 100% oleogel cocoa creams were not as good as the 50% oleogel cocoa creams. Peroxide value of the BWO oleogels were highly increased during storage.

HOSO and 5% BWO had the highest acidity value in terms of % oleic acid that exceeded the maximum limit according to Codex Alimentarius (1999) at the end of the storage. In addition, results of the BWO oleogels was closer to the maximum limits. None of the cocoa creams was exceeded the maximum limit.

Among the oleogels and cocoa creams the hardest sample was found palm oil coco cream with significant difference ($p < 0.05$). Although none of the oleogel cocoa cream was not as high as the palm oil CWO8 and BWO8 cocoa creams may acceptable.

When the rheological properties were analyzed, all samples were found as frequency dependent. Because $G' > G''$, all samples except HOSO cocoa cream were accepted as elastic characteristics.

As it was understood from these experiments, generally wax concentration % replacement amount and wax type affect the physical and chemical properties of the food products. However, while replacement of palm oil with the oleogel decreased saturated fat content remarkably, none of the sample in this study could provide the same physical and chemical properties of the palm oil. Different type of waxes and concentrations may be analyzed to find the best oleogel properties.

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APPENDICES

A. TABLES

Table A.1. *SFC Ratio of the Oils and Oleogels at 4 °C*

Sample	Ratio
Palm Oil	0.47±0.03 ^a
HOSO	0.00±0.00 ^b
5% Beeswax Oleogel	0.02±0.00 ^b
8% Beeswax Oleogel	0.03±0.00 ^b
5% Carnauba Wax Oleogel	0.02±0.00 ^b
8% Carnauba Wax Oleogel	0.03±0.00 ^b

Table A.2. *SFC Ratio of the Cocoa Creams at 4 °C*

Sample	Ratio
Palm Oil Cocoa Cream	0.59±0.02 ^a
HOSO Cocoa Cream	0.32±0.00 ^c
50% Oleogel BWO5 Cocoa Cream	0.38±0.02 ^b
50% Oleogel BWO8 Cocoa Cream	0.38±0.00 ^b
50% Oleogel CWO5 Cocoa Cream	0.36±0.02 ^{bc}
50% Oleogel CWO8 Cocoa Cream	0.38±0.00 ^b

Table A.3. *SFC Ratio of the Oils and Oleogels at 25 °C*

Sample	Ratio
Palm Oil	0.16±0.00 ^a
HOSO	0.00±0.00 ^e
5% Beeswax Oleogel	0.01±0.00 ^d
8% Beeswax Oleogel	0.02±0.00 ^{bc}
5% Carnauba Wax Oleogel	0.02±0.00 ^{cd}
8% Carnauba Wax Oleogel	0.03±0.00 ^b

Table A.4. *SFC Ratio of the Cocoa Creams at 25 °C*

Sample	Ratio
Palm Oil Cocoa Cream	0.42±0.00 ^a
HOSO Cocoa Cream	0.35±0.01 ^b
50% Oleogel BWO5 Cocoa Cream	0.30±0.01 ^{cde}
50% Oleogel BWO8 Cocoa Cream	0.31±0.00 ^{cd}
50% Oleogel CWO5 Cocoa Cream	0.31±0.00 ^{bc}
50% Oleogel CWO8 Cocoa Cream	0.31±0.01 ^{bc}

Table A.5. *SFC Ratio of the Oils and Oleogels at 35 °C*

Sample	Ratio
Palm Oil	0.05±0.00 ^a
HOSO	0.00±0.00 ^d
5% Beeswax Oleogel	0.01±0.00 ^{cd}
8% Beeswax Oleogel	0.02±0.01 ^b
5% Carnauba Wax Oleogel	0.01±0.00 ^{bc}
8% Carnauba Wax Oleogel	0.02±0.00 ^b

Table A.6. *SFC Ratio of the Cocoa Creams at 35 °C*

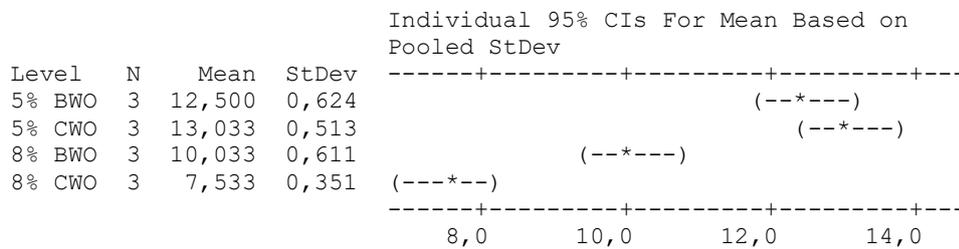
Sample	Ratio
Palm Oil Cocoa Cream	0.26±0.00 ^b
HOSO Cocoa Cream	0.37±0.01 ^a
50% Oleogel BWO5 Cocoa Cream	0.25±0.01 ^b
50% Oleogel BWO8 Cocoa Cream	0.26±0.01 ^b
50% Oleogel CWO5 Cocoa Cream	0.25±0.01 ^b
50% Oleogel CWO8 Cocoa Cream	0.26±0.01 ^b

B. STATISTICAL ANALYSIS

Table B.1. *Analysis of Variance for CFT of the Oleogels*

Source	DF	SS	MS	F	P
sample	3	57,402	19,134	66,55	0,000
Error	8	2,300	0,287		
Total	11	59,702			

S = 0,5362 R-Sq = 96,15% R-Sq(adj) = 94,70%



Pooled StDev = 0,536

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
5% CWO	3	13,0333	A
5% BWO	3	12,5000	A
8% BWO	3	10,0333	B
8% CWO	3	7,5333	C

Means that do not share a letter are significantly different.

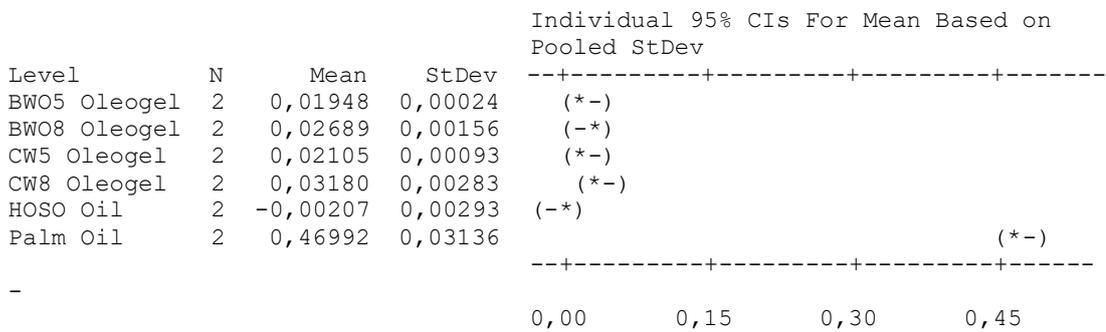
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of sample

Individual confidence level = 98,74%

Table B.2. Analysis of Variance of SFC ratio of the Oils and Oleogels at 4 °C

Source	DF	SS	MS	F	P
sample	5	0,339588	0,067918	406,17	0,000
Error	6	0,001003	0,000167		
Total	11	0,340591			

S = 0,01293 R-Sq = 99,71% R-Sq(adj) = 99,46%



Pooled StDev = 0,01293

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
Palm Oil	2	0,46992	A
CW8 Oleogel	2	0,03180	B
BW08 Oleogel	2	0,02689	B
CW5 Oleogel	2	0,02105	B
BW05 Oleogel	2	0,01948	B
HOSO Oil	2	-0,00207	B

Means that do not share a letter are significantly different.

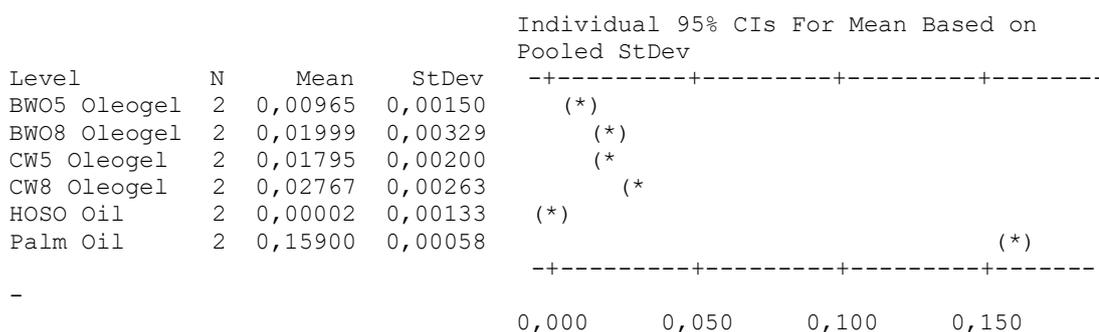
Tukey 95% Simultaneous Confidence Intervals
All Pairwise Comparisons among Levels of sample

Individual confidence level = 99,27%

Table B.4. Analysis of Variance of SFC ratio of the Oils and Oleogels at 25 °C

Source	DF	SS	MS	F	P
sample	5	0,0354266	0,0070853	1627,90	0,000
Error	6	0,0000261	0,0000044		
Total	11	0,0354528			

S = 0,002086 R-Sq = 99,93% R-Sq(adj) = 99,86%



Pooled StDev = 0,00209

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
Palm Oil	2	0,15900	A
CW8 Oleogel	2	0,02767	B
BW08 Oleogel	2	0,01999	B C
CW5 Oleogel	2	0,01795	C D
BW05 Oleogel	2	0,00965	D
HOSO Oil	2	0,00002	E

Means that do not share a letter are significantly different.

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

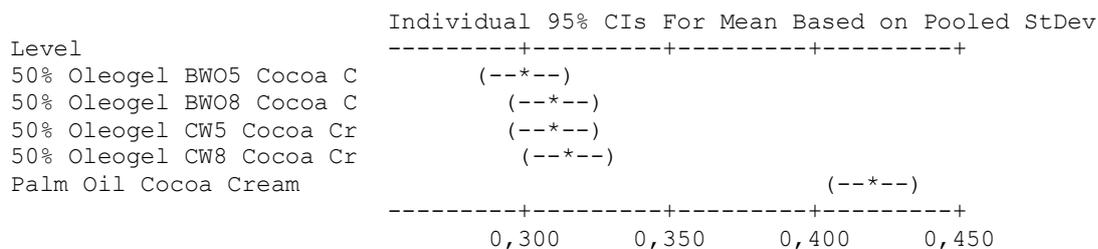
Individual confidence level = 99,27%

Table B.5. Analysis of Variance of SFC ratio of the all cocoa creams at 25°C

Source	DF	SS	MS	F	P
sample	9	0,0355084	0,0039454	44,53	0,000
Error	10	0,0008860	0,0000886		
Total	19	0,0363944			

S = 0,009413 R-Sq = 97,57% R-Sq(adj) = 95,37%

Level	N	Mean	StDev
50% Oleogel BW05 Cocoa C	2	0,30104	0,00934
50% Oleogel BW08 Cocoa C	2	0,30916	0,00321
50% Oleogel CW5 Cocoa Cr	2	0,31102	0,00250
50% Oleogel CW8 Cocoa Cr	2	0,31288	0,00995
HOSO Cocoa Cream	2	0,34769	0,00763
Palm Oil Cocoa Cream	2	0,42070	0,00075



Pooled StDev = 0,00941

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
Palm Oil Cocoa Cream	2	0,42070	A
50% Oleogel CW8 Cocoa Cream	2	0,31288	B C
50% Oleogel CW5 Cocoa Cream	2	0,31102	B C
50% Oleogel BW08 Cocoa Cream	2	0,30916	C D
50% Oleogel BW05 Cocoa Cream	2	0,30104	C D E

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 = 99,73%

Table B.6. Analysis of Variance of SFC ratio of the Oils and Oleogels at 35

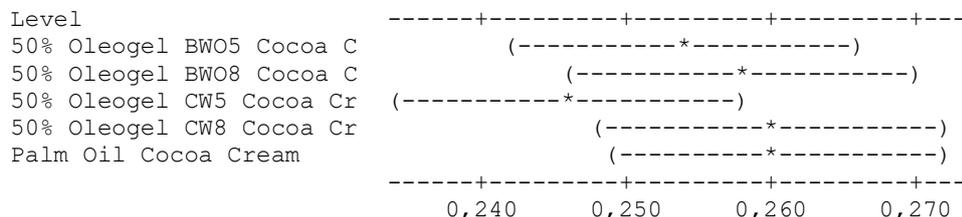
°C

Source	DF	SS	MS	F	P
sample	5	0,0028515	0,0005703	54,39	0,000
Error	6	0,0000629	0,0000105		
Total	11	0,0029144			

S = 0,003238 R-Sq = 97,84% R-Sq(adj) = 96,04%

50% Oleogel CW8 Cocoa Cr 2 0,26029 0,00719
 Palm Oil Cocoa Cream 2 0,26043 0,00348

Individual 95% CIs For Mean Based on
 Pooled StDev



Pooled StDev = 0,00656

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
Palm Oil Cocoa Cream	2	0,260432	A
50% Oleogel CW8 Cocoa Cream	2	0,260285	A
50% Oleogel BW08 Cocoa Cream	2	0,258222	A
50% Oleogel BW05 Cocoa Cream	2	0,254250	A
50% Oleogel CW5 Cocoa Cream	2	0,246372	A

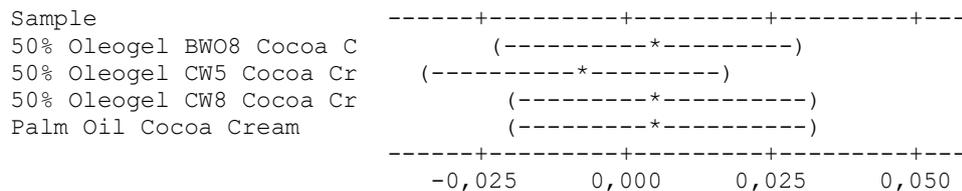
Means that do not share a letter are significantly different.

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

Individual confidence level = 98,98%

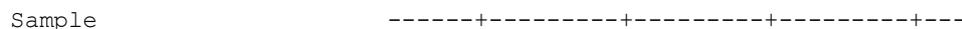
Sample = 50% Oleogel BW05 Cocoa Cream subtracted from:

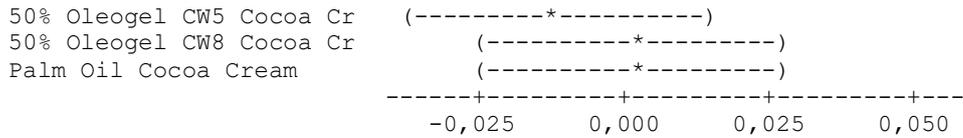
Sample	Lower	Center	Upper
50% Oleogel BW08 Cocoa C	-0,022314	0,003973	0,030260
50% Oleogel CW5 Cocoa Cr	-0,034164	-0,007877	0,018410
50% Oleogel CW8 Cocoa Cr	-0,020251	0,006036	0,032323
Palm Oil Cocoa Cream	-0,020104	0,006182	0,032469



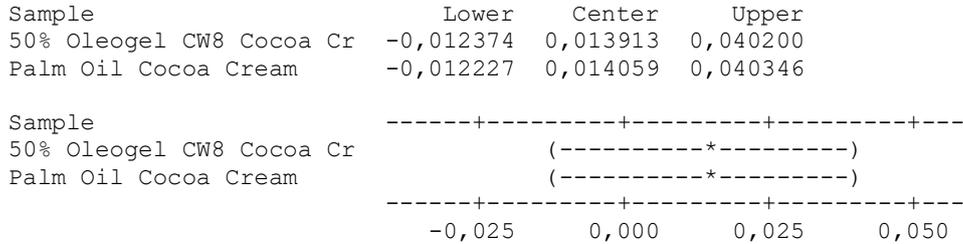
Sample = 50% Oleogel BW08 Cocoa Cream subtracted from:

Sample	Lower	Center	Upper
50% Oleogel CW5 Cocoa Cr	-0,038137	-0,011850	0,014437
50% Oleogel CW8 Cocoa Cr	-0,024224	0,002063	0,028350
Palm Oil Cocoa Cream	-0,024077	0,002210	0,028496





Sample = 50% Oleogel CW5 Cocoa Cream subtracted from:



Sample = 50% Oleogel CW8 Cocoa Cream subtracted from:

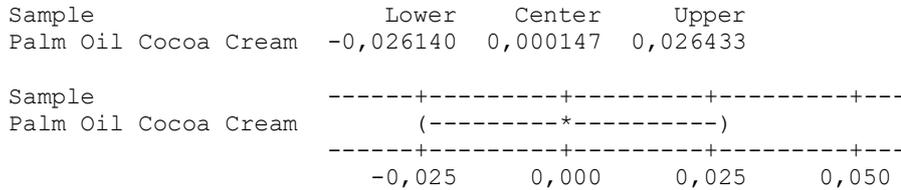
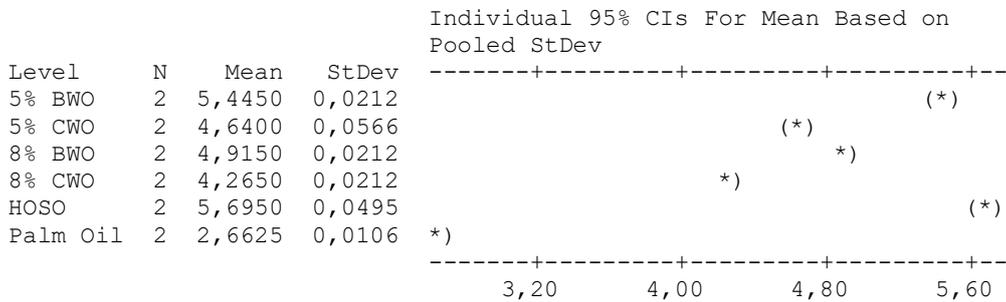


Table B.8. Analysis of Variance of Peroxide Values of Oils and Oleogels

Source	DF	SS	MS	F	P
Sample	5	11,75984	2,35197	1984,09	0,000
Error	6	0,00711	0,00119		
Total	11	11,76696			

S = 0,03443 R-Sq = 99,94% R-Sq(adj) = 99,89%



Pooled StDev = 0,0344

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
HOSO	2	5,6950	A
5% BWO	2	5,4450	B
8% BWO	2	4,9150	C
5% CWO	2	4,6400	D
8% CWO	2	4,2650	E
Palm Oil	2	2,6625	F

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 = 99,27%

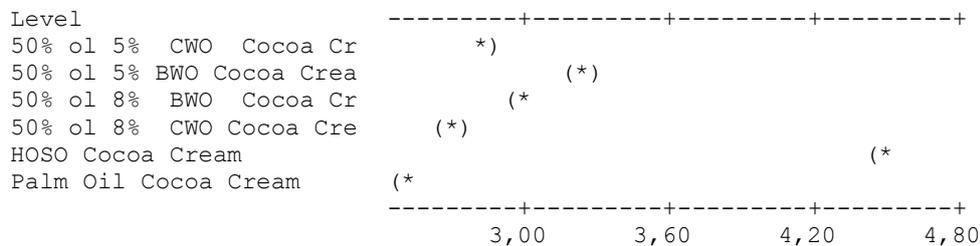
**Table B.9. Analysis of Variance of Peroxide Values of the Oils and 50 %
Cocoa Creams**

Source	DF	SS	MS	F	P
Sample	5	5,004967	1,000993	1225,71	0,000
Error	6	0,004900	0,000817		
Total	11	5,009867			

S = 0,02858 R-Sq = 99,90% R-Sq(adj) = 99,82%

Level	N	Mean	StDev
50% ol 5% CWO Cocoa Cr	2	2,8500	0,0141
50% ol 5% BWO Cocoa Crea	2	3,2350	0,0495
50% ol 8% BWO Cocoa Cr	2	2,9800	0,0141
50% ol 8% CWO Cocoa Cre	2	2,7000	0,0000
HOSO Cocoa Cream	2	4,4750	0,0354
Palm Oil Cocoa Cream	2	2,5000	0,0283

Individual 95% CIs For Mean Based on Pooled StDev



Pooled StDev = 0,0286

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
HOSO Cocoa Cream	2	4,4750	A
50% ol 5% BWO Cocoa Cream	2	3,2350	B
50% ol 8% BWO Cocoa Cream	2	2,9800	C
50% ol 5% CWO Cocoa Cream	2	2,8500	D
50% ol 8% CWO Cocoa Cream	2	2,7000	E
Palm Oil Cocoa Cream	2	2,5000	F

Means that do not share a letter are significantly different.

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

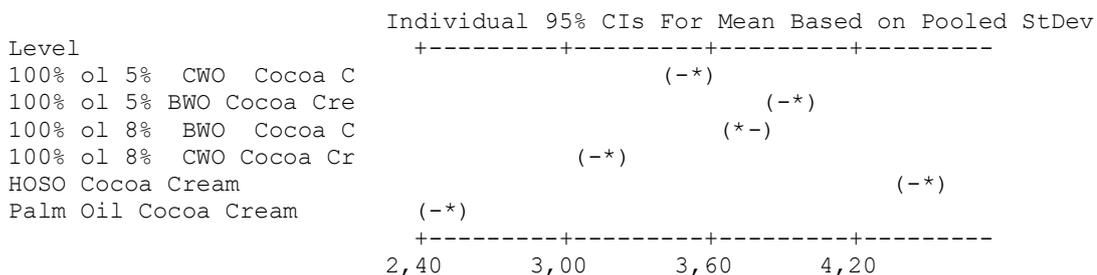
Individual confidence level = 99,27%

Table B.10. Analysis of Variance of Peroxide Values of the Oils and 100% Cocoa Creams

Source	DF	SS	MS	F	P
Sample	5	4,59460	0,91892	367,57	0,000
Error	6	0,01500	0,00250		
Total	11	4,60960			

S = 0,05 R-Sq = 99,67% R-Sq(adj) = 99,40%

Level	N	Mean	StDev
100% ol 5% CWO Cocoa C	2	3,5200	0,0283
100% ol 5% BWO Cocoa Cre	2	3,9550	0,0212
100% ol 8% BWO Cocoa C	2	3,7350	0,0212
100% ol 8% CWO Cocoa Cr	2	3,1750	0,1061
HOSO Cocoa Cream	2	4,4750	0,0354
Palm Oil Cocoa Cream	2	2,5000	0,0283



Pooled StDev = 0,0500

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
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HOSO Cocoa Cream	2	4,4750	A
100% ol 5% BWO Cocoa Cream	2	3,9550	B
100% ol 8% BWO Cocoa Cream	2	3,7350	C
100% ol 5% CWO Cocoa Cream	2	3,5200	D
100% ol 8% CWO Cocoa Cream	2	3,1750	E
Palm Oil Cocoa Cream	2	2,5000	F

Means that do not share a letter are significantly different.

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

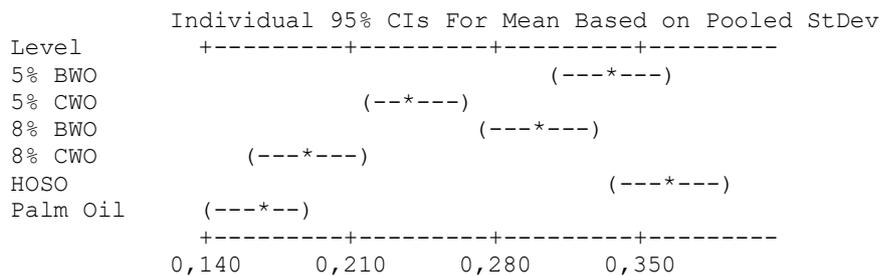
Individual confidence level = 99,27%

Table B.11. Analysis of Variance of Acidity of the Oils and Oleogels

Source	DF	SS	MS	F	P
Sample	5	0,064742	0,012948	57,55	0,000
Error	6	0,001350	0,000225		
Total	11	0,066092			

S = 0,015 R-Sq = 97,96% R-Sq(adj) = 96,26%

Level	N	Mean	StDev
5% BWO	2	0,33500	0,00707
5% CWO	2	0,24000	0,01414
8% BWO	2	0,30000	0,00000
8% CWO	2	0,19000	0,01414
HOSO	2	0,36500	0,02121
Palm Oil	2	0,16500	0,02121



Pooled StDev = 0,01500

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
HOSO	2	0,36500	A

5% BWO	2	0,33500	A B
8% BWO	2	0,30000	B
5% CWO	2	0,24000	C
8% CWO	2	0,19000	C D
Palm Oil	2	0,16500	D

Means that do not share a letter are significantly different.

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

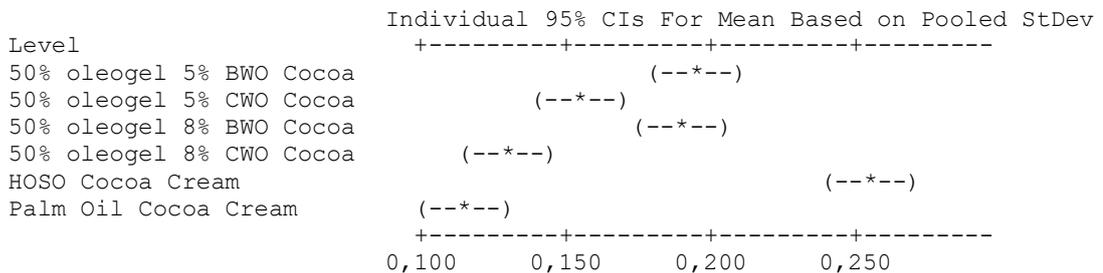
Individual confidence level = 99,27%

**Table B.12. Analysis of Variance of Acidity of the Oils and 50% Oleogel
Cocoa Creams**

Source	DF	SS	MS	F	P
Sample	5	0,026067	0,005213	52,13	0,000
Error	6	0,000600	0,000100		
Total	11	0,026667			

S = 0,01 R-Sq = 97,75% R-Sq(adj) = 95,88%

Level	N	Mean	StDev
50% oleogel 5% BWO Cocoa	2	0,19500	0,00707
50% oleogel 5% CWO Cocoa	2	0,15500	0,00707
50% oleogel 8% BWO Cocoa	2	0,19000	0,01414
50% oleogel 8% CWO Cocoa	2	0,13000	0,01414
HOSO Cocoa Cream	2	0,25500	0,00707
Palm Oil Cocoa Cream	2	0,11500	0,00707



Pooled StDev = 0,01000

Grouping Information Using Tukey Method

Sample	N	Mean	Grouping
HOSO Cocoa Cream	2	0,25500	A
50% oleogel 5% BWO Cocoa Cream	2	0,19500	B
50% oleogel 8% BWO Cocoa Cream	2	0,19000	B C
50% oleogel 5% CWO Cocoa Cream	2	0,15500	C D
50% oleogel 8% CWO Cocoa Cream	2	0,13000	D E
Palm Oil Cocoa Cream	2	0,11500	E

Means that do not share a letter are significantly different.

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

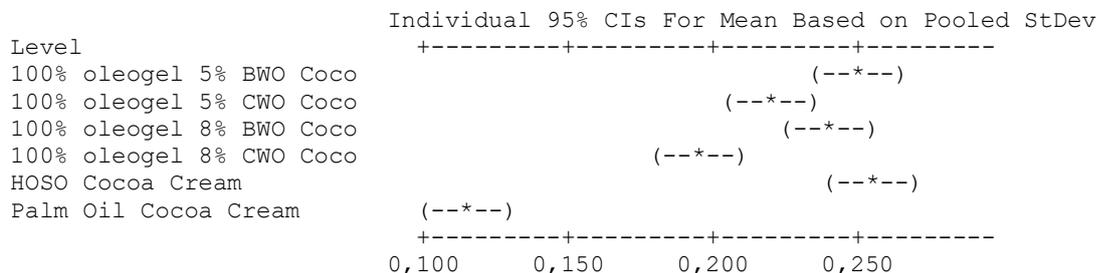
Individual confidence level = 99,27%

Table B.13. Analysis of Variance of Acidity of the Oils and 100% Oleogel Cocoa Creams

Source	DF	SS	MS	F	P
Sample	5	0,0276750	0,0055350	94,89	0,000
Error	6	0,0003500	0,0000583		
Total	11	0,0280250			

S = 0,007638 R-Sq = 98,75% R-Sq(adj) = 97,71%

Level	N	Mean	StDev
100% oleogel 5% BWO Coco	2	0,25000	0,01414
100% oleogel 5% CWO Coco	2	0,22000	0,00000
100% oleogel 8% BWO Coco	2	0,24000	0,00000
100% oleogel 8% CWO Coco	2	0,19500	0,00707
HOSO Cocoa Cream	2	0,25500	0,00707
Palm Oil Cocoa Cream	2	0,11500	0,00707



Pooled StDev = 0,00764

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 = 98,98%

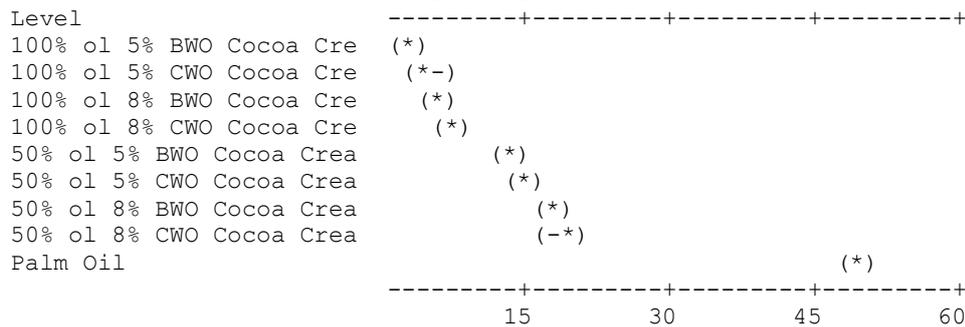
Table B.15. Analysis of Variance of Hardness of Oils and Oleogel Cocoa Creams

Source	DF	SS	MS	F	P
sample	8	4810,12	601,27	344,57	0,000
Error	18	31,41	1,74		
Total	26	4841,53			

S = 1,321 R-Sq = 99,35% R-Sq(adj) = 99,06%

Level	N	Mean	StDev
100% ol 5% BWO Cocoa Cre	3	3,410	0,613
100% ol 5% CWO Cocoa Cre	3	5,167	0,702
100% ol 8% BWO Cocoa Cre	3	6,600	0,608
100% ol 8% CWO Cocoa Cre	3	7,367	0,153
50% ol 5% BWO Cocoa Crea	3	13,942	1,040
50% ol 5% CWO Cocoa Crea	3	15,218	0,622
50% ol 8% BWO Cocoa Crea	3	18,582	2,165
50% ol 8% CWO Cocoa Crea	3	18,846	1,214
Palm Oil	3	49,879	2,610

Individual 95% CIs For Mean Based on Pooled StDev



Pooled StDev = 1,321

Grouping Information Using Tukey Method

sample	N	Mean	Grouping
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Palm Oil	3	49,879	A
50% ol 8% CWO Cocoa Cream	3	18,846	B
50% ol 8% BWO Cocoa Cream	3	18,582	B
50% ol 5% CWO Cocoa Cream	3	15,218	B C
50% ol 5% BWO Cocoa Cream	3	13,942	C
100% ol 8% CWO Cocoa Cream	3	7,367	D
100% ol 8% BWO Cocoa Cream	3	6,600	D E
100% ol 5% CWO Cocoa Cream	3	5,167	D E
100% ol 5% BWO Cocoa Cream	3	3,410	E

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 = 99,75%