CHEMICAL AND ENZYMATIC INTERESTERIFICATION OF TALLOW WITH DIFFERENT OILS

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ABSTRACT

CHEMICAL AND ENZYMATIC INTERESTERIFICATION OF TALLOW WITH DIFFERENT OILS

The purpose of this study is to manufacture structured lipids by enzymatic and chemical interesterification of tallow with corn, canola and safflower oils individually and to investigate the effects of several process parameters on various chemical and physical properties of structured lipids. Moreover, collection of Fourier-transform mid infrared (FT-MIR) and near infrared (FT-NIR) spectra during interesterification process is also aimed in order to monitor the processes and to construct chemometric models for the prediction of chemical and physical properties of the interesterified products. Both enzymatic and chemical interesterification provided modification of the properties of tallow. The blend ratio is the most significant factor among the parameters investigated for both types of interesterification. Longer reaction time for enzymatic interesterification caused undesirable changes in physical properties of fats. Interesterified lipids have generally low trans fatty acids and they tend to have lower consistencies and solid fat contents compared to their physical blends and the tallow; as a result, they also acquired better spreadable and plastic behaviors. The structured lipids produced with chemical interesterification of tallow with corn oil have better physical properties, higher oxidative stability and lower free fatty acid content compared to structured lipids produced with other vegetable oils. Chemical and physical properties of interesterified fats could be predicted accurately with chemometric analysis of FT-NIR spectra.

ÖZET

HAYVAN İÇYAĞININ FARKLI YAĞLARLA ENZİMATİK VE KİMYASAL OLARAK İNTERESTERİKASYONU

Bu çalışmanın amacı, hayvan içyağı ile mısır, kanola ve aspir yağlarının ayrı ayrı enzimatik ve kimyasal interesterifikasyonu ile yapılandırılmış bir yağ elde edilmesidir. Bu amaçla, yapılandırılmış yağın mono, di ve trigliserit kompozisyonu, serbest yağ asitliği, oksidatif stabilitesi, yağ asidi kompozisyonu, katı yağ içeriği, erime ve yumuşama noktası, kıvam, kristal yapı, renk gibi özellikleri belirlenecektir. Ayrıca, interesterifikasyon işlemi süresince Fourier-transform infrared (FTIR ve FTNIR) ile spektral ölçümler alınarak, interesterifikasyon periyodu incelenecektir. İnteresterifikasyon süresinin, sıvı yağ çeşidinin katalist oranının ve karışım oranının etkisini belirlemek üzere veriler tek ve çok değişkenli istatistiksel yöntemlerle analiz edilecektir. Hem kimyasal hem de enzimatik interesterifikasyon reaksiyonları iç yağın fiziksel ve kimyasal özelliklerini modifiye edebilmiştir. Her iki reaksiyon tipi için de, karışım oranı önemli bir faktör olarak tespit edildi. Enzimatik interesterifikasyon süresinin uzun tutulması, yapılandırılmış yağların fiziksel özelliklerini negatif yönde etkilemiştir. İç yağ ve mısır yağının kimyasal interesterifiye edilmesi ile yapılandırılmış yağlar diğer örneklere daha düzgün fiziksel özellikler, daha yüksek oksidatif stabilite ve daha düşük serbest yağ asitliği göstermektedir. Yapılandırılmış yağların genellikle trans yağ içerikleri düşüktür. İnteresterifikasyon işlemi ile iç yağın katı yağ içerikleri ve kıvam değerleri düşürülerek, yağa daha plastik ve sürülebilir özellik kazandırılmıştır. Ayrıca, FT-NIR spectral ölçümlerle oluşturulan modellerle, yapılandırılmış yağların fiziksel ve kimyasal özellikleri tahmin edilebilmiştir.

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LIST OF ABBREVIATIONS

BR	Blend Ratio
CA	Canola oil
CI	Chemical Interesterification
CC	Catalyst Concentration
CO	Corn Oil
CP	Central Point
DAG	Diacylglycerol
EI	Enzymatic Interesterification
FFA	Free Fatty Acid
FTIR	Fourier Transform Middle Infrared Spectroscopy
FTNIR	Fourier Transform Near Infrared Spectroscopy
MAG	Monoacylglycerol
MP	Melting Point
MUFA	Monounsaturated fatty acid
ND	Not Detected
OS	Oxidative Stability
OT	Oil Type
PCA	Principal Component Analysis
PLS	Partial Least Square
PUFA	Polyunsaturated fatty acid
SA	Safflower oil
SFA	Saturated fatty acid
SFC	Solid Fat Content
SMP	Slip Melting Point
TAG	Triacylglycerol
TFA	Trans fatty acid
T	Tallow

CHAPTER 1

INTRODUCTION

Fats and oils are consumed as food themselves or used as ingredients due to their nutritional and physical properties (O'Brien 2000). However, naturally present fats and oils are not always suitable for food processes. Fatty acid compositions, fatty acid distributions, and ratio of saturated to unsaturated fatty acids, melting points, crystallization behaviors, storage stabilities, nutritional values, caloric values and health-promoting effects of fats and oils differ from each other due to their sources, processes etc. Therefore, in many cases appropriate modification should be carried out to provide desirable characteristics to lipids. The structured lipids are the products that have been modified in terms of their original composition and/or distribution of fatty acids in the glycerol backbone (Foresti and Ferreira 2010). The modifications can be achieved by either chemical or enzymatic interesterification. There are several examples of the use of this process in the modification of the properties of tallow. It was found out that the interesterification of tallow with rapeseed oil reduced the resistance of the products to thermal oxidation (Ledóchowska et al. 1998). Another study of interesterification of tallow and sunflower oil showed that the physical properties of tallow could be improved as a result of this process (Rodríguez et al. 2001).

In this thesis study, structured lipids were manufactured by both enzymatic and chemical interesterification of tallow with corn, canola and safflower oils. Effects of blend ratio, catalyst concentration and oil type on the chemical (triacylglycerol composition, free fatty acid content, fatty acid composition) and the physical properties (solid fat content, melting and softening point, consistency, color and texture, crystal morphology) of structured lipids produced with chemical interesterification were investigated by univariate and multivariate statistical analysis of the data and the results are discussed in 4th chapter. Since chemical interesterification of corn-tallow blends resulted in more desirable properties processes of these blends were monitored by determining their chemical and physical properties with respect to reaction time and both mid-infrared and near-infrared spectra were also collected during the processes. Results of this process monitoring for chemical interesterification were evaluated in Chapter 5 while chapter 6 deals with the monitoring of the enzymatic interesterification of the same

system. Infrared spectroscopic data which were obtained in characterization and process monitoring parts of the structured lipids were used in the differentiation of the samples with respect to processes and process parameters in Chapters 4-6. In addition, these data in combination with multivariate regression techniques were also used in the prediction of the chemical and physical properties of structured lipids and the results were discussed in Chapter 7.

CHAPTER 2

LITERATURE VIEW

2.1. Tallow

Tallow is an animal fat that may be rendered from beef, mutton fat or processed from suet. Commercial tallow may be also derived from other animals, such as lard and pigs (Thomas 2002).

Tallow is solid at room temperature and can have a solid hard fat portion at ordinary temperatures with a yellowish white color. It is not soluble in cold alcohol, but can dissolve in boiling alcohol, chloroform, ether and the essential oils. The hardness and melting-point of tallow depend on provender, age, animal type and health of the animal. Beef tallow is considered as a commercially low-value fat since it is not suitable for direct human consumption due to its high melting point, narrow plastic range, and low levels of polyunsaturated fatty acids (Kowalski et al. 2004). Tallow has not an optimal consistency at ambient temperature, thus its use in several food products is limited. High melting and slip melting points (about 40-60 °C) are the other handicaps that prevent the direct usage of tallow in food processes. Thus, tallow should be modified in order to obtain fats with desirable properties for its use in edible products. Tallow is a mixture of solid fats, palmitin, stearin and olein (Bhattacharyya and Bhattacharyya, & De 2000) and its fatty acid composition is presented in Table 2.1.

Table 2.1 Fatty acid composition of tallow
(Source: Mattson and Lutton 1958)

Saturated fatty acids	**Percentage**
Palmitic acid (C16:0)	26
Stearic acid (C18:0)	14
Myristic acid (C14:0)	3
Monounsaturated fatty acids:	
Oleic acid (C18:1n9)	47
Polyunsaturated fatty acids	
Linoleic acid (C18:2n6)	3
Linolenic acid (C183n:3)	1

Beef tallow is considered as a less valuable fat and not suitable due to its high melting point and low level of polyunsaturated fatty acids. However, minor quantities of beef tallow may be used for edible purposes such as frying fats or shortenings. For this purpose, tallow should be modified before use. One of the possible methods of tallow modification is interesterification with vegetable oils (Kowalski et al. 2005). Chemical or enzymatic interesterification means alteration of both the physical and nutritional properties of fat. A study about interesterification of tallow and sunflower oil mixtures showed that an appropriate method of interesterification improved the physical properties of tallow, whereas blending did not significantly modify it (Rodríguez et al. 2001). In another study, tallow and rapeseed oil mixture was interesterified and it was found out that the interesterification reduced the resistance of products to thermal oxidation (Ledóchowska et al. 1998).

2.2. Corn Oil

Corn or maize oil is extracted from the germ of corn, and it is widely used in food industry. It is an important key ingredient of margarine and other processed foods. Corn oil is mainly composed of 13% monounsaturated, 60% polyunsaturated, and 25%saturated fats. It consists of mostly linoleic acid and vitamin E. It is a rich source of ω-6 series, which help to regulate blood cholesterol levels and eicosanoid synthesis, and to lower blood pressure (Moreau 2011).

Corn oil has desired quality due to its pleasant, slightly nutty, and slightly sweet flavor. It has a long shelf life due to its higher oxidative stability. Desirable flavor and the oxidative stability of corn oil increases both consumer demand and its applications in the food industry. Corn oil is especially preferred in deep frying due to its smoke point of 450°F (Rodrigues and Gioielli 2003; Orthoefer et al. 2003).

2.3. Safflower Oil

Safflower (*Carthamus tinctorius L.*) is a highly branched, herbaceous, thistle-like annual plant. It is a minor crop and approximately 600,000 tons are produced commercially in more than sixty countries worldwide. It is commercially cultivated for extraction of vegetable oil from its seeds (Blum et al. 1966).

Safflower oil is flavorless and colorless, and nutritionally very similar to sunflower oil. It is mainly used in cosmetics and as cooking oil, in salad dressings and for the production of margarine. It may also be consumed as a nutritional supplement. For frying process, safflower oil can compete with other vegetable oils due to its high smoke point (Zohary et al. 2012).

Different kinds of oils may be produced from two types of safflower. One of them is high in monounsaturated fatty acid (oleic acid) and the other is high in polyunsaturated fatty acid (linoleic acid). The ratio of fatty acids is given in Table 2.2.

The fatty acid composition of oil among the species is not considerably different, indicating that seed oil of safflower is possibly suitable for human consumption and industrial purposes (Sabzalian et al. 2008).

Table 2.2 Fatty acid percentages of vegetable oil
(Source: Sabzalian et al. 2008; Lin et al 2013; Moreau 2011)

Fatty acids	Corn Oil	Safflower oil	Canola oil
Palmitic acid (C16:0)	13	7	4
Stearic acid (C18:0)	3	3	2
Oleic acid (C18:1n9)	52	14	56
Linoleic acid (C18:2n6)	31	75	26
Linolenic (C18:3n3)	1	1	10

In dietary use, high-linoleic safflower oil has been preferred due to its effect on increased adiponectin level, a protein that helps regulating blood glucose levels and fatty acid breakdown (Nagao et al. 2003). In addition, increased omega-6 linoleic acid amount from safflower oil in diet caused significant reduction in total cholesterol (Ramsden et al. 2013).

Safflower oil is susceptible to oxidation due to its high iodine value, high linoleic acid content and low content of gamma tocopherol. Under unsuitable conditions, safflower oil may oxidize more rapidly than other domestic liquid oils (Blum et al. 1966).

2.4. Canola Oil

Canola oil is a low erucic acid rapeseed oil. It is the third largest vegetable oil produced by volume after palm and soybean oil (Copeland et al. 2012).

Canola oil contains low level (7%) of saturated fatty acids (SFAs); substantial amounts of monounsaturated fatty acids (MUFAs) and polyunsaturated fatty acids (PUFAs), including 56% oleic acid, 26% linoleic acid, and 10% alpha-linolenic acid (ALA), plant sterols (0.53–0.97%) and tocopherols (700–1,200 ppm) (Lin et al. 2013).

It is very stable at high frying temperatures; so that, it is suitable for frying process. Furthermore, canola oil can be also used in salad dressings and could be blended with other oils, in margarines and shortenings (Eskin et al. 1991).

2.5. Structured Lipids

Naturally present lipids have different properties in terms of their fatty acid compositions, fatty acid distributions, and ratio of saturated to unsaturated fatty acids, melting points, crystallization behaviors, storage stabilities, nutritional values, caloric values and health-promoting effects. The original form of some lipids might not be suitable for specific purposes; in these cases modification in their structure could provide desired attributes to the product (Martin et al. 2010).

Modification means any alteration in the structure of the naturally occurring lipids. Structured lipids (SLs) are defined as the lipids that have been chemically or enzymatically modified from their natural form including triacylglycerols (TAGs), diacylglycerols, monoacylglycerols and phospholipids. This modification can be carried out by incorporation of new fatty acids, by restructuring the fatty acids positions, or the fatty acid profile from the natural state, or by the synthesis of new TAGs (Ribeiro et al. 2009).

In general, SLs refer to TAGs containing mixtures of both short- (SCFAs) or medium-chain fatty acids (MCFAs) and long-chain fatty acids (LCFAs) in the same glycerol molecule (Lee and Akoh 1998).

2.5.1. Synthesis of structured lipids

The production of the SLs can be achieved by chemical or enzymatic interesterification and methods of synthesis should be chosen depending on the desired properties of the product. SLs can be produced to acquire regiospecific location of fatty acids or to have random location in a glycerol backbone. Regiospecific location of fatty

acids can be carried out by enzyme catalyzed reactions by regiospecific lipases, and randomized distribution of fatty acids can be obtained by non-specific lipases or by chemical interesterification. Consequently, interesterification makes possible the rearrangement of existing acyl groups or incorporation of new fatty acids to create novel properties (Xu et al. 2006).

2.5.1.1. Chemical interesterification

Chemical interesterification has long been used in the production of SLs. The commonly used chemical synthesis of SLs is transesterification. In transesterification, mixture of medium chain and long chain TAGs are hydrolyzed and then re-esterification of released fatty acids takes place. Chemical interesterification is conducted under relatively mild conditions with chemical catalysts. Chemical randomization can be acquired at 60-90^{o}C, even at 30^{o}C, depending on the oils used. However, the reaction time can vary and it increases at low temperatures. Alkali metals or alkali metal alkylates are used as catalysts. When the catalysts are used, the process requires high temperature and anhydrous conditions. Chemical interesterification may result in desired randomized TAG molecular species. The selection and the amount of catalyst, reaction temperature and time and the substrate molar ratios are important parameters for a product design (Hœy and Xu 2001).

Chemical interesterification is widely used in the production of trans-free plastic fats to replace hydrogenation technology. Chemical interesterification can also be used to produce some commercial products with nutritional purposes (Smith et al. 1994).

2.5.1.2. Enzymatic interesterification

Enzymatic interesterification is a general term including the reactions between an ester and a fatty acid, an alcohol, or another ester catalyzed by a lipase enzyme. Therefore, information regarding the TAG lipases, interesterification reactions, factors that affect enzymatic process and product yield will be covered in this section.

2.5.1.2.1. Lipases used in enzymatic interesterification

TAG lipases belong to the class of hydrolases that act on the carboxylic ester bonds (Rajendran 2009). These enzymes have the ability of both the hydrolysis of esters and acyl-transfer reactions such as esterification (acid and alcohol), transesterification (alcohol and ester), interesterification (ester and acid) and transfer of acyl groups from esters to other nucleophiles such as amines, thiols or hydroperoxides (Alcántara 1998). They may remain dissolved in oil water interfaces under the natural conditions and they hydrolyze the TAGs that have low solubility in the water. In the presence of trace amounts of water, they can reverse the reaction and lead to esterification and formation of glycerides from the fatty acids and glycerols (Rajendran 2009).

Microbial lipases are both regiospecific and fatty acid specific. They are used in the esterification and transesterification reactions (Gupta et al. 2003). Certain lipases show positional specificity toward ester bonds in positions sn-1,3 of the TAG and this is due to the inability of lipases to act on sn-2 position of TAGs due to steric hindrance. Steric hindrance prevents the binding of the fatty acid in sn-2 position to the active site of the enzyme (Macrae and How 1988).

In an interesterification reaction by a 1,3-specific lipase, initially a mixture of TAGs, 1,2- and 2,3-diacylglycerols, and free fatty acids are produced. Then, acyl migration takes place due to prolonged reaction periods which cause the formation of 1,3-diacylglycerols and also allows some randomization of the fatty acids existing at the sn-2 position of the TAGs (Rajendran 2009).

A variety of specific lipases are available and they are used in the construction of specific structured lipids due to their regiospecificity or stereospecificity. Regiospecific lipases are listed in Table 2.3. There are also a few lipases available possessing sn-2 specificity or sn-1 and sn-3 specificity. The specificity of lipases varies due to microenvironmental conditions (Pabai et al. 1995).

2.5.1.2.2. Factors affecting enzymatic process and product yield

The factors that affect both enzymatic process and product are namely pH, water content, temperature, substrate composition and substrate molar ratio, reaction time, lipase content and type of solvent, etc. It is important to realize that in almost all cases

the reaction conditions are different and particular for the enzymatic interesterification studies in the literature. Therefore, it is difficult to make a comparison of the results since the enzymes have different activities and selectivity depending on their concentration, the reaction medium, pH, temperature and other parameters, which highly affect enzyme properties and acyl migrations (Rodriguesa and Fernandez-Lafuente 2010).

The water content is an important factor which determines the shift in reaction equilibrium toward hydrolysis or ester synthesis. Low water activity is essential for ester synthesis. However, very low water activity prevents all reactions, therefore, lipases need a certain amount of water to remain hydrated for enzymatic activity (Briand et al. 1994).

The pH value affects the catalytic activity of lipases. Enzymes may show different activities at different pH ranges due to their origin and the ionization state of residues in their active sites. Lipases can be active in a wide pH range, from 4 to 10 and for most lipases optimum pH lies between 7 and 9 (Malcata et al. 1992).

Lipases may show different sensitivity to different solvents. Polarity of solvent determines the catalytic activity of the enzyme. Moreover, the solubility of the reactants, presence of chemical interference, solvent density, viscosity, surface tension, toxicity, flammability, waste disposal, and cost are other factors that must be taken into consideration in enzymatic interesterification process (Malcata et al. 1992).

Temperature is another significant parameter for the enzymatic interesterification. Temperature should be controlled adequately for a reproducible assay of enzyme-catalyzed reactions. The optimum temperature for most immobilized lipases is in the range of 30 to 62^{o}C, whereas it is slightly lower for free lipases (Malcata et al. 1992). Generally, increasing the temperature enhances the interesterification. However, if the temperature is so high, it can damage the enzyme structure and cause reduction in reaction rate. In some cases, high temperature could be needed to provide substrate solubility as in solvent-free systems but it is not necessary in organic solvents included systems in which substrates are readily solubilized (Jimenez et al. 2017).

Product accumulation is another factor that affects the rate of reaction. The production and accumulation of high amount free fatty acids reduce the reaction rate due to acidification of micro-aqueous phase surrounding the lipase (Jimenez et al. 2017).

Table 2.3 Specific lipases for the production of specific structured triacylglycerols (Source: Xu 2000)

Lipase source	Fatty acid specificity	Regio specificity
Aspergillus niger	S, M, L	1, 3 >> 2
Candida lipolytica	S, M, L	1, 3 > 2
Humicola lanuginosa	S, M, L	1, 3 >> 2
Mucor javanicus	M, L	>> S 1, 3 > 2
Rhizomucor miehei	S > M, L	1 > 3 >> 2
Pancreatic	S > M, L	1, 3
Pre-gastric	S, M	>> L 1, 3
Penicillium camembertii	MAG, DAG > TAG	1, 3
Penicillium roquefortii	S, M	>> L 1, 3
Rhizopus delemar	M, L >> S	1, 3 >> 2
Rhizopus javanicus	M, L > S	1, 3 > 2
Rhizopus japonicus	S, M, L	1, 3 > 2
Rhizopus niveus	M, L > S	1, 3 > 2
Rhizopus oryzae	M, L > S	1, 3 >>> 2
Pseudomonas fluofescens	M, L > S	1, 3 > 2
Pseudomonas sp.	S, M, L	1, 3 > 2
Rhizopus arrhizus	S, M > L	1, 3

*Abbrevations: S=short chain fatty acids; M= medium chain fatty acids, L=long chain fatty acids

2.5.1.2.3. Enzymatic acidolysis

Acidolysis is the transfer of an acyl group between an acid and an ester. Esters can be TAGs, diacylgycerols, monoacylglycerols, glycerol-phospholipids, alkyl fatty acid esters, etc. and acids can be fatty acids or other acids (Xu 2003). It is an effective method of combining free fatty acids into TAGs. Enzymatic acidolysis reaction is a reversible reaction. The reaction takes place in two steps: hydrolysis and esterification. Diacylglycerols are considered as the reaction intermediates. The new fatty acids in the system are incorporated into TAGs during hydrolysis and esterification takes place until the reaction reaches an equilibrium (Xu 2003).

sn 1,3 specific lipases can selectively catalyze exchange reaction at the sn-1 and sn-3 positions while leaving the sn-2 acyl group unchanged. This provides an opportunity to produce functional lipids with special purposes for the fatty acid types located in 1,3- or 2-positions. By this way, many functional lipids, such as cocoa butter equivalents, human milk fat replacers or structured lipids containing different fatty acids have been developed (Baljit et al. 2002).

2.5.1.2.4. Enzymatic alcoholysis

Alcoholysis is an esterification reaction between an alcohol and an ester. Alcoholysis is also a reversible reaction. The starting ester can be acylglycerols, TAGs or alkyl esters and or alcohols including glycerol, methanol, ethanol, or sterol. Enzymatic alcoholysis has been widely used in the production of partial acylglycerols such as monoacylglycerols and diacylglycerols, and biodiesels such as fatty acid methyl esters or ethyl esters (Xu 2003).

2.5.1.2.5. Enzymatic transesterification

Transesterification is the exchange of acyl groups between two esters, namely, two TAGs (Figure 2.1). Enzymatic transesterification is an alternative method for the modification of oils and fats (Xu 2003). Transesterification is predominantly conducted to change the physical properties of individual fats and oils or physical blends by altering the positional distribution of fatty acids in the TAGs (Jimenez et al. 2017). This reaction has been used to produce margarines, shortenings and other structured lipids with specific functions (Lai et al. 2000).

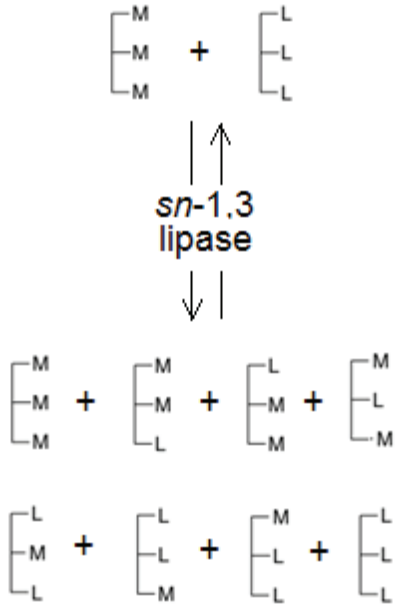

Figure 2.1 Enzymatic transesterification between two triacylglycerols (MMM and LLL) with sn-1,3 specific lipases (Jimenez et al. 2017).

2.5.1.3. Comparison of chemical and enzymatic interesterification

The main difference between chemical and enzymatic interesterification is the randomization of the fatty acids. Chemical interesterification provides new physical properties to the modified lipids by the random incorporation or the restructuring of acyl residues of TAGs. On the contrary, enzymatic interesterification leads to the attachment of specific fatty acids to specific positions of TAG structure to produce new products (Martin et al. 2010). Each reaction type includes advantages and disadvantages. Chemical interesterification is an old technology which has been widely used in the food industry (Xu 2000). Generally, chemical catalysts are cheaper compared to lipases. Moreover, chemical interesterification has lower cost and capital investment than enzymatic interesterification. The main disadvantages of chemical reaction are harsh process conditions and non-specificity of the catalysts. Therefore, chemical interesterification usually is not suitable for the production of specific TAGs due to non-positional specificity. On the other hand, the specificity of enzymes leads to design of structured lipids with various end-use properties. The most important advantages of enzymes for the production of structured lipids are;

- usage of lipases under milder reaction conditions,
- utilization in natural reaction systems,
- reducing environmental pollution,
- availability of lipases from a wide range of sources,
- capability to improve lipases by genetic engineering,
- the use of lipases for the production of particular biomolecules (Mohamed et al. 1993).

2.5.2. Importance of structured lipids in food industry

The thermal properties, crystallization and melting profile are the most important physical properties of lipids in the food industry. For example, salad oils should stay liquid during storage in a refrigerator or frying oils and oils used as food coating should not have solid components. On the other hand, frying oils should have appropriate levels of solid fat at refrigeration, ambient and at mouth temperatures (Gunstone 2006).

Nutritional changes are important in terms of maintenance of good health and treatment of diseases. The total level of fat in a food is important for consumers due to its calorie value and health concerns involving illnesses such as cardiovascular disease. In addition, an appropriate balance between saturated, monounsaturated and polyunsaturated fatty acids is necessary for good health (Rodrigues and Gioielli 2003). Therefore, the nutritional properties of lipids can also be modified to improve their health effects.

The chemical properties of lipids are quite important for their applications in food products. For this reason, characteristics fats and oils used in food systems should be optimized in order to obtain more desirable properties. For example, oxidative stability is one of the most important parameters that affects the shelf life of foods. Therefore, foods containing lipids that are more stable to oxidation would have longer shelf life (Hamam and Shahidi 2008). Several chemical properties of lipids could be also modified with restructuring.

The low-caloric values lipids have been recently attracted the attention of both food manufacturers and consumers. Low-caloric values lipids could be characterized by a mixture of short-chain fatty acids (SCFA) and/or medium chain fatty acids (MCFA) and long chain fatty acids (LCFA) in the same glycerol molecule. Low calorie is obtained by reducing caloric content of SCFA or MCFA compared to LCFA. These reduced calorie lipids could be used in baking chips, dips, coatings, bakery and dairy products, or as a cocoa butter replacer (Hamam and Shahidi 2008).

Human milk fat substitutes are another important application area for the structured lipids. Human milkfat supplies about 50–60% of dietary calories, and approximately 98% of the fat is in the form of TAG with a unique fatty acid distribution (López-López et al. 2002; Morea et al. 2003). The major saturated fatty acid is palmitic acid which is about 20–25% of the total fatty acids. The palmitic acid is generally located at sn-2 position of TAG whereas oleic acid is mainly placed in sn-1,3 positions. However, cow's milk fat or other vegetable oils contains palmitic acid located predominantly at sn-1,3 positions (Nelson and Innis 1999). Therefore, human milk fat substitutes can be synthesized by an interesterification using a sn-1,3-specific lipase from different lipid sources.

The daily intake of trans fatty acids (TFA) strictly regulated worldwide to less than 2.2 g. Moreover, food manufacturers are also required to label the TFA content clearly on their products by Food and Drug Administration (FDA) in USA (Farmani et

al. 2007). Therefore, oil-fat modification technologies have been improved to produce products with low or zero trans-fat. For this purpose, interesterification is a potential alternative method which rearranges fatty acids on the glycerol backbone in order to develop desirable chemical properties of fats (Li et al. 2018).

2.6. Infrared Spectroscopy

Infrared (IR) spectroscopy is a technique which is based on the measurement of IR radiation reflected from, transmitted or absorbed by a sample. Generally, the absorption of IR radiation is related to the changes of vibrational or rotational energy states of molecules. The types of vibrations are stretching (change in inter-nuclear distance) and bending (changes in the valence angle). The IR signal of a molecule is relative to square of the change of dipole moment that occurs by vibrational motion of the molecule. Spectral regions of IR spectra are divided into three regarding the wavenumber ranges as near infrared (12500-4000 $cm^{-1)}$, middle infrared (4000-400 cm^{-1}) and far infrared (400-10 cm^{-1}). While the middle infrared spectra measure normal vibrational transitions, near infrared detects the overtones of molecules and far infrared spectra explores normal vibrations of weak bonds and bonds of heavy atoms. When the samples are exposed to infrared radiation, the molecules absorb radiation selectively at specific wavelengths which causes the change in dipole moment of the sample molecules. The vibrational energy of molecules is transferred from ground state to excited state and the frequency of the absorption peak is determined by the vibrational energy gap. The intensity of absorption peaks depends on the change of dipole moment and the possibility of the transition of energy levels. Therefore, it is possible to obtain abundant structural information for a molecule by analyzing the infrared spectrum (Kaya and Huck 2017; Gunesakaran 2000, Ozaki et al. 2006).

IR spectra may supply significant information about the individual components of complex mixtures. Moreover, Fourier transform infrared (FT-IR and FT-NIR) spectroscopy supports the capability of quantitative analysis of IR spectroscopy, especially when the spectra is evaluated with multivariate statistical analysis techniques. IR spectroscopy is a promising tool for the analyses of fats and oils, with the advantages of being fast, non-destructive, and easy-to-use; moreover, minimum or no sample preparation is required before the analysis. In the scientific literature, there are many

examples of IR spectroscopy applications to determine various properties of fats and oils (Cascant et al. 2018; Gertz and Behmer 2014; Hocevar et al. 2012; Ozdemir et al. 2018; Van Der Voort, et al. 1996).

The wavenumber regions of FT-NIR and FT-IR spectra of lipids presents different chemical stretching of molecules in the lipid structure. In FT-NIR spectra, absorption bands between 6055 and 5345 cm^{-1} is mainly related to the first overtone of C-H stretching in fatty acid molecules (Blanco et al. 2004). The absorption peak in the 5345-4562 cm^{-1} region is ascribable to the combination band of O-H and C=O stretching of ester groups (RCOOR). The peaks between 7397-6661 cm^{-1} corresponds to the first overtone of the O-H bond of mono- and diglycerides (Blanco et al. 2004; Chang et al. 2005; Knothe 2000).

In FT-IR spectra, the region in between 1500-800 cm^{-1} which is also called as fingerprint region receives more attention. This region includes C-O-C vibration in esters, C-H bending and stretching vibrations, and the second overtone of C=O and -OH in fatty acid structure (Chang et al. 2005; Moh et al. 1999).

These spectral methods also provide characterization of fats and oils with the help of multivariate statistical techniques such as partial least square (PLS) and principal component analysis (PCA), which establish the differences between the samples, or allow the prediction of measured parameters.

For instance, in a study about the determination of iodine number and fatty acid profile of pig fat samples, the results of the multivariate calibration models showed that FT-NIR spectroscopy is able to accurately predict these properties (Foca et al. 2016). The suitability of IR spectroscopy in monitoring lipase-catalyzed interesterification of bulky fats was also demonstrated (Chang et al. 2005). Mid-IR spectroscopy in combination with chemometric techniques such as PLS regression was successfully employed for the determination of the composition of waste frying oils including soybean oil, palm oil, and hydrogenated vegetable fat (Hocevar et al. 2012). In another study, the possibility of monitoring hydrogenation process of soybean oil by a compact near-IR spectrometer and the suitable data elaboration was demonstrated (Pereira et al. 2018).

2.7. Multivariate Statistical Methods

Multivariate analysis implicates a set of techniques which are used in the analysis of data sets with more than one variable. The most common projection methods of

multivariate data analysis used in fats and oils, especially for classification and authentication, are principal component analysis (PCA), partial least square analysis (PLS), and discriminant analysis techniques (linear discriminant analysis (LDA) and partial least-squares discriminant analysis (PLS-DA). PCA can be used in unsupervised learning problems to discover and visualize the patterns in high-dimensional data sets when there is no specific response variable. LDA is a supervised algorithm which takes the class label into consideration. LDA is able to reduce 'dimensionality' while preserving as much of the class discrimination information at the same time. It can project the data points on a line so that clusters are separated as much as possible, with each cluster having a relative distance to a centroid. While PCA determines whether a new data point belong to a part of the group of data points from the training set or not LDA determines how to classify a new observation out of a group of classes. PLS is a supervised, quick, efficient and optimal regression method which is based on covariance. It is generally recommended to be used where the number of variables is high, and where it is likely that the explanatory variables are correlated. PLS-DA is similar to classical PLS regression where the response variable is a categorical one expressing the class membership of the statistical units. Therefore, PLS-DA does not allow other response variables in comparison to the one for defining the groups of individuals (Eriksson et al. 2001; Brereton 2003; Gan et al. 2005).

2.7.1. Principal component analysis (PCA)

The principal component analysis is a way of identifying and expressing the data to reveal their similarities and differences. It is also known as a variable reduction procedure. PCA is very useful if the number of the data is large and there is some redundancy in variables. The means of redundancy is that some of the variables are correlated with one another. This redundancy makes possible to reduce the observed variables into a smaller number of principal components (PCs) that takes into account of most of the variance in the observed variables (Eriksson et al. 2001).

Principal components (PC) provide description of the information with considerably few variables than originally presented. PC is defined as a linear combination of optimally weighted observed variables. "Optimally weighted" refers to the fact that the observed variables are weighted in such a way that the resulting

components account for a maximal amount of variance in the data set. The loadings are the coefficients of the original variables which define each PC (Brereton 2003). The categorization of the data could be examined by a score plot which is formed by plotting the latent variables. In score plots, the horizontal axis indicates the scores of first PC and the vertical one presents the second PC. By constructing PCA models the inter-relationships between different variables, sample patterns, groupings, similarities or differences could be observed (Gan et al. 2005). The application of PCA with different methods, in particular, combining with IR spectroscopy has growing interest in classification, characterization and authentication studies of lipids and oils in recent years.

2.7.2. Partial least-squares (PLS) analysis

The principle of PLS analysis is the determination of the components in the input matrix (X) that describe the relevant variations in the input variables, and have maximal correlation with the target value in Y, but without including the variations that are irrelevant or noisy (Rezzi et al. 2005). Since the first PLS component is usually not enough to describe the variation in the Y data, second PLS component describes the remaining variation. The second PLS component is a line orthogonal to first one which improves the description of X data and provides good correlation with Y remained after first component (Eriksson et al. 2001).

In the scientific literature, there are many examples of PLS models combined with IR spectroscopy applications to determine various properties of fats and oils (Cascant et al. 2018; Gertz and Behmer 2014; Hocevar et al. 2012; Ozdemir et al. 2018; Van Der Voort et al. 1996).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

The tallow used in interesterification was purchased from Güler Sucukları Et Mamülleri Ltd. Şti (Sivas, Turkey) and it was obtained immediately after slaughter from two different types of calves (Montofan and Holstein) of 2 years old and stored at -20^{o}C. Canola, safflower and corn oils were obtained from local market. Sodium methoxide was provided by a local oil factory. Lipase enzyme from *Thermomyces lanuginosus* was obtained from Sigma–Aldrich (St. Louis, MO). All other reagents and solvents are of analytical or chromatographic grade.

3.2. Production of Structured Lipids

In the production of structured lipids, three different factorial design was employed in order to investigate the effects of process parameters on physical and chemical properties of interesterified fats. In this part, production methods that used for manufacturing structured lipids were explained.

3.2.1. Chemical Interesterification Process

Tallow was liquefied in a microwave oven (Arçelik MD 674, Turkey) and stirred after cartilage was removed. The liquefied tallow was stored at -20°C until chemical interesterification. The liquefied tallow was mixed with safflower, corn and canola oils individually. A full factorial design was employed to evaluate the effects of catalyst (CH_3NaO) concentration (0.75-0.875-1%, w/w), oil type, and blend ratio (60:40, 70:30 and 80:20, w/w) on structured lipids. Thirty different blends were prepared according to an experimental design (Table 3.1). A hundred g of a blend was placed in 500 mL flask and dried under 185 mPa vacuum in a rotary evaporator (Laborato 4000 Heidolph, Germany) at 90 °C with magnetic stirring at 100 rpm for 30 min. The reaction was

initiated by adding the chemical catalyst at certain levels provided in experimental design. Chemical interesterification was performed in a rotary evaporator at 90 °C with stirring at 100 rpm and samples were removed from the system after 30 min. The product was washed with 5% phosphoric acid (H_3PO_4) twice in order to inactivate sodium methoxide and re-washed with 10% NaCl to remove impurities. Product, catalyst, NaCl and phosphoric acid were separated from each other by the help of a separation funnel. After that, the product was filtered through a vacuum filtration unit with 400 mm pore size filter paper (Macherey-Nagel, Düren, Germany) and washed with hot water for three times to remove all residues. The structured lipid and water were also separated from each other by a separation funnel. The structured lipid was taken into 500 mL flask and traces of water was evaporated under 185 mPa vacuum in a rotary evaporator at 90 °C with stirring at 100 rpm for 30 min.

3.2.2. Monitoring of Chemical Interesterification Process

Since the structured lipids chemically interesterified with corn oil have better physical properties, higher oxidative stability and lower free fatty acid (FFA) content compared to other structured lipids a new set of chemically interesterified lipids were manufactured by using corn oil for the process monitoring part of the study. Tallow from two different types of calves (Montofan and Holstein, at the age of 2) was purchased from the local market. Corn oil also was obtained from a local market. A full factorial mixed design was employed to evaluate the effects of reaction time (0, 10, 20, 30 min), and blend ratio (60:40, 70:30 and 80:20) on structured lipids. Fifteen different blends were prepared according to an experimental design (Table 3.2). Since the previous results indicated that the catalyst concentration had generally no significant effect on the samples, constant catalyst concentration of 0.75% of sodium methoxide was used in monitoring experiments. The interesterification procedure explained in Section 3.2.1 was also used in the production of new set of chemically interesterified lipids for monitoring.

Table 3.1 Full factorial design of chemical interesterification process for tallow- oil mixtures

No	Sample Name*	Blend Ratio	Oil Type	Catalyst Concentration (%)
1	CO61	60-40	CO	0.75
2	CO71	70-30	CO	0.75
3	CO81	80-20	CO	0.75
4	CA61	60-40	CA	0.75
5	CA71	70-30	CA	0.75
6	CA81	80-20	CA	0.75
7	SA61	60-40	SA	0.75
8	SA71	70-30	SA	0.75
9	SA81	80-20	SA	0.75
10	CO62	60-40	CO	0.875
11	CO72	70-30	CO	0.875
12	CO82	80-20	CO	0.875
13	CA62	60-40	CA	0.875
14	CA72	70-30	CA	0.875
15	CA82	80-20	CA	0.875
16	SA62	60-40	SA	0.875
17	SA72	70-30	SA	0.875
18	SA82	80-20	SA	0.875
19	CO63	60-40	CO	1
20	CO73	70-30	CO	1
21	CO83	80-20	CO	1
22	CA63	60-40	CA	1
23	CA73	70-30	CA	1
24	CA83	80-20	CA	1
25	SA63	60-40	SA	1
26	SA73	70-30	SA	1
27	SA83	80-20	SA	1
28	CP1	70-30	CO	0.875
29	CP2	70-30	CO	0. 875
30	CP3	70-30	CO	0.875

* CP: Central Point; CA: canola oil; CO: corn oil; SA: safflower oil

Table 3.2 Full factorial experimental design for the chemical interesterification process monitoring of tallow-corn oil

No	Sample Name*	Blend ratio	Time
1	C60	60:40	0
2	C70	70:30	0
3	C80	80:20	0
4	C61	60:40	10
5	C71	70:30	10
6	C81	80:20	10
7	C62	60:40	20
8	C72	70:30	20
9	C82	80:20	20
10	C63	60:40	30
11	C73	70:30	30
12	C83	80:20	30
13	MCP1	70:30	20
14	MCP2	70:30	20
15	MCP3	70:30	20

* MCP:Central Point

3.2.3. Enzymatic interesterification process

The structured lipids previously produced with chemical interesterification of corn oil and tallow have better physical and physical and chemical properties compared to samples with other oils. Therefore, corn oil was chosen as a substrate in the production of enzymatically interesterified lipids. The liquefied tallow was mixed with corn oil in different weight ratios of 60:40, 70:30 and 80:20. A full factorial experimental design was employed to evaluate the effects of reaction time (0, 3, and 6, 9, 12 h), and blend ratio (60:40, 70:30 and 80:20) on structured lipids (Table 3.3), and 18 different blends were prepared. A hundred g of the blend was placed in 500 mL flask and dried under 185 MPa vacuum in a rotary evaporator (Laborato 4000 Heidolph, Germany) at 90°C with magnetic stirring at 100 rpm for 30 min. The reaction was initiated by adding 10% (w/w) enzyme (Lipozyme TL IM) at 55°C (Ronne et al. 2005). Enzymatic interesterification was performed in a shaking incubator at 55°C with stirring at 120 rpm (Sartorious, Certomat B5-1, Germany). Reaction was stopped by denaturation of lipase enzyme in a shaking water bath at 80 °C for 30 min. The denatured enzyme was removed by vacuum filtration.

Table 3.3 Full factorial experimental design of enzymatic interesterification of tallow-corn oil

No	Sample Name*	Time (h)	Blend ratio
1	E60	0	60:40
2	E63	3	60:40
3	E66	6	60:40
4	E69	9	60:40
5	E612	12	60:40
6	E70	0	70:30
7	E73	3	70:30
8	E76	6	70:30
9	E79	9	70:30
10	E712	12	70:30
11	E80	0	80:20
12	E83	3	80:20
13	E86	6	80:20
14	E89	9	80:20
15	E812	12	80:20
16	ECP1	6	70:30
17	ECP2	6	70:30
18	ECP3	6	70:30

* ECP1, ECP2, and ECP3: Central Points

3.3. Chemical Analysis of Structured Lipids

The official methods that are used in the chemical analysis of interesterified lipids were explained.

3.3.1. Fatty Acid Composition

The fatty acid composition of samples was determined after converting them into their corresponding fatty acid methyl esters (FAME). For this purpose, 0.4 g sample was weighted into 100 mL erlenmeyer flask and dissolved in 4 mL isooctane. 0.2 mL of 2 M methanolic KOH was added, and the mixture was vortexed for 30 s. Following incubation

in a dark place for 6 min, solution was titrated with 1 N HCl using methyl orange as an indicator. After waiting for 25-30 min for separation of phases, upper clear phase was filtered into vials for gas chromatography (GC) analysis (IUPAC 1987). Specifications of GC instrument used in the analysis and the analysis conditions for GC as well as the column information are provided in Table 3.4.

Table 3.4 Chromatographic method for the analysis of fatty acid methyl esters

Instrumentation	
Chromatographic system	Agilent 6890 GC
Inlet	Split/splitless
Detector	FID
Automatic sampler	Agilent 7683
Column	100 m x 0.25 mm ID, 0.2 μm HP-88 (J&W112-88A7)
Liner	Split liner (p/n 5183-4647)
Experimental Conditions of GC-FID	
Inlet temperature	225° C
Injection volume	1μL
Split ratio	1/50
Carrier gases	Helium
Head pressure	1 mL/min constant flow
Oven temperature	60 °C, 1 min, 3°C/min, 170°C 15 min, 10°C/min, 230 °C 15 min, 3°C/min.
Detector temperature	260 °C

3.3.2. Oxidative Stability

The oxidation induction time was determined by the Rancimat method and measured with the Rancimat apparatus (873 Biodiesel, Metrohm, Switzerland) (T = 120 °C; air flow rate = 20 L/h) (Uncu and Ozen 2015).

Three g of sample was placed inside the glass reaction vessel for the measurement. Carrier medium was selected as deionized water. Reaction temperature was set to a constant value of 120 °C for both columns of Rancimat apparatus with a constant 20 L/h air flow. Stability was expressed as the oxidation induction time (h).

3.3.3. Free Fatty Acid Determination

Titrimetric method specified in European Commission Regulations No.2568/91 (EEC 1991) was used in free fatty acid (FFA) value determination of the products. 150 mL diethyl ether-ethanol (1:1) mixture was neutralized with KOH with the addition of phenolphthalein. 10 g sample was dissolved in 75 mL diethyl ether-ethanol solution. The sample solution was titrated with 0.1 mol/L solution of KOH until the indicator changed color.

Acidity was expressed as percentage of oleic acid with the equation given below:

$$V \times c \times \frac{M}{1000} \times \frac{100}{m} = \frac{V \times c \times M}{10 \times m}$$

where:

V = the volume of titrated KOH solution used in milliliters;

c = the exact concentration in moles per liter of the titrated solution of KOH used;

M = the molar weight in grams per mole of the acid used to express the result (=282);

m = the weight in grams of the sample

3.3.4. Mono-, -Di- and Triacylglycerol Content Determination

Mono- di- and triacylglycerol content of structured lipids were analyzed according to AOCS Cd11C-93 (2002) method by column chromatography. The column was prepared using 30 g of silica gel slurry with petroleum ether. 0.9 g of fat sample was weighed and dissolved in 3 mL of chloroform. Dissolved sample was transferred to the top of the column by washing with 3 mL of chloroform for three times. The sample was eluted using 250 mL solvent for each fraction, as shown below,

a. Fraction-I (triglycerides)-250 mL 10% diethyl ether in petroleum ether

b. Fraction-II (diglycerides)-250 mL 25% diethyl ether in petroleum ether

c. Fraction-III (monoglycerides)-250 mL 100% diethyl ether

The fractions were collected separately in a flask and solvents were evaporated in a rotary evaporator at 50 °C. The flasks were dried until a constant weight was obtained.

Calculations:

a. Fraction I = $\frac{\text{mass of Fraction I}}{\text{mass of sample}} \times 100$

b. Fraction II = $\frac{\text{mass of Fraction II}}{\text{mass of sample}} \times 100$

c. Fraction III = $\frac{\text{mass of Fraction III}}{\text{mass of sample}} \times 100$

3.4. Physical Analysis of Structured Lipids

The official methods that are used in the physical analysis of interesterified lipids were explained.

3.4.1. Crystal Morphology

The polymorphic forms of fat crystals in the structured lipids were determined by X-ray diffraction (Philips, Holland) using Cu as anode material (k = 1.54056 A°, voltage 45 kV, tube current 40 mA, fixed 1.0-,1.0-, and 0.76-mm divergence, anti-scatter and receiving slits). Samples were scanned from 4 to 50° (2θ scale) at a rate of 2.0°/min. The analyses were performed at ambient temperature.

3.4.2. Color Measurement

The color of structured lipids was determined by measuring CIE L* (lightness), a* (redness), and b (yellowness) with a color measurement device (Minolta, Japan). ΔE values were calculated considering tallow itself as a standard.

3.4.3. Determination of Melting Point

The melting temperatures of structured lipids were measured with a differential scanning calorimeter (DSC, Q10 TA Instruments, Crawley, UK). All samples were kept at 4°C for 24 h prior to measurements. Samples (9–10 mg) were placed in hermetically sealed aluminum pans. DSC analyses were carried out from 20 to −40°C and from −40 to 80°C at a scan rate of 10°C/min with respect to an empty pan (Rodríguez et al. 2001). Data analysis was performed with IFESTOS software (developed by Dr. Dimitrios Fessas

of University of Milan), calculating the melting points at different percentages of melted crystals (85, 90, and 95%). A correlation between temperature and percentages of lipid crystals was obtained and melting points were determined at a certain percentage.

3.4.4. Determination of Slip Melting Points

Slip melting points (SMP) were determined according to AOCS method Cc 3-25 (AOCS 1989). First, samples were heated to 60 °C in an oven for complete melting of the crystals. Capillary tubes filled with melted sample were chilled at 4°C overnight before being immersed in a beaker of distilled water at ambient temperature. The water was heated at a rate of 1.2 °C/min and the temperature at which the column of fat rose in the tube was recorded as slip melting point. This experiment was carried out twice for each sample.

3.4.5. Consistency Measurements

Consistency of samples was determined via penetration tests using a 45^{o} acrylic cone fitted to a constant speed texture analyzer (TA.XT plus, UK). Samples were heated to 60°C in an oven for complete melting of the crystals and conditioned in 50 mL glass beakers. Tempering was allowed to occur for 24 h in a commercial refrigerator (4°C) and then for 24 h in an oven with controlled temperature (4, 10, 15, 25°C). Used test parameters were penetration depth of 0.4 cm with 0.2 cm/sec speed for 5 sec testing time (Silvia et al. 2009). Measurements were performed in triplicate and consistency was calculated as “yield value” according to the following equation (Haighton 1959):

$$C = \frac{KW}{P^{1.6}}$$

where C is the yield value (MPa), K is a constant depending on the cone angle (4700–a dimensional), W is the compression force (N), and p is the penetration depth (cm).

3.4.6. Determination of Solid Fat Content

Solid fat content (SFC) was determined by a nuclear magnetic resonance (NMR) spectrometer (Bruker, USA) according to the AOCS Official Method Cd 16b-93 (1999). 5-8 mg of sample was weighted in NMR tubes and melted at 80^{o}C. The melted samples were recrystallized at 0^{o}C for 30 min. The recrystallized lipids were stabilized for 30 min at each measuring temperature at 10, 20, 30 and 35^{o}C before measuring liquid signal.

3.5. Infrared Spectroscopic Analysis

The instrumental analysis methods for both near and middle infrared spectra analysis were explained in this section.

3.5.1. FT-NIR Spectroscopy Analysis

FT-NIR spectra were acquired both on melted and solid structured lipids with an MPA spectrometer (Bruker Optics, Milan, Italy). After melting the samples in a temperature-controlled oven at 60 °C, they were transferred to a water bath at 60 °C. FT-NIR spectra were acquired in transflectance (1 mm pathlength) mode by a fiber optic probe inserted directly into the sample. A spectral range of 12500–3600 cm^{-1} was used, with 8 cm^{-1} resolution, and 32 scans for both background and samples. For measurements on solid samples, melted lipids were poured in disposable glass vials (8 mm pathlength) and incubated overnight at 25 °C in a temperature-controlled oven. FT-NIR spectra were then collected in transmission mode by using the same analytical conditions applied to the melted samples. All spectra were acquired in duplicate.

3.5.2. FT-IR Spectroscopy Analysis

FT-IR spectra were acquired both on melted and solid structured lipids with a Vertex 70 spectrometer (Bruker Optics, Milan, Italy) controlled by OPUS software (v. 6.5 Bruker Optics, Ettlingen, Germany). Spectra were collected over the mid-IR range of 4000-700 cm^{-1} at 4 cm^{-1} resolution by using a single reflection ATR cell and 32 scans for

both samples and background. Measurements were replicated twice on samples prepared as already reported for FT-NIR analysis.

3.6. Statistical Analysis

The data were analyzed by univariate (ANOVA) statistical analysis technique to investigate the effect of the oil types, blend ratio, reaction time and catalyst concentration on chemical and physical properties of the structured lipids by a software (MODDE 11, MKS Umetrics, Umea, Sweeden). To investigate the effect of processing parameters for interesterification process principal component analysis (PCA) was used.

Since FT-IR and FT-NIR data are complex and the simple univariate analysis methods are not sufficient, more multivariate analysis techniques are required. Therefore, raw FT-NIR and FT-IR spectral data were transferred to a multivariate data analysis software (SIMCA 14.1, MKS Umetrics, Umea, Sweeden). Four data matrices with 75 samples including vegetable oils (4), tallow (2), interesterified lipids (60), and non-esterified blends (9) were constructed with FT-NIR and FT-IR data for both melted and solid samples. Besides the whole mid-IR (4000-700 cm^{-1}) and NIR (12500–3600 cm^{-1}) ranges following selected ranges were also used in order to keep the most informative and less noisy segments of the spectra:

a) FT-NIR: 9002-4497 cm^{-1}
b) FT-IR: 3051-2599 and 2052-597 cm^{-1}

For all matrices, the replicated spectra were averaged prior to the application of various pre-processing techniques including standard normal variate (SNV), multiplicative scatter correction (MSC), first (d1) and second-order (d2) derivatives. The partial least square regression (PLS) analysis was applied to each pre-treated data matrices in order to predict both chemical and physical properties of interesterified lipids. Models were validated by both external and cross-validation. The best models were selected based on the following figures of merit: determination coefficient (R^2), root mean square error of calibration (RMSEC) and validation (RMSECV), number of latent variables. In order to improve the models data fusion sets were also composed. NIR (12000-3999 cm^{-1}) and mid-IR (3992-597 cm^{-1}) spectral data combined together separately for solid and melted form of lipids for the prediction of chemical and physical

properties of interesterified fats. For the best models of data fusion sets external validation prediction was performed as well.

CHAPTER 4

CHEMICAL INTERESTERIFICATION OF TALLOW WITH VEGETABLE OILS

4.1. Characterization of Chemical Properties of Chemically Interesterified Lipids

Free fatty acid (FFA) value, fatty acid profile and mono (MAG), di (DAG) and triacylglycerol (TAG) content of structured lipids produced with chemical interesterification of tallow with different vegetable oils according to experimental design provided in Material & Method section were determined. Data were analyzed by univariate (ANOVA) and multivariate statistical analysis (PCA) techniques to investigate the effects of oil type (sunflower, safflower and canola oils), blend ratio (60:40, 70:30 and 80:20) and catalyst concentrations (0.75, 0.875 and 1%).

4.1.1. Fatty Acid Profile of Chemically Interesterified Lipids

The fatty acid compositions of blends and structured lipids are given in Table 4.1-4.3. Tallow is very rich in terms of saturated fatty acids while vegetable oils used in modification of tallow have high amounts of mono and polyunsaturated fatty acids.

The major fatty acids in all products are palmitic, stearic, oleic, and linoleic acids. The fatty acid compositions of interesterified products are in agreement with the previous studies (Meng et al. 2011; Kowalski et al. 2004).

The predominant fatty acids for the samples interesterified with canola oil are oleic, stearic and palmitic acids. While the amount of oleic acid decreased with the increased ratio of tallow in blends, stearic and palmitic acid concentrations increased. Vegetable oils are rich in terms of oleic acid while stearic and palmitic acids are among the major fatty acids of tallow; therefore, this trend was expected and it was also observed in the other studies (Meng et al. 2010).

The predominant fatty acids are linoleic, stearic, oleic and palmitic acids in the products that are interesterified with safflower oil. The initial amount of linoleic acid in

safflower oil was 75.15% and it decreased to 32.83%, 25.58%, and 18.25% by blending with tallow at 60:40, 70:30 and 80:20 ratios, respectively. There has not been any significant change in the amount of oleic, palmitic and stearic acids before and after interesterification.

While corn oil has 30% oleic acid blending and interesterifying with tallow resulted in products with a range of 33.51-36.57% oleic acid. However, interesterified corn-tallow products have much lower linoleic acid concentration (12.90-29.71%) compared to corn oil (54.59%) itself regardless of catalyst and oil concentration due to lower linoleic acid concentration (2.97%) of tallow. The amount of palmitic and stearic acid did not change either by blending or interesterifying with respect to corn oil composition.

As in the previous studies, chemical interesterification of tallow did not result in formation of significant amounts of trans fatty acids (Meng et al. 2010). Generally, the amount of fatty acids in trans form is less than 1% except canola oil containing samples. Safflower and corn oils themselves have trans fatty acid (TFA) content of less than 1% and their interesterified forms have slightly higher percentages of trans fats. Canola oil itself, on the other hand, has higher content of TFAs (2.5%) compared to other oils and interesterified samples containing canola oil have lower trans-fat content compared to oil itself. These results indicate that these structured lipids are suitable for the production of low trans-fat containing shortenings, margarines and frying fats (Figure 4.1).

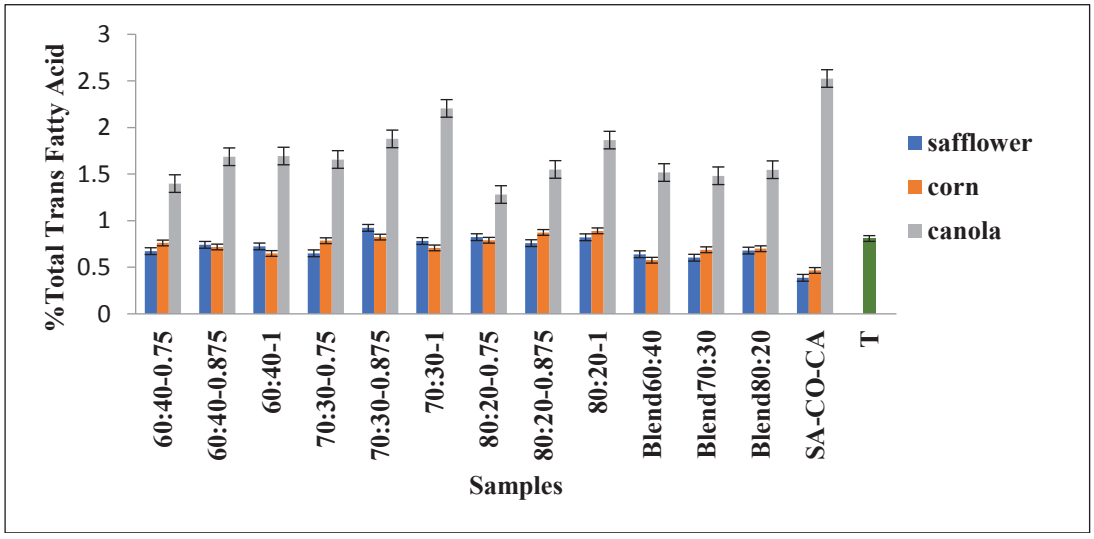

Figure 4.1 Total trans fatty acid contents of the samples

Table 4.1 Fatty acid profile (%) of blends and structured lipids produced with canola oil

	Blend/structured lipid*												
Fatty acid	CA61	CA62	CA63	CA71	CA72	CA73	CA81	CA82	CA83	CA60	CA70	CA80	CA
C14:0	1.09	1.08	1.11	1.22	1.32	1.28	1.33	1.32	1.41	1.06	1.27	1.51	ND
C14:1	0.27	0.26	0.36	0.31	0.33	0.32	0.28	0.35	0.35	0.15	0.19	0.15	ND
C15:0	0.83	0.21	0.21	0.25	0.27	0.26	0.25	0.28	0.29	0.21	0.24	0.28	ND
C16:0	15.07	15.47	15.07	16.39	17.28	17.03	18.71	18.42	18.65	15.55	16.70	18.75	4.94
C16:1	0.76	0.75	0.80	0.76	0.85	0.78	0.82	0.89	0.84	0.73	0.88	0.98	0.16
C17:0	0.62	0.64	0.62	0.72	0.77	0.71	0.73	0.69	0.74	0.70	1.31	0.83	ND
C17:1	0.24	0.23	0.25	0.27	0.28	0.26	0.14	0.24	0.23	0.19	0.25	0.26	ND
C18:0	19.20	19.93	19.06	21.32	21.71	22.98	26.68	25.86	26.65	22.08	22.03	25.00	1.50
C18:1n9c	45.32	44.27	42.97	43.51	42.16	41.02	39.77	40.59	39.24	42.63	42.76	41.13	57.82
C18:1n9t	1.22	1.38	1.45	1.23	1.28	1.39	0.95	1.12	0.97	1.45	1.41	1.42	2.53
C18:2n6t	0.18	0.31	0.24	0.43	0.60	0.82	0.33	0.43	0.90	0.07	0.08	0.13	ND
C18:2n6c	10.65	11.44	12.38	9.68	9.51	9.65	7.46	7.60	7.48	11.24	9.64	7.17	24.22
C20:0	0.39	0.43	0.53	0.33	0.26	0.31	0.24	0.23	0.40	0.45	0.36	0.28	ND
C18:3n6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:1n9	3.49	3.31	3.57	2.67	2.69	2.57	1.83	1.97	1.66	3.30	2.68	1.88	7.71
C18:3n3	0.86	0.61	1.75	0.78	0.58	0.55	0.41	0.40	0.21	0.20	0.22	0.25	1.13

*Abbreviations are provided in Materials & Methods section

Standard deviation for C14:0=±0.02, C14:1=0, C15:0=±0.01, C16:0=±0.21, C16:1=±0.04, C17:0=±0.04, C17:1=±0.02, C18:0=±0.25, C18:1n9c=±0.49, C18:1n9t=±0.04, C18:2n6t=±0.02, C18:2n6c=±0.59,C20:0=±0.02, C18:3n6=0, C20:1n9=±0.02,C18:3n3=±0.01 (calculated from CPs)

Table 4.2 Fatty acid profile (%) of blends and structured lipids with tallow and safflower oil

	SA61	SA62	SA63	SA71	SA72	SA73	SA81	SA82	SA83	SA60	SA70	SA80	SA	T
C14:0	0.96	1.07	1.09	1.32	1.23	1.17	1.35	1.41	1.37	1.15	1.33	1.46	0.07	1.80
C14:1	0.07	0.08	0.06	0.12	0.13	0.18	0.20	0.16	0.22	0.24	0.27	0.37	ND	0.45
C15:0	0.13	0.19	0.18	0.19	0.24	0.24	0.24	0.21	0.26	0.17	0.23	0.28	ND	0.35
C16:0	15.68	15.73	15.44	17.55	17.08	17.67	18.48	18.44	17.99	15.95	17.51	18.46	6.55	22.50
C16:1	0.60	0.67	0.72	0.79	0.80	0.76	0.88	0.93	0.90	0.74	0.83	0.91	0.08	1.07
C17:0	0.73	0.64	0.62	0.72	0.75	0.82	0.95	0.78	0.81	0.56	0.62	0.75	ND	0.94
C17:1	0.14	0.16	0.20	0.27	0.21	0.21	0.26	0.25	0.27	0.13	0.26	0.23	ND	0.30
C18:0	25.54	20.66	19.23	22.66	22.67	25.39	25.46	24.86	24.87	19.77	22.67	25.68	2.91	32.10
C18:1n9c	24.31	26.99	27.39	30.97	29.77	27.37	31.78	32.94	32.44	27.14	29.48	32.47	14.06	36.20
C18:1n9t	0.60	0.69	0.70	0.56	0.73	0.68	0.72	0.66	0.74	0.64	0.60	0.68	0.39	0.74
C18:2n6t	0.08	0.06	0.02	0.09	0.20	0.10	0.10	0.10	0.08	ND	ND	ND	ND	0.07
C18:2n6c	30.42	32.26	33.54	23.87	25.29	24.61	18.47	18.50	19.22	32.83	25.59	18.26	75.16	2.97
C20:0	0.43	0.35	0.32	0.30	0.32	0.31	0.60	0.29	0.29	0.26	0.21	0.14	0.38	0.13
C18:3n6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:1n9	0.19	0.23	0.29	0.31	0.33	0.25	0.22	0.21	0.23	0.20	0.22	0.18	0.22	0.20
C18:3n3	0.16	0.23	0.22	0.26	0.28	0.23	0.30	0.28	0.31	0.21	0.20	0.12	0.19	0.23

*Abbreviations are provided in Materials & Methods section

Standard deviation for C14:0=±0.02, C14:1=0, C15:0=±0.01, C16:0=±0.21, C16:1=±0.04, C17:0=±0.04, C17:1=±0.02, C18:0=±0.25, C18:1n9c=±0.49, C18:1n9t=±0.04, C18:2n6t=±0.02, C18:2n6c=±0.59,C20:0=±0.02, C18:3n6=0, C20:1n9=±0.02,C18:3n3=±0.01 (calculated from CPs)

Table 4.3 Fatty acid profile (%) of blends and structured lipids with corn oil

	CP1	CP2	CP3	CO61	CO62	CO63	CO71	CO72	CO73	CO81	CO82	CO83	CO60	CO70	CO80	CO
C14:0	1.23	1.23	1.27	1.01	1.03	1.03	1.24	1.19	1.22	1.41	1.46	1.45	0.88	1.22	1.43	ND
C14:1	0.31	0.32	0.31	0.25	0.27	0.26	0.17	0.28	0.18	0.20	0.21	0.19	0.22	0.31	0.36	ND
C15:0	0.21	0.23	0.21	0.19	0.19	0.19	0.22	0.20	0.23	0.24	0.25	0.26	0.16	0.23	0.27	ND
C16:0	18.50	18.74	19.00	17.52	17.31	17.30	18.44	17.90	18.75	19.56	19.73	19.60	16.51	18.66	19.91	11.23
C16:1	0.83	0.78	0.87	0.71	0.72	0.73	0.86	0.81	0.86	0.95	1.00	0.99	0.58	0.75	0.89	0.08
C17:0	0.67	0.60	0.70	0.48	0.54	0.48	0.69	0.72	0.70	0.84	0.85	0.87	0.47	0.63	0.79	ND
C17:1	0.22	0.20	0.25	0.21	0.17	0.17	0.23	0.26	0.25	0.29	0.28	0.27	0.16	0.24	0.28	ND
C18:0	21.40	22.01	21.80	18.46	18.29	18.40	21.03	21.55	21.75	24.82	24.41	25.20	16.18	22.00	25.49	1.87
C18:1n9c	35.97	34.95	35.99	34.79	35.28	35.18	35.53	34.83	35.85	36.04	36.57	36.30	33.51	34.71	35.46	30.56
C18:1n9t	0.69	0.60	0.67	0.69	0.65	0.60	0.71	0.74	0.63	0.70	0.73	0.76	0.54	0.61	0.62	0.47
C18:2n6t	0.05	0.08	0.09	0.07	0.07	0.05	0.08	0.08	0.08	0.09	0.14	0.13	0.04	0.08	0.08	0.00
C18:2n6c	18.96	19.25	17.88	24.55	24.39	24.52	19.69	20.42	18.47	13.80	13.28	12.90	29.71	19.54	13.45	54.59
C20:0	0.21	0.27	0.23	0.30	0.30	0.29	0.31	0.29	0.29	0.30	0.28	0.31	0.31	0.28	0.25	ND
C18:3n6	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
C20:1n9	0.45	0.48	0.44	0.55	0.57	0.58	0.53	0.51	0.48	0.48	0.48	0.46	0.59	0.47	0.40	0.96
C18:3n3	0.30	0.27	0.30	0.22	0.23	0.24	0.26	0.21	0.27	0.29	0.32	0.30	0.16	0.28	0.32	0.24

*Abbreviations are provided in Materials & Methods section

Standard deviation for C14:0=±0.02, C14:1=0, C15:0=±0.01, C16:0=±0.21, C16:1=±0.04, C17:0=±0.04, C17:1=±0.02, C18:0=±0.25, C18:1n9c=±0.49, C18:1n9t=±0.04, C18:2n6t=±0.02, C18:2n6c=±0.59,C20:0=±0.02, C18:3n6=0, C20:1n9=±0.02,C18:3n3=±0.01 (calculated from CPs)

Among all samples, the structured lipids interesterified with canola oil had the higher percentage of monounsaturated fatty acids (MUFA) since canola oil, itself, had the highest MUFA content among vegetable oils used in this study. As expected, MUFA% decreased from 51.3% to 43.8% with a change of tallow composition from 60 to 80% in structured lipids prepared with canola oil. The products with safflower oil had lower MUFA% and this ratio varied between 25-35%. According to Figure 4.2 which shows MUFA amounts of the samples, interesterification process did not cause sharp changes in the percentages of MUFAs for all samples.

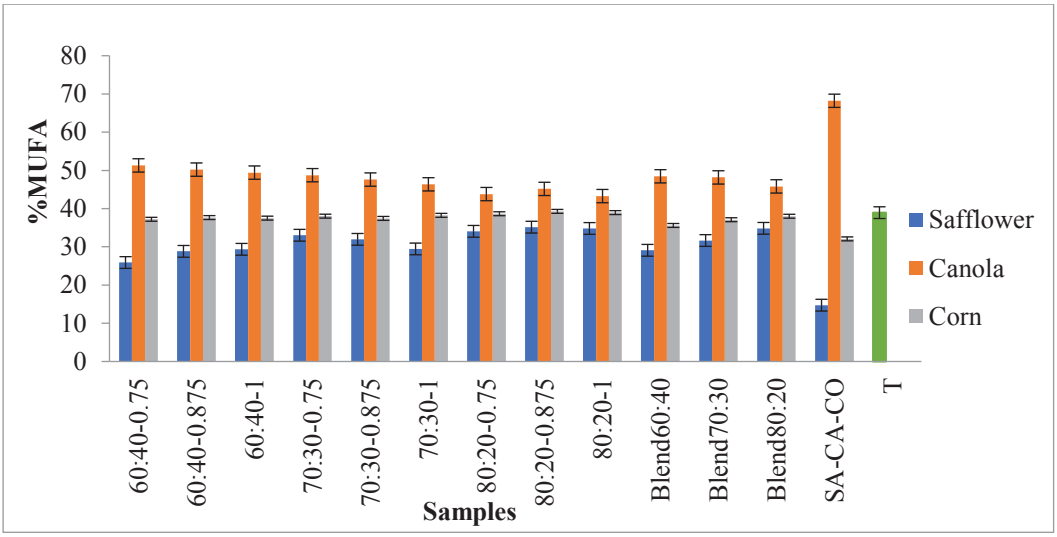

Figure 4.2 Monounsaturated fatty acid (MUFA) contents of the samples

Samples interesterified with safflower oil are rich in terms of polyunsaturated fatty acids (PUFA) since the original PUFA content of safflower oil is also high (Figure 4.3). Saturated fatty acid content (SFA) of tallow (57.79%) decreased both by chemical interesterification and blending and the effect of catalyst concentration was not significant for SFA content (Figure 4.4).

ANOVA results (App. A7) indicated that all the models constructed for MUFA%-PUFA%-SFA% and TFA% were significant with non-significant lack of fit at 95% confidence interval. Normality and residuals were also checked for the models. All oil types including canola, corn and safflower oils have significant effect on MUFA content of the samples as ANOVA table indicated. Moreover, the interactions between blend ratio-safflower and canola oils are also important for this model. Figure 4.5-4.7 shows the effect of significant parameters on fatty acid composition of structured lipids.

When the blend ratio of tallow increased, MUFA content of the samples interesterified with safflower oil also increased. However, opposite trend was observed for the structured lipids interesterified with canola oil samples (Figure 4.5). Increasing blend ratio leads to slight decreases in MUFA content of samples. Almost all factors have remarkable effect on PUFA content of the samples (App. A7). With the increase in blend ratio of tallow, PUFA content of interesterified fats decreased regardless of oil type (Figure 4.6). The ANOVA table reveals that blend ratio and interaction of blend ratio-safflower oil have considerable effect on SFA content of structured lipids. Higher concentrations of tallow result in structured lipids with higher SFA (Figure 4.7). The model constructed for TFAs of interesterified fats showed that catalyst concentration, oil type and interaction between catalyst concentration-oil type are significant (Figure 4.8). The interaction of catalyst concentration with oil type is more substantial when canola oil is used. The higher amounts of TFAs in canola oil samples come from canola oil itself. Since high temperature treatments are applied during canola oil refining mostly in the deodorization step in order to eliminate the intense bad odor of this oil, higher amounts of TFAs form.

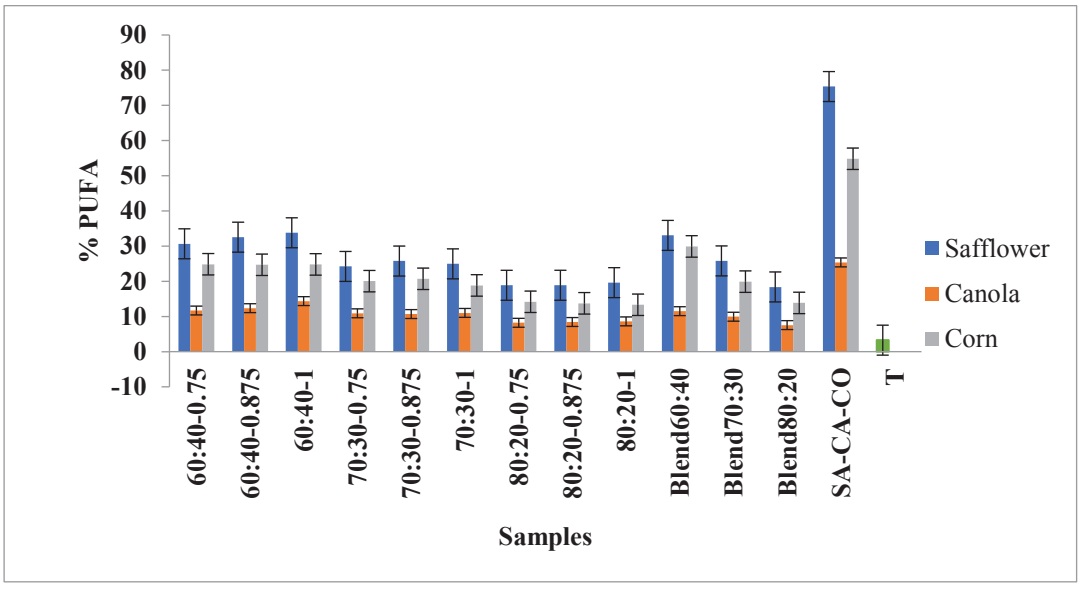

Figure 4.3 Polyunsaturated fatty acid (PUFA) contents of the samples

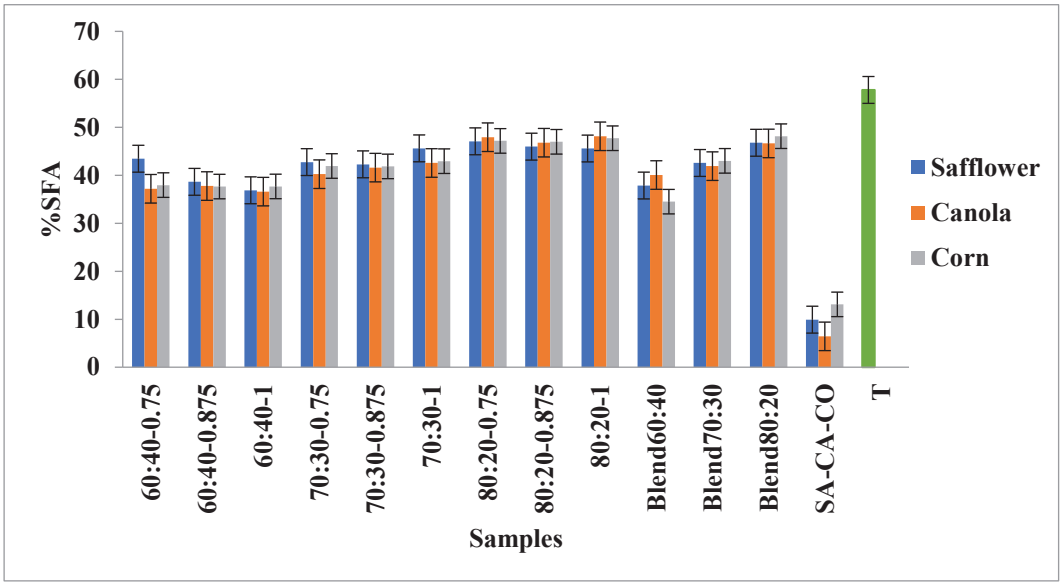

Figure 4.4 Saturated fatty acid (SFA) contents of the samples

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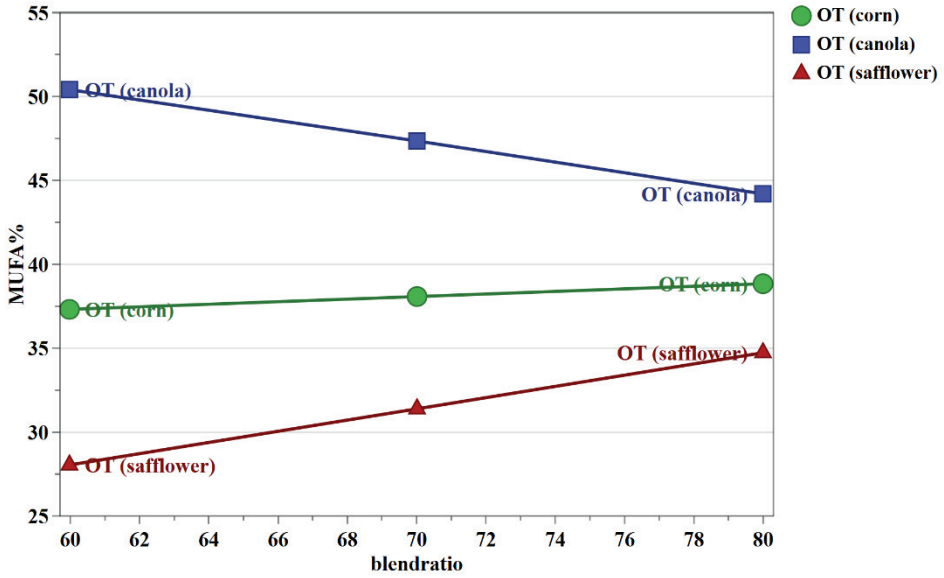

Figure 4.5 Interaction plot showing the effect of blend ratio x oil type on monounsaturated fatty acid (MUFA) content of structured lipids

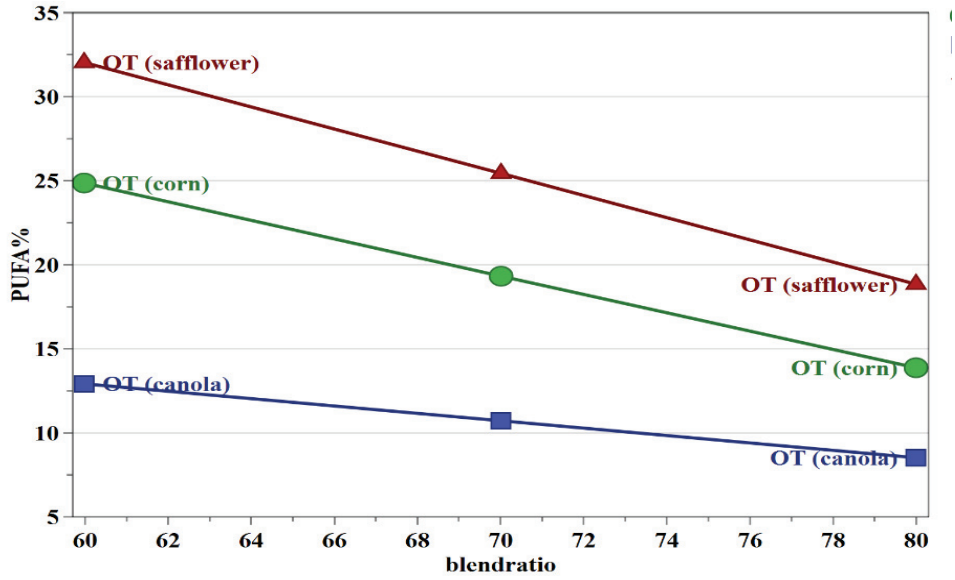

Figure 4.6 Interaction plot showing the effect of blend ratio x oil type on polyunsaturated fatty acid (PUFA) content of structured lipids

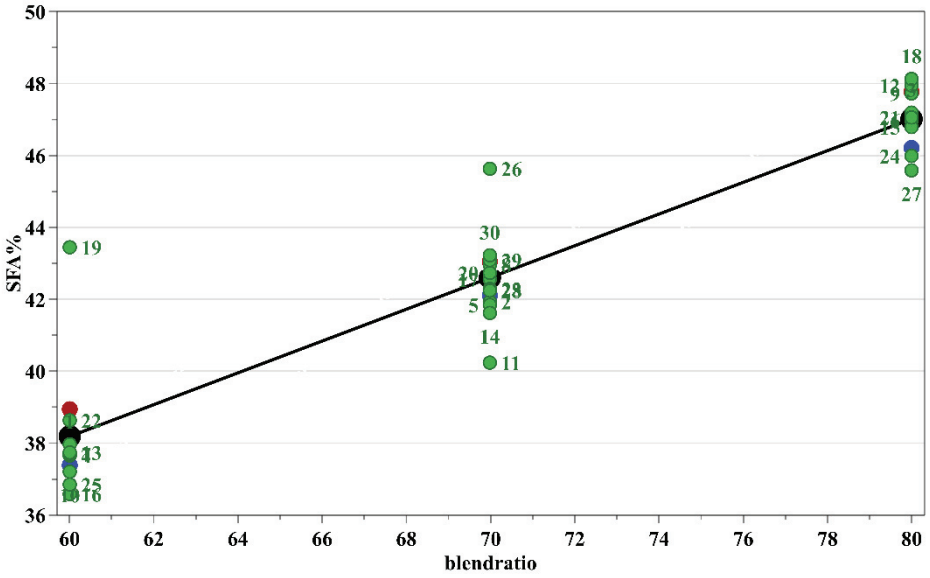

Figure 4.7 Main effect plot for blend ratio on saturated fatty acid (SFA) content of structured lipids

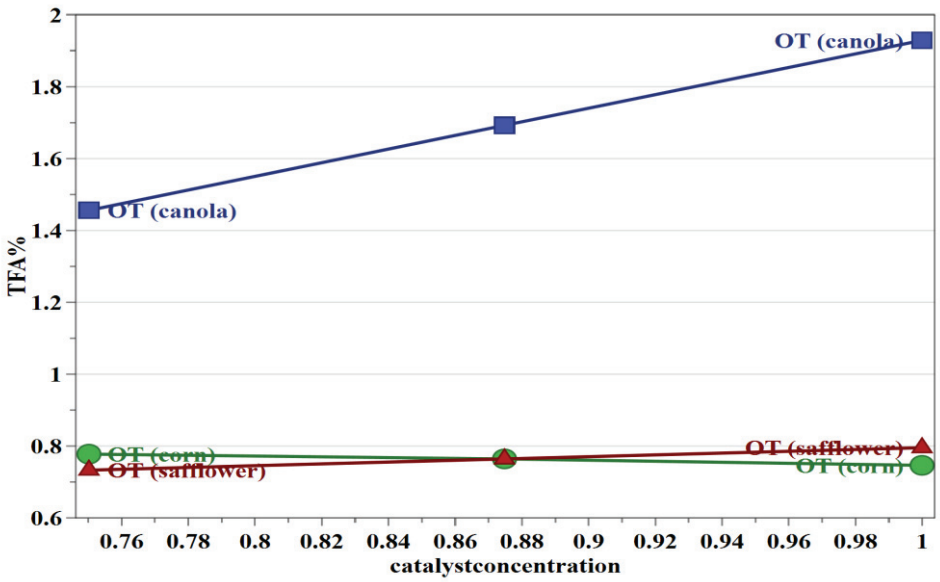

Figure 4.8 Interaction plot showing the effect of catalyst concentration x oil type on trans fatty acid (TFA) content of structured lipids

4.1.2. Oxidative Stability of Chemically Interesterified Lipids

The oxidation induction time determined from Rancimat measurement was used as an index of the oxidative stability. The oxidation induction times of the samples are given in Table 4.4. The oxidation induction time of tallow is 4.81 h while blends without interesterification have a range of induction times of 7.98-12.25 h. In general, oxidation induction times of interesterified samples decreased compared to starting blends. There are some fluctuations in between samples depending on catalyst concentrations. However, 1% CH_3NaO concentration mostly led to formation of structured lipids with low oxidation induction times regardless of blend ratio especially for corn and canola oils (Figure 4.9).

As it is seen in Figure 4.10, there has been a drastic decrease in oxidation induction times of the samples produced with safflower oil after chemical interesterification process compared to tallow. This result is in accordance with the previous studies, which also observed a decrease in oxidative stability after the chemical interesterification of beef tallow with rapeseed oil (Kowalski et al. 2004). In fact, the induction time for the sample with 60:40 ratio and 0.75% catalyst concentration was as low as 0.71 h. This result could be due to the high linoleic acid and low tocopherol content of safflower oil. Under unsuitable conditions, safflower oil may be oxidized more rapidly than other vegetable

oils. Therefore, the samples interesterified with safflower has lower oxidative stability (Blum 1966). Solely, the structured lipid having 70:30 ratio and 0.75% catalyst concentration had the highest induction time (3.16 h) compared to other samples interesterified with safflower oil.

Table 4.4 Oxidation induction times (h) of samples

Sample	OS (h)	Sample	OS (h)	Sample	OS (h)
SA61	0.71	CO61	7.48	CA61	6.43
SA62	1.55	CO62	7.76	CA62	6.37
SA63	1.12	CO63	4.78	CA63	2.71
SA71	3.16	CO71	3.82	CA71	8.49
SA72	2.84	CO72	6.77	CA72	7.55
SA73	0.9	CO73	6.85	CA73	5.55
SA81	1.08	CO81	7.3	CA81	4.72
SA82	3.09	CO82	7.4	CA82	4.25
SA83	2.17	CO83	5.34	CA83	2.92
SA60	2.83	CO60	6.73	CA60	5.73
SA70	3.17	CO70	8.51	CA70	7.74
SA80	4.2	CO80	10	CA80	9.06
SA	2.04	CP1	10.92	CA	4.51
T	4.81	CP2	12.25		
CO	4.98	CP3	7.98		

*Abbreviations are provided in Materials & Methods section
Standard deviation of chemical measurements: OS=±1.78, (calculated from CPs)

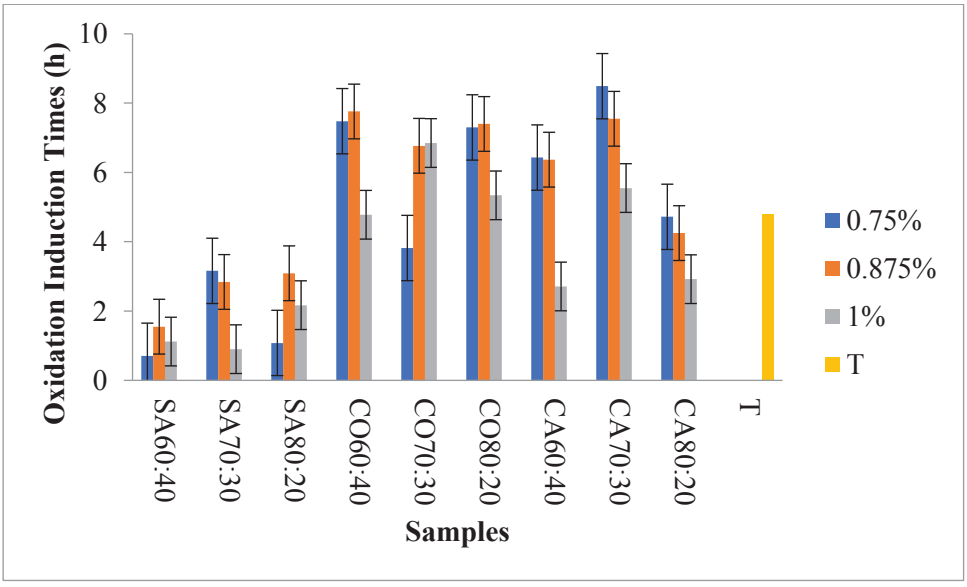

Figure 4.9 Oxidation induction times of structured lipids at different catalyst concentrations

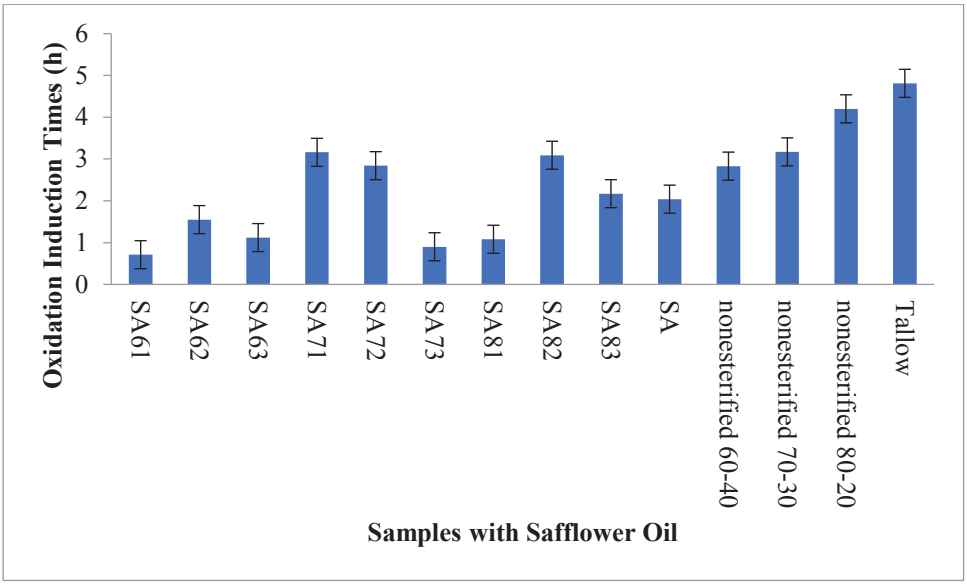

Figure 4.10 Oxidation induction times of samples with safflower oil

Figure 4.11 reveals that some of tallow samples interesterified with canola oil had better oxidative stability than tallow itself. It was observed that as the percentage of tallow in blends increased, oxidative stability decreased. Same trend was also observed in the chemical interesterification of beef tallow with canola oil (Liu et al. 2009; Martin et al. 2010). Canola oil and tallow have comparable oxidation induction times although they have quite different fatty acids profile. Presence of tocopherols in canola oil can be associated with longer induction times. Canola oil contains approximately 700 ppm tocopherol which can improve oxidative stability (Przybylski et al. 2005). The highest value of the induction time among all interesterified samples was observed as 8.49 h for sample containing canola oil at 70:30 ratio with a catalyst concentration of 0.75%.

The oxidation induction time values of the blends with corn oil are generally higher than tallow (Figure 4.12). Oxidative stabilities of the structured lipids at 60:40 ratio with 0.75% and 0.875% catalyst concentrations have the highest oxidative stabilities of 7.48 and 7.76 h among samples with corn oil. This result can also be associated with the presence of tocopherol and β-carotene in corn oil.

The statistical analysis results for oxidative stability are given in App. A7. ANOVA results indicated that constructed model was significant with non-significant lack of fit. Normality and residuals were checked for the model. The ANOVA table reveals that blend ratio and catalyst concentration are not significant for this model (App. A1) meaning that both factors do not affect the oxidative stabilities of structured lipids.

However, model showed that oil type is the only significant factor. Generally, corn oil samples have better oxidative stabilities compared to other oil types. In addition, safflower oil is the most considerable one due to its higher negative effect on oxidative stability followed by canola oil (Figure 4.13).

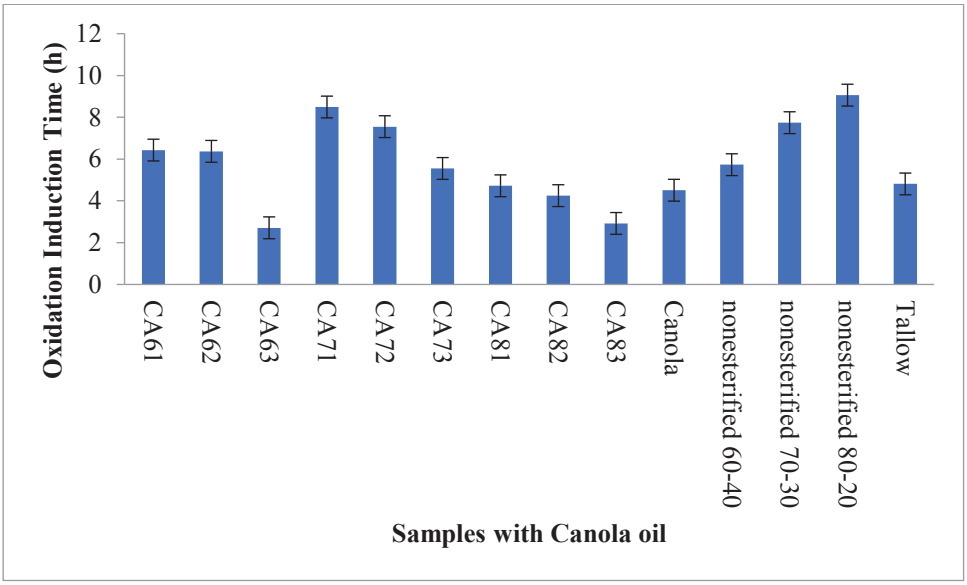

Figure 4.11 Oxidation induction times of samples with canola oil

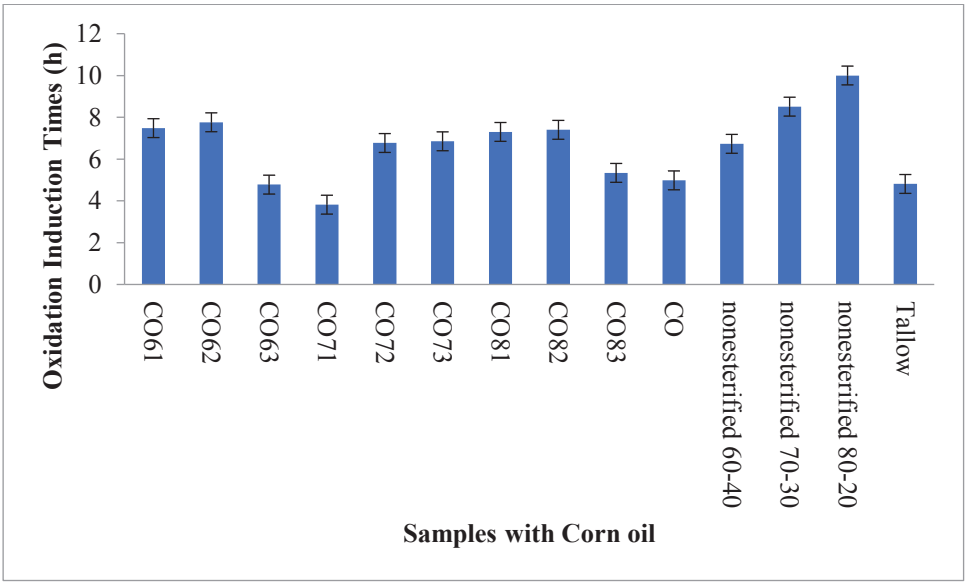

Figure 4.12 Oxidation induction times of samples with corn oil

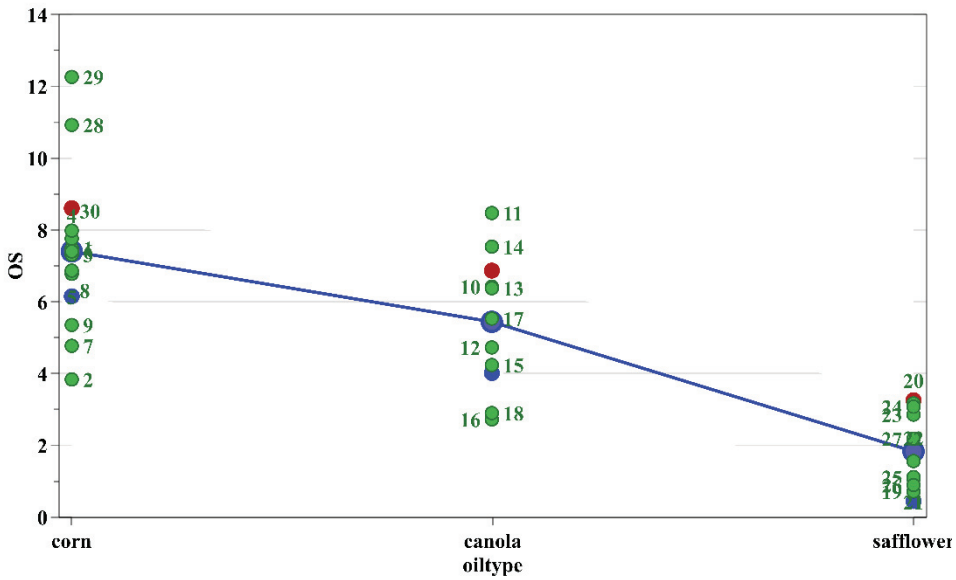

Figure 4.13 The main effect plot of oil type on oxidative stability (OS) of structured lipids

4.1.3. Free Fatty Acid Content of Chemically Interesterified Lipids

The free fatty acid (FFA) content was determined in order to obtain a measure about the hydrolytic rancidity of both interesterified samples and blends. The FFA% of the samples are provided in Table 4.5. The acidity is expressed as percentage of oleic acid.

The FFA% of tallow is 1.15% while blends without interesterification have an acidity range of 0.6-1.5%. Generally, FFA% of interesterified lipids increased compared to starting blends. There are some fluctuations in between samples depending on catalyst concentrations (Figure 4.14). However, 1% catalyst concentration led to formation of structured lipids with higher FFA% especially for corn oil-tallow samples.

As it could be seen in Figure 4.15, there is a drastic increase in FFA% of samples with safflower oil after chemical interesterification process compared to tallow, safflower oil and their blends. This result is in accordance with the previous studies, which observed an increase in free fatty acid content after chemical interesterification (Kowalska et al. 2005; Hoshina et al. 2004). It was reported that the higher amount of catalysts in the reaction medium caused the formation of higher amounts of FFA and monoacylglycerols (MAG) + diacylglycerols (DAG), and lower content of triacylglycerols (TAG) (Ledóchowska and Wilczyńska 1998).

Table 4.5 Free fatty acid (FFA) percentages (% oleic acid) of the chemically interesterified lipids, blends, vegetable oils and tallow

Sample	%FFA	Sample	%FFA	Sample	%FFA
SA61	2.42	**CO61**	2.74	**CA61**	2.23
SA62	1.08	**CO62**	3.11	**CA62**	3.39
SA63	1.68	**CO63**	3.90	**CA63**	4.12
SA71	2.60	**CO71**	2.15	**CA71**	2.7
SA72	3.25	**CO72**	2.48	**CA72**	2.9
SA73	3.26	**CO73**	3.88	**CA73**	2.37
SA81	1.60	**CO81**	2.33	**CA81**	1.56
SA82	3.4	**CO82**	3.04	**CA82**	2.5
SA83	3.23	**CO83**	3.85	**CA83**	2.56
SA60	0.65	**CO60**	0.62	**CA60**	1.03
SA70	1.31	**CO70**	0.63	**CA70**	0.68
SA80	0.46	**CO80**	0.76	**CA80**	0.77
SA	0.23	**CO**	0.09	**CA**	0.09
T	1.15	**CP1**	3.03		
		CP2	2.97		
		CP3	1.87		

*Abbreviations are provided in Materials & Methods section
Standard deviation of chemical measurements: FFA=±0.53, (calculated from three CPs)

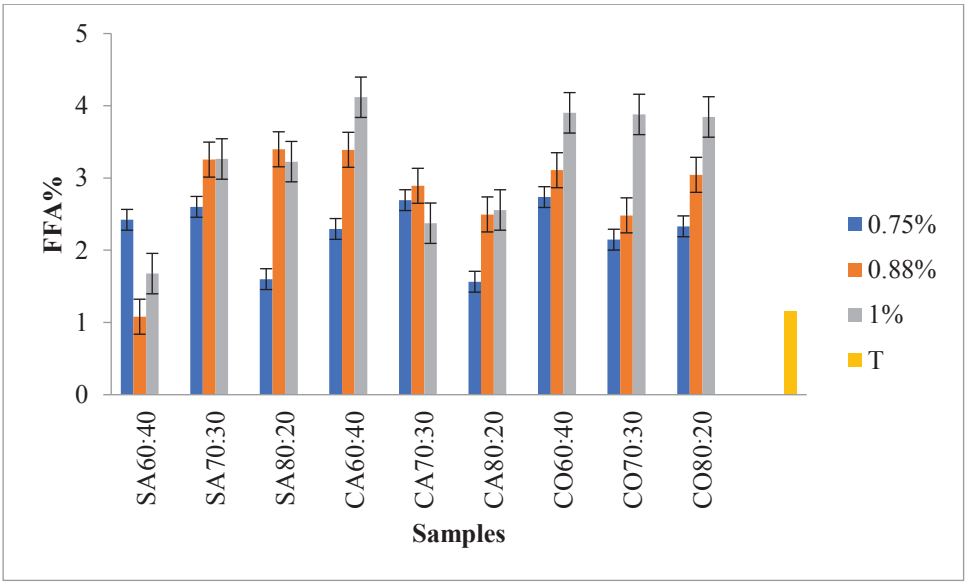

Figure 4.14 Free fatty acid percentages (FFA%) of structural lipid samples at different catalyst concentrations

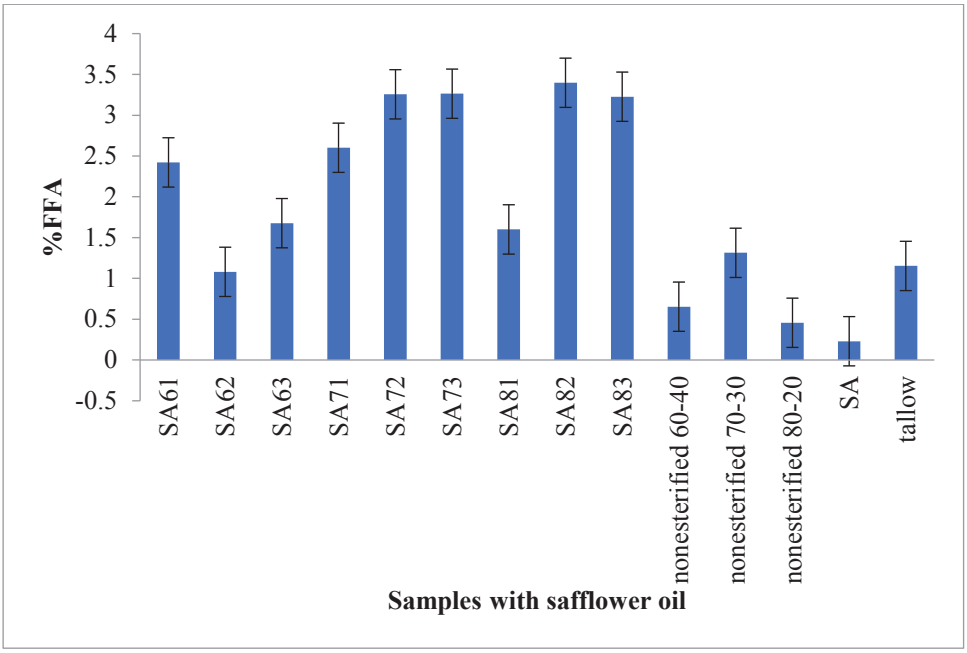

Figure 4.15 Free fatty acid percentages (FFA%) of the samples with safflower oil

Figure 4.16 reveals that most of the samples interesterified with canola oil had lower FFA content than the other structured lipids. Interesterified tallow-canola oils have higher FFA% compared to blends regardless of tallow amount.

Generally, FFA% of the blends with corn oil is lower than tallow since corn oil have a very low FFA% (Figure 4.17). However, the samples interesterified with corn oil have higher FFA% than their blends. This result could be explained better with respect to the MAG, DAG and TAG contents of the samples.

Appendix 1 shows the statistical analysis results for FFA%. ANOVA results indicated that constructed model was significant with non-significant lack of fit at 95% confidence interval. Catalyst concentration is the most important factor affecting FFA% as ANOVA table indicated. The use of chemical catalyst at higher concentrations gave rise to higher FFA% of interesterified lipids (Figure 4.18). The interaction between blend ratio and oil type is a less significant for this model (App. A7).

4.1.4. Mono, Di, and Triacylglycerol Contents of Chemically Interesterified Lipids

Mono, di and triacylglycerol (MAG, DAG and TAG) contents of structured lipids were determined in order to better understand the modifications in the glycerol backbone

that occurred during chemical interesterification processes. MAG, DAG and TAG contents of the samples are expressed in relative percentages of overall content (Table 4.6). The results are in accordance with the previous studies, which observed a decrease in TAG% after interesterification of tallow and sunflower oil (Kowalska et al. 2005; Ledóchowska and Wilczyńska 1998).

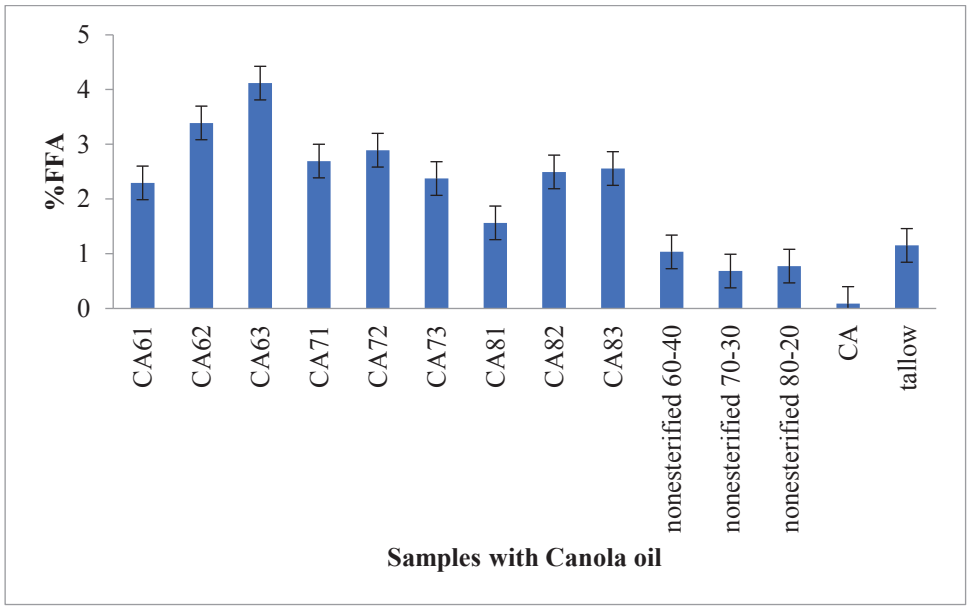

Figure 4.16 Free fatty acid percentages (FFA%) of the samples with canola oil

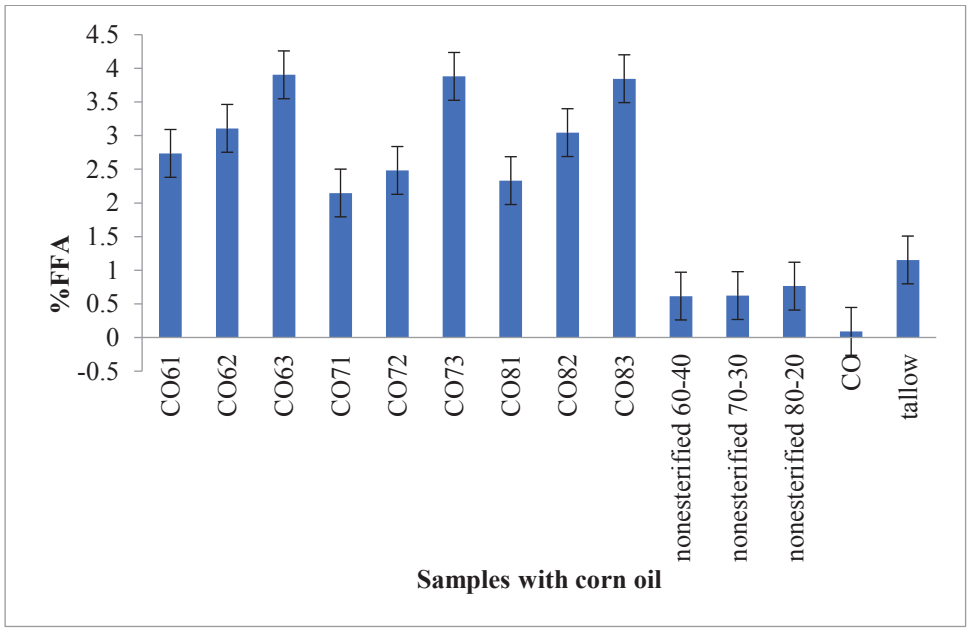

Figure 4.17 Free fatty acid percentages (FFA%) of samples with corn oil

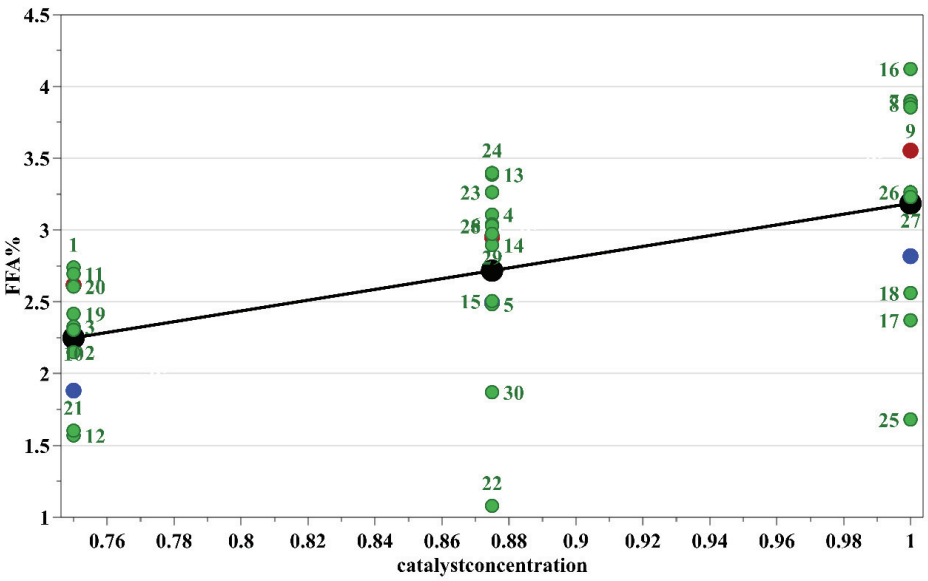

Figure 4.18 The main effect plot for catalyst concentration (%) on free fatty acid (FFA%) of structured lipid samples

The TAG% of tallow is approximately 98% while blends without interesterification have slightly lower TAG% values. Both blending and chemical interesterifications caused an increase in MAG and DAG contents of the samples. Generally, TAG% of chemically interesterified lipids decreased compared to starting blends. There are some fluctuations in between samples depending on the catalyst concentrations (Figure 4.19). However, 0.875% CH_3Na concentration mostly led to formation of structured lipids with higher TAG% (Figure 4.19).

Interesterified tallow-canola oils have, in general, lower TAG% compared to other structured lipids. As it can be seen from Figure 4.20 samples having low TAG content have higher DAG% values as expected. In addition, MAG% of samples with safflower oil is higher compared to other lipids (Figure 4.21).

Appendix 7 shows the statistical analysis results for TAG content. ANOVA results indicated that constructed model was insignificant with non-significant lack of fit. Normality and residuals were checked for the model. Examination of the significance levels of main factors and their interactions shows that catalyst concentration, oil type and blend ratio did not significantly affect TAG% of chemically interesterified lipids.

Table 4.6 Relative percentages of triacylglycerol (TAG), diacylglycerol (DAG) and monoacylglycerol (MAG) of the samples

Sample	TAG%	DAG%	MAG%	Sample	TAG%	DAG%	MAG%	Sample	TAG%	DAG%	MAG%
SA61	79.91	0.38	17.17	**CA61**	79.57	6.32	10.16	**CO61**	52.62	13.45	9.5
SA62	89.76	0.07	14.16	**CA62**	83.98	8.52	7.48	**CO62**	89.32	4.77	3.31
SA63	82.75	1.19	15.4	**CA63**	63.56	8.4	10.25	**CO63**	82.75	9.08	3.74
SA71	78.53	4.32	14.34	**CA71**	76.08	9.26	8.06	**CO71**	89.65	1.75	6.48
SA72	84.43	2.86	12.66	**CA72**	73.07	5.06	10.08	**CO72**	81.38	11.03	0.89
SA73	83.34	4.33	8.51	**CA73**	70.29	11.22	10.01	**CO73**	82.89	5.3	11.73
SA81	76.72	6.6	10.2	**CA81**	64.87	9.76	8.49	**CO81**	81.62	2.09	10.8
SA82	84.19	2.7	10.12	**CA82**	84.76	5.48	9.68	**CO82**	82.02	1.44	12.37
SA83	81.22	1.92	15.72	**CA83**	82.3	2.01	8.48	**CO83**	81.31	5.93	9.71
SA60	88.3	0.45	6.19	**CA60**	91.02	6.74	1.69	**CP1**	77.51	14.37	6.96
SA70	82.37	0.98	13.42	**CA70**	94.41	4.83	0.17	**CP2**	82.88	7.52	9.45
SA80	81.24	1.04	4.7	**CA80**	96.29	2.46	1.4	**CP3**	74.25	8.42	6.15
SA	90.36	2.58	1.14	**CA**	74.87	1.91	4.2	**CO60**	85.51	5.91	0.26
CO	92.06	2.01	0.35	**T**	97.94	0.48	0.92	**CO70**	86.84	2.68	4.05
								CO80	85.52	0.57	8.48

*Abbreviations are provided in Materials & Methods section

Standard deviation of chemical measurements: TAG%=±3.56, DAG%=±3.04, MAG%=±1.4 (calculated from CPs)

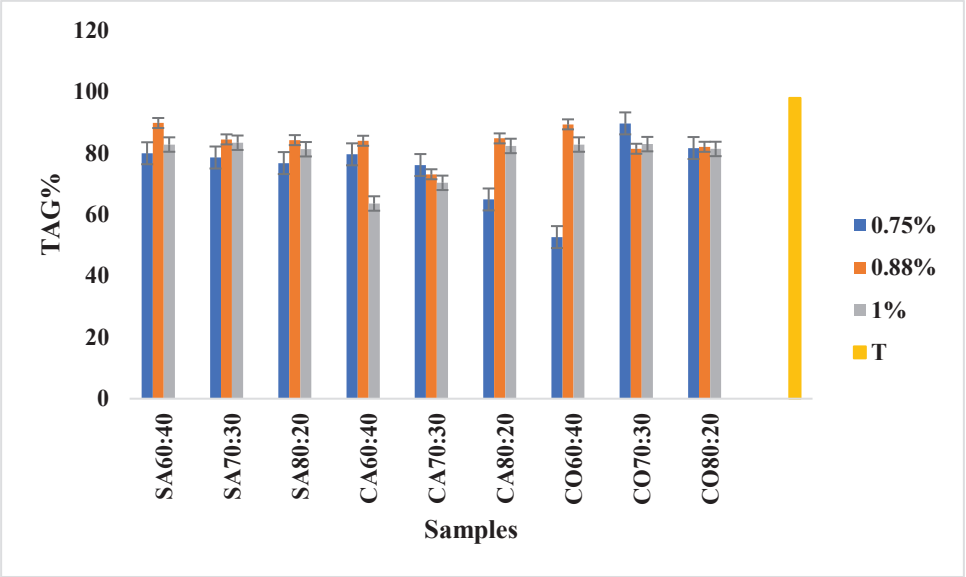

Figure 4.19 Triacylglycerol content (TAG%) of chemically interesterified samples at various catalyst concentrations

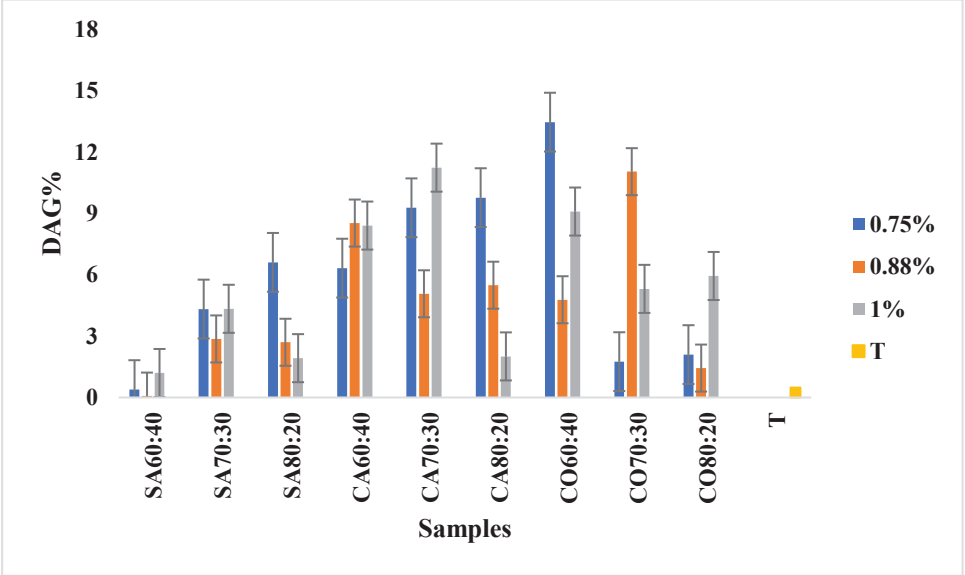

Figure 4.20 Diacylglycerol content (DAG%) of chemically interesterified samples at various catalyst concentrations

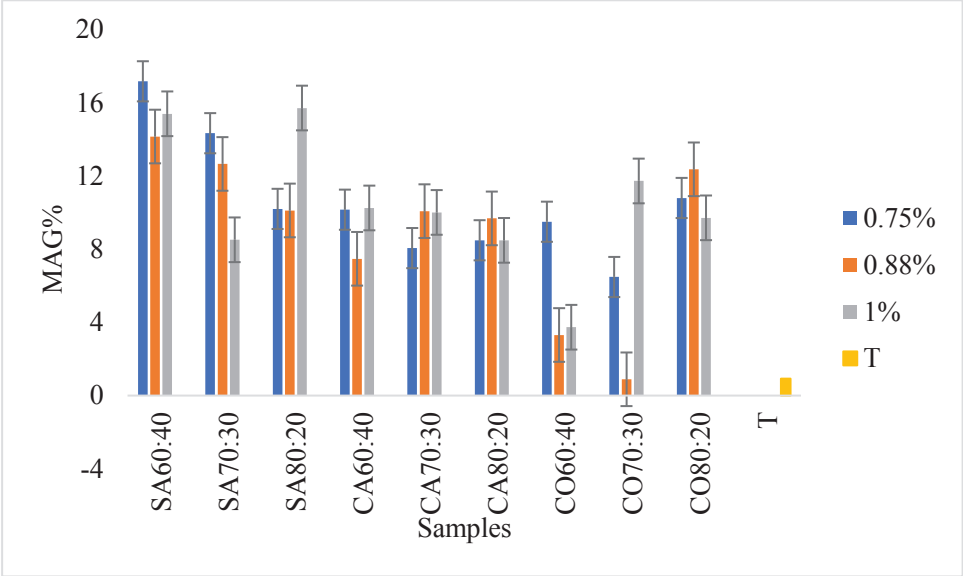

Figure 4.21 Monoacylgycerol content (MAG%) of chemically interesterified samples at various catalyst concentrations

Same statistical analyses were also performed for DAG and MAG% of chemically interesterified lipids. ANOVA results indicated that constructed model was significant with non-significant lack of fit (App. A7). The main effect plot of DAG% confirms the significance of oil type (safflower) for this model (Figure 4.22). Generally, structured lipids interesterified with safflower oil have lower DAG% values compared to the samples interesterified with other oil types. Additionally, the interaction between oil type (safflower) and blend ratio is less significant ($p \leq 0.05$) for the DAG content model.

Oil type, particularly corn and safflower oils, and their interactions with blend ratio have important effect on MAG% of structured lipids. When the blend ratio is increased, MAG content of the samples interesterified with corn oil increases also. However, an opposite trend holds for the structured lipids with safflower oil (Figure 4.23).

In order to better understand the changes in TAG, DAG and MAG content after the chemical interesterification reaction correlation between FFA% and MAG+DAG content of interesterified lipids is evaluated (Figure 4.24). The correlation coefficient is calculated and found as 0.69. There is an increasing trend between FFA content and MAG+DAG% of the samples (Figure 4.24). Generally, the samples having higher amounts of MAG+DAG content, also have higher FFA% as Figure 4.24 indicated. Therefore, the increase in both FFA% and MAG+DAG% can be associated with the activity of chemical catalyst that snatch fatty acids from their original place and then by random distribution of fatty acids in TAG backbone of new structured lipids.

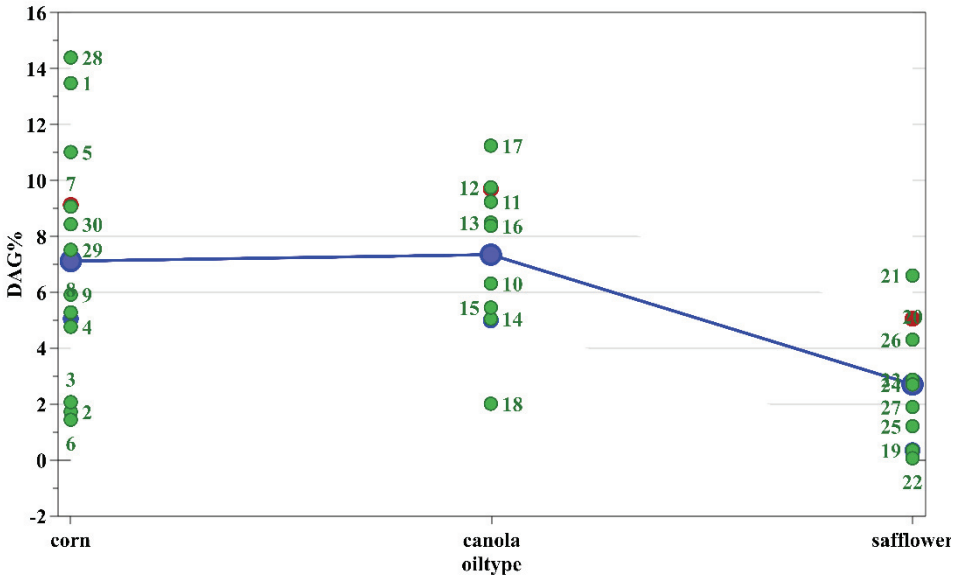

Figure 4.22 Main effect plot of oil type of chemically interesterified samples for diacylglycerol content (DAG%)

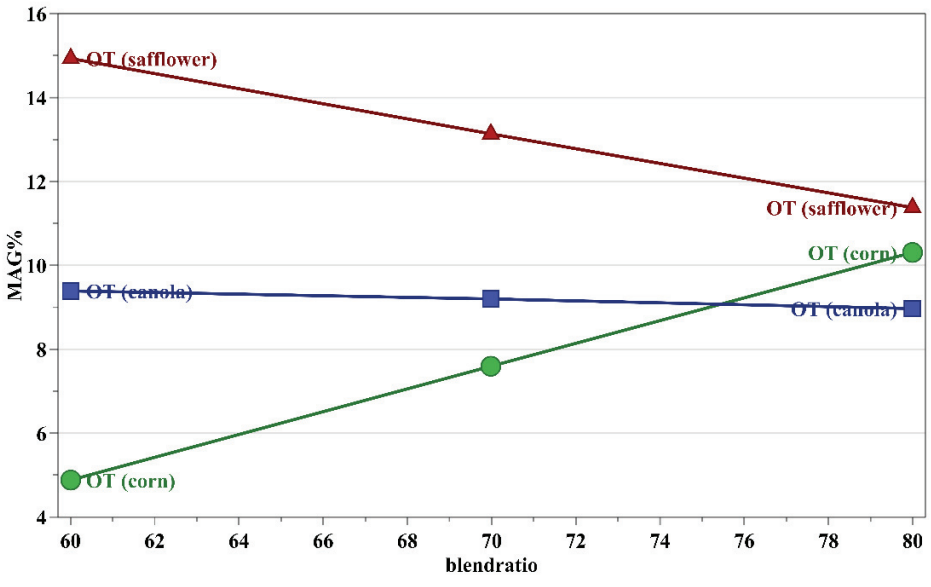

Figure 4.23 Interaction plot showing the effect of oil type x blend ratio of chemically interesterified samples for monoacylglycerol content (MAG%)

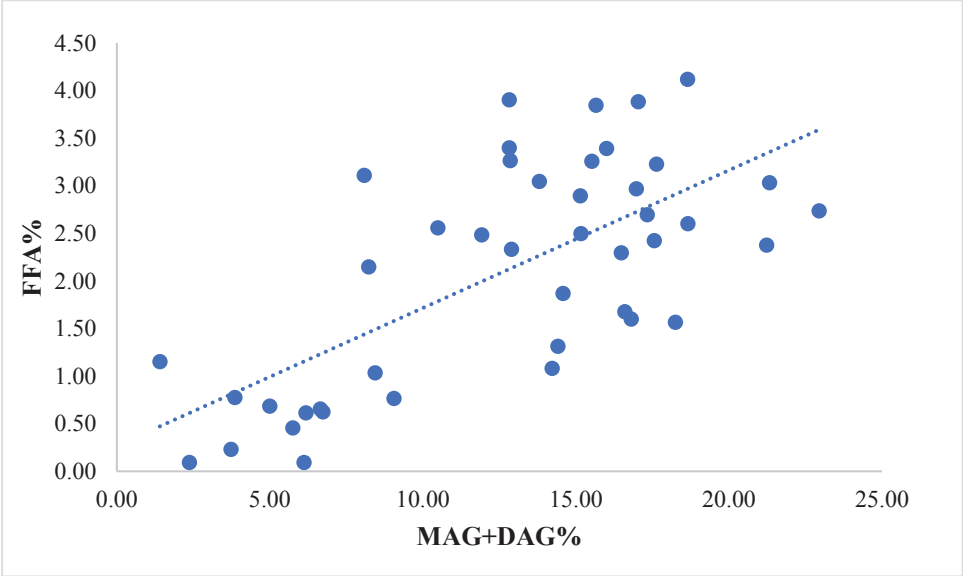

Figure 4.24 Free fatty acid content (FFA%) versus monoacylglycerol and diacylglycerol content (MAG+DAG%) of structured lipids

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To analyze the chemical data of chemically interesterified lipids principal component analysis (PCA) was also applied. The model was constructed by using all measured chemical parameters with 5 PCs, R^2 = 0.84, and Q^2 = 0.42. There is a clear discrimination of the samples with respect to the oil type (Figure 4.25). While the samples containing canola oil located at the right part of ellipse, the samples with corn oil placed just right of the center and safflower containing ones are further in the left. Therefore, a discrimination with respect to first principal component was obtained as far as the oil type is concerned. This discrimination is mostly resulted from higher TFA and MUFA contents of canola oil samples as observed in Figure 4.26. In addition, the structured lipids containing 40% safflower and corn oils placed at the bottom part of the left quartile due to their higher content of PUFA and especially linoleic acid amount. Moreover, groupings among the samples having different blend ratios are observed. The samples with 80% tallow are mostly located at the top of the ellipse regardless of oil type since they have higher SFA% (Figure 4.26). Samples having 60% tallow was in the lower part of ellipse and 70% tallow containing samples are placed in the middle. Therefore, a separation based on blend ratio is also possible with respect to second principal component. As a result, multivariate analysis of the chemical properties data indicated that type of the oil type and blend ratio caused differences in the chemical properties of the produced interesterified products.

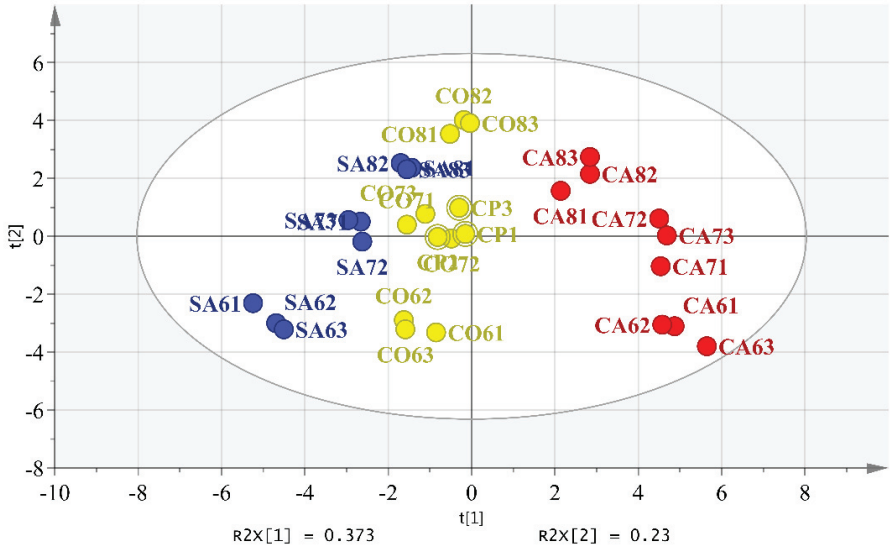

Figure 4.25 Score plot of the PCA model constructed by using all chemical parameters of chemically interesterified lipids (CP1-2-3=70% tallow & 30%corn oil; 0.875% catalyst concentration, samples were colored with respect to oil type)

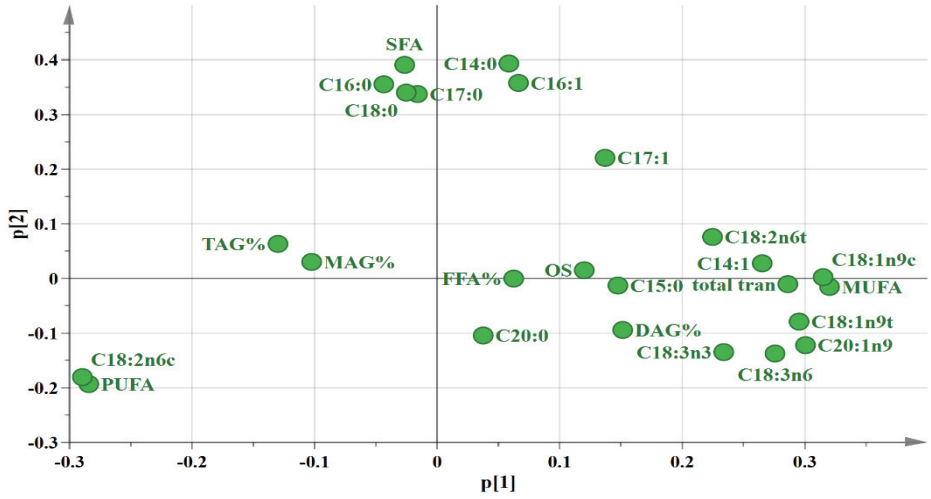

Figure 4.26 Loading plot of the PCA model constructed by using all chemical parameters of chemically interesterified lipids

4. 2. Characterization of Physical Properties of Chemically Interesterified Lipids

The crystal morphology, color, melting (MP) and slip melting points (SMP), consistency and solid fat content (SFC) of the structured lipids produced with chemical

interesterification of tallow with different vegetable oils according to experimental design provided in Material and Methods section were determined. Data were analyzed by univariate (ANOVA) and multivariate statistical analysis (PCA) techniques to investigate the effects of oil type (sunflower, safflower and canola oils), blend ratio (60:40, 70:30 and 80:20) and catalyst concentrations (0.75, 0.875 and 1%).

4.2.1 Crystal Morphology of Chemically Interesterified Lipids

The polymorphic forms of fat crystals in the structured lipids were defined by X-ray diffraction based on the determination of the long and short spacings of the crystals (Figure 4.27). The short spacing of α form appears near 4.15 Å, and of the β' form is placed between 4.2 and 3.8 Å and of the β form at 4.6 Å (single strong spacing). Levels of β' and β crystals in mixtures were estimated by the relative intensity of the short spacings at 3.8, 4.2, and 4.6 Å as the equation indicated below:

$$\lambda = 1.54 \text{ Å}$$

$$2d * \sin\theta = 1.54 * \text{Å}$$

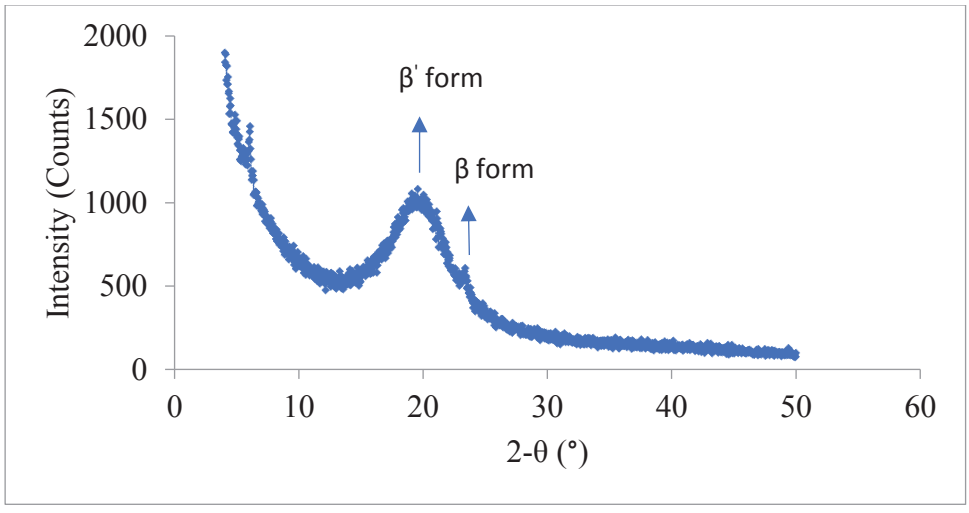

Figure 4.27 Diffractograms for short spacings and long spacings of lipid interesterified with canola oil

Table 4.7 Polymorphic forms of structured lipids, blends and tallow

Sample*	Crystals	Sample*	Crystals	Sample*	Crystals
CA61	β	SA61	β+β'	CO61	β+β'
CA62	β	SA62	β	CO62	β+β'
CA63	β+β'	SA63	β+β'	CO63	β
CA71	β	SA71	β+β'	CO71	β+β'
CA72	β'	SA72	β+β'	CO72	β+β'
CA73	β+β'	SA73	β+β'	CO73	β+β'
CA81	β'	SA81	β+β'	CO81	β
CA82	β'	SA82	β+β'	CO82	β+β'
CA83	β'	SA83	β'	CO83	β+β'
CA60	β+β'	SA60	β+β'	CO60	β+β'
CA70	β+β'	SA70	β+β'	CO70	β+β'
CA80	β+β'	SA80	β+β'	CO80	β+β'
Tallow	β+β'			CP1	β+β'
				CP2	β+β'
				CP3	β+β'

*Abbreviations are provided in Materials & Methods section

The polymorphic forms of structured lipids and blends are provided in Table 4.7. Tallow contains mixtures of β and β' forms. α forms were not found in neither structured lipids nor blends. The non-interesterified blends also contain both β and β' forms together. After chemical interesterification, β and β' forms were also mostly present together especially for the structured lipids produced with safflower and corn oils. Therefore, chemical interesterification did not cause important changes in the polymorphic structures of lipids produced from safflower and corn oils. This result is in accordance with the previous studies in which tallow and palm oil were interesterified with different vegetable oils (Jeung et al. 2008; Meng et al. 2010). Samples with canola oil have a more mixed profile and have also either β'or β form individually as shown in Figure 4.27. In general, lower blend ratio and catalyst concentration combination (CA61, CA62, CA71) resulted in formation of β crystals while higher blend ratio and catalyst combination (CA63, CA72, CA81, CA82, CA83) caused formation of β' crystals for canola oil containing samples. CA81, CA82, CA83, SA83 are the samples which contain only β' polymorphic form and the β' crystal form is important in bakery industry due to its good aeration properties and smooth texture. Therefore, these lipids can be applicable as alternatives for bakery fats.

4.2.2 Color Properties of Chemically Interesterified Lipids

The lightness (L), redness (a) and yellowness (b) values of the chemically interesterified samples were measured and then total color difference (ΔE) were calculated considering tallow itself as a standard. The L, a, b and ΔE values of the chemically interesterified samples are listed in Table 4.8. The lightness value of tallow is 79.42, redness is -1.91 and yellowness is 2.85. Both chemical interesterification and blending caused small decreases in the lightness of the samples with respect to tallow. Among all oil types corn oil containing samples have lower L values compared to other structured lipids. Generally, a and b values of the samples increased compared to tallow itself after chemical interesterification and blending (Table 4.8). As observed in Figure 4.28, oil concentration at 40% increased total color difference. The higher ratio of corn oil created higher total color difference particularly. This result can be associated with β-carotene content of corn oil.

The Appendix 8 shows the statistical analysis results for color measurements. As explained in Materials and Methods section a full factorial design was used and oil type, oil concentration and catalyst concentrations were the investigated factors. ANOVA results indicated that constructed model for total color difference is significant at $p<0.05$ with non-significant lack of fit. Normality and residuals were also checked for the model. Blend ratio and oil type (corn) are the significant factors for ΔE of the chemically interesterified lipids (App. A8). In addition, blend ratio and oil type interaction for ΔE is also significant ($p<0.05$). Although increasing blend ratio leads to decreases in ΔE of corn oil samples, ΔE of safflower oil samples increased (Figure 4.29).

4.2.3 Melting Points of Chemically Interesterified Fats

Beef tallow is relatively a less valuable fat and not very suitable for food applications especially due to its high melting point. One of the purposes of the interesterification of tallow with different vegetable oils is to reduce the melting and softening points of tallow. Since the thermal properties of edible fats could be characterized by melting and crystallization profile, melting temperatures of the structured lipids were measured by a differential scanning calorimeter.

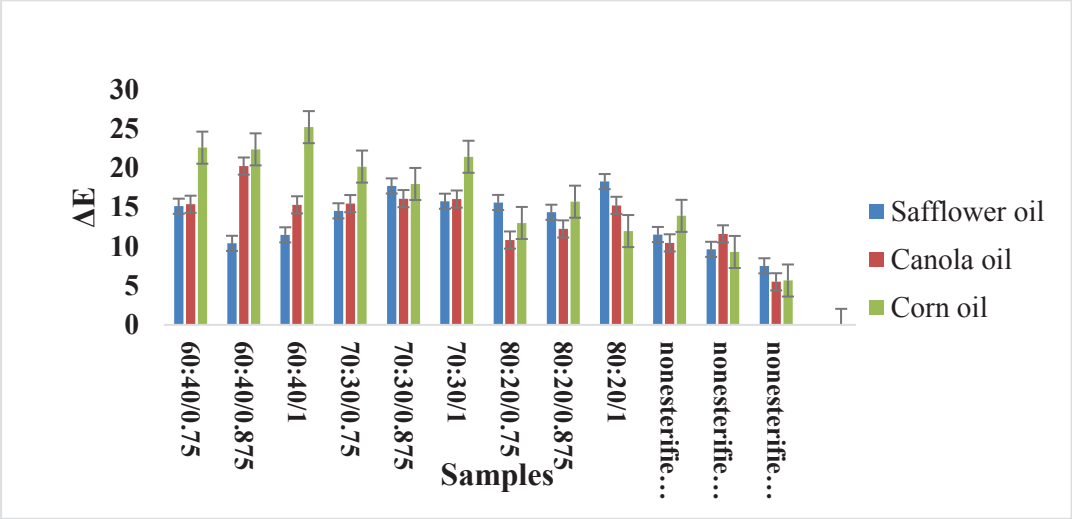

Figure 4.28 Total color difference of the samples

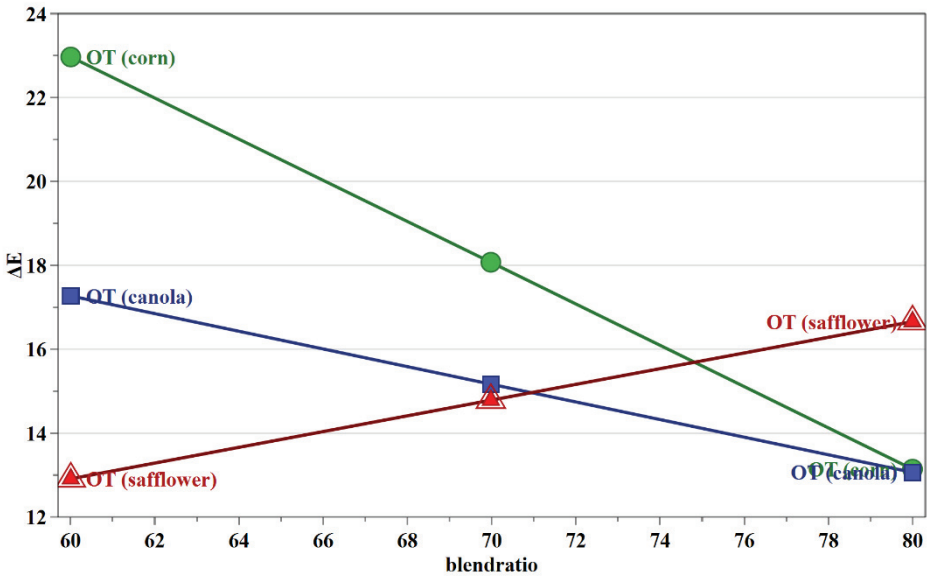

Figure 4.29 Interaction plot showing the effect of blend ratio x oil type for total color difference of structural lipids

Table 4.8 L, a, b and ΔE color values of the samples

Sample	L	a	b	ΔE	Sample	L	a	b	ΔE	Sample	L	a	b	ΔE
SA61	66.52	-3.73	10.52	15.11	**CA61**	68.38	-3.65	13.39	15.36	**CO61**	59.93	-4.01	14.05	22.57
SA62	69.43	-4.67	3.51	10.38	**CA62**	62.51	-3.35	13.86	20.23	**CO62**	60.59	-3.89	14.74	22.35
SA63	69.41	-4.41	7.85	11.46	**CA63**	66.26	-4.30	10.25	15.28	**CO63**	57.58	-3.97	15.21	25.18
SA71	65.00	-3.61	2.60	14.52	**CA71**	67.44	-3.65	12.45	15.44	**CO71**	63.36	-4.50	14.76	20.16
SA72	65.31	-4.37	13.25	17.69	**CA72**	66.63	-3.84	12.41	16.08	**CO72**	67.59	-3.16	16.29	17.94
SA73	68.12	-5.31	13.29	15.74	**CA73**	67.65	-4.02	13.52	16.02	**CO73**	63.14	-3.24	16.70	21.41
SA81	64.01	-4.08	3.72	15.59	**CA81**	68.94	-3.77	0.99	10.80	**CO81**	69.74	-4.65	11.06	12.98
SA82	68.68	-4.02	12.13	14.34	**CA82**	73.18	-5.32	12.79	12.22	**CO82**	71.84	-4.21	16.40	15.69
SA83	63.06	-5.06	10.30	18.24	**CA83**	65.16	-5.41	6.90	15.22	**CO83**	70.40	-4.33	10.30	11.94
SA60	69.35	-3.25	-2.56	11.51	**CA60**	70.44	-3.65	-2.20	10.44	**CO60**	66.04	-4.38	0.07	13.88
SA70	71.07	-2.56	-1.89	9.62	**CA70**	69.40	-2.48	-2.95	11.59	**CO70**	70.62	-3.37	0.26	9.28
SA80	72.91	-2.47	-0.88	7.52	**CA80**	75.00	-2.31	-0.37	5.48	**CO80**	74.23	-3.30	1.10	5.65
Tallow	79.42	-1.91	2.85	0.00						**CP1**	65.78	-3.68	15.85	18.92
										CP2	66.74	-4.29	15.15	17.82
										CP3	70.25	-4.15	5.81	9.89

*Abbreviations are provided in Materials & Methods section

Standard deviation of color measurements: L=±1.92, a=±0.26, b=±4.58, ΔE=±4.03 (calculated from CPs)

Various TAG profiles were created in the produced structured lipids throughout the reaction. Since each TAG has its own melting point, it is difficult to define a specific melting point for fats with heterogeneous TAG composition. For this reason, it is more appropriate to express melting point as a function of a given percentage of melted crystals. In this study, 85, 90 and 95% of melted crystals were considered and the melting temperatures at these ratios (MP85%, MP90%, MP95%) were measured as shown in Table 4.9.

As expected, higher crystal percentage corresponded to higher melting temperature. As it could be seen in Table 4.9, tallow has really high melting temperatures. After blending tallow with different oils, there are small decreases in melting points of the samples. However, after chemical interesterification sharp decreases in melting points of the structured lipids were observed regardless of oil type. These changes in melting points are in accordance with the previous studies (Ribeiro et al. 2017; Meng et al. 2011; Liu and Lampert 1999). The β' form of crystals has a high melting point between 17–69 °C and the melting point of β form is 32-78 °C depending on chain length of fatty acids (Nas et al. 2001). The interesterified fats were more likely to have β and β′ crystal types together and melting points of the samples were relevant to melting point of crystal types. Therefore, it is clear that polymorphic structure of interesterified lipids is highly related to melting points of the structured lipids.

Figure 4.30 shows the DSC melting profile of chemically interesterified samples of canola-tallow blend and at least three endothermic peaks with shoulders across a wide temperature range can be observed. These peaks are related to the melting of different TAG crystals. Peak 1, associated with TAGs of vegetable oils, is very low, needing very little energy to melt; this peak can be attributed to the presence of a high content of mono- and polyunsaturated fatty acids. Peak 2 represents middle-melting TAG species probably formed during interesterification reaction or non-esterified blends. Peak 3 belongs to high-melting TAG species associated with saturated TAGs deriving from tallow. In addition, red line presents percentage of melted fat crystals (Figure 4.30).

The melting temperatures of interesterified lipids increased by gradual increasing of percentage of crystals in lipid structure. All the samples containing 60% tallow have lower MP85% regardless of oil type. When the ratio of tallow raised from 60% to 80%, a clear increase was also observed in melting points at all melting percentages (Figure 4.31-32-33).

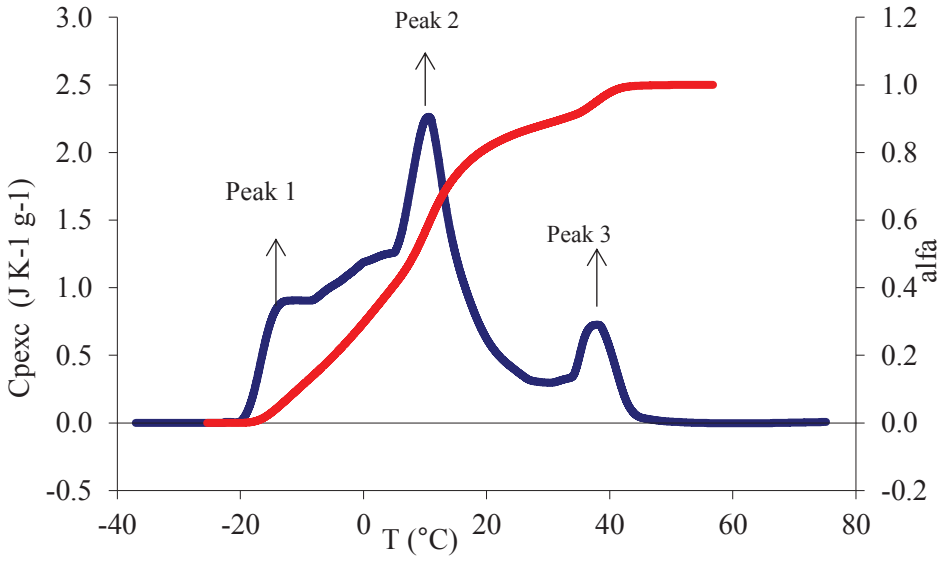

Figure 4.30 DSC thermogram of interesterified canola oil-tallow sample

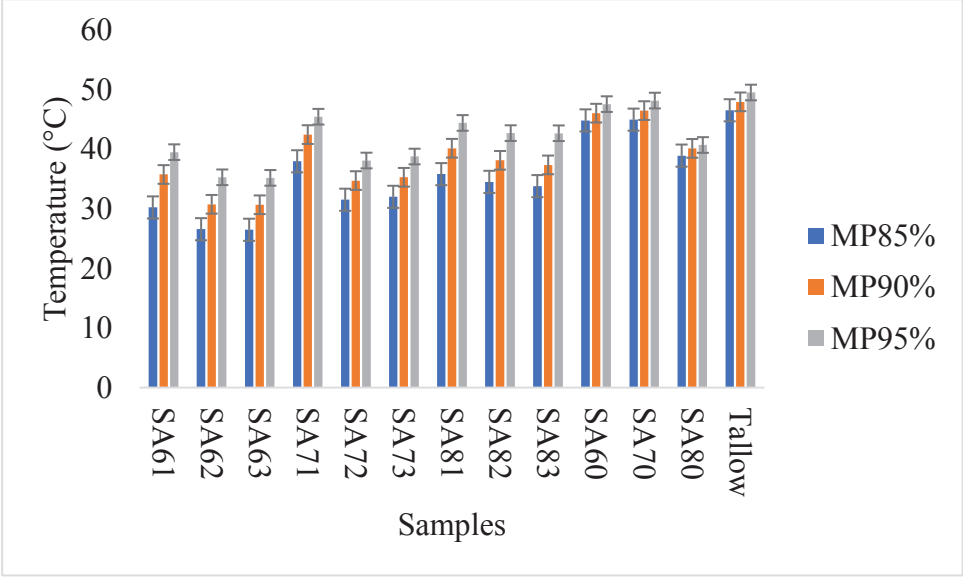

Figure 4.31 Melting temperatures of safflower oil samples at different percentages of melting

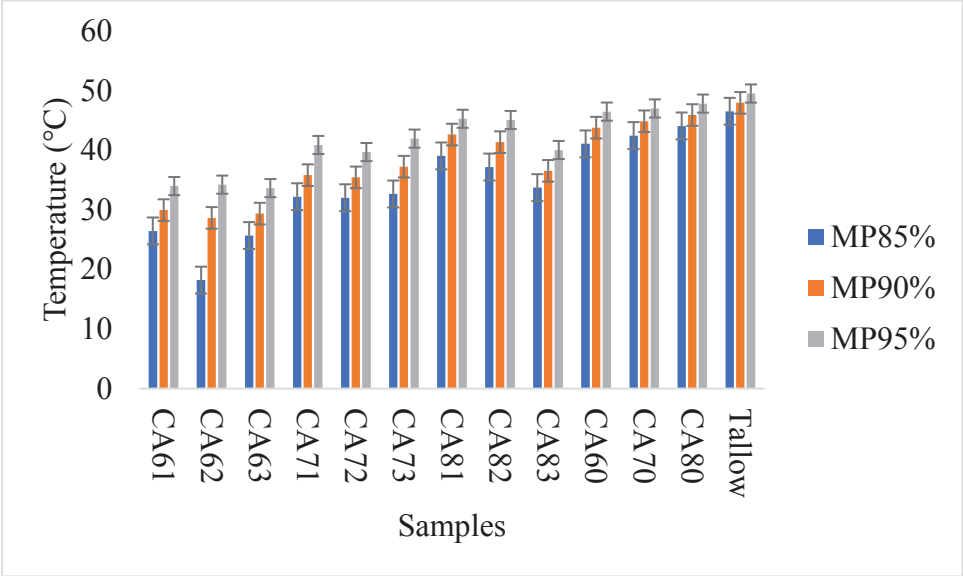

Figure 4.32 Melting temperatures of canola oil samples at different percentages of melting

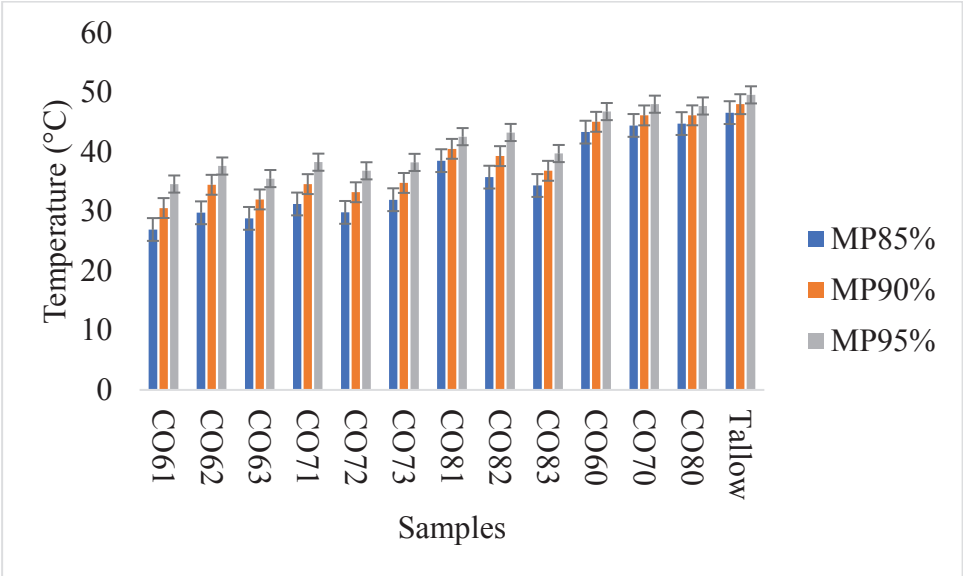

Figure 4.33 Melting temperatures of structured lipids with corn oil at different percentages of melting

Table 4.9 Melting points of chemically interesterified samples and tallow at various percentages of melted crystals

Sample	MP85%	MP90%	MP95%	Sample	MP85%	MP90%	MP95%	Sample	MP85%	MP90%	MP95%
SA61	30.25	35.79	39.54	**CA61**	26.48	29.98	34.01	**CO61**	26.94	30.54	34.58
SA62	26.60	30.78	35.32	**CA62**	18.22	28.67	34.25	**CO62**	29.75	34.45	37.60
SA63	26.50	30.70	35.22	**CA63**	25.70	29.38	33.67	**CO63**	28.81	31.99	35.50
SA71	38.00	42.49	45.49	**CA71**	32.23	35.84	40.91	**CO71**	31.23	34.56	38.25
SA72	31.55	34.76	38.13	**CA72**	32.05	35.47	39.72	**CO72**	29.82	33.20	36.80
SA73	32.03	35.32	38.80	**CA73**	32.68	37.27	41.98	**CO73**	31.94	34.76	38.21
SA81	35.84	40.19	44.45	**CA81**	39.08	42.66	45.31	**CO81**	38.50	40.49	42.54
SA82	34.55	38.17	42.73	**CA82**	37.21	41.39	45.11	**CO82**	35.74	39.27	43.24
SA83	33.83	37.40	42.71	**CA83**	33.75	36.57	40.07	**CO83**	34.33	36.80	39.70
SA60	44.87	46.09	47.60	**CA60**	41.10	43.82	46.52	**CO60**	43.30	45.03	46.75
SA70	45.00	46.52	48.20	**CA70**	42.50	44.89	47.04	**CO70**	44.42	46.10	47.99
SA80	38.96	40.17	40.74	**CA80**	44.11	45.94	47.86	**CO80**	44.73	46.12	47.68
Tallow	46.57	47.99	49.55					**CP1**	32.72	35.70	38.85
								CP2	33.11	36.34	39.72
								CP3	37.17	40.88	43.95

*Abbreviations are provided in Materials & Methods section

Standard deviation of melting points: MP85=±2.01, MP90=±2.31, MP95=±2.23 (calculated from CPs)

The ANOVA results of melting points given in Appendix 8 showed that the models at all melting percentages are significant at $p<0.05$ with non-significant lack of fit. Normality and residuals were also checked for the model. Blend ratio has the most prominent effect on melting temperatures of chemically interesterified lipids (App. A8). Figure 4.34 presents the main effect as blend ratio on MP85% for chemically interesterified samples. The MPs reach maximum values, when the blend ratio of tallow is 80% as it could be seen in Figure 4.34. Same trend was also observed at the other percentages of melting. However, catalyst concentration has only important impact on melting temperatures at 90 and 95% of melting. The increasing catalyst concentration leads to small decreases in MP95% of interesterified fats (Figure 4.35).

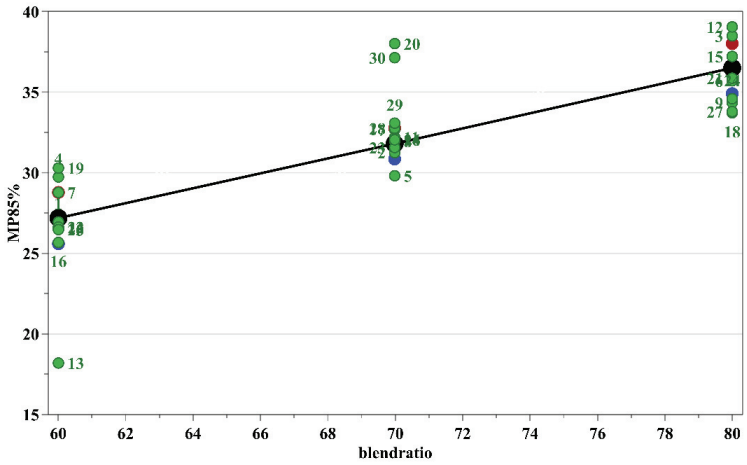

Figure 4.34 Main effect plot of blend ratio on MP85% of chemically interesterified fats

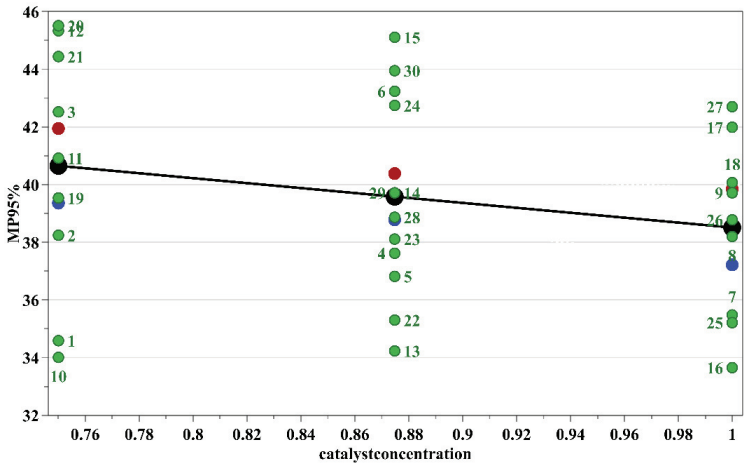

Figure 4.35 Main effect plot of catalyst concentration on MP95% of chemically interesterified fats

4.2.4 Slip Melting Point of Chemically Interesterified Lipids

The slip melting points (SMP) of chemically interesterified lipids are provided in Table 4.10. Interesterification reactions, as expected, caused a decline in SMPs of structured lipids in comparison to the tallow and the non-interesterified blends due to the randomization of TAG structure (Oliveira et al. 2017; Kowalska et al. 2007). SMP of tallow is 46.95 ºC while SMP of chemically interesterified samples varies between the temperature range of 34.25-42.80 ºC.

Figure 4.36 shows the SMP of chemically interesterified lipids graphically and it indicates that when the tallow is interesterified with safflower oil, SMP of the samples decreased with respect to catalyst concentration at 60% blend ratio. However, at higher blend ratios effect of catalyst concentration was not that significant. When corn oil is used in chemical interesterification, there is no change in SMP of structured lipids depending on catalyst concentration. However, SMP of the samples interesterified with canola oil slightly increased with increasing catalyst concentration at 60:40 and 70:30 ratios and decreased at 80:20 ratio (Figure 4.36).

Table 4.10 Slip melting points of chemically interesterified lipids

	SMP (ºC)				
SA61	39.10	**CA61**	32.75	**CO61**	35.15
SA62	37.20	**CA62**	32.60	**CO62**	35.10
SA63	31.80	**CA63**	35.30	**CO63**	34.25
SA71	40.15	**CA71**	37.65	**CO71**	35.20
SA72	36.95	**CA72**	38.85	**CO72**	36.90
SA73	37.30	**CA73**	39.30	**CO73**	38.10
SA81	41.65	**CA81**	42.80	**CO81**	40.15
SA82	39.35	**CA82**	40.05	**CO82**	39.80
SA83	39.75	**CA83**	36.10	**CO83**	39.60
SA60	44.00	**CA60**	43.85	**CP1**	39.45
SA70	44.55	**CA70**	45.25	**CP2**	38.50
SA80	45.75	**CA80**	45.95	**CP3**	41.70
Tallow	46.95			**CO60**	43.20
				CO70	45.10
				CO80	45.95

*Abbreviations are provided in Materials & Methods section
Standard deviation of SMP=±1.34 (calculated from CPs)

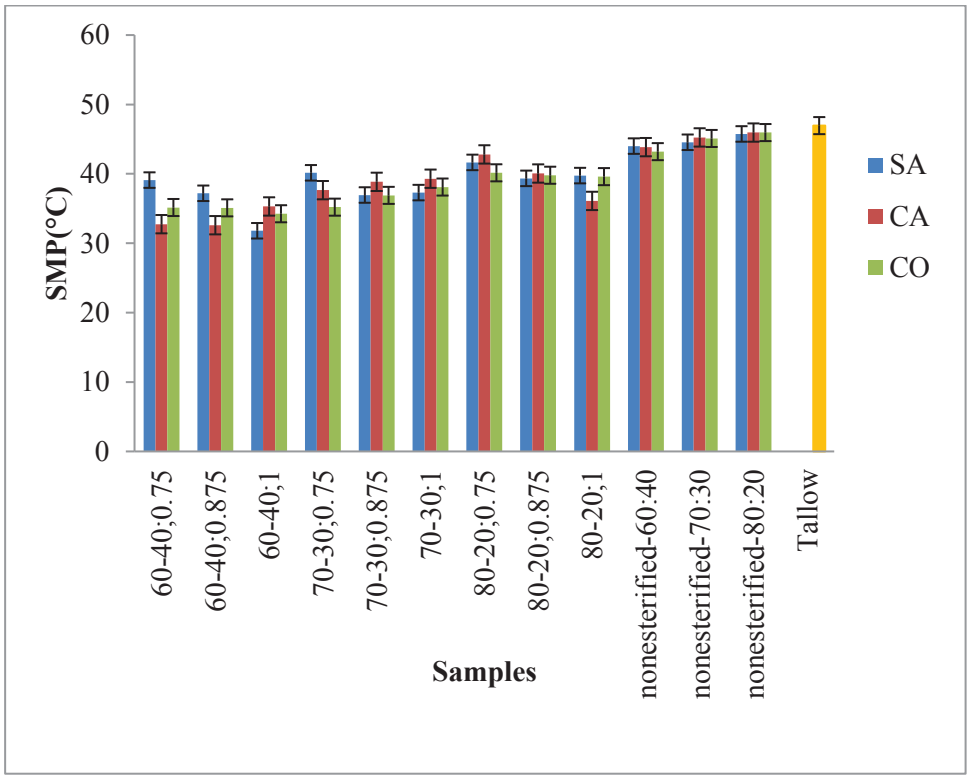

Figure 4.36 Slip melting points of the samples

The statistical analysis results for SMP of chemically interesterified samples are given in Appendix 8. ANOVA results indicated that constructed model is significant at $p<0.05$ with non-significant lack of fit. Blend ratio is the only significant factor for SMP of the chemically interesterified lipids. The main effect plot also confirms this result stated above (Figure 4.37). Slip melting points of interesterified fats increase by increasing tallow concentration.

As regards to the relationship between SMP and MP, the correlations were calculated. It was observed that MP85 and MP90 values were either lower or equal to SMPs, whereas temperatures corresponding to 95% melted crystals were higher than SMPs (Figure 4.38-40). Therefore, it can be inferred that SMP measured by the open capillary method roughly corresponds to the melting of 85-90% of the fat sample. Therefore, a good correlation between SMP and MP95 was obtained ($r = 0.88$).

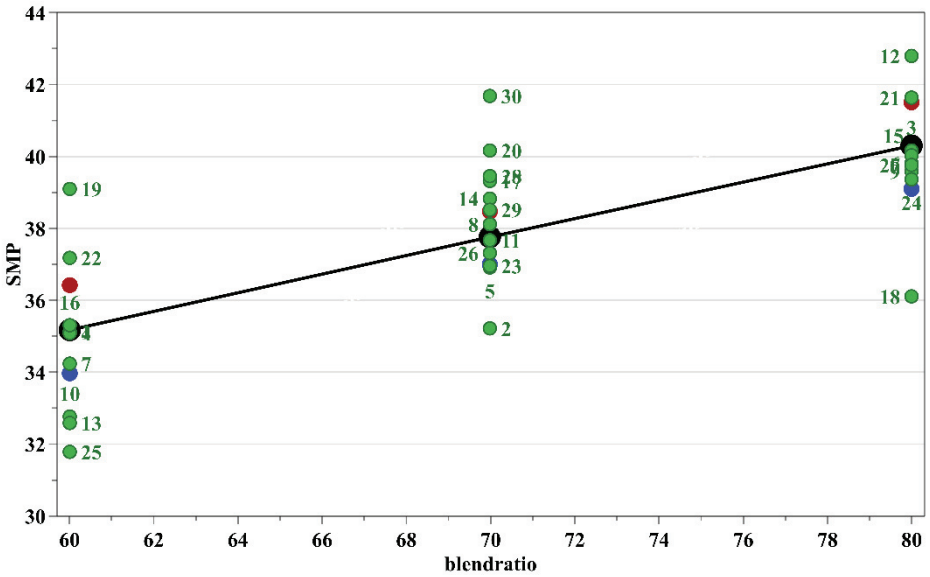

Figure 4.37 Main effect plot of blend ratio on SMP of chemically interesterified fats

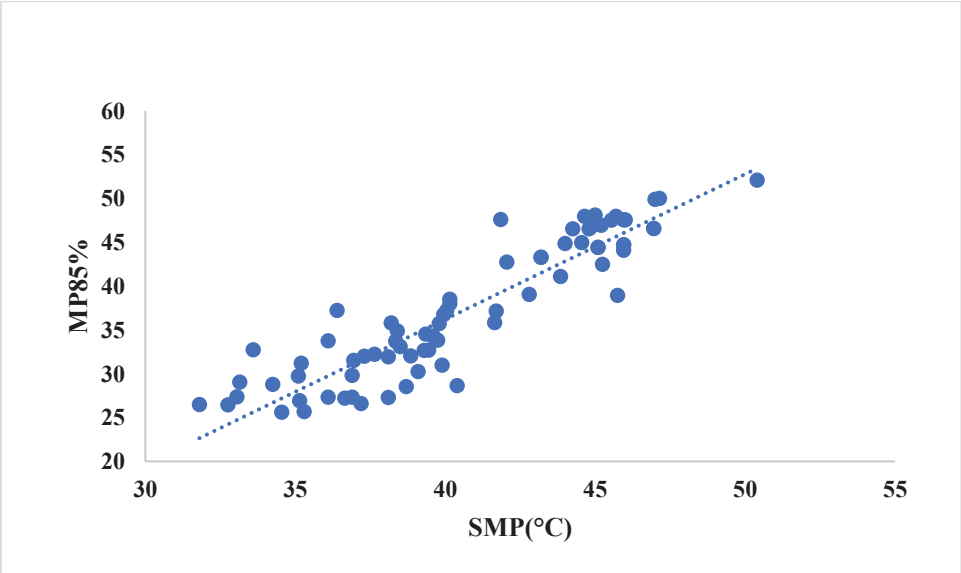

Figure 4.38 MP85% versus SMP of chemically interesterified lipids

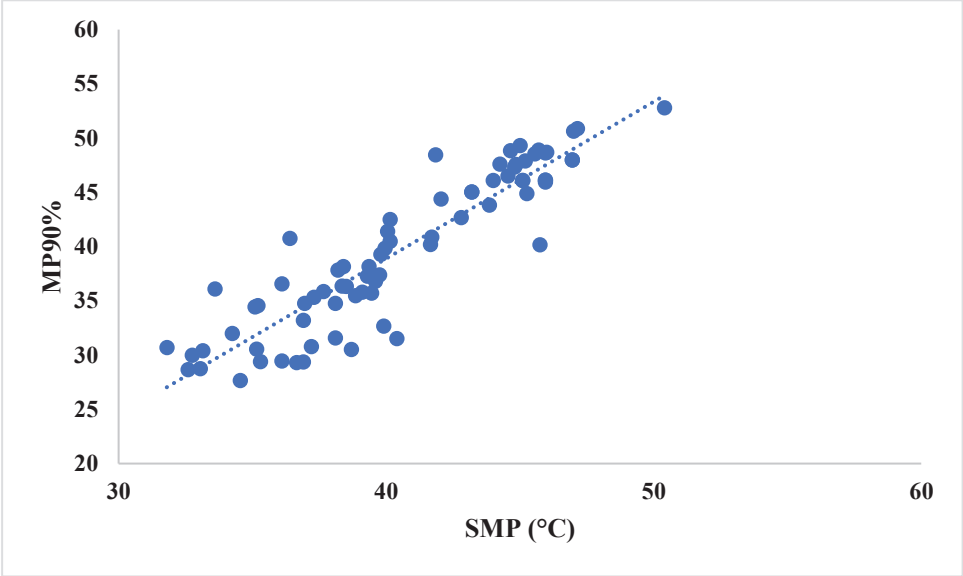

Figure 4.39 MP90% versus SMP of chemically interesterified lipids

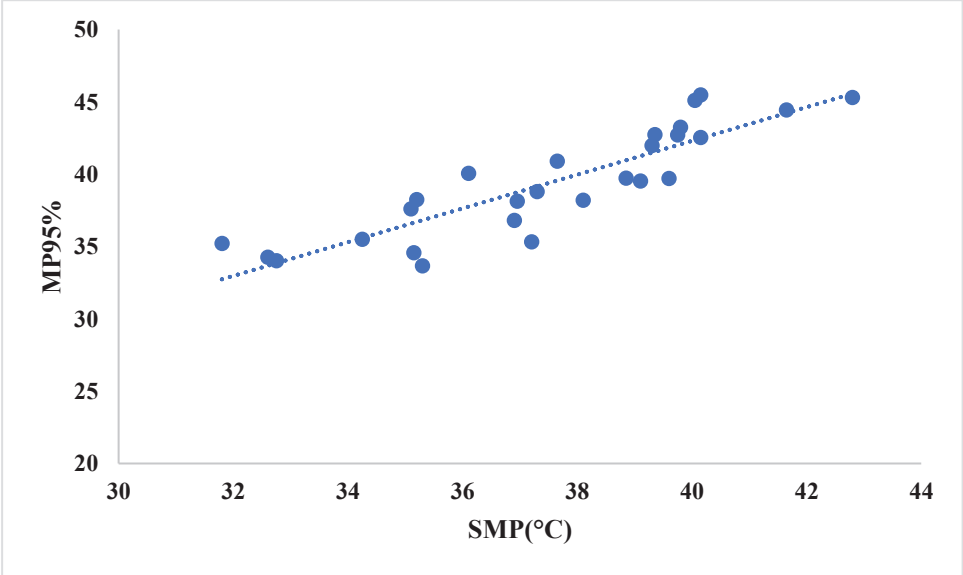

Figure 4.40 MP95% versus SMP of chemically interesterified lipids

4.2.5 Consistency of Chemically Interesterified Lipids

Consistency measurement is important for classification of the fat plasticity. Consistency of the samples was determined by Texture Analyzer over the temperature range of 4-25 °C. The consistency was calculated as ‘‘yield value’’ (MPa) and results for chemically interesterified samples are given in Table 4.11. The consistency of all samples

decreased clearly as a function of temperature. This result can be associated with the gradual melting of crystals that generate more fragile crystalline networks. The same behavior was also observed in the previous studies (Silvia et al. 2009; Bezzera et al. 2017; Oliviera et al. 2017). The consistency of tallow (385.93-69.85 MPa) was quite higher than both interesterified lipids and non-interesterified blends at all temperatures. Consistency of blends increased with the increasing amounts of tallow in the blends. However, interesterified lipids tend to show lower consistency values compared to their physical blends regardless of oil type and catalyst concentration. This decrease in the consistency of interesterified lipids is attributed to higher amounts of UUU of TAGs produced by chemical interesterification. In addition, differences in polymorphic structure and aggregation behavior which led to alteration in the structure of the fat crystal network of tallow can change the consistency (Marangoni and Rousseau 1998; Silvia et al. 2009).

Generally, fats with consistencies of 9.8-98 MPa are considered spreadable. If the consistency is between 19.6 and 78.4 MPa products are more suitable for plastic and spreadable purposes and they are considered to be very hard above 147 MPa (Haighton 1959). Therefore, chemical interesterification allowed manufacturing of the structured lipids which have better plastic and spreadable properties compared to tallow.

As it could be seen in Figure4.41, 42 and 43 consistencies of most of the samples interesterified with safflower oil were not measurable at 25 °C; therefore, these structured lipids are viscous at ambient temperature. The consistency of SA61, SA72, SA63 could not be determined at all testing temperatures, since these lipids are highly viscous at refrigeration temperature. The other lipids interesterified with safflower oil are spreadable and plastic.

Most of the samples interesterified with canola oil can be considered as spreadable and plastic (Figure 4.44-4.46). The consistency values decreased to the levels suitable for spreadability with the increasing temperature. However, the consistency of CA82, CA83 were very high at 4 °C, and these lipids can be classified as hard. Moreover, these structured lipids contains β' polymorphic forms which have higher melting points also.

Interesterified samples produced with corn oil also resulted in products mostly with spreadable and plastic properties (Figure 4.47-4.49). The consistency of CO81 and CO82 were very high at 4 and 10 °C, and these lipids can be classified as hard. However, the consistency values decreased to the appropriate levels for spreadability as the temperature increased.

Table 4.11 Consistency values of chemically interesterified lipids and tallow

	Consistency (Mpa)												
	4°C	10°C	15°C	25°C		4°C	10°C	15°C	25°C		4°C	10°C	15°C	25°C
SA61	0.00	0.00	0.00	0.00	**CA61**	55.94	11.81	3.23	1.52	**CO61**	44.97	12.61	9.30	2.34
SA62	37.82	3.60	0.00	0.00	**CA62**	17.46	42.18	0.00	0.00	**CO62**	33.21	11.97	3.46	0.00
SA63	0.00	0.00	0.00	0.00	**CA63**	40.59	20.45	6.75	3.64	**CO63**	19.93	4.22	0.00	0.00
SA71	48.03	12.04	9.24	0.00	**CA71**	77.16	24.36	3.46	2.22	**CO71**	76.22	30.23	9.50	6.54
SA72	0.00	0.00	0.00	0.00	**CA72**	64.13	21.78	4.43	4.23	**CO72**	76.14	35.27	6.34	2.08
SA73	411.08	78.05	55.16	0.00	**CA73**	612.49	158.38	153.14	3.34	**CO73**	28.73	14.81	0.00	0.00
SA81	668.90	164.73	103.09	6.79	**CA81**	68.42	42.75	12.73	16.12	**CO81**	127.13	53.43	10.38	25.01
SA82	66.57	36.81	29.38	5.80	**CA82**	174.25	51.99	19.60	10.71	**CO82**	302.19	339.40	105.96	5.16
SA83	136.04	69.51	11.11	0.00	**CA83**	121.97	71.50	47.15	0.00	**CO83**	88.33	72.67	15.60	0.00
SA60	116.14	0.00	0.00	0.00	**CA60**	61.49	35.31	13.45	10.74	**CP1**	59.44	19.44	5.50	7.03
SA70	150.92	42.13	14.88	11.63	**CA70**	78.94	49.03	16.75	14.35	**CP2**	69.37	17.07	29.23	11.37
SA80	140.36	88.02	42.81	26.02	**CA80**	159.64	91.23	32.69	22.93	**CP3**	52.51	28.23	9.10	8.49
Tallow	385.93	224.52	87.57	69.85						**CO60**	54.89	16.62	10.23	9.08
										CO70	97.62	70.13	26.40	9.60
										CO80	164.09	101.37	39.69	20.16

*Abbreviations are provided in Materials & Methods section

Standard deviation of consistency at 4 °C=±6.92, at 10 °C= ±4.8, at 15 °C=±10.44, at 25 °C=±1.8, (calculated from CPs)

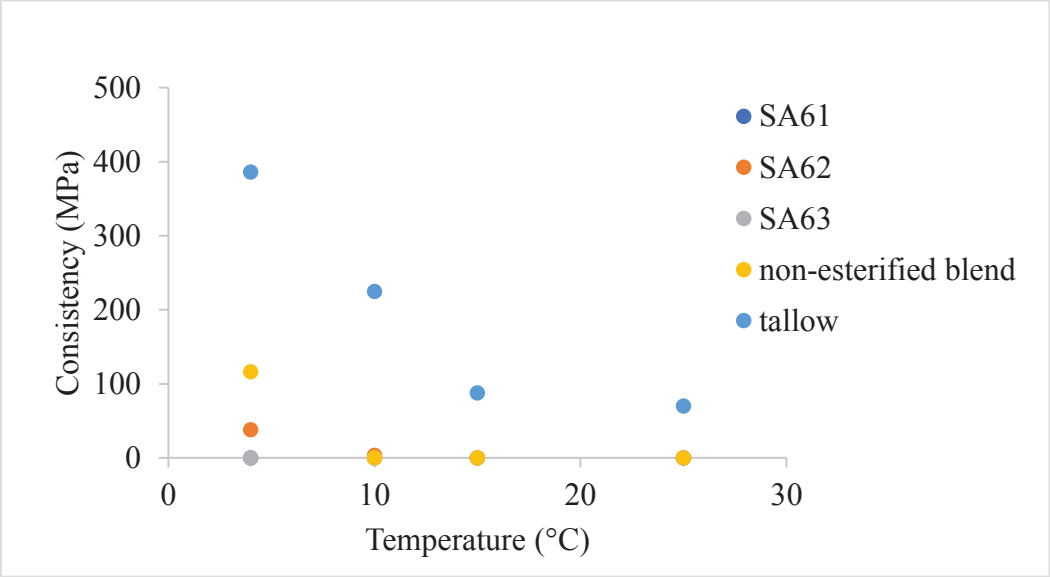

Figure 4.41 Consistency of the samples interesterified with safflower oil and tallow at 60:40 ratio (%)

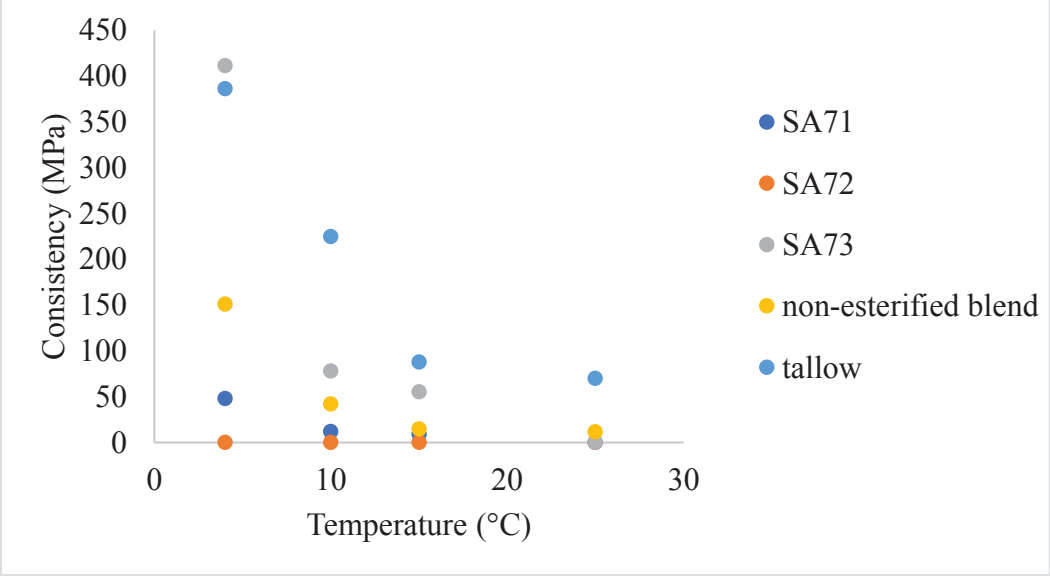

Figure 4.42 Consistency of samples interesterified with safflower oil and tallow at 70:30 ratio (%)

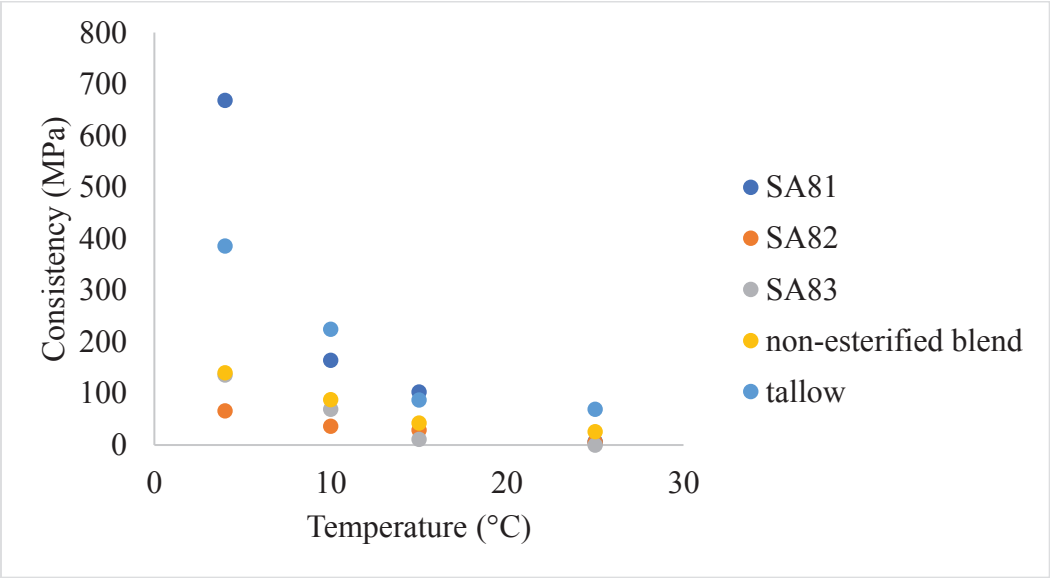

Figure 4.43 Consistency of samples interesterified with safflower oil and tallow at 80:20 ratio (%)

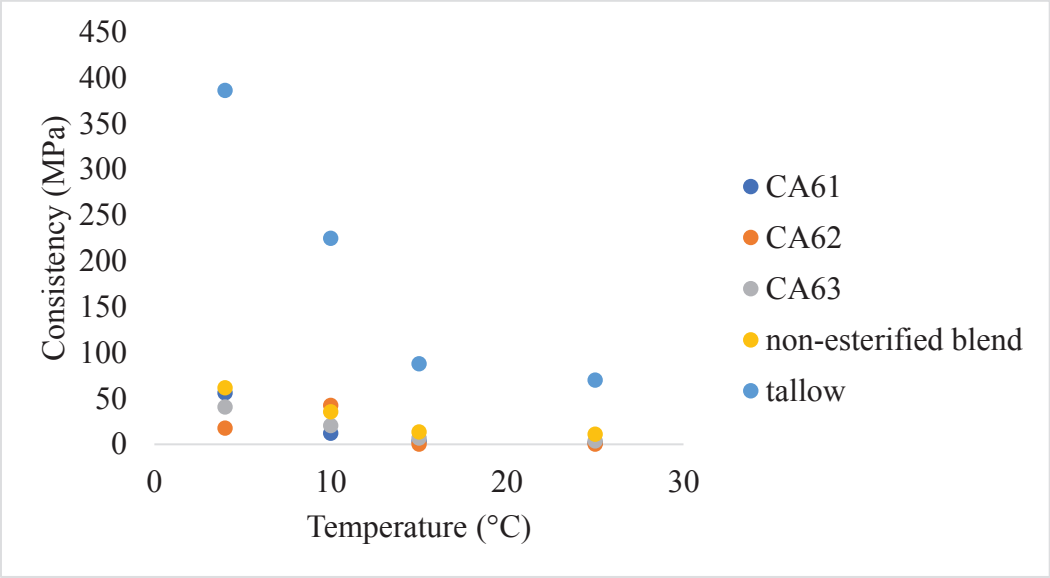

Figure 4.44 Consistency of samples interesterified with canola oil and tallow at 60:40 ratio (%)

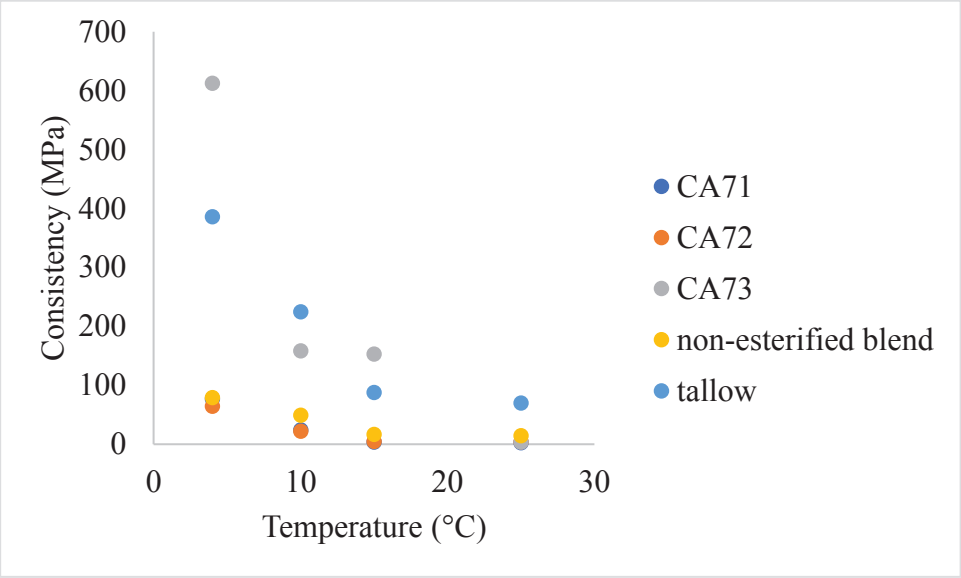

Figure 4.45 Consistency of samples interesterified with canola oil and tallow at 70:30 ratio (%)

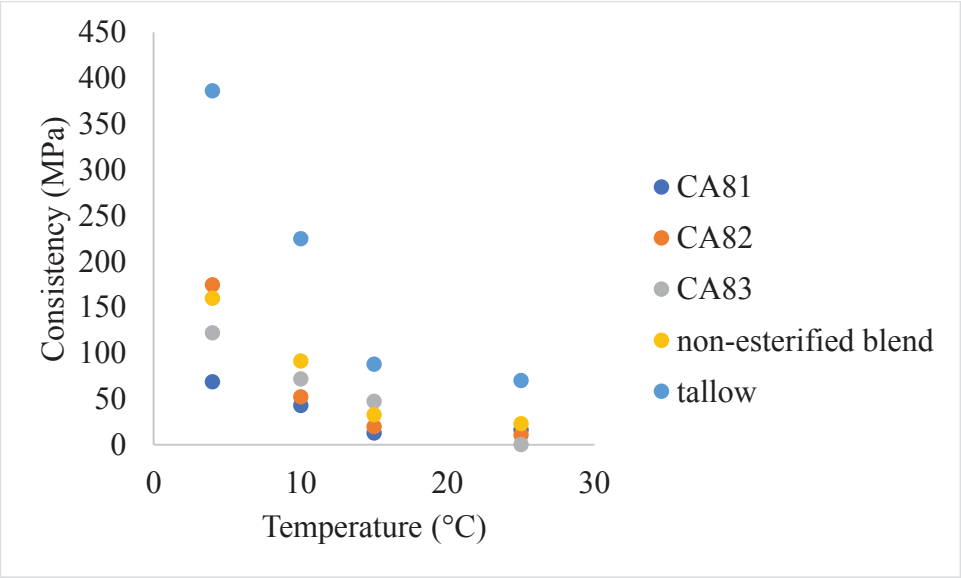

Figure 4.46 Consistency of samples interesterified with canola oil and tallow at 80:20 ratio (%)

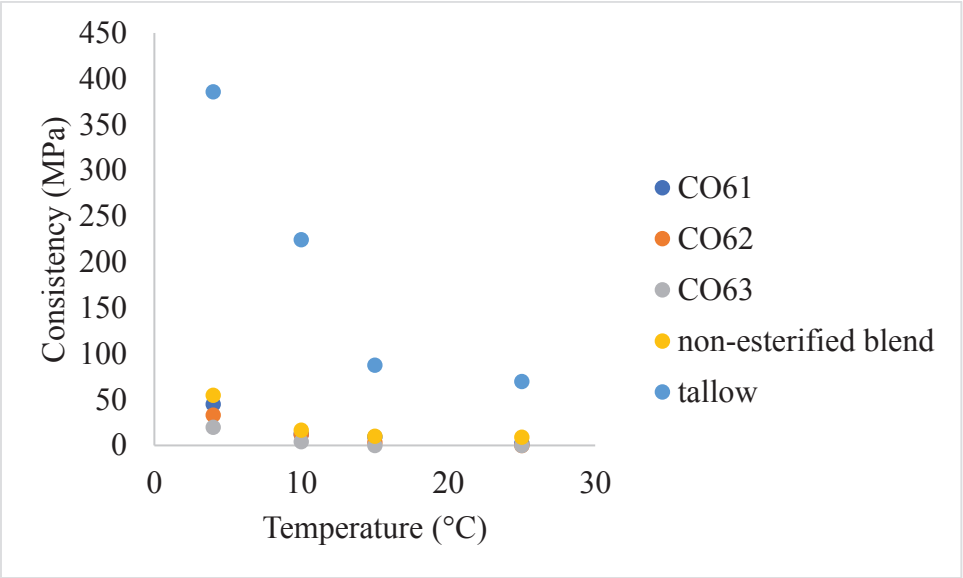

Figure 4.47 Consistency of samples interesterified with corn oil and tallow at 60:40 ratio (%)

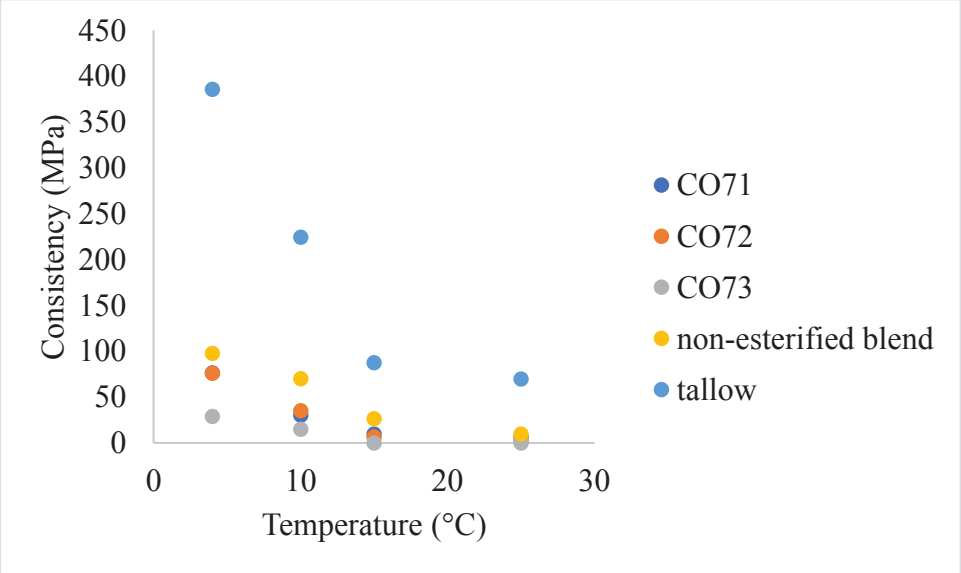

Figure 4.48 Consistency of samples interesterified with corn oil and tallow at 70:30 ratio (%)

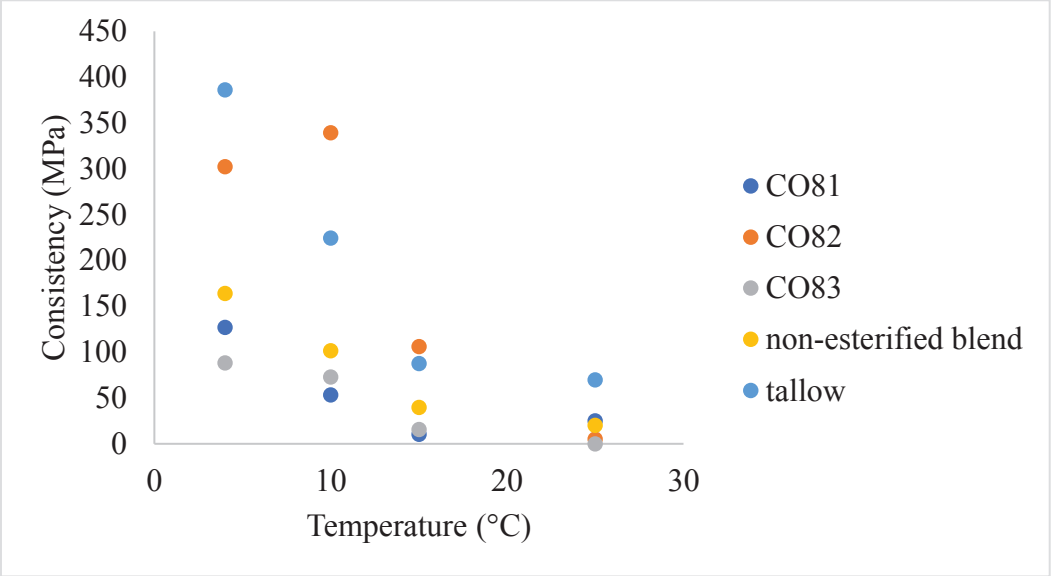

Figure 4.49 Consistency of samples interesterified with corn oil and tallow at 80:20 ratio (%)

Appendix 8 shows the statistical analysis results for consistency at all temperatures. ANOVA results indicated that constructed models were insignificant at 4,10 and 15 °C at $p<0.05$. Although the models are found as insignificant, ANOVA table reveals that blend ratio has important impact on consistency at these temperatures. The model for consistency at 25 ºC is significant with non-significant lack of fit. Blend ratio, catalyst concentration and their interactions are the most prominent factors for this model (App. A8). In addition, ANOVA table indicated that oil type especially safflower oil highly affects the consistency of structured lipids at 25°C.

Figure 4.50 shows the effect of blend ratio-catalyst concentration interaction on consistency at 25 ºC. The consistency increases at low and moderate catalyst concentration with increasing blend ratio and this increase is more significant at the lowest catalyst concentration as Figure 4.50 indicated. The highest catalyst concentration did not cause much change in consistency at 25 ºC.

Figure 4.51 shows the effect of oil type on consistency at 25 ºC. The structured lipids interesterified with safflower oil have lower consistency values compared to other oil types.

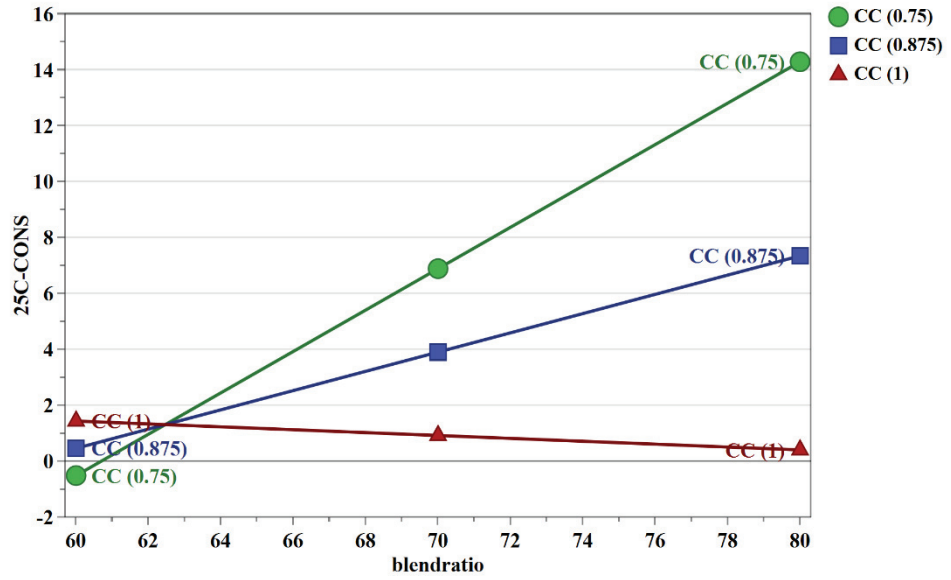

Figure 4.50 Interaction plot showing the effect of blend ratio and catalyst concentration for consistency at 25 ºC of structural lipids

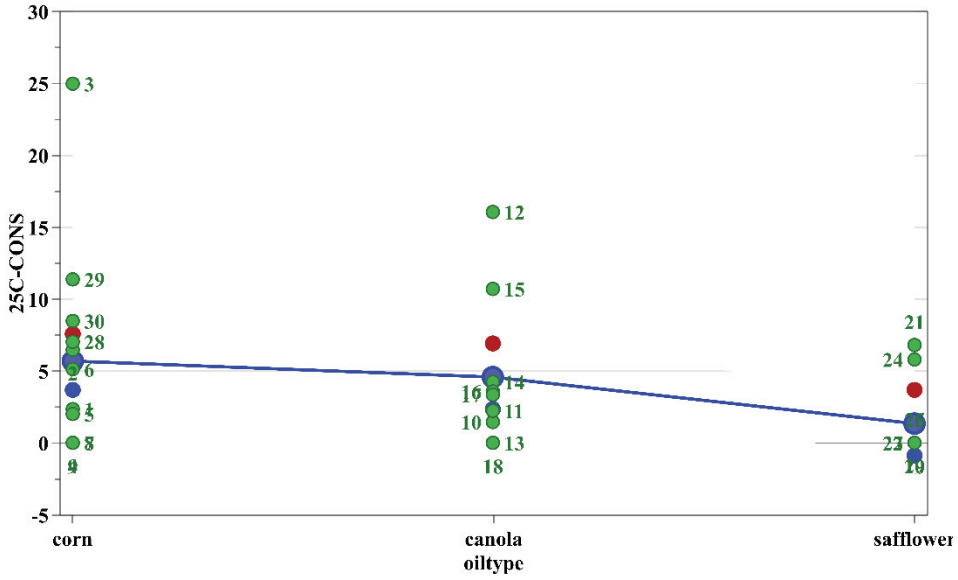

Figure 4.51 Main effect plot of oil type of chemically interesterified samples for consistency at 25 ºC

4.2.6 Solid Fat Content of Chemically Interesterified Lipids

Solid fat content (SFC) is a measure of the percentage of fat in crystalline (solid) phase to total fat across a temperature gradient. SFC is an important parameter for the evaluation of possible applications of structured lipids which is based on physical properties. SFC percentage of samples was determined by Nuclear Magnetic Resonance spectroscopy at 4 different temperatures and the data are given in Table 4.12. SFC of both interesterified lipids and non-interesterified blends were determined over the temperature range of 10–35 °C. It was observed that raising the temperature caused a marked decrease in the value of SFC regardless of oil type, blend ratio and catalyst concentration. SFC profiles of non-interesterified blends in different proportions have increasing trend with the increasing amounts of tallow in the blends. Interesterified lipids tend to have lower SFC% values compared to their physical blends. Same trends were found out in the interesterification of tallow and palm oils (Meng et al. 2010; Karabulut et al. 2004). The decrease in the SFC of interesterified lipids could be attributed to decreased proportion of the high-melting TAGs and medium chain TAGs in the structure of lipids. This decrease in SFC with respect to increase in temperature is expected as in other studies (Fauzi et al. 2013; Bezzera et al. 2017; Oliveira et al. 2017). In addition, the decrease of SFC in tallow and non-esterified blends can be associated with the alteration in TAGs structure caused by chemical interesterification and melting temperature of crystals. However, above 20 °C more plastic behavior was observed for both blends and structured lipids.

As the tallow ratio was increased, SFC of the samples increased particularly for the lipids interesterified with safflower oil. Increase in catalyst concentration did not sifnificantly affect SFC for safflower containing samples (Figure 4.52, 4.53 and 4.54). There is a decrease in SFC of all samples as a function of temperature as expected.

For the lipids interesterified with canola oil, SFC of the samples increased as well with an increase in tallow ratio. When the catalyst concentration was raised, SFC did not change significantly regardless of blend ratio for samples with canola oil (Figure 4.55, 4.56 and 4.57). There is a decrease in SFC of samples as a function of temperature.

As in other oils increase in tallow ratio in tallow-corn oil blends caused an increase in SFC. In addition, catalyst concentration did not have much affect on SFC (Figure 4.58, 4.59 and 4.60).

Table 4.12 Solid fat content (%) of chemically interesterified lipids and tallow

	Solid fat content (%)													
	10°C	**20°C**	**30°C**	**35°C**		**10°C**	**20°C**	**30°C**	**35°C**		**10°C**	**20°C**	**30°C**	**35°C**
CA61	22.9	11.1	4	1.6	**SA61**	20.7	10.7	4.7	2.1	**CO61**	20.4	10.7	4.4	1.6
CA62	18.2	8.7	3.1	0.9	**SA62**	25.3	17.7	7.9	4	**CO62**	22.3	11	4.8	2
CA63	23.1	11.2	3.8	1.4	**SA63**	20.8	10.4	4.5	1.9	**CO63**	20.6	9.8	4	1.6
CA71	34.6	18.8	7.4	4	**SA71**	27.2	18.7	9.5	5.9	**CO71**	22	12	5.2	2.8
CA72	36.5	19.5	7.6	3.9	**SA72**	27.1	15.3	7.1	3.8	**CO72**	20.8	10.4	4.5	1.9
CA73	30.3	17.1	7.3	3.8	**SA73**	29.4	17.2	7.9	4.5	**CO73**	29.2	15.9	7.3	3.6
CA81	38.1	26.7	14.2	9.3	**SA81**	37.5	24.6	12	7.2	**CO81**	39.8	26.1	12.6	7.2
CA82	43.6	27.7	12.7	7.2	**SA82**	36.6	24	11.7	6.9	**CO82**	38.9	26.1	12.4	7
CA83	37.6	23.5	10.6	5.7	**SA83**	34	21.5	9.8	5.7	**CO83**	35.6	23.3	11.4	6.4
CA60	33.9	22.4	12.5	8.4	**SA60**	33.5	22.6	12.2	8.2	**CO60**	27.8	17.5	9.4	6.2
CA70	39.5	26.5	14.8	9.8	**SA70**	39.2	26.6	15	9.9	**CO70**	40.1	26.6	15	10.1
CA80	47.1	31.7	18.2	12.2	**SA80**	46.6	31.6	18.11	12	**CO80**	46.8	32.4	18.5	12.6
Tallow	51.1	42.7	24	17.3						**CP1**	29.3	17	7.6	4
										CP2	35.7	20.1	8.4	4.5
										CP3	27.7	20.2	10.7	6.5

*Abbreviations are provided in Materials & Methods section

Standard deviation of solid fat content: at 10 °C=±3.46, at 15 °C=±1.49, at 30 °C=±1.31, at 35 °C=±1.08 (calculated from CPs)

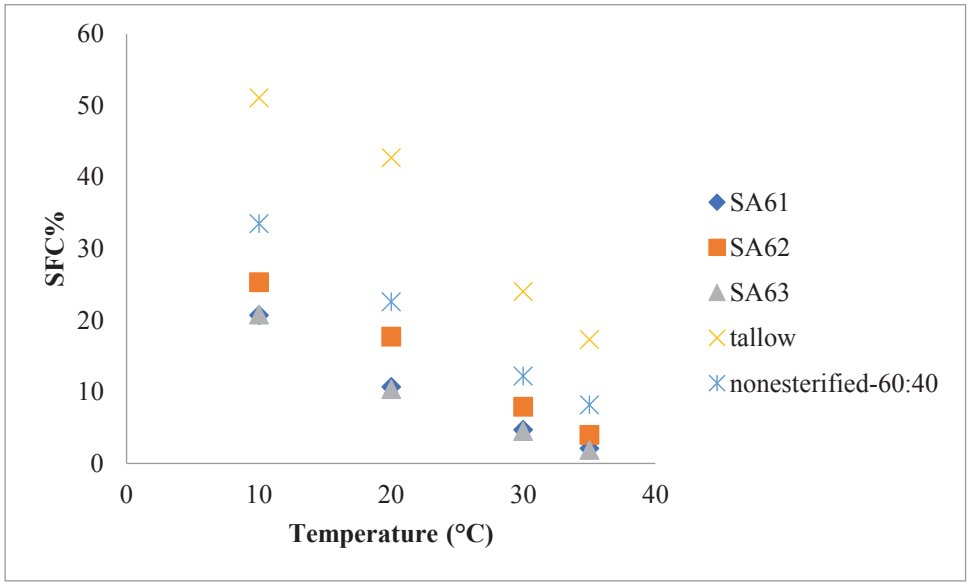

Figure 4.52 Solid fat content (SFC%) versus temperature for the samples interesterified with safflower oil and 60% tallow

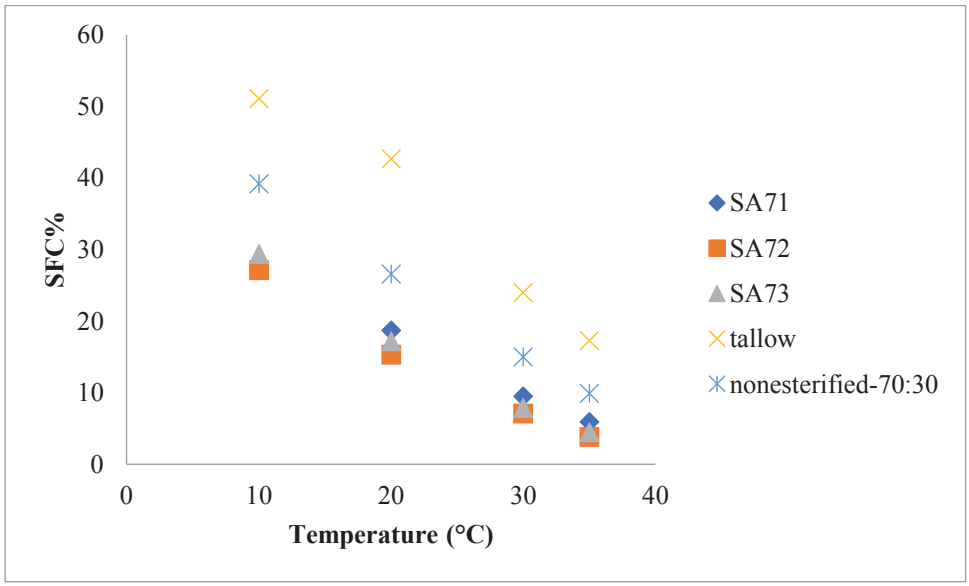

Figure 4.53 Solid fat content (SFC%) versus temperature for the samples interesterified with safflower oil and 70% tallow

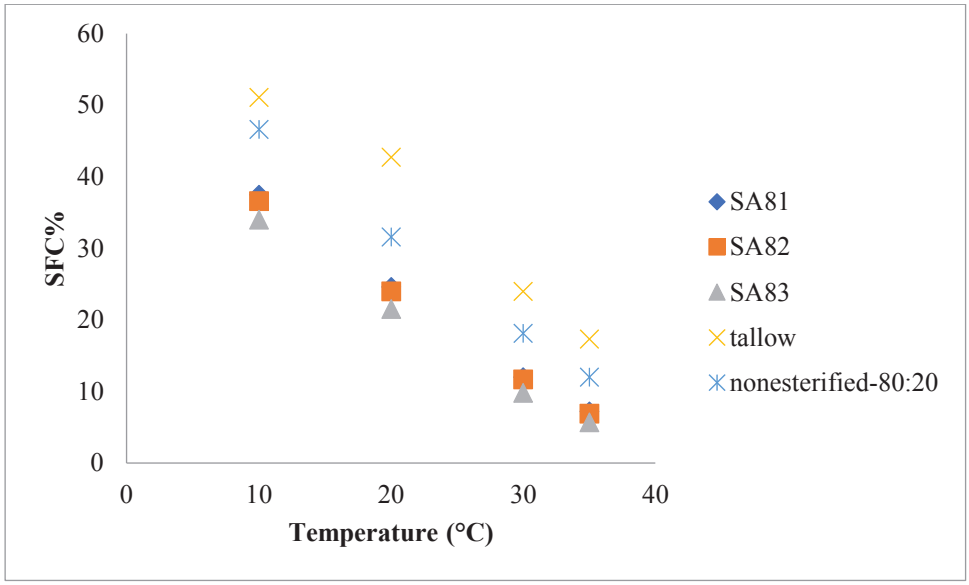

Figure 4.54 Solid fat content (SFC%) versus temperature for the samples interesterified with safflower oil and 80% tallow

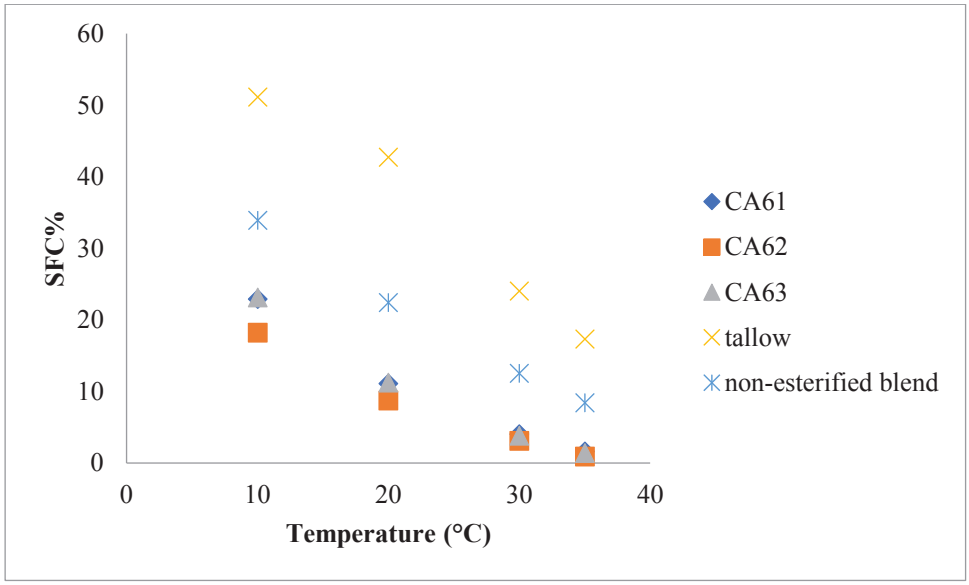

Figure 4.55 Solid fat content (%) versus temperature for the samples interesterified with canola oil and 60% tallow

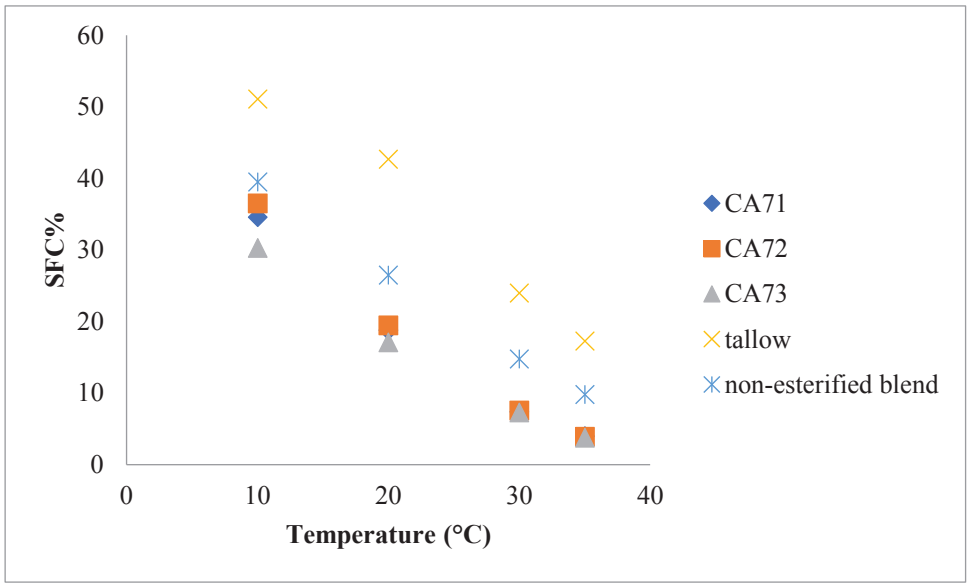

Figure 4.56 Solid fat content (SFC%) versus temperature for the samples interesterified with canola oil and 70% tallow

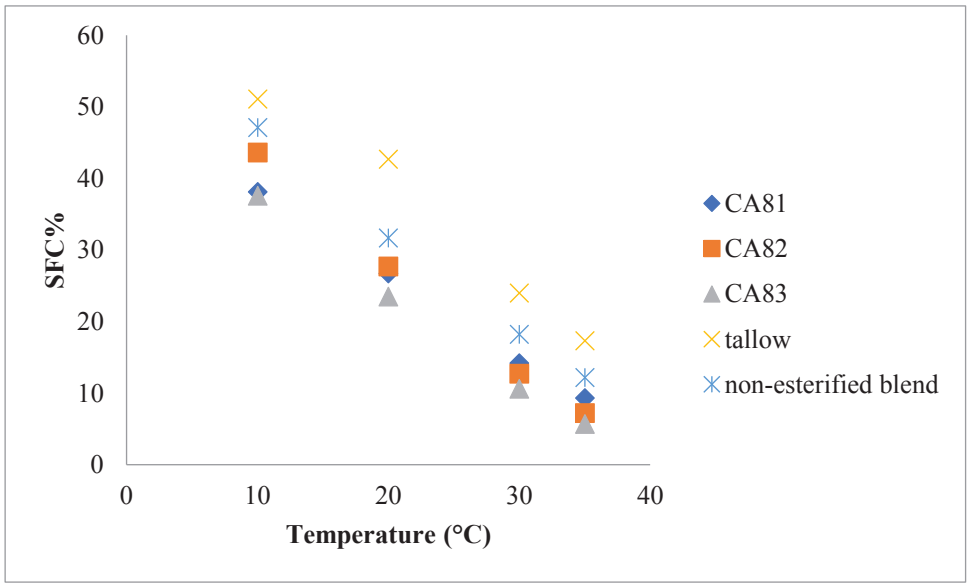

Figure 4.57 Solid fat content (SFC%) versus temperature for the samples interesterified with canola oil and 80% tallow

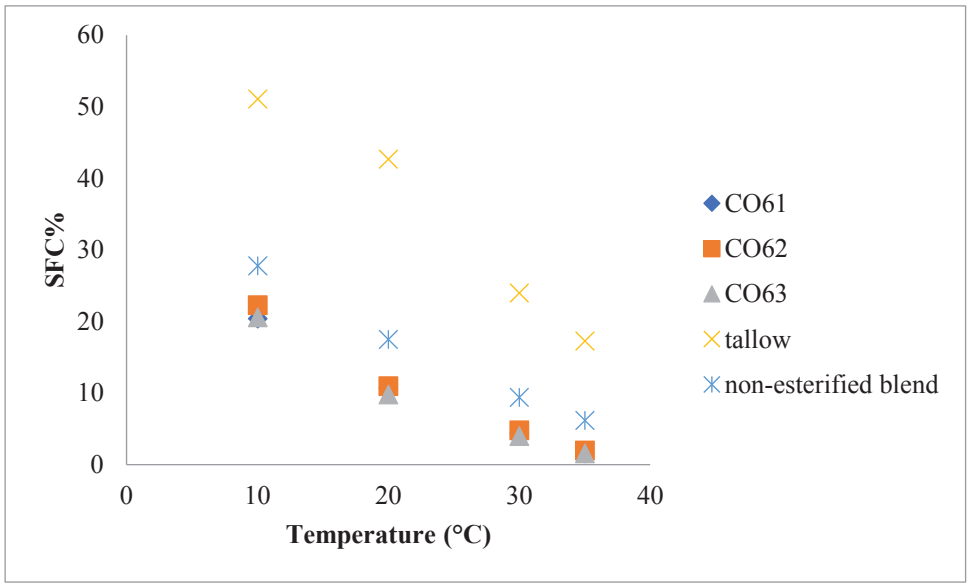

Figure 4.58 Solid fat content (SFC%) versus temperature for the samples interesterified with corn oil and 60% tallow

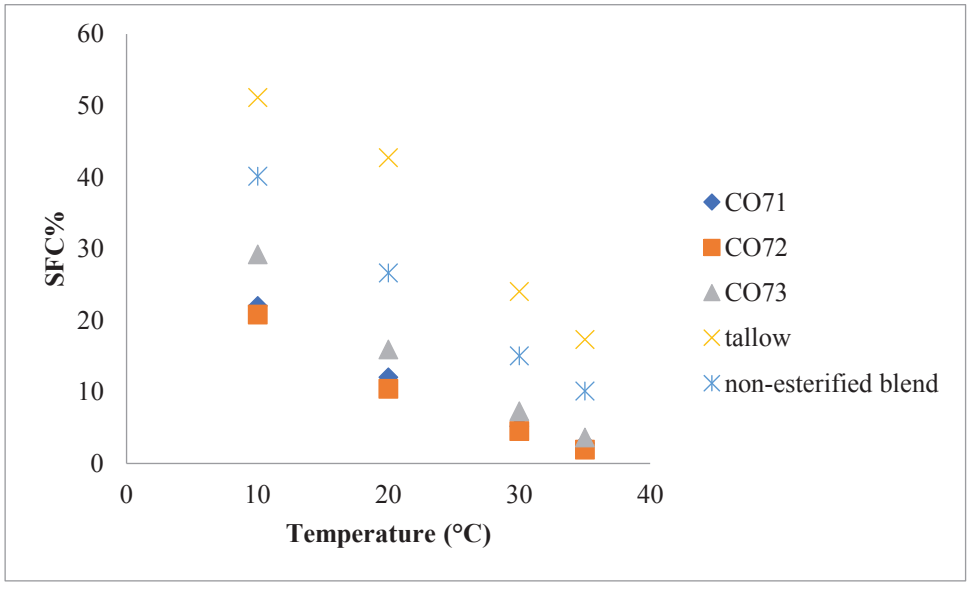

Figure 4.59 Solid fat content (SFC%) versus temperature for the samples interesterified with corn oil and 70% tallow

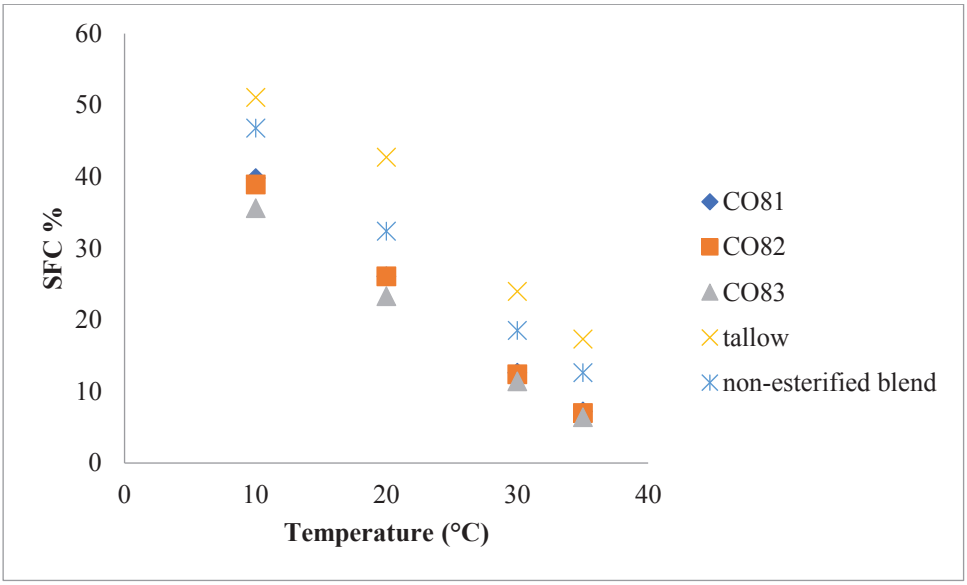

Figure 4.60 Solid fat content (SFC%) versus temperature for the samples interesterified with corn oil and 80% tallow

Appendix 8 shows the statistical analysis results for solid fat content of interesterified lipids at all temperatures. ANOVA results indicated that constructed models were significant with non-significant lack of fit at all temperatures. The ANOVA table reveals that blend ratio is the only effective factor for this model (App. A8) meaning that blend ratio highly affects the SFC of structured lipids in the temperature range of 10-35 °C. SFC% of samples increased regardless of oil type when the amount of tallow increased for all temperatures and the effect of blend ratio on SFC is shown for 10°C in Figure 4.61.

The correlations were tried to be established in between consistency and solid fat content results of interesterified lipids at a constant measurement temperature (10 °C). No correlation was found between consistency and SFC of samples at 10 °C. However, good correlations were obtained between melting points (MP85-90-95%) and solid fat content (30-35 °C) of structured lipids (r=0.86-0.89). Figure 4.62 presents the relationship between MP85% -SFC at 35 °C and reveals that most of the samples have MP85% lower than 35 °C. This means that 85% percent of fat crystals are melted below 35 °C. In addition, when the melting point rises above 35 °C, SFC of the samples increases as well (Figure 4.62).

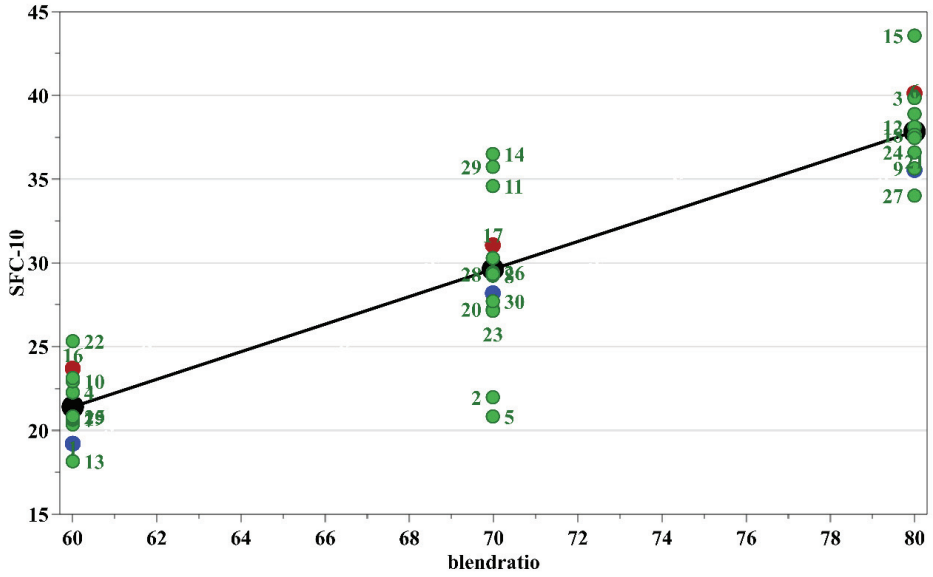

Figure 4.61 Main effect plot of blend ratio of chemically interesterified samples for SFC% at 10 ºC

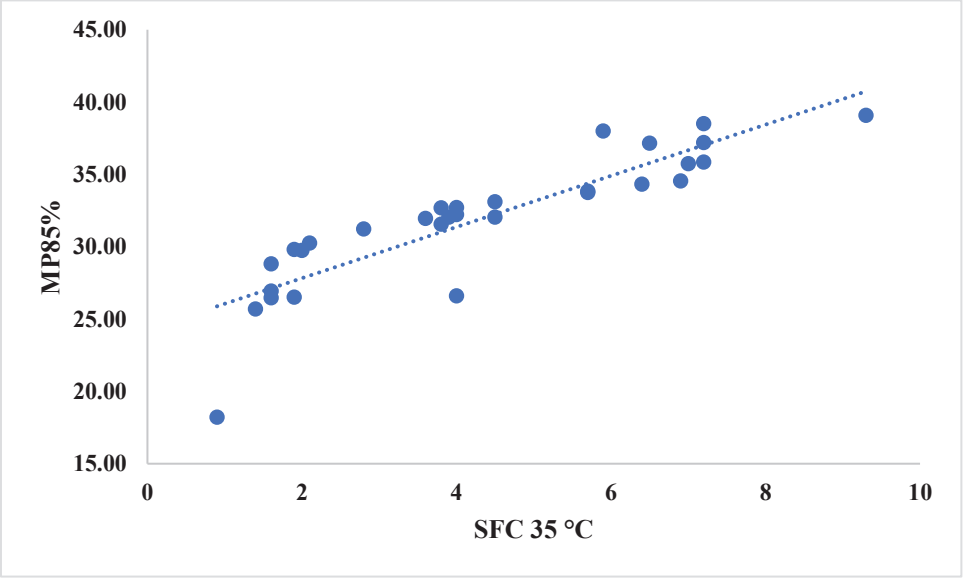

Figure 4.62 MP85% versus SFC at 35 °C of chemically interesterified lipids

In order to better analyze the physical data of chemically interesterified lipids principal component analysis (PCA) was also used. The model was constructed by using all measured physical parameters with 2 PCs, $R^2 = 0.72$, and $Q^2 = 0.56$. There is not a clear discrimination of the samples with respect to the oil type (Figure 4.63). However, the samples containing 60% of tallow located at the left part of quartile while the structured lipids with 80% placed in the right quartile. Samples having 70% tallow are

generally placed between those two percentages. Therefore, there is a rough discrimination between the samples with respect to their blend ratio as far as the physical properties are concerned. Discrimination between 60% and 80% tallow containing samples mostly resulted from higher SFC, SMP and melting point of samples as observed in loading plot (Figure 4.62). Moreover, some of the samples with 80% and 70% tallow grouped at the bottom of the ellipse regardless of oil type since they have higher consistency values (Figure 4.63-64).

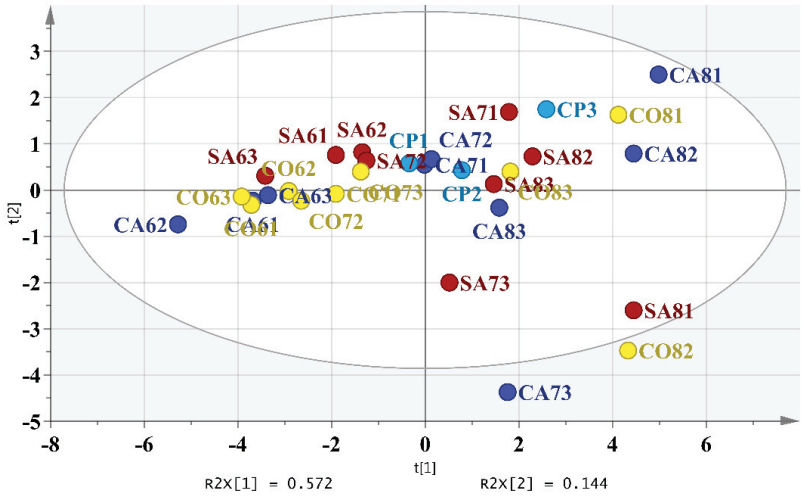

Figure 4.63 Score plot of the PCA model constructed by using all physical parameters of chemically interesterified lipids (CP1-2-3=70% tallow & 30%corn oil; 0.875% catalyst concentration, samples were colored with respect to oil type)

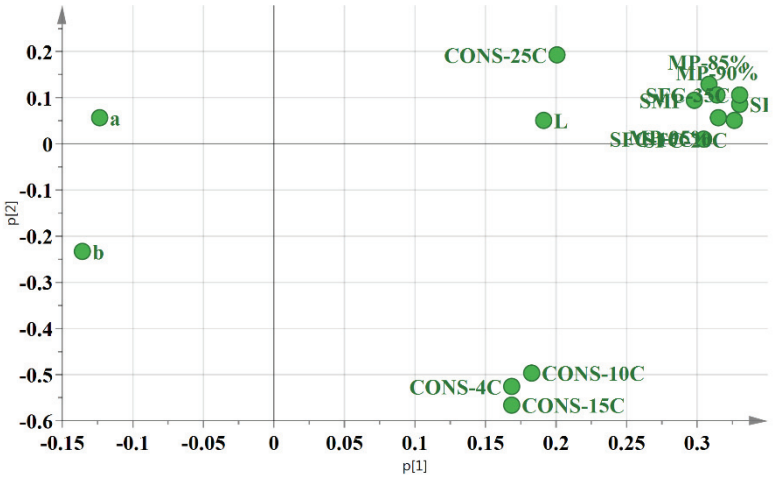

Figure 4.64 Loading plot of the PCA model constructed by using all physical parameters of chemically interesterified lipids

In order to better characterize chemically interesterified lipids a PCA model was also constructed by using all data including both chemical and physical results with 5 PCs, R^2=0.80, and Q^2=0.45. There is a clear separation of the samples with respect to the oil type according to this model (Figure 4.65). Samples produced with canola oil located at the right part of ellipse while the structured lipids with safflower oil placed at left part of ellipse. Samples containing corn oil are generally placed between them. Discrimination of canola oil containing samples is mostly resulted from higher TFA and MUFA and OS of these samples as observed in loading plot (Figure 4.66). Moreover, canola oil containing samples grouped together in between them according to blend ratio since the amount of saturated and unsaturated fatty acids changed by increasing ratio of tallow (Figure 4.66). The interesterified lipids containing safflower oil separated from other lipids due to their higher PUFA and MAG content. The groupings in between samples according to blend ratio were also observed. Corn oil samples mostly separated from the others due to their higher consistency values as loading plot confirmed. The results of PCA models were in accordance with ANOVA results. Generally, the catalyst concentration does not have remarkable effect on the chemical and the physical properties of structured lipids. Blend ratio is the most significant factor followed by oil type. Among the interesterified fats, samples produced with corn oil are discriminated from the others due to their better physical and chemical properties.

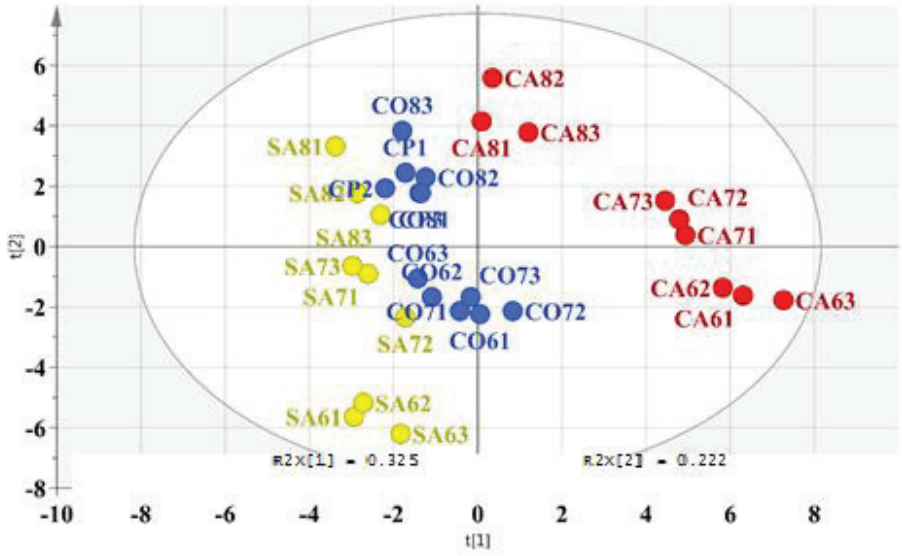

Figure 4.65 Score plot of the PCA model constructed by using all parameters of chemically interesterified lipids (CP1-2-3=70% tallow & 30% corn oil; 0.875% catalyst concentration, samples were colored with respect to oil type)

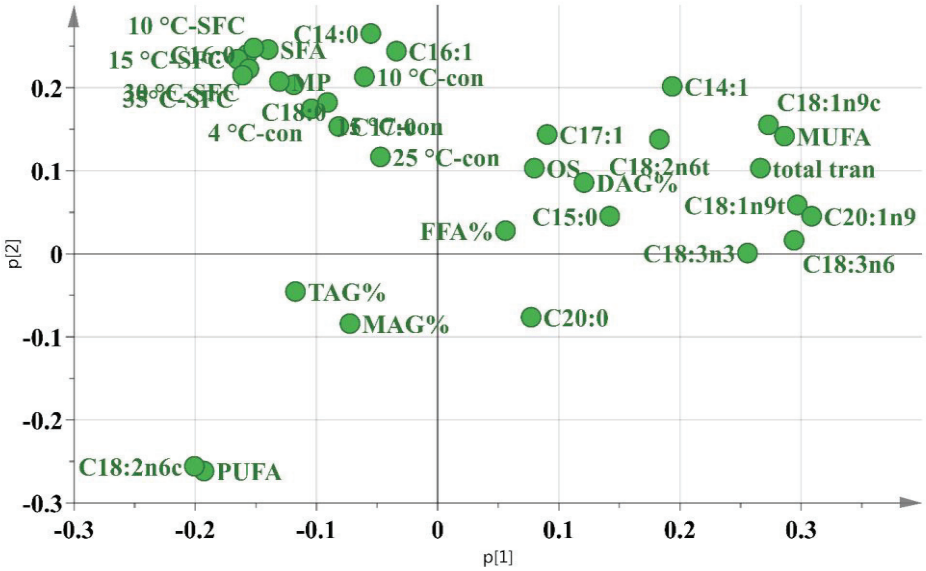

Figure 4.66 Loading plot of the PCA model constructed by using all parameters of chemically interesterified lipids

4.3. Near and Mid-Infrared Spectroscopic Characterization

In order to characterize chemically interesterified fats, spectral data were also collected by mid (FT-IR) and near infrared (FT-NIR) spectrometers. FT-NIR and FT-IR spectra were acquired both on melted and solid form of structured lipids. The principal component analysis (PCA) was applied to the spectral data of interesterified lipids to investigate the differences between the samples. Four different PCA models were constructed with FT-IR and FT-NIR spectra of solid and melted forms of the samples. The model which was constructed by using melted NIR spectra had 3 PCs, $R^2 = 0.99$, and $Q^2 = 0.99$. Although not perfect there is some discrimination of samples with respect to the oil type (Figure 4.67). Most of the corn oil containing samples were located at the left part of quartile while the structured lipids with safflower oil placed in the right quartile except one. Samples produced with canola oil are generally placed between those two percentages.

The model was constructed by using solid NIR spectra with 2 PCs, $R^2 = 0.99$, and $Q^2 = 0.98$. There is not a clear discrimination of samples with respect to the oil type (Figure 4.68). However, the samples containing corn oil mostly located together at the

right upper part of quartile. In addition, samples having 80% tallow are generally placed at the right bottom quartile regardless of oil type.

The PCA model obtained from IR melted spectra contains 7 PCs with R^2=0.99 and Q^2=0.98. PCA score plot was also plotted by coloring samples according to their blend ratio (Figure 4.69). Discrimination of samples with respect to the blend ratio is not very clear again. However, samples containing 60% of tallow located around upper part quartile while the structured lipids with 80% tallow placed in the bottom.

The PCA model of FT-IR solid spectra with 9 PCs, R^2=0.99 and Q^2=0.98 did not show any discrimination according process parameters. However, most of the samples produced with 0.75% catalyst concentration come together at the left part of the quartile (Figure 4.70).

The results of PCA models were in accordance with ANOVA results. Generally, catalyst concentration does not have remarkable effect on chemical and physical properties of structured lipids. Blend ratio is the most significant factor followed by oil type. Among the interesterified fats, samples produced with corn oil are discriminated from the others due to their better physical and chemical properties.

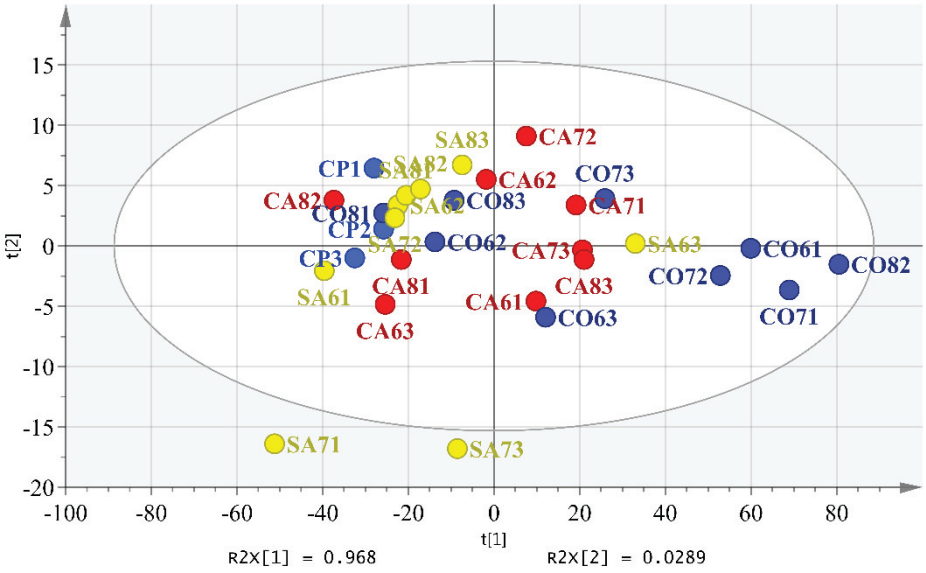

Figure 4.67 Score plot of the PCA model constructed by using melted spectra of FT-NIR of chemically interesterified lipids (CP1-2-3=70% tallow & 30% corn oil; 0.875% catalyst concentration, samples were colored with respect to oil type)

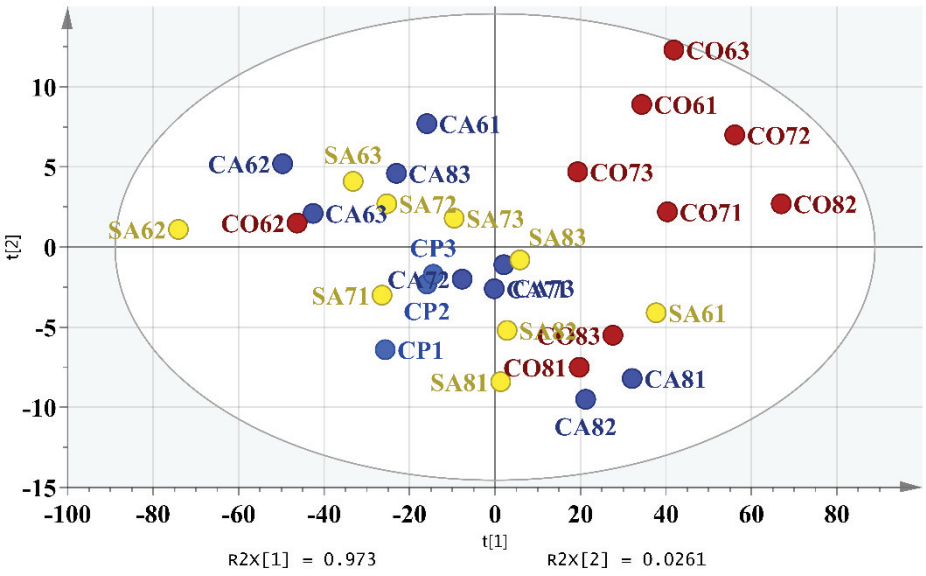

Figure 4.68 Score plot of the PCA model constructed by using solid spectra of FT-NIR chemically interesterified samples (CP1-2-3=70% tallow & 30% corn oil; 0.875% catalyst concentration, samples were colored with respect to oil type)

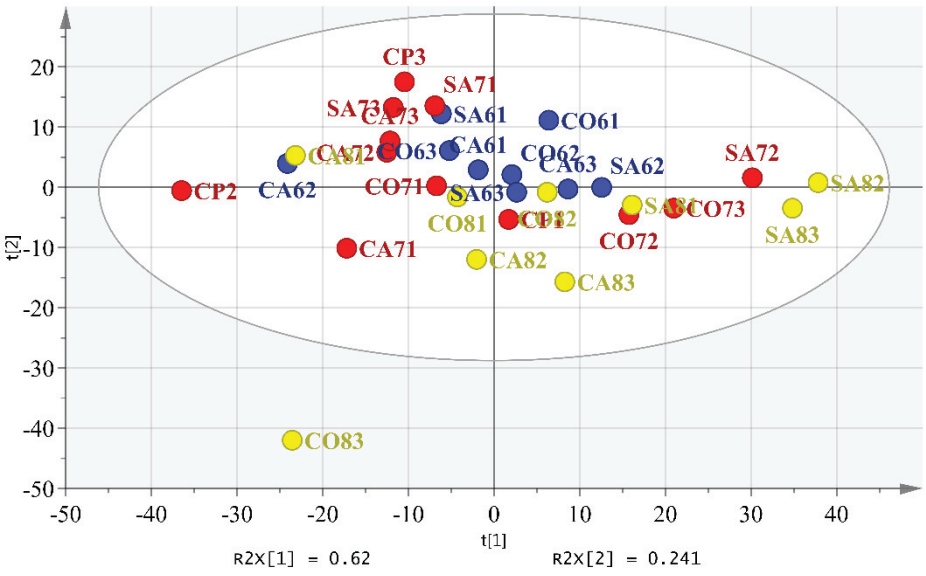

Figure 4.69 Score plot of the PCA model constructed by using melted spectra of FT-IR of chemically interesterified lipids (CP1-2-3=70% tallow & 30% corn oil; 0.875% catalyst concentration, samples were colored with respect to blend ratio)

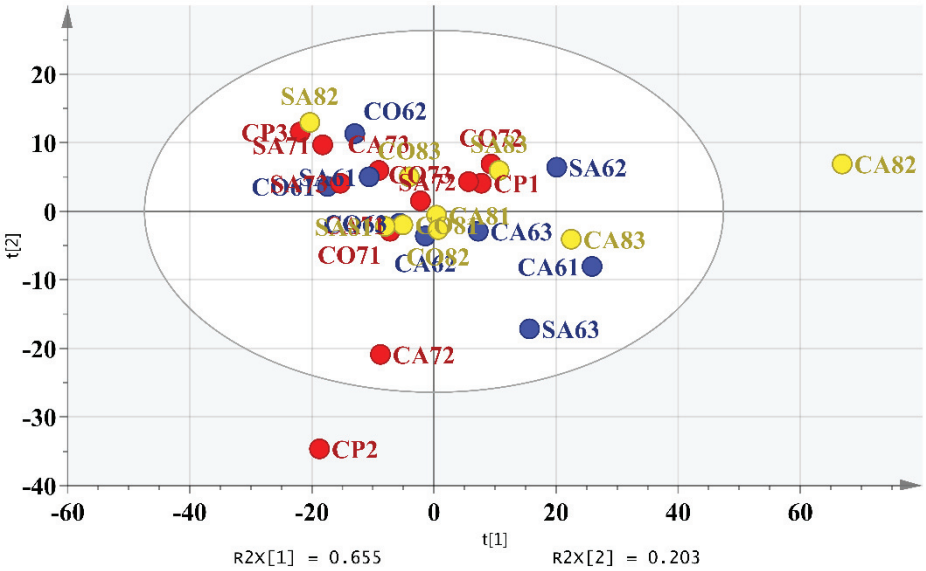

Figure 4.70 Score plot of the PCA model constructed by using solid spectra of FT-IR of chemically interesterified lipids (CP1-2-3=70% tallow & 30% corn oil; 0.875% catalyst concentration, samples were colored with respect to blend ratio)

CHAPTER 5

MONITORING OF THE CHEMICAL INTERESTERIFICATION OF TALLOW-CORN OIL BLENDS

5.1. Changes in the Chemical Properties of the Structured Lipids During the Chemical Interesterification

As explained in Chapter 4, the structured lipids produced with chemical interesterification of tallow with corn oil have better physical properties, higher oxidative stability and lower free fatty acid (FFA) content compared to the other structured lipids. Therefore, a new set of chemically interesterified lipids was manufactured by tallow and corn oil including reaction time as a parameter for the process monitoring part of the study. Details of the process and experimental parameters are explained in the Materials & Methods section. Since the previous results indicated that catalyst concentration had generally no significant effect on the properties of the samples, constant catalyst concentration of 0.75% (w/w) of sodium methoxide was used in the process monitoring study. The same chemical properties were determined, and the data were analyzed by univariate (ANOVA) and multivariate statistical analysis (PCA) techniques to investigate the effects of blend ratio (60:40, 70:30, 80:20 w/w) and reaction time (0, 10, 20, 30 min).

5.1.1. Fatty Acid Profiles during the Chemical Interesterification

The fatty acid compositions of the chemically interesterified samples during the process are given in Table 5.1. Compositions of the products are in agreement with the previous studies (O'Brien 2008; Meng et al. 2011; Kowalski et al. 2004). The major fatty acids in all products are oleic, palmitic, stearic and linoleic acids. In general, interesterification reactions did not cause sharp changes in the amounts of fatty acids of the structured lipids. Oleic and palmitic acid contents did not change either by blending

or interesterifying. However, there is a decrease in linoleic acid concentration of the interesterified samples with increasing blend ratio of tallow.

As in the previous researches, chemical interesterification of tallow did not cause formation of trans fatty acids (TFA) (Liu et al. 2010). Corn oil itself has TFA content of less than 1% and its interesterified forms with tallow have slightly higher percentages of trans fats. Structured lipids have a TFA content in the range of 0.59-1.25% and the amount of fatty acids in the trans form is less than 1% for all interesterified samples except the one produced with 60:40 ratio and interesterified for 10 min. These results indicate that these structured lipids are suitable for the production of low trans-fat containing shortenings, margarines and frying fats (Figure 5.1).

Neither blending nor chemical interesterification with corn oil at three different reaction time did not change the monounsaturated fatty acid (MUFA) composition of tallow-corn mixtures and the amounts were not also different compared to tallow itself (Figure 5.2). The chemical interesterification did not cause any important changes in polyunsaturated (PUFA) ratio of the samples as well (Figure 5.3). Saturated fatty acid (SFA) content of tallow (57.79%) decreased by chemical interesterification with corn oil. However, SFA% of structured lipids did not change during chemical interesterification (Figure 5.4).

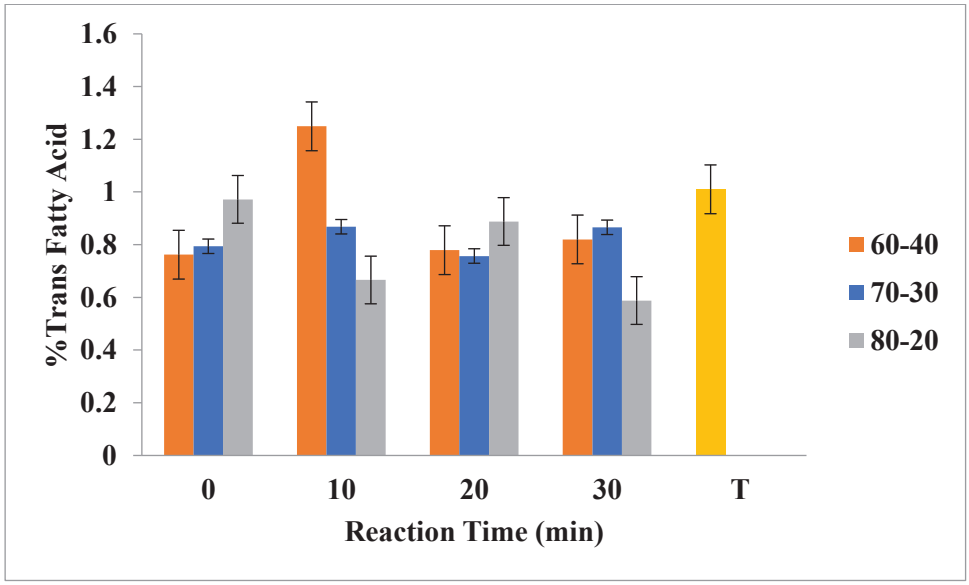

Figure 5.1 Trans fatty acid contents of tallow-corn oil samples during interesterification

Table 5.1 Percentages of individual fatty acids of chemically interesterified samples during chemical interesterification

Samples	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n9c	C18:1n9t	C18:2n6t	C18:2n6c	C20:0	C20:1n9	C18:3n3
C60	1.62	0.26	0.33	19.09	0.63	0.83	0.17	21.93	28.12	0.62	0.14	24.94	0.62	0.44	0.27
C61	1.66	ND	ND	19.55	ND	0.85	ND	23.42	27.84	1.25	ND	25.43	ND	ND	ND
C62	1.58	0.28	0.33	18.65	0.60	0.84	0.16	23.75	27.59	0.61	0.17	24.11	0.65	0.44	0.26
C63	1.55	0.27	0.32	18.22	0.57	0.84	0.15	24.07	27.78	0.66	0.16	24.04	0.66	0.45	0.27
C70	1.88	0.28	0.37	20.02	0.74	0.95	0.19	25.99	27.96	0.63	0.16	19.59	0.62	0.40	0.24
C71	1.84	0.30	0.38	20.00	0.74	0.95	0.19	25.14	28.14	0.65	0.22	20.21	0.60	0.41	0.25
C72	1.87	0.30	0.39	20.50	0.71	0.99	0.21	26.89	27.42	0.59	0.17	18.78	0.59	0.37	0.23
C73	1.89	0.28	0.40	20.05	0.72	0.99	0.18	27.70	27.35	0.69	0.18	18.35	0.61	0.38	0.25
C80	2.29	0.30	0.41	21.35	0.86	1.12	0.25	29.17	27.76	0.76	0.21	14.36	0.59	0.32	0.25
C81	2.12	ND	0.43	21.10	0.79	1.10	ND	31.85	27.29	0.67	ND	14.06	0.60	ND	ND
C82	2.21	0.30	0.41	20.90	0.81	1.08	0.21	31.18	27.38	0.70	0.18	13.47	0.58	0.35	0.23
C83	2.03	ND	0.50	20.37	0.81	1.15	ND	31.83	27.75	0.59	ND	14.26	0.73	ND	ND
MCP1	2.15	0.39	0.51	22.67	0.80	1.00	ND	24.41	26.70	0.61	ND	20.22	0.55	ND	ND
MCP2	2.22	ND	0.40	21.81	0.79	1.00	ND	26.14	26.82	0.63	ND	19.23	0.60	0.36	ND
MCP3	2.03	0.36	0.39	20.85	0.76	0.93	0.20	23.95	27.50	0.73	ND	21.09	0.58	0.40	0.25
T	2.57	0.45	0.54	22.75	1.01	1.37	0.28	39.09	28.24	0.72	0.29	2.14	0.56	ND	ND
CO	ND	ND	ND	ND	ND	ND	ND	2.06	30.994	0.564	ND	54.725	0.757	0.884	0.411

*Abbreviations are provided in Materials & Methods section

Standard deviation for C14:0 = ±0.08, C14:1 = ±0.18, C15:0 = ±0.06, C16:0 = ±0.74, C16:1 = ±0.02, C17:0 = ±0.03, C17:1 = ±0.09, C18:0 = ±0.94, C18:1n9c = ±0.35, C18:1n9t =± 0.05, C18:2n6t = 0, C18:2n6c = ±0.76, C20:0 = ±0.02, C20:1n9 = ±0.18, C18:3n3 = ±0.12 (calculated from CPs)

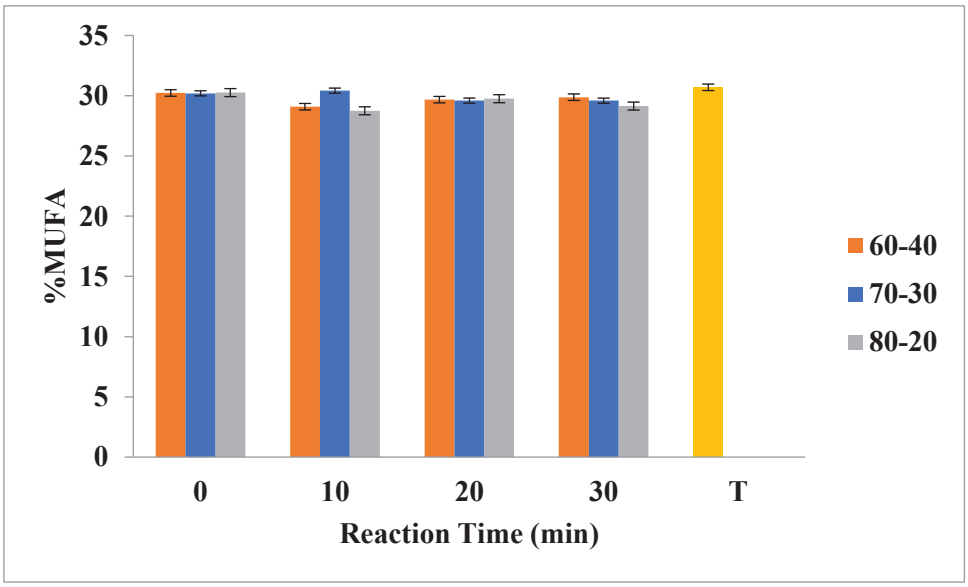

Figure 5.2 Monounsaturated fatty acid (MUFA) contents of tallow-corn oil samples during interesterification

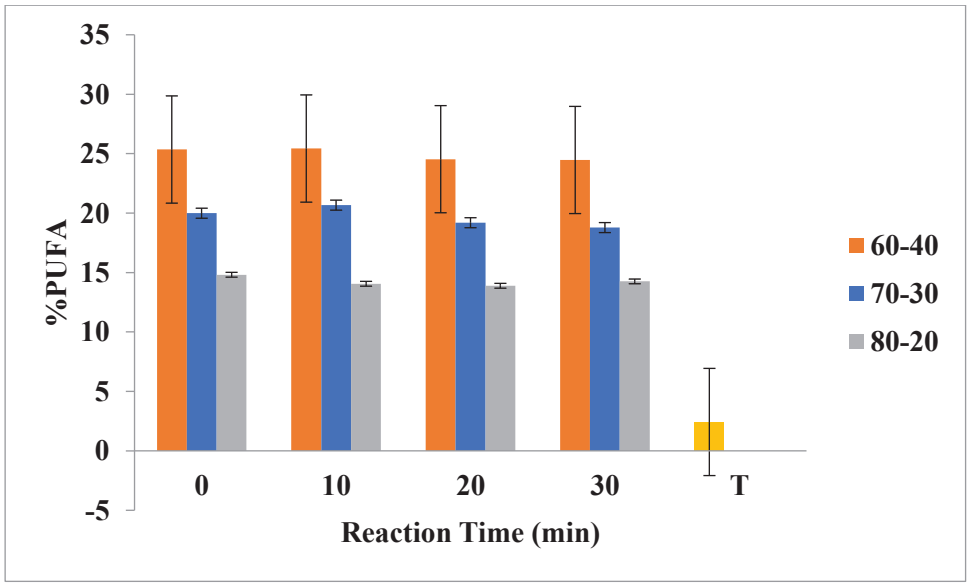

Figure 5.3 Polyunsaturated fatty acid (PUFA) contents of tallow-corn oil samples during interesterification

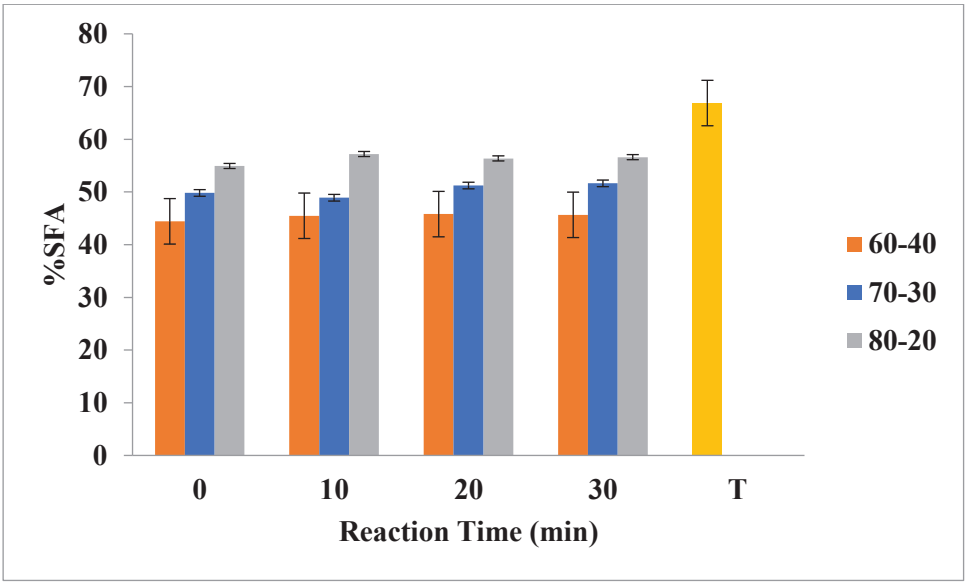

Figure 5.4 Saturated fatty acid (SFA) contents of tallow-corn oil samples during interesterification

ANOVA results (App. 9) indicated that while the models constructed for PUFA% and SFA% were significant with non-significant lack of fit at 95% confidence interval, the models for MUFA% and TFA% were found insignificant. Normality and residuals were also checked for the models. Reaction time did not have any prominent effect on neither PUFA nor SFA content of samples. The ANOVA table reveals that only blend ratio has considerable effect on SFA and PUFA contents of structured lipids. Figure 5.5-5.6 shows the effect of significant parameters on fatty acid composition of structured lipids. With the increase in blend ratio of tallow, PUFA content of interesterified fats decreased (Figure 5.5). However, opposite trend was observed for SFA of the structured lipids. Higher concentrations of tallow resulted in structured lipids with higher SFA (Figure 5.6). These changes in SFA and PUFA content of produced lipids depending on the blend ratio are related to the compositional differences between the tallow and corn oils since tallow has high content of SFA and corn oil is rich in terms of PUFA.

5.1.2. Oxidative Stabilities during the Chemical Interesterification

Oxidative stabilities (OS) in terms of the oxidation induction times of the samples are listed in Table 5.2. While the oxidation induction time of tallow is 10.2 h blends without interesterification have a range of induction times of 6.84-9.63 h. Chemical

interesterification caused a decrease in oxidative stabilities of corn-tallow samples compared to initial blends. This result is in accordance with the previous studies, which observed a decrease in OS after chemical interesterification (Kowalski et al. 2004; Hoshina et al. 2004). Oxidation induction times of the samples containing 70% tallow did not significantly change during chemical interesterification. However, OS for the structured lipids with low and high tallow concentrations fluctuated throughout the chemical interesterification (Figure 5.7).

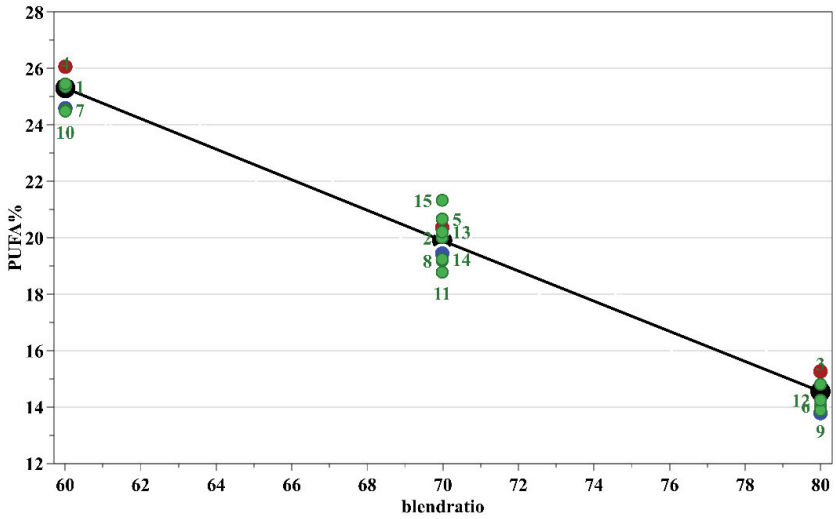

Figure 5.5 Main effect plot for blend ratio on polyunsaturated fatty acid (PUFA) content of structured lipids

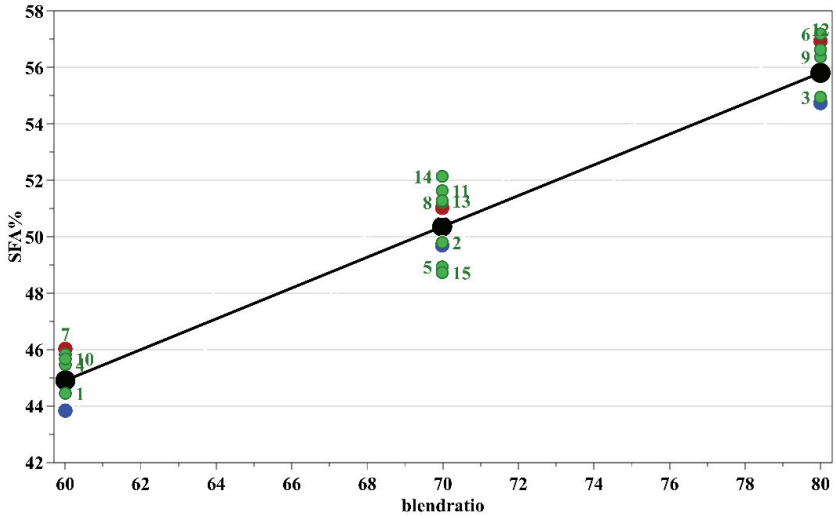

Figure 5.6 Main effect plot for blend ratio on saturated fatty acid (SFA) content of structured lipids

Table 5.2 Oxidative stabilities (OS) in terms of induction times (h) for chemically interesterified lipids

Sample	OS (h)
C60	6.84
C61	5.73
C62	6.52
C63	5.62
C70	6.48
C71	7.09
C72	6.81
C73	6.95
C80	9.63
C81	8.36
C82	8.75
C83	7.47
MCP1	7.04
MCP2	7.89
MCP3	7.54
CO	4.42
T	10.20

*Abbreviations are provided in Materials & Methods section
Standard deviation for OS = ±0.35 (calculated from CPs)

The statistical analysis results for OS data of the chemically interesterified samples are given in App 9. ANOVA results indicated that constructed model was significant with non-significant lack of fit. Normality and residuals were checked for the model. The ANOVA table reveals that only blend ratio has significant effect on OS (Figure 4.4). This means that OS of structured lipids do not change significantly during the chemical interesterification since time is not a significant factor. With the increase in tallow concentration OS of structured lipids increased also (Figure 5.8).

5.1.3. Free Fatty Acid Content during Chemical Interesterification

The free fatty acid percentages (FFA%) in terms of oleic acid content of the samples are provided in Table 5.3. The FFA% of tallow is 0.67% while the blends without interesterification have a range of 0.42-0.66% as oleic acid. Generally, FFA% of interesterified lipids (0.9-2.67%) are higher than their starting blends.

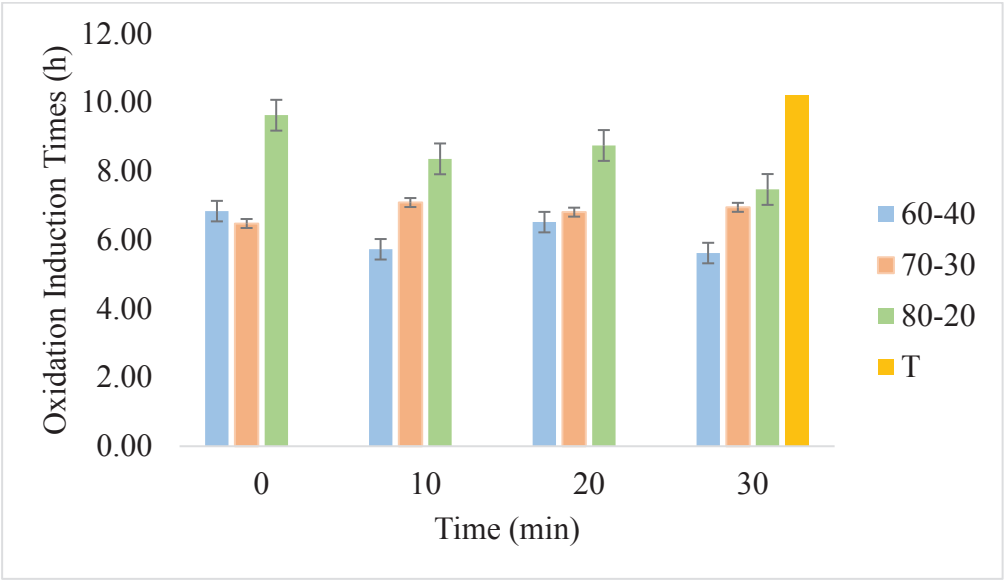

Figure 5.7 Oxidation induction times of tallow-corn oil samples during chemical interesterification

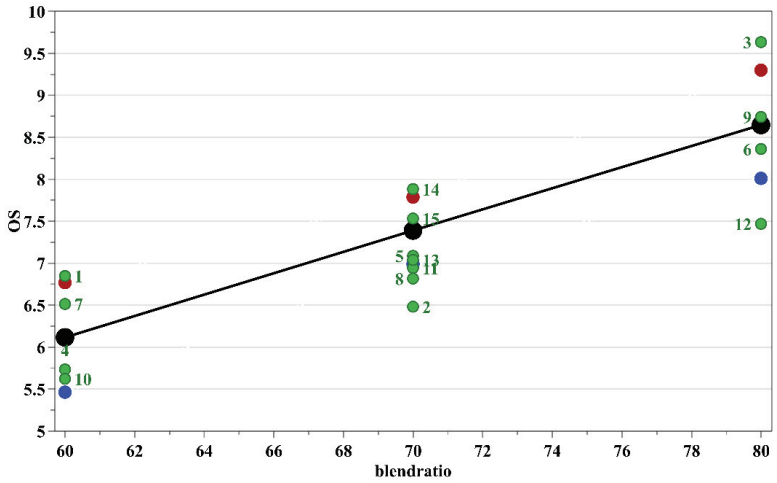

Figure 5.8 The main effect plot for blend ratio on oxidative stability (OS) of structured lipids

Table 5.3 Free fatty acid percentages (% oleic acid) of the chemically interesterified lipids during reaction

Sample	FFA%
C60	0.66
C61	1.56
C62	2.67
C63	2.08
C70	0.42
C71	0.92
C72	2.21
C73	1.16
C80	0.61
C81	1.47
C82	1.04
C83	1.52
MCP1	0.90
MCP2	2.34
MCP3	1.48
CO	0.14
T	0.67

*Abbreviations are provided in Materials & Methods section
Standard deviation for FFA measurement = ±0.59 (calculated from CPs)

As it could be seen in Figure 5.9, there has been a drastic increase and fluctuation in FFA% of samples during chemical interesterification. The samples having 60:40 and 70:30 blend ratios have a similar trend with regard to the FFA content during interesterification process. FFA% increased up to 20 min and then decreased for both ratios. However, fluctuations in FFA% was observed for the sample with 80:20 ratio. This fluctuation can be associated with the activity of chemical catalyst. Throughout the interesterification reactions, the catalyst acts on fatty acids of the triacylglycerol molecules and lead to the formation of diacylglycerol and monoacylglycerol molecules. Therefore, increases and fluctuations in FFA% could be observed during reaction time (Kowalski et al. 2004; Hoshina et al. 2004).

ANOVA results for FFA content of chemically interesterified samples indicated that constructed model could be considered as significant with non-significant lack of fit. Normality and residuals were checked for the model. The ANOVA table reveals that the reaction time is the only important factor for this model (App. 9). This is also supported

by the main effect plot which shows an increase in the FFA content of the samples during the chemical interesterification reaction (Figure 5.10).

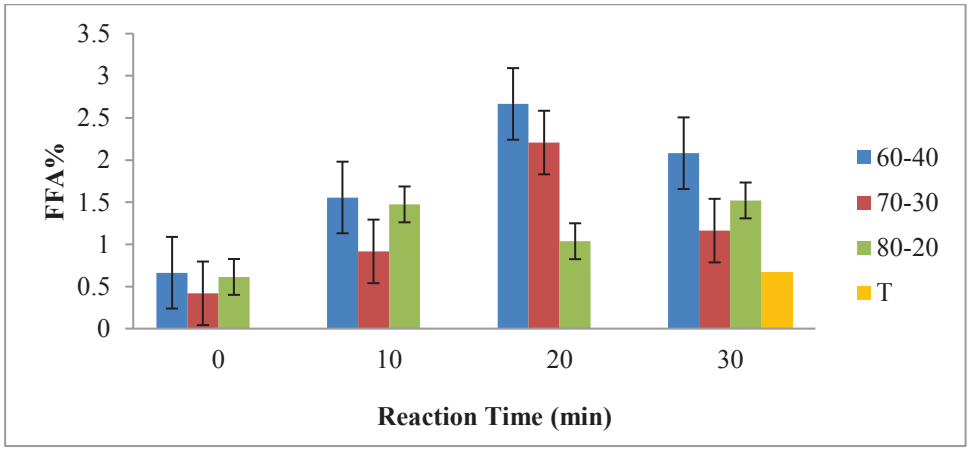

Figure 5.9 Free fatty acid percentages (FFA%) of tallow-corn oil samples during interesterification

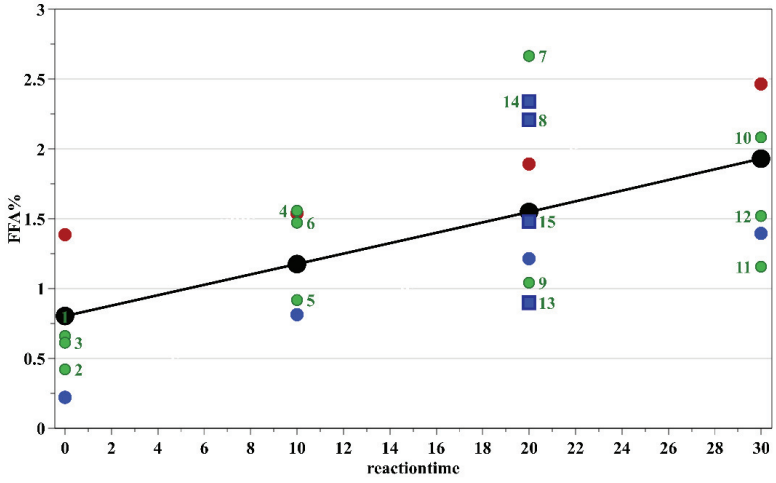

Figure 5.10 The main effect plot for the reaction time on free fatty acid content (FFA%) of structured lipid samples

5.1.4. Mono, Di, and Triacylglycerol Contents during the Chemical Interesterification

Mono, di and triacylglycerol (MAG, DAG and TAG) contents of the structured lipids were determined in order to better understand the changes in the glycerol backbone

that occurred during reaction time. MAG, DAG and TAG contents of the samples are expressed in relative percentages of the overall content (Table 5.4). The results are in accordance with the previous studies, which observed a decrease in TAG% after interesterification (Kowalska et al. 2005; Ledóchowska and Wilczyńska 1998).

The TAG% of tallow is approximately 98% while blends without interesterification have slightly lower TAG% values. Both blending and chemical interesterification caused an increase in MAG and DAG contents of the samples. Generally, TAG% of chemically interesterified lipids were lower than starting blends. TAG content of the chemically interesterified lipids decreased throughout the interesterification process (Figure 5.11). Although DAG contents of the samples with low and moderate tallow ratio increased during the reaction, DAG% of samples with high tallow concentration decreased (Figure 5.12). Same trend was also observed for MAG content of the samples up to 20 min reaction time. After that point MAG% of the samples decreased with 30 min of interesterification process. This drop is more significant when the tallow concentration is high (Figure 5.13).

In order to better understand the changes in TAG, DAG and MAG content during chemical interesterification reaction correlation between FFA% and TAG content of interesterified lipids is also evaluated (Figure 5.14). The correlation coefficient is calculated and found as -0.62. There is a decreasing trend between FFA content and TAG% of the samples during interesterification reaction (Figure 5.14). Generally, the samples having higher amounts of TAG content, have lower FFA% as Figure 5.14 indicated. The increase in FFA% and decrease in TAG% with time confirmed that the chemical interesterification reaction had been started and continued.

The statistical analysis results for TAG, DAG, and MAG% of the samples are given in App. 9. ANOVA results indicated that constructed models were significant with insignificant lack of fit. Normality and residuals were checked for the models. The ANOVA table reveals that only reaction time is a significant factor for the model of TAG (App. 9). As the reaction time is increased, TAG% of the samples decreased particularly (Figure 5.15). The ANOVA table shows that both reaction time and blend ratio are significant factors for DAG (App 9). As Figure 5.16 and 5.17 indicated there is also an increase in DAG content with the increase in the blend ratio and the reaction time. Same statistical analyses were also performed for MAG% of chemically interesterified lipids and according to the ANOVA table the interaction between the reaction time and the blend ratio is significant for this model. Figure 5.18 shows the effect of blend ratio-time

interaction on MAG%. While MAG content increased with increasing reaction time at 60:40 ratio opposite trend was observed for the highest ratio.

Table 5.4 Relative percentages of mono, di and triacylglycerol (MAG, DAG and TAG) of the samples

Samples	TAG%	DAG%	MAG%
C60	94.58	2.24	2.85
C61	92.16	2.36	4.95
C62	88.37	3.92	6.94
C63	87.01	3.52	6.22
C70	94.66	1.54	2.33
C71	94.23	2.55	4.71
C72	87.43	4.21	6.09
C73	84.71	8.84	3.10
C80	92.58	3.19	8.70
C81	92.65	8.83	6.20
C82	90.21	7.66	5.09
C83	87.06	6.36	2.07
MCP1	91.18	4.04	4.00
MCP2	89.09	5.51	4.84
MCP3	90.10	5.49	3.62
T	97.74	0.23	0.33
CO	92.39	2.46	0.42

*Abbreviations are provided in Materials & Methods section
Standard deviation for: TAG% =± 0.85, DAG% =± 0.69, MAG% = ±0.51 (calculated CPs)

To analyze the chemical data of the chemically interesterified lipids throughout the reaction principal component analysis (PCA) was also applied. The model was constructed by using all measured chemical parameters with 7 principal components (PC), $R^2 = 0.96$, and $Q^2 = 0.29$. There is a clear discrimination of the samples with respect to blend ratio (Figure 5.19). While the samples containing 80% tallow located at the right part of the ellipse, samples with 70% tallow placed just right of the center and samples containing 60% tallow are further in the left. Therefore, a discrimination with respect to the first PC was obtained as far as the blend ratio is concerned. This discrimination mostly resulted from higher PUFA and MUFA contents of the samples as observed in Figure 5.19. Moreover, the samples with 80% tallow mostly located at the right of the ellipse regardless of oil type since they have higher SFA% (Figure 5.20). Therefore, multivariate analysis of chemical data indicated that blend ratio caused differences in the chemical properties of the products.

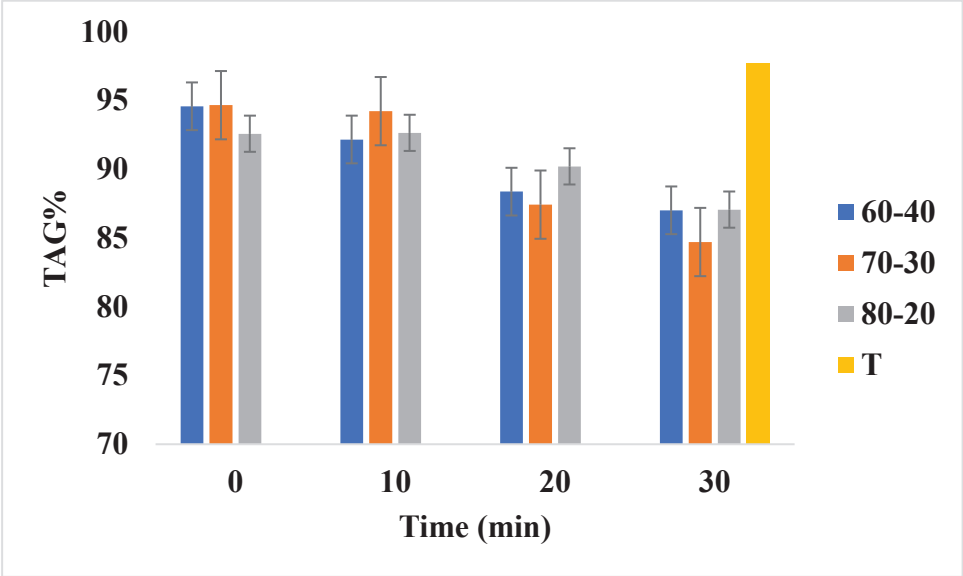

Figure 5.11 Triacylglycerol percentage (TAG%) of tallow-corn oil samples during chemical interesterification

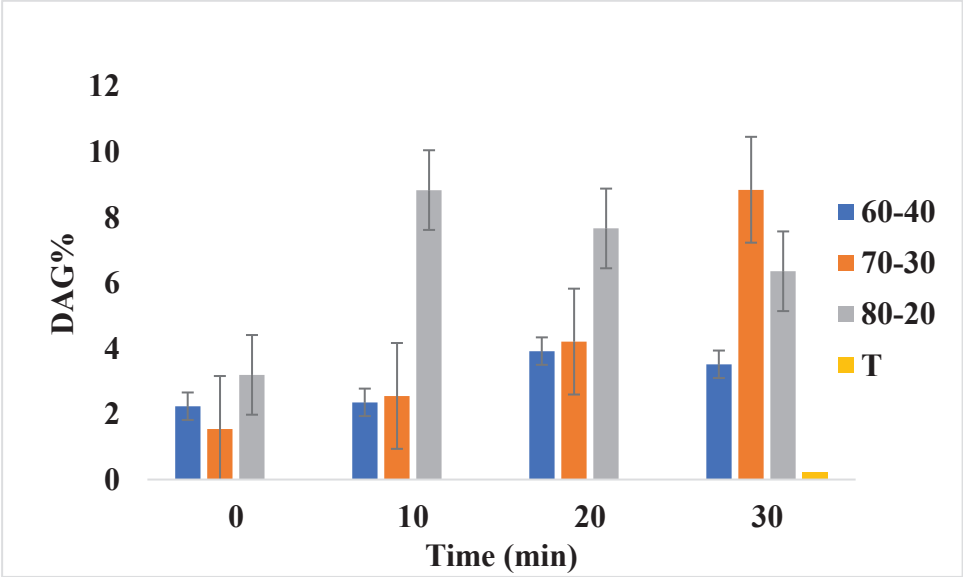

Figure 5.12 Diacylglycerol percentage (DAG%) of tallow-corn oil samples during chemical interesterification

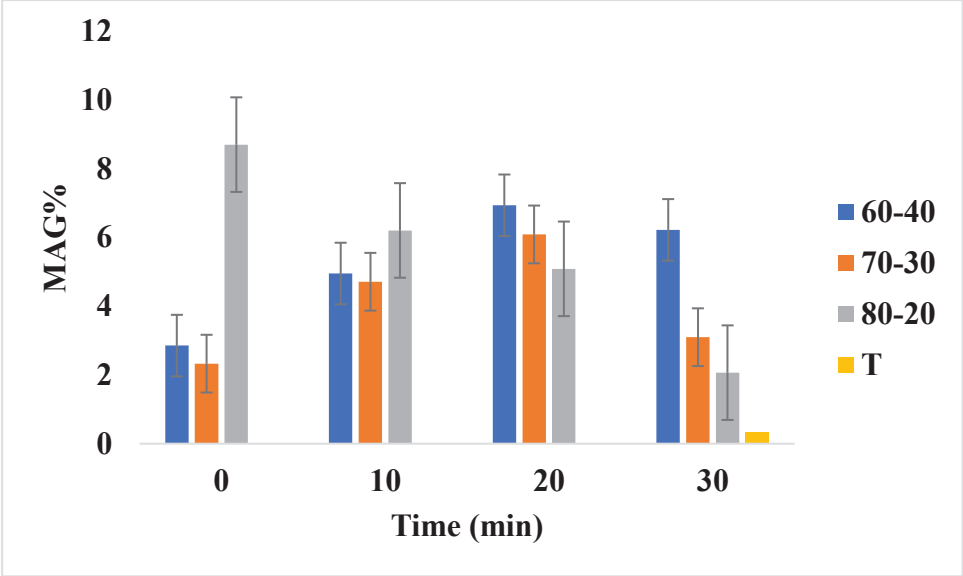

Figure 5.13 Monoacylglycerol percentage (MAG%) of tallow-corn oil samples during chemical interesterification

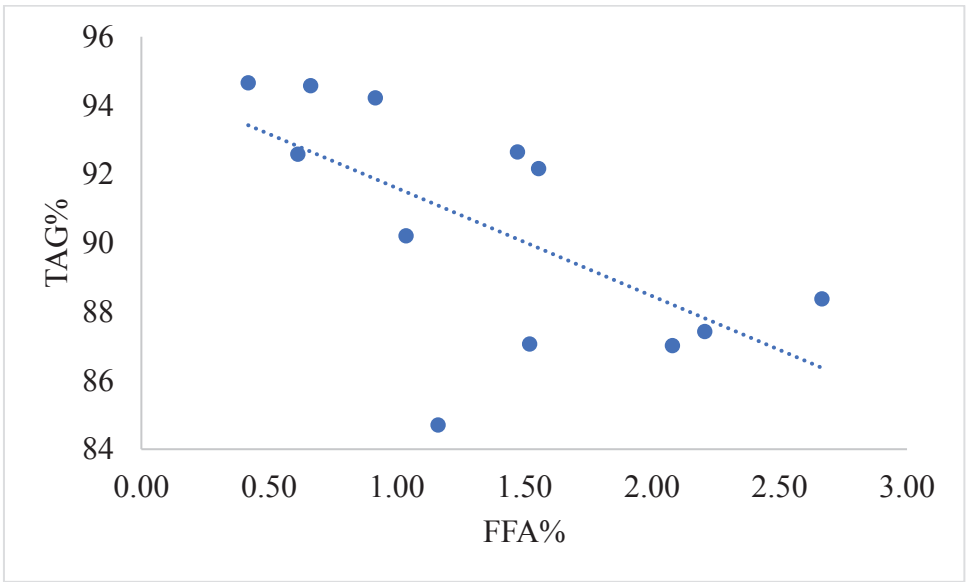

Figure 5.14 Free fatty acid content (FFA%) versus triacylglycerol (TAG%) of structured lipids

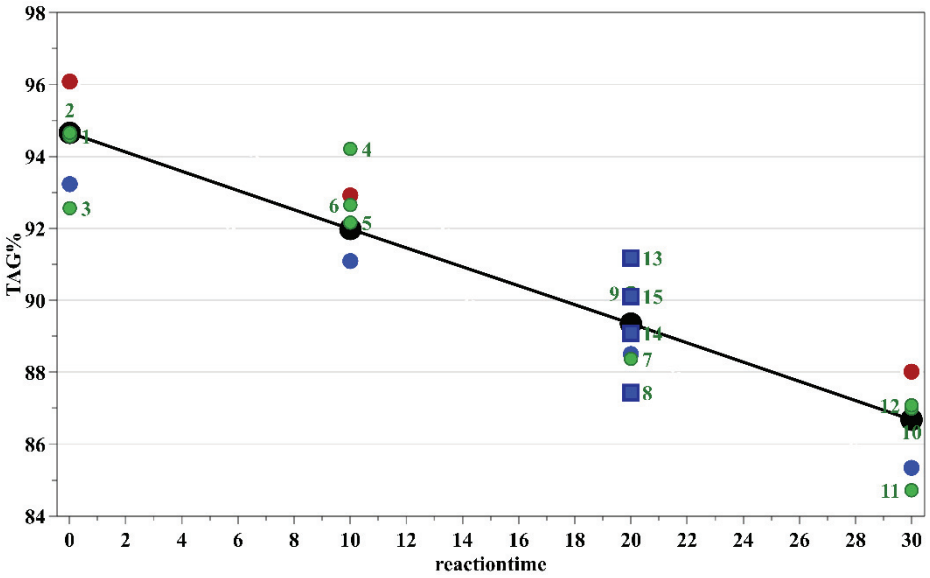

Figure 5.15 Main effect plot for the reaction time on triacylglycerol content (TAG%) of chemically interesterified samples

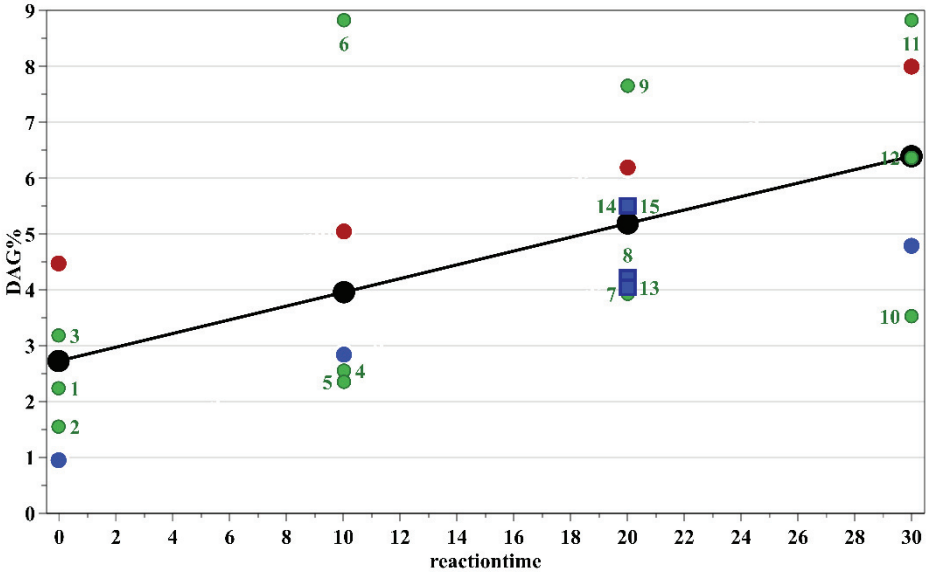

Figure 5.16 Main effect plot for the reaction time on diacylglycerol content (DAG%) of chemically interesterified samples

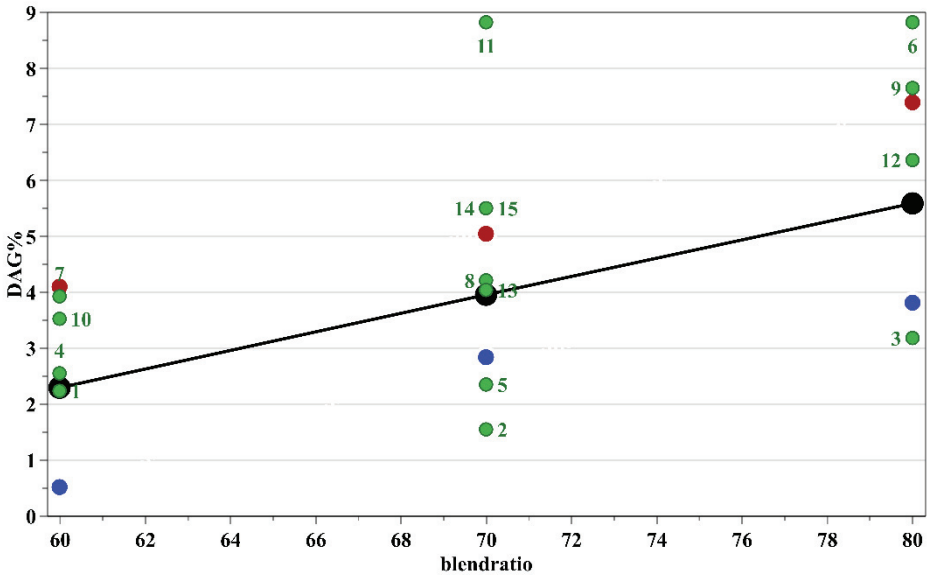

Figure 5.17 Main effect plot for the blend ratio on diacylglycerol content (DAG%) of chemically interesterified samples

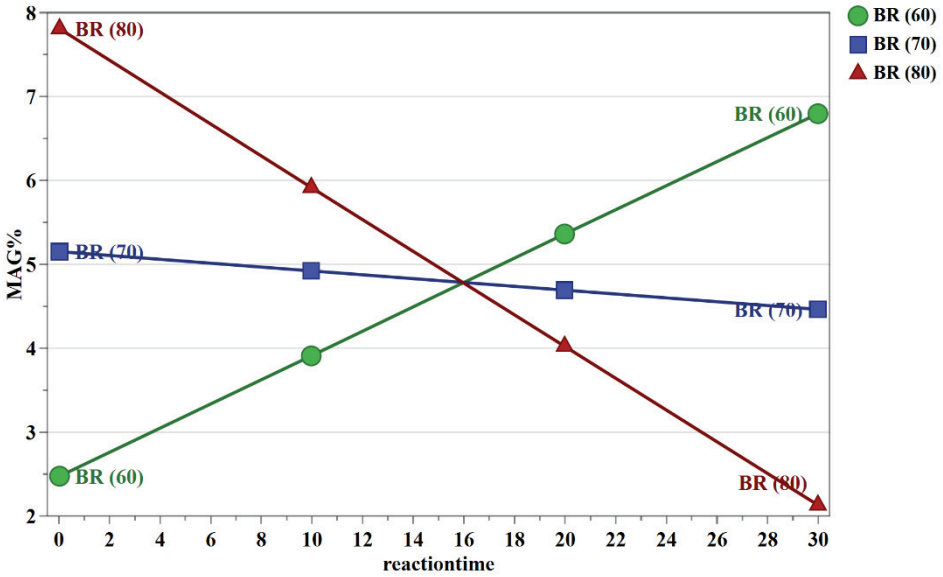

Figure 5.18 Interaction plot showing the effect of reaction time x blend ratio on monoacylglycerol content (MAG%) of chemically interesterified samples

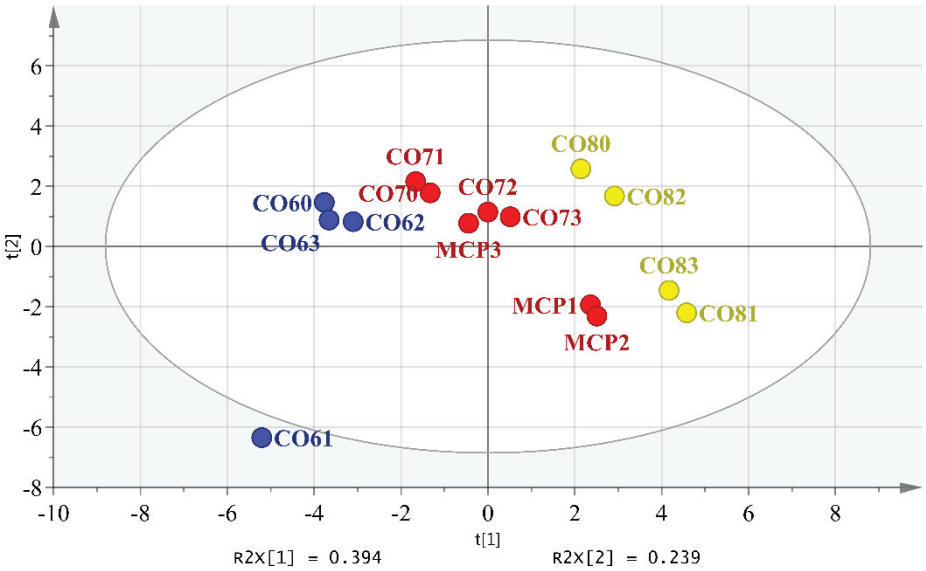

Figure 5.19 Score plot of the PCA model constructed by using all chemical parameters of chemically interesterified lipids throughout the reaction (MCP1-2-3=70% tallow-20 min, coloring was done with respect to blend ratio)

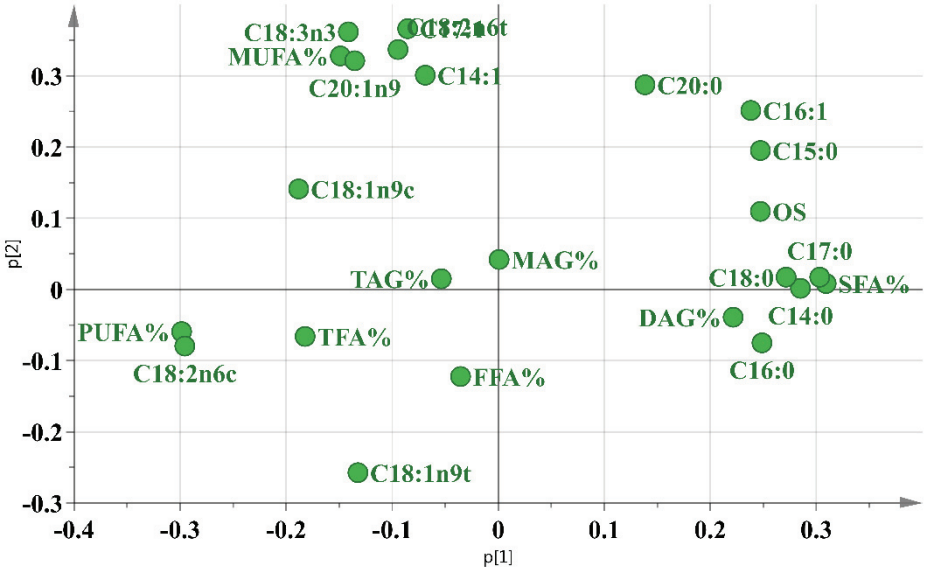

Figure 5.20 Loading plot of the PCA model constructed by using all chemical parameters of chemically interesterified lipids throughout reaction

5.2. Change in the Physical Properties of the Structured Lipids During the Chemical Interesterification

The same physical properties were determined, and the data were analyzed by univariate (ANOVA) and multivariate statistical analysis (PCA) techniques to investigate

the effects of blend ratio (60:40, 70:30, 80:20 w/w) and reaction time (0, 10, 20, 30 min) throughout chemical interesterification reactions.

5.2.1 Crystal Morphology during the Chemical Interesterification

The polymorphic forms of the structured lipids and blends are provided in Table 5.5. Tallow contains only β' form and α forms were not found in neither structured lipids nor blends. During the chemical interesterification reaction of tallow-corn oil and with blending, polymorphic structure of lipids did not change depending on the blend ratio except the samples coded as C70 and C72. After the chemical interesterification, β+β' forms were present together in the rest of the samples. Therefore, chemical interesterification caused little changes in the polymorphic structures of lipids with respect to the reaction time. This result is in accordance with the previous studies in which mostly β+β' crystal forms were shown together (Jeung et al. 2008; Liu et al. 2010). The β' form of the crystal is important in bakery industry due to its good aeration properties and smooth texture. Therefore, these lipids have potential to be used as bakery fats.

Table 5.5 Polymorphic forms of structured lipids and tallow

Samples	Crystals
C60	β+β'
C61	β+β'
C62	β+β'
C63	β+β'
C70	β
C71	β+β'
C72	β'
C73	β+β'
C80	β+β'
C81	β+β'
C82	β+β'
C83	β+β'
MCP1	β+β'
MCP2	β+β'
MCP3	β+β'
T	β'

*Abbreviations are provided in Materials & Methods section

5.2.2. Color Properties during the Chemical Interesterification

The lightness (L), redness (a) and yellowness (b) values of the chemically interesterified samples were measured and then the total color differences (ΔE) were calculated considering tallow itself as a standard. The L, a, b and ΔE values of the chemically interesterified samples during the reaction are listed in Table 5.6. The lightness value of tallow is 80.71, the redness is -2.24 and the yellowness is 3.42. Both blending and chemical interesterification throughout the reaction caused decreases in the lightness of the samples with respect to tallow. Thirty minutes of reaction time resulted in lower L values compared to initial blends. Generally, a and b values of the samples increased compared to tallow itself after the chemical interesterification and with blending (Table 5.6). As could be observed in Figure 5.21, when the oil concentration is 40%, the ΔE values of the samples decreased up to 20 min reaction time and then increased. The opposite trend was observed when the oil concentration is decreased to 30%.

Table 5.6 L, a, b and ΔE color values of chemically interesterified lipids during interesterification

Sample	L	a	b	ΔE
C60	70.48	-3.11	2.01	10.36
C61	71.20	-2.75	7.27	3.13
C62	60.18	-3.42	11.46	2.02
C63	65.59	-4.00	8.27	10.28
C70	79.28	-2.50	6.19	6.65
C71	74.43	-2.63	5.55	12.88
C72	74.23	-2.61	7.83	22.08
C73	75.55	-2.80	6.97	7.85
C80	78.79	-2.30	4.03	9.66
C81	73.73	-1.50	14.22	15.98
C82	74.96	-2.33	11.18	6.30
C83	69.33	-0.95	16.38	17.29
MCP1	72.38	-2.47	3.61	8.34
MCP2	65.41	-2.06	14.24	18.74
MCP3	70.01	-2.57	3.76	10.71
T	80.71	-2.24	3.42	0.00

*Abbreviations are provided in Materials & Methods section
Standard deviation for L=±2.89, a=±0.22, b=±4.98, ΔE=±4.45 (calculated from CPs)

The Appendix 10 shows the statistical analysis results for color measurements. ANOVA results indicated that constructed model for the total color difference is not significant at $p<0.05$ with non-significant lack of fit. Normality and residuals were also checked for the model. Although the model is insignificant, reaction time has some effect on ΔE of the chemically interesterified lipids (App. 10). Increasing reaction time leads to increases in ΔE of the samples (Figure 5.22).

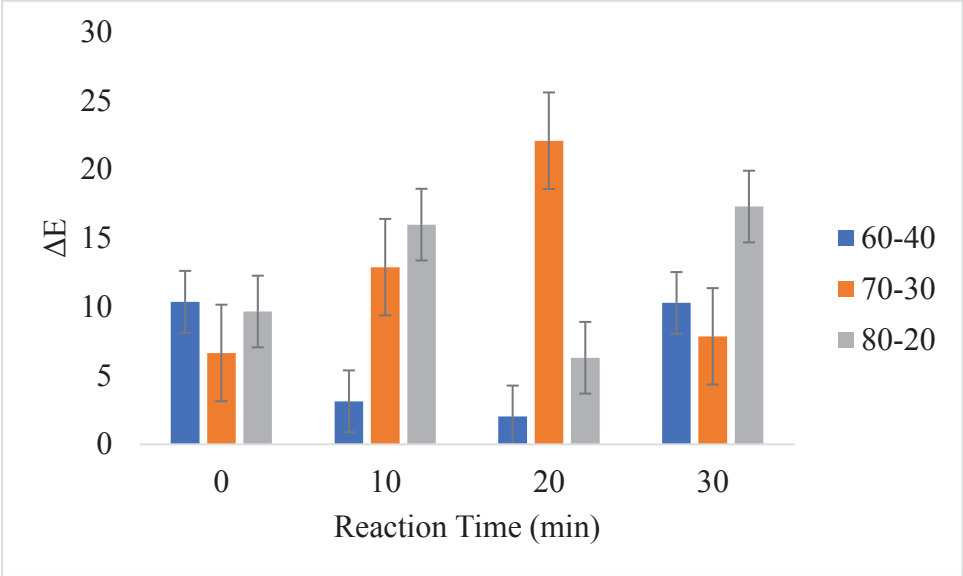

Figure 5.21 Total color difference of the tallow-corn oil samples during the chemical interesterification

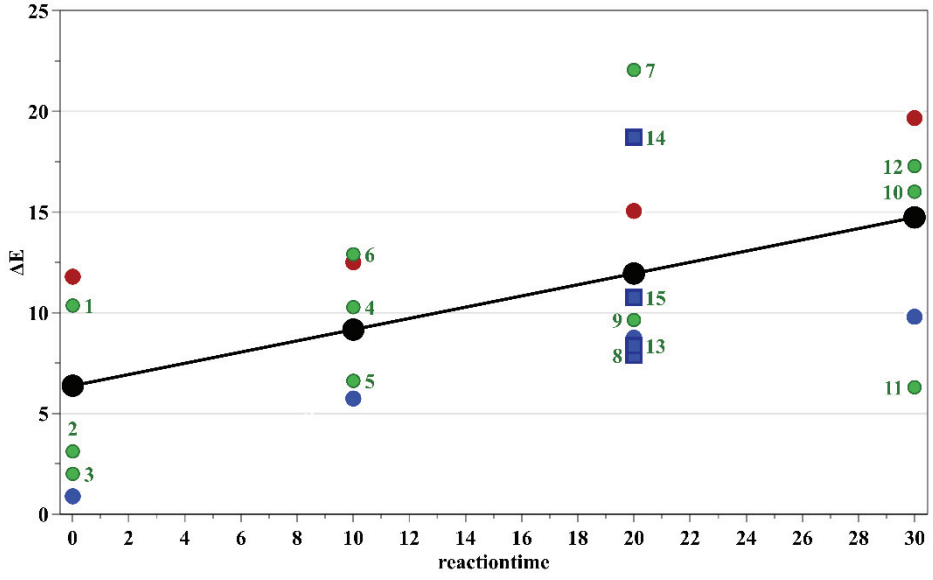

Figure 5.22 Main effect plot of reaction time on ΔE of chemically interesterified samples

5.2.3. Melting Points during the Chemical Interesterification

As explained in Chapter 4, melting points of the samples were expressed as a function of a given percentage (85, 90 and 95%) of melted crystals since various TAG profiles were created throughout the reaction and each TAG has its own melting point,. The melting temperatures at 85, 90 and 95% of melted crystals (MP85%, MP90%, MP95%) are provided in Table 5.7. As expected, the higher crystal percentage corresponded to the higher melting temperature. As it could be seen in Table 5.7, tallow samples have really high melting temperatures (52.10-53.74 °C). At the beginning of the reaction, just after blending tallow with corn oil, there are small decreases in the melting points of the samples. However, during the chemical interesterification sharp decreases in the melting points of the structured lipids were observed regardless of the blend ratio. These changes in the melting points are in accordance with the previous studies (Ribeiro et al. 2017; Meng et al. 2011; Liu and Lampert 1999). The β' form of the crystals has a high melting point between 17–69°C and the melting point of β form is 32-78 °C depending on the chain length of the fatty acids (Nas et al. 2001). The β and β′ crystal type is formed throughout the reaction and the melting points of the samples could be associated with the melting points of these crystal types.

Table 5.7 Melting points of chemically interesterified samples at various percentages of melted crystals during reaction time

Sample	MP85%	MP90%	MP95%
C60	47.99	48.82	49.94
C61	46.56	47.60	48.88
C62	34.91	38.16	41.96
C63	47.63	48.46	49.56
C70	49.90	50.62	51.62
C71	47.99	48.90	50.05
C72	47.55	48.53	49.63
C73	47.57	48.62	49.87
C80	50.02	50.86	51.93
C81	46.75	47.57	48.57
C82	46.94	47.89	49.11
C83	46.55	47.32	48.34
MCP1	47.57	48.70	50.10
MCP2	42.77	44.39	46.18
MCP3	48.13	49.30	50.73
T	52.10	52.79	53.74

*Abbreviations are provided in Materials & Methods section
Standard deviation of MP85 = ±2.41, MP90 = ±2.19, MP95 = ±2.01 (calculated from CPs)

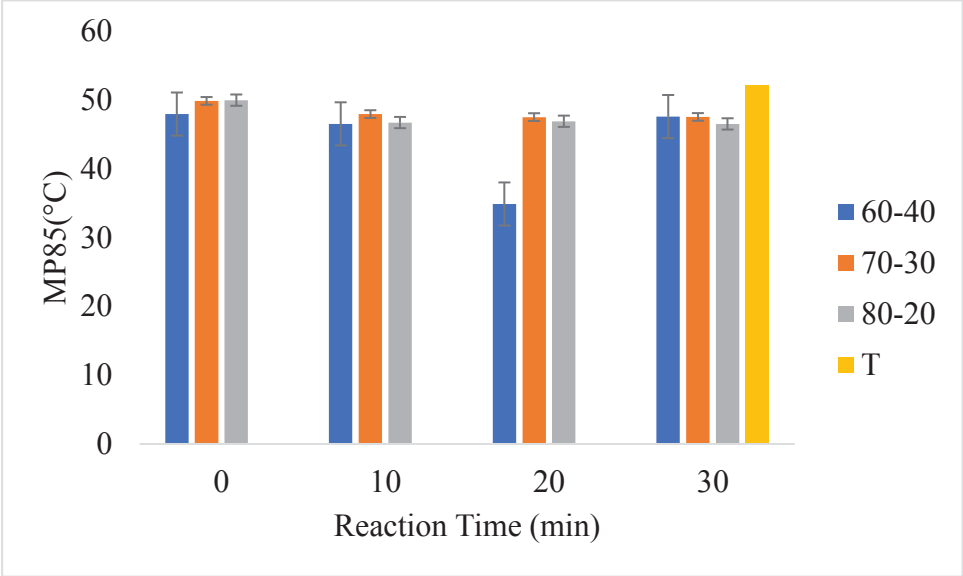

Figure 5.23 Melting temperatures of the tallow-corn oil samples at 85% of melting during chemical interesterification

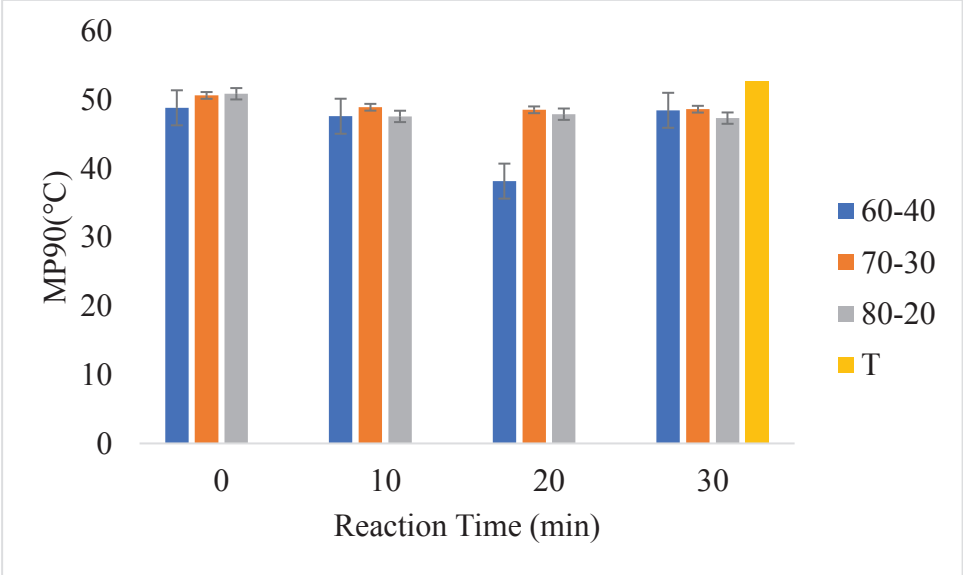

Figure 5.24 Melting temperatures of the tallow-corn oil samples at 90% of melting during chemical interesterification

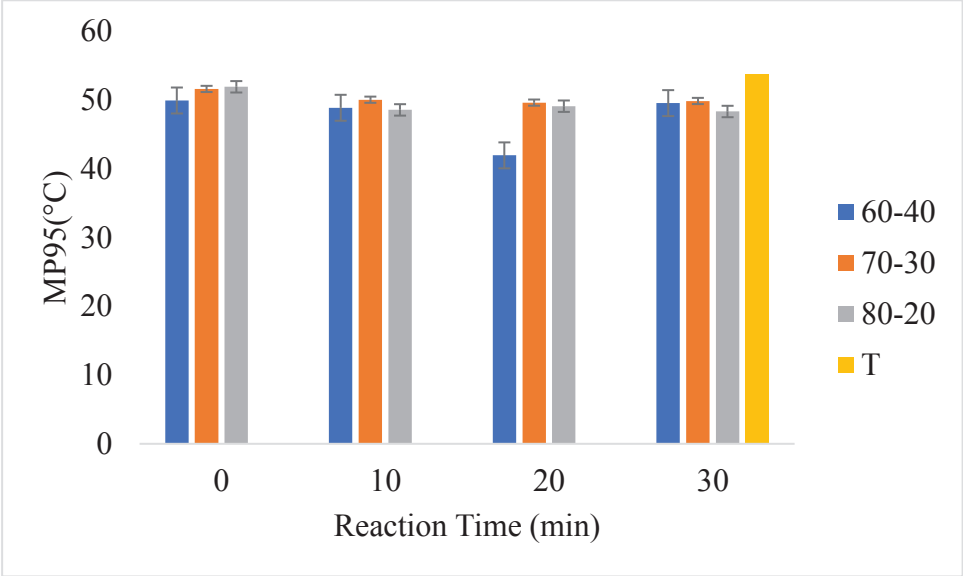

Figure 5.25 Melting temperatures of the tallow-corn oil samples at 95% of melting during chemical interesterification

The melting temperatures of the interesterified lipids slightly increased by the gradual increase of the percentage of crystals in lipid structure. The melting temperatures did not significantly change during chemical interesterification reaction. There is a small decrease in melting point at 20 min of reaction time for the only sample containing 60% tallow (Figure 5.23-24-25).

Appendix 10 shows the statistical analysis results for melting points. ANOVA results indicated that constructed models were insignificant with non-significant lack of fit. Normality and residuals were checked for the model. Examination of the significance levels of the main factors and their interactions shows that blend ratio and reaction time did not significantly affect the melting points of chemically interesterified lipids.

5.2.4. Slip Melting Points during the Chemical Interesterification

The ranges of the slip melting points (SMP) of the chemically interesterified lipids throughout the reaction are provided in Table 5.8. Interesterification reactions caused a decline in SMPs of structured lipids compared to initial blends and tallow. SMP of tallow is 50.4°C and the SMP of the blends are in the range of 44.65-47.15°C. Chemically interesterified samples have SMPs of 38.40-45.95°C. During the chemical interesterification reaction, SMP of the samples containing 60% tallow decreased up to 20 min of reaction time (Figure 5.26). After that point it slightly increased. The samples

interesterified with 70 and 80% tallow have almost the same SMP throughout the interesterification process.

Table 5.8 Slip melting points of the chemically interesterified lipids during the reaction

Sample	SMP (°C)
C60	44.65
C61	44.25
C62	38.40
C63	41.85
C70	47.00
C71	45.70
C72	45.55
C73	45.95
C80	47.15
C81	44.85
C82	45.20
C83	44.80
MCP1	46.00
MCP2	42.05
MCP3	45.00
T	50.40

*Abbreviations are provided in Materials & Methods section
Standard deviation of SMP=±1.68 (calculated from CPs)

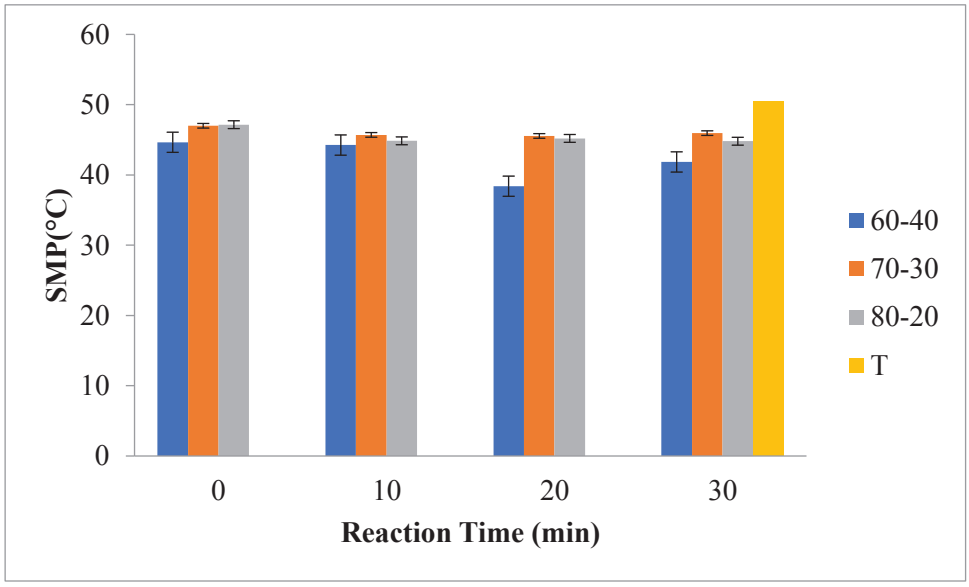

Figure 5.26 Slip melting points (SMP) of the samples during the chemical interesterification

Appendix 10 shows the statistical analysis results for the SMP of the chemically interesterified samples during the process. ANOVA results indicated that constructed model is not significant at $p<0.05$ with non-significant lack of fit. Normality and residuals were also checked for the model. Although the model is insignificant, blend ratio has an effect on the SMPs of the chemically interesterified lipids (App. 10). When the tallow concentration was increased, SMPs of the samples slightly increased as Figure 5.27 indicated.

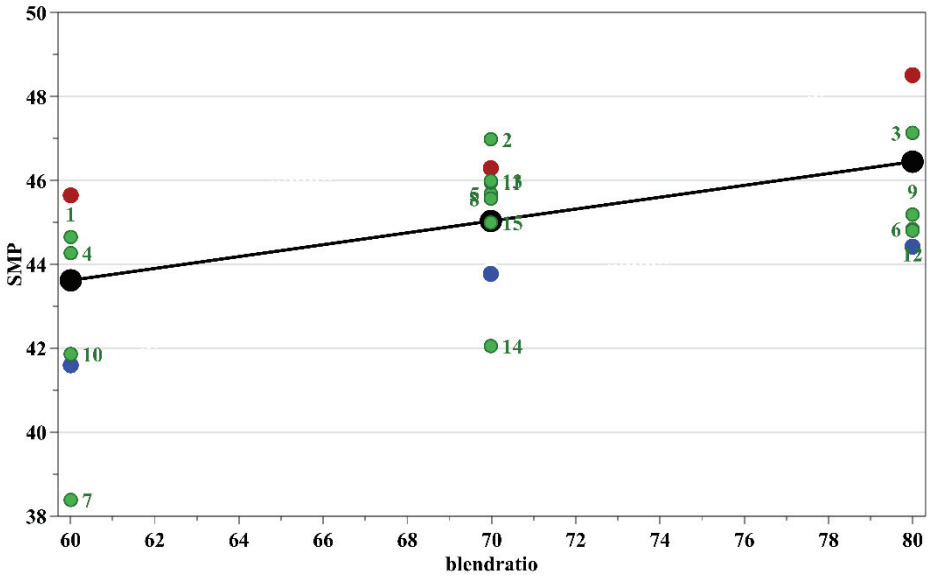

Figure 5.27 Main effect plot of blend ratio on SMPs of chemically interesterified fats

5.2.5. Consistency during the Chemical Interesterification

The consistency was calculated as ‘‘yield value’’ (MPa) at four temperatures and the results for the samples during the chemical interesterification process are given in Table 5.9. The consistencies of all samples decreased clearly as a function of temperature. This result can be associated with the gradual melting of the crystals that generate more fragile crystalline networks. The same behavior was also observed in the previous studies (Silvia et al. 2009; Bezzera et al. 2017; Oliviera et al. 2017). The consistency of tallow was quite higher than both interesterified lipids and initial blends at all temperatures. Consistencies of the blends in different proportions increased with the increasing amounts

of tallow in the blends. However, after the chemical interesterification lower consistency values were obtained compared to the physical blends regardless of the reaction time.

Table 5.9 Consistency values of chemically interesterified lipids during the reaction

Sample	Consistency (MPa)			
	4°C	**10°C**	**15°C**	**25°C**
C60	134.40	72.76	49.25	37.88
C61	109.44	50.98	38.33	18.29
C62	105.70	35.36	28.88	25.71
C63	70.81	54.89	36.32	25.81
C70	167.18	113.04	77.12	54.12
C71	79.29	59.63	46.90	45.95
C72	120.65	75.89	45.39	24.93
C73	109.45	74.86	68.14	66.54
C80	275.59	185.31	119.29	67.65
C81	166.02	146.70	97.72	87.13
C82	94.89	92.70	75.84	61.88
C83	115.28	98.20	77.82	71.67
CP1	474.16	312.27	295.98	293.42
CP2	255.91	199.76	176.85	109.61
CP3	328.82	320.48	255.92	68.21
T	603.02	435.71	332.75	181.66

*Abbreviations are provided in Materials & Methods section
Standard deviation of consistency at 4°C = ±90.72, at 10°C = ±55.07, at 15°C = ±49.5, at 25°C = ±97.88, (calculated from CPs)

Consistencies of the samples during the chemical interesterification of tallow with corn oil at various ratios are shown in Figure 5.28-29-30. As it could be seen from these plots most of the samples chemically interesterified with corn oil can be considered as spreadable and plastic.

Appendix 10 shows the statistical analysis results for the consistencies of the samples during the process. ANOVA results indicated that constructed models were insignificant with insignificant lack of fit at all temperatures. Therefore, it could be concluded that there are not any important differences in the consistencies of corn-tallow interesterified samples produced using different parameters during the chemical interesterification.

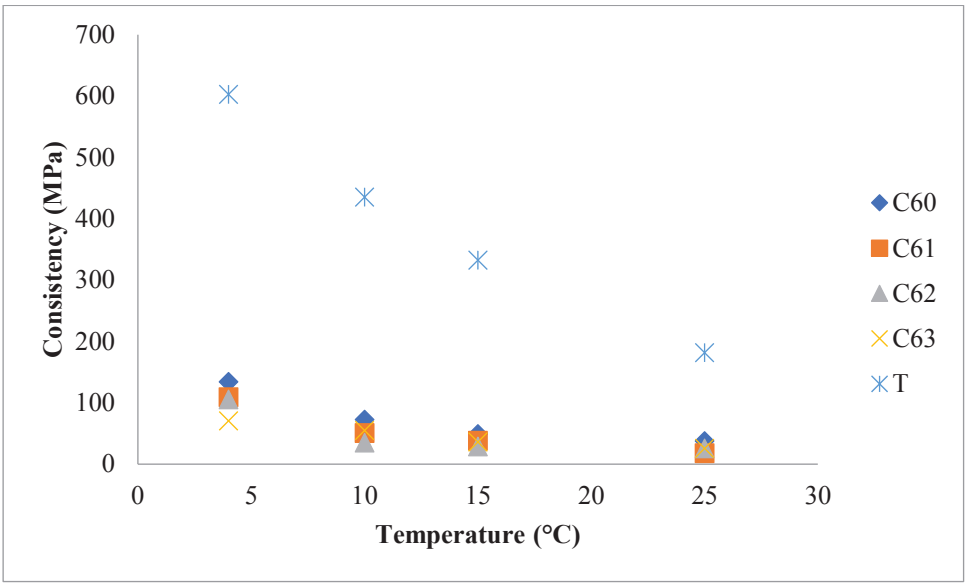

Figure 5.28 Consistencies of the tallow-corn oil samples with 60:40 ratio during the chemical interesterification

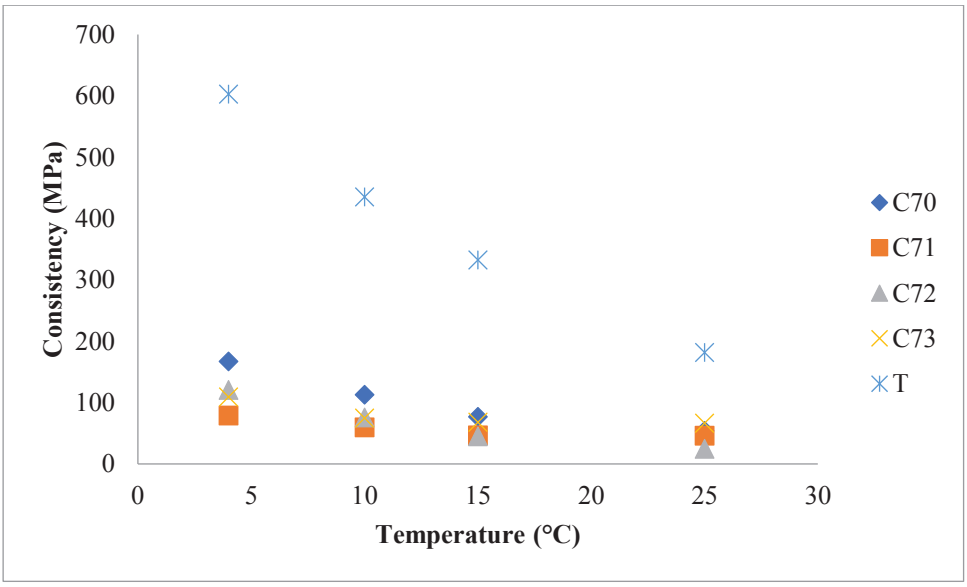

Figure 5.29 Consistencies of the tallow-corn oil with 70:30 ratio samples during the interesterification

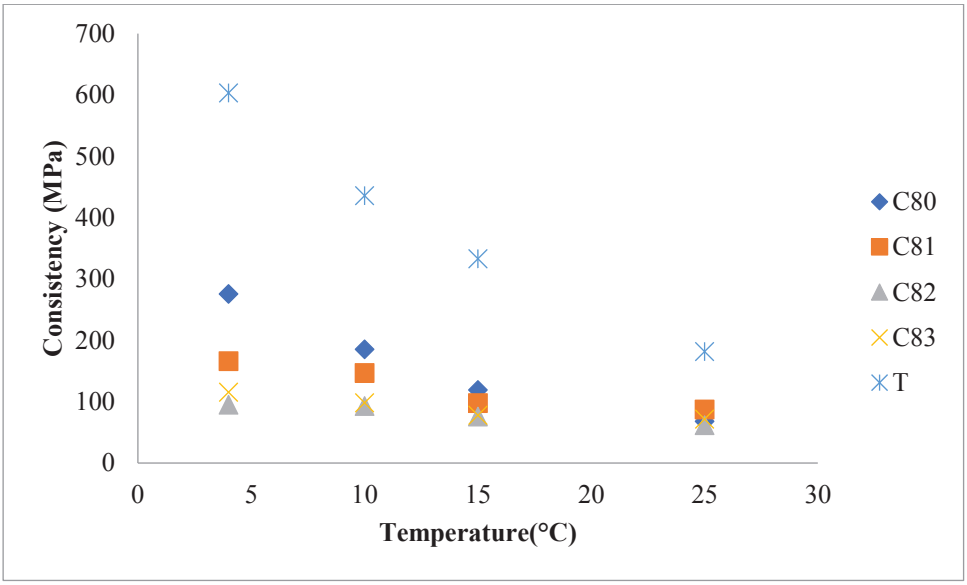

Figure 5.30 Consistencies of the tallow-corn oil samples with 80:20 ratio during the interesterification

5.2.6. Solid Fat Content during the Interesterification

Solid fat content (SFC) of the samples was determined by Nuclear Magnetic Resonance spectroscopy at 4 different temperatures during the chemical interesterification and the data are given in Table 5.10. SFC of both interesterified lipids and initial blends were determined over the temperature range of 10–35°C. It was observed that raising the temperature caused an important decrease in the value of SFC regardless of the reaction parameters. SFC profiles of non-interesterified blends in different proportions have an increasing SFC trend with the increasing amounts of tallow in the blends. Interesterified lipids have lower SFC% values compared to their physical blends. Same trends were also found out in the previous studies (Meng et al. 2010; Karabulut et al. 2004). The decrease in the SFC of interesterified lipids could be attributed to decreased proportion of the high-melting TAGs and medium chain TAGs in the structure of the lipids. This decrease in SFC with respect to the increase in the temperature was expected as in other studies (Fauzi et al. 2013; Bezzera et al. 2017; Oliveira et al. 2017). In addition, the decrease of SFC compared to tallow and non-esterified blends can be associated with decrease in TAG content and change in melting temperature of the crystals caused by blending with corn oil. SFC of the structured lipids slightly decreased throughout the chemical interesterification. However, there is an increase in SFC% of the

chemically interesterified samples at all temperatures at 30 min reaction time (Figure 5.31-32-33). In addition, more plastic behavior was observed for both blends and structured lipids regardless of reaction time above 20°C.

Table 5.10 Solid fat content (%) of chemically interesterified lipids during interesterification

	SFC%			
Sample	**10°C**	**20°C**	**30°C**	**35°C**
C60	45.40	32.50	20.50	14.90
C61	42.10	29.20	17.50	12.00
C62	33.00	21.10	10.10	6.00
C63	36.70	26.40	14.80	10.00
C70	54.20	40.90	26.00	18.90
C71	50.40	36.00	21.60	15.40
C72	46.50	34.70	20.60	14.70
C73	48.00	36.90	21.90	15.30
C80	62.00	44.20	29.60	21.60
C81	61.00	42.00	24.50	16.50
C82	61.90	44.80	24.90	17.10
C83	58.70	43.00	23.20	15.70
MCP1	48.10	34.40	20.50	14.10
MCP2	43.80	32.10	15.50	9.90
MCP3	43.80	30.70	18.10	12.60
T	69.20	69.00	42.50	33.80

*Abbreviations are provided in Materials & Methods section
Standard deviation of solid fat content: at 10 °C = ±2.03, at 15 °C =± 1.53, at 30 °C = ±2.04, at 35 °C = ±1.74 (calculated from CPs)

The statistical analysis results for SFC of the chemically interesterified samples at all temperatures for the process monitoring are given in App. 10. ANOVA results indicated that constructed models were significant with non-significant lack of fit at all temperatures ($p<0.05$). Normality and residuals were checked for the model. Examination of the significance levels of the main factors reveals that both blend ratio and reaction time are significant factors for the models (App. 10). As the blend ratio is increased, SFC of the samples increased particularly (Figure 5.34). Figure 5.35 shows that SFC% of structured lipids decreases as the reaction time is increased.

In order to better understand the physical properties data of the chemically interesterified lipids throughout the reaction, principal component analysis (PCA) was also used. The model was constructed by using all measured physical parameters with 4 PCs, $R^2 = 0.95$, and $Q^2 = 0.74$. Although it is not very clear a separation of the samples

with respect to the blend ratio could still be observed (Figure 5.36). While the samples containing 80% tallow located at the right part of the ellipse, samples with 60% tallow placed just right of the center and samples containing 70% tallow are in between them. Samples having 70 and 80% ratios are quite close to each other and it is very hard to separate them. Although not very obvious this discrimination is mostly resulted from the higher SFC, MP and SMP values of the samples as observed in the loading plot (Figure 5.36). Moreover, the central points (MCP) are mostly located at the upper part of the ellipse since they have higher consistency values (Figure 5.37). Therefore, multivariate analysis of the physical data indicated that the blend ratio caused some differentiation in the physical properties of the products while the reaction time did not.

To better analyze the chemically interesterified lipids during reaction a PCA model was also constructed by using all the data including both chemical and physical results with 4 PCs, R^2=0.83, and Q^2=0.46. There is a clear separation of the samples with respect to the blend ratio (Figure 5.38). Samples containing 60% tallow were located at the left part of ellipse while the structured lipids with 80% tallow placed at the right part of ellipse. Samples with 70% tallow concentration are generally placed between them. Discrimination of the samples containing 60% tallow is mostly resulted from higher TFA, MUFA and PUFA and FFA% of samples as observed in loading plot (Figure 5.39). The interesterified lipids produced with 80% tallow separated from other lipids due to higher SFC, SMP and MP. Moreover, there is not any discrimination with respect to reaction time. The results of PCA models were in accordance with ANOVA results Generally, the reaction time does not have remarkable effect on the chemical and the physical properties of structured lipids. Blend ratio is the most significant factor. However, reaction time is important since the non-interesterified blends (CO60, CO80) are placed separately from interesterified blends (Figure 5.38). This shows interesterification caused changes in the properties of corn oil-tallow blends.

5.3. Near and Mid-Infrared Spectroscopic Characterization of the Structured Lipids During the Chemical Interesterification

In order to characterize the structured lipids during the chemical interesterification spectral data were also collected by mid (FT-IR) and near infrared (FT-NIR) spectrometers. FT-NIR and FT-IR spectra were acquired both on melted and solid forms of the structured lipids. The principal component analysis (PCA) was applied to the

spectral data of the interesterified lipids to investigate the differences between the samples. Four different PCA models were constructed with FT-IR and FT-NIR spectra of the solid and melted forms of the samples.

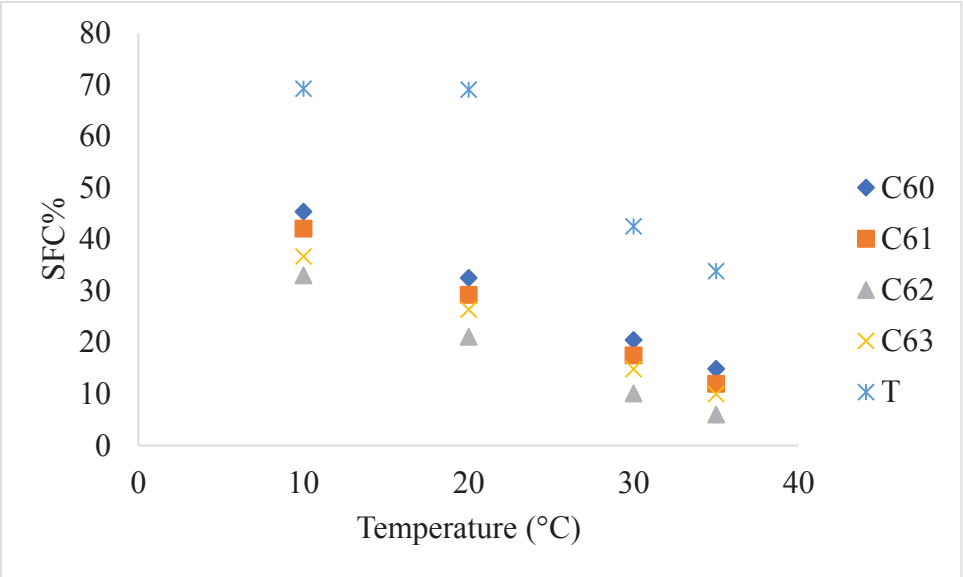

Figure 5.31 Solid fat content (SFC%) versus temperature for the samples interesterified with 60% tallow

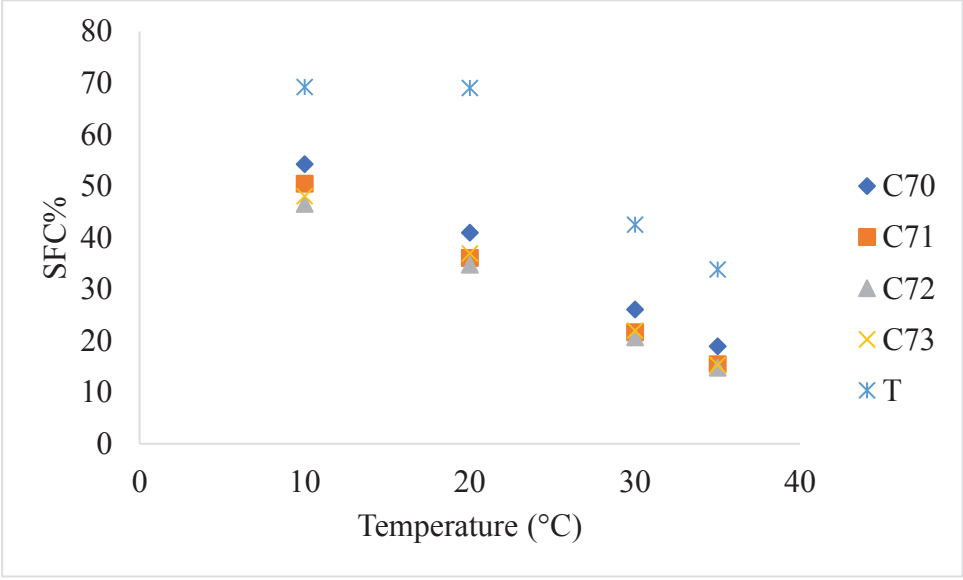

Figure 5.32 Solid fat content (SFC%) versus temperature for the samples interesterified with 70% tallow

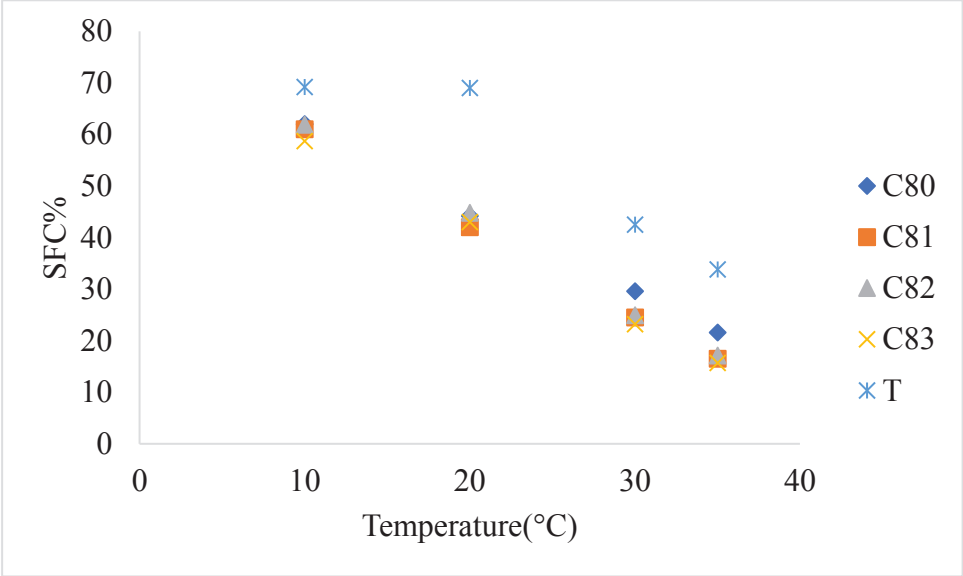

Figure 5.33 Solid fat content (SFC%) versus temperature for the samples interesterified with 80% tallow

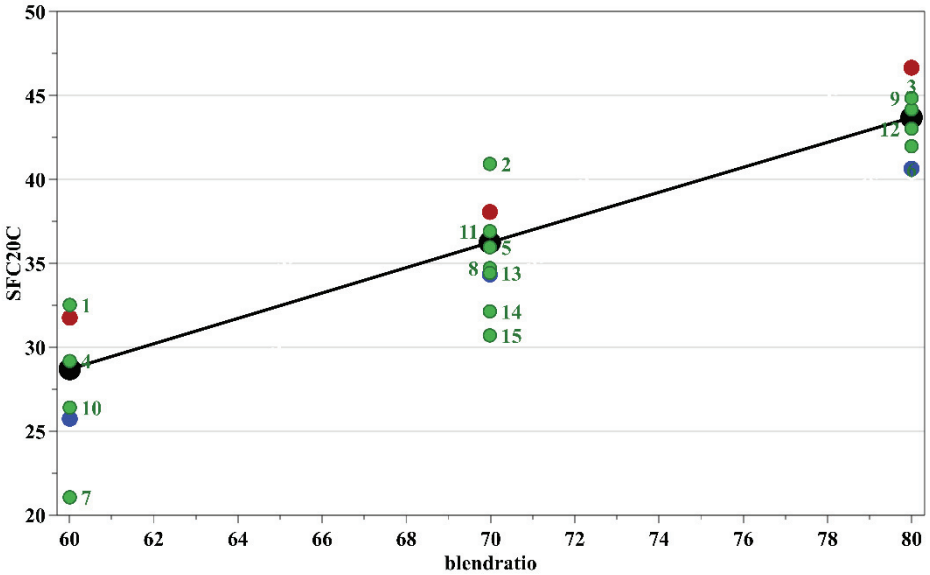

Figure 5.34 Main effect plot of blend ratio on solid fat content (SFC) of tallow-corn oil samples during the interesterification at 20 ºC

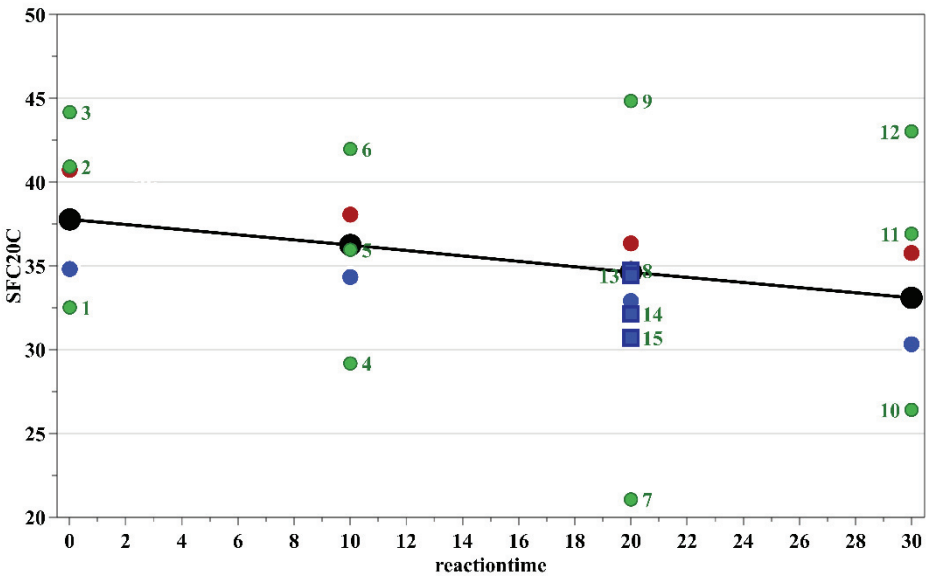

Figure 5.35 Main effect plot of reaction time on solid fat content (SFC) of tallow-corn oil samples during the interesterification at 20 ºC

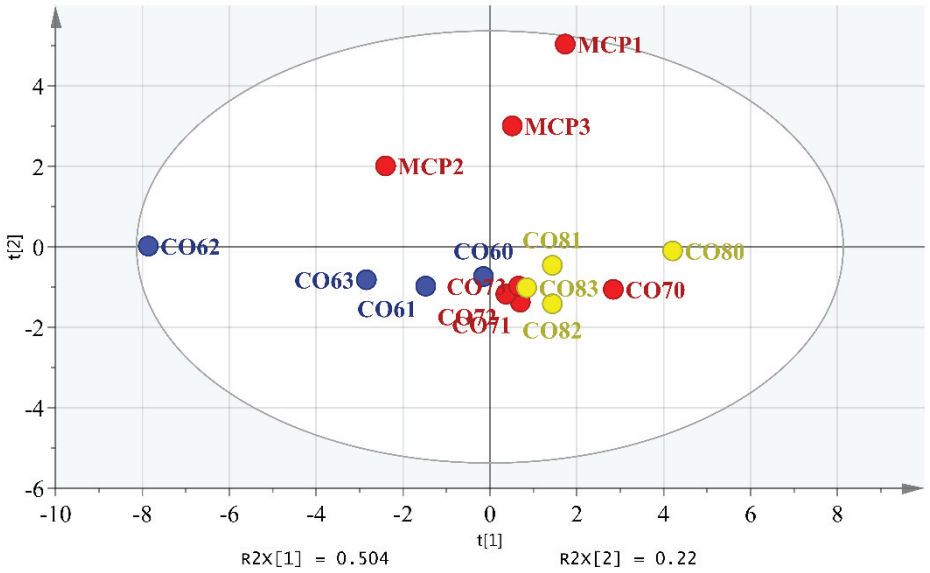

Figure 5.36 Score plot of the PCA model constructed by using all physical parameters of the chemically interesterified lipids throughout the reaction (MCP1-2-3=70% tallow-20 min, samples colored with respect to blend ratio)

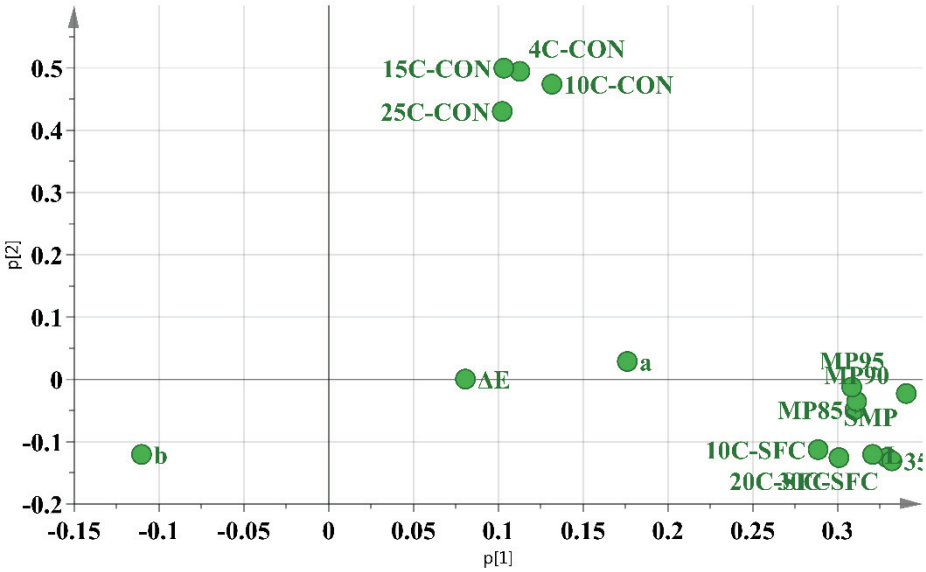

Figure 5.37 Loading plot of the PCA model constructed by using all physical parameters of the chemically interesterified lipids throughout the reaction

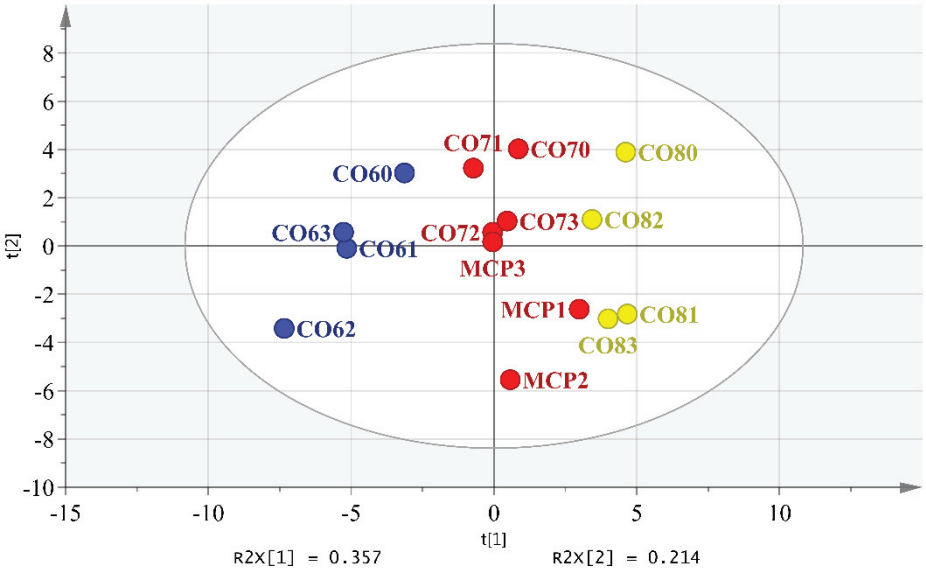

Figure 5.38 Score plot of the PCA model constructed by using all parameters of the chemically interesterified lipids throughout the reaction(MCP1-2-3=70% tallow-20 min, samples colored with respect to blend ratio)

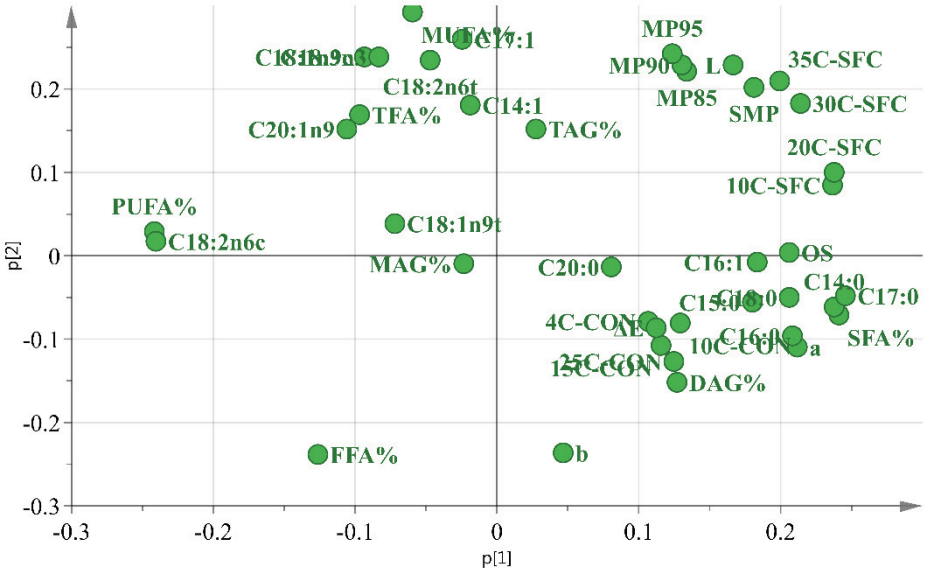

Figure 5.39 Loading plot of the PCA model constructed by using all parameters of the chemically interesterified lipids throughout the reaction

The model which was constructed by using the melted NIR spectra had 3 PCs, R^2 = 0.99, and Q^2 = 0.99. There is no discrimination of the samples with respect to the blend ratio and the reaction time (Figure 5.40). Most of the samples were located around the center of the ellipse. Samples containing 80% of tallow were divided into two groups with respect to the reaction time. While the initial blend and 10 minute interesterified product were placed at the bottom part of the left quartile, 20 and 30 minutes samples located at the upper part of the right quartile.

The model was also constructed by using solid NIR spectra with 4 PCs, R^2 = 0.99, and Q^2 = 0.99. There is not a clear discrimination of the samples with respect to the blend ratio (Figure 5.41). Samples containing 70% of tallow were divided into two groups according to the reaction time. While the initial blend and 10 minute interesterified product were placed at the left quartile, samples produced with 20 and 30 minutes reaction located at the right part as Figure 5.39 indicated.

The PCA model obtained from FT-IR melted spectra contains 6 PCs with R^2=0.99 and Q^2=0.97. PCA score plot was plotted by coloring the samples according to the reaction time (Figure 5.42). Discrimination of the samples with respect to the blend ratio is not observed again. However, most of the samples containing 70% of tallow located around the right part of the quartile while the others were placed in the left. The PCA model of FT-IR solid spectra with 6 PCs, R^2=0.99 and Q^2=0.98 did not show any

discrimination according to the process parameters (Figure 5.43). Although FT-IR and FT-NIR spectral analysis did not result in clear discrimination with respect to blend ratio and reaction time for the samples non-interesterified samples are generally separated from interesterified samples in all score plots. Therefore, it could be concluded that interesterified samples have different properties compared to physical blends as the multivariate statistical analysis of chemical and physical properties data also showed.

The results of PCA models for the analysis of chemical and physical properties data are in accordance with the conclusions obtained from ANOVA. Generally, both the reaction time and the blend ratio have some but not very considerable effects on the several of the chemical and the physical properties of the structured lipids. However, there is not a clear discrimination of the samples according to neither the blend ratio nor the reaction time as the score plots of IR spectral models confirmed.

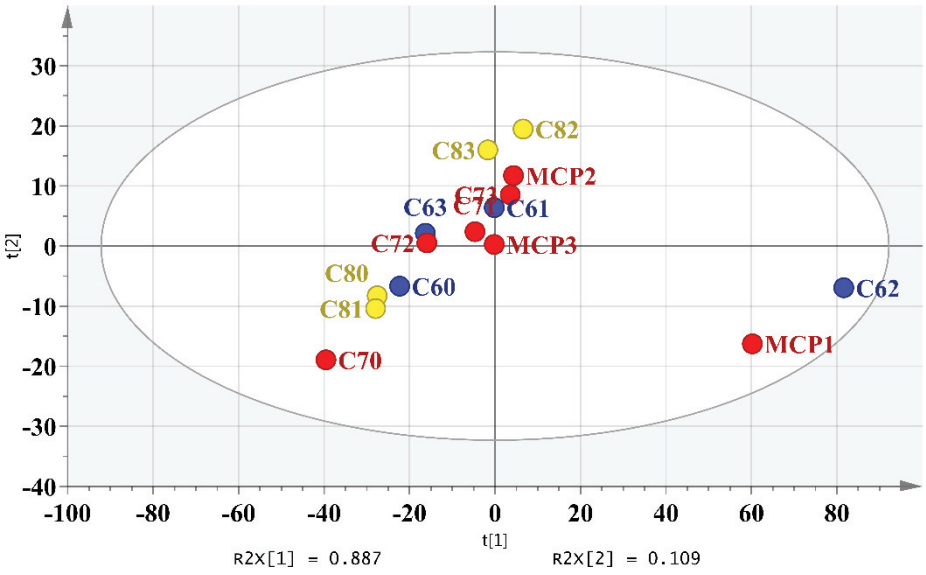

Figure 5.40 Score plot of the PCA model constructed by using melted spectra of FT-NIR of chemically interesterified lipids during the reaction (MCP1-2-3=70% tallow-20 min, samples are colored with respect to the blend ratio)

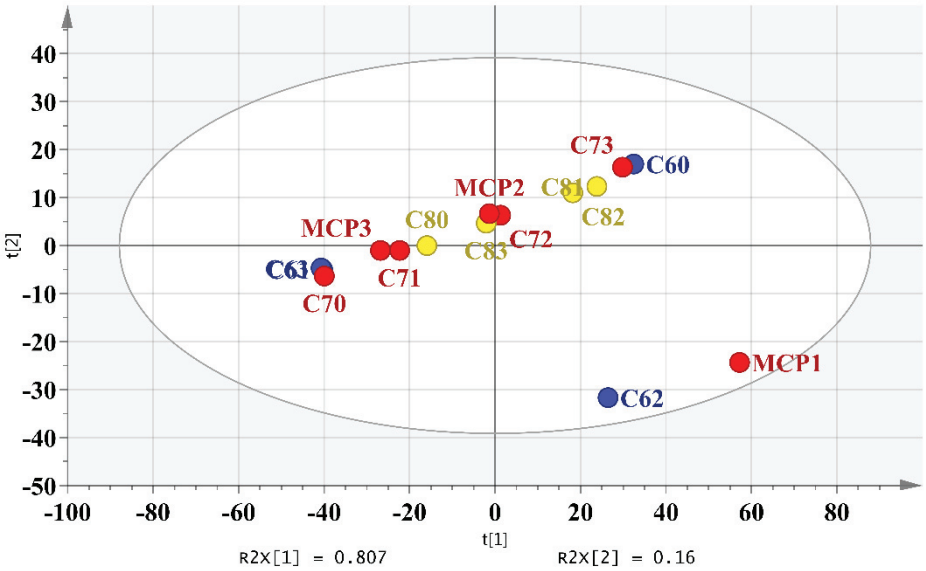

Figure 5.41 Score plot of the PCA model constructed by using solid spectra of FT-NIR of chemically interesterified lipids during the reaction (MCP1-2-3=70% tallow-20 min, samples are colored with respect to the blend ratio)

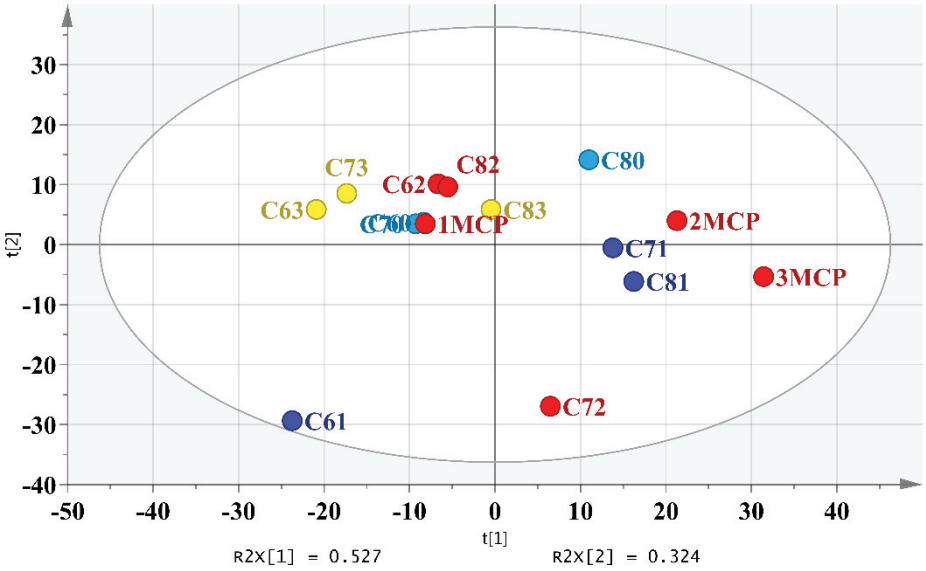

Figure 5.42 Score plot of the PCA model constructed by using melted spectra of FT-IR of chemically interesterified lipids during the reaction (MCP1-2-3=70% tallow-20 min, samples are colored with respect to the reaction time)

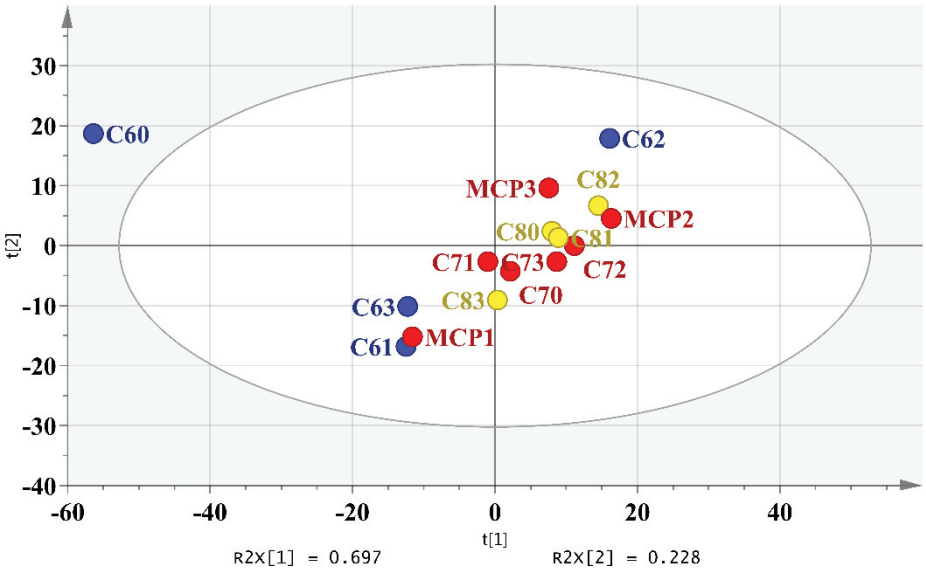

Figure 5.43 Score plot of the PCA model constructed by using solid spectra of FT-IR of chemically interesterified lipids during the reaction (MCP1-2-3=70% tallow-20 min, samples are colored with respect to the blend ratio)

CHAPTER 6

ENZYMATIC INTERESTERIFICATION OF BEEF TALLOW WITH CORN OIL

6.1. Chemical Analysis of Enzymatically Interesterified Lipids

As previously discussed, the structured lipids produced with chemical interesterification of corn oil and tallow have better physical and chemical properties; therefore, corn oil was also chosen as a substrate in the production of enzymatically interesterified lipids. A full factorial design as provided in Materials & Methods section was employed to evaluate the effects of reaction time (0, 3, and 6, 9, 12 h), and blend ratio (60:40, 70:30 and 80:20) on the properties of the structured lipids. The same chemical properties as in the previous chapters were measured, and the data were analyzed by univariate (ANOVA) and multivariate statistical analysis (PCA) techniques to investigate the effects of these parameters. In addition, spectral data were also collected by mid (FT-IR) and near infrared (FT-NIR) spectrometers to observe the differences between the interesterified lipids during enzymatic interesterification.

6.1.1. Fatty Acid Profile of Interesterified Lipids

The fatty acid compositions of enzymatically interesterified lipids during the process are given in Table 6.1. The major fatty acids in all products are oleic, palmitic, stearic and linoleic acids. Generally, interesterification reactions did not cause sharp changes in the amounts of fatty acids of the structured lipids as observed in the previous studies (Svensson and Adlercreutz 2008; Rønne et al. 2005; Silva et al. 2009).

The lipase enzyme from *Thermomyces lanuginosus* was used in the enzymatic interesterification reaction and this enzyme has a regioselectivity for fatty acids located at the sn-1 and sn-3 positions of triacylglycerol (TAG) molecule. Since the main purpose of the research is the modification of tallow by interesterification, selectivity of the enzyme for these positions does not have a major importance in this study but it was still of interest to understand and enlighten the reaction mechanism. While the fatty acids in

the sn-1 and sn-3-positions could have been changed due to regiospecifity of Lipozyme TL IM throughout the enzymatic interesterification, the fatty acids in the sn-2 position could stay as it is. However, the previous studies revealed that the enzyme was not perfectly regioselective for sn-1,3 position (Svensson and Adlercreutz 2008; Rønne et al. 2005) and there were also significant changes in the fatty acid composition in the sn-2 position after 6 hours of reaction (Svensson and Adlercreutz 2008).

In the current study, enzymatic interesterification did not cause any important changes in polyunsaturated fatty acid (PUFA) ratios of the samples (Figure 6.1). Saturated fatty acid (SFA) content of tallow (57.79%) decreased both by blending and enzymatic interesterification with corn oil (Figure 6.2). There is a sharp increase in PUFA% and decrease in SFA% of samples containing 70% tallow after 12 h reaction time (Figure 6.1 and 2). In addition, there are small fluctuations in monounsaturated fatty acid percentage (MUFA%) of enzymatically interesterified lipids during the process (Figure 6.3). These changes can be associated with the activity of the lipase enzyme. In general, tallow has SFAs located in sn-2 position (Forssell et al. 1992). Therefore, while SFAs were kept at sn-2 position, the MUFAs and PUFAs were presumably released from their positions throughout the enzymatic interesterification which causes increases of MUFA or PUFA of the samples. The Food and Agricultural Organization/World Health Organization (FAO/WHO) and the European Union Committee advise that the minimum polyunsaturated to saturated fatty acid ratio (PUFA/SFA) should be 1 for controlling the saturated fat consumption and encouraging the intake of MUFA and PUFA. While the PUFA/SFA ratio of the enzymatically interesterified lipids and physical blends ranged between 0.27-0.87 and 0.29-0.87, respectively tallow has the ratio of 0.06. This result indicated that PUFA content of tallow was increased by both blending and enzymatic interesterification reaction.

As in the previous researches, enzymatic interesterification of tallow with corn oil did not cause formation of trans fatty acids (TFA) (Foglia et al. 1993; Forssell et al. 1992). Structured lipids having 60:40 and 80:20 ratios have similar trends regarding the TFA content, and the highest TFA ratios for these blends were observed at 9 h and after that. However, the enzymatic interesterification for 3 h resulted in high TFA content for 70:30 ratio and a gradual decline was observed after 3 h. The amount of fatty acids in trans form is less than 1% for several of the interesterified products. Corn oil itself has TFA content of less than 1% and their interesterified forms have slightly higher percentages of

trans fats. These results indicate that these structured lipids are suitable for the production of low trans containing shortenings, margarines and frying fats (Figure. 6.4).

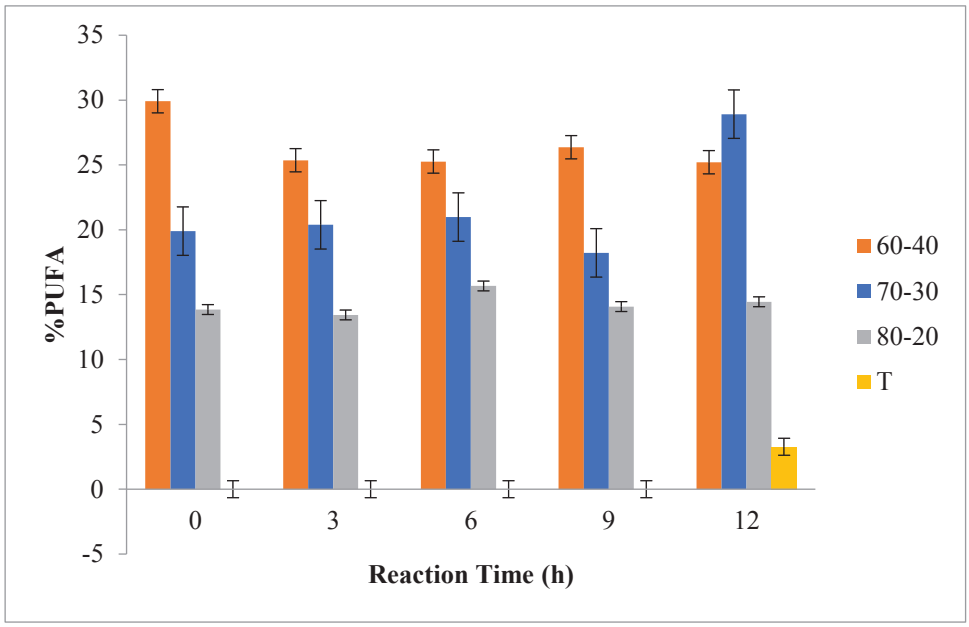

Figure 6.1 Percentages of polyunsaturated fatty acids (PUFA) of the structured lipids during the enzymatic interesterification with respect to blend ratio and reaction time

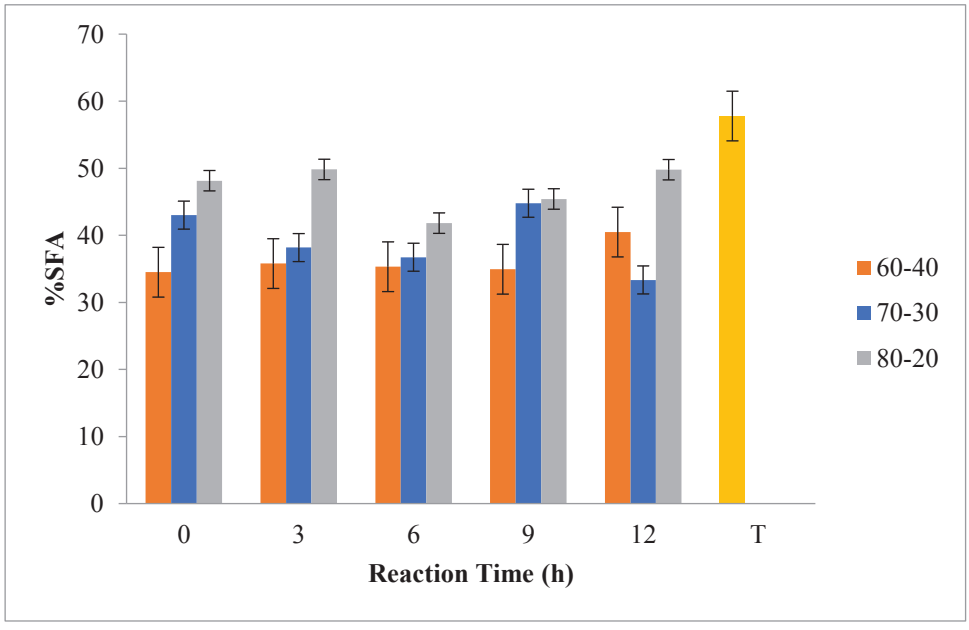

Figure 6.2 Percentages of saturated fatty acids (SFA) of the structured lipids during the enzymatic interesterification with respect to blend ratio and reaction time

Table 6.1 Percentages of individual fatty acids of enzymatically interesterified samples, corn oil and tallow

Samples	C14:0	C14:1	C15:0	C16:0	C16:1	C17:0	C17:1	C18:0	C18:1n9c	C18:1n9t	C18:2n6t	C18:2n6c	C20:0	C20:1n9	C18:3n3
ECP1	1.43	0.38	0.23	16.90	0.94	0.71	0.28	20.30	37.74	0.04	0.06	20.07	0.28	0.50	0.21
ECP2	1.46	0.38	0.22	16.41	0.99	0.66	0.27	18.50	38.56	0.17	0.03	21.39	0.26	0.51	0.20
ECP3	1.46	0.34	0.19	16.68	1.01	0.69	0.27	19.77	38.28	0.12	0.04	20.10	0.25	0.67	0.18
E60	0.88	0.22	0.16	16.51	0.58	0.47	0.16	16.18	33.51	0.54	0.04	29.71	0.31	0.59	0.16
E63	1.14	0.29	0.18	16.33	0.81	0.59	0.24	17.25	36.29	0.68	0.06	25.06	0.30	0.55	0.24
E66	1.14	0.29	0.19	15.79	0.82	0.61	0.22	17.30	36.88	0.65	0.07	24.96	0.30	0.56	0.23
E69	1.22	ND	ND	16.41	0.99	0.61	ND	16.41	36.10	1.03	ND	26.36	0.29	0.59	ND
E612	1.76	0.23	0.29	18.59	0.80	0.76	0.21	18.76	31.12	1.44	0.06	24.97	0.33	0.52	0.18
E70	1.22	0.31	0.23	18.66	0.75	0.63	0.24	22.00	34.71	0.61	0.08	19.54	0.28	0.47	0.28
E73	1.44	0.36	0.22	16.63	0.99	0.69	0.27	18.92	38.06	1.26	ND	20.18	0.28	0.51	0.20
E76	1.50	ND	ND	16.26	1.15	0.70	ND	18.27	39.37	1.08	ND	20.98	ND	0.77	ND
E79	1.24	0.19	0.21	17.31	0.87	0.80	0.26	24.85	34.57	0.58	ND	18.00	0.38	0.44	0.23
E712	2.33	ND	ND	16.24	ND	ND	ND	14.77	37.74	ND	ND	28.92	ND	ND	ND
E80	1.43	0.36	0.27	19.91	0.89	0.79	0.28	25.49	35.46	0.62	0.08	13.45	0.25	0.40	0.32
E83	1.49	0.21	0.23	18.51	0.98	0.90	0.27	28.18	34.88	0.11	0.06	12.94	0.52	0.37	0.44
E86	1.54	0.26	0.25	17.32	1.02	0.77	0.24	21.61	40.56	0.09	0.06	15.33	0.32	0.42	0.28
E89	1.55	0.23	0.24	18.05	1.45	0.83	0.30	24.37	37.42	0.67	0.10	13.70	0.38	0.43	0.28
E812	2.29	0.40	0.38	20.42	1.09	1.00	0.30	25.27	32.84	0.75	0.18	14.04	0.42	0.39	0.23
T	1.80	0.45	0.35	22.52	1.07	0.94	0.30	32.06	36.18	0.74	0.07	2.97	0.13	0.20	0.23
CO	ND	ND	ND	11.23	0.08	ND	ND	1.87	30.56	0.47	ND	54.59	ND	ND	0.96

*Abbreviations are provided in Materials & Methods section

Standard deviation for C14:0=±0.01, C14:1=±0.02, C15:0=±0.02, C16:0=±0.2, C16:1=±0.03, C17:0=±0.02, C17:1=0, C18:0=±0.76, C18:1n9c=±0.34, C18:1n9t=±0.05, C18:2n6t=±0.01, C18:2n6c=±0.61, C20:0=±0.02, C20:1n9=±0.01,C18:3n3=±0.01,C20:1n9c=±0.08 (calculated from CPs)

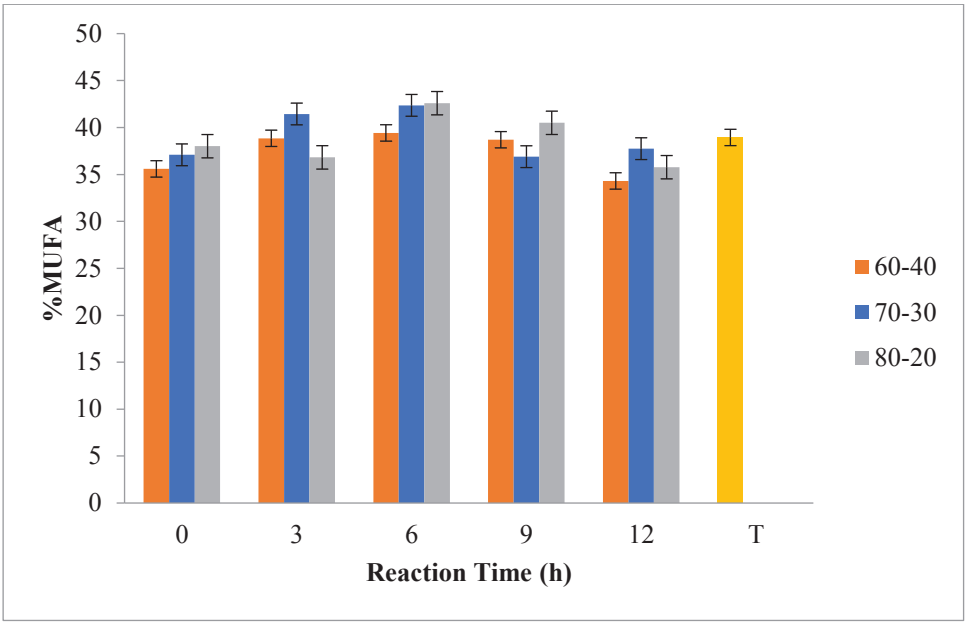

Figure 6.3 Percentages of monounsaturated fatty acids (MUFA) of the structured lipids during the enzymatic interesterification with respect to blend ratio and reaction time

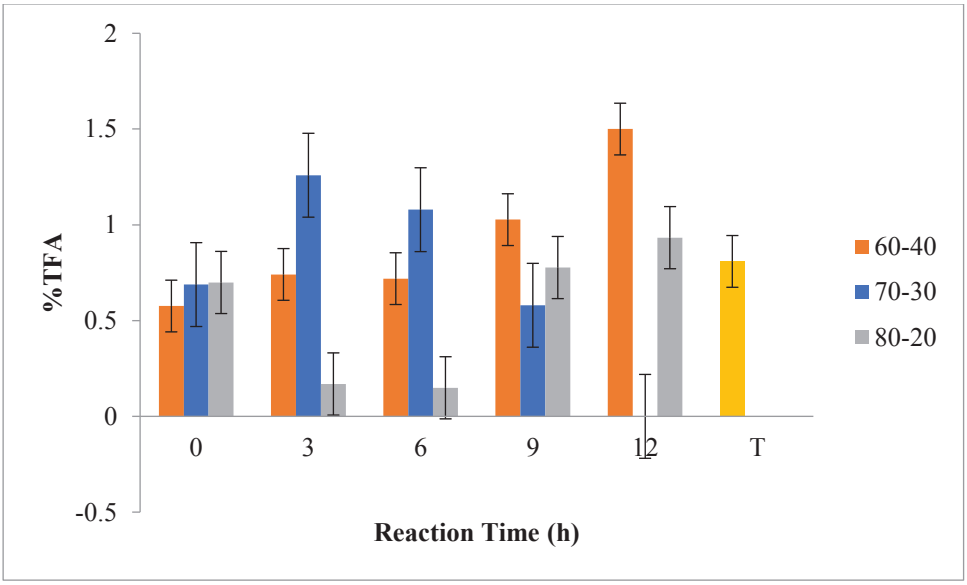

Figure 6.4 Percentages of trans fatty acids (TFA) of the structured lipids during the enzymatic interesterification with respect to blend ratio and reaction time

ANOVA results (App. 11) indicated that while the models constructed for PUFA% and SFA% were significant with non-significant lack of fit at 95% confidence interval, the models for MUFA% and TFA% were found insignificant. Normality and residuals were also checked for the models. Reaction time did not have any prominent

effect on the fatty acid contents of the samples. The ANOVA table reveals that blend ratio has important effect on SFA and PUFA content of the structured lipids. While the increase in blend ratio of tallow led to decrease in PUFA content of the interesterified fats, SFA content of the enzymatically interesterified lipids increased respectively as Figure 6.5 and 6 indicated.

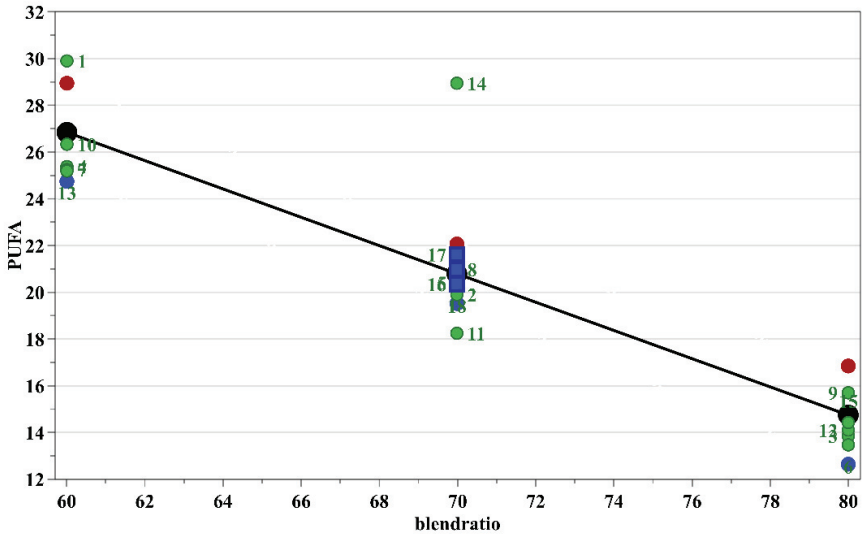

Figure 6.5 Main effect plot for the blend ratio on polyunsaturated fatty acid content (PUFA%) of enzymatically interesterified samples

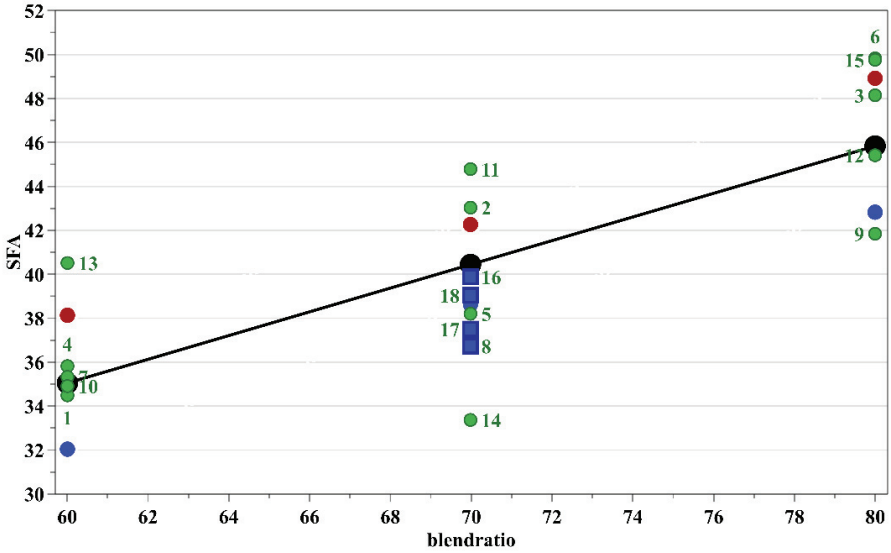

Figure 6.6 Main effect plot for the blend ratio on saturated fatty acid content (SFA%) of enzymatically interesterified samples

6.1.2. Oxidative Stability of Interesterified Lipids

The oxidation induction times of the enzymatically interesterified samples are shown in Table 6.2. The oxidation induction time of tallow is 4.81 h and while blends without interesterification have a range of induction times of 6.73-10 h. Oxidative stability decreased with interesterification and the enzymatically interesterified samples have lower oxidative stabilities (0.6-3.93 h) in comparison to chemically interesterified lipids (3.82-7.76 h). Oxidation induction times of lipids decreased regardless of blend ratio especially after 6 h reaction time (Figure.6.7).

In the previous studies, the decrease in oxidative stability of interesterified fats compared to the initial mixture was also observed in general (Bryś et al. 2014; Martin et al. 2010; Kowalska et al. 2014). The methods that are used in the production or purification of structured lipids, oil sources, presence of antioxidants during the manufacturing are among the main factors that affect the oxidative stability of structured lipids. Moreover, the structure of the triacylglycerol (TAG) including fatty acid composition and positional distribution on the glycerol backbone, as well as the interaction of these factors, have important impact on the oxidative stability of the structured products. In the present study, since the enzyme used in interesterification reaction (Lipozyme TL IM) has regiospecifity on sn-1,3 bonds of TAG molecules, the final products would not be glycerols but sn-2 monoglyceride instead. In general, tallow has SFAs located in sn-2 position (Forssell et al. 1992). Therefore, while SFAs were kept at sn-2 position, the MUFAS and PUFAs were presumably released from their positions throughout the enzymatic interesterification which causes decreases in oxidation induction time of structured lipids. This decrease is more remarkable between 3-6 hours of reaction. However, after 6 hours of reaction, there are some increases in oxidation induction time of the samples which can be associated with the rearrangement of PUFAs in di- and triacylglycerol molecules. It can be suggested that 3 h reaction time could be more suitable for enzymatical interesterification of tallow if only the oxidative stability of interesterified products would be considered.

Univariate statistical analysis was also performed for oxidative stability data of enzymatically interesterified samples (App. 11). ANOVA results indicated that constructed model was significant with significant lack of fit. Normality and residuals were checked for the model. The ANOVA table reveals that only reaction time has

significant effect on the oxidative stabilities of the samples (App 11). Increasing reaction time has negative effect on the oxidative stability of the samples (Figure 6.8).

Table 6.2 Oxidation induction times (h) of corn oil and tallow and enzymatically interesterified lipids during reaction

Sample	Oxidation induction time (h)
E60	6.73
E63	3.12
E66	0.60
E69	1.38
E612	2.80
E70	8.51
E73	1.80
E76	0.82
E79	2.08
E712	1.81
E80	10.00
E83	3.39
E86	1.93
E89	1.84
E812	1.90
ECP1	0.89
ECP2	1.10
ECP3	1.31
T	4.81
CO	4.98

*Abbreviations are provided in Materials & Methods section
Standard deviation for OS=±0.17 (calculated from CPs)

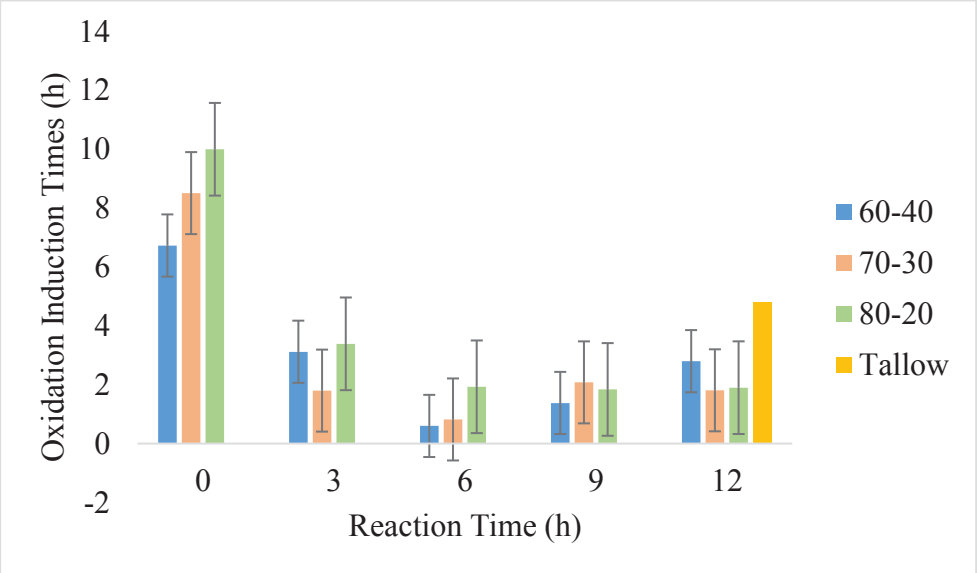

Figure 6.7 Oxidation induction times of tallow-corn oil samples during the enzymatic interesterification process with respect to blend ratio and reaction time

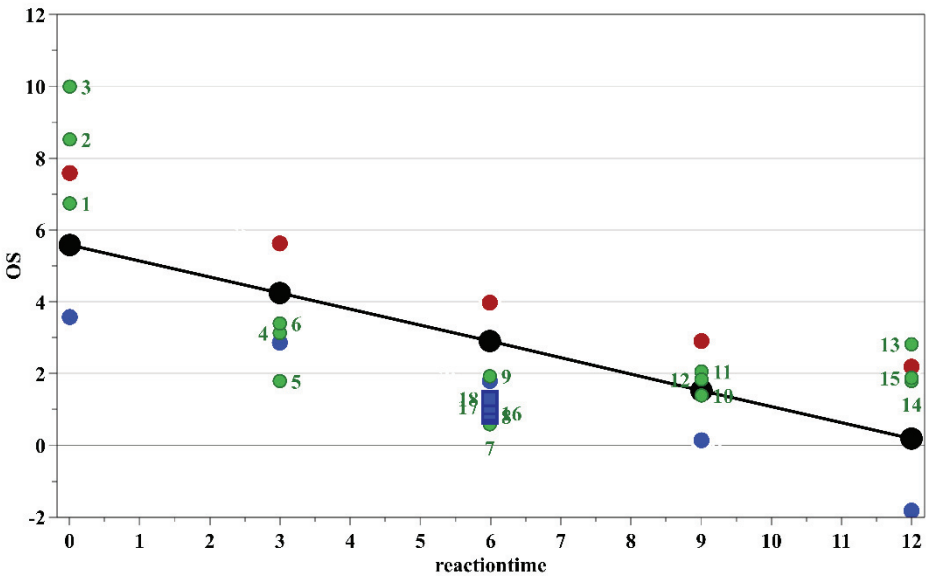

Figure 6.8 Main effect plot for the blend ratio on oxidative stability (OS) of enzymatically interesterified samples

6.1.3. Free Fatty Acid Content of Interesterified Lipids

The free fatty acid percentages (FFA%) of the samples are listed in Table 6.3. The acidity is expressed as the percentage of oleic acid. The FFA% of tallow is 1.15% while blends without interesterification have a range of 0.62-0.76% as oleic acid. Generally, FFA % of interesterified lipids increased sharply compared to starting blends. It means that neutralization should be applied to samples after enzymatic interesterification.

As it could be seen in Figure. 6.9, there has been a drastic increase and fluctuations in FFA% of the samples depending on their blend ratio during enzymatic interesterification. The fluctuations can be associated with the activity of the enzyme. Throughout interesterification reactions, the enzyme acts on fatty acids of the TAG molecules and lead to formation of diacylglycerol and monoacylglycerol molecules. Therefore, increases and fluctuations in FFA% could be observed during reaction time. This increase was also observed in the previous studies (Kowalska et al. 2014; Rønne et al. 2005).

Table 6.3 Free fatty acid percentages (% oleic acid) of corn oil, tallow and the enzymatically interesterified lipids during reaction

Sample	FFA%
E60	0.62
E63	12.76
E66	19.00
E69	5.67
E612	12.47
E70	0.62
E73	17.81
E76	25.64
E79	20.03
E712	12.60
E80	0.76
E83	11.15
E86	15.38
E89	20.50
E812	20.75
ECP1	21.89
ECP2	24.35
ECP3	23.24
T	1.15
CO	0.09

*Abbreviations are provided in Materials & Methods section
Standard deviation for FFA=±1.01 (calculated from CPs)

ANOVA results for the enzymatically interesterified samples indicated that constructed model was insignificant with significant lack of fit. Normality and residuals were checked for the model. Although the model is not significant, reaction time has still important effect on FFA content as ANOVA table indicated (App. 11). This is also supported by the main effect plot. The FFA content of the samples increased slightly during the enzymatic interesterification reaction (Figure. 6.10).

6.1.4. Mono, di, and triacylglycerol contents of interesterified lipids

Mono, di and triacylglycerol (MAG, DAG and TAG) contents of the structured lipids were determined to examine the changes in the glycerol backbone that occurred by the action of Lipozyme TL IM during interesterification. MAG, DAG and TAG contents

of the samples are expressed in relative percentages of the overall content (Table 6.4). The results are in accordance with the previous studies, which observed a decrease in TAG% after interesterification (Kowalska et al. 2005; Ledóchowska and Wilczyńska 1998; Kowalska et al. 2014).

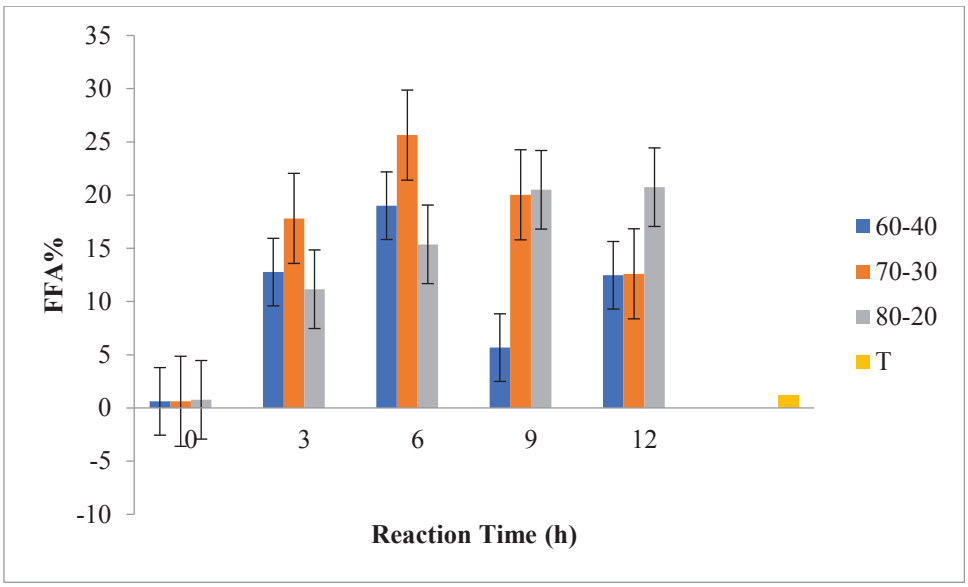

Figure 6.9 Free fatty acid percentages (FFA%) versus reaction time of the enzymatically interesterified lipids with respect to blend ratio

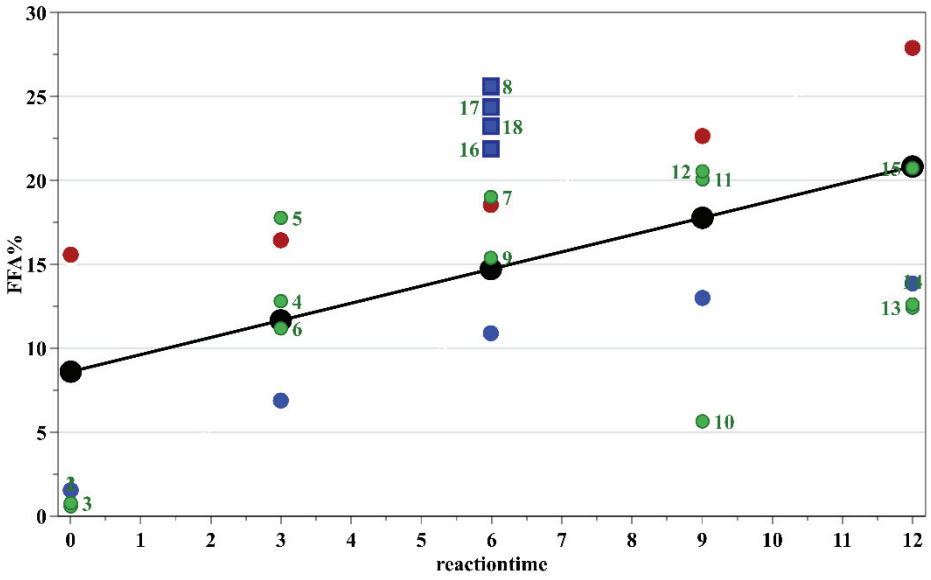

Figure 6.10 The main effect plot for reaction time on free fatty acid (FFA%) of the enzymatically interesterified samples

The TAG% of tallow is approximately 98% while blends without interesterification have slightly lower TAG% values. Both blending and enzymatic interesterification caused an increase in MAG and DAG contents of the samples. Generally, TAG% of enzymatically interesterified lipids were lower than their starting blends.

As it could be seen in Figure. 6.11, there has been a drastic decrease in TAG% of the samples up to 6 h of enzymatic interesterification process. After that point, there are some fluctuations in TAG% of the samples with respect to their blend ratio. Although DAG content of the samples increased up to 9 h reaction time, after 12 h of reaction DAG% of samples decreased (Figure 6.12). Same trend was also observed for MAG content of the samples up to 6 h reaction time. After that point MAG% of samples decreased up to 12 h of interesterification process (Figure 6.13). These changes in TAG, DAG, and MAG content of the interesterified lipids could be explained with the activity of Lipozyme TL IM. The decrease in TAG content and the increase of DAG and MAG up to 6 h of reaction confirms that the enzyme works effectively. It means that the enzyme attacks the fatty acid located at sn1,3 positions of TAGs and provide the formation of MAG and DAG. With the increase in reaction time, the decrease in DAG and MAG contents reveals that the fatty acids are snatched from their positions by the enzyme and they participate in the production of new TAG molecules. Moreover, the fluctuations in TAG after 6 h of reaction supports this explication.

In order to better understand the enzymatic interesterification reaction a correlation between FFA% and DAG+MAG content of interesterified lipids is also evaluated (Figure 6.14). Pearson correlation coefficient is calculated and found as less than 1 (P =0.9) and correlation coefficient is also good (R^2 = 0.82). There is an increasing trend between FFA content and DAG+MAG % of the samples during enzymatic interesterification reaction (Figure 6.14). Generally, the samples having higher FFA%, have also higher amount of DAG+MAG content as Figure 6.14 indicated. The increase in both FFA% and DAG+MAG content with time confirmed that the enzyme showed activity. The enzyme released the fatty acids from their specific positions and caused an increase in both FFA% and DAG+MAG%.

The similar trend was also observed in another study and the increase in FFA and MAG+DAG contents was correlated with temperature, the time of reaction and the enzyme concentration. Moreover, it was commented that the decrease in TAG was inversely proportional with the same factors (Kowalski et al. 2004).

Table 6.4 Relative percentages of triacylglycerol (TAG), diacylglycerol (DAG) and monoacylglycerol (MAG) of the samples

Samples	TAG%	DAG%	MAG%
E60	85.51	5.91	0.26
E63	66.96	21.74	11.93
E66	54.37	26.12	15.93
E69	68.41	14.00	12.07
E612	70.60	15.62	13.05
E70	86.84	2.68	4.05
E73	69.00	22.99	7.76
E76	49.84	30.39	22.32
E79	56.47	31.43	18.69
E712	56.35	26.08	17.30
E80	85.52	0.57	8.48
E83	61.84	19.44	12.84
E86	63.17	24.78	11.47
E89	48.04	21.15	17.74
E812	63.16	16.66	17.27
ECP1	56.67	24.32	11.04
ECP2	61.19	22.63	11.76
ECP3	60.25	22.67	9.21
T	97.94	0.48	0.92

*Abbreviations are provided in Materials & Methods section
Standard deviation for: TAG% = ±1.95, DAG% = ±0.79, MAG% = ±1.08 (calculated from CPs)

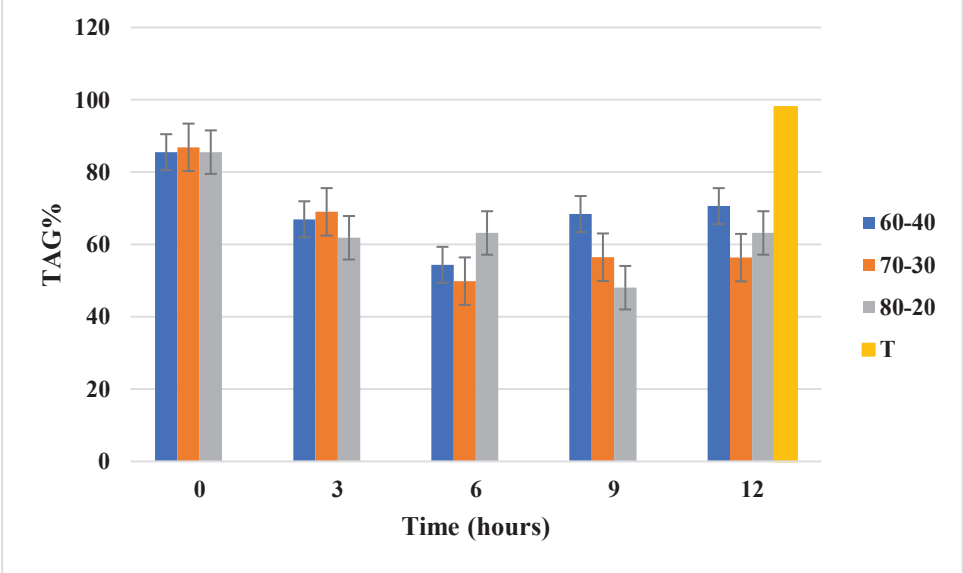

Figure 6.11 Triacylglycerol percentages (TAG%) of the structured lipids during enzymatic interesterification reaction with respect to blend ratio and reaction time

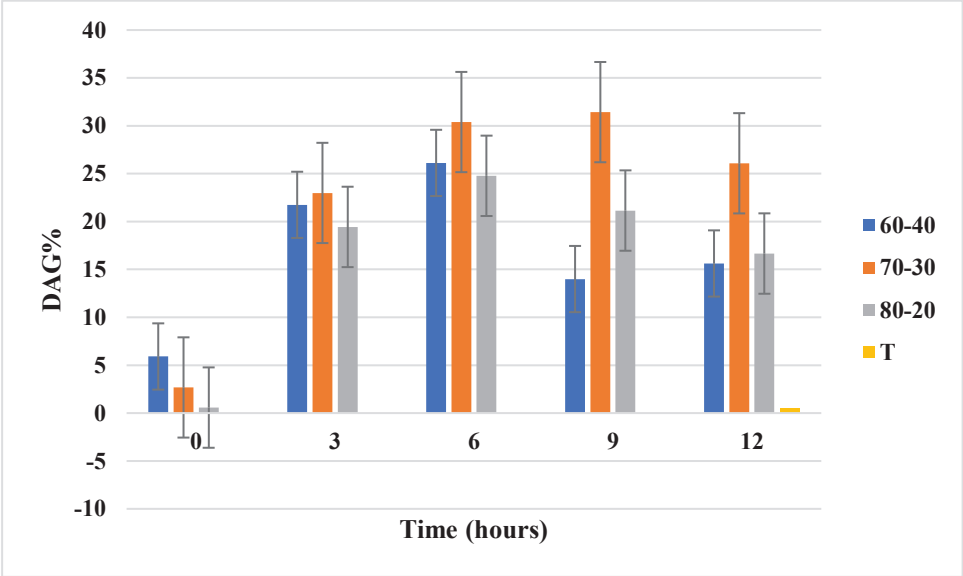

Figure 6.12 Diacylglycerol percentage (DAG%) of the structured lipids during enzymatic interesterification reaction with respect to blend ratio and reaction time

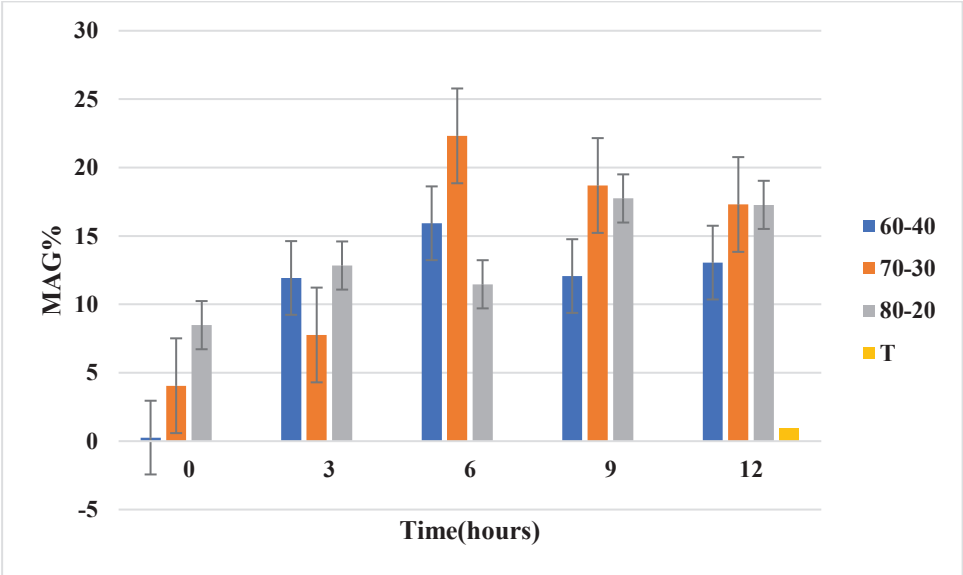

Figure 6.13 Monoacylglycerol percentage (MAG%) of the structured lipids during enzymatic interesterification reaction with respect to blend ratio and reaction time

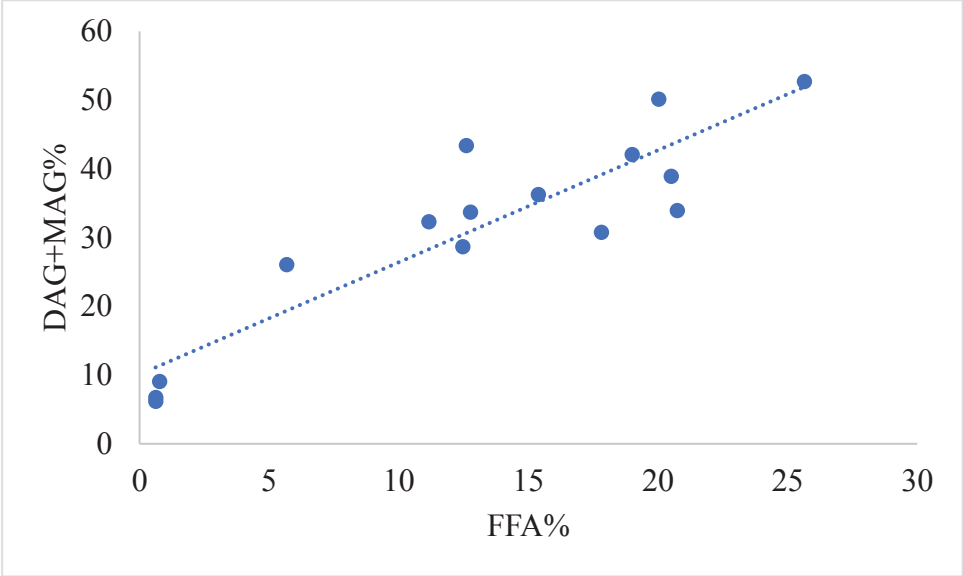

Figure 6.14 Free fatty acid content (FFA%) versus mono and diacylglycerol content (DAG+MAG%) of the structured lipids

The statistical analysis results for TAG-DAG-MAG% of the samples are given in App. 11. ANOVA results indicated that while the models constructed for TAG and MAG were significant, the model for DAG was insignificant. Normality and residuals were checked for the models. The ANOVA table reveals that only reaction time is a significant factor for the models (App 11). As the reaction time is increased, TAG% of samples decreased particularly (Figure. 6.15). Although the model for DAG is not significant, ANOVA table reveals that reaction time has premoninat effect on DAG of interesterified lipids. As Figure 6.16 indicated with increase in reaction time there is an increase in DAG content. In addition, MAG% also increased with the rise in reaction time (Figure. 6.17).

To analyze the chemical properties data of the enzymatically interesterified lipids throughout the reaction principal component analysis (PCA) was also applied. The model was constructed by using all measured chemical parameters with 7 PCs, $R^2 = 0.6$, and $Q^2 = 0.2$. There is some discrimination of the samples with respect to blend ratio (Figure. 6.18). While the samples containing 80% tallow are located at the right part of the ellipse, some of the samples with 70% tallow are placed just around the center and the samples containing 60% tallow are further in the upper left quartile. Therefore, a discrimination with respect to the first principal component was obtained as far as the blend ratio is concerned. This discrimination mostly resulted from higher PUFA and SFA content of the samples as observed in Figure 6.19. Moreover, the samples with 80% tallow mostly

located at the right side of the ellipse since they have higher SFA% (Figure 6.19). In addition, physical blends (E60, E70, E80) are separated from their interesterified forms meaning that interesterfied products have different chemical properties than blends.

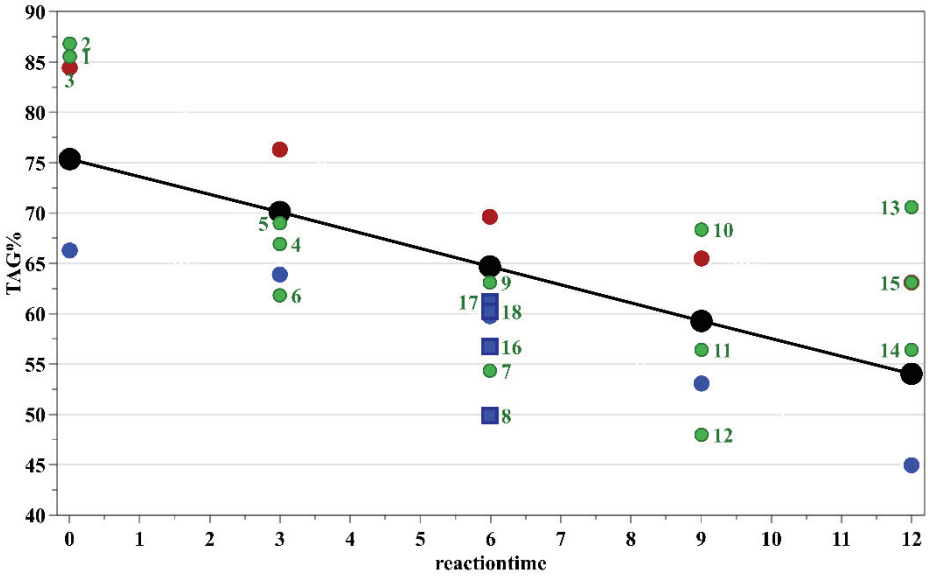

Figure 6.15 Main effect plot of reaction time of enzymatically interesterified samples for triacylglycerol content (TAG%)

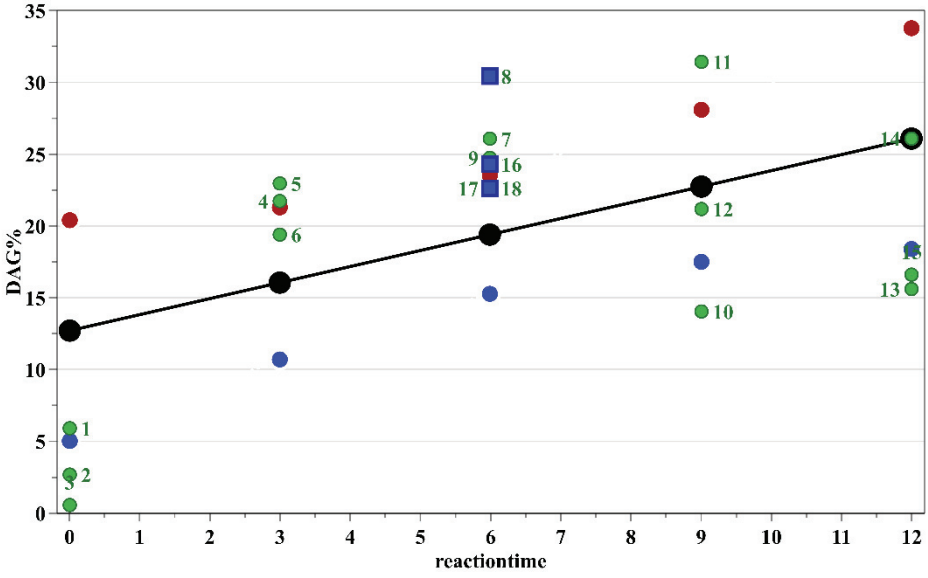

Figure 6.16 Main effect plot of reaction time of enzymatically interesterified samples for diacylglycerol content (DAG%)

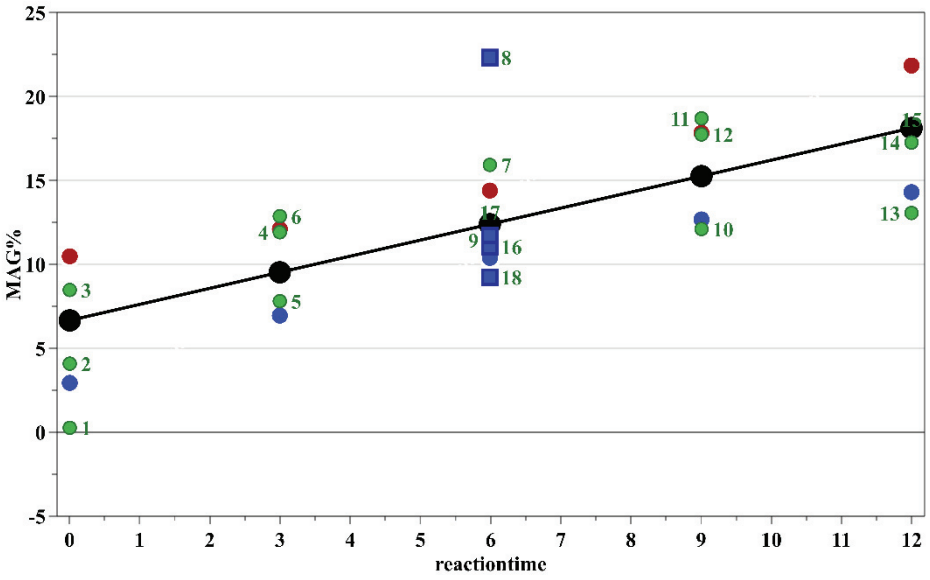

Figure 6.17 Main effect plot of reaction time of enzymatically interesterified samples for monoacylglycerol content (MAG%)

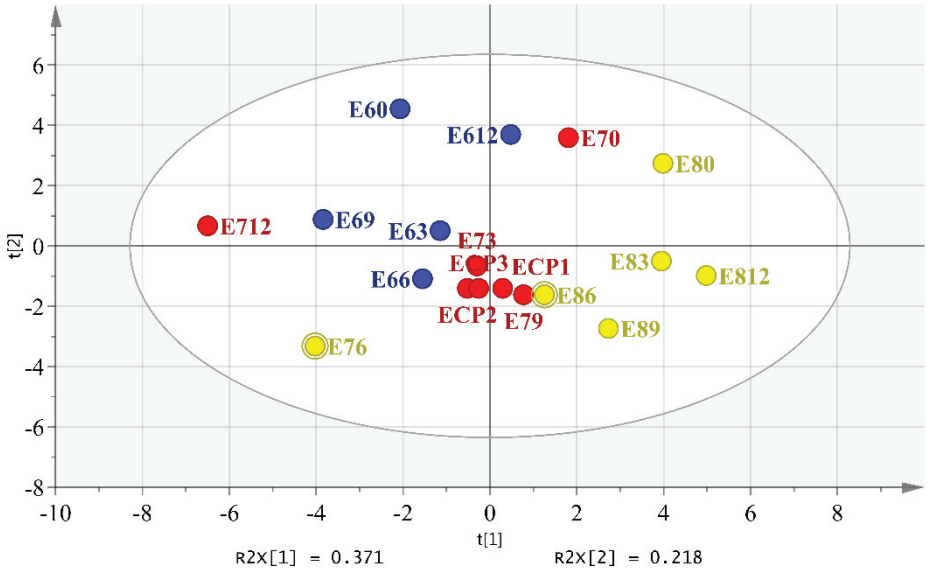

Figure 6.18 Score plot of the PCA model constructed by using all chemical parameters of enzymatically interesterified lipids throughout reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the blend ratio)

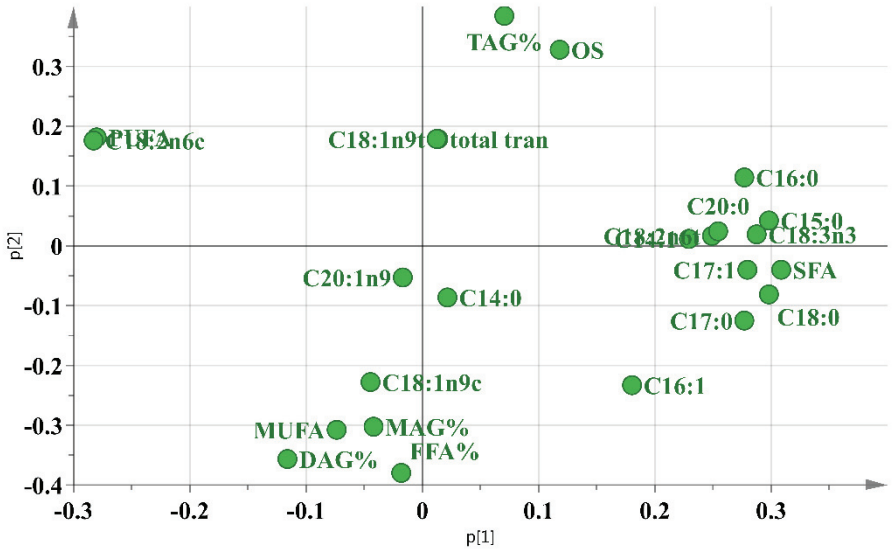

Figure 6.19 Loading plot of the PCA model constructed by using all chemical parameters of enzymatically interesterified lipids throughout reaction

6.2. Physical Properties of Structured Lipids During Enzymatic Interesterification

The same physical properties as in the previous chapters were measured, and the data were analyzed by univariate (ANOVA) and multivariate statistical analysis (PCA) techniques to investigate the effects blend ratio and reaction time on these parameters. In addition, spectral data were also collected by mid (FT-IR) and near infrared (FT-NIR) spectrometers to observe the differences between the interesterified lipids during enzymatic interesterification.

6.2.1. Crystal Morphology of Enzymatically Interesterified Lipids

The polymorphic forms of the structured lipids and blends are provided in Table 6.5. Same crystal types were also observed in the previous studies (Li et al. 2018; Liu et al. 2009; Jin et al. 2008). Tallow contains mixtures of β and β' forms dominated by β' form. α forms were only observed in the enzymatically structured lipids E712 and E812 which are the samples having long reaction times. The non-interesterified blends also contain both β and β' forms but dominated by β form. However, after enzymatic interesterification only β form existed in most of the samples. However, long reaction

times resulted in different polymorphs: sample having 60:40 blend ratio with 12 h reaction time have β+β' and 70:30 and 80:20 blend ratios with the same reaction time have β+ α and α polymorphs, respectively.

Table 6.5 Polymorphic forms of tallow and the structured lipids during enzymatic interesterification

Samples	Crystal Type
E60	β+β'
E63	β
E66	β
E69	β
E612	β+β'
E70	β+β'
E73	β
E76	β
E79	β
E712	α+β
E80	β+β'
E83	β'
E86	β
E89	β
E812	α
ECP1	β
ECP2	β
ECP2	β
T	β+β'

*Abbreviations are provided in Materials & Methods section

6.2.2. Color Properties of Enzymatically Interesterified Lipids

The lightness (L), redness (a) and yellowness (b) values of the enzymatically interesterified samples were measured and then total color difference (ΔE) were calculated considering tallow itself as a standard. The L, a, b and ΔE values of the enzymatically interesterified samples during the reaction are listed in Table 6.6. The lightness value of tallow is 80.71, redness is -2.24 and yellowness is 3.42. Both enzymatic interesterification at all reaction times and blending caused decreases in the lightness of the samples with respect to tallow and the lightness of the interesterified lipids were also lower than initial blends. L values of the interesterified lipids increased with increasing reaction time. Moreover, twelve hours of reaction time resulted in higher L values regardless of blend ratio. Generally, a and b values of the samples decreased compared to tallow itself after enzymatic interesterification (Table 6.6). Generally, the ΔE values of

the samples increased up to 6 h reaction time and then decreased respectively as Figure 6.20 indicated.

Table 6.6 L, a, b and ΔE color values of tallow, non-esterified blends and enzymatically interesterified lipids during reaction

Sample	L	a	b	ΔE
E60	66.04	-4.38	0.07	13.88
E63	52.83	-3.25	-1.06	26.90
E66	50.37	-3.20	0.56	29.16
E69	53.65	-3.64	-0.18	26.00
E612	59.69	-3.49	1.09	19.86
E70	70.62	-3.37	0.26	9.28
E73	55.61	-3.34	-0.60	24.10
E76	57.04	-3.23	0.39	22.55
E79	55.71	-3.09	-1.48	24.13
E712	64.43	-3.08	2.20	15.05
E80	74.23	-3.30	1.10	5.65
E83	58.57	-3.86	-2.09	21.51
E86	58.10	-2.83	-1.28	21.73
E89	63.26	-3.28	0.05	16.46
E812	68.48	-3.10	1.67	11.07
ECP1	60.36	-3.00	0.37	19.24
ECP2	63.46	-2.94	1.55	16.04
ECP3	61.01	-2.63	0.16	18.61
T	79.42	-1.91	2.85	0.00

*Abbreviations are provided in Materials & Methods section
Standard deviation for L=±1.34, a=±0.16, b=±0.61, ΔE=±1.39 (calculated from CPs)

The Appendix 12 shows the statistical analysis results for color measurements. ANOVA results indicated that constructed model for total color difference is not significant at $p<0.05$ with non-significant lack of fit. Normality and residuals were also checked for the model. Although the model is insignificant, blend ratio could be considered as an important factor for ΔE of the interesterified lipids (App. 12). Increasing the reaction time leads to decreases in ΔE of the samples (Figure 6.21).

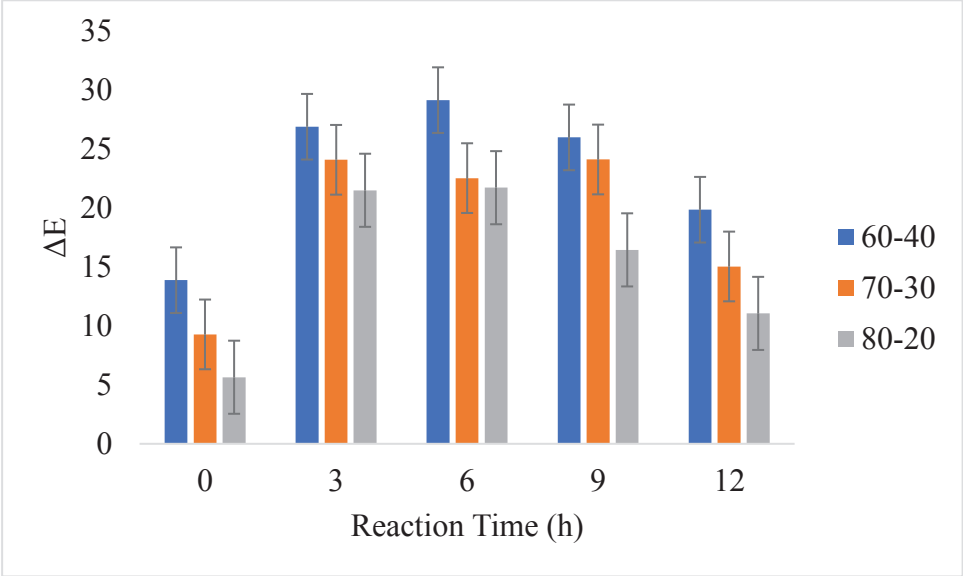

Figure 6.20 Total color difference of the samples during enzymatic interesterification with respect to blend ratio and reaction time

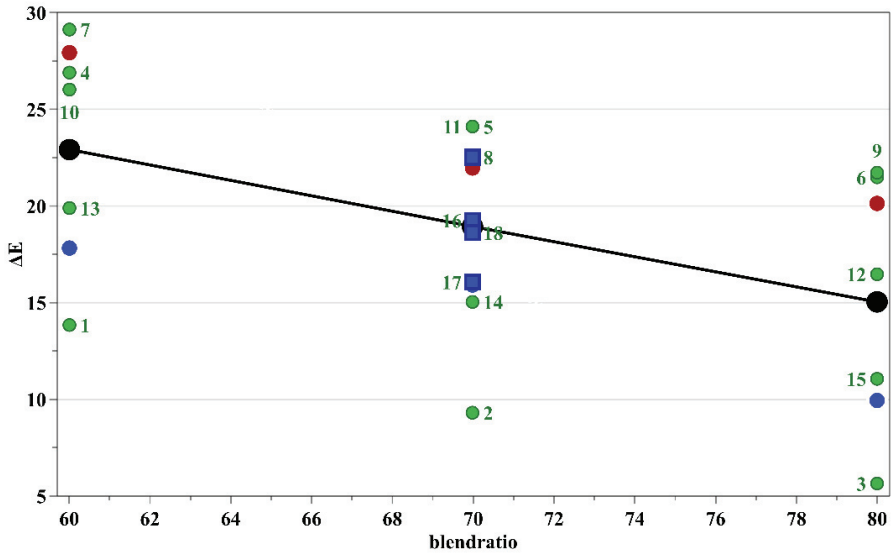

Figure 6.21 Main effect plot of reaction time of enzymatically interesterified samples for ΔE

6.2.3. Melting Points of Enzymatically Interesterified Fats

The melting points of the enzymatically interesterified samples are also expressed as a function of a given percentage (85, 90 and 95%) of melted crystals since different TAG profiles were created throughout the enzymatic interesterification and each TAG

has its own melting point. The melting temperatures at 85, 90 and 95% of melted crystals (MP85%, MP90%, MP95%) are provided in Table 6.7. As expected, higher crystal percentages corresponded to higher melting temperatures. As it could be seen in Table 6.7, tallow has really high melting temperatures (46.6-49.5 °C). Before starting reaction, blending of tallow with corn oil caused slight decreases in melting points of the samples. In addition, enzymatic interesterification also resulted in further decreases in melting points of the products. These changes in melting points are in accordance with the previous studies (Engellman et al. 2018; Jin et al. 2008). Generally, the melting temperatures of interesterified lipids slightly increased by gradual increasing of percentage of crystals in lipid structure. (Figure 6.22, 23, 24). The melting points of the samples decreased up to 6 h of reaction, after that point the MPs of the interesterified lipids slightly increased (Figure 6.22, 23, 24). Same trend was also observed in TAG profile of enzymatically interesterified lipids. Therefore, it could be interpreted that the enzyme released fatty acids from TAG structure up to 6 h of reaction, after that fatty acids are placed in newly formed TAG backbone. The increase in DAG and MAG content up to 6 h reaction time also supports this mechanism. The correlations between melting points and TAG (r =0.92) and DAG+MAG (r =-0.91) agree with this mechanism. As TAG content of the interesterified samples increased, the MP85 of the samples increased also (Figure.6.25). However, the MP85 of the structured lipids decreased with increasing DAG+MAG content as Figure 6.26 indicated. Same trends were also observed at other percentages of melting. Moreover, melting points of the samples were relevant to melting points of crystal types (β and β′) which formed throughout the reaction.

Appendix 12 shows the statistical analysis results for melting points. ANOVA results indicated that constructed models were insignificant at $p \leq 0.05$ with non-significant lack of fit. Normality and residuals were checked for the model. Although the models were insignificant, examination of the significance levels of the main factors and their interactions shows that only blend ratio has some effect on MPs of enzymatically interesterified lipids. The MPs at all melting percentages decreased slightly with increasing amount of tallow concentration as Figure 6.27-28-29 revealed.

Table 6.7 Melting points of tallow and the enzymatically interesterified samples during the reaction at various percentages of melted crystals

Sample	MP85	MP90	MP95
E60	43.3	45	46.7
E63	27.3	31.6	39.4
E66	25.6	27.7	29.6
E69	36.8	39.8	42.5
E612	37.2	40.8	43.2
E70	44.4	46.1	48
E73	35.8	37.8	40
E76	27.4	28.8	30.2
E79	27.3	29.5	31.4
E712	29	30.4	32.2
E80	44.7	46.1	47.7
E83	28.6	31.6	38.2
E86	33.7	36.3	38.6
E89	28.5	30.5	32.3
E812	31	32.7	34.3
ECP1	32.7	36.1	39.1
ECP2	27.2	29.3	31.2
ECP3	27.3	29.4	31.3
T	46.6	48	50

*Abbreviations are provided in Materials & Methods section
Standard deviation of MP85 = ±2.57, MP90 =± 3.18, MP95 = ±3.68 (calculated from CPs)

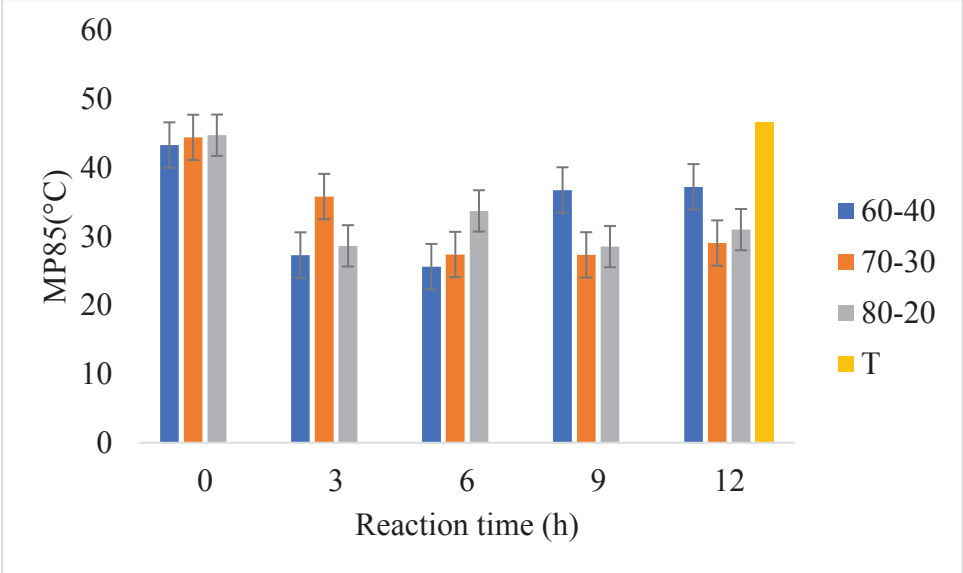

Figure 6.22 Melting temperatures of the samples at 85% of melting with respect to reaction time and blend ratio

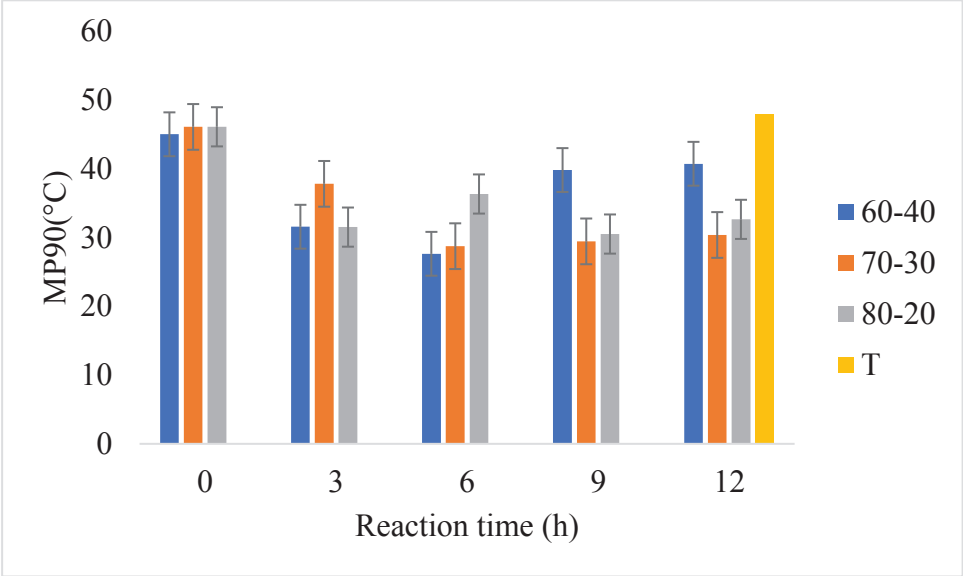

Figure 6.23 Melting temperatures of the samples at 90% of melting with respect to reaction time and blend ratio

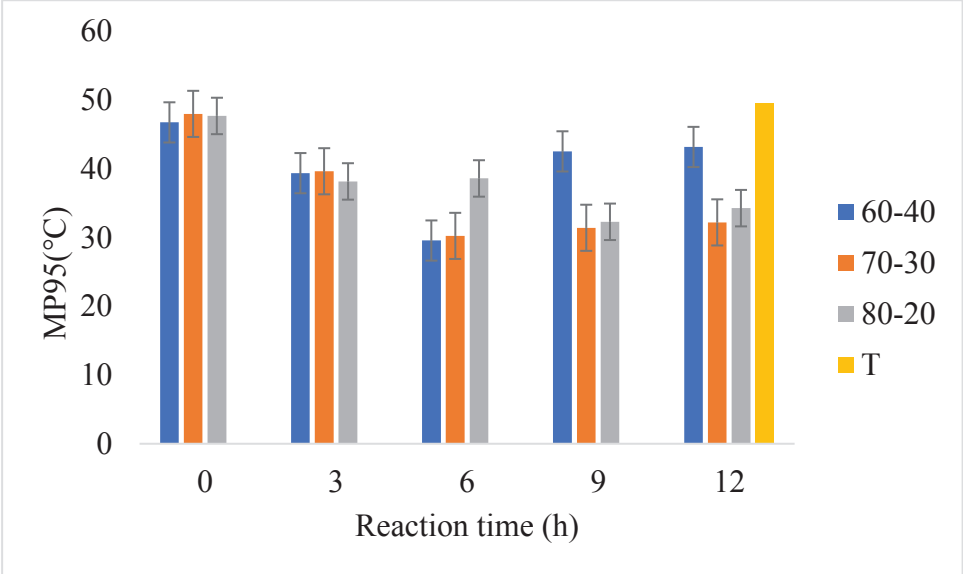

Figure 6.24 Melting temperatures of the samples at 95% of melting with respect to reaction time and blend ratio

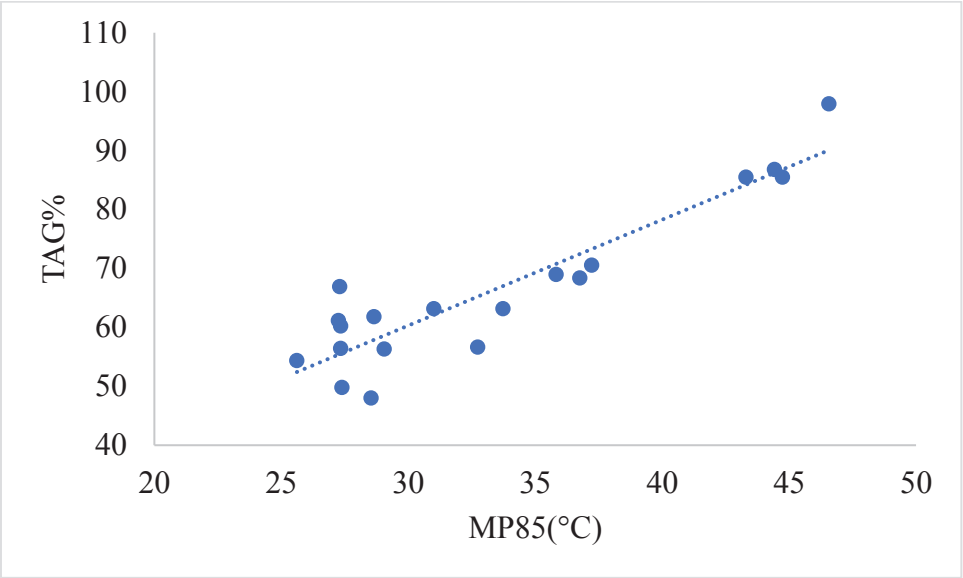

Figure 6.25 MP85% versus triacylglycerol content (TAG%) of the structured lipids

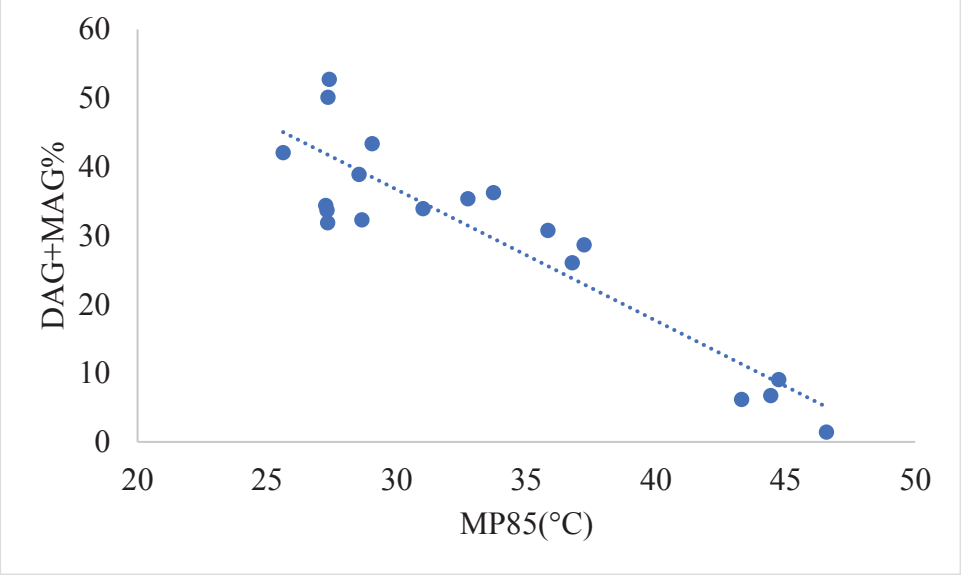

Figure 6.26 MP85% versus mono and diacylglycerol content (DAG+MAG%) of structured lipids

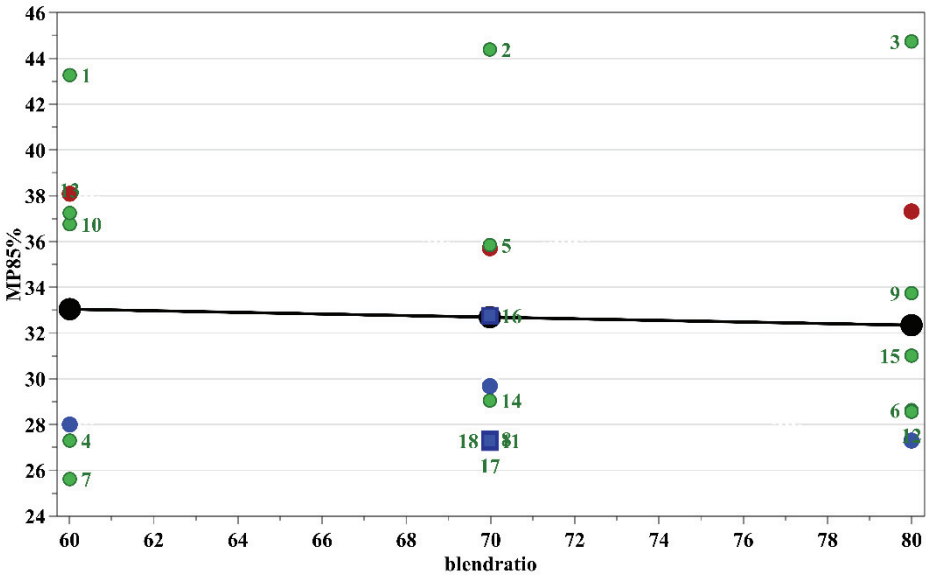

Figure 6.27 Main effect plot of blend ratio on MP85% of the enzymatically interesterified fats

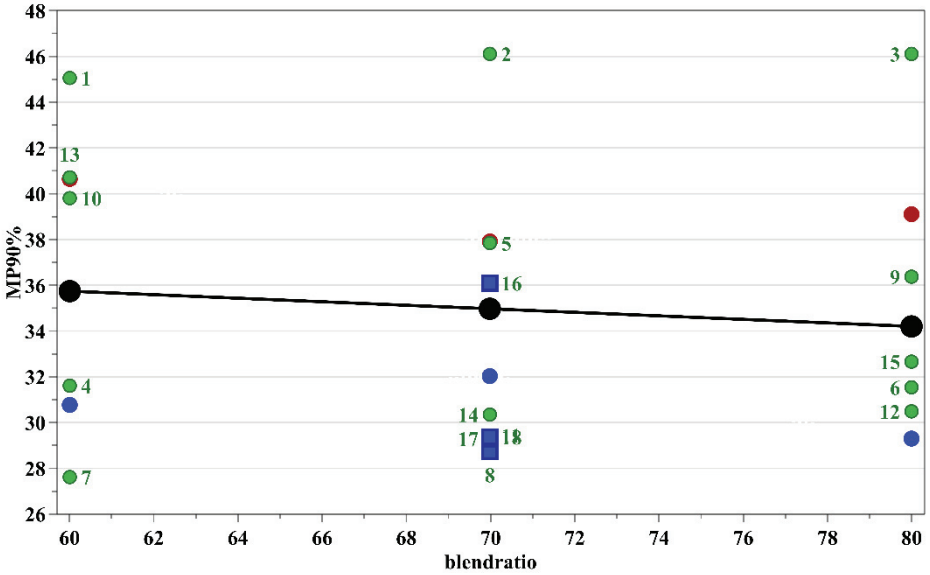

Figure 6.28 Main effect plot of blend ratio on MP90% of the enzymatically interesterified fats

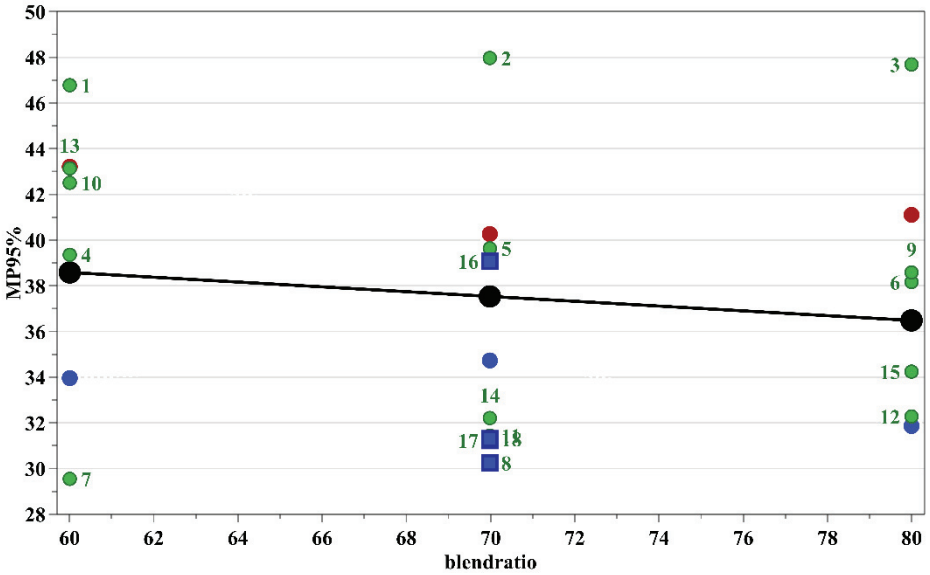

Figure 6.29 Main effect plot of blend ratio on MP95% of the enzymatically interesterified fats

6.2.4. Slip Melting Point of Enzymatically Interesterified Lipids during Reaction Time

The ranges of slip melting points (SMP) of the enzymatically interesterified lipids throughout the reaction are provided in Table 6.8. Enzymatic interesterification reactions caused decline in SMPs of structured lipids compared to initial blends and tallow. Same decline has also been observed in the previous researches (Bhattacharyya et al. 2000; Kowalska et al. 2015; Kowalska et al. 2014). While SMP of tallow is 46.95 °C SMP range of enzymatically interesterified samples is 33.05-45.95 °C and for the non-esterified blends this range is 43.2-45.95 °C.

For the enzymatic interesterification of tallow with only corn oil, parameters investigated were blend ratio and reaction time and their effect on SMP is shown graphically in Figure 6.30. As it is seen from this figure, SMP of the enzymatically interesterified samples decreased up to 6 h reaction time regardless of blend ratio. After that point, there are some fluctuations in SMP of the samples. In first 6 h of interesterification, an increase in TAG content and a decrease in DAG+MAG content were observed along with a rise in SMP of the samples. The correlations between SMPs and TAG (r =0.87) and DAG+MAG (r =-0.90) contents are satisfactory. As TAG content of the interesterified samples increased, SMP of the samples increased also (Figure 6.31).

However, SMP of the structured lipids decreased with increasing DAG+MAG content as Figure 6.32 indicated. Therefore, SMP of the samples could be associated with TAGs that were restructured during enzymatic interesterification reactions. Moreover, the correlations between melting points at different melting percentages and SMP were tried to be established and the correlations at different melting percentages were found to be similar (r =0.81, r =-0.80, r =-0.81). It is clear that SMP is relevant to all melting points at different melting percentages as Figure 6.33 confirmed.

Table 6.8 Slip melting points (SMP) of the enzymatically interesterified lipids during reaction

Sample	SMP (°C)
E60	43.20
E63	38.10
E66	34.55
E69	39.95
E612	36.40
E70	45.10
E73	38.20
E76	33.05
E79	36.10
E712	33.15
E80	45.95
E83	40.40
E86	38.35
E89	38.70
E812	39.90
ECP1	33.60
ECP2	36.65
ECP3	36.90
T	46.95

*Abbreviations are provided in Materials & Methods section
Standard deviation of SMP = ±1.5 (calculated from CPs)

Appendix 12 shows the statistical analysis results for SMP of the enzymatically interesterified samples. ANOVA results indicated that constructed model could be considered as significant at $p<0.05$ with non-significant lack of fit. Normality and residuals were also checked for the model. Time is the significant factor for SMP of the enzymatically interesterified lipids (App. 12). The main effect plot also confirms the results stated above (Figure 6.34). With the increase in reaction time, SMPs of the samples decreased as Figure 6.34 indicated.

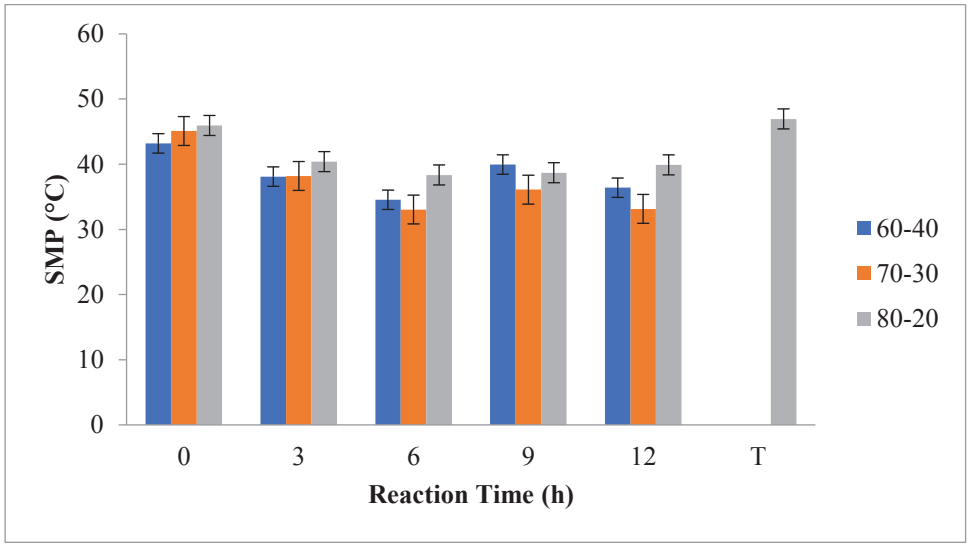

Figure 6.30 Slip melting points (SMP) of the samples with respect to blend ratio and reaction time

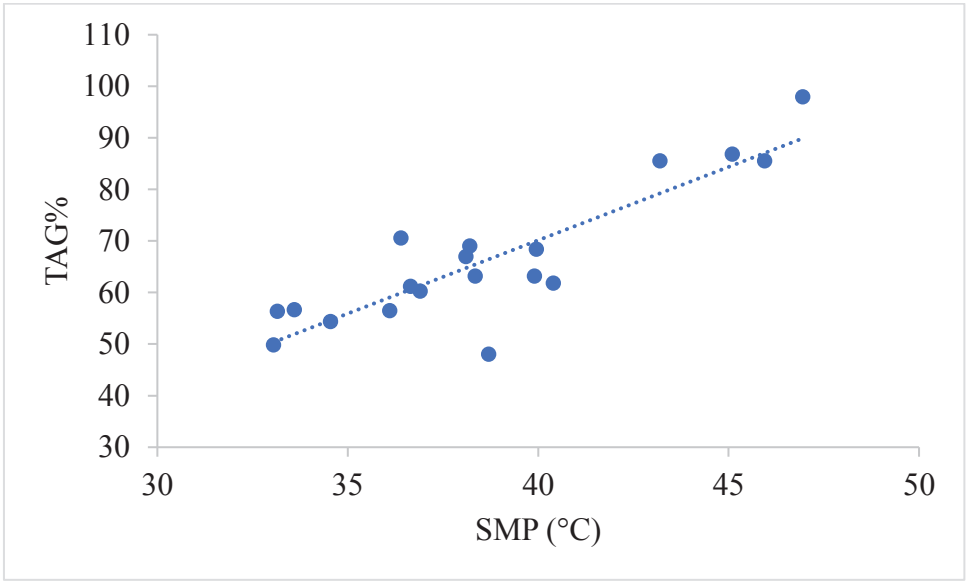

Figure 6.31 Slip melting point (SMP) versus triacylglycerol (TAG%) content of the structured lipids

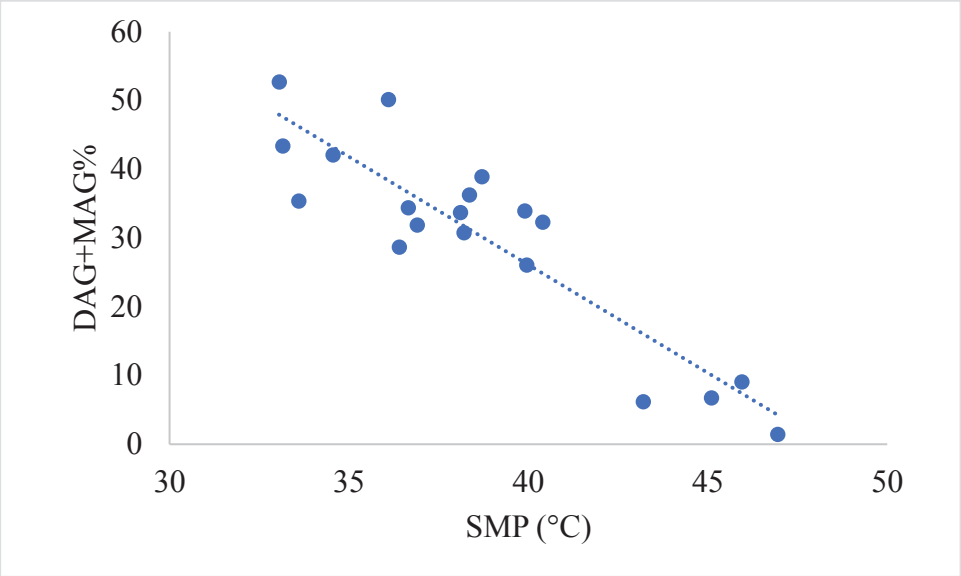

Figure 6.32 Slip melting point (SMP) versus mono and diacylglycerol content (DAG+MAG%) of structured lipids

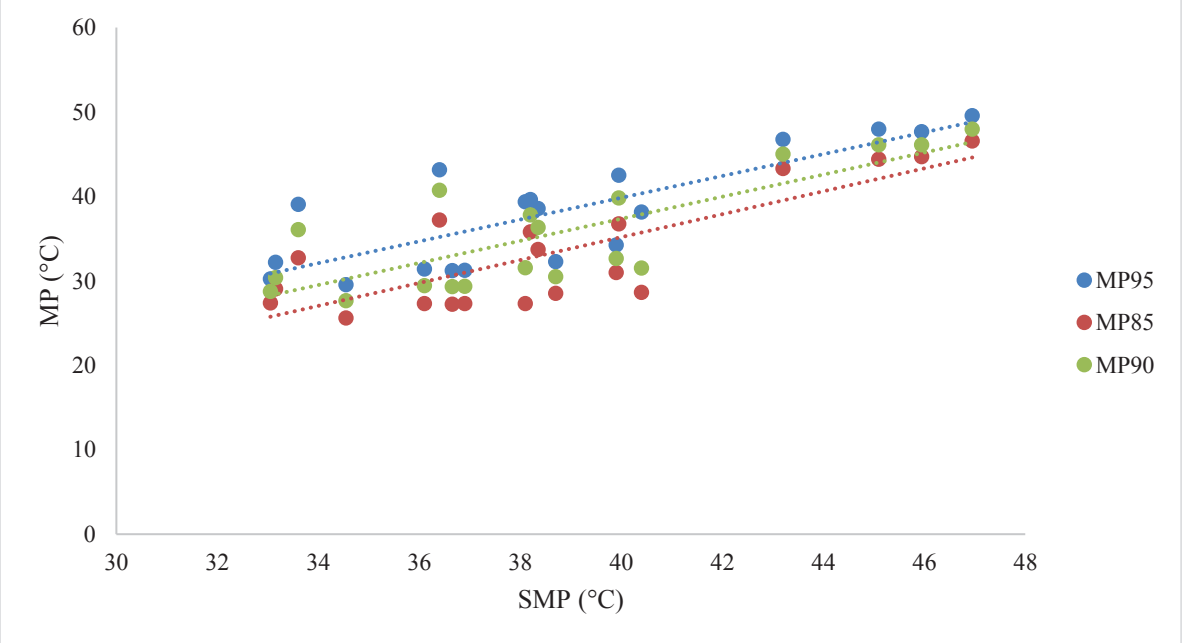

Figure 6.33 Slip melting point (SMP) versus melting points (MP) at various % of melted crystals of the structured lipids

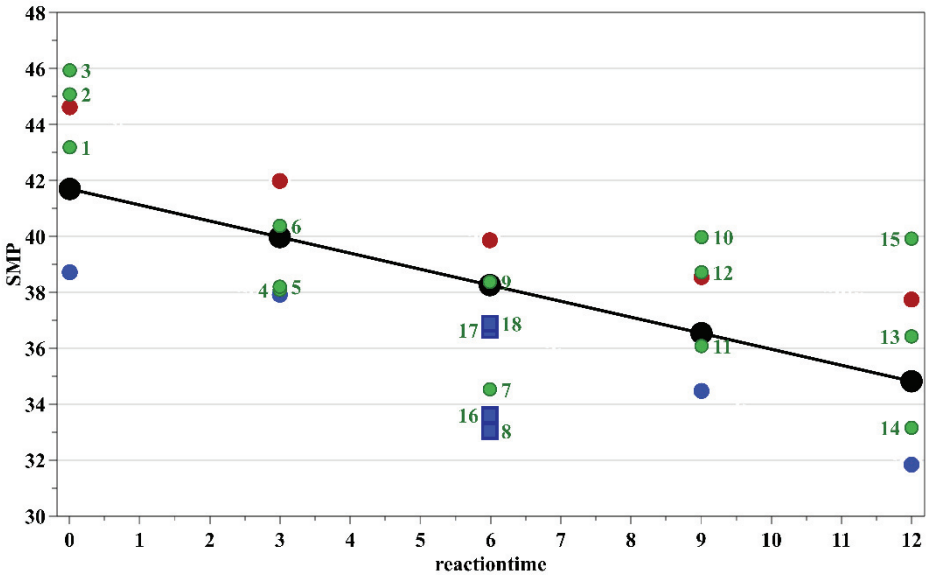

Figure 6.34 Main effect plot of reaction time on SMPs of enzymatically interesterified fats

6.2.5. Consistency of Enzymatically Interesterified Lipids

The consistency was calculated as ‘‘yield value’’ (MPa) and the results for the samples during enzymatic interesterification process are presented in Table 6.9. The consistency of all samples decreased clearly as a function of temperature. This result can be associated with the gradual melting of crystals that generate more fragile crystalline networks. The same behavior was also observed in the previous studies (Silvia et al. 2009; Bezzera et al. 2017). Changes in the consistency of the enzymatically interesterified tallow-corn samples with various blend ratios are shown with respect to reaction times in Figure 6. 35-37. As it could be seen from these figures consistency of the enzymatically interesterified samples was measurable at all temperatures. The consistency of the structured lipids interesterified with 80% tallow are less than the interesterified lipids containing 60 and 70% tallow. Twelve-hour reaction time caused sharp increases in consistency levels of the samples including 60 and 70% of tallow at 4 and 10 °C. However, the sample interesterified with 80% tallow had the same consistency value at 4 °C with its initial blend after 12 h reaction time. These lipids can be classified as hard; however, the consistency values decreased to the levels suitable for spreadability with the increasing temperature.

Table 6.9 Consistency values of tallow, non-interesterified blends and the enzymatically interesterified lipids during reaction

	Consistency (MPa)			
Sample	**4°C**	**10°C**	**15°C**	**25°C**
E60	54.89	16.62	10.23	9.08
E63	27.22	11.11	4.31	4.07
E66	24.71	12.09	4.49	3.77
E69	103.78	12.63	7.57	6.92
E612	761.93	282.86	12.50	10.81
E70	97.62	70.13	26.40	9.60
E73	56.79	21.12	13.90	7.61
E76	39.33	14.96	10.83	9.18
E79	464.47	54.28	36.52	18.06
E712	1055.07	783.73	608.35	69.80
E80	164.09	101.37	39.69	20.16
E83	112.59	55.57	50.80	20.37
E86	116.34	54.25	12.25	11.17
E89	131.54	51.14	21.61	17.84
E812	164.11	88.52	58.33	27.99
ECP1	57.63	19.77	13.98	12.11
ECP2	70.46	54.40	52.96	24.40
ECP3	150.71	99.66	40.06	26.55
T	385.93	224.52	87.57	69.85

*Abbreviations are provided in Materials & Methods section
Standard deviation of consistency at 4°C = ±41.19, at 10°C = ±32.71, at 15°C = ±16.22, at 25°C =± 6.36, (calculated from CPs)

Appendix 12 shows the statistical analysis results for the consistency of the enzymatically interesterified fats. ANOVA results indicated that only the model constructed at 4 °C was significant with significant lack of fit. The models for consistency at 10,15, 25 °C were not significant. Therefore, it could be concluded that there are not important differences in consistency of structured lipids with regard to blend ratio and reaction time at 10, 15, 25 °C during enzymatic interesterification. The main effect plot reveals that time affects the consistency of structured lipids at 4°C (Figure 6.38). Figure 6.38 shows that as reaction time increases, consistency of samples increases also.

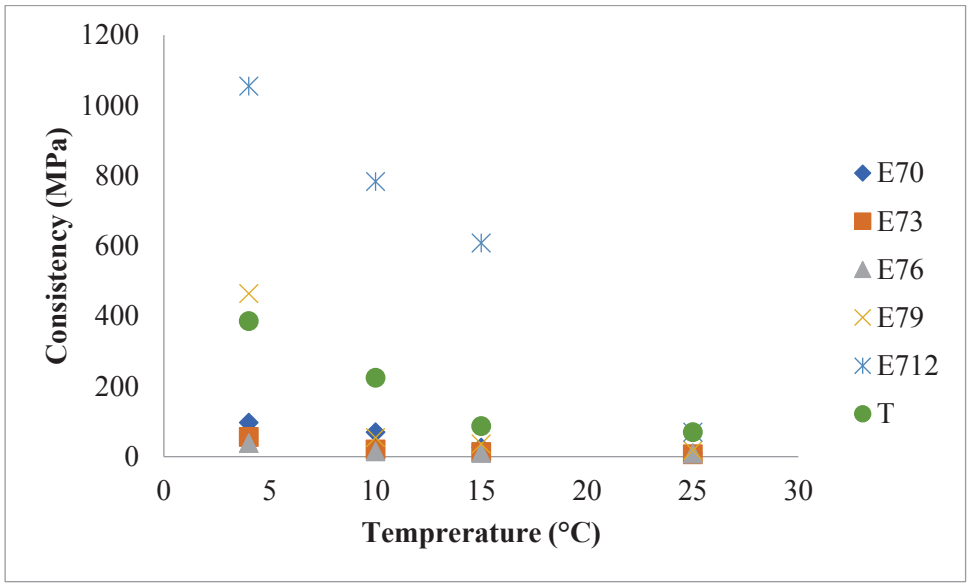

Figure 6.36 Consistency of the samples at 70:30 ratio (%) during interesterification reaction time

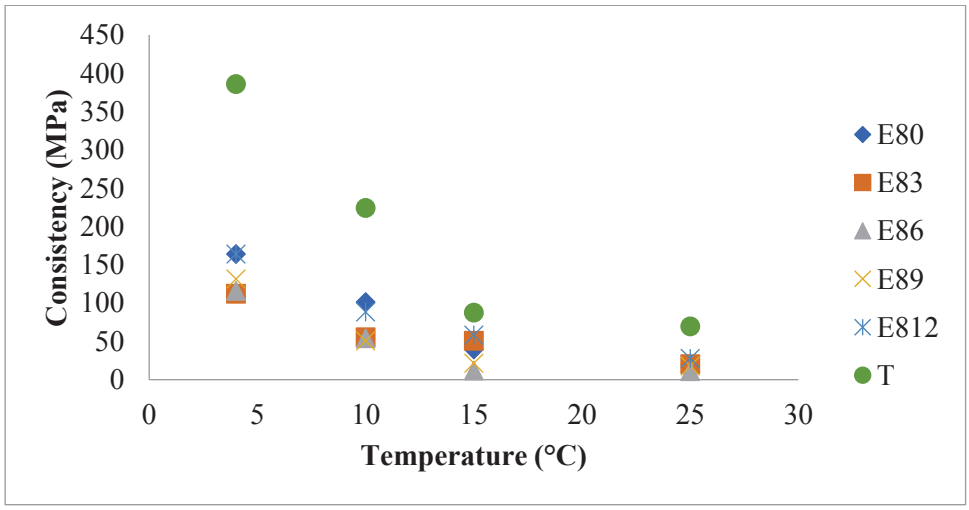

Figure 6.37 Consistency of the samples at 80:20 ratio (%) during interesterification reaction time

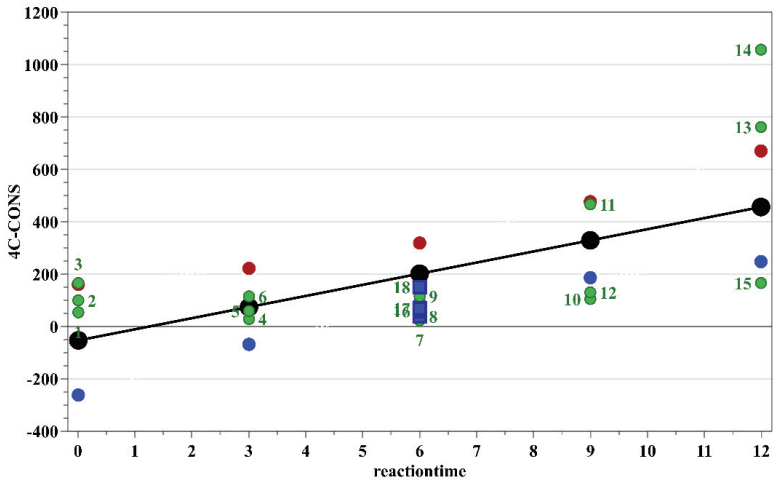

Figure 6.38 Main effect plot of reaction time on consistency at 4°C of enzymatically interesterified fats

6.2.6. Solid Fat Content of Enzymatically Interesterified Lipids during Reaction

Solid fat content (SFC) is a measure of the percentage of fat in crystalline (solid) phase to total fat across a temperature gradient. The SFC is an important parameter to decide on the appropriateness of the lipid for the possible applications. SFC percentages of the samples was determined with a Nuclear Magnetic Resonance spectroscopy at 4 different temperatures and the data are listed in Table 4.4. SFC of both interesterified lipids and non-interesterified blends were determined over the temperature range of 10–35 °C. It was observed that raising temperature caused a marked decrease in the value of SFC regardless of reaction parameters. SFC profiles of non-interesterified blends in different proportions have an increasing trend with the increasing amounts of tallow in the blends. Interesterified lipids tend to have lower SFC% values compared to their physical blends. Same trends were observed in the previous studies (Chang et al. 2005; Jin et al. 2008; Kowalska et al. 2015; Li et al. 2018). The decrease in the SFC of interesterified lipids could be attributed to decreased proportion of the high-melting TAGs and medium chain TAGs in the structure of lipids. This decrease in SFC with respect to increase in temperature was similar to other studies (Fauzi et al. 2013; Bezzera et al. 2017; Oliveira et al. 2017). In addition, lower SFC of structured lipids compared to both tallow and non-interesterified blends can be associated with the alteration of TAGs structure and melting temperature of different crystals.

Solid fat content of structured lipids slightly decreased throughout the enzymatic interesterification process. However, there is a sharp increase in SFC% of structured lipids at all temperatures after 12 hours of reaction time (Figure 6.39-41).

Appendix 12 presents the statistical analysis results for SFC of the enzymatically interesterified samples at different temperatures. ANOVA results indicated that constructed model for SFC at 35 °C was significant with significant lack of fit. The effect plot reveals that reaction time is the only significant factor for this model (Figure 6.42) meaning that time highly affects the SFC of structured lipids at 35 °C. As the reaction time is increased, SFC of the samples decreased particularly (Figure 6.42). The models of SFC at 10 and 30 °C were found insignificant with significant lack of fit as ANOVA table showed (App. 12). Although the model constructed for SFC at 10 °C is not significant, ANOVA table still reveals that blend ratio has prominent effect on SFC of structured lipids at 10 °C. Increasing blend ratio caused a rise in SFC at 10 °C as Figure 6.43 confirmed. Moreover, the model at 20 °C could be considered as significant since the p-value is lower. The main effect plot of the model displayed that blend ratio has important effect on SFC of samples at 20 °C and SFC% of enzymatically interesterified lipids increased with increasing reaction time (Figure 6.44).

In order to better understand the physical properties data of the enzymatically interesterified lipids throughout the reaction principal component analysis (PCA) was also applied. The model was constructed by using all measured physical parameters with 3 PCs, $R^2 = 0.88$, and $Q^2 = 0.58$. There is some separation of the samples with respect to reaction time (Figure 6.45). While the non-esterified samples are located at the right part of the ellipse, samples produced in 6 h reaction time are placed just left of the center and 3 and 9 h samples are in between them. Samples belonging to 3, 6 and 9 h of reaction time are quite close to each other. Moreover, the structured lipids produced in 12 h reaction time are located at the right upper part of the quartile. Therefore, some discrimination with respect to first principal component was obtained as far as the reaction time is concerned. This discrimination is mostly resulted from higher SFC, MP and SMP values of non-esterified samples as observed in Figure 6.46. The multivariate analysis of the physical properties data indicated that reaction time caused differences in the physical properties of the products. Since the non-interesterified blends are separately placed in the score plot it could be concluded that physical properties of interesterified blends are different compared to non-esterified ones. In addition, physical properties of the

interesterified samples produced after 12 h reaction time is closer to non-esterified blends than 3, 6 and 9 h reaction time.

Table 6.10 Solid fat content (%) of tallow, non-interesterified blends and enzymatically interesterified lipids during reaction

	SFC%			
Sample	**10°C**	**20°C**	**30°C**	**35°C**
E60	27.8	17.5	9.4	6.2
E63	22.1	12.1	5.6	2.1
E66	20.6	11.2	0.2	0.1
E69	21	12.7	6.8	3.8
E612	25.9	17	8.9	5.2
E70	40.1	26.6	15	10.1
E73	25	16.5	5.2	2.8
E76	25.7	16.4	0.9	1.2
E79	24.3	15.2	2.6	0.5
E712	33.2	25.7	10	0.4
E80	46.8	32.4	18.5	12.6
E83	20.2	16.7	8.2	3.9
E86	29.5	17.9	4.6	0.7
E89	35.7	23.3	7.4	2.1
E812	39.7	28.6	13	0.5
ECP1	28.1	19.2	5.2	0.7
ECP2	27.9	20.8	5.2	0.8
ECP3	29.3	19	4.4	0.4
T	51.1	42.7	24	17.3

*Abbreviations are provided in Materials & Methods section
Standard deviation of solid fat content: at 10 °C =± 0.62, at 15 °C = ±0.81, at 30 °C =± 0.38, at 35 °C = ±0.17 (calculated from three CPs)

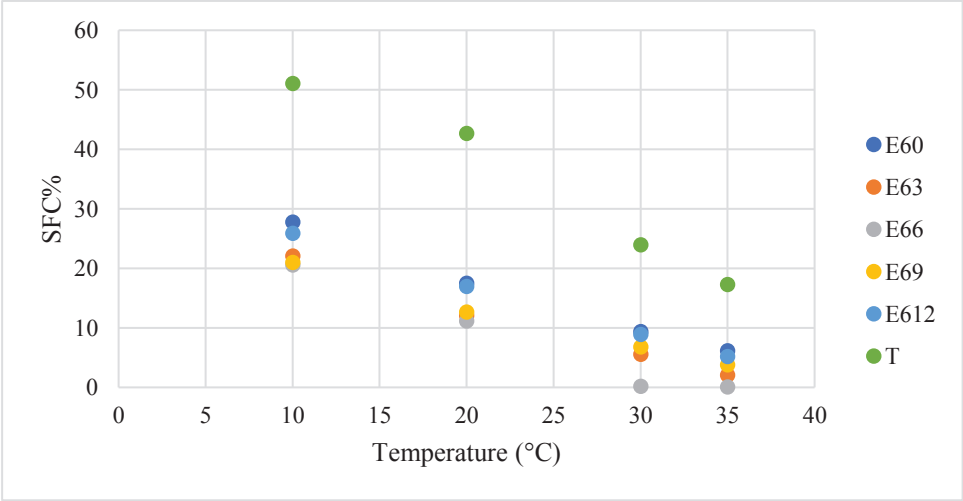

Figure 6.39 Solid fat content (SFC%) versus temperature for the samples with 60% tallow enzymatically interesterified at different reaction times

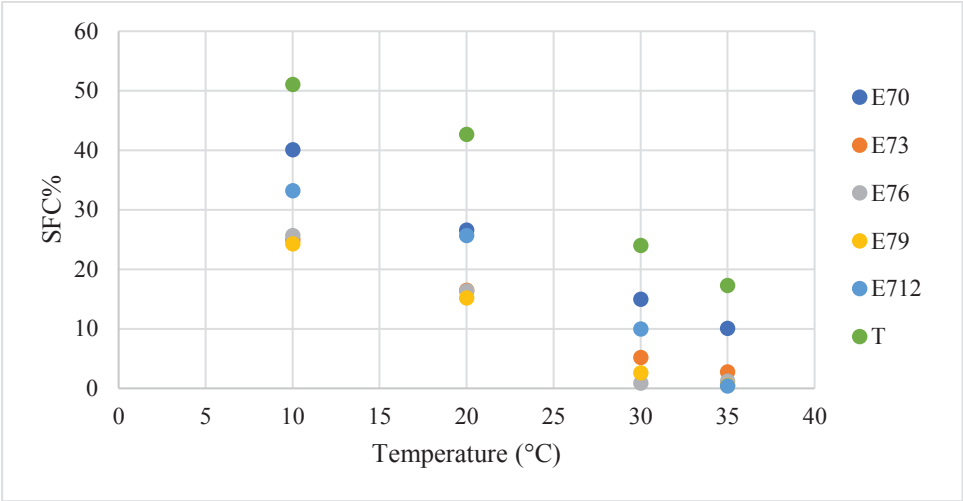

Figure 6.40 Solid fat content (SFC%) versus temperature for the samples with 70% tallow enzymatically interesterified at different reaction times

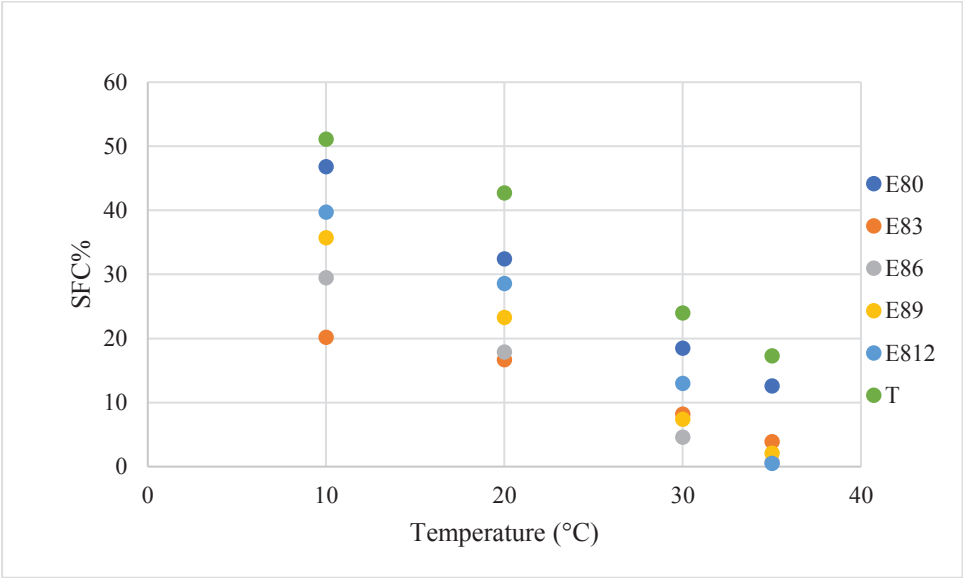

Figure 6.41 Solid fat content (SFC%) versus temperature for the samples with 80% tallow enzymatically interesterified at different reaction times

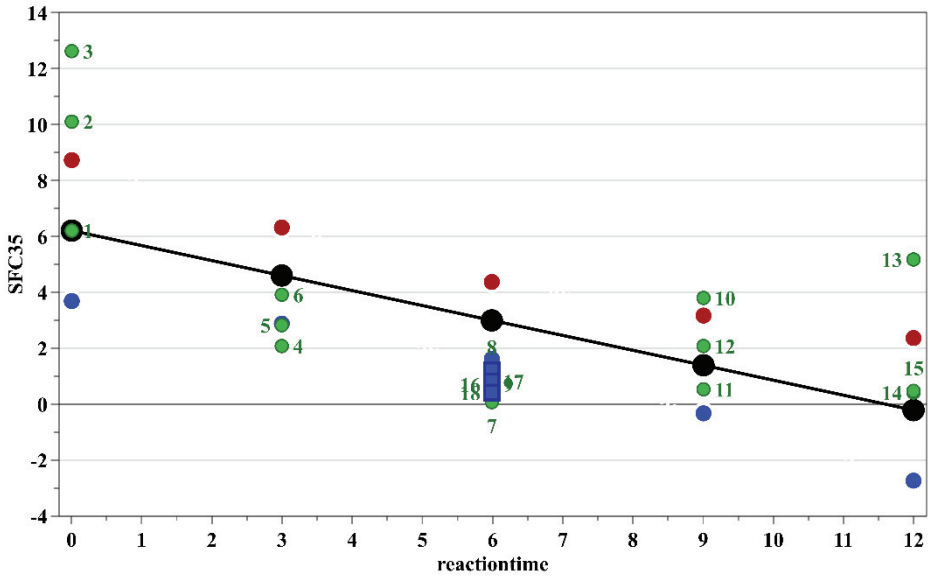

Figure 6.42 Main effect plot of reaction time of enzymatically interesterified samples during reaction for SFC% at 35 ºC

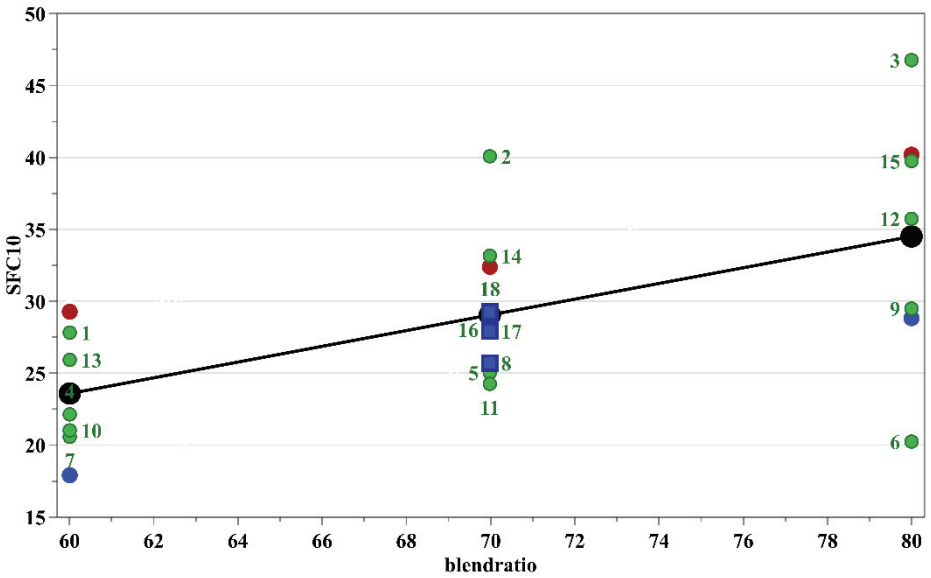

Figure 6.43 Main effect plot of blend ratio of enzymatically interesterified samples during reaction for SFC% at 10 ºC

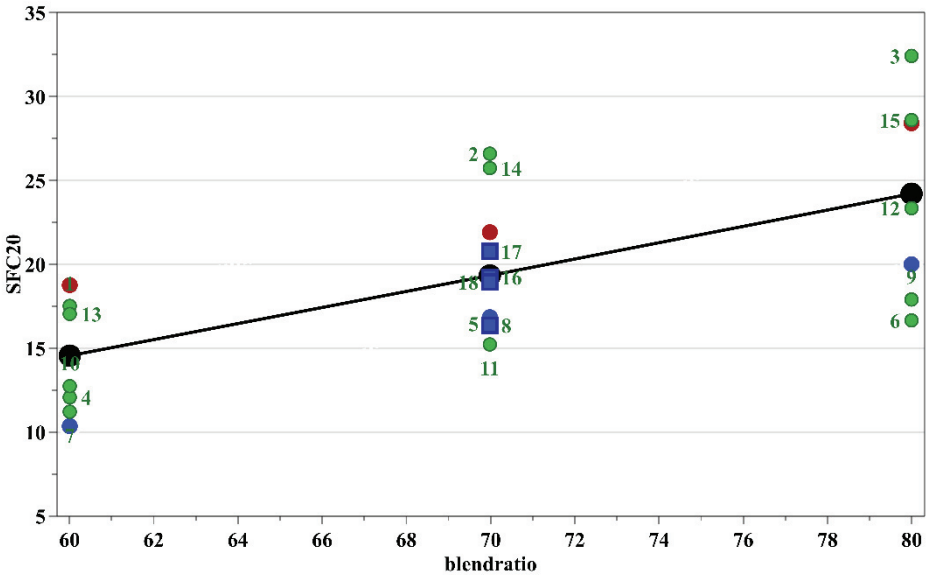

Figure 6.44 Main effect plot of blend ratio of enzymatically interesterified samples during reaction for SFC% at 20 ºC

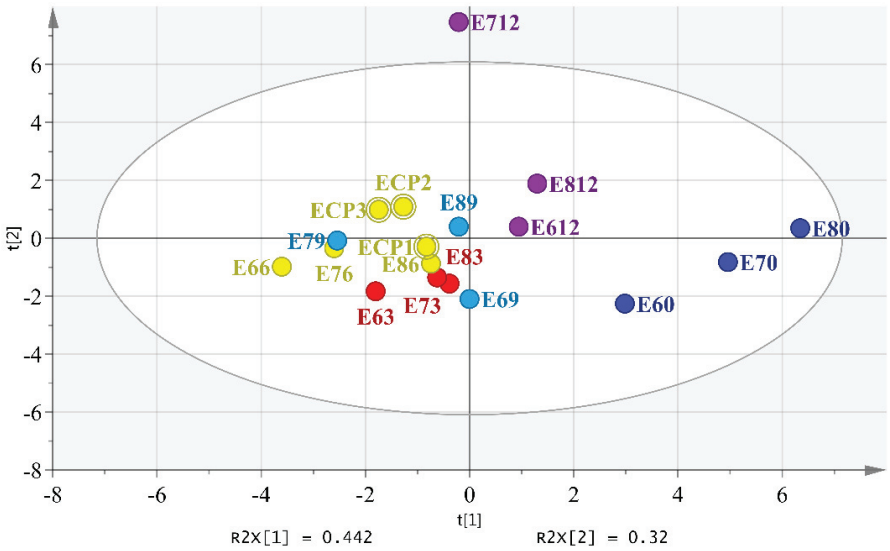

Figure 6.45 Score plot of the PCA model constructed by using all physical parameters of enzymatically interesterified lipids throughout reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the reaction time)

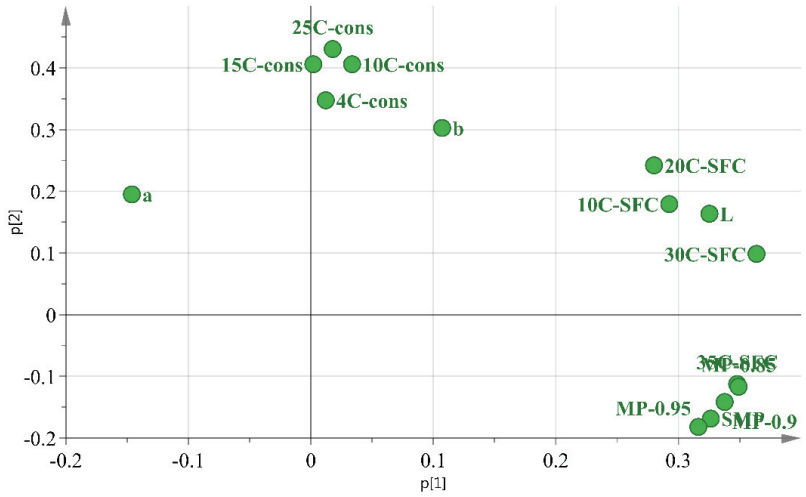

Figure 6.46 Loading plot of the PCA model constructed by using all physical parameters of enzymatically interesterified lipids throughout reaction

Principal component analysis (PCA) was also applied to whole data including both physical and chemical properties. The model was constructed with 4 PCs, $R^2 = 0.82$, and $Q^2 = 0.25$. There is a separation of the samples with respect to reaction time (Figure 6.47). While the non-esterified samples located at the left upper part of ellipse, samples produced in 3, 6, 9 h reaction time placed right bottom of the center and 12 h samples are in between them. It seems that there is a reverse trend in the properties of structured lipids at 12 h reaction time and these samples are getting closer to non-esterified blends instead of moving farther apart. As the loading plot shows the structured lipids produced in 3, 6, 9 h reaction time are separated from non-esterified and 12 h samples due to their chemical properties (Figure 6.48). The multivariate analysis of the whole data also confirmed that reaction time is an important parameter for the enzymatic interesterification reaction.

6.3. Near and Mid-Infrared Spectroscopic Characterization of the Structured Lipids During the Enzymatic Interesterification

In order to characterize the structured lipids during the enzymatic interesterification spectral data were also collected with mid (FT-IR) and near infrared (FT-NIR) spectrometers. FT-NIR and FT-IR spectra were acquired both with melted and solid forms of the structured lipids. The principal component analysis (PCA) was applied to the spectral data of the interesterified lipids to investigate the differences between the

samples. Four different PCA models were constructed with FT-IR and FT-NIR spectra of the solid and melted forms of the samples.

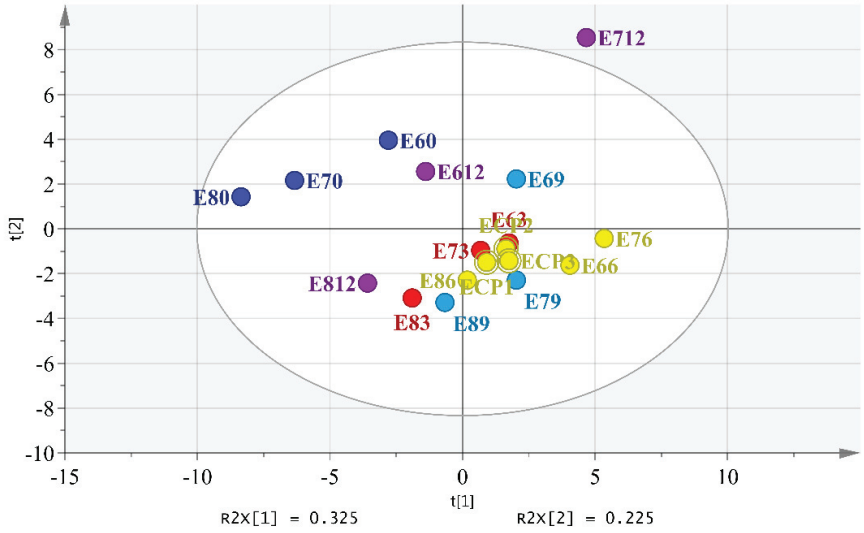

Figure 6.47 Score plot of the PCA model constructed by using chemical and physical properties data of the enzymatically interesterified lipids throughout reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the reaction time)

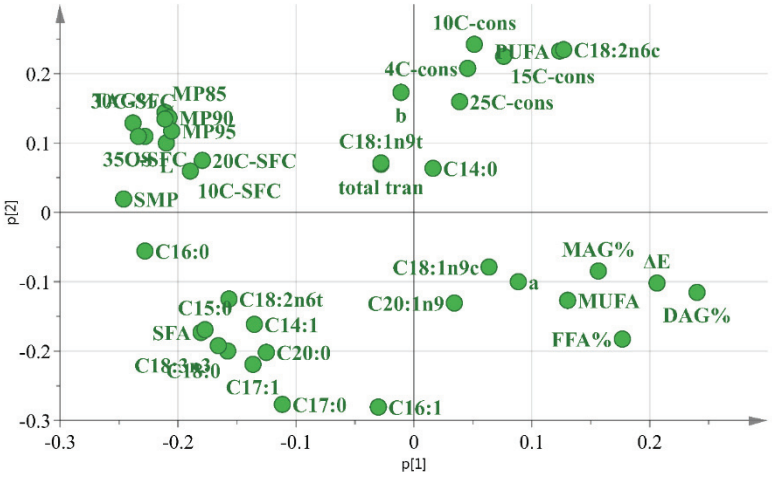

Figure 6.48 Loading plot of the PCA model constructed by using chemical and physical properties data of the enzymatically interesterified lipids throughout reaction

The model which was constructed by using the melted NIR spectra had 3 PCs, R^2 = 0.99, and Q^2 = 0.99. According to score plot of PCA model non-interesterified blends

are separated from interesterified samples (Figure 6.49). However, discrimination of interesterified samples with respect to blend ratio and reaction time is not clear.

The model was also constructed by using solid NIR spectra with 3 PCs, $R^2 = 0.99$, and $Q^2 = 0.99$. Score plot shows that all interesterified lipids are separated from initial blends regardless of blend ratio (Figure 6.50). Although separation of the interesterified samples is not very clear interesterified lipids containing 60 and 80% tallow could be differentiated from each other. Furthermore, samples containing 80% of tallow are divided into two groups according to the reaction time.

The PCA model obtained from FT-IR melted spectra contains 2 PCs with R^2=0.9 and Q^2=0.85. PCA score plot was plotted by coloring the samples according to the reaction time (Figure 6.51). Discrimination of the initial blends from the interesterified samples is observed again. However, samples having a reaction time of 9 h are located around the left part of the quartile together while the others were placed further in the right.

The PCA model of FT-IR solid spectra with 6 PCs, R^2=0.99 and Q^2=0.96 did not show a good discrimination according to the process parameters. The initial blends separated from interesterified samples. Moreover, 3 hour samples placed together at the right bottom part of the quartile (Figure 6.52).

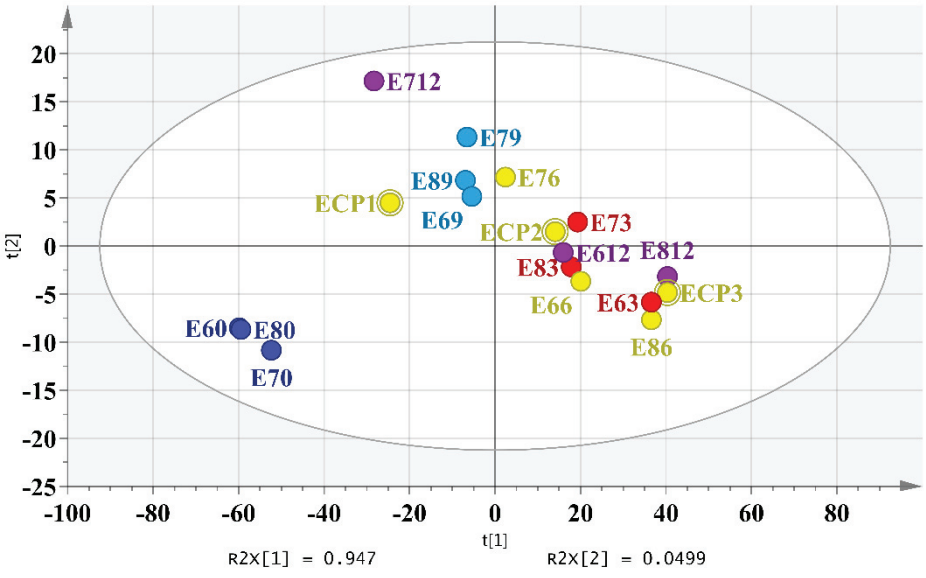

Figure 6.49 Score plot of the PCA model constructed by using melted spectra of FT-NIR of enzymatically interesterified lipids during the reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the reaction time)

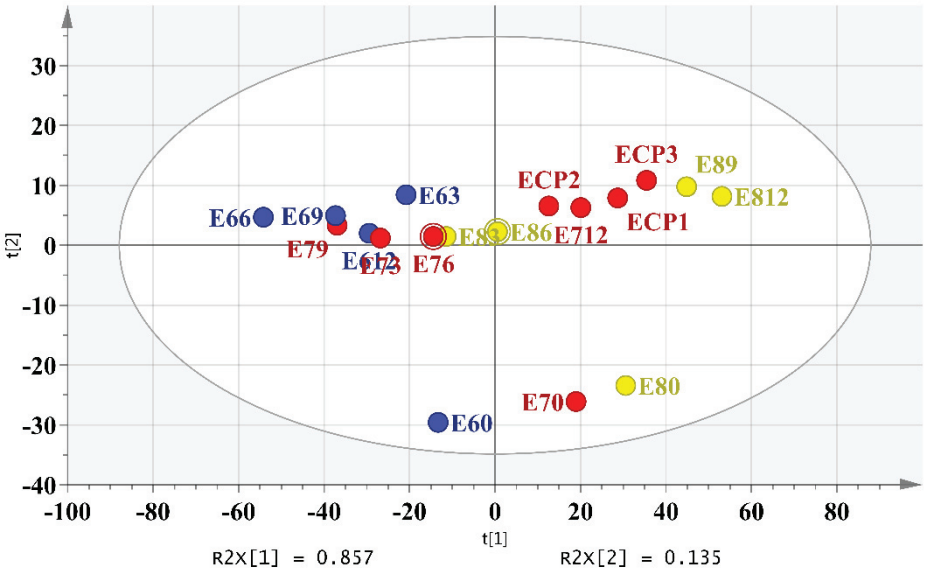

Figure 6.50 Score plot of the PCA model constructed by using solid spectra of FT-NIR of enzymatically interesterified lipids during the reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the blend ratio)

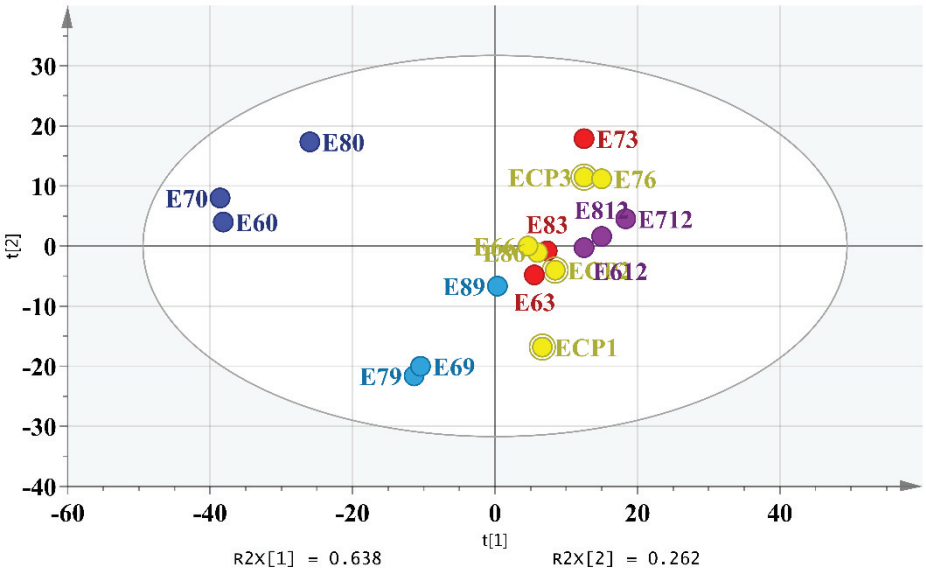

Figure 6.51 Score plot of the PCA model constructed by using melted spectra of FT-IR of enzymatically interesterified lipids during the reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the reaction time)

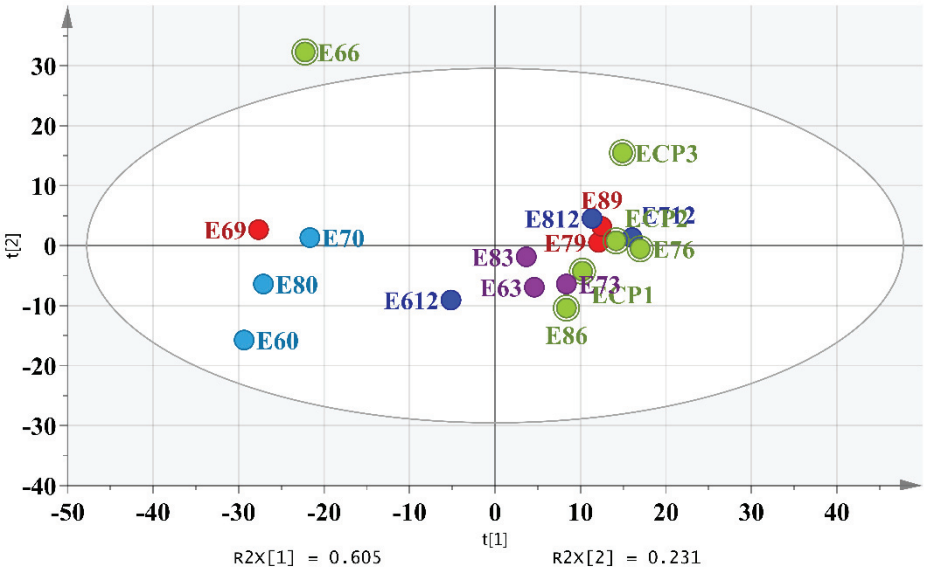

Figure 6.52 Score plot of the PCA model constructed by using solid spectra of FT-IR of enzymatically interesterified lipids during the reaction (ECP1-2-3=70% tallow-6 h, samples are colored with respect to the reaction time)

The results of PCA models using chemical and physical properties data are in accordance with the conclusions obtained from ANOVA. Generally, both the reaction time and the blend ratio have effects on the several chemical and the physical properties of the structured lipids. Although it is not perfect some discrimination with respect to blend ratio and reaction time is observed using chemical and physical properties of the samples. IR data generally provided separation of interesterified and non-interesterified blends.

6.4. Comparison of Chemical and Enzymatic Interesterification Reactions

Although, the enzymatic interesterification can be achieved under milder reaction conditions in comparison to the chemical reaction, chemical interesterification showed better performance in the current study. The results of chemical and physical analysis of structured lipids indicated that the chemical interesterification can modify tallow more effectively compared to the enzymatic interesterification. The structured lipids produced by enzymatic interesterification have higher FFA content and lower oxidative stability compared to chemically interesterified fats. The solid fat content of the samples produced

by enzymatic interesterification is less than the structured lipids manufactured by chemical interesterification. The enzymatically interesterified fats have higher consistency values. Moreover, melting temperature ranges and slip melting temperatures of samples are close to each other regardless of reaction type. In general, 12 h of enzymatic interesterification resulted in structured lipids with worse chemical and physical properties. The enzyme used in this study was sn-1,3 specific lipase. Therefore, enzymatic interesterification of tallow can be also tried with a sn-2 specific enzyme in order to obtain better end products.

CHAPTER 7

PREDICTION OF CHEMICAL AND PHYSICAL PARAMETERS OF STRUCTURED LIPIDS WITH INFRARED SPECTROSCOPY

Since the data obtained from Fourier transform infrared (FT-IR) and Fourier transform near infrared (FT-NIR) spectroscopy are complex and the simple quantitative analysis methods are not sufficient, more sophisticated multivariate analysis techniques are required to extract information from them. Multivariate regression analysis techniques could be used to estimate the amounts of parameters determined with standard analysis methods from the spectral data. In this study, measured chemical and physical properties of the structured lipids were tried to be predicted from the collected FT-IR and FT-NIR spectra of the samples using partial least square regression (PLS) method. For this purpose, 4 data matrices with 75 samples including vegetable oils (4), tallow (2), interesterified lipids (60), and non-interesterified blends (9) were constructed with FT-NIR and FT-IR spectra of both melted and solid samples. The following wavenumber ranges were selected in order to keep the most informative and less noisy segments of the spectra:

-FT-NIR: 9002-4497 cm^{-1}
-FT-IR: 3051-2599 and 2052-597 cm^{-1}.

7.1. Infrared Spectral Profiles of Structured Lipids

The reduced (without non-informative wavenumber regions) FT-NIR and FT-IR spectra of melted and solid interesterified lipids are shown in Figure 7.1. In FT-NIR spectra, absorption bands between 6055 and 5345 cm^{-1} appeared to be highly significant. This region is mainly related to the first overtone of C-H stretching in fatty acid molecules (Blanco et al. 2004). The absorption peak in the 5345-4562 cm^{-1} region is ascribable to the combination band of O-H and C=O stretching of ester groups (RCOOR). The region 7397-6661 cm^{-1} corresponds to the first overtone of the O-H bond of mono- and diglycerides that might be produced as intermediates and by-products during

interesterification reactions (Blanco et al. 2004; Chang et al. 2005; Knothe, 2000). FT-NIR spectra of solid samples showed higher absorbance values and baseline trend in comparison to melted samples, probably due to scattering effects caused by the fat crystals (Chang et al. 2005).

For both solid and melted FT-IR spectra, more attention was paid to the fingerprint region (1500-800 cm^{-1}). This region includes C-O-C vibration in esters, C-H bending and stretching vibrations, and the second overtone of C=O and -OH in fatty acid structure (Chang et al. 2005; Moh et al. 1999). Melted and solid sample spectra were more similar than in the case of FT-NIR region, because scattering effects are less important in FT-IR region (Doyle 1995). Moreover, the very little amount of sample used for FT-IR spectra collection and the absence of a temperature control, made melted samples solidify during measurements, thus decreasing differences with respect to previously crystallized samples.

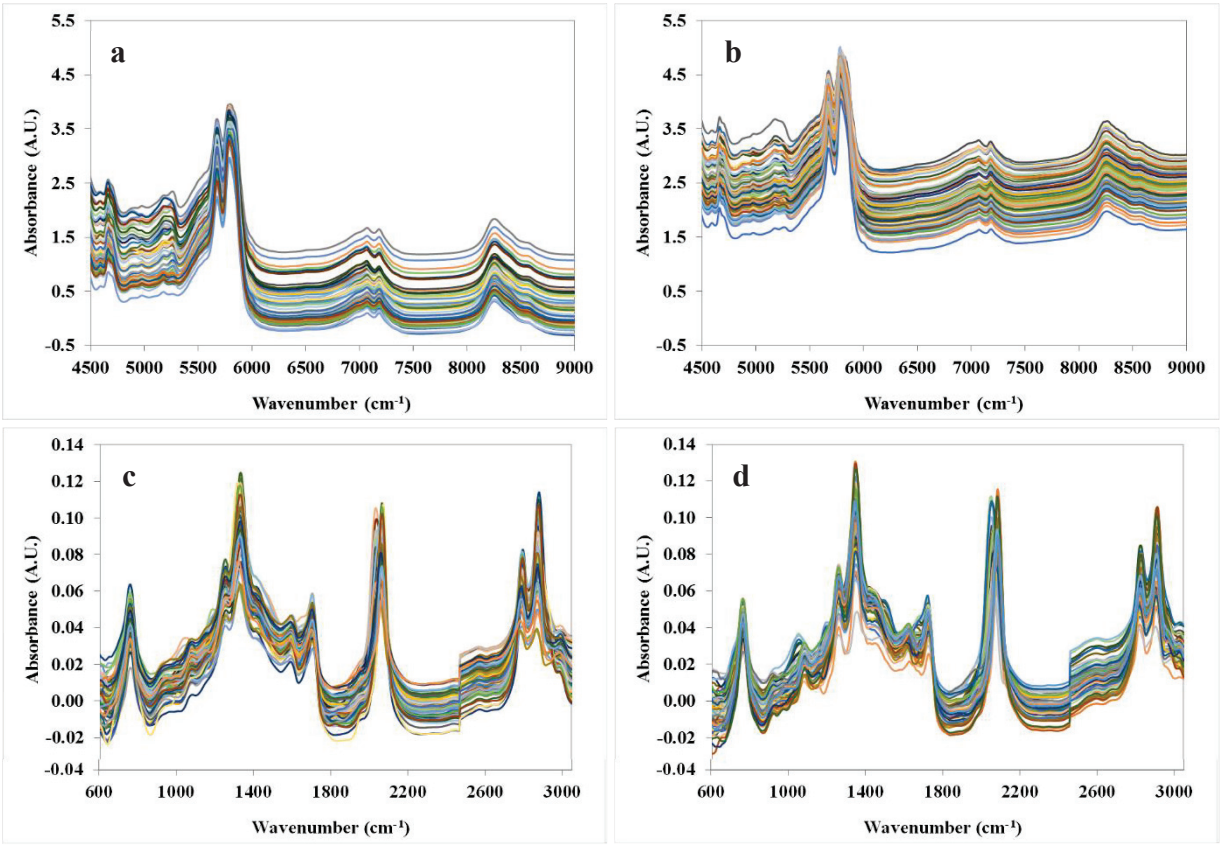

Figure 7.1 Infrared spectra of structured lipids: a) FT-NIR spectra of melted samples; b) FT-NIR spectra of solid samples; c) FT-IR spectra of melted samples; d) FT-IR spectra of solid samples

7.2. Prediction of Chemical and Physical Parameters from Near Infrared Spectra with Partial Least Square Analysis

The capability of predicting chemical and physical properties of interesterified fats from NIR spectra was investigated by PLS analysis. All regression models were developed by using FT-NIR spectra of both solid and melted samples individually. The replicated spectra were averaged prior to the application of various pre-processing techniques including; standard normal variate (SNV), multiplicative scatter correction (MSC), first (d1) and second-order (d2) derivatives. PLS analysis was applied to each pre-treated data matrices in order to predict the chemical and physical properties of the interesterified lipids. Moreover, the models were also validated by external and cross-validation procedures. PLS analysis results are summarized in terms of significant principal components (PCs), coefficients of determination in calibration (R^2_{cal}), cross-validation (R^2_{cv}) and prediction (R^2_{pred}), root mean square errors of calibration (RMSEC), validation (RMSECV) and prediction (RMSEP) in Table 7.1 and 7.2. For each response variable, the best models were chosen based on lower number of PCs, higher R^2, and lower errors. The FT-NIR spectra collected on solid samples provided better prediction models compared to the models constructed with melted samples. Therefore, only the models generated by NIR-solid spectra are discussed here. In particular, since d2 pre-treated data provided higher determination coefficients with lower RMSEC and RMSECV, d2 pre-treated models are chosen as the best models and explained in detail.

The chemical properties including oxidative stability (OS), free fatty acid (FFA) content, mono- (MUFA), and polyunsaturated fatty acid (PUFA) contents, saturated (SFA), and trans fatty acid (TFA) contents, mono (MAG), -di (DAG) and triacylglycerol (TAG) contents were provided as separate responses (Y matrices) and the models were developed for each of these responses.

The PLS analysis for prediction of OS was performed by relating FT-NIR spectral data as X variables and oxidation induction times as Y variables. The model contains three significant components (PCs). The regression coefficient of the model determined with calibration set was found as 0.71 and (Table 7.1). RMSEP value was calculated with the external validation set as 2.97. According to comparison criteria, if R^2 is in between 0.66 and 0.80 approximate predictions, in between 0.81 and 0.90 good predictions and larger than 0.90 excellent predictions could be obtained (Tamaki and Mazza 2011).

Therefore, the model for OS by FT-NIR yielded in approximate prediction as Table 7.1 indicated.

The PLS model for FFA content showed a good predictive ability with higher R^2_{cal}=1, R^2_{cv}=0.95 and R^2_{pred} =0.88 and lower RMSE values. Although the model constructed for MUFA has high regression coefficient of calibration R^2_{cal}=0.95, regression coefficient of cross validation and prediction is lower (R^2_{cv}=0.6 and R^2_{pred} =0.51). It means that the MUFA content could not be very well predicted with FT-NIR spectra. The model for PUFA provided better prediction compared to the model of MUFA with higher regression coefficient of prediction (Table 7.1). For the prediction of SFA content of interesterified lipids, regression coefficient of calibration set was found as 0.96 (Figure 7.2 and Table 7.1). The model has lower error values (RMSEC=1.16 and RMSECV=2.37). RMSEP value was also calculated with the validation set as 5.38 and the model could be regarded as excellent according to the comparison criteria. The PLS regression of trans fatty acids with 7 PCs has lower regression coefficient of cross validation and prediction (R^2_{cv}=0.4 and R^2_{pred} =0.1) meaning that TFA of structured lipids could not be well estimated from NIR spectra. This can be associated with narrow range of the trans fatty acids of structured lipids. The PLS models for TAG and DAG have good capability of prediction due to higher R^2 and lower RMSE values (Table 7.1). However, the model constructed for MAG has lower regression coefficient of cross validation and prediction which decreases the reliability of prediction ability of the model (R^2_{cv}=0.47and R^2_{pred} =0.49).

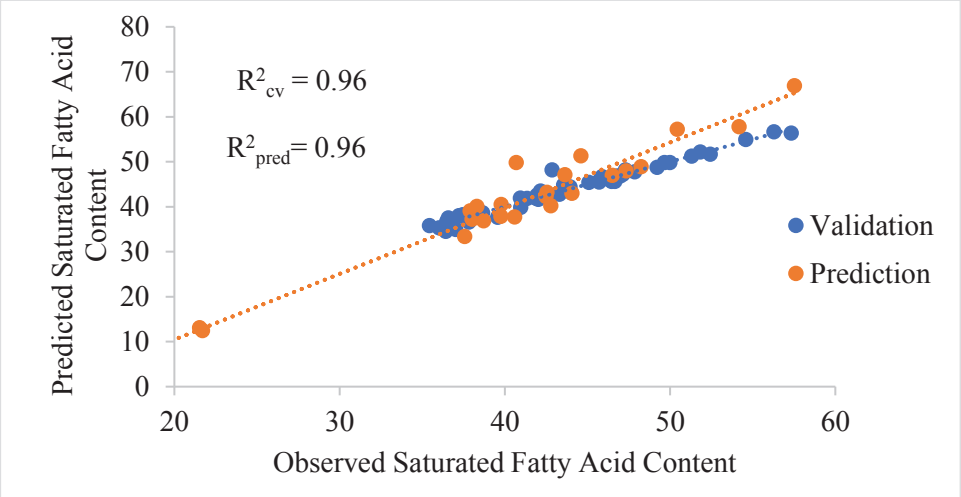

Figure 7.2 PLS regression curve for measured vs. predicted SFA values of interesterified lipids

Table 7.1 Statistical parameters of developed PLS models for the prediction chemical properties of structured lipids by FT-NIR data acquired on solid samples

Chemical Parameters	PCs	R^2_{cal}	R^2_{cv}	R^2_{pred}	RMSEC	RMSECV	RMSEP	Equation
OS	3	0.71	0.35	0.4	1.71	2.4	2.97	$y=x-1.83*10^{-7}$
FFA	6	1	0.95	0.88	0.51	1.69	2.32	$y=x-4.23*10^{-7}$
MUFA	6	0.95	0.56	0.51	1.43	3.3	6.93	$y=x+1.13*10^{-6}$
PUFA	5	0.88	0.36	0.89	2.22	4.3	5.4	$y=x+7.19*10^{-9}$
SFA	6	0.96	0.76	0.96	1.16	2.37	5.38	$y=x+8.21*10^{-8}$
TFA	7	0.89	0.18	0.4	0.16	0.35	0.41	$y=x+4.59*10^{-8}$
TAG	5	0.94	0.66	0.65	3.27	6.87	6.99	$y=x+6.15*10^{-7}$
DAG	6	0.98	0.85	0.84	1.21	2.84	3.24	$y=x+5.03*10^{-7}$
MAG	5	0.92	0.47	0.49	1.56	3.21	4.11	$y=x-5.95*10^{-7}$

*Abbreviations are provided in Materials & Methods section

The physical properties including slip melting point (SMP), melting points (MP), consistency and solid fat content (SFC) at different temperatures and total color difference were provided as separate responses (Y matrices) and the models were developed for each of these responses separately. The limited errors and the high determination coefficients (R^2_{cal}=0.99, R^2_{cv}=0.89) make PLS model useful for SMP prediction of the structured lipids. The PLS regression curve for SFC at 30 °C obtained with the FT-NIR spectra is given in Figure 7.3.

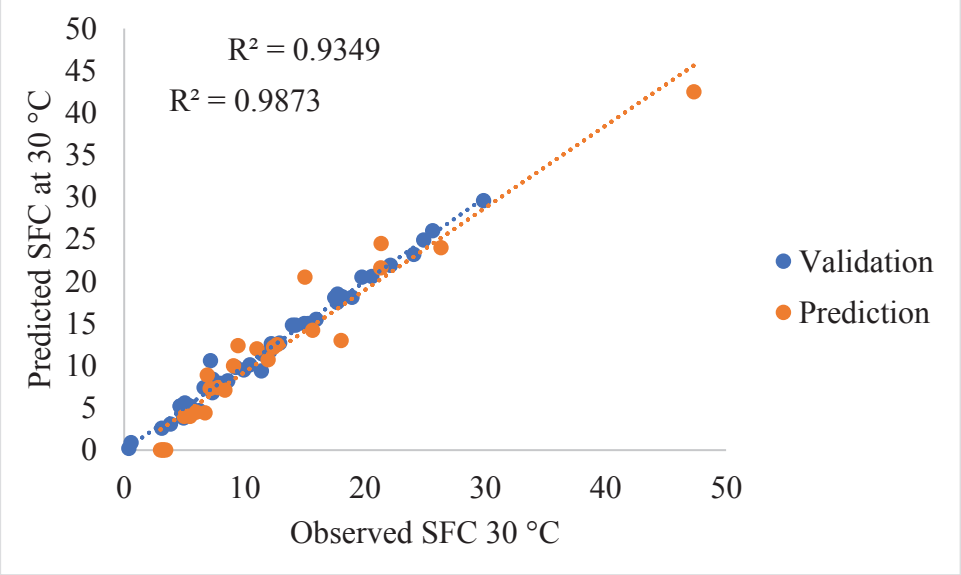

Figure 7.3 PLS regression curve for measured vs. predicted SFC at 30 °C values of interesterified lipids

Although the regression coefficient of prediction (R^2_{pred} =0.30-0.37) and RMSEP (13.32-15.63) values are not good enough, all the models constructed for the prediction of melting points based on FT-NIR spectra have high R^2_{cal} values and R^2_{cv} of the models are also satisfactory (Table 7.2).

The FT-NIR spectra is not able to predict the consistency of interesterified lipids at 4 °C. Moreover, the models for consistency at 10 and 15 °C do not have good prediction ability (Table 7.2). Only the model based on consistency of interesterified lipids at 25 °C provided high determination coefficients (1 and 0.67 in calibration and cross-validation, respectively) with RMSEC and RMSECV of 1.89 and 14.59, respectively. The analytical method used for consistency measurement has high standard deviation. Therefore, PLS regression could not predict very well the consistencies of interesterified lipids.

SFC of the structured lipids was also analyzed with PLS regression. The models of SFC at different temperatures was tested with both calibration and validation sets and excellent predictions were observed. R^2_{cal}, R^2_{cv}, R^2_{pred} are mostly larger than 0.90, and RMSE values are lower as Table 7.2 showed. The model created for total color difference (ΔE) contains three PCs and the correlation coefficients of calibration and validation are 0.91 and 0.63, respectively (Table 7.2). It was demonstrated that FT-NIR analysis of the interesterified products exhibited higher correlations with conventional methods and the good prediction models were observed for SFC and dropping point of the samples (Chang et al. 2005; Zhang et al. 2006). The authors also demonstrated that FT-NIR analysis of the interesterified products in solid form exhibited higher correlations with conventional methods in comparison to FT-NIR spectroscopy applied to liquid samples or FT-IR spectroscopy of both solid and melted fats.

In the case of prediction of chemical properties of interesterified lipids, FT-NIR spectroscopy in combination with PLS regression is successful for the determination of FFA, DAG, PUFA and SFA. Additionally, the physical properties of interesterified fats including SMP and SFC could be very well predicted by FT-NIR spectra.

7.3. Prediction of Chemical and Physical Parameters from Middle Infrared Spectra with Partial Least Square Analysis

The capability of predicting chemical and physical properties of interesterified fats by PLS regression from middle infrared (mid-IR) spectra was also investigated. All regression models were developed by using FT-IR spectra of both solid and melted samples individually. The various transformations of spectral data including; SNV, MSC, d1 and d2 derivatives were also used. PLS analysis was applied to each pre-treated data matrices in order to predict the chemical and physical properties of the interesterified lipids. The PLS results are summarized in Table 7.3 and 7.4 and significant components (PCs), coefficients of determination in calibration (R^2_{cal}), cross-validation (R^2_{cv}) and prediction (R^2_{pred}), root mean square errors of calibration (RMSEC), validation (RMSECV) and prediction (RMSEP) are listed. Same criteria as used for NIR predictions were also applied for choosing the FT-IR models. As observed in the FT-NIR solid spectra, the FT-IR spectra collected from solid samples also provided better prediction capability compared to melted samples.

Table 7.2 Statistical parameters of developed PLS models for the prediction physical properties of structured lipids by FT-NIR data acquired on solid samples

Physical Properties	PCs	R^2_{cal}	R^2_{cv}	R^2_{pred}	RMSEC	RMSECV	RMSEP	Equation
SMP	6	0.99	0.89	0.4	0.48	1.27	14.4	$y=x-3.48*10^{-6}$
MP85	4	0.94	0.74	0.37	2.03	3.87	13.32	$y=x-1.21*10^{-6}$
MP90	4	0.95	0.7	0.3	1.65	3.44	14.67	$y=x-3.23*10^{-7}$
MP95	5	0.96	0.64	0.3	1.33	3.09	15.63	$y=x-8.44*10^{-7}$
Consistency at 4 °C	5	0.66	-0.15	0.19	71.11	125.01	295.72	$y=x-7.59*10^{-6}$
Consistency at 10 °C	7	0.98	0.37	0.14	9.23	37.02	187.85	$y=x+3.52*10^{-7}$
Consistency at 15 °C	4	0.9	0.31	0.16	16.18	34.94	133.99	$y=x+2.79*10^{-7}$
Consistency at 25 °C	7	1	0.67	0.53	1.89	14.59	49.7	$y=x-5.28*10^{-7}$
SFC at 10 °C	3	0.94	0.75	0.9	3.03	5.73	5.89	$y=x+1.42*10^{-6}$
SFC at 20 °C	6	0.98	0.81	0.96	1.34	3.76	3.65	$y=x-1.29*10^{-6}$
SFC at 30 °C	4	0.99	0.89	0.94	0.85	2.27	2.91	$y=x-1.03*10^{-6}$
SFC at 35°C	3	0.98	0.9	0.88	0.8	1.92	3.09	$y=x-1.97*10^{-7}$
ΔE	3	0.91	0.63	0.16	2.01	4.01	8.14	$y=x-4.76*10^{-7}$

*Abbreviations are provided in Materials & Methods section

Therefore, the models generated by MIR-solid spectra are explained in this section. Moreover, the models constructed by d2 transformation data are chosen as the best due to higher determination coefficients with lower RMSEC and RMSECV values.

The chemical properties including OS, FFA content, MUFA and PUFA, SFA, and TFA contents, MAG, DAG and TAG compositions were provided as separate responses (Y matrices) and models were developed for each of these responses again.

The PLS regression of OS contains five significant components (PCs). The regression coefficient of the model determined with calibration set was found as 0.95 (Table 7.3). Although the RMSEC and RMSECV values are low, regression coefficient of cross validation and prediction is below 0.66 (R^2_{cv}=0.40 and R^2_{pred}=0.14) which reduces the prediction capacity of the model.

The PLS model for FFA content with 4 PCs have an excellent predictive potential with high R^2_{cal}=0.99, R^2_{cv}=0.93 and R^2_{pred} =0.82 and low RMSE values (Table 7.3). Although the model constructed for MUFA has high regression coefficient of calibration R^2_{cal}=0.88, regression coefficient of cross validation is lower and R^2_{pred} equals to 0. It means that the PLS model for MUFA content is bad and MUFA amount could not be predicted with FT-IR spectra. In addition, FT-IR spectra is not able to predict the PUFA content of structured lipids by PLS regression. For the prediction of SFA content of interesterified lipids, regression coefficient of calibration set was found as 0.96 (Table 7.3). The model has lower error values (RMSEC=1.11 and RMSECV=3.64). RMSEP value was also calculated with the validation set as 8.46 and the model could be regarded as satisfactory according to the comparison criteria. The PLS regression of the model for TFA resulted in average regression coefficient of calibration 0.71 and lower RMSE values. However, lower value of regression coefficient of both cross validation and prediction (R^2_{cv}=0.01and R^2_{pred} =0.003) makes the model less credible. Not very good model of TFA and PUFA can be associated with the narrow range of the data. Despite lower R^2_{cv} and R^2_{pred} values, the PLS models for TAG and MAG could be presumed as satisfactory due to higher R^2_{cal} and lower RMSE values (Table 7.3). Additionally, the model constructed for DAG has regression coefficient of calibration, cross validation and prediction values in the suggested range of comparison criteria. Therefore, it can be concluded that DAG content of interesterified fats could be predicted well by PLS regression (Figure 7.4).

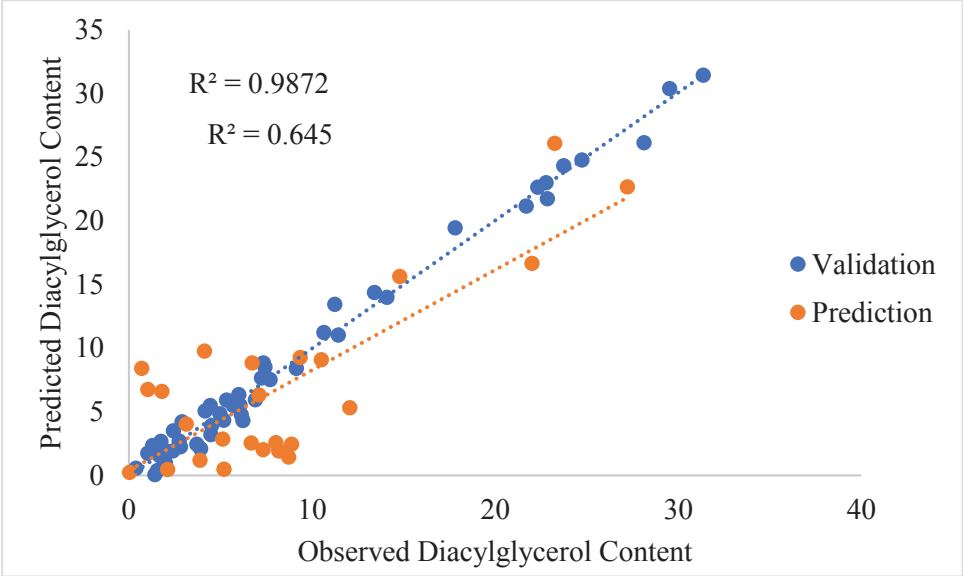

Figure 7.4 PLS regression curve for measured vs. predicted DAG values of interesterified lipids

The physical properties results including SMPs, MPs, consistency and SFCs at different temperatures and total color difference were also predicted form FT-IR spectra by PLS regression. The PLS regression models for the physical properties of interesterified lipids obtained with the FT-IR spectra is given in Table 7.4.

The PLS model for SMP obtained with the FT-IR spectra with 3 PCs and the high determination coefficients makes this model reasonable for SMP prediction of the structured lipids. Although the regression coefficient of cross validation (R^2_{cv}=0.52-0.55) and RMSEP (8.74-13.42) is not good enough, all the models constructed for the prediction of MPs based on FT-IR spectra are satisfactory (Table 7.4). The FT-IR spectra is not able to predict the consistency of interesterified lipids at 4 °C as well. Moreover, the models for consistency at other temperatures are not reliable due to lower values of R^2_{pred} and R^2_{cv} and higher RMSEP values (62.19-190.27). The lower performances of these models can be due to the very limited amount of sample used for spectra acquisition, possibly not representing the real structure of crystallized lipids and higher standard deviation of the analytical method.

The models of SFC at different temperatures was tested with both calibration and validation sets and generally good prediction abilities were observed. For the prediction of SFC at 20 °C of interesterified lipids, regression coefficient of calibration set was found as 0.94 (Figure 7.5 and Table 7.4). The model has lower error values (RMSEC=2.53 and RMSECV=5.91). RMSEP value was also calculated with the validation set as 10 and the

model could be regarded as good according to the comparison criteria. The model created for total color difference (ΔE) with three PCs and the correlation coefficients of calibration and prediction are 0.84 and 0.04 are not satisfactory (Table 7.4). These PLS results are in agreement with a study about the monitoring of lipase-catalyzed interesterification of lard and rapeseed oil (Brys et al. 2005).

Among the prediction models of chemical properties of the interesterified lipids, FT-IR spectroscopy is satisfactory for FFA and DAG. Moreover, the physical properties of interesterified fats including MPs, SMP and SFC could be predicted by FT-IR spectra as well.

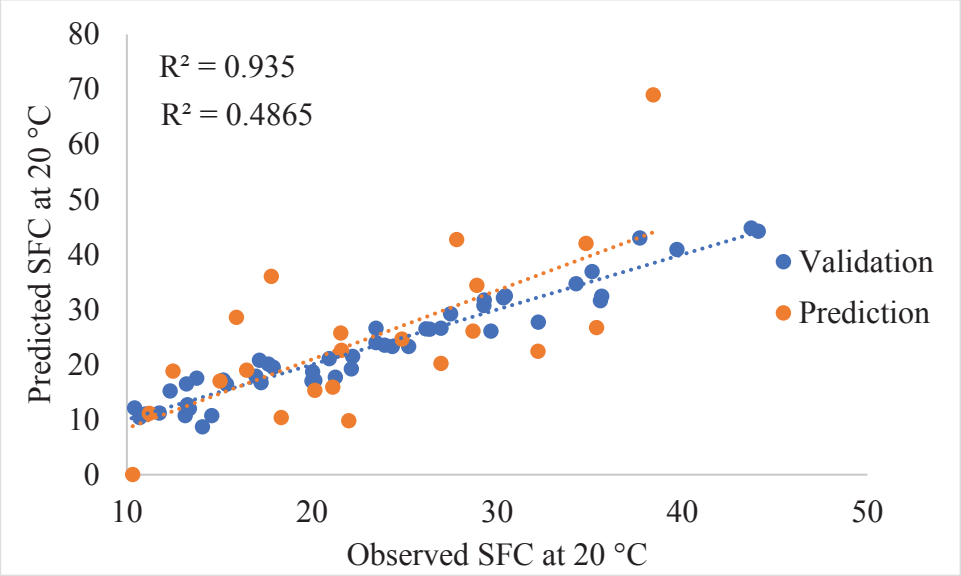

Figure 7.5 PLS regression curve for measured vs. predicted SFC at 20 °C values of interesterified lipids

7.4. Prediction of Chemical and Physical Parameters of Interesterified Lipids by Combining Near and Middle Infrared Spectra with Partial Least Square Analysis

In order to improve the prediction ability of the PLS regression models from infrared spectra, middle and near spectral data were combined together. All regression models were developed by using solid samples. The various transformations of spectral data including; SNV, MSC, d1 and d2 derivatives were also applied again and the models constructed by d2 transformation data were chosen as the best ones due to higher determination coefficients with lower RMSEC and RMSECV.

Table 7.3 Statistical parameters of developed PLS models for the prediction chemical properties of structured lipids by FT-IR data acquired on solid samples

Chemical Parameters	PCs	R^2_{cal}	R^2_{cv}	R^2_{pred}	RMSEC	RMSECV	RMSEP	Equation
OS	5	0.95	0.4	0.14	0.73	2.66	3.16	$y=x-7.62*10^{-6}$
FFA	4	0.99	0.93	0.82	0.82	2.01	3.1	$y=x-3.26*10^{-7}$
MUFA	4	0.88	0.2	0	2.3	5.68	14.75	$y=x+4.26*10^{-7}$
PUFA	5	0.93	-0.19	0.36	1.73	7.38	14.05	$y=x-5.45*10^{-7}$
SFA	4	0.96	0.61	0.81	1.11	3.64	8.46	$y=x+3.25*10^{-6}$
TFA	3	0.79	0.01	0.03	0.21	0.43	0.66	$y=x-8.53*10^{-8}$
TAG	3	0.88	0.49	0.43	4.78	9.21	9.98	$y=x+3.11*10^{-6}$
DAG	6	0.99	0.73	0.65	1.08	4.33	4.49	$y=x+7.31*10^{-7}$
MAG	3	0.8	0.31	0.59	2.35	4.4	3.55	$y=x-6.40*10^{-7}$

*Abbreviations are provided in Materials & Methods section

Table 7.4 Statistical parameters of developed PLS models for the prediction physical properties of structured lipids by FT-IR data acquired on solid samples

Physical Parameters	PCs	R^2_{cal}	R^2_{cv}	R^2_{pred}	RMSEC	RMSECV	RMSEP	Equation
SMP	3	0.85	0.61	0.51	1.62	2.48	14.2	$y=x+2.52*10^{-6}$
MP85	4	0.92	0.52	0.73	2.29	5.39	8.74	$y=x-8.14*10^{-7}$
MP90	4	0.94	0.55	0.74	1.84	4.49	9.69	$y=x-1.13*10^{-6}$
MP95	3	0.87	0.55	0.59	2.29	4.05	13.42	$y=x-3.6*10^{-6}$
Consistency at 4 °C	6	0.77	-0.48	0.04	59.45	163.41	308.81	$y=x+5.65*10^{-6}$
Consistency at 10 °C	3	0.74	0.04	0.10	30.14	55.60	190.27	$y=x-2.53*10^{-6}$
Consistency at 15 °C	3	0.75	0.04	0.09	25.31	46.41	142.27	$y=x+6.78*10^{-8}$
Consistency at 25 °C	3	0.73	0.09	0.27	12.94	21.87	62.19	$y=x-6.83*10^{-7}$
SFC at 10 °C	4	0.94	0.50	0.64	3.04	7.55	10.72	$y=x+2.27*10^{-6}$
SFC at 20 °C	4	0.94	0.53	0.59	2.53	5.91	10	$y=x-3.36*10^{-7}$
SFC at 30 °C	3	0.87	0.62	0.61	2.60	4.21	6.16	$y=x-1.02*10^{-6}$
SFC at 35 °C	3	0.87	0.58	0.61	2.08	3.46	5.02	$y=x-4.04*10^{-7}$
ΔE	3	0.84	0.20	0.04	2.69	5.70	8.85	$y=x-2.77*10^{-7}$

*Abbreviations are provided in Materials & Methods section

The PLS regression models for the chemical and the physical properties of interesterified lipids obtained from data fusion are listed in Table 7.5 and Table 7.6. As it could be seen from the tables, generally data fusion slightly improved the predictability of the models. For instance, the PLS model with combined data for OS have higher R^2_{cal} and R^2_{cv} in comparison to the models obtained from FT-IR and FT-NIR spectra. However, regression coefficient of prediction is still very low. In particular, the models constructed for FFA, SFA and DAG contents (Figure 7.6) are benefited from the data fusion. Since these models have R^2_{cal} mostly larger than 0.90, it could be stated that combining FT-IR and FT-NIR spectra improved predictability of these chemical properties for interesterified lipids. The PLS models for the physical properties of the interesterified lipids are also enhanced by combining the data. Especially, the regression coefficient of the data fusion models belonging to SFC and MPs are higher compared to FT-IR spectral models (Figure 7.7). However, PLS models for consistency of structured lipids did not improve with the combined data. The PLS regression models for consistency at all temperatures still have larger RMSE values and low R^2_{pred} and R^2_{cv} as Table 7.6 showed.

The application of FT-NIR and FT-IR spectroscopy for the prediction of physical and chemical properties of the interesterified lipids was investigated. The use of PLS regression analysis coupled with d2 pre-treatments provided satisfactory models. Both FT-IR and FT-NIR analysis of the interesterified products in solid form exhibited good correlations with conventional methods. However, FT-NIR spectroscopy shows better performance compared to FT-IR spectroscopy. Among the chemical parameters, SFA, FFA and DAG contents could be very well predicted. In addition, the models constructed for SFC and MPs of interesterified lipids have higher prediction ability. The best models for MP prediction were calculated using FT-NIR spectra since higher amount of sample which is more representative of the whole fat compared to FT-IR analysis was used. Similarly, only FT-NIR spectra provided good prediction models for consistency at 25 °C. In general, chemical properties were predicted better than physical properties as expected since infrared profile of the samples is the result of vibrations of chemical bonds. While some physical properties could be affected from chemical composition others could be also related to other structural properties.

Moreover, creating data fusion with both FT-IR and FT-NIR spectra improved the performance of these regression models. Therefore, IR spectroscopy techniques can be used for monitoring the changes of fats during interesterification, thus providing

producers of the structured fats with rapid and non-destructive techniques as good alternatives to the traditional analytical methods.

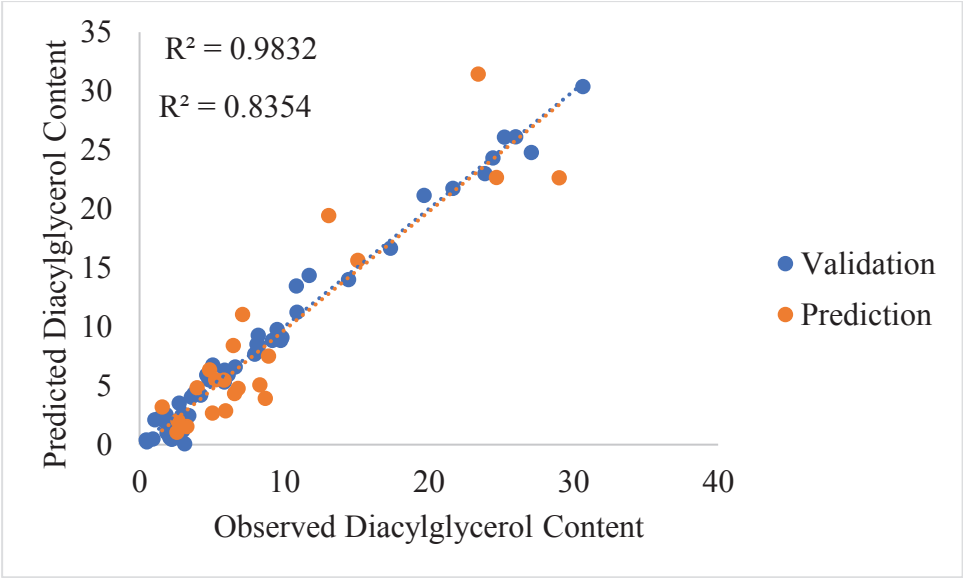

Figure 7.6 PLS regression curve of data fusion for measured vs. predicted DAG values of interesterified lipids

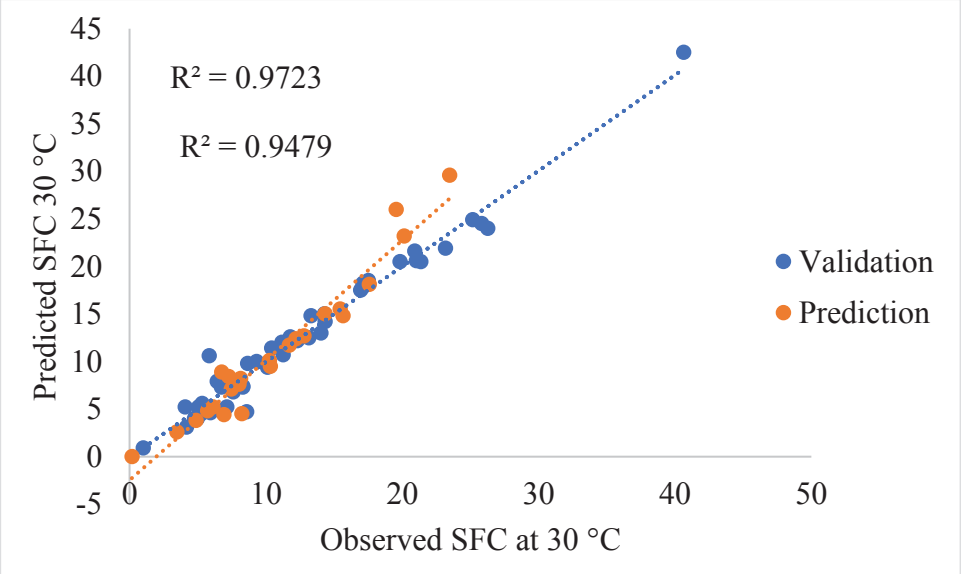

Figure 7.7 PLS regression curve of data fusion for measured vs. predicted SFC at 30 °C values of interesterified lipids

Table 7.5 Statistical parameters of developed PLS models for the prediction of chemical properties of structured lipids by both FT-NIR and FT-IRdata acquired on solid samples

Chemical Parameters	PCs	R^2_{cal}	R^2_{cv}	R^2_{pred}	RMSEC	RMSECV	RMSEP	Equation
OS	4	0.91	0.48	0.06	0.93	1.88	3.05	$y=x+5.02*10^{-7}$
FFA	5	1.00	0.90	0.97	0.51	2.29	1.66	$y=x+3.12*10^{-7}$
MUFA	5	0.95	0.00	0.54	1.81	7.88	4.95	$y=x+5.46*10^{-8}$
PUFA	4	0.91	0.41	0.95	2.89	6.10	4.08	$y=x-5.75*10^{-8}$
SFA	2	0.93	0.84	0.95	2.69	4.21	2.58	$y=x+1.25*10^{-6}$
TFA	3	0.74	0.07	0.36	0.25	0.47	0.36	$y=x-5.51*10^{-8}$
TAG	5	0.98	0.62	0.57	2.11	7.35	7.64	$y=x+1.18*10^{-6}$
DAG	5	0.98	0.81	0.79	1.13	3.05	3.65	$y=x-3.21*10^{-7}$
MAG	6	0.98	0.56	0.32	0.75	3.18	4.63	$y=x+3.64*10^{-7}$

*Abbreviations are provided in Materials & Methods section

Table 7.6 Statistical parameters of developed PLS models for the prediction of physical properties of structured lipids by both FT-NIR and FT-IR data acquired on solid samples

Physical Properties	PCs	R^2_{cal}	R^2_{cv}	R^2_{pred}	RMSEP	RMSECV	RMSEP	Equation
SMP	2	0.91	0.79	0.92	2.87	4.41	3.41	$y=x+1.03*10^{-8}$
MP85	5	0.98	0.82	0.92	1.49	4.57	4	$y=x-1.19*10^{-6}$
MP90	6	0.99	0.83	0.91	0.86	4.39	4.32	$y=x-1.75*10^{-6}$
MP95	6	0.99	0.80	0.92	0.89	4.56	4.37	$y=x-1.34*10^{-6}$
Consistency at 4 °C	8	1.00	0.36	0.02	11.15	185.94	183.05	$y=x+8.15*10^{-6}$
Consistency at 10 °C	5	0.98	0.49	0.14	20.61	116.27	81.31	$y=x+1.22*10^{-5}$
Consistency at 15 °C	6	0.90	0.50	0.26	8.55	93.16	39.29	$y=x+3.26*10^{-6}$
Consistency at 25 °C	3	0.99	0.61	0.53	5.72	35.45	18.69	$y=x-3.85*10^{-7}$
SFC at 10 °C	2	0.91	0.82	0.88	4.13	6.03	5.21	$y=x-4.62*10^{-8}$
SFC at 20 °C	5	0.99	0.83	0.92	1.25	4.91	3.21	$y=x+2.87*10^{-7}$
SFC at 30 °C	3	0.98	0.89	0.95	1.33	3.19	2.2	$y=x-8.44*10^{-7}$
SFC at 35°C	6	1.00	0.86	0.92	0.33	2.84	2.04	$y=x+3.87*10^{-7}$
ΔE	8	1.00	0.63	0.53	0.43	4.67	4.73	$y=x+8.69*10^{-7}$

*Abbreviations are provided in Materials & Methods section

CHAPTER 8

CONCLUSIONS

New products with better physical properties were produced by chemical interesterification of tallow in combination with vegetable oils. Generally, the blend ratio is the most significant factor that affects the properties of the end products. 1% catalyst concentration used in the process have negative effect on the chemical properties of the structured lipids. The samples produced with canola oil have higher contents of trans fatty acids compared to the others. Oxidative stabilities of the samples containing safflower oil is lower than other structured lipids. Interesterified lipids tend to have lower consistencies and solid fat contents in comparison with their physical blends and the tallow, therefore, they also acquired better spreadable and plastic behaviors. Mostly β' crystal form is observed for the produced products. Interesterified lipids produced from corn oil have more desirable properties (higher oxidative stability, lower free fatty acid content, more plastic properties) compared to other oils; therefore, they can be suggested as alternative lipid sources for bakery industry due to their possible good aeration properties and smooth texture.

Because of the favorable properties of the chemically interesterified corn oil-tallow mixtures interesterification process was also monitored for these samples with respect to blend ratio and reaction time. Generally, blend ratio is the significant factor and the reaction time does not generally have remarkable effect on the chemical and the physical properties of the structured lipids. The chemical and physical properties of the structured lipids throughout the chemical interesterification process indicated that 10 min reaction time is not enough for rearrangement of fatty acids in the triacylglycerol backbone. However, there is not a clear difference in between the samples produced with 20 and 30 reaction time. Therefore, reducing reaction time to 20 min could be suggested for chemical interesterification of tallow and corn oil.

Corn oil and tallow were also used as substrates in the production of the enzymatically interesterified lipids. The enzymatic interesterification caused sharp decreases in oxidation induction time of structured lipids. However, after 6 hours of reaction, there are some increases in oxidation induction time of the samples which can be associated with the rearrangement of polyunsaturated fatty acids in di- and

triacylglycerol backbone. Generally, free fatty acid value of the enzymatically interesterified lipids increased sharply compared to starting blends. This means that neutralization should be applied to samples after the enzymatic interesterification. The enzymatic interesterification of tallow with corn oil did not cause formation of trans fatty acids. As opposed to chemical interesterification, the long reaction times resulted in different polymorphs. In general, reaction time longer than 6 h have a trend changing effect on the several physical properties such as melting point, slip melting point and solid fat content. The univariate and multivariate analyses of the results confirmed that reaction time is highly important for the enzymatic interesterification reaction. It was observed that 12 h reaction time caused negative effect on the chemical and the physical properties of the structured lipids.

The structured lipids manufactured by the interesterification of tallow could be used in bakery industry since these lipids have desired β and β' polymorphic forms and low trans fatty acid contents. Some of the interesterified lipids could be suggested as frying fats due to their higher oxidative stabilities. Moreover, these structured lipids can be utilized as alternative products instead of margarines or butterfat due to their good spreadability and plastic properties.

Mid-infrared and near infrared spectra of all structured lipids were collected and were used in differentiation of the samples and in the prediction of chemical and physical properties. In general, infrared spectral data evaluated with chemometric methods agree with univariate statistical analysis in terms of the identification of significant factors affecting the properties of interesterified lipids. FT-NIR estimated saturated fatty acid, free fatty acid and diacylglycerol contents, solid fat content and slip melting point very well and prediction ability of FT-IR for the same parameters are also good. Moreover, combining FT-IR and FT-NIR spectral data improved the performance of these regression models. Therefore, IR spectroscopic techniques can be used for monitoring the changes of fats during interesterification, thus providing producers of structured fats with rapid and non-destructive techniques as good alternatives to the traditional analytical methods.

As the further study, fatty acids located at sn-2 position of triacylglycerol structure should be investigated in order to better understand the enzymatic interesterification mechanism. The chemical interesterification could be also performed by the addition of individual fatty acids, organic acids or amino acids to modify the tallow for different applications. Future studies should be focused on the collection of FT-NIR spectra of

more samples at different interesterification stages in order to improve the developed models and propose in-line applications for industrial processing.

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APPENDIX A

SUPPLEMENTARY MATERIALS

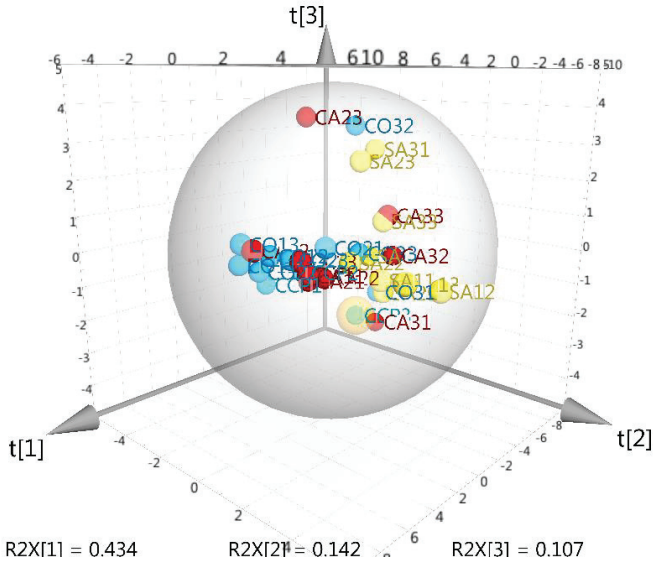

Figure A.1 3D Score plot of the PCA model constructed by using all parameters of the chemically interesterified lipids (CP1-2-3=70% tallow-, samples colored with respect to oil type)

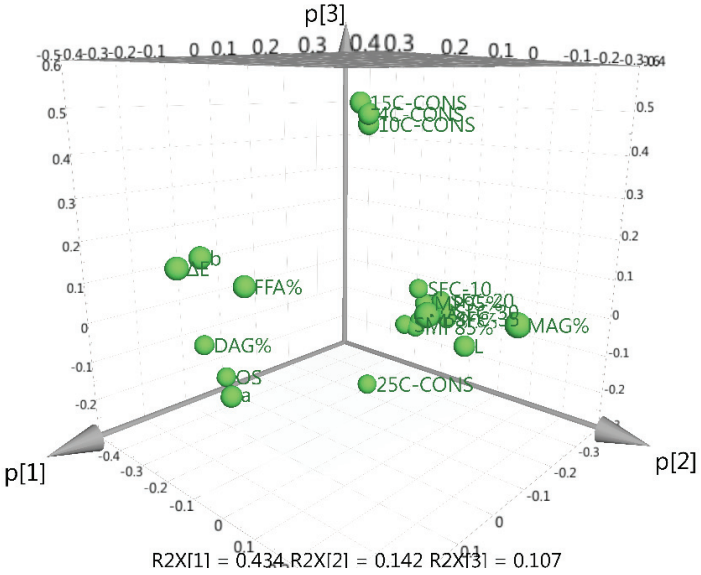

Figure A.2 3D Loading plot of the PCA model constructed by using all parameters of the chemically interesterified lipids

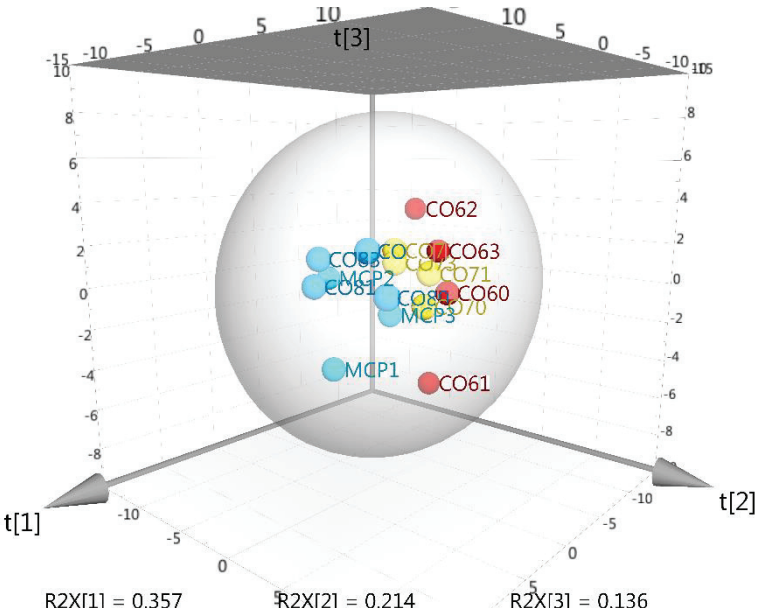

Figure A.3 3D Score plot of the PCA model constructed by using all parameters of the chemically interesterified lipids throughout the reaction (MCP1-2-3=70% tallow-20 min, samples colored with respect to reaction time)

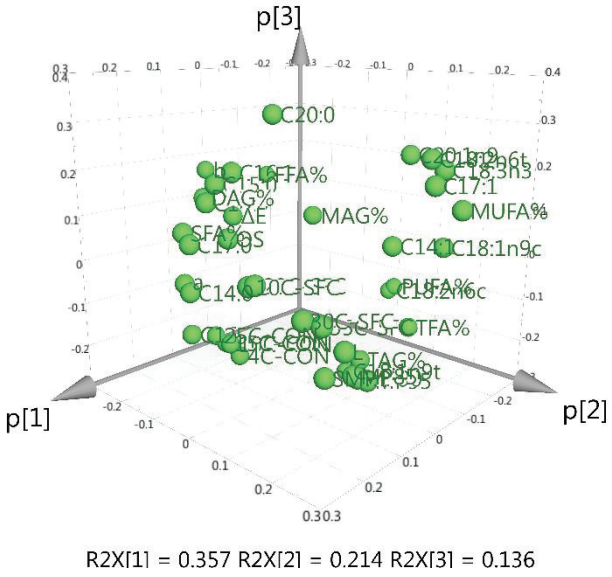

Figure A.4 3D Loading plot of the PCA model constructed by using all parameters of the chemically interesterified lipids throughout the reaction

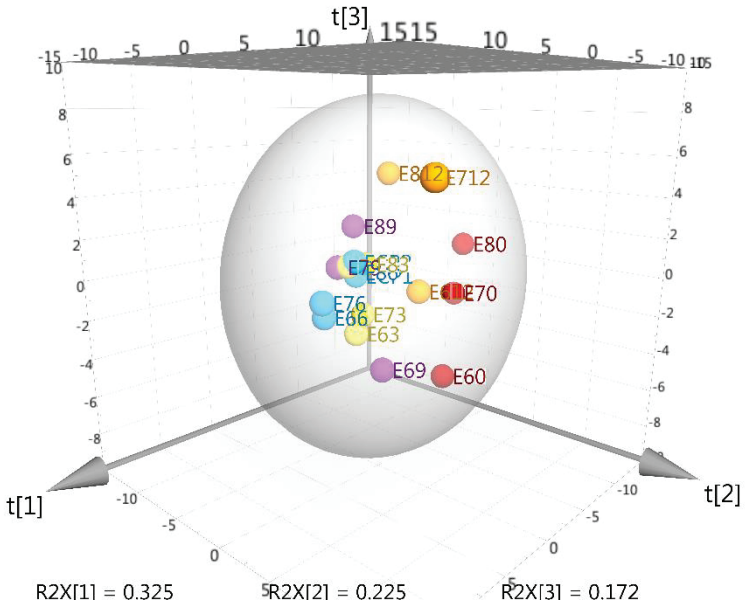

Figure A.5 3D Score plot of the PCA model constructed by using all parameters of the enzymatically interesterified lipids throughout the reaction (ECP1-2-3=70% tallow-6 h, samples colored with respect to reaction time)

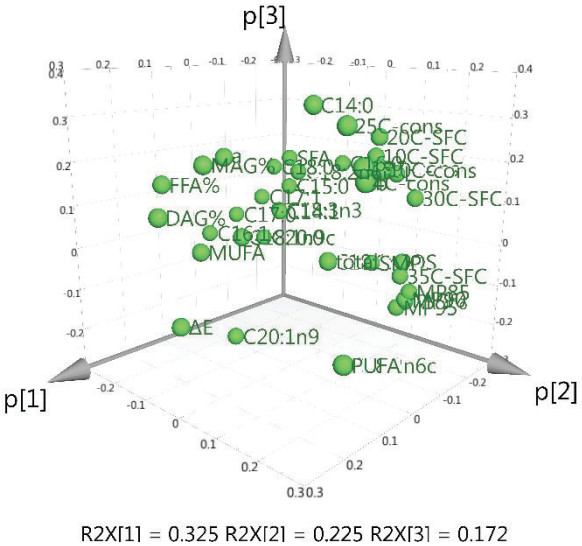

Figure A.6 3D Loading plot of the PCA model constructed by using all parameters of the enzymatically interesterified lipids throughout the reaction

Table A.7 ANOVA table of chemical parameters for chemically interesterified lipids

	MUFA%	PUFA%	SFA%	TFA%	OS	FFA%	MAG%	DAG%	TAG%
p value of model	0.00	0.00	0.00	0.00	0.00	0.02	0.01	0.08	0.72
p-value of lack of fit	0.25	0.97	0.14	0.11	0.80	0.49	0.86	0.50	0.10
R^2	0.99	1.00	0.92	0.95	0.68	0.57	0.62	0.48	0.23
R_{adj}^2	0.98	0.99	0.89	0.93	0.54	0.38	0.45	0.25	-0.11
Q^2	0.96	0.99	0.79	0.87	0.35	-0.15	0.14	-0.13	-1.38
p value of factors									
BR	0.18	0.00	0.00	0.27	0.94	0.79	0.70	0.33	0.68
CC	0.42	0.04	0.70	0.01	0.22	0.00	0.89	0.75	0.40
Oil Type									
corn	0.00	0.00	0.87	0.00	0.00	0.14	0.00	0.11	0.75
canola	0.00	0.00	0.15	0.00	0.32	0.95	0.29	0.09	0.11
safflower	0.00	0.00	0.11	0.00	0.00	0.18	0.00	0.00	0.18
p-value of interactions									
BR*CC	0.69	0.02	0.13	0.31	0.64	0.36	0.22	0.55	0.87
BR*OT(corn)	0.20	0.00	0.42	0.40	0.95	0.80	0.01	0.07	0.37
BR*OT(canola)	0.00	0.00	0.07	0.27	0.41	0.02	0.62	0.86	0.99
BR*OT(safflower)	0.00	0.00	0.01	0.79	0.45	0.01	0.04	0.05	0.38
CC*OT(corn)	0.33	0.00	0.44	0.03	0.63	0.19	0.85	0.51	0.46
CC*OT(canola)	0.07	0.31	0.31	0.00	0.25	0.78	0.64	0.75	0.40
CC*OT(safflower)	0.38	0.04	0.08	0.21	0.49	0.30	0.78	0.73	0.91

Table A.8 ANOVA table of physical parameters for chemically interesterified lipids

	Consistency				Solid Fat Content				Melting Points			SMP	ΔE
	4°C	10°C	15°C	25°C	10°C	20°C	30°C	35°C	MP85%	MP90%	MP95%		
p value-model	0.40	0.27	0.35	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
p-value-lack of fit	0.00	0.00	0.03	0.76	0.98	0.98	0.99	1.00	0.77	0.92	0.91	0.61	0.95
R^2	0.33	0.38	0.35	0.78	0.83	0.85	0.86	0.86	0.79	0.79	0.77	0.66	0.65
R_{adj}^2	0.03	0.10	0.06	0.68	0.75	0.79	0.80	0.80	0.70	0.69	0.67	0.51	0.49
Q2	-0.81	-0.41	-0.64	0.47	0.68	0.73	0.75	0.75	0.54	0.54	0.49	0.04	0.32
p value-factors													
BR	0.04	0.01	0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
CC	0.68	0.62	0.41	0.00	0.87	0.42	0.24	0.11	0.09	0.03	0.04	0.13	0.50
Oil Type													
corn	0.31	0.74	0.53	0.04	0.24	0.28	0.55	0.37	0.28	0.97	0.33	0.87	0.01
canola	0.76	0.92	0.59	0.40	0.05	0.41	0.73	0.66	0.15	0.42	0.79	0.37	0.26
safflower	0.52	0.68	0.96	0.01	0.36	0.84	0.37	0.21	0.65	0.45	0.24	0.46	0.10
p-value-interactions													
BR*CC	0.41	0.83	0.71	0.00	0.50	0.41	0.23	0.13	0.32	0.28	0.41	0.61	0.46
BR*OT(corn)	0.80	0.20	0.91	0.29	0.80	0.56	0.70	0.91	0.35	0.37	0.32	0.94	0.00
BR*OT(canola)	0.45	0.20	0.57	0.88	0.42	0.28	0.13	0.13	0.03	0.05	0.13	0.46	0.67
BR*OT(safflower)	0.32	0.99	0.65	0.23	0.30	0.10	0.06	0.11	0.18	0.26	0.60	0.50	0.00
CC*OT(corn)	0.53	0.70	0.44	0.02	0.58	0.55	0.32	0.25	0.36	0.23	0.30	0.15	0.98
CC*OT(canola)	0.16	0.34	0.06	0.45	0.62	0.77	0.63	0.42	0.90	0.62	0.65	0.64	0.68
CC*OT(safflower)	0.42	0.57	0.23	0.10	0.96	0.76	0.60	0.72	0.30	0.10	0.14	0.06	0.66

Table A.9 ANOVA table of chemical parameters for chemically interesterified lipids for monitoring of the process

	OS	FFA%	TAG%	DAG%	MAG%	MUFA%	PUFA%	SFA%	TFA%
p value-model	0.00	0.06	0.00	0.01	0.01	0.50	0.00	0.00	0.47
p-value-lack of fit	0.36	0.78	0.73	0.09	0.38	0.70	0.94	0.94	0.07
R^2	0.76	0.48	0.85	0.61	0.60	0.19	0.98	0.96	0.20
R_{adj}^2	0.69	0.34	0.80	0.50	0.49	-0.03	0.97	0.94	-0.02
Q^2	0.37	0.15	0.69	0.08	0.28	-0.37	0.97	0.94	-0.60
p value-factors									
BR	0.00	0.16	0.67	0.01	0.73	0.60	0.00	0.00	0.32
reaction time	0.16	0.02	0.00	0.01	0.51	0.18	0.13	0.07	0.28
BR*time	0.41	0.40	0.29	0.77	0.00	0.65	0.71	0.98	0.56

Table A.10 ANOVA table of physical parameters for chemically interesterified lipids for monitoring of the process

	Consistency				Solid Fat Content				Melting Points			SMP	ΔE
	4°C	10°C	15°C	25°C	10°C	20°C	30°C	35°C	MP85%	MP90%	MP95%		
p value-model	0.85	0.70	0.83	0.81	0.00	0.00	0.00	0.00	0.45	0.45	0.45	0.08	0.17
p-value-lack of fit	0.76	0.76	0.80	0.99	0.31	0.23	0.45	0.48	0.24	0.29	0.39	0.50	0.54
R2	0.07	0.12	0.07	0.08	0.93	0.87	0.77	0.72	0.21	0.21	0.21	0.45	0.36
R2 adj	-0.19	-0.12	-0.18	-0.17	0.91	0.84	0.70	0.64	-0.01	-0.01	-0.01	0.30	0.18
Q2	-0.36	-0.23	-0.34	-0.23	0.85	0.74	0.57	0.47	-0.62	-0.57	-0.50	0.11	-0.07
p value-factors													
BR	0.52	0.28	0.40	0.40	0.00	0.00	0.00	0.00	0.23	0.25	0.29	0.04	0.27
reaction time	0.73	0.97	0.77	0.69	0.00	0.05	0.01	0.01	0.29	0.27	0.23	0.12	0.05
BR*time	0.65	0.69	0.86	0.97	0.16	0.18	0.74	0.81	0.92	0.99	0.89	0.54	0.68

Table A.11 ANOVA table of chemical parameters for enzymatically interesterified lipids

	OS	FFA%	TAG%	DAG%	MAG%	MUFA%	PUFA%	SFA%	TFA%
p value-model	0.02	0.14	0.05	0.20	0.01	0.81	0.00	0.00	0,84
p-value-lack of fit	0.00	0.01	0.13	0.08	0.93	0.06	0.02	0.06	0.72
R^2	0.48	0.32	0.42	0.28	0.54	0.07	0.81	0.62	0.46
R_{adj}^2	0.37	0.17	0.30	0.12	0.44	-0.14	0.77	0.54	0.34
Q^2	-0.19	-0.26	-0.38	-0.70	0.20	-1.11	0.65	0.23	-0.58
p value-factors									
BR	0.53	0.46	0.45	0.98	0.27	0.41	0.00	0.00	0,54
reaction time	0.00	0.04	0.01	0.04	0.00	0.64	0.51	0.89	0,77
BR*time	0.41	0.35	0.50	0.56	0.86	0.87	0.38	0.46	0,63

Table A.12 ANOVA table of physical parameters for enzymatically interesterified lipids

	Consistency				Solid Fat Content				Melting Points			SMP	ΔE
	4°C	**10°C**	**15°C**	**25°C**	**10°C**	**20°C**	**30°C**	**35°C**	**MP85%**	**MP90%**	**MP95%**		
p value-model	0.03	0.23	0.49	0.17	0.12	0.06	0.40	0.02	0.19	0.17	0.08	0.05	0.21
p-value-lack of fit	0.01	0.01	0.00	0.20	0.01	0.05	0.08	0.00	0.09	0.18	0.31	0.21	0.08
R2	0.46	0.26	0.15	-0.19	0.33	0.40	0.19	0.52	0.28	0.30	0.37	0.42	0.27
R_{adj}^2	0.34	0.10	-0.03	0.30	0.19	0.28	0.01	0.41	0.13	0.14	0.24	0.29	0.11
Q^2	0.03	-0.22	-0.47	0.15	-0.52	-0.29	-0.84	-0.12	-0.63	-0.56	-0.30	-0.20	-0.64
p value-factors													
BR	0.70	0.98	0.75	0.18	0.02	0.01	0.18	0.78	0.85	0.68	0.56	0.29	0.06
reaction time	0.01	0.06	0.14	0.07	0.63	0.87	0.36	0.01	0.05	0.05	0.02	0.01	0.40
BR*time	0.17	0.48	1.00	0.91	0.84	0.98	0.58	0.05	0.37	0.31	0.26	0.89	0.85

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Aktas, A. B., Ozen, B., Tokatli, F., & Sen, I. (2014). Phenolics profile of a naturally debittering olive in comparison to regular olive varieties. Journal of the Science of Food and Agriculture, 94(4), 691-698.