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Chemical and physical properties of fats produced by chemical interesterification of tallow with vegetable oils

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SUMMARY: This study aims at manufacturing structured lipids by chemical interesterification (CI) of beef tallow with corn, canola and safflower oils individually at various tallow blend ratios (60, 70, 80%) and catalyst concentrations (0.75, 0.875, 1%). Several physical and chemical properties of interesterified products were determined and data were analyzed using univariate and multivariate statistical techniques. Interesterified lipids were more spreadable and showed plastic behavior due to their lower consistency and solid fat contents. Decreases in melting points to a temperature range of 26.5-45.5 °C regardless of oil type were observed. Interesterified fats displayed mostly β' and β'+β crystal forms. The CI of tallow did not result in the formation of significant amounts of *trans*-fatty acids. Samples interesterified with corn oil had lower free fatty acid contents (1.87-3.9%) and higher oxidation induction times (3.82-12.25h) than other lipids. Therefore, fats containing corn oil-tallow could be used in the baking industry due to their potentially good aeration properties and smooth texture.

KEYWORDS: *Chemical interesterification; Chemical properties; Physical properties; Tallow; Vegetable oils*

RESUMEN: ***Propiedades químicas y físicas de las grasas producidas mediante interesterificación química de sebo con aceites vegetales.*** Este estudio tiene como objetivo la fabricación de lípidos estructurados mediante interesterificación química (IQ) de sebo de res con aceites de maíz, canola y cártamo individualmente en varias relaciones de la mezcla (60% -70% -80%) y concentraciones de catalizador (0,75, 0,875, 1%). Se determinaron varias propiedades físicas y químicas de los productos interesterificados y los datos se analizaron con técnicas estadísticas univariante y multivariantes. Los lípidos interesterificados son más extensibles y tienen un comportamiento plástico debido a su menor consistencia y contenido de grasa sólida. Se observaron disminuciones en los puntos de fusión a un rango de temperatura de 26,5-45,5 °C, independientemente del tipo de aceite. Las grasas interesterificadas muestran principalmente formas cristalinas β 'y β' + β. La IQ de sebo no dio lugar a la formación de cantidades significativas de ácidos grasos *trans*. Las muestras interesterificadas con aceite de maíz tienen un contenido de ácidos grasos libres más bajo (1,87-3,9%) y tiempos de inducción de oxidación más altos (3,82-12,25 h) que otros lípidos. Por lo tanto, las grasas que contienen sebo-aceite de maíz podrían usarse en la industria de la panadería debido a sus posibles buenas propiedades de aireación y textura suave.

PALABRAS CLAVE: *Aceites vegetales; Interesterificación química; Propiedades físicas; Propiedades químicas; Sebo*

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1. INTRODUCTION

Fats and oils are significant ingredients in food products due to their nutritional and functional properties. The fatty acid compositions, fatty acid distributions, and saturated/unsaturated fatty acids ratio, melting points, crystallization behaviors, storage stabilities, nutritional values, and health-promoting effects of fats and oils vary because of factors such as raw material sources, processing type and conditions and some naturally present fats and oils are not always suitable for food processes due to their properties. Appropriate modifications could be carried out to add desirable characteristics to the lipids so that they can be available to be used in a wider spectrum (Martin *et al.,* 2010; Segura and Jachmanián, 2020). One of these modification techniques is the well-known chemical interesterification method which provides new chemical and physical properties to lipids by altering their triacylglycerol composition, therefore changing the crystal morphology of the lipids (Riberio *et al.,* 2009; Zhu *et al.,* 2019). Chemical interesterification is carried out by the breaking of fatty acid groups from the mixture of fats and a random re-esterification onto the glycerol backbone. The reaction is catalyzed by alkali metals or alkali metal alkylates at high temperatures under vacuum (Zhang *et al.,* 2019). There are various applications for chemical interesterification process in the literature which have been used to improve the properties of several fats (Naeli *et al.,* 2017; Oliveria *et al.,* 2017; Tourchi *et al.,* 2019).

Beef tallow is one of the important by-products of the meat industry. It has limited commercial use due to its hard structure, high melting point and low levels of polyunsaturated fatty acids (PUFA); therefore, modifications are needed to widen its application area. Chemical interesterification can be applied to provide desirable characteristics to the tallow (Kowalska *et al.,* 2005; Zhang *et al.,* 2018). In a study on the chemical interesterification of tallow with sunflower oil, it was observed that the ratio of tallow in the blend, catalyst concentration, and reaction temperature had significant effects on the melting point of the product (Rodriguez *et al.*, 2001). Interesterified fats produced with beef tallow and rapeseed oil have higher free fatty acid, monoacylgycerol and diacylglycerol contents, and shorter oxidation induction times than a non-esterified blend (Kowalska *et al.*, 2007; Kowalski *et al.*, 2004). The solid fat content of the structured lipids produced by the chemical interesterification of beef tallow with canola oil decreased to the desired levels compared to the physical blends and these products consisted mainly of the β' polymorphic form together with a small content of β form (Meng *et al.,* 2010).

As a by-product of the meat indusrty, tallow, having limited usage in food applications due to its properties, could benefit from interesterification by widening its applications in the food industry; however, there are a few studies in the literature about this. To the best of our knowledge, safflower and corn oils have not been used together with tallow to modify the properties of this fat. Depending on compositional factors, different types of fat/oil blends in general result in fats having different physical and chemical properties after chemical interesterification. In this study, beef tallow with high saturated fatty acid content was blended with various vegetable oils with high monounsaturated (canola oil) and polyunsaturated (safflower and corn oils) fatty acids. With this study, it is intended to compare several important properties of fats produced through chemical interesterification of tallow together with three different oils which are easily available and economical. The present study has a purpose to modify several chemical and physical properties of tallow blended with corn, canola and safflower oils individually through the chemical interesterification process. In addition, it is also aimed to evaluate the effects of the oil types, the blend ratios and the catalyst concentrations on the chemical (free fatty acid content, fatty acid composition, mono, di, and triacylglycerol composition, oxidative stability) and the physical properties (solid fat content, consistency, melting point and crystal morphology) of the produced products using both univariate and multivariate statistical analysis techniques.

2. MATERIALS AND METHODS

2.1. Fat samples and reagents

Beef tallow used for chemical interesterification reactions was obtained from two different breeds of 2-year-old calves (Montafon and Holstein) immediately after slaughter and stored at -20 °C. Canola, safflower and corn oils were obtained from the local market. A sodium methoxide (CH_3NaO) catalyst (Solem Kimya, Turkey), was provided by

a local oil processing plant. All other reagents and solvents were of analytical or chromatographic grade and obtained from Sigma (Sigma-Aldrich, Germany).

2.2. Chemical interesterification process

A full factorial design was employed to evaluate the effects of catalyst concentration (0.75-0.875-1%, w/w), oil type (safflower, corn and canola oils), and tallow-to-oil blend ratio (60:40, 70:30 and 80:20, w/w) on the properties of interesterified lipids. Catalyst concentration and blend ratio were chosen according to preliminary trials and studies in the literature (Meng *et al*., 2010, Kowalski *et al*., 2004). A modified procedure from the literature was used for the chemical interesterification process (Kowalski *et al.,* 2004). Thirty different blends were prepared according to an experimental design (Table 1). An oil blend was dried under 185 mPa vacuum in a rotary evaporator (Laborato 4000 Heidolph, Germany) at 90 °C with stirring at 100 rpm for 30 min, then chemical catalyst was added at certain levels provided in the experimental design. 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 with the aid of a separation funnel. The product was then filtered through a vacuum filtration unit with 400 mm pore size filter paper (Macherey-Nagel, Düren, Germany) and washed with hot water three times to remove all residues. The structured lipid and water were also separated from each other by a separation funnel. The traces of water were evaporated under 185 mPa vacuum in the same rotary evaporator at 90 °C with stirring at 100 rpm for 30 min.

2.3. Chemical analyses of interesterified lipids

2.3.1. Free fatty acid (FFA) content

The titrimetric method specified in AOCS method Ca 5a-40 (American Oil Chemists' Society, 2017) was used for the FFA determination of the products. Acidity was expressed as percentage of oleic acid.

2.3.2. Mono- (MAG), -di-(DAG) and triacylglycerol (TAG) content determination

MAG, DAG and TAG contents of the structured lipids were determined according to the AOCS Cd11C-93 (American Oil Chemists' Society, 2017) method by column chromatography. Sample was dissolved in chloroform and transferred to the column by washing with chloroform three times. Then, 250 mL of 10, 25 and 100% (v/v) diethyl ether in petroleum ether were used for the elution of TAG, DAG and MAG fractions, respectively. The fractions were collected separately in a flask and solvents were evaporated in a rotary evaporator (Laborato 4000 Heidolph, Germany) at 50 °C. The flasks were dried until constant weight. Percentages of mass fractions were calculated for each part.

2.3.3. Oxidative stability

The oxidation induction time was determined with Rancimat apparatus (873 Biodiesel, Metrohm, Switzerland). The sample was placed inside the glass reaction vessel for the measurement. The carrier medium was deionized water and the reaction temperature was set to 120 °C for both columns with a constant 20 L/h air flow. Stability was expressed as the oxidation induction time (h).

2.3.4. Fatty acid composition

The fatty acid composition of the samples was determined after converting them into their corresponding fatty acid methyl esters (FAME) according to a procedure provided by IUPAC (1987). Chromatographic analyses were performed with a GC (Agilent 6890) equipped with an auto-sampler, a split/splittless (1:50) injector and an FID detector. An HP 88 capillary column (100 m x 0.25 mm ID x 0.2μm) was used in the analyses. Conditions for GC analysis are described in a previous study (Meng *et al.*, 2010). A 37-component mixture of FAME (Sigma) was used as the standard.

2.4. Physical analyses of interesterified lipids

2.4.1. Crystal morphology

Polymorphic forms of the fat crystals were determined with X-ray diffraction (Philips, Holland) using Cu as anode material (k = 1.54056 Å, 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.

Table 1. Chemical properties of the structured lipids produced

Parameters				Chemical Properties[a]							
Sample	**Oil Type**	**Tallow-oil Blend ratio**	**Catalyst Conc. %**	**OS (h)**	**FFA%**	**TAG%**	**DAG+MAG%**	**MUFA%**	**PUFA%**	**SFA%**	**TFA%**
SA61	Safflower	60-40	0.75	0.71	2.42	79.91	17.55	25.89	30.65	43.46	0.67
SA62	Safflower	60-40	0.875	1.55	1.08	89.76	14.23	28.82	32.54	38.64	0.74
SA63	Safflower	60-40	1	1.12	1.68	82.75	16.59	29.35	33.78	36.87	0.72
SA71	Safflower	70-30	0.75	3.16	2.6	78.53	18.66	33.03	24.22	42.75	0.65
SA72	Safflower	70-30	0.875	2.84	3.25	84.43	15.52	31.96	25.76	42.28	0.92
SA73	Safflower	70-30	1	0.9	3.26	83.34	12.84	29.45	24.94	45.61	0.78
SA81	Safflower	80-20	0.75	1.08	1.6	76.72	16.8	34.06	18.87	47.08	0.82
SA82	Safflower	80-20	0.875	3.09	3.4	84.19	12.82	35.15	18.87	45.98	0.76
SA83	Safflower	80-20	1	2.17	3.23	81.22	17.64	34.80	19.61	45.58	0.82
SA60	Safflower	60-40	0	2.83	0.65	88.3	6.64	29.09	33.04	37.87	0.64
SA70	Safflower	70-30	0	3.17	1.31	82.37	14.4	31.65	25.78	42.57	0.60
SA80	Safflower	80-20	0	4.2	0.46	81.24	5.74	34.84	18.38	46.78	0.68
CO61	Corn	60-40	0.75	7.48	2.74	52.62	22.95	37.21	24.84	37.96	0.76
CO62	Corn	60-40	0.875	7.76	3.11	89.32	8.08	37.65	24.70	37.65	0.72
CO63	Corn	60-40	1	4.78	3.9	82.75	12.82	37.52	24.80	37.68	0.65
CO71	Corn	70-30	0.75	3.82	2.15	89.65	8.23	38.03	20.03	41.94	0.79
CO72	Corn	70-30	0.875	6.77	2.48	81.38	11.92	37.44	20.71	41.86	0.82
CO73	Corn	70-30	1	6.85	3.88	82.89	17.03	38.24	18.83	42.94	0.71
CO81	Corn	80-20	0.75	7.3	2.33	81.62	12.89	38.65	14.18	47.17	0.79
CO82	Corn	80-20	0.875	7.4	3.04	82.02	13.81	39.27	13.74	46.98	0.87
CO83	Corn	80-20	1	5.34	3.85	81.31	15.64	38.95	13.33	47.72	0.89
CO60	Corn	60-40	0	6.73	0.62	85.51	6.17	35.59	29.91	34.50	0.58
CO70	Corn	70-30	0	8.51	0.63	86.84	6.73	37.09	19.90	43.02	0.69
CO80	Corn	80-20	0	10	0.76	85.52	9.05	38.00	13.85	48.15	0.70
CP1	Corn	70-30	0.875	10.92	3.03	77.51	21.33	38.47	19.30	42.23	0.73
CP2	Corn	70-30	0.875	12.25	2.97	82.88	16.97	37.33	19.59	43.08	0.67
CP3	Corn	70-30	0.875	7.98	1.87	74.25	14.57	38.52	18.27	43.21	0.75
CA61	Canola	60-40	0.75	6.43	2.23	79.57	16.48	51.30	11.69	37.20	1.40
CA62	Canola	60-40	0.875	6.37	3.39	83.98	16	50.20	12.36	37.76	1.69
CA63	Canola	60-40	1	2.71	4.12	63.56	18.65	49.41	14.37	36.59	1.69
CA71	Canola	70-30	0.75	8.49	2.7	76.08	17.32	48.74	10.90	40.23	1.66
CA72	Canola	70-30	0.875	7.55	2.9	73.07	15.14	47.60	10.68	41.60	1.88
CA73	Canola	70-30	1	5.55	2.37	70.29	21.23	46.34	11.01	42.56	2.21
CA81	Canola	80-20	0.75	4.72	1.56	64.87	18.25	43.80	8.20	47.93	1.28
CA82	Canola	80-20	0.875	4.25	2.5	84.76	15.16	45.15	8.43	46.79	1.55
CA83	Canola	80-20	1	2.92	2.56	82.3	10.49	43.27	8.59	48.13	1.87
CA60	Canola	60-40	0	5.73	1.03	91.02	8.43	48.45	11.50	40.05	1.52
CA70	Canola	70-30	0	7.74	0.68	94.41	5	48.16	9.93	41.91	1.48
CA80	Canola	80-20	0	9.06	0.77	96.29	3.86	45.81	7.55	46.64	1.55
CA	Canola			4.51	0.09	74.87	6.11	68.21	25.35	6.43	2.53
SA	Safflower			2.04	0.23	90.36	3.72	14.74	75.35	9.91	0.39
CO	Corn			4.98	0.09	92.06	2.36	32.07	54.83	13.10	0.47
T	Tallow			4.81	1.15	97.94	1.4	38.94	3.27	57.79	0.81

[a] OS: oxidative stability, FFA: free fatty acid, TAG: triacylglycerol content, DAG: diacylglycerol content, MAG: monoacylglycerol content, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid, TFA: *trans* fatty acid.
Standard deviations OS:1.78, FFA:0.53, TAG%:3.56, DAG%:3.04, MAG%:1.4, MUFA%:0.55, PUFA%:0.57, SFA%:0.44, TFA%:0.04 (standard deviation for each measurement is calculated from three replicates of central points in the experimental design)

2.4.2. Determination of melting point

The melting points of the structured lipids were measured with a differential scanning calorimeter (DSC, Q10 TA Instruments, Crawley, UK). All samples were conditioned at 4 °C for 24 h prior to measurements. Samples were placed in hermetically sealed aluminum pans and 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 (Rodriguez *et al.,* 2001). Data analysis was performed with DSC TRIOS software (TA Instruments, Crawley, UK).

2.4.3. Consistency measurements

Sample consistency was determined with a penetration test using a 45° acrylic cone fitted to a constant speed texture analyzer (TA.XT plus, UK). Samples were conditioned at 60 °C in an oven for complete melting of the crystals. Tempering was allowed to occur for 24 h in a commercial refrigerator (4 °C), then for 24 h in an oven with controlled temperature (4, 10, 15, 25 °C). Test parameters were penetration depth of 0.4 cm with 0.2 cm/s speed for 5 s testing time (Silva *et al.,* 2009). Consistency was calculated as "yield value" (Haighton, 1959).

2.4.4. Determination of solid fat content (SFC)

The solid fat content of lipids was determined by a nuclear magnetic resonance (NMR) spectrometer (Bruker, USA) according to AOCS Official Method Cd 16b-93 (American Oil Chemists' Society, 2017). Samples were melted at 80 °C and recrystallized at 0 °C for 30 min. Then, they were stabilized for 30 min at various temperatures (10, 20, 30 and 35 °C) before measuring the liquid signal.

2.5. Data Analysis

The data were analyzed according to the univariate (ANOVA) statistical analysis technique to investigate the effects of the oil type, blend ratio and catalyst concentration on the chemical and the physical properties of the structured lipids with software (MODDE 11, MKS Umetrics, Sweeden). To investigate the effects of the same parameters, principal component analysis (PCA) was also used as the multivariate statistical analysis tool (SIMCA 14.1, MKS Umetrics, Sweeden). PCA is generally used to find a relationship between all factors and responses and can extract useful information from multivariate data. Score and loading plots are very helpful in understanding the characteristics of systems or processes. While the score plots show the positioning of the samples in different groups, the loading plot confirms the groupings of these samples by supporting with responses. R^2 and Q^2 values prove degree of fitness and predictability of the constructed models, respectively.

3. RESULTS AND DISCUSSIONS

3.1. Chemical Properties of the Interesterified Lipids

3.1.1. Free fatty acid, mono, di and triacylglycerol contents

The results from the chemical analysis of the produced interesterified samples are listed in Table 1. As can be seen from the table, the FFA content in the tallow is 1.15% while the blends without interesterification have an acidity range of 0.46-1.5%. In general, the FFA percentages of the interesterified lipids (1.08-4.12%) were higher compared to the starting blends. This result is in accordance with previous studies, where an increase in FFA content was observed after chemical interesterification (Hoshino *et al.,* 2004*;* Kowalska *et al.,* 2005). There are some fluctuations among the samples depending on the catalyst concentration; although 1% catalyst concentration led to the formation of structured lipids with higher FFA%, especially for the corn oil-tallow samples. With the increse in catalyst concentration, there would be more FFA released from TAG, DAG and MAG structures. The correlation graphic of DAG + MAG and FFA also supports this result (Figure 1). It was also reported that the higher amount of catalysts in the reaction medium caused the formation of higher amounts of FFA and MAG + DAG, and a lower TAG content (Ledochowska and Wilczynska, 1998). ANOVA was used to investigate the effects of the factors on the chemical parameters of the structured lipids (Table 2). As the ANOVA table indicated, catalyst concentration is the most important factor affecting FFA value and the use of a chemical catalyst at higher concentrations resulted in higher FFA%.

The TAG content in tallow is approximately 98% while the blends without interesterification have lower TAG% (81.24-96.29%) values (Table 1). Both blending and chemical interesterification

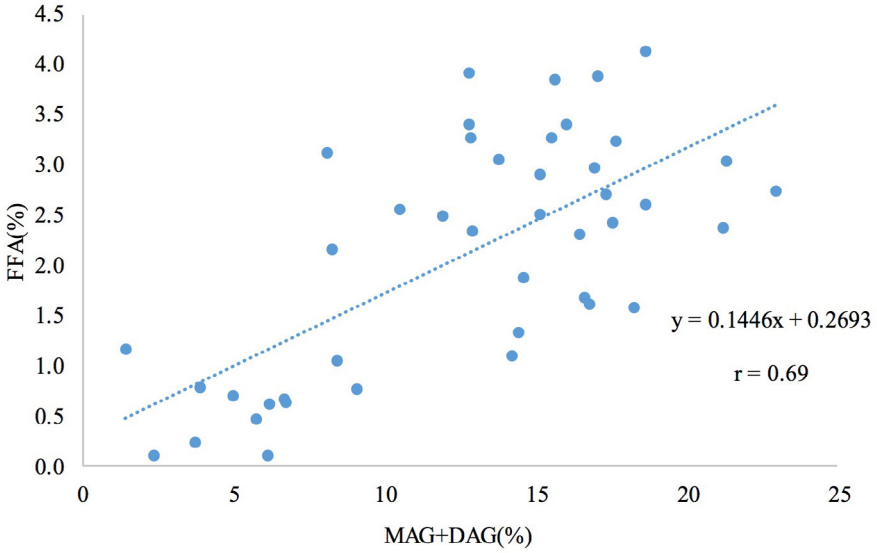

FIGURE 1. Free fatty acid content (FFA) versus monoacylglycerol and diacylglycerol contents (MAG+DAG) of structured lipids

caused an increase in MAG and DAG contents in the samples. The TAG content of the interesterfied lipids varied between 52.62-89.76% and all interesterfied fats containing canola oil had lower TAG contents compared to their starting blends. Fluctuations in TAG contents in the samples exist depending on the catalyst concentrations; although 0.875% CH_3NaO concentration mostly led to the formation of the structured lipids with higher TAG%. As expected, the structured lipids with low TAG contents had higher DAG and MAG values. A correlation between FFA and MAG + DAG contents in the interesterified lipids was evaluated in order to better understand the changes in TAG, DAG and MAG contents after the chemical interesterification reaction (Figure 1) and the 'r' value was calculated as 0.69. Although this value was not especially high, there was still an increasing trend between FFA and MAG + DAG contents in the samples. Generally, samples having higher amounts of MAG + DAG content, also have higher FFA%. Therefore, the increase in both FFA% and MAG + DAG% could be associated with the activity of the chemical catalyst that snatched the fatty acids from their original place and then random distribution of the fatty acids were provided in the TAG backbone of the newly structured lipids. According to ANOVA, models developed for DAG and MAG were significant with non-significant lack of fit at 95% confidence interval (Table 2). Examination of the significance levels of the main factors and their interactions shows that the catalyst concentration, the oil type and the blend ratio did not significantly affect the TAG% of the chemically interesterified lipids (Table 2). The ANOVA table confirms the significance of the oil type (safflower) for the model of DAG%. Generally, the structured lipids interesterified with the safflower oil have lower DAG% values compared to the samples interesterified with the other oil types. Additionally, the interaction between the oil type (safflower) and the blend ratio have some significance ($p \leq 0.05$) for the DAG content model. Oil type, particularly corn and safflower oils, and their interactions with the blend ratio have important effects on the MAG% of structured lipids. Increasing the blend ratio (increasing tallow amount in the blend) resulted in an increase in the MAG contents for corn oil; however, MAG contents decreased slightly with increasing blend ratio for the interesterified lipids with safflower oil according to the interaction plot (not shown).

3.1.2. Oxidative stability

The oxidation induction time of the tallow was 4.81 h while the non-interesterified blends showed a range of induction times from 7.98-12.25 h (Table 1). In general, the oxidation induction times of the interesterified samples decreased compared to their starting blends. Among all the samples, the tallow

interesterified with corn oil had the highest oxidation induction times (3.82-12.25 h). Varying the catalyst concentration resulted in ups and downs in the oxidative stability of the samples. However, 1% CH_3NaO concentration mostly led to the formation of structured lipids with low oxidation induction times regardless of the blend ratio. There was a drastic decrease in the oxidation induction times of the samples produced with safflower oil after the CI process compared to tallow itself. This result is in accordance with the previous studies, which also observed a decrease in oxidative stability after the chemical interesterfication of beef tallow interesterified with rapeseed oil (Hoshino *et al.,* 2004*;* Kowalska *et al.,* 2007; Kowalski *et al.,* 2004). The decrease in oxidative stability could be due to the high linoleic acid and low tocopherol content of safflower oil (240-670 mg/kg) (Codex, 2011). On the other hand, some of the tallow samples interesterified with canola oil had better oxidative stability than tallow itself. The presence of tocopherols in canola oil can be associated with longer induction times. According to the literature, canola oil could contain tocopherols up to in the range of 430-2680 mg/kg, which could have an effect on the improvement in the oxidative stability (Codex, 2011). The ANOVA table reveals that the blend ratio and the catalyst concentration were not significant for oxidative stability, meaning that neither factor affected the oxidative stability of the structured lipids (Table 2). The model showed that oil type was the only significant factor. Generally, corn oil samples have better oxidative stability compared to the other oil types while products which contain safflower oil are the least stable ones against oxidation.

3.1.3. Fatty acid composition

The major fatty acid in safflower and corn oils are linoleic acid (75.12 and 54.59%, respectively) while canola oil is rich in terms of oleic acid (57.82%), a

Table 2. ANOVA table of chemical parameters for chemically interesterified lipids

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

[a] OS: oxidative stability, FFA: free fatty acid, TAG: triacylglycerol content, DAG: diacylglycerol content, MAG: monoacylglycerol content, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acid, SFA: saturated fatty acid, TFA: *trans* fatty acid.
[b] tallow-oil blend ratio; [c] catalyst concentration; [d] oil type

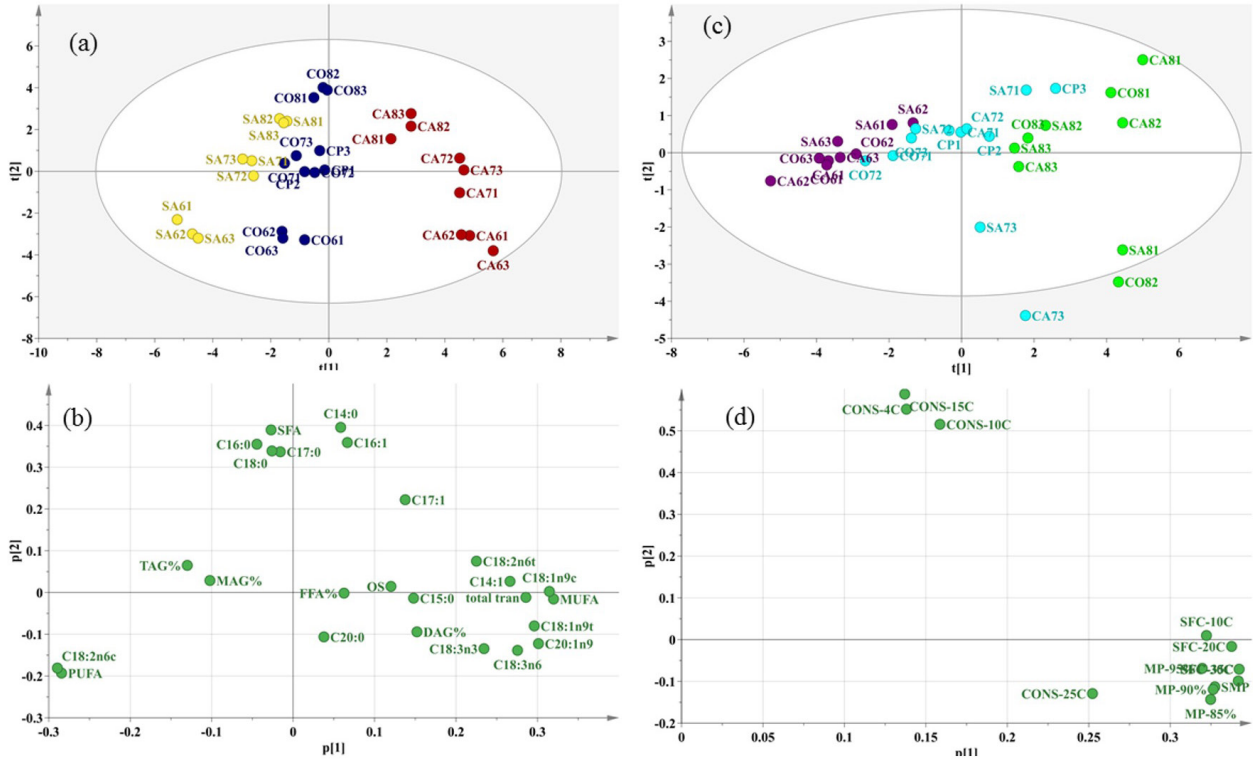

FIGURE 2. (a) Score plot (coloring is done with respect to oil types. Yellow: safflower, dark blue: corn, red: canola oils) and (b) loading plot obtained from principal component analysis for chemical parameters (c) Score plot (coloring is done with respect to tallow to oil blend ratios. Purple: 60:40, light blue: 70:30, green: 80:20) and (d) loading plot obtained with principal component analysis for physical parameters

monounsaturated fatty acid. The main fatty acids in tallow, on the other hand, are palmitic (22.50%), stearic (32.10%) and oleic (36.20%) acids. The MUFA, PUFA and SFA contents in the blends of interesterified products were in the ranges as determined by their blend ratio and intial compositions of tallow and oils without any significant changes as expected and this was also confirmed by ANOVA (Table 1). As in previous studies (Meng *et al*., 2011), the chemical interesterification of of tallow did not result in the formation of significant amounts of *trans* fatty acids. Generally, the amount of fatty acids in the *trans* form is less than 1%, except for samples which contain canola oil (Table 1). Safflower and corn oils themselves have *trans* fatty acid contents of less than 1% and their interesterified forms have slightly higher percentages of *trans* fats. Canola oil used in blending with tallow, on the other hand, has a higher content of *trans* fatty acids (2.5%) compared to the other oils and the interesterified samples containing canola oil have lower *trans*-fat contents compared to the oil itself. Since high temperature treatments are applied during canola oil refining mostly in the deodorization step to eliminate the intense bad odor of this oil, higher amounts of *trans* fatty acids form during this process. The model constructed for the *trans* fatty acids of interesterified fats showed that catalyst concentration and oil type and interaction between oil type and catalyst concentration were the significant factors. Due to higher *trans* fatty acid content in canola oil itself, interesterified products containing canola oil also had higher *trans* fatty acid contents with respect to other samples and this is the reason for the differences between *trans* fatty acid contents in fats containing different oils. In addition, increasing catalyst concentration resulted in an increase in *trans* fatty acid contents in fats which contain canola oil according to statistical analysis. However, most of the products are within acceptable limits.

3.1.4. Combination of chemical parameters

Since many parameters were determined in the study, chemical data were combined and analyzed with a multivariate statistical analysis technique, principal

component analysis (PCA), to obtain a better view of the process. The model was constructed by using all measured chemical parameters with 5 principal components (PC), R^2=0.84, and Q^2=0.42. There was a clear discrimination among the samples with respect to oil type (Figure 2a). While the samples containing canola oil were located at the right part of the ellipse samples with corn oil were placed just right of the center and safflower-containing ones were further to the left. Therefore, a discrimination with respect to the first principal component (PC) was obtained for the oil type. This discrimination mostly resulted from the higher *trans* fatty acid and MUFA contents in canola oil samples as observed in the loading plot (Figure 2b). In addition, the structured lipids containing 40% safflower and corn oils were placed at the bottom part of left quartile due to their higher PUFA contents, especially linoleic acid content. Moreover, groupings among the samples with different blend ratios were observed. The samples with 80% tallow were mostly located at the top of the ellipse regardless of the oil type since they presented higher saturated fatty acid (SFA) contents (Figure 2a). The samples containing 60% tallow were in the lower part of the ellipse and 70% tallow-containing samples were placed in the middle. Therefore, a separation based on the blend ratio was also possible with respect to the second PC. As a result, multivariate analysis of the chemical data indicated that the type of oil and the blend ratio caused differences in the chemical properties of the interesterified products.

3.2. Physical Properties of the Interesterified Lipids

3.2.1. Crystal morphology

The physical properties of the samples are provided in Table 3. As far as the crystal morphology is concerned, tallow contained mixtures of β and β' forms. α forms were not found in either structured lipids nor blends. The non-interesterified blends also contained both β and β' forms together. After the chemical interesterification, β and β' forms were also mostly present together especially for the structured lipids containing safflower and corn oils. Therefore, the interesterification did not cause important changes in the polymorphic structures of the lipids produced from safflower and corn oils. This result was in accordance with previous studies related to beef tallow-palm oil interesterified fats and other similar products (Lee *et al.,* 2008*;* Meng *et al.,* 2010). In general, a lower tallow-oil blend ratio and lower catalyst concentration combination (CA61, CA62, CA71) resulted in the formation of only β crystals while a higher blend tallow-oil ratio and catalyst concentration combination (CA72, CA81, CA82, CA83) caused the formation of β' crystals alone for canola oil-containing samples (Table 3). SA83, along with CA72, CA81, CA82, and CA83 were the samples which contained only the β' polymorphic form and this crystal form is important in the baking industry due to its aeration properties and smooth texture. Therefore, these lipids can be used as alternatives for bakery fats.

3.2.2. Melting point

The melting point range of the tallow was quite high (46.5-49.5 °C) and there were small decreases in the melting points of the samples due to blending the tallow with different oils. However, after the interesterfication sharp decreases in the melting points of the structured lipids were observed regardless of the oil type. These changes in the melting points were in accordance with the results of previous studies (Meng *et al.,* 2011; Morselli Riberio *et al.,* 2017). All samples containing 60% tallow presented lower melting points regardless of the oil type. When the tallow ratio was raised from 60 to 80%, a clear increase was also observed in the melting points. 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 the chain length of the fatty acids (Martin *et al.,* 2010). The interesterified fats were more likely to have β and β′ crystal types together and the melting points of the samples were relevant to the melting point of the crystal types. Therefore, it was clear that polymorphic structure of the interesterified lipids was highly related to the melting points of the structured lipids. The ANOVA results for melting point (Table 4) show that the model is significant at $p < 0.05$ with non-significant lack of fit. Blend ratio had the most prominent effect on the melting points of the chemically interesterified lipids. Melting points reached maximum values when the blend ratio of tallow was 80%. Although not to the extent of blend ratio, catalyst concentration had some significance on the melting points of the structured lipids and a slight decrease in melting points was observed with increasing catalyst concentration.

TABLE 3. Physical properties of the structured lipids produced

Parameters						Physical properties							
Sample	Oil type	Tallow-oil Blend ratio	Catalyst conc. (%)	Crystal morphology	Melting point (°C)	Consistency (MPa)				Solid fat content (%)			
						4 °C	10 °C	15 °C	25 °C	10 °C	15 °C	30 °C	35°C
SA61	Safflower	60-40	0.75	β+β'	30.3-39.5	nd	nd	nd	nd	20.7	10.7	4.7	2.1
SA62	Safflower	60-40	0.875	β	26.6-35.3	37.82	3.6	nd	nd	25.3	17.7	7.9	4
SA63	Safflower	60-40	1	β+β'	26.5-35.2	nd	nd	nd	nd	20.8	10.4	4.5	1.9
SA71	Safflower	70-30	0.75	β+β'	38-45.5	48.03	12.04	9.24	nd	27.2	18.7	9.5	5.9
SA72	Safflower	70-30	0.875	β+β'	31.6-38.1	nd	nd	nd	nd	27.1	15.3	7.1	3.8
SA73	Safflower	70-30	1	β+β'	32.0-38.8	411.08	78.05	55.16	nd	29.4	17.2	7.9	4.5
SA81	Safflower	80-20	0.75	β+β'	35.8-44.5	668.9	164.73	103.09	6.79	37.5	24.6	12	7.2
SA82	Safflower	80-20	0.875	β+β'	34.6-42.4	66.57	36.81	29.38	5.8	36.6	24	11.7	6.9
SA83	Safflower	80-20	1	β'	33.8-42.7	136.04	69.51	11.11	nd	34	21.5	9.8	5.7
SA60	Safflower	60-40	0	β+β'	44.9-47.6	116.14	nd	nd	nd	33.5	22.6	12.2	8.2
SA70	Safflower	70-30	0	β+β'	45-48.2	150.92	42.13	14.88	11.63	39.2	26.6	15	9.9
SA80	Safflower	80-20	0	β+β'	39-40.7	140.36	88.02	42.81	26.02	46.6	31.6	18.11	12
CO61	Corn	60-40	0.75	β+β'	26.9-34.6	44.97	12.61	9.3	2.34	20.4	10.7	4.4	1.6
CO62	Corn	60-40	0.875	β+β'	29.8-37.6	33.21	11.97	3.46	nd	22.3	11	4.8	2
CO63	Corn	60-40	1	β	28.8-35.5	19.93	4.22	nd	nd	20.6	9.8	4	1.6
CO71	Corn	70-30	0.75	β+β'	31.2-38.3	76.22	30.23	9.5	6.54	22	12	5.2	2.8
CO72	Corn	70-30	0.875	β+β'	29.8-36.8	76.14	35.27	6.34	2.08	20.8	10.4	4.5	1.9
CO73	Corn	70-30	1	β+β'	31.9-38.2	28.73	14.81	nd	nd	29.2	15.9	7.3	3.6
CO81	Corn	80-20	0.75	β	38.5-42.5	127.13	53.43	10.38	25.01	39.8	26.1	12.6	7.2
CO82	Corn	80-20	0.875	β+β'	35.7-43.2	302.19	339.4	105.96	5.16	38.9	26.1	12.4	7
CO83	Corn	80-20	1	β+β'	34.3-39.7	88.33	72.67	15.6	nd	35.6	23.3	11.4	6.4
CO60	Corn	60-40	0	β+β'	43.3-46.8	54.89	16.62	10.23	9.08	27.8	17.5	9.4	6.2
CO70	Corn	70-30	0	β+β'	44.4-48	97.62	70.13	26.40	9.60	40.1	26.6	15	10.1
CO80	Corn	80-20	0	β+β'	44.7-47.7	164.09	101.37	39.69	20.16	46.8	32.4	18.5	12.6
CP1	Corn	70-30	0.875	β+β'	32.7-38.9	59.44	19.44	5.5	7.03	29.3	17	7.6	4
CP2	Corn	70-30	0.875	β+β'	33.1-39.7	69.37	17.07	29.23	11.37	35.7	20.1	8.4	4.5
CP3	Corn	70-30	0.875	β+β'	37.2-44	52.51	28.23	9.1	8.49	27.7	20.2	10.7	6.5
CA61	Canola	60-40	0.75	β	26.5-34.0	55.94	11.81	3.23	1.52	22.9	11.1	4	1.6
CA62	Canola	60-40	0.875	β	18.2-34.3	17.46	42.18	nd	nd	18.2	8.7	3.1	0.9
CA63	Canola	60-40	1	β+β'	25.7-33.7	40.59	20.45	6.75	3.64	23.1	11.2	3.8	1.4
CA71	Canola	70-30	0.75	β	32.2-40.9	77.16	24.36	3.46	2.22	34.6	18.8	7.4	4
CA72	Canola	70-30	0.875	β'	32.1-39.7	64.13	21.78	4.43	4.23	36.5	19.5	7.6	3.9
CA73	Canola	70-30	1	β+β'	32.7-42	612.49	158.38	153.14	3.34	30.3	17.1	7.3	3.8
CA81	Canola	80-20	0.75	β'	39.1-45.3	68.42	42.75	12.73	16.12	38.1	26.7	14.2	9.3
CA82	Canola	80-20	0.875	β'	37.2-45.1	174.25	51.99	19.6	10.71	43.6	27.7	12.7	7.2
CA83	Canola	80-20	1	β'	33.8-40.1	121.97	71.5	47.15	nd	37.6	23.5	10.6	5.7
CA60	Canola	60-40	0	β+β'	41.1-46.5	61.49	35.31	13.45	10.74	33.9	22.4	12.5	8.4
CA70	Canola	70-30	0	β+β'	42.5-47	78.94	49.03	16.75	14.35	39.5	26.5	14.8	9.8
CA80	Canola	80-20	0	β+β'	44.1-47.9	159.64	91.23	32.69	22.93	47.1	31.7	18.2	12.2
T	Tallow			β+β'	46.6-49.6	385.93	224.52	87.57	69.85	51.1	42.7	24	17.3

Standard deviations Melting point: 2.18, Consistency at 4 °C:6.92, at 10 °C:4.8 at 15 °C:10.44, at 25 °C:1.80, Solid fat content at 10 °C:3.46, at 15 °C:1.49, at 30 °C:1.31, at 35 °C:1.08, nd: could not be determined. (standard deviation for each measurement is calculated from three replicates of central points in the experimental design)

3.2.3. Consistency

The consistency of all the samples decreased clearly as a function of temperature (Table 3). This result can be associated with the gradual melting of the crystals that generated more fragile crystalline networks. The same behavior was observed in previous studies (Bezerra *et al.,* 2017; Oliveria *et al.,* 2017; Silva *et al.,* 2009). The consistency of tallow (69.85-385.93 MPa) was quite higher than both the interesterified lipids and the non-interesterified blends at all temperatures (Table 3). The consistency of the blends in different proportions increased with increasing amounts of tallow in the blends. However, the interesterified lipids presented lower consistency values compared to their physical blends regardless of the oil type and the catalyst concentration. This decrease in the consistency of the interesterified lipids could be attributed to higher amounts of unsaturated fatty acid composition in all positions of (UUU) TAGs produced by interesterification. In addition, differences in polymorphic structure and aggregation behavior which led to the alteration in the structure of the fat crystal network of tallow could change the consistency (Silva *et al.,* 2009). Generally, fats with consistency 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 classified as very hard at above 147 MPa (Haighton, 1959). Most of the samples interesterified with canola oil could be considered as spreadable and plastic. The consistency values decreased to the levels suitable for spreadability with the increasing temperature. However, the consistency of the samples with canola oil of high blend ratio and high catalyst concentration (CA82 and CA83) were very high at 4 °C, and these lipids could be classified as hard. Moreover, these structured lipids contain β' polymorphic forms which have higher melting points. The consistency of most of the samples interesterified with safflower oil was not measurable at 25 °C; therefore, these structured lipids were viscous at ambient temperature. Moreover, the structured lipids with safflower oil had lower melting points which also explained the lower consistency values of these lipids. Interesterified samples produced with corn oil also resulted in products with mostly spreadable and plastic properties. The statistical analysis results for the consistency at all temperatures indicated that constructed models were insignificant at 4, 10 and 15 °C at $p < 0.05$ (Table 4). Although the models were found insignificant, the ANOVA table reveals that the blend ratio had an important impact on the consistency at these temperatures (Table 4). The model for consistency at 25 ºC was significant with non-significant lack of fit. The blend ratio and the catalyst concentration were the most prominent factors for this model and higher blend ratio meant higher consistency while the opposite effect was true for the catalyst concentration. In addition, oil type, especially safflower oil, highly affected the consistency of the structured lipids at 25 °C since safflower oil-containing interesterified lipids had lower consistency.

3.2.4. Solid fat content

The solid fat contents of both the interesterified lipids and the non-interesterified blends were determined over the temperature range of 10–35 °C (Table 3). As expected, it was observed that raising the temperature caused a marked decrease in solid fat content regardless of the oil type, the blend ratio or the catalyst concentration. The solid fat content profiles of non-interesterified blends in different proportions showed increasing trends with the increasing amounts of tallow in the blends. Interesterified lipids tended to have lower solid fat content values compared to their physical blends. Same trends were also observed in previous studies (Karabulut *et al.,* 2004; Meng *et al.,* 2010). A decrease in the solid fat content of the interesterified lipids could be attributed to the decreased proportion of the high-melting TAGs and médium-chain TAGs in the structured lipids. This decrease in solid fat content with respect to the increase in temperature was expected and has been reported in other studies (Bezerra *et al.,* 2017; Fauzi *et al.,* 2013; Oliveria *et al.,* 2017). In addition, the decrease in solid fat content in tallow and non-esterified blends could be associated with the alteration in the TAG structure caused by the interesesterfication and the melting temperature of crystals. However, a more plastic behavior was observed for both blends and structured lipids above 20 °C. The ANOVA table revealed that the blend ratio is the only effective factor for solid fat content in the temperature range of 10-35 °C. The solid fat content in the samples increased regardless of the oil type when the amount of tallow increased for all temperatures (Table 4).

TABLE 4. ANOVA table of physical parameters for chemically interesterified lipids

	Melting Point	Consistency			Solid Fat Content				
		4 °C	10 °C	15 °C	25 °C	10 °C	20 °C	30 °C	35 °C
p value-model	0.00	0.40	0.272	0.354	0.00	0.00	0.00	0.00	0.00
p-value-lack of fit	0.91	0.00	0.002	0.033	0.759	0.98	0.98	0.99	1.00
R^2	0.80	0.33	0.38	0.35	0.78	0.83	0.85	0.86	0.86
R^2 adj	0.71	0.03	0.10	0.06	0.68	0.75	0.79	0.80	0.80
Q^2	0.58	-0.81	-0.41	-0.64	0.47	0.68	0.73	0.75	0.75
p value-factors									
BR[a]	***0.00***	***0.04***	***0.01***	***0.04***	***0.00***	***0.00***	***0.00***	***0.00***	***0.00***
CC[b]	***0.04***	0.68	0.62	0.41	0.01	0.87	0.42	0.24	0.11
OT[c]									
corn	0.91	0.31	0.74	0.53	***0.04***	0.24	0.28	0.55	0.37
canola	0.35	0.76	0.92	0.59	0.40	***0.05***	0.41	0.73	0.66
safflower	0.41	0.52	0.68	0.96	***0.01***	0.36	0.84	0.37	0.21
p-value-interactions									
BR*CC	0.31	0.41	0.83	0.71	***0.01***	0.50	0.41	0.23	0.13
BR*OT(corn)	0.32	0.81	0.20	0.91	0.29	0.80	0.56	0.70	0.91
BR*OT(canola)	***0.04***	0.45	0.19	0.57	0.88	0.42	0.28	0.13	0.13
BR*OT(safflower)	0.28	0.32	0.99	0.65	0.23	0.30	0.10	0.06	0.11
CC*OT(corn)	0.27	0.53	0.70	0.44	***0.02***	0.58	0.55	0.32	0.25
CC*OT(canola)	0.71	0.16	0.34	0.06	0.45	0.62	0.77	0.63	0.42
CC*OT(safflower)	0.15	0.42	0.57	0.23	0.10	0.96	0.76	0.60	0.72

[a] tallow-oil blend ratio; [b] catalyst concentration; [c] oil type

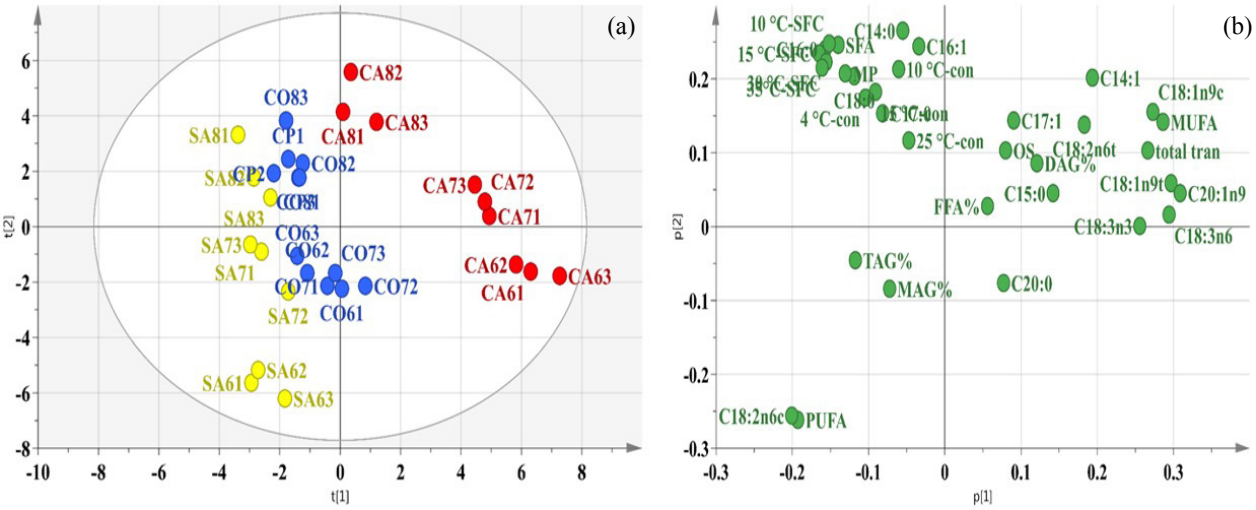

FIGURE 3. (a) Score plot (coloring is done with respect to oil types. Yellow: safflower, dark blue: corn, red: canola oils) and (b) loading plot obtained from principal component analysis for all data

3.2.5. Combination of physical and other parameters

The principal component analysis was also used to investigate the data pertaining to the physical properties of the interesterified lipids. The model was constructed by using all the measured physical parameters with 2 principal components, R^2=0.85, and Q^2=0.69. There is some discrimination among the samples with respect to the blend ratio (Figure 2c). Samples containing 60% tallow were located at the left part of the quartile while the structured lipids with 80% were placed to the right of the quartile. Samples with 70% tallow generally placed between them. Therefore, there was a rough discrimination among the samples depending

on their blend ratio as far as the physical properties are concerned. Discrimination between 60% and 80% tallow-containing samples mostly resulted from the higher solid fat contents and melting points of samples as observed in the loading plot (Figure 2d). Moreover, some of the samples with 80% and 70% tallow were grouped at the bottom of the ellipse regardless of the oil type since they had higher consistency (Figure 2d). The PCA score plot of the physical properties data did not show any separation with respect to the oil type or the catalyst concentration.

In order to better characterize chemically interesterified lipids, a PCA model was also constructed using all data including both chemical and physical properties of the 5 PCs, R^2=0.80, and Q^2=0.45. According to the score plot, there was a clear separation of the samples with respect to oil type (Figure 3a). Samples containing canola oil were located at the right part of ellipse while the interesterified fats with safflower oil were placed at the left part of ellipse. Samples containing corn oil were placed between them. Discrimination of canola oil-containing samples mostly resulted from the higher *trans* fatty acids and MUFA contents and oxidative stability of the samples as observed in the loading plot (Figure 3b). Moreover, canola oil samples were grouped together in between them with respect to their blend ratios since the amount of saturated and unsaturated fatty acids changed by increasing the ratio of tallow (Figure 3b). The interesterified lipids containing safflower oil were separated from other lipids due to their higher PUFA and MAG contents. Corn oil samples were differentiated from the rest since they had higher consistency values as the loading plot confirmed. Groupings in between the interesterified samples according to blend ratio were also observed. The results of the PCA models were in accordance with ANOVA results. Generally, the catalyst concentration did not have a remarkable effect on the chemical and physical properties of the structured lipids while blend ratio and oil type were the significant factors. Among the interesterified fats, samples produced with corn oil were discriminated from the others due to their more desirable physical and chemical properties.

4. CONCLUSIONS

Chemical interesterification served to manufacture new products from tallow in combination with vegetable oils with better spreadable and plastic behaviors. In general, the tallow-oil blend ratio was the most significant factor that affected the end product. The 1% catalyst concentration used in the process had a negative effect on the chemical properties of the structured lipids. As a first study in the literature that uses tallow and corn oil blends in chemical interesterification process, it can be concluded that interesterified lipids produced from corn oil had more desirable properties (higher oxidative stability, lower FFA%, more plastic properties) compared to products containing canola and safflower oils. Consequently, structured tallow and corn oil combinations (60-80% tallow) produced using CI with a catalyst at 0.75-0.875% levels can be particularly suggested as alternative lipid sources for the baking industry due to their possible promising aeration properties and smooth texture.

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