Effects of processing parameters on chemical and physical properties of enzymatically interesterified beef tallow-corn oil blends

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Running title: Enzymatic interesterification of tallow-corn oil

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Abstract

Purpose of this study is to manufacture structured lipids by enzymatic interesterification of beef tallow with corn oil and to investigate effects of process parameters on chemical and physical properties of products. Full factorial design was constructed by considering blend ratio and reaction time as process parameters. Enzymatic interesterification was catalyzed with sn-1,3 specific lipase. Structured lipids have higher free fatty acid content and lower oxidative stability compared to initial blends. Interesterification did not cause trans fatty acid formation and interesterified lipids mostly contained β crystals. Solid fat content and slip melting point decreased up to 6 h of interesterification; however, longer reaction times have negative effects on these parameters. Statistical analyses results confirmed that reaction time is highly important for enzymatic interesterification.

Practical applications: Some of interesterified lipids can be utilized as alternatives to margarines or butterfat due to their lower trans fatty acid content and crystal morphology.

Keywords: tallow, corn oil, enzymatic interesterification, sn-1,3 specific lipase

1 Introduction

Beef tallow is considered as a by-product of meat industry. It has relatively a high melting point range (40-60 °C); therefore, it is classified as a hard fat. Beef tallow contains low levels of polyunsaturated fatty acids (PUFA), which limits its use in food industry as well as in direct human consumption (Kowalski et al., 2004). Thus, tallow needs to be modified in order to obtain a product with desirable properties. One of the possible methods for tallow modification is interesterification with vegetable oils (Kowalski et al., 2005). Both chemical and enzymatic interesterification reactions are able to alter the physical and chemical properties of fats. Chemical interesterification provides new properties to the modified lipids by the random incorporation or the restructuring of acyl residues of triacylglycerols (TAG) by the help of a chemical catalyst. However, enzymatic interesterification (EI) leads to the attachment of specific fatty acids to specific positions of TAG structure to produce novel products by lipase enzyme (Martin et al., 2010). 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 is produced. Then, acyl migration takes place due to prolonged reaction periods that cause the formation of 1,3-diacylglycerols and this reaction also allows some randomization of the fatty acids existing at the sn-2 position of the TAGs (Xu, 2000; Rajendran, 2009).

There are several studies using this process in the modification of tallow. In a previous study, enzymatic interesterification of tallow with sunflower and soybean oils did not cause formation of trans fatty acids (Foglia et al., 1993). The enzymatic interesterification of beef tallow and rapeseed oil resulted in lower thermal resistant properties with respect to the non-esterified tallow (Kowalska et al., 2015). 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). Lipids produced by enzymatic interesterification of beef tallow with soybean and palm oils were classified as low trans-fat margarines due to their desirable physico-chemical properties and polymorphs (Li et al., 2018). It was not encountered any study in the literature regarding the production of structured lipids using enzymatic interesterification of beef tallow with corn oil.

The aim of this research is to modify beef tallow by enzymatic interesterification with corn oil and to determine the effects of process parameters (i.e., blend ratio and reaction time) on various chemical (free fatty acids, fatty acid profile, and oxidative stability) and physical (slip melting point, solid fat content, and polymorphic behavior) properties of the structured lipids.

2 Materials and methods

2.1 Fat samples and reagents

Two different breeds of 2-years old calves (Montafon and Holstein) were the sources of beef tallow used in interesterification reactions and it was obtained immediately after slaughter and stored at -20ºC. Corn oil was obtained from a local market. Lipase enzyme from *Thermomyces lanuginosus* (Lipozyme TL IM) was obtained from Sigma–Aldrich (St. Louis, MO). All other reagents and solvents are of analytical or chromatographic grade and were obtained from Sigma (Sigma-Aldrich, Germany).

2.2 Enzymatic interesterification process

A full factorial experimental design was employed to evaluate the effects of reaction time (0, 3, 6, 9, and 12 h) and tallow to corn oil blend ratio (60:40, 70:30 and 80:20) on chemical and physical properties of structured lipids (Table 1-2), as a result 18 different blends

including 3 central points were prepared. The liquefied tallow was mixed with corn oil at given ratios. 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 with stirring at 120 rpm (Sartorious, Certomat B5-1, Germany). Reaction was stopped by denaturation of lipase enzyme by keeping the samples in a shaking water bath at 80 °C for 30 min. The denatured enzyme was removed by vacuum filtration.

2.3 Chemical property analyses

2.3.1 Free fatty acid content

Titrimetric method specified in AOCS standard official method Ca 5a-40 was used in free fatty acid (FFA) determination of the products (AOCS, 1989). The analyses were performed twice. Acidity was expressed as percentage of oleic acid.

2.3.2 Mono, di and triacylglycerol content determination

Mono- (MAG), di- (DAG) and triacylglycerol (TAG) contents of structured lipids were analyzed according to AOCS Cd11C-93 method by column chromatography (AOCS, 2002).

2.3.3 Oxidative stability

Rancimat apparatus was used in measurement of the oxidation induction time (873 Biodiesel, Metrohm, Switzerland). Sample was placed inside the glass reaction vessel for the measurement. Carrier medium was deionized water and 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) (Uncu & Ozen, 2016).

2.3.4 Fatty acid composition

Fatty acid composition of the samples was determined after converting them into the corresponding fatty acid methyl esters (FAME). Chromatographic analyses were performed with a GC (Agilent 6890) equipped with an auto-sampler, a split/splitless (1:50) injector and a FID detector. An HP 88 capillary column (100 m x 0.25 mm ID x 0.2 μm) was used in the analyses. Conditions for GC analysis was described in a previous paper (Meng et al., 2010). Supelco 37 Component Mix was used as standard (Sigma-Aldrich, Germany).

2.4 Physical property analyses

2.4.1 Determination of slip melting points

Slip melting point (SMP) was determined according to AOCS Official method Cc 3-25 (AOCS, 1989). The analyses were replicated twice.

2.4.2 Determination of solid fat content

Solid fat content (SFC) was determined by a nuclear magnetic resonance (NMR) spectrometer (Bruker, USA) according to AOCS Official Method Cd 16b-93 (AOCS, 2017). Samples were melted at 80 ºC and recrystallized at 0 ºC for 30 min. Then, they were stabilized for 30 min at various temperatures (10, 20, 30 and 35 ºC) before measuring the liquid signal.

2.4.3 Crystal morphology

The polymorphic structures of 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 at ambient temperature. The analyses were replicated twice.

2.5 Statistical analyses

The analytical data were analyzed by the analysis of variance (ANOVA) in order to investigate the effect of blend ratio, reaction time and their interaction on chemical and physical properties of the structured lipids (MODDE 11 software, MKS Umetrics, Umea, Sweeden). To investigate the effects of processing parameters on interesterification process the principal component analysis (PCA) was also used (SIMCA 14.1, MKS Umetrics, Umea, Sweeden). Constructed models were defined in terms of number of principal components (PC), R^2 and Q^2 (a measure showing predictive ability of the model).

3 RESULTS AND DISCUSSION

3.1 Chemical properties

The fatty acid composition of the interesterified samples during the process is given in Table 1. The major fatty acids in all interesterified lipids are oleic, palmitic, stearic, and linoleic acids. In general, EI did not cause sharp changes in the amounts of fatty acids of the structured lipids during reaction period and similar result was also observed in previous studies (Rønne et al., 2005; Svensson & Adlercreutz 2008; Silva et al., 2009). As in the former researches, enzymatic interesterification of tallow with corn oil did not cause formation of trans-fatty acids (TFA) (Forssell et al., 1992; Foglia et al., 1993). Tallow-corn oil blends had 0.6-0.7% trans-fat content before interesterification. The range of trans fatty acids for lipids during interesterification varied from 0.1 to 1.3%. Only three out of 15 interesterified products had 1% or higher concentrations of trans-fatty acids. These results indicate that produced structured lipids are mostly suitable for the manufacturing of low-trans containing shortenings, margarines and frying fats. Saturated fatty acid content (SFA) of tallow (57.8%)

153 decreased with EI with corn oil. Moreover, there were small fluctuations in monounsaturated
154 fatty acid (MUFA) percentages of enzymatically interesterified lipids during the process.
155 These changes can be associated with the activity of the sn-1,3 lipase enzyme. In general,
156 tallow has SFAs located in sn-2 position (Forssell et al., 1992). Therefore, while SFAs were
157 kept at sn-2 position, the MUFA and PUFA were presumably released from their positions
158 throughout the EI, thus causing increases in MUFA or PUFA of the samples. The Food and
159 Agricultural Organization/World Health Organization (FAO/WHO) and the European Union
160 Committee advise that the minimum PUFA/SFA ratio should be 1 for controlling the
161 saturated fat consumption and encouraging the intake of MUFA and PUFA. While the
162 PUFA/SFA ratio of the enzymatically interesterified lipids ranged between 0.3-0.9, tallow
163 had a ratio of 0.1. This result indicated that PUFA content of tallow was increased by both
164 blend ratio and EI reaction. ANOVA results (Table 3) indicated that while the models
165 constructed for PUFA% and SFA% were significant with significant lack of fit at 95%
166 confidence interval, the models for MUFA% and TFA% were found not significant. Reaction
167 time did not have any prominent effect on the fatty acid contents of the samples. The ANOVA
168 table reveals that blend ratio has important effect on SFA and PUFA content of the structured
169 lipids (Table 3). While the increase in blend ratio of tallow led to a decrease in PUFA content
170 of the interesterified fats, SFA content of the enzymatically interesterified lipids increased as
171 expected.

172 Enzymatically interesterified samples had lower oxidative stabilities (0.6-3.9 h) in
173 comparison to initial non-esterified blends. Oxidation induction times of lipids decreased
174 regardless of blend ratio especially after 6 h reaction time (Table 2). In previous studies, the
175 decrease in oxidative stability of interesterified fats compared to the initial mixture was also
176 generally observed (Martin et al., 2010; Bryś et al., 2014; Kowalska et al., 2014). The

177 methods that are used in the production or purification of structured lipids, the oil sources,
178 and the presence of antioxidants during the manufacturing are among the main factors that
179 affect the oxidative stability of structured lipids. Moreover, the structure of the TAGs,
180 including fatty acid composition and positional distribution on the glycerol backbone as well
181 as the interaction of these factors, has important impact on the oxidative stability of the
182 structured products. In the present study, since the enzyme used in interesterification reaction
183 (Lipozyme TL IM) has regiospecifity on sn-1,3 bonds of TAG molecules, reaction products
184 would not be glycerol but sn-2 MAGs. Since, tallow has SFAs located in sn-2 position,
185 MUFAs and PUFAs were presumably the ones that are released from their positions
186 throughout the enzymatic interesterification, causing decrease in oxidation induction time of
187 structured lipids. This decrease is more remarkable between 3-6 h of reaction. However, after
188 6 h of reaction, there were some increases in oxidation induction time of the samples, which
189 can be associated with the rearrangement of PUFAs in DAG and TAG molecules. It can be
190 suggested that 3 h reaction time could be more suitable for EI of tallow if sole consideration
191 would be the oxidative stability of interesterified products. ANOVA results for oxidative
192 stability indicated that the constructed model was significant with significant lack of fit;
193 however, it still reveals the significance of reaction time on the oxidative stabilities of the
194 samples and causes a decrease in oxidation induction time (Table 3).

195 Free fatty acid percentages (FFA%) of the samples are listed in Table 2. FFA% of
196 tallow was 1.2% while blends without interesterification have a FFA range of 0.6-0.8%.
197 Generally, FFA% of interesterified lipids (5.7-24.4%) increased sharply compared to starting
198 blends. This indicates that neutralization should be applied to samples after enzymatic
199 interesterification. There was a drastic increase in FFA% up to 6 h regardless of blend ratio;
200 however, fluctuations for this value was observed after 6 h depending on the blend ratio. The

fluctuations can be associated with the activity of the enzyme. Throughout interesterification reactions, enzyme acts on fatty acids of the TAG molecules and leads to formation of DAG and MAG molecules. Therefore, increases and fluctuations in FFA% could be observed during reaction time. Increasing trend was also observed in previous studies (Rønne et al., 2005; Kowalska et al. 2014). According to the ANOVA table, the model constructed for FFA% is not significant, even if reaction time resulted to have an important effect on FFA content (Table 3).

MAG, DAG and TAG contents of the structured lipids were determined to examine changes in the glycerol backbone that occurred by the action of Lipozyme TL IM during interesterification. MAG, DAG and TAG contents are expressed in relative percentages of the overall content (Table 2). The results are in accordance with previous studies that observed a decrease in TAG% after interesterification (Ledóchowska & Wilczyńska, 1998; Kowalska et al., 2005; Kowalska et al., 2014). Generally, TAG% of enzymatically interesterified lipids were lower than their starting blends. There was a drastic decrease in TAG% up to 6 h of EI process. After that point, fluctuations in TAG% were observed with respect to the blend ratio. Although DAG content of the samples increased up to 9 h reaction time, later on DAG% decreased (Table 2). The same trend was also observed for MAG content of the samples up to 6 h reaction time and MAG% decreased after that point (Table 2). These changes in TAG, DAG, and MAG contents of the interesterified lipids could be explained again with the activity of Lipozyme TL IM. The decrease in TAG content and the increase of DAGs and MAGs up to 6 h of reaction confirms that the enzyme works effectively, attacking the fatty acids located at sn1,3 positions of TAGs and providing the formation of MAGs and DAGs. With the increase in reaction time, decrease in DAG and MAG contents revealed that the fatty acids are snatched from their positions by the enzyme

225 and they participate in the production of new TAG molecules. Moreover, the fluctuations in
226 TAGs after 6 h of reaction supports this explanation.

227 For a better evaluation of EI reaction, a correlation between FFA% and DAG+MAG
228 contents of interesterified lipids was also evaluated (Fig. 1). The Pearson correlation
229 coefficient r was 0.90, thus indicating a linearly increasing trend between FFA content and
230 DAG+MAG% of the samples during EI reaction. Increase in both FFA% and DAG+MAG%
231 is also an indication of the activity of the enzyme. The enzyme released the fatty acids from
232 their specific positions and caused an increase in both FFA% and DAG+MAG% (Fig. 1). A
233 similar trend was also observed in another study (Kowalski et al., 2004) and the increase in
234 FFA and MAG+DAG contents was correlated with the reaction temperature and time, and
235 the enzyme concentration. Moreover, it was commented that the decrease in TAG% was
236 inversely proportional with the same factors. ANOVA results indicated that while the models
237 constructed for TAG% and MAG% were significant, the model for DAG% was not
238 significant. The ANOVA table reveals that only reaction time was a significant factor for the
239 models (Table 3).

240 **3.2 Physical properties**

241 Slip melting point, solid fat content and crystal morphology of initial blends and structured
242 lipids with various tallow to corn oil blend ratios and EI reaction times were determined.
243 Results are presented in Table 2. EI caused a decline in SMPs of structured lipids compared
244 to initial blends and tallow (Table 2). The same decline has also been observed in previous
245 researches (Bhattacharyya et al., 2000; Kowalska et al., 2014; Kowalska et al., 2015). While
246 SMP of tallow was 47.0 °C, SMP range of enzymatically interesterified samples was 33.1-
247 45.9 °C and for the non-esterified blends this range was 43.2-45.9 °C. 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 the first 6 h of interesterification, a decrease in TAG content and an increase in DAG+MAG content were observed along with a rise in SMP of the samples. The correlation between SMPs and TAGs ($r = 0.87$) was satisfactory. As TAG content of the interesterified samples decreased, SMP of the samples decreased too (Fig. 2). Therefore, SMP of the samples could be associated with TAGs that were restructured during enzymatic interesterification reactions. ANOVA results indicated a significant ($p<0.05$) model for SMP, with non-significant lack of fit and a significant effect of reaction time (Table 3).

Solid fat content (SFC) is a measure of the percentage of fat in crystalline (solid) phase to total fat across a temperature gradient and is an important parameter to decide on the appropriateness of lipids for a possible application. SFC data of both interesterified lipids and non-interesterified blends over the temperature range of 10–35 °C are listed in Table 2. As expected, raising temperature caused a marked decrease in the SFC values regardless of the reaction parameters. SFC profiles of non-interesterified blends had an increasing trend with the increasing amounts of tallow in the blends. Interesterified lipids tended to have lower SFC values compared to their physical blends. Similar trends were also observed in 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 and medium chain TAGs in the structure of lipids. In addition, lower SFC of structured lipids compared to both tallow and non-interesterified blends can be associated with the alteration of TAG structure and the melting temperature of different crystals. SFC of structured lipids slightly decreased throughout the EI process. However, there was a sharp increase in SFC of structured lipids at all temperatures after 12 h of reaction time. ANOVA

results indicated that only the constructed model for SFC at 35 °C was significant even if with a significant lack of fit. Although model is not that good it still indicates that reaction time and the interaction of time x blend ratio were the significant factors meaning that time highly affects the SFC of structured lipids at 35 °C (Table 3).

The polymorphic forms of the structured lipids and blends are provided in Table 2. The same crystal types were also observed in previous studies (Li et al., 2018; Meng et al, 2011; 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 EI only β form existed in most of the samples. However, long reaction times resulted in different polymorphs: sample having 60:40 blend ratio after 12 h reaction time had β+β' forms, while 70:30 and 80:20 blend ratios with the same reaction time had β+ α and α polymorphs, respectively. The β' form of the crystals had a high melting point, between 17 and 69 °C, whereas the melting point of the β form was 32-78 °C depending on the chain length of the fatty acids (Akoh, 2017). Generally, the β and β′ crystal types were formed throughout the reaction and the SMPs of the samples were linked to the melting points of these crystal types.

3.3 Principal Component Analysis

Principal component analysis (PCA) was applied to whole data including all measured physical and chemical properties. The model was constructed with 6 PCs, $R^2 = 0.96$, and $Q^2 = 0.62$. While first PC explains 44% of variation the second PC accounted for 24% of variation. According to the score plot, there is a rough separation of the samples according to the reaction time (Fig. 3a). While the non-esterified samples located at the left upper part

of ellipse, samples produced in 3, 6, and 9 h of reaction time are placed right bottom of the center and 12 h samples are towards non-esterified blends. 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. Therefore, reaction times longer than 6 h is not necessary for this application. 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 since they have higher MUFA, FFA, MAG and DAG contents (Fig. 3b). On the other hand, TAG content, OS, SMP and SFC of initial blends are higher compared to interesterified samples with reaction times of 3, 6 and 9 h. The sample containing 70% tallow and interesterified for 12 h (E712) is separately placed on the right side of ellipse in the score plot due to its low trans-fat content (Fig. 3a). The multivariate analysis of the whole data also confirmed that reaction time is an important parameter for the enzymatic interesterification reaction.

CONCLUSION

Tallow and corn oil were 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 h of reaction, there were some increases in oxidation induction time of the samples that can be associated with the rearrangement of PUFAs in DAG and TAG backbone. Generally, FFA content of the enzymatically interesterified lipids increased significantly compared to starting blends. This means that neutralization should be applied to the samples after the enzymatic interesterification. The enzymatic interesterification of tallow with corn oil did not cause formation of trans-fatty acids. In general, reaction time longer than 6 h had a trend changing effect on the several

physical properties. The univariate and multivariate analyses of the results confirmed that reaction time is highly important for the EI reaction. It was observed that 12 h reaction time caused negative effect on the chemical and physical properties of the structured lipids. The structured lipids manufactured by the enzymatic interesterification of tallow and corn oil could be used in bakery industry since these lipids have desired β and β' polymorphic forms and low trans-fatty acid contents. Moreover, these structured lipids can be utilized as alternative products instead of margarines or butterfat.

Conflict of interest

The authors declare that they have no conflict of interest.

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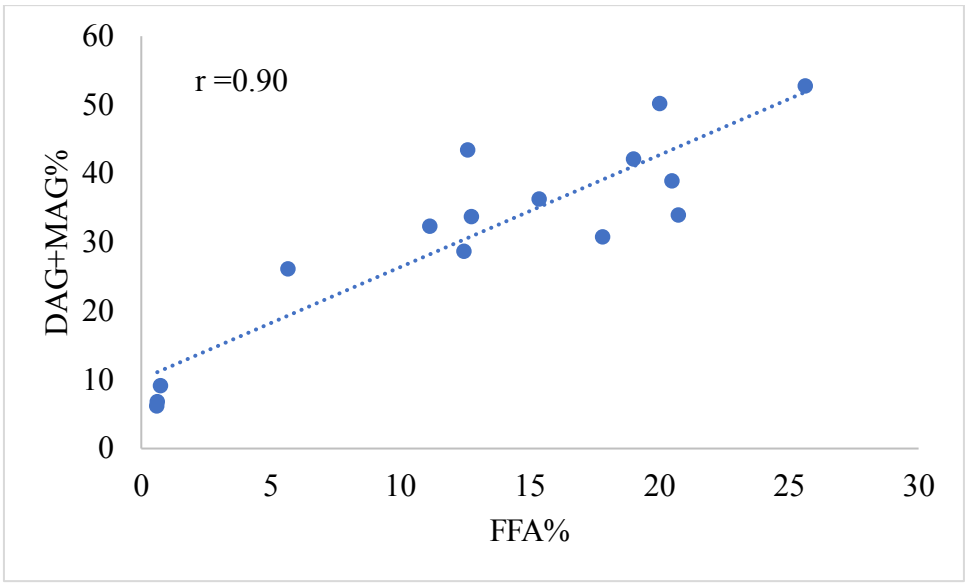
CAPTIONS TO FIGURES

Fig. 1. Correlation of free fatty acid content (FFA%) and mono and diacylglycerol content (DAG+MAG%) of the structured lipids obtained by enzymatic interesterification of tallow and corn oil

Fig. 2. Correlation of slip melting points (SMP) and triacylglycerol contents (TAG%) of the structured lipids obtained by enzymatic interesterification of tallow and corn oil

Fig. 3. a) Score and b) loading plots of the PCA model constructed by using chemical and physical parameters of enzymatically interesterified lipids obtained with tallow and corn oil (in Fig. 3a samples are colored with respect to the blend ratio; see Tables 1-2 for sample codes and abbreviations)



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436 Fig. 1

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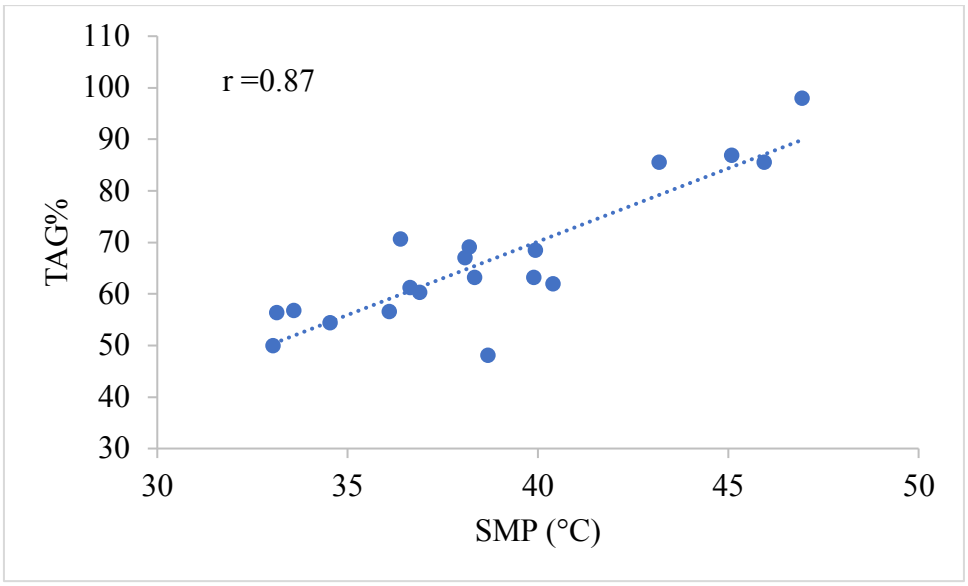

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441 Fig. 2

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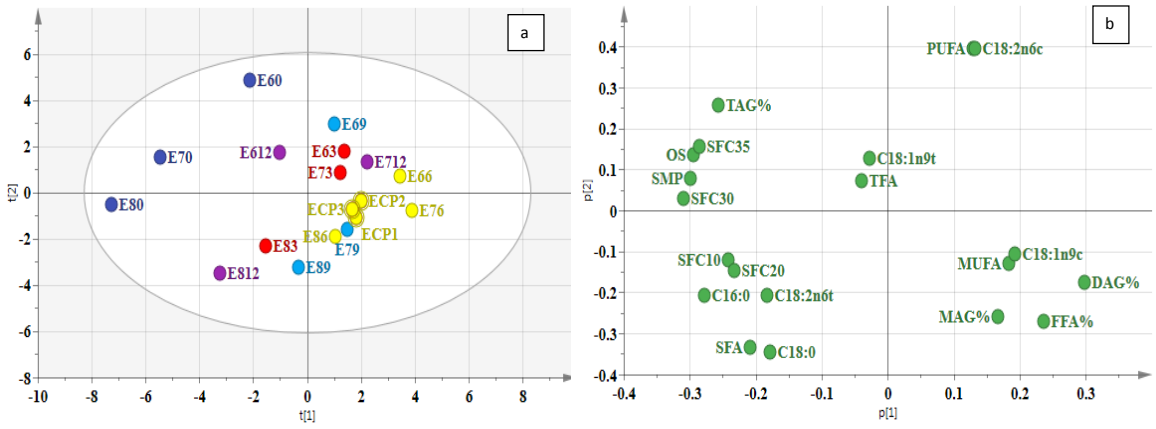

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445 Fig. 3

Table 1 Fatty acid profile of the blends and enzymatically interesterified lipids obtained with tallow and corn oil

			Fatty Acid Relative Concentration (%)*										
Tallow to corn oil ratio (%)	**Reaction Time (h)**	**Sample Code**	**MUFA**	**PUFA**	**SFA**	**TFA**	**C16:0**	**C18:0**	**C18:1n9c**	**C18:1n9t**	**C18:2n6t**	**C18:2n6c**	**PUFA/SFA**
60	**0**	**E60**	35.6	29.9	34.5	0.6	16.5	16.2	33.5	0.5	0.0	29.7	0.9
70	**0**	**E70**	37.1	19.9	43.0	0.7	18.7	22.0	34.7	0.6	0.1	19.5	0.5
80	**0**	**E80**	38.0	13.9	48.1	0.7	19.9	25.5	35.5	0.6	0.1	13.5	0.3
60	**3**	**E63**	38.9	25.4	35.8	0.7	16.3	17.3	36.3	0.7	0.1	25.1	0.7
70	**3**	**E73**	41.4	20.4	38.2	1.3	16.6	18.9	38.1	1.3	0.0	20.2	0.5
80	**3**	**E83**	36.8	13.4	49.8	0.2	18.5	28.2	34.9	0.1	0.1	12.9	0.3
60	**6**	**E66**	39.4	25.3	35.3	0.7	15.8	17.3	36.9	0.7	0.1	25.0	0.7
70	**6**	**E76**	42.4	21.0	36.7	1.1	16.3	18.3	39.4	1.1	0.0	21.0	0.6
80	**6**	**E86**	42.6	15.7	41.8	0.1	17.3	21.6	40.6	0.1	0.1	15.3	0.4
60	**9**	**E69**	38.7	26.4	34.9	1.0	16.4	16.4	36.1	1.0	0.0	26.4	0.8
70	**9**	**E79**	36.9	18.2	44.8	0.6	17.3	24.9	34.6	0.6	0.0	18.0	0.4
80	**9**	**E89**	40.5	14.1	45.4	0.8	18.1	24.4	37.4	0.7	0.1	13.7	0.3
60	**12**	**E612**	34.3	25.2	40.5	0.7	18.6	18.8	31.1	1.4	0.1	25.0	0.6
70	**12**	**E712**	37.7	28.9	33.3	0.0	16.2	14.8	37.7	0.0	0.0	28.9	0.9
80	**12**	**E812**	35.8	14.5	49.8	0.9	20.4	25.3	32.8	0.8	0.2	14.0	0.3
70	**6**	**ECP1****	39.9	20.3	39.8	0.1	16.9	20.3	37.7	0.0	0.1	20.1	0.5
70	**6**	**ECP2****	40.9	21.6	37.5	0.2	16.4	18.5	38.6	0.2	0.0	21.4	0.6
70	**6**	**ECP3****	40.7	20.3	39.0	0.2	16.7	19.8	38.3	0.1	0.0	20.1	0.5
		Tallow	38.9	3.3	57.8	0.5	22.5	32.1	30.6	0.5	0.1	3.0	0.1
		Corn oil	32.01	54.8	13.1	0.5	11.2	1.9	30.6	0.5	0.00	54.6	4.2

MUFA: monounsaturated fatty acid,; PUFA: polyunsaturated fatty acid; SFA: saturated fatty acid; TFA: trans fatty acid

* Standard deviation values for MUFA=±0.43%; PUFA=±0.61%; SFA=±0.97%; TFA=±0.04%; C16:0=±0.2%, C18:0=±0.76%, C18:1n9c=±0.34%, C18:1n9t=±0.05%, C18:2n6t=±0.01%, C18:2n6c=±0.61% (calculated from ECPs)

**ECP=central point

Table 2 Results of chemical and physical analyses of the blends and enzymatically interesterified lipids obtained with tallow and corn oil

Tallow to corn oil ratio (%)	Reaction Time (h)	Sample Code	OS* (h)	FFA* (%)	TAG* (%)	DAG* (%)	MAG* (%)	SFC* (10°C)	SFC* (20°C)	SFC* (30°C)	SFC* (35°C)	SMP* (°C)	Crystal Morphology
60	**0**	**E60**	6.7	0.6	85.5	5.9	0.3	27.8	17.5	9.4	6.2	43.2	β+β'
70	**0**	**E70**	8.5	0.6	86.8	2.7	4.1	40.1	26.6	15.0	10.1	45.1	β+β'
80	**0**	**E80**	10.0	0.8	85.5	0.6	8.5	46.8	32.4	18.5	12.6	46.0	β+β'
60	**3**	**E63**	3.1	12.8	67.0	21.7	11.9	22.1	12.1	5.6	2.1	38.1	β
70	**3**	**E73**	1.8	17.8	69.0	23.0	7.8	25.0	16.5	5.2	2.8	38.2	β
80	**3**	**E83**	3.4	11.2	61.8	19.4	12.8	20.2	16.7	8.2	3.9	40.4	β'
60	**6**	**E66**	0.6	19.0	54.4	26.1	15.9	20.6	11.2	0.2	0.1	34.6	β
70	**6**	**E76**	0.8	25.6	49.8	30.4	22.3	25.7	16.4	0.9	1.2	33.1	β
80	**6**	**E86**	1.9	15.4	63.2	24.8	11.5	29.5	17.9	4.6	0.7	38.4	β
60	**9**	**E69**	1.4	5.7	68.4	14.0	12.1	21.0	12.7	6.8	3.8	40.0	β
70	**9**	**E79**	2.1	20.0	56.5	31.4	18.7	24.3	15.2	2.6	0.6	36.1	β
80	**9**	**E89**	1.8	20.5	48.0	21.1	17.7	35.7	23.3	7.4	2.1	38.7	β
60	**12**	**E612**	2.8	12.5	70.6	15.6	13.1	25.9	17.0	8.9	5.2	36.4	β+β'
70	**12**	**E712**	1.8	12.6	56.3	26.1	17.3	33.2	25.7	10.0	0.4	33.2	α+β
80	**12**	**E812**	1.9	20.7	63.2	16.7	17.3	39.7	28.6	13.0	0.5	39.9	α
70	**6**	**ECP1****	0.9	21.9	56.7	24.3	11.0	28.1	19.2	5.2	0.7	33.6	β
70	**6**	**ECP2****	1.1	24.4	61.2	22.6	11.8	27.9	20.8	5.2	0.8	36.7	β
70	**6**	**ECP3****	1.3	23.2	60.3	22.7	9.2	29.3	19.0	4.4	0.4	36.9	β
		Tallow	4.8	1.2	97.9	0.5	0.9	51.1	42.7	24.0	17.3	47.0	β+β'
		Corn oil	4.9	0.1	92.1	2.1	0.4						

OS: oxidative stability; FFA: free fatty acid; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; SFC: solid fat content; SMP: slip melting point

* Standard deviation values for OS=±0.17; FFA=±1.01; TAG=±1.95; DAG=±0.79; MAG=±1.08, SFC10°C=±0.62, SFC20°C =±0.81, SFC30°C =±0.38, SFC35°C =±0.17, SMP=±1.5 (calculated from ECPs)

**ECP=central point

Table 3 ANOVA table showing the effect of process parameters on some chemical and physical properties of structured lipids produced by enzymatic interesterification of beef tallow with corn oil

	ANOVA Table													
	OS	**FFA**	**TAG**	**DAG**	**MAG**	**MUFA**	**PUFA**	**SFA**	**TFA**	**SFC (10°C)**	**SFC (20°C)**	**SFC (30°C)**	**SFC (35°C)**	**SMP**
p value-model	**0.02**	0.14	**0.05**	0.20	**0.01**	0.81	**0.00**	**0.00**	0.84	0.12	**0.06**	0.40	**0.02**	**0.05**
p-value-lack of fit	**0.00**	**0.01**	0.13	0.08	0.93	0.06	**0.02**	**0.06**	0.72	0.01	0.05	0.08	**0.00**	0.21
R^2	0.48	0.32	0.42	0.28	0.54	0.07	0.81	0.62	0.46	0.33	0.40	0.19	0.52	0.42
R_{adj}^2	0.37	0.17	0.30	0.12	0.44	-0.14	0.77	0.54	0.34	0.19	0.28	0.01	0.41	0.29
p value-factors														
blend ratio	0.53	0.46	0.45	0.98	0.27	0.41	**0.00**	**0.00**	0.54	**0.02**	**0.01**	0.18	0.78	0.29
reaction time	**0.00**	**0.04**	**0.01**	**0.04**	**0.00**	0.64	0.51	0.89	0.77	0.63	0.87	0.36	**0.01**	**0.01**
BR*RT	0.41	0.35	0.50	0.56	0.86	0.87	0.38	0.46	0.63	0.84	0.98	0.58	**0.05**	0.89

OS: Oxidative stability; FFA: free fatty acidity; TAG: triacylglycerol; DAG: diacylglycerol; MAG: monoacylglycerol; MUFA: monounsaturated fatty acids; PUFA: polyunsaturated fatty acids; SFA: saturated fatty acids; TFA: trans-fatty acids; SFC: solid fat content; SMP: slip melting point