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

Ayse Burcu Aktas[1] | Banu Ozen[2] | Cristina Alamprese[3]

[1]Food Engineering Department, Cumhuriyet University, Sivas, Turkey

[2]Food Engineering Department, Izmir Institute of Technology, Urla-Izmir, Turkey

[3]Department of Food, Environmental and Nutritional Sciences (DeFENS), Università degli Studi di Milano, Milan, Italy

Correspondence
Banu Ozen, Food Engineering Department, Izmir Institute of Technology, Urla-Izmir, Turkey.
Email: banuozen@iyte.edu.tr

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Abstract

The purpose of this study was to improve some physical and chemical characteristics of tallow through enzymatic interesterification process with corn oil and to investigate effects of process parameters on chemical and physical properties of obtained products. Full factorial design was constructed using blend ratio and reaction time as process parameters. Enzymatic interesterification was catalyzed with sn-1,3-specific lipase. Interesterified lipids have higher free fatty acid content and lower oxidative stability compared to initial blends. Interesterification did not cause trans-fatty acid formation and products mostly contained β crystals. Solid fat content and slip melting point decreased up to 6 hr 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.

1 | INTRODUCTION

Beef tallow is considered as both a by-product of meat industry and an ingredient for meat products. 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, Tarnowska, Gruczynska, & Bekas, 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, Tarnowska, & Gruczynska, 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, Reglero, & Señoráns, 2010). In an interesterification reaction with 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, Palanisamy, & Thangavelu, 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, Petruso, & Feairheller, 1993). The enzymatic interesterification of beef tallow and rapeseed oil resulted in lower melting properties with respect to the nonesterified tallow (Kowalska, Gruczynska, & Kowalska, 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, Castro, Salinas, López, & Miranda, 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). In general, interesterified products have more desirable properties due to their lower melting temperature, wider range solid-to-fat ratio, and modified crystallization behavior and they could have different applications in areas such as bakery products (Rohm, Schäper, & Zahn, 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 having high polyunsaturated fatty acid content although it is a readily available and economical type of edible oil. Although plant oils used in interesterification processes are mostly rich in terms of unsaturated fatty acids their minor components might also affect the properties of manufactured fats.

The aim of this research was to modify chemical and physical characteristics of beef tallow by enzymatic interesterification with corn oil in order to widen its spectrum of usage in food industry. Another purpose was 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 obtained interesterified products.

2 | MATERIALS AND METHODS

2.1 | Fat samples and reagents

Two different breeds of 2-year-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 −20°C. 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 hr) and tallow-to-corn oil blend ratio (60:40, 70:30, and 80:20) on the chemical and physical properties of structured lipids (Tables 1–2), as a result 18 different blends including 3 central points were prepared. The tallow liquified in a microwave oven was mixed with corn oil at given ratios. The reaction was initiated by adding 10% (wt/wt) enzyme (Lipozyme TL IM) at 55°C (Rønne, Yang, Mu, Jacobsen, & Xu, 2005). Enzymatic interesterification was performed in a shaking incubator with stirring at 120 rpm (Sartorious, Certomat B5-1, Germany). The reaction was stopped through 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, 1989b). 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 the AOCS Cd11C-93 method using column chromatography (AOCS, 2002).

2.3.3 | Oxidative stability

Rancimat apparatus was used in the 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 (hr) (Uncu & Ozen, 2015).

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 × 0.25 mm ID × 0.2 μm) was used in the analyses. Conditions for GC analysis were described in a previous paper (Meng, Liu, Shan, Jin, & Wang, 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, 1989a). The analyses were replicated twice.

2.4.2 | Determination of solid fat content

Solid fat content (SFC) was determined using a nuclear magnetic resonance (NMR) spectrometer (Bruker, USA) according to AOCS Official Method Cd 16b-93 (AOCS, 1999). Samples were melted at

TABLE 1 Fatty acid profile of the blends, enzymatically interesterified lipids, 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

Abbreviations: 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.

***The first digit represents blend ratio and the further digit(s) represent(s) reaction time.

TABLE 2 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

Abbreviations: DAG, diacylglycerol; FFA, free fatty acidity; MAG, monoacylglycerol; MUFA, monounsaturated fatty acids; OS, oxidative stability; PUFA, polyunsaturated fatty acids; SFA, saturated fatty acids; SFC, solid fat content; SMP, slip melting point; TAG, triacylglycerol; TFA, trans-fatty acids.
Bold values indicate significant parameters at $p \leq 0.05$.

80°C and recrystallized at 0°C for 30 min. Then, they were stabilized for 30 min at various temperatures (10°C, 20°C, 30°C, and 35°C) before measuring the liquid signal.

2.4.3 | Crystal morphology

Polymorphic structures of structured lipids were determined using 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 at ambient temperature. The analyses were replicated twice.

2.5 | Statistical analyses

Analytical data were analyzed using analysis of variance (ANOVA) in order to investigate the effect of blend ratio, reaction time, and their interaction ($p < 0.05$) on the chemical and physical properties of the structured lipids (MODDE 11 software, MKS Umetrics, Umea, Sweden). To investigate the effects of processing parameters on the interesterification process, the principal components analysis (PCA) was also used (SIMCA 14.1, MKS Umetrics, Umea, Sweden). 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 compositions of the interesterified samples, tallow, and corn oil during the process are 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 dramatic changes in the amounts of fatty acids of the structured lipids during the reaction period and similar results were also observed in previous studies (Pang, Ge, Cao, Cheng, & Jiang, 2019; Rønne, Yang, Mu, Jacobsen, & Xu, 2005; Segura & Jachmanián, 2020; Silva et al., 2009; Svensson & Adlercreutz, 2008;). 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). 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%) decreased through EI with corn oil. Moreover, there were small fluctuations in monounsaturated fatty acid (MUFA) percentages of enzymatically interesterified lipids during the process. These changes can be associated with the activity of the sn-1,3 lipase enzyme. In general, tallow has SFAs located in the sn-2 position (Forssell et al., 1992). Therefore, while SFAs were kept at the sn-2 position, the MUFA and PUFA were presumably released from their positions throughout the EI, thus causing increases in 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 PUFA/SFA ratio 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 ranged between 0.3 and 0.9, tallow had a ratio of 0.1. This result indicated that PUFA content of tallow was increased by both blend ratio and EI reaction. ANOVA results (Table 2) indicated that while the models constructed for

PUFA% and SFA% were significant with significant lack of fit at 95% confidence interval, the models for MUFA% and TFA% were found not significant. Reaction time did not have any prominent effect on the fatty acid contents of the samples. The ANOVA table reveals that blend ratio has an important effect on SFA and PUFA content of the structured lipids (Table 2). While the increase in blend ratio of tallow led to a decrease in PUFA content of the interesterified fats, SFA content of the enzymatically interesterified lipids increased as expected.

Enzymatically interesterified samples had lower oxidative stabilities (0.6–3.9 hr) in comparison to initial nonesterified blends. Oxidation induction times of lipids decreased regardless of blend ratio especially after 6 hr of reaction time (Table 3). In previous studies, the decrease in oxidative stability of interesterified fats compared to the initial mixture was also generally observed (Bryś, Wirkowska, Górska, Ostrowska-Ligęza, & Bryś, 2014; Kowalska, Żbikowska, & Kowalski, 2014; Li et al., 2018; Martin et al., 2010; Pang et al., 2019). The methods that are used in the production or purification of structured lipids, the oil sources, and the presence of antioxidants during the manufacturing are among the main factors that affect the oxidative stability of structured lipids. Moreover, the structure of the TAGs, including fatty acid composition and positional distribution on the glycerol backbone as well as the interaction of these factors, has an important impact on the oxidative stability of the structured products. In the present study, since the enzyme used in the interesterification reaction (Lipozyme TL IM) has regiospecificity on sn-1,3 bonds of TAG molecules, reaction products would not be glycerol but sn-2 MAGs. Since tallow has SFAs located in the sn-2 position, MUFAs and PUFAs were presumably the ones that were released from their positions throughout the enzymatic interesterification, causing a decrease in the oxidation induction time of structured lipids. This decrease is more remarkable between 3 and 6 hr of reaction. However, after 6 hr of reaction, there were some increases in the oxidation induction time of the samples, which can be associated with the rearrangement of PUFAs in DAG and TAG molecules. It can be suggested that 3 hr of reaction time could be more suitable for EI of tallow if sole consideration would be the oxidative stability of interesterified products. ANOVA results for oxidative stability indicated that the constructed model was significant with significant lack of fit; however, it still reveals the significance of reaction time on the oxidative stabilities of the samples and causes a decrease in the oxidation induction time (Table 2).

Free fatty acid percentages (FFA%) of the samples are listed in Table 3. FFA% of tallow was 1.2% while blends without interesterification have a FFA range of 0.6%–0.8%. Generally, FFA% of interesterified lipids (5.7%–24.4%) increased sharply compared to starting blends. This indicates that neutralization should be applied to samples after enzymatic interesterification. There was a drastic increase in FFA% up to 6 hr regardless of blend ratio; however, fluctuations for this value was observed after 6 hr 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 the 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 (Kowalska et al., 2014; Li et al., 2018; Rønne et al., 2005). 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 2).

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 3). The results are in accordance with previous studies that observed a decrease in TAG% after interesterification (Kowalski, Tarnowska, & Gruczynska, 2005; Kowalska et al., 2014; Ledóchowska & Wilczyńska, 1998; Pang et al., 2019; Segura & Jachmanián, 2020). Generally, TAG% of enzymatically interesterified lipids were lower than their starting blends. There was a drastic decrease in TAG% up to 6 hr 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 hr of reaction time, later on DAG% decreased (Table 3). The same trend was also observed for the MAG content of the samples up to 6 hr of reaction time and MAG% decreased after that point (Table 3). 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 hr 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, a decrease in DAG and MAG contents revealed 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 TAGs after 6 hr of reaction support this explanation.

For a better evaluation of EI reaction, a correlation between FFA% and DAG + MAG contents of interesterified lipids was also evaluated (Figure 1). The Pearson correlation coefficient, r was 0.90, thus indicating a linearly increasing trend between FFA content and DAG + MAG% of the samples during EI reaction. An increase in both FFA% and DAG + MAG% is also an indication of the activity of the enzyme. The enzyme released the fatty acids from their specific positions and caused an increase in both FFA% and DAG + MAG% (Figure 1). A similar trend was also observed in another study (Kowalski et al., 2004) and the increase in FFA and MAG + DAG contents was correlated with the reaction temperature and time, and the enzyme concentration. Moreover, it was commented that the decrease in TAG% was inversely proportional with the same factors. ANOVA results indicated that while the models constructed for TAG% and MAG% were significant, the model for DAG% was not significant. The ANOVA table reveals that only reaction time was a significant factor for the models (Table 2).

TABLE 3 Results of chemical and physical analyses of the blends, enzymatically interesterified lipids, 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						

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

*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.

***The first digit represents blend ratio and the further digit(s) represent(s) reaction time.

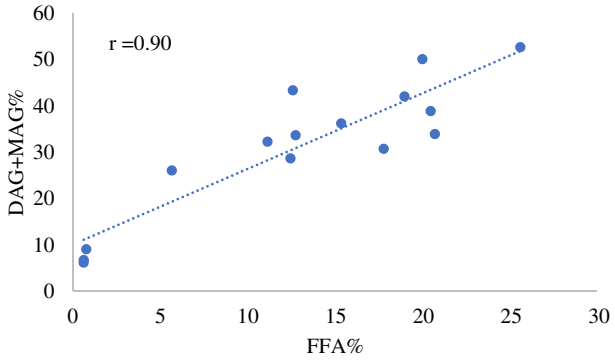

FIGURE 1 Correlation of free fatty acid content (FFA%) and mono- and diacylglycerol content (DAG + MAG%) of the structured lipids obtained by enzymatic interesterification of tallow and corn oil

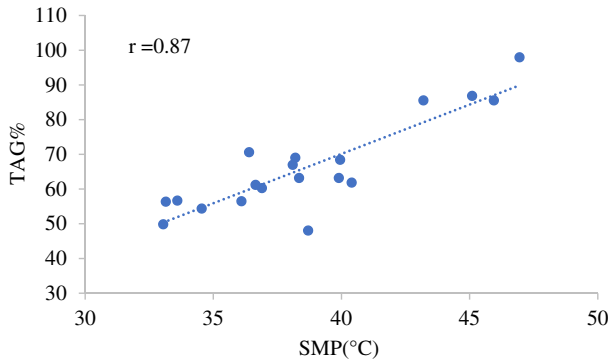

FIGURE 2 Correlation of slip melting points (SMP) and triacylglycerol contents (TAG%) of the structured lipids obtained by enzymatic interesterification of tallow and corn oil

3.2 | Physical properties

Slip melting point, solid fat content, and crystal morphology of initial blends and structured lipids with various tallow-to-corn oil blend ratios and EI reaction times were determined. Results are presented in Table 3. EI caused a decline in SMPs of structured lipids compared to initial blends and tallow (Table 3). The same decline has also been observed in previous researches (Bhattacharyya, Bhattacharyya, & De, 2000; Kowalska et al., 2014, 2015, 2019). While SMP of tallow was 47.0°C, SMP range of enzymatically interesterified samples was 33.1°C –45.9°C and for the nonesterified blends this range was 43.2°C–45.9°C. SMP of the enzymatically interesterified samples decreased up to 6 hr of reaction time regardless of blend ratio. After that point, there are some fluctuations in SMP of the samples. In the first 6 hr 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 (Figure 2). Therefore, SMP of the samples could be associated with TAGs that were restructured during enzymatic interesterification reactions. ANOVA results indicated a significant (p < .05) model for SMP, with nonsignificant lack of fit and a significant effect of reaction time (Table 2).

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 noninteresterified blends over the temperature range of 10°C–35°C are listed in Table 3. As expected, raising temperature caused a marked decrease in the SFC values regardless of the reaction parameters. SFC profiles of noninteresterified 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, Lai, Zhang, Søndergaard, & Xu, 2005; Jin, Zhang, Shan, Liu, & Wang, 2008; Kowalska et al., 2015; Li et al., 2018; Pang et al., 2019; Segura & Jachmanián, 2020). 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 noninteresterified 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 hr 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 × blend ratio were the significant factors meaning that time highly affects the SFC of structured lipids at 35°C (Table 2).

The polymorphic forms of the structured lipids and blends are provided in Table 3. The same crystal types were also observed in previous studies (Jin et al., 2008; Li et al., 2018; Meng et al., 2011; Zhang, Lee, Zhou, & Wang, 2019). 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 noninteresterified 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 hr of 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°C–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 components analysis

Principal components 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.

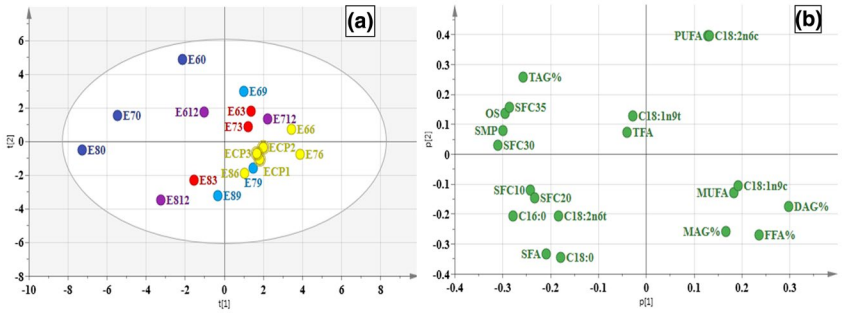

FIGURE 3 (a) Score and (b) loading plots of the PCA (6 PCs, R^2 = 0.96, Q^2 = 0.62) model constructed by using chemical and physical parameters of enzymatically interesterified lipids obtained with tallow and corn oil (in Figure 3a samples are colored with respect to the blend ratio; see Tables 1-2 for sample codes and abbreviations)

According to the score plot, there is a rough separation of the samples according to the reaction time (Figure 3a). While the nonesterified samples (E60, E70, and E80) were located at the left upper part of ellipse, samples produced in 3 (E63, E73, E83), 6 (E66, E76, E86), and 9 hr (E69, E79, E89) of reaction time are placed right at the bottom of the center and 12 hr samples (E612, E712, E812) are towards nonesterified blends. It seems that there is a reverse trend in the properties of structured lipids at 12 hr of reaction time and these samples are getting closer to nonesterified blends instead of moving farther apart. Therefore, reaction times longer than 6 hr are not necessary for this application. As the loading plot shows, the structured lipids produced in 3, 6, and 9 hr of reaction time are separated from nonesterified and 12 hr samples since they have higher MUFA, FFA, MAG, and DAG contents (Figure 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 hr. The sample containing 70% tallow and interesterified for 12 hr (E712) is separately placed on the right side of ellipse in the score plot due to its low trans-fat content (Figure 3a). The multivariate analysis of the whole data also confirmed that reaction time is an important parameter for the enzymatic interesterification reaction.

4 | CONCLUSION

Tallow and corn oil were used as substrates in the production of the enzymatically interesterified lipids. The enzymatic interesterification caused sharp decreases in the oxidation induction time of structured lipids. However, after 6 hr of reaction, there were some increases in the 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 times longer than 6 hr had a trend-changing effect on 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 hr of reaction time caused a 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 along with modified melting and SFC properties. These types of modifications in the physical and chemical properties of fats caused by the interesterification process result in increased elasticity, hardness, and density with the capability of incorporation of more air in dough structures (Rohm et al., 2018). Moreover, these structured lipids can be utilized as alternative products instead of margarines or butterfat.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

ORCID

Banu Ozen https://orcid.org/0000-0002-0428-320X

REFERENCES

Akoh, C. C. (2017). *Food lipids: Chemistry, nutrition, and biotechnology*, Boca Raton, FL: CRC Press.

AOCS. (1989a). AOCS method Cc 3-25. slip melting point, standard open tube melting point. In *Official methods and recommended practices of the AOCS* (5th Ed.). Champaign, IL: The American Oil Chemists' Society.

AOCS (1989b). AOCS method Ca 5a-40. Determination of free fatty acid content. In *Official methods and recommended practices of the AOCS* (4th ed.). Champaign, IL: The American Oil Chemists' Society.

AOCS (1999). AOCS method Cd 16b–93. Determination of solid fat content. In *Official methods and recommended practices of the aocs* (3rd ed.). Champaign, IL: The American Oil Chemists' Society.

AOCS. (2002). AOCS method Cd 11bc-93. Determination of mono-di-triacylglycerol content. In *Official methods and recommended practices of the AOCS* (3rd ed.). Champaign, IL: The American Oil Chemists' Society.

Bhattacharyya, S., Bhattacharyya, D. K., & De, B. K. (2000). DK Bhattacharyya, Modification of tallow fractions in the preparation of edible fat products. *European Journal of Lipid Science and Technology*, *102*(5), 323–328. https://doi.org/10.1002/(SICI)1438-9312(200005)102:5<323:AID-EJLT323>3.0.CO;2-N

Bryś, J., Wirkowska, M., Górska, A., Ostrowska-Ligęza, E., & Bryś, A. (2014). Application of the calorimetric and spectroscopic methods in analytical evaluation of the human milk fat substitutes. *Journal of Thermal Analysis and Calorimetry*, *118*(2), 841–848. https://doi.org/10.1007/s10973-014-3893-1

Chang, T., Lai, X., Zhang, H., Søndergaard, I., & Xu, X. (2005). Monitoring lipase-catalyzed interesterification for bulky fat modification with

FT-IR/NIR spectroscopy. *Journal of Agricultural and Food Chemistry*, *53*(26), 9841–9847. https://doi.org/10.1021/jf051892v

Foglia, T. A., Petruso, K., & Feairheller, S. H. (1993). Enzymatic interesterification of tallow-sunflower oil mixtures. *Journal of the American Oil Chemists' Society*, *70*(3), 281–285. https://doi.org/10.1007/BF02545309

Forssell, P., Kervinen, R., Lappi, M., Linko, P., Suortti, T., & Poutanen, K. (1992). Effect of enzymatic interesterification on the melting point of tallow–rapeseed oil (LEAR) mixture. *Journal of the American Oil Chemists' Society*, *69*(2), 126–129. https://doi.org/10.1007/BF02540561

Jin, Q., Zhang, T., Shan, L., Liu, Y., & Wang, X. (2008). Melting and solidification properties of palm kernel oil, tallow, and palm olein blends in the preparation of shortening. *Journal of the American Oil Chemists' Society*, *85*(1), 23–28. https://doi.org/10.1007/s11746-007-1152-3

Kowalska, D., Gruczynska, E., & Kowalska, M. (2015). The effect of enzymatic interesterification on the physico-chemical properties and thermo-oxidative stabilities of beef tallow stearin and rapeseed oil blends. *Journal of Thermal Analysis and Calorimetry*, *120*(1), 507–517. https://doi.org/10.1007/s10973-014-3869-1

Kowalska, M., Woźniak, M., Krzton-Maziopa, A., Tavernier, S., Pazdur, Ł., & Żbikowska, A. (2019). Development of the emulsions containing modified fats formed via enzymatic interesterification catalyzed by specific lipase with various amount of water. *Journal of Dispersion Science and Technology*, *40*(2), 192–205. https://doi.org/10.1080/01932691.2018.1467328

Kowalska, M., Żbikowska, A., & Kowalski, B. (2014). Enzymatically modified fats based on mutton tallow and rapeseed oil suitable for fatty emulsions. *Journal of the American Oil Chemists' Society*, *91*(10), 1703–1710. https://doi.org/10.1007/s11746-014-2512-4

Kowalski, B., Tarnowska, K., & Gruczynska, E. (2005). The properties of the mixture of beef tallow and rapeseed oil with a high content of tallow after chemical and enzymatic interesterification. *Grasas Y Aceites*, *56*(4), 267–275. https://doi.org/10.3989/gya.2005.v56.i4.92

Kowalski, B., Tarnowska, K., Gruczynska, E., & Bekas, W. (2004). Chemical and enzymatic interesterification of a beef tallow and rapeseed oil equal-weight blend. *European Journal of Lipid Science and Technology*, *106*(10), 655–664. https://doi.org/10.1002/ejlt.200400973

Ledóchowska, E., & Wilczyńska, E. (1998). Comparison of the oxidative stability of chemically and enzymatically interesterified fats. *Lipid/Fett*, *100*(8), 343–348. https://doi.org/10.1002/(SICI)1521-4133(199808)100:8<343:AID-LIPI343>3.0.CO;2-1

Li, Y., Zhao, J., Xie, X., Zhang, Z., Zhang, N., & Wang, Y. (2018). A low trans margarine fat analog to beef tallow for healthier formulations: Optimization of enzymatic interesterification using soybean oil and fully hydrogenated palm oil. *Food Chemistry*, *255*, 405–413. https://doi.org/10.1016/j.foodchem.2018.02.086

Martin, D., Reglero, G., & Señoráns, F. J. (2010). Oxidative stability of structured lipids. *European Food Research and Technology*, *231*(5), 635–653. https://doi.org/10.1007/s00217-010-1324-5

Meng, Z., Liu, Y. F., Jin, Q. Z., Huang, J. H., Song, Z. H., Wang, F. Y., & Wang, X. G. (2011). Comparative analysis of lipid composition and thermal, polymorphic, and crystallization behaviors of granular crystals formed in beef tallow and palm oil. *Journal of Agricultural and Food Chemistry*, *59*(4), 1432–1441. https://doi.org/10.1021/jf103875f

Meng, Z., Liu, Y., Shan, L., Jin, Q., & Wang, X. (2010). Reduction of graininess formation in beef tallow-based plastic fats by chemical interesterification of beef tallow and canola oil. *Journal of the American Oil Chemists' Society*, *87*(12), 1435–1442. https://doi.org/10.1007/s11746-010-1627-5

Pang, M., Ge, Y., Cao, L., Cheng, J., & Jiang, S. (2019). Physicochemical properties, crystallization behavior and oxidative stabilities of enzymatic interesterified fats of beef tallow, palm stearin and camellia oil blends. *Journal of Oleo Science*, *68*(2), 131–139. https://doi.org/10.5650/jos.ess18201

Rajendran, A., Palanisamy, A., & Thangavelu, V. (2009). Lipase catalyzed ester synthesis for food processing industries. *Brazilian Archives of Biology and Technology*, *52*(1), 207–219. https://doi.org/10.1590/S1516-89132009000100026

Rodríguez, A., Castro, E., Salinas, M. C., López, R., & Miranda, M. (2001). Interesterification of tallow and sunflower oil. *Journal of the American Oil Chemists' Society*, *78*(4), 431–436. https://doi.org/10.1007/s11746-001-0280-5

Rohm, H., Schäper, C., & Zahn, S. (2018). Interesterified fats in chocolate and bakery products: A concise review. *LWT*, *87*, 379–384. https://doi.org/10.1016/j.lwt.2017.08.076

Rønne, T. H., Yang, T., Mu, H., Jacobsen, C., & Xu, X. (2005). Enzymatic interesterification of butterfat with rapeseed oil in a continuous packed bed reactor. *Journal of Agricultural and Food Chemistry*, *53*(14), 5617–5624. https://doi.org/10.1021/jf050646g

Segura, N., & Jachmanián, I. (2020). Zero-trans fats by enzymatic interesterification of blends beef tallow/rice bran oil. *OCL*, *27*, 4.

Silva, R. C., Cotting, L. N., Poltronieri, T. P., Balcão, V. M., de Almeida, D. B., Goncalves, L. A. G., … Gioielli, L. A. (2009). The effects of enzymatic interesterification on the physical-chemical properties of blends of lard and soybean oil. *LWT-Food Science and Technology*, *42*(7), 1275–1282. https://doi.org/10.1016/j.lwt.2009.02.015

Svensson, J., & Adlercreutz, P. (2008). Identification of triacylglycerols in the enzymatic transesterification of rapeseed and butter oil. *European Journal of Lipid Science and Technology*, *110*(11), 1007–1013. https://doi.org/10.1002/ejlt.200800074

Uncu, O., & Ozen, B. (2015). Prediction of various chemical parameters of olive oils with Fourier transform infrared spectroscopy. *LWT-Food Science and Technology*, *63*(2), 978–984. https://doi.org/10.1016/j.lwt.2015.05.002

Xu, X. (2000). Production of specific-structured triacylglycerols by lipase-catalyzed reactions: A review. *European Journal of Lipid Science and Technology*, *102*(4), 287–303. https://doi.org/10.1002/(SICI)1438-9312(200004)102:4<287:AID-EJLT287>3.0.CO;2-Q

Zhang, Z., Lee, W. J., Zhou, H., & Wang, Y. (2019). Effects of chemical interesterification on the triacylglycerols, solid fat contents and crystallization kinetics of palm oil-based fats. *Food & Function*, *10*(11), 7553–7564. https://doi.org/10.1039/C9FO01648A

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