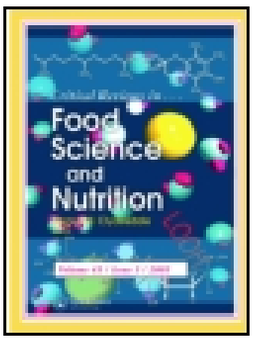

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REVIEW

Evaluation of enzymatic interesterification in structured triacylglycerols preparation: a concise review and prospect

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ABSTRACT

Enzymatic interesterification (EIE) is one of the emerging technologies in the specialty fats industry. EIE has several advantages over the conventional chemical interesterification method, such that the process has higher flexibility and efficiency, is environmentally friendly and the immobilized enzyme can be recycled besides of the lower requirement for substrate's acid value. The physical properties and nutritional qualities of the fats and oils are modified after EIE, depending on the change in the position of fatty acids on the triacylglycerol (TAG) molecules. Evaluation of the interesterification reaction are important and useful in terms of its technological applications. This paper summarizes the conventional methods and the advancement for evaluating EIE processes, e.g., determination of the change in slip melting points, solid fat contents, TAG with equivalent carbon numbers, and *sn*-2 fatty acid compositions of the end product. Nonetheless, these methods are not comprehensive because during the EIE process, acyl migration occurs. A novel and convenient evaluation model which is based on the fatty acid distribution on the glycerol-backbone is proposed as a perspective. This model can be employed to monitor the interesterification degree and acyl migration during a regiospecific EIE process, which serves as a reaction rule that can be employed to control and optimize the EIE process, thereby producing structured TAG with desired properties.

KEYWORDS

Acyl migration; interesterification; structured triacylglycerols

Introduction

Fats and oils are widely utilized for both food application and industrial uses. However, the technological application of most edible fats and oils are restrained due to the natural triacylglycerol (TAG) structures which are unable to provide desirable characteristics to the fat products. The physical, chemical and nutritional properties of the fat products are associated to the fatty acid compositions and the stereochemistry of the TAG (Farfán et al. 2013). Physicochemical properties of a particular TAG are dependent on the fatty acid chain length, degree of saturation, and the position of fatty acid on the glycerol backbone. To enhance the technological traits of the natural edible fats and oils, a process known as interesterification (IE) can be performed to modify the properties of TAG (Rohm, Schäper, and Zahn 2018; Sharma and Lokesh 2012). IE process occurs in two stages; initial hydrolysis and a subsequent esterification process. At the end of the process, a redistribution of the two fatty acids within a TAG or the exchange of the fatty acids between TAGs, can be observed (Wirkowska-Wojdyła et al. 2016). Following IE, alteration in the TAG molecular structures causes modification in terms of melting/dropping point (Aktas et al. 2019; Hu, Xu, and Yu 2017b; Shi et al. 2015; Xie and Zang 2016), crystallization behavior (Dollah et al. 2015; Lopez-Hernandez et al. 2007; Pang et al. 2019; Zhu et al. 2017), solid fat content (de Paula et al. 2018; Ebrahimi, Farmani, and Bahmaei 2017; Kowalski et al. 2004; Oliveira et al. 2017; Zhu, Weng, et al. 2018), thermal properties (Dollah et al. 2015; Hu, Xu, and Yu 2017a; Pang et al. 2019; Zhou et al. 2019) and the oxidative stability (Bogevik et al. 2018; Bryś et al. 2019; Kowalska, Gruczynska, and Kowalska 2015; Kowalska et al. 2014; Wang et al. 2015) of the fat products. *Trans* isomers are not produced during the IE process as a result of the unaltered degree of unsaturation in the fatty acid (Shi et al. 2015), making it a desirable technique in the structured lipid industry.

There are two common pathways to perform IE, i.e., chemical interesterification (CIE) and enzymatic interesterification (EIE). CIE is commonly practiced in the industry and this process randomly rearranges the fatty acid positions on the glycerol backbone using chemical catalysts (Norizzah, Nur Azimah, and Zaliha 2018). Such random reaction is ideal in producing structured TAGs and the procedures required for performing CIE are rather easy and have been extensively employed in the literatures (Zhang et al. 2019;

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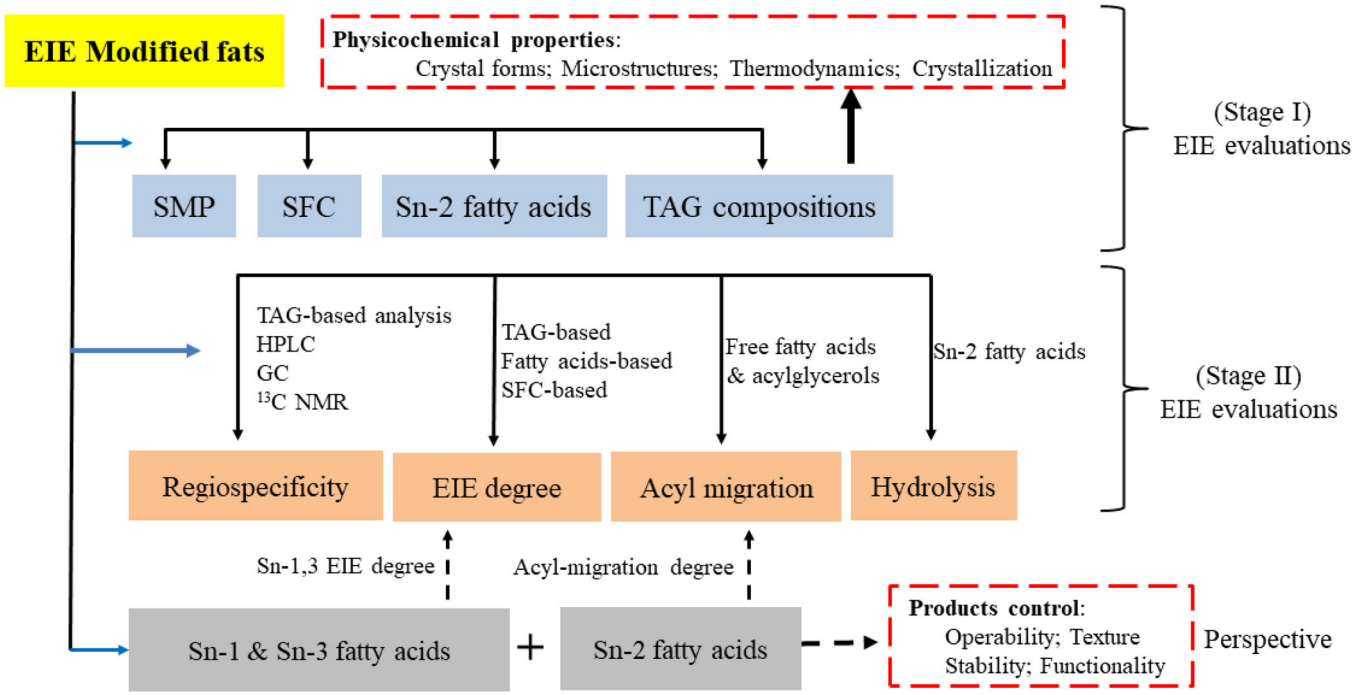


Figure 1. Schematic view for evaluations of EIE modification. EIE, enzymatic interesterification; TAGs, triacylglycerols; SMP, slip melting point; SFC, solid fats content.

Zhang et al. 2017). On the contrary, EIE is receiving more attention as an alternative lipid modification method. The enzymatic method has several advantages over the traditional CIE for the enzymes have high acyl exchange reaction efficiency, reaction process is simple, flexible, does not involve the use of hazardous chemicals and formation of less by-products (Svensson and Adlercreutz 2011; Norizzah, Nur Azimah, and Zaliha 2018). Furthermore, EIE is performed at lower reaction temperatures (40–90 °C) comparing to that of CIE process (210–260 °C). Degradation of the thermolabile bioactive compounds and fatty acids is inhibited during EIE and therefore, end products with better nutritional quality and shelf life can be obtained (Bryś et al. 2019; Danthine et al. 2014). In comparison to the CIE process that lacks specificity, the use of specific lipases during EIE allows for better specificity and control over the IE reaction (Lee et al. 2015; Moore and Akoh 2017), producing end products with desired physical and nutritional properties. Lipids with distinctive properties can be obtained from EIE process, depending on the type of enzyme catalysts (*sn*-1,3-specific or non-specific) and the reaction conditions. Process that is catalyzed using specific enzymes can result in the incorporation of a targeted fatty acid into a specific position on the glycerol backbone. The physical and nutritional properties of the interesterified products are reliant on the type and position of the fatty acids on the TAG molecule. Thus, studies on IE reaction mechanisms including the randomization, occurrence of acyl migration during the reaction and the produced TAG molecular species, is crucial.

Although the effect of sn-1,3 specific lipase on the ester bond at sn-2 position is not pronounce, it has positional selectivity for the acyl ester bond at the sn-1 and sn-3 positions on the glycerol backbone. Thus, its potential for industrialization has attracted the attention from the food industry for the production of cocoa butter with main composition of 1,3-distearoyl-2-oleoyl-sn-glycerol (sn-StOSt), human milk fat substitute with main composition of 1,3-dioleoyl-2-palmitoyl-sn-glycerol (sn-OPO) and base materials for the production of food specialty fats. Theoretically, the position of the fatty acid at sn-2 remained unchanged when the IE is catalyzed using sn-1,3-specific lipase. Nevertheless, in actual catalytic process, modification of the sn-2 fatty acid attributed to acyl migration, has been reported. It has been reported that acyl migration occurs alongside with the IE process even in regiospecific reactions, which causes randomization in the *sn*-2 position. Especially under extreme reaction conditions, acyl migration is promoted, producing complete or partially randomized products. Not only the specificity of the EIE process is affected by acyl migration, the physical, chemical and nutritional properties of the final product will also be affected. It is important to study the EIE mechanism and pathways of acyl migration in reactions catalyzed using sn-1,3 specific lipase, which is of great significance to the application of enzymatically directed controlled IE technology. The establishment of IE process evaluation methods is important and has become a key problem to be addressed for industrial application. Arrangement of fatty acyl group on the glycerol backbone is known to be influenced by the interesterification degree (ID), and therefore, evaluation of the IE process can be established via this method. Methods to evaluate the ID and acyl-migration degree are beneficial as a mean to keep the reaction under control and produce interesterified products which are of 1,3-regiospecific. This article aims to provide a review on the current available approaches used to monitor

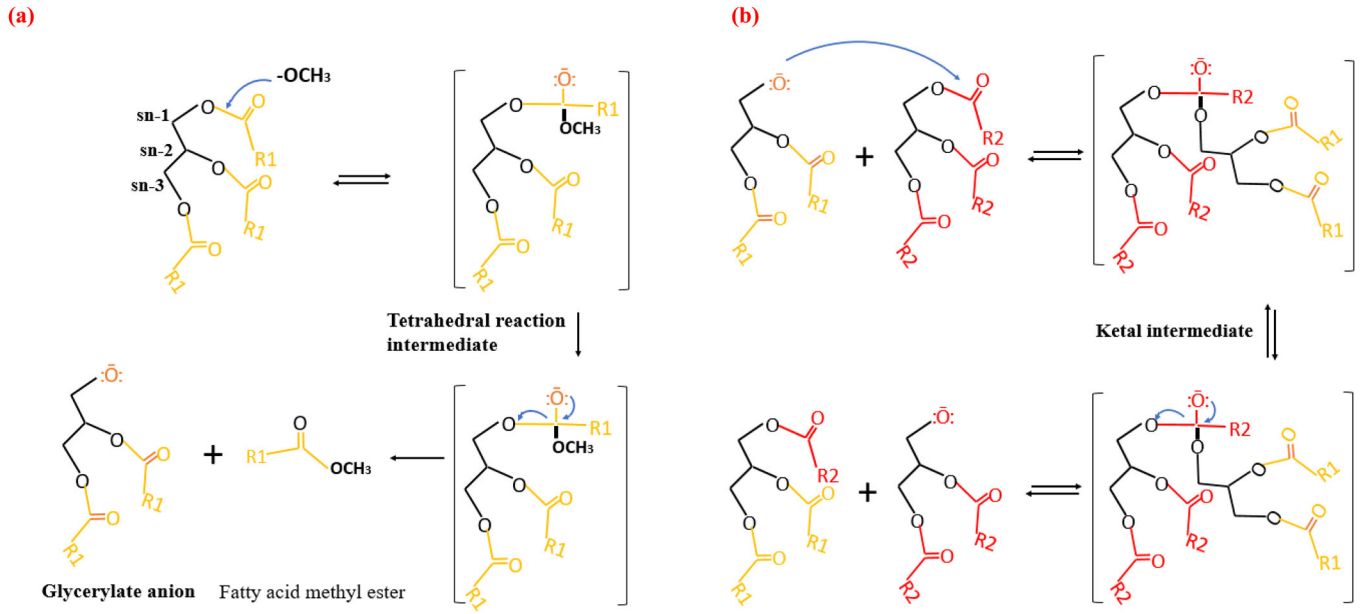


Figure 2. Carbonyl addition interesterification reaction mechanism (R1, R2 are fatty acyl groups) where (a) is the formation of diacylglycerol anion intermediate product and (b) is an intermolecular interesterification reaction.

the IE process and also to provide a perspective on the possible alternative methodology (Figure 1).

IE reaction and substrates for EIE

IE reaction can be categorized into intramolecular and intermolecular reaction. The production of interesterified fats and oils is mainly through the chemical catalysis method whereby chemical catalysts such as alkaline sodium methoxide (CH_3ONa) is used to promote the random rearrangement of fatty acids to achieve final state of equilibrium (Norizzah, Nur Azimah, and Zaliha 2018). Presently, two types of IE mechanisms are identified, namely, carbonyl addition and Claisen condensation. Figure 2 shows the carbonyl addition mechanism. Using CH_3ONa-catalyzed intermolecular IE as an example, the nucleophilic methoxy ion (CH_3O^-) attacks the positively-charged carbonyl atom in TAG to form a tetrahedral intermediate. Glycerylate anion, which is an intermediate product, and a fatty acid ester, are obtained as the products from the subsequent intra- or intermolecular carbonyl reaction, which continues until thermodynamic equilibrium has been reached (Rousseau, Ghazani, and Marangoni 2017). The glycerylate anion then attacks another activated TAG ester bond to form an intermediate complex, dissociating the glycerylate anion and the newly formed TAG molecule before completing the IE process. This process repeats until the positions of all fatty acyl groups have been altered. In a CIE process, and in this case, the use of CH_3ONa catalyst that does not possess positional specificity, the reaction is completely randomized, and it is therefore a challenge to obtain TAG with targeted structure via controlling the process conditions.

Various fats and oils obtained from plants, animals and microbes, can be used as a base oil for IE reaction. For instance, the development of structured TAG using palm oil and its fractions is a hot topic in the lipid industry. Tallow, fish oil, lard, and some other new oils such as the *Acer truncatum* seed oil, wing bean oil, basa catfish oil, and mango kernel oil, are all in favor to be used for IE. Some of the recent IE performed on various oil sources are shown in Table 1. Application of these oils in the preparation of specialty fats can improve the physicochemical characteristics and add value to the end product. Oils which are thermo-oxidatively stable such as the high oleic sunflower oil (more than 88% oleic acid), after IE, exhibit great value for food application in the preparation of bakery products, margarine, infant food, and frozen desserts (Kadivar et al. 2014). In general, the physicochemical properties of oil are prioritized over the fatty acid composition as a criterion when selecting the base oil for IE; the former influences the operational characteristics of the interesterified fats (melting point, crystallization and texture) whereas the latter affects the nutritional characteristics. Chemical compositions of the fats such as the type of fatty acids and their distribution along the glycerol backbone influence the physical characteristics. On the other hand, the nutritional value and metabolic activity of the lipid mixture can be changed by incorporating fatty acids with different chain lengths or by modifying their positions on the glycerol molecule. Interesterified fats produced from EIE using various oil substrates and their applications are shown in Table 2. The interesterified fats are commonly applied in food systems as specialty fats such as cocoa butter substitute, shortening, fats for fast-frozen food, infant formula and human milk fat substitute.

Practical application value

One of the original intentions of performing sn-1,3-specific lipase-catalyzed IE is to control the random distribution of acyl groups at sn-1 and sn-3 positions for producing structured TAG with enhanced physical and chemical properties. However, under the influence of external factors (reaction

Table 1. Current new base oils for interesterification modifications to produce advanced interesterified fats.

Base oils	Advantages	References
Coix seed oil	Coix seed oil is high in unsaturated fatty acids (Cl8:1, C18:2)	Xu et al. 2018
Cinnamomum camphora seed oil	Cinnamomum camphora seed oil mainly contains medium-chain fatty acids (C10:0, C12:0)	Xu et al. 2018
Flaxseed oil	Flaxseed oil is rich in ω-3 and ω-6 fatty acids	Khodadadi and Kermasha 2014
Anhydrous milk fats	Milk fats are natural plastic fat, which contains >400 fatty acids participating in the structure of TAGs with different molecular masses and a wide range of crystallization and melting temperatures.	Viriato et al. 2018
Patawa oil, *Moringa oleifera* seed oil, camellia seed oil	These oils are similar to olive oil, which is rich in monounsaturated fatty acid (C18:1)	Dollah et al. 2015; Oliveira et al. 2017; Ruan et al. 2014
Buriti oil (*Mauritia flexuosa* L.)	Buriti oil is a source of positive minor compounds (carotenoids etc.)	Speranza et al. 2018
Irvingia gabonensis seed oil	*Irvingia gabonensis* seed oil contains high quantities of C14:0 and C12:0	Yamoneka et al. 2018
Perilla oil	Perilla oil has a high amount of α-linolenic acid (ALA, C18:3)	Zhao et al. 2013
Mango kernel oil	Mango kernel oil TAG species are mainly symmetrical monounsaturated.	Jin et al. 2019
crambe abyssinica oil	*crambe abyssinica* oil has high erucic acids (C22:1)	Guedes et al. 2014
Heterotrophic microalgal oil	Heterotrophic microalgal oil is a good dietary source of long-carbon chain polyunsaturated fatty acids (C22:6, n-3)	Bogevik et al. 2018

Table 2. Approaches for enzymatic interesterification of different substrates to obtain interesterified fats for various applications.

Substrates (selected mass ratios)	Reactor (selected reaction temperature [°C] and time [h])	Enzyme	Applications	References
Fully hydrogenated soybean oil + rice bran stearin + coconut oil (5:5:2)	Shaking water bath (65 °C, 24 h)	*Rhizomucor miehei*	Trans-free shortening	Shi et al. 2015
Palm mid fraction + hardened soybean oil (1:2)	Orbital shaking reactor (60–70 °C, 4 h)	*Thermomyces lanoginosus*	Cocoa butter equivalent	Soekopitojo 2009
Irvingia gabonensis seed fat + *Dacryodes edulis pulp oil* (9:1)	Stirring reactor (70 °C, 16 h)	*Thermomyces lanoginosus*	Cocoa butter alternative	Yamoneka et al. 2018
Heterotrophic microalgal oil + rapeseed oil (10:7)	Shaking reactor (60 or 80 °C, 8 h)	*Rhizomucor miehei* or *Candida Antarctica*	Low-saturation triacylglycerols	Bogevik et al. 2018
Tea seed oil + solid fractions of tea seed oil (43:100)	Stirring reactor (60 °C, 8 h)	*Thermomyces lanoginosus*, immobilised	Cocoa butter replacer	Zarringhalami et al. 2010
Fully hardened soybean oil + sunflower oil with elevated oleic acid content (1:0.43)	stirring reactor (70 °C, 24 h)	*Thermomyces lanoginosus*	Zero trans shortening fats	Li et al. 2010
Palm stearin + soybean oil (7:3)	Stirring reactor (60 °C, 5 h)	*Thermomyces lanuginosus* immobilized	Fast-frozen food fats	Zhu, Liu, et al. 2018
Lard + rapeseed oil + fish oil (7:2:1)	Shaking water bath (50 °C, 4 h)	*Rhizomucor miehei* immobilized	Cookies fats	Wirkowska-Wojdyła et al. 2016
Acertruncatum oil + palm stearin + palm kernel oil (3:6:1)	Stirring reactor (60 °C, 9 h)	*Rhizomucor miehei* immobilized	Margarine fats	Hu, Xu, and Yu 2017b
Hard palm mid fraction + mango kernel fats + cocoa butter (2:6:2)	Stirring reactor (75 °C, 5 h)	*Rhizomucor miehei* immobilized	Chocolate fats	Jin et al. 2018
Mutton tallow + hemp oil (1:1) + water (10%–25%)	Shaking water bath (60 °C, 6 h)	*Rhizomucor miehei*	Emulsion modified fats	Kowalska et al. 2019
Palm stearin + soybean oil (5:5–7:3)	Fluidized bed reactor (60 °C, 1.57 and 0.40 mL/min, not converted to residence time)	*Thermomyces lanuginosus* immobilised	Fast-frozen food fats	Zhu et al. 2017
ARA-rich fungal oil from *Mortierella alpine* + caprylic/capric triacylglycerols (1:1 mol/mol)	Stirring reactor (90 °C, 3 h)	*Candida Antarctica*, immobilized	Infant formula	Korma et al. 2018
Thistle milk oil + lard (2:8)	Thermostatic shaker (70 °C, 2–6 h)	*Rhizomucor miehei*	Human milk fats substitutes	Bryś et al. 2019
Palm stearin + fish oil (1:1–3:1–1:3)	Stirred tank reactor (60 °C, 6–24 h)	*Thermomyces lanuginosus* immobilised	Human milk fats substitutes	Ghosh et al. 2016
Soybean oil + fully hydrogenated palm oil (4:3)	Stirring reactor (95 °C, 4.08 h)	*Candida Antarctica B,* immobilised	low trans margarine fat analogs to beef tallow	Li et al. 2018
Cocoa nut oil + sesame oil + Tween 40 (50–70:50–30:0.5)	Stirring reactor (45–65 °C, 16–48 h)	*Rhizomucor miehei*	Structure lipids	Sivakanthan, Jayasooriya, and Madhujith 2019

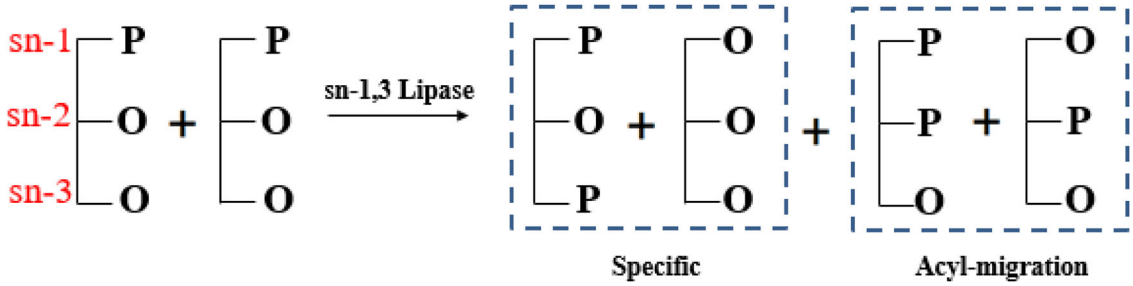


Figure 3. Production of sn-POO through interesterification reaction catalyzed using sn-1,3 specific lipase.

time and temperature, amount of lipase, type of immobilized carrier, and etc.), acyl migration at sn-2 is promoted. For example, in an ideal positional specific IE of sn-POO (1-palmitoyl-2,3-dioleoyl-sn-glycerol), there is no change of fatty acid at sn-2 and only sn-POP (1,3-dipalmitoyl-2-oleoyl-sn-glycerol) and OOO (triolein) are produced. In practice, acyl migration at sn-2 occurs under the effect of various external factors, forming sn-PPO (1,2-dipalmitoyl-3-oleoyl-sn-glycerol) and sn-OPO (1,3-dioleoyl-2-palmitoyl-sn-glycerol) (Figure 3).

Inhibition of acyl migration

By using sn-1,3 specific lipase, preparation of symmetrical TAG with saturated fatty acids at sn-1,3 and unsaturated fatty acid at sn-2 can be achieved, i.e., TAG with n-SUS structure, whereby S represented saturated fatty acyl and U represented unsaturated fatty acyl. For instance, sn-StOSt and sn-POP have the characteristics of high solid fat content at low temperature (10–15 °C), enable shape retention, and rapidly melt in the mouth when consumed (35–40 °C) (Jahurul et al. 2014). By controlling the external conditions, occurrence of acyl migration can be inhibited which will result in the production of interesterified fats with high content of sn-SUS TAG that has enhanced physical and chemical properties. This interesterified product shows important applications as food specialty fat, e.g. replacement for natural cocoa butter in the preparation of chocolate and confectionery.

Promotion of acyl migration

In vegetable oils, saturated fatty acids are naturally distributed in sn-1 and sn-3. During the sn-1,3 positional specific catalysis process, migration of sn-2 acyl group can be promoted, thereby increasing the formation of symmetrical TAG structure with unsaturated fatty acids at sn-1,3 and saturated fatty acid at sn-2, i.e., TAG with sn-USU structure. The metabolic pathway of sn-2 fatty acid differs from that of fatty acids at sn-1 and sn-3. For example, sn-OPO TAG, which is an important component in human milk fat substitute, saturated fatty acid positioned at sn-2 can promote the digestion and absorption of fatty acids and calcium in the human body. Migration of acyl group can be promoted by controlling the external conditions, thereby enhancing the nutritional properties of vegetable oils as base materials for milk powder formulation.

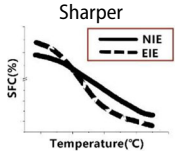
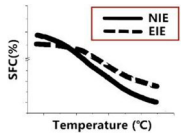
Hence, the application of controlled IE technology on the basis of the application of sn-1,3 specific lipase has a large potential application value in the processing and production of functional food specialty fats.

EIE evaluation methods

Slip melting point

Slip melting point (SMP) is an important parameter which is of great significance for monitoring the ID for industrial production (Ruan et al. 2014). SMP is defined as the temperature at which the fat or oil column in a capillary tube rises (Shi et al. 2015). AOCS Official Method (AOCS Official Method Cc 3-2525 1997) is commonly used to determine the SMP of physical blends before and after EIE. After EIE, SMP of the physical blend differs from the substrate and this is due to the re-distribution of the fatty acids in the TAG (Adhikari et al. 2012; Sharma and Lokesh 2012). SMP is influenced by its fatty acid compositions, fatty acid chain lengths, the saturation ratio of fatty acids, the complexity of TAG, and the position of fatty acids on the glycerol backbone (Zhao, Hu, Zhu, Li, et al. 2014). A re-distribution of TAG following EIE modification increases the end product's SMP (from 38.5 to 52.2 °C), while most other properties decrease after EIE reactions (Table 3). The alteration of SMP is reported to be contributed by the increment in the tri-saturated (S3) TAG content (Dian et al. 2019). Similarly, Dollah et al. (2015) also reported on the increase in SMP and SFC of enzymatically interesterified blend consists of *Moringa oleifera* seed oil/palm olein at high temperature due to rise in the concentration of TAG with high melting points. In general, SMP of an enzymatically interesterified oil in a system consisting of only one type of vegetable oil increases (Berry 2019); only a minor change in SMP of interesterified vegetable oils rich in saturated fatty acids (coconut oil, palm oil) and animal fats (lard and butter); and a reduction in SMP of interesterified oil blend consisting of oils with significantly different melting points. As noted by Zhang et al., the SMP of oil blend consisting of palm mid fraction and beef tallow increased after IE, attributed to the increment of S3 TAG content in palm mid fraction (Zhang et al. 2018). Hence, interesterified fats can be used to balance the saturated and unsaturated fatty acid content and increase the SFC of a system at 25–40 °C for the preparation of food specialty fats (Zhang et al. 2018). In most of the EIE of oil blends, a decrease in the SMP was

Table 3. Change in the physiochemical properties in various oils before and after EIE catalyzed by *sn*-1,3 specific lipase.

	SMP (°C)		Trend	SFC and TAG		
				Sharper 	Smoother 	
Substrates	Physical blend	After IE				References
Rice bran stearin + fully hydrogenated soybean oil + coconut oil (5:5:2)	62.0 ± 0.7	46.4 ± 0.3	↓	StStSt, StPSt/PStSt↓ POO, OOO ↑	—	Shi et al. 2015
Palm stearin + *acertruncatum* oil + palm kernel oil (6:3:1)	50.7 ± 0.3	37.0 ± 0.3	↓	S3↓, S2U↑, SU2↑; U3↓	—	Hu, Xu, and Yu 2017b
Coconut oil + sesame oil (5:5)	20.8 ± 0.3	18.9 ± 0.3	↓	—	—	Sivakanthan et al. 2019
Fully hydrogenated expanded press soybean oil + cold press corn oil (5:5)	63.2 ± 1.4	49.6 ± 1.0	↓	StStSt, StPSt/PStSt↓, StOSt, StOP, StOO; POO↑	—	Yu et al. 2018
Heterotrophic microalgal oil + rapeseed oil (10:7)	46	35	↓	PPP/MPSt, PPSt ↓, PPO, POO ↑	—	Bogevik et al. 2018
Highly hydrogenated soybean oil + *cinnamomum camphora* seed oil + perilla oil (8:2:10)	50.51	39.05	↓	PStSt, StStSt LnLnL/ LnLLn ↓, PStP, StStL/StLSt, StOO/ OStO, OLLn/ LnLO/OLnL ↑	—	Zhao et al. 2013
Palm stearin + *Akebia trifoliata* variety *Australis* seed oil (7:3)	49.30 ± 0.27	37.60 ± 0.20	↓	POP/PPO, PPP/PLSt/ PStL/StPL, StStL/ StLSt ↓, PLO/OPL/ POL, PPL/PLP, POO/OPO ↑		Zhao et al. 2014
Palm oil (itself)	38.5	52.2	↑	—	S3 (PPP)↑, S2U (POP)↓, SU2 ↓U3 ↑	Dian et al. 2019
Rice bran oil + palm stearin + coconut oil (5:5:4)	34.3 ± 1.1	27.5 ± 1.1	↓	LOO, POO, POP, PPP ↓, PLP, LLO/ LaPO ↑		Adhikari et al. 2010
Beef tallow stearin + rapeseed oil (4:6)	46.8 ± 0.8	29.8 ± 0.6	↓	—	—	Kowalska et al. 2015
Cocoa butter (itself)	35.0	50.0	↑	—	POSt, POP, StOSt ↓, POO, StOO, StStP, StPP, PPP, StStSt ↑	Berry 2019
Moringa oleifera seed oil + palm olein (70:30)	—	—	—		S3 ↑, S2U ↓, SU2 ↑	Dollah et al. 2015

S, saturated fatty acids; U, unsaturated fatty acids; S3, tri-saturated TAGs; S2U, di-saturated TAGs; SU2, mono-saturated TAGs; U3, tri-unsaturated TAGs; M, myristic acid; P, palmitic acid; St, stearic acid; O, oleic acid. L, linoleic acid; Ln, linolenic acid.

reported (Sarafhana Dollah et al. 2016; Lopez-Hernandez et al. 2007; Reena, Reddy, and Lokesh 2009; Ribeiro et al. 2018; Zhu et al. 2017), and this can be due to the incorporation of tri-unsaturated (U3) liquid oils into the base oil formulation, resulting in the change of the concentration of S3 TAG and therefore, decreasing the SMP.

Solid fat content

Natural oils exist as a mixture of solid and liquid at room temperature. The physical properties and sensory attributes (plasticity, shape retention, and melt-in-the-mouth properties) of fat products are influenced by the amount of fat crystals, which is also known as the solid fat content (SFC). In the oil processing industry, SFC is commonly used as a parameter for palm oil extraction and also as an index in the production of plastic fats such as the cocoa butter, margarine, and butter. During IE, modification in the S3 and di-saturated (S2U) TAG content in fats and oils can be reflected by their SFC profile. Change in SMP and SFC values after EIE is attributed to the modification of the type and content of TAG species, i.e., the S3, S2U and di-unsaturated (SU2) TAG, which all these species have distinctive melting points (Table 3). During CIE, concentration of SU2-TAG increased significantly when soybean oil was added into the oil blend while the blend consisted of palm kernel oil showed a reduction in the SU2-TAG content. The SFC profile can be improved by adding sufficient amount of palm mid fraction to expand the plasticity range (Zhang et al. 2019). Attributes affected by the change in the SFC profile are as follows:

Table 4. Methods used to determine the TAGs species after EIE modification.

Equipment	Column	Results	References
HPLC + ELSD	RP-C18 column (5 μm, 4 × 250 mm)	Individual chromatographic TAG peaks were identified by comparing peak elution time with those of the TAG standard (OLL, OLO, OOO, PMP, PPP, PPSt, PLL, PLO, POO, StOO, PLP, POP and POSt)	Dian et al. 2019
HPLC + ELSD	RP-C18 column (5 μm, 4.6 × 250 mm)	ECN, regiospecificity of the fatty acids in TAGs was not determined	Biswas et al. 2018; Shi et al. 2015; Yu et al. 2018; Zhu et al. 2018
HPLC + RID	RP-C18 column (5 μm, 4 × 250 mm)		Jin et al. 2018; Norizzah et al. 2018
HPLC + ELSD	C18 column (5 μm, 3.9 × 150 mm)		Shin, Heo, and Lee 2019; Zhao et al. 2013
HPLC + ELSD	C18 column (5 μm, 4.6 × 150 mm)		Zhu et al. 2017
UPLC + Q-TOF + MS	Acquity UPLC BEH C18 column (i.d. 2.1 × 50 mm, 1.9 μm) (in ESI positive-ion mode)		Abed et al. 2018
	Acquity UPLC BEH C18 (i.d. 2.1 × 100 mm, 1.7 μm)		Bogevik et al. 2018
UPC^2 + Q-TOF + MS	Acquity UPC^2 BEH 2-EP column (150 × 3.0 mm, 1.7 μm)		Hu, Xu, and Yu 2017a
UHR-TOF + MS	Zorbax Eclipse Plus C18 column (1.8 mm, 2.1 × 100 mm)	ECN, regio-isomers of OPO and PPO were determined	Chen et al. 2019
Binary solvent HPLC + APCI + MS	A silver-modified cation exchange column (Luna SCX, Phenomenex, 150 × 2.0 mm, 5μm, 100 Å)	POP/PPO, POS/PSO, SOS/SSO and POO/OPO, SOO/OSO were separated, identified and quantified respectively	Santoro et al. 2018; Sun et al. 2018
GC + FID	RTX-65TG (30 m × 0.25 mm, 0.1 μm film thickness)	C28-C54: the number behind C means the carbon number of the triacylglycerol while the acid distribution in the TAG was not identified.	Hu, Xu, and Yu 2017b; Zhang et al. 2019
	DB-17HT (50%–methyl phenylpolysiloxane, 15 m × 0.25 mm i.d., 0.15 mm film)		Morselli Ribeiro et al. 2017; Speranza et al. 2018

HPLC, high performance liquid chromatography; ELSD, evaporative light scattering detector; RP, reverse-phase; RID, refractive index detector; UPLC-Q-TOF-MS, ultra-performance liquid chromatography quadrupole time-of-flight mass spectrometry; UPC^2-Q-TOF-MS (ultra-performance convergence chromatography quadrupole time-of-flight mass spectrometry; GC, gas chromatography; FID, flame ionization detector; APCI, atmospheric pressure chemical ionization; ECN, equivalent carbon number.

Plasticity

SFC at 4 and 10 °C indicate the spreadability of the product when refrigerated. The plastic range of fats is at SFC of 15%–35% and at this range, shortenings with desirable spreadability can be obtained (Pang et al. 2019; Rao et al. 2001). Plasticity of fats is also desirable for good aeration properties in the end products.

Shape retention

SFC between 20 and 22 °C determine the stability and resistance of fat products to oil exudation. In this case, the ideal SFC shall not be less than 10% (Oliveira et al. 2017; Xu et al. 2018).

Melt-in-the-mouth properties (steep SFC profile)

SFC between 33 and 38 °C influence the impression and sensation of sandiness of fat products in the mouth (a waxy mouth-feel can be perceived when the SFC is above 3.5% at 33.3 °C). In margarine, it is desirable to have high SFC to provide suitable crystal structure at room temperature and low SFC at high temperature, so that it can melt easily in the mouth when consumed (de Paula et al. 2018; Zhu, Liu, et al. 2018). Not only the functional properties of a product are affected by the SFC, the polymorphism and structure of the fat crystals are also controlled by the SFC. Compatibility of different fats and oils can be established by determining the SFC (eutectic or partial crystallization and the iso-solid profile). Presently, the SFC profiles at different temperatures can be obtained directly using a nuclear magnetic resonance spectroscopy (*p*-NMR).

TAG compositions and ID

Physical and sensorial attributes of fat products such as the hardness, spreadability and palatability, are related to the TAG compositions. Even with a minor change in the TAG structure, the crystallization profile and crystal polymorphism will be modified. When heat is applied during the process (e.g. deodorization, CIE, EIE), positional isomers are produced which then induces the change of physical properties in the fat products. At high temperatures, formation of asymmetric TAG is facilitated, which then affects the crystallization behavior. Therefore, it is vital to monitor and reduce the formation of the aforesaid undesirable compounds during the process in order to control the quality of products (Santoro et al. 2018). Various types of TAG are available depending on the different types of fatty acids that are bounded to the glycerol backbone. TAG in natural fats and oils have almost similar physicochemical properties and existed in large number of isomers and positional isomers. Therefore, procedure to separate TAG compounds has become the key for analyzing the TAG fingerprint spectrum in edible oil.

Chromatography and mass spectrometry (MS) are widely used to separate the TAG in edible oils, and these techniques are rapid and efficient. Chromatographic analysis can

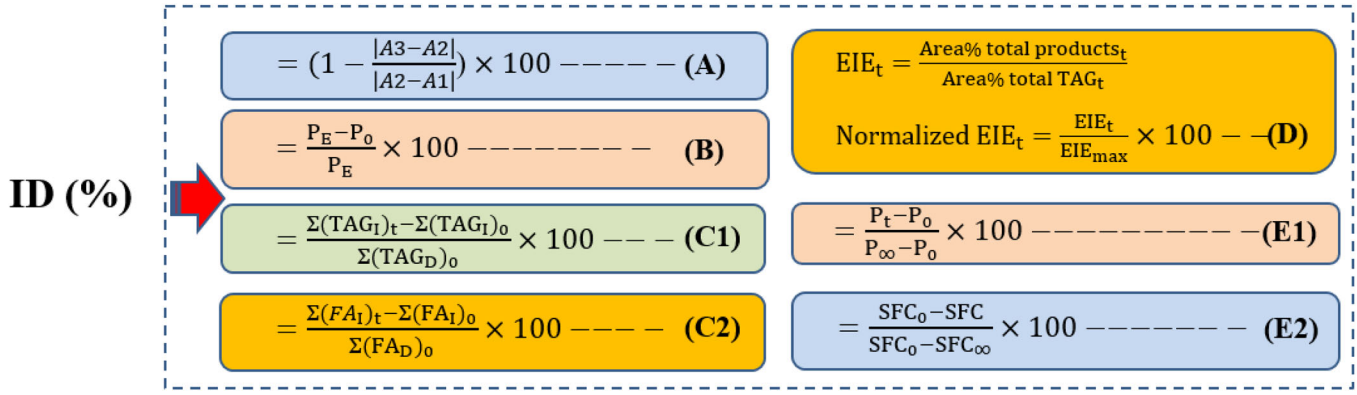


Figure 4. The evaluations equations of EIE modification. Notes: A (where A_1 is the TAG content in the initial blend [starting mixture], A_3 is the experimental TAG content in the interesterified product and A_2 is the theoretical completely random TAG content in interesterified product [calculated using the law of probability and the typical fatty acid composition in the blend; Li et al. 2018]); B (where P_E and P_0 are the area percentage of ECN [24, 26 and 28] of the blend before and after EIE, respectively; Yang et al. 2014); C1 (where TAG_I is the concentration [mM] of the TAG, whose concentration increased during the reaction; TAG_D is the concentration [mM] of the TAG, whose concentration decreased during the reaction. The index "t" and "0" represent the concentrations of TAG at a given reaction time and at the initial reaction, respectively; Morselli Ribeiro et al. 2017; Nunes et al. 2011; Paula et al. 2015); C2 (where FA_I is the % area of fatty acids which increased during the reaction, FA_D is the % area of fatty acids, which decreased during the reaction, subscripts T and 0 represent the area % of fatty acids at a given reaction time and at the beginning of the reaction, respectively; Sivakanthan, Jayasooriya, and Madhujith 2019); D (where total products are the sum of LLP, POL, PLP, and LOL and total TAG is the sum of LLL, POP, LLP, POL, PLP, and LOL. The subscript *t* indicates the sampling time. EIE_{max} was the maximal value of EIE observed and represented equilibrium involving the *sn*-1,3 positions; Cao et al. 2016; Svensson and Adlercreutz 2011); E1 (where P_t is the peak ratio at time *t*, $P\infty$ is the peak ratio at equilibrium, and P_0 is the peak ratio at time 0. During the reaction, the peaks TAG changed the most. Therefore, the peak ratio of ECN 44 and 48 was used for monitoring the process; Zhang, Smith, and Adler-Nissen 2004); E2 (where SFC_0 is the SFC at time 0 [in the blend], SFC is the SFC at time "t" of the reaction and $SFC\infty$ is the SFC at the equilibrium stage; Neeharika et al. 2015).

be divided into gas chromatography (GC) and high-performance liquid chromatography (HPLC). GC separates TAG based on the TAG carbon number, while reversed-phase liquid chromatography analysis is based on equivalent carbon number (ECN). ECN can be calculated by determining the differences in between the carbon number and the total number of double bonds in the TAG. However, it is a challenge to isolate TAG positional isomers, especially when the number of double bonds is similar and the only difference is their position within the molecule. Most of the TAG isomers cannot be efficiently separated and analyzed using GC or HPLC (Table 4), with the exception for individual TAG. Therefore, analysis for TAG positional isomers is still an existing challenge in lipid analysis. Other than the analysis for isomerism, TAG analysis based on the total carbon number has progressed considerably. At present, rapid analysis of glycerols is mostly carried out using GC by identifying the peak retention time. Using a non-polar high-temperature GC column (filled with dimethylpolysiloxane), peaks generated are related to the boiling points of the TAG whereby the TAG with high boiling point has a long retention time.

EIE reaction can be monitored directly by analyzing the TAG compositions since the molecular structure of TAG is altered during the reaction. In combination with MS, the relative molecular mass distribution of the TAG mixture can be acquired for the determination of degree of IE (ID). Li et al. determined the ID by calculating the change in the TAG before and after the reaction using the following equation (Figure 4a) (Li et al. 2018). It was found that the EIE of oil blend consisting of soybean oil and fully hydrogenated palm oil catalyzed by Lipozyme 435 had the highest ID (97.0 ± 0.68%). Yang et al. used Lipozyme TL IM to catalyze the IE of oil blend consisting of soybean oil and medium-chain fatty acid glycerols. The change in the rate of IE was determined using HPLC by measuring the change in the TAG composition using the ECN of 24, 26, 28 (Yang et al. 2014). The amount of ECN can be used to monitor the ID by comparing the ECN before and after IE. The ID of the reaction is shown in Figure 4b. Nues et al. performed regression analysis to fit the response function using the experimental data (Figure 4c1) and found that the experimental results are in good agreement with the predicted values (Nunes et al. 2011). ID can also be determined using the equation reported by Sivakanthan with slight modifications (Sivakanthan, Jayasooriya, and Madhujith 2019). Change in the fatty acid is also considered to be another way to calculate the ID (Figure 4c2). Cao et al. applied the equation that is shown in Figure 4d to investigate and compare the catalytic activity of different enzymes on the IE of TAG and reported that the randomization of IE can be monitored using the ID (Cao et al. 2016). For instance, Svensson et al. used the equation to monitor the ID and reported that when the reaction was performed for less than 1–2 h, the fatty acid at *sn*-2 was not modified due to the alteration in *sn*-1,3 during the EIE has already been completed by then (Svensson and Adlercreutz 2011). Paula et al. evaluated the ID (Figure 4c) during IE of milk fat and soybean oil catalyzed by *R. oryzae* lipase under different reaction times (2, 4, and 6 h) in a basket-type stirred tank reactor. A maximum ID value of 9.19 ± 0.60% was achieved at 4 h and as the reaction time was extended, the ID decreased to 7.31 ± 0.99% (Paula et al. 2015). Zhang et al. also studied the effect of reaction time on the conversion degree based on ECN during EIE (Figure 4e1) and found that the ECN conversion degree increased with reaction time (Zhang, Smith, and Adler-Nissen 2004). Neeharika et al. modified the equation (Figure 4e2) to evaluate the ID according to the change in SFC values (Neeharika et al. 2015).

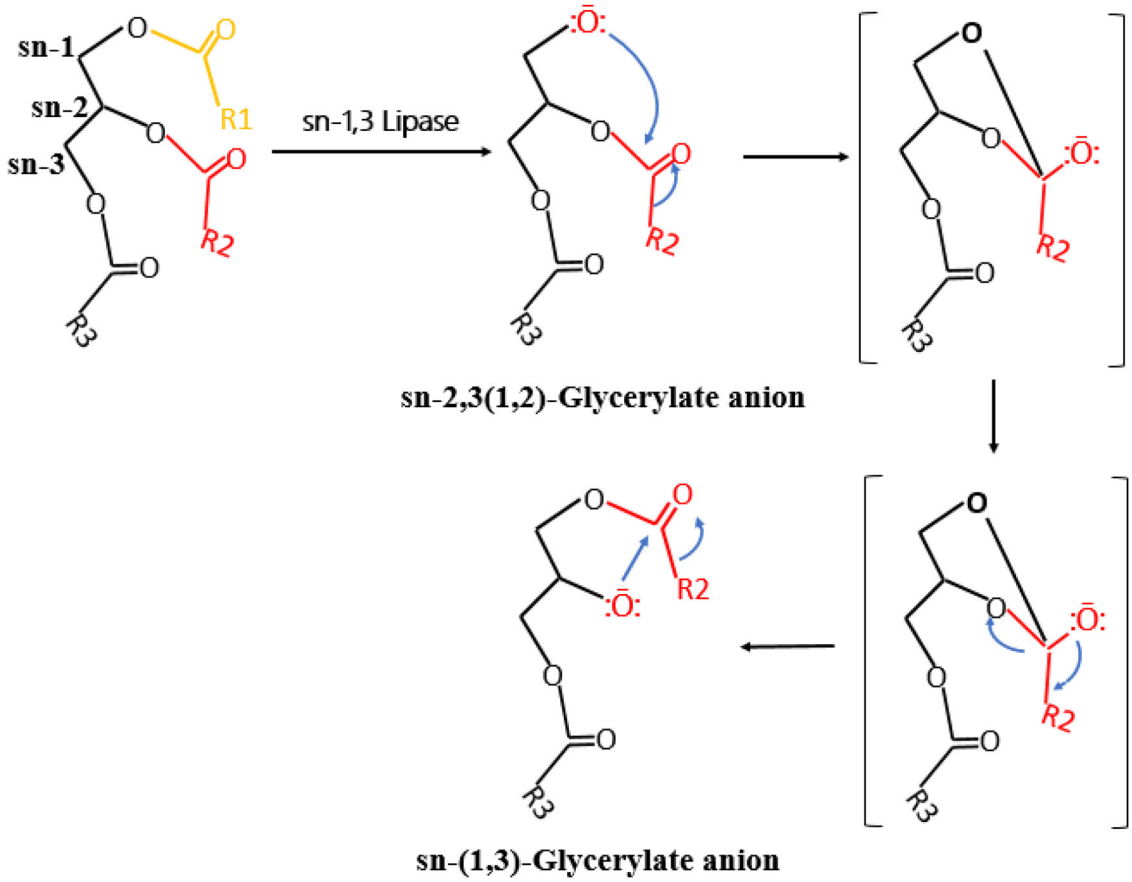


Figure 5. Mechanism of acyl migration at sn-2 during interesterification reaction (R1, R2, R3, are the fatty acyl groups).

Sn-2 fatty acid and acyl migration

Re-distribution of acyl group during IE changes the physicochemical characteristics of oils such as the SFC, melting point, crystal structure, texture, and also the nutritional properties. Properties of fats are dependent on the fatty acid compositions and TAG structures. The position of fatty acids in the TAG plays an important role when it comes to modifying the lipids for improved digestion and absorption. The theoretical premise of fatty acid position distribution research is that the metabolic pathway of *sn*-2 fatty acid on TAG is different from that of *sn*-1,3 (Karupaiah and Sundram 2007). By analyzing the fatty acid at *sn*-2, it is possible to understand the fatty acid distribution characteristics of TAG and also to determine the mechanism of the IE process. Kowalska et al. reported that when Novozyme 435 was used as the catalyst, some positional randomization of fatty acids in TAG was observed. The *sn*-2 percentage data showed that the distribution of fatty acid was near random (approximately 33.3% each at *sn*-1, 2, and 3) (Kowalska, Gruczynska, and Kowalska 2015; Yang et al. 2014).

Common method for determining the fatty acid positional distribution at *sn*-2 and *sn*-1,3 is by first hydrolyzing the ester bonds at *sn*-1,3 in the TAG using pancreatic lipase. The products obtained from lipolysis are then isolated using thin layer chromatography (TLC). The *sn*-2 monoacylglycerol (MAG) band is then scraped off and its lipids were extracted into diethyl ether and subsequently used for fatty acid analysis by using GC (Supplementary Figure 1a). The working equation is as follows (Ilyasoglu 2017; Lee et al. 2010; Zhao, Hu, Zhu, Li, et al. 2014):

$$\mathrm{sn}(1,3)\% = (3 \times \mathrm{total}\% - \mathrm{sn}(2)\%)/2 \quad (1)$$

Measurement of fatty acid at *sn*-2 can also be done by using ^{13}C NMR. The ^{13}C NMR signals are clearly distinguished into unsaturated fatty acids (170.88 ppm) and saturated fatty acids (170.91 ppm) esterified at the *sn*-2 position, unsaturated fatty acids (171.29 ppm) and saturated fatty acids (171.32 ppm) esterified at the sn-1,3 positions (Supplementary Figure 1b). These differences are caused by the double bond inductive effects on the carbonyl group (Lopes et al. 2016; Silva et al. 2012). ^{13}C NMR was also reported to be used in regiospecific analysis, especially for evaluating the positional (*sn*-2 and *sn*-1,3) saturated and unsaturated fatty acid compositions (Kok, Chuah, and Cheng 2018; Morselli Ribeiro et al. 2017).

In an ideal 1,3-specific IE reaction, the fatty acid composition in the *sn*-2 position remains unaltered. However, modification in the *sn*-2-fatty acid is often observed during EIE process in practice and under certain conditions, a completely randomized fatty acid distribution is obtained. Svensson et al. studied randomization in the *sn*-2 position in TAG and found that during the reaction, palmitic acid migrated to *sn*-2 while oleic acid was removed from the *sn*-2 position (Svensson and Adlercreutz 2011). After 24 h, acyl groups at *sn*-2 were completely randomized and the total composition in the TAG were reassembled. When *sn*-1,3 regioselective lipases are used, fatty acid at *sn*-2 theoretically

remained unmodified. However in reality, alteration of fatty acid at *sn*-2 is observed which are proposed to be caused by acyl migration (Cao et al. 2016). In the structured lipid industry, the preparation of human milk fat substitutes and cocoa butter equivalent inclined to the use of enzymes which are of high regioselectivity to prevent the occurrence of acyl migration. Conversely, nonselective enzymes are used during the production of margarine fats as occurrence of acyl migration is deemed to be desirable and beneficial as all the fatty acid positions in the TAG must be accessible. Yang et al. reported on the similar ID in a reaction catalyzed by Lipozyme TL IM (immobilized *Thermomyces lanuginosus*) and Lipozyme RM IM (immobilized *Rhizomucor miehei*), and the *sn*-1,3 specific Lipozyme TL IM. The ID was measured by observing the change of C16:0 and C18:2 at *sn*-2 (Yang et al. 2014). As the reaction time increases, the concentration of C16:0 at *sn*-2 increased and this increment was significantly higher in the reaction that was catalyzed by Lipozyme RM IM. It was suggested that Lipozyme TL IM has a lower specificity for *sn*-1,3. Zhao et al. reported that although Lipozyme TL IM enzyme is a *sn*-1,3 positional-specific lipase, the fatty acid composition at *sn*-2 in the product was altered after IE (Zhao, Hu, Zhu, Bai, et al. 2014). This phenomenon was attributed to the occurrence of acyl migration as a side reaction.

In an EIE process that is catalyzed by *sn*-1,3 specific enzyme, acyl migration is influenced by the high reaction temperature and long reaction time (Mohd Hassim, Ismail, and Mat Dian 2018; Oh et al. 2009; Sivakanthan, Jayasooriya, and Madhujith 2019; Svensson and Adlercreutz 2011). Cao et al. indicated that both the type of lipase (*T. lanuginosus*) and the assisting material (silica), influenced the fatty acid exchange at *sn*-2 (Cao et al. 2016). During a TAG-TAG EIE reaction, application of silica gel promoted the manifestation of acyl migration. The acyl exchange of fatty acids at *sn*-1,3 was considerably faster compared to that at *sn*-2 when the reaction was catalyzed by using Lipozyme TL IM. Nonetheless, a completely randomized distribution is possible when the reaction is performed for 24 h.

Theoretically, sn-1,3 specific lipase reacts with the carbonyl group at sn-1 and sn-3 ester bonds in TAG to produce an intermediate product, sn-2,3(1,2)- glycerylate anions (Figure 5). This intermediate product then attacks the carbonyl group at sn-1 and sn-3 which is activated by other TAG, achieving positional-specific IE reaction. However, external conditions promote sn-2,3(1,2)- glycerylate anions to attack the intermolecular-carbonyl group at sn-2 and produce an intermediate sn-1,3-glycerylate anion. When sn-1,3-glycerylate anions interacted with another activated sn-1 or sn-3 carbonyl which then undergo carbonyl addition reaction, the position of acyl group at sn-2 is changed. Acyl migration weakens the specificity of the sn-1,3- specific lipase and the degree of acyl migration is inferred to be affected by the reaction temperature, reaction time, lipase and etc. Acyl migration is a side reaction that occur alongside IE, depending on the external factors, it can be promoted or inhibited. At present, there is only few reports on the mechanism of acyl migration during IE catalyzed by sn-1,3-specific lipase (Lopes et al. 2016). Some studies evaluated the degree of acyl migration by monitoring the content of a particular fatty acid at the sn-2 position (Liu et al. 2017), and this method has a drawback for being able to evaluate only on a particular type of fatty acid (not universal). In summary, there is still insufficient study on the evaluation methods on the degree of acyl migration and the effect of external factors on acyl migration.

A perspective on *sn*-fatty acid-derived reaction degree evolution methods

Determination of the change in the physicochemical properties of interesterified oil or the change in TAG (based on the total carbon number and ECN), neither method can offer actual measurement of the ID during a reaction catalyzed using sn-1,3 specific lipase, in which the effect of acyl migration on the ID, has not been fully considered. If the application of sn-1,3 specific lipase does not cause acyl migration during the IE, only the exchange of acyl group occurs at the sn-1 and sn-3 positions on the glycerol skeleton and this is promoted by external conditions, the evaluation of the degree of acyl migration can then be done through monitoring the change of fatty acid at the sn-2 position.

The ID of a sn-1,3 specific lipase catalyzed reaction cannot be accurately determined and therefore it is interesting to propose a potential evaluation method. External conditions not only affect the IE rate, but also lead to the occurrence and development of acyl migration, thus weakening the specificity of the sn-1,3 specific lipase and affects the yield and structure of TAG. Thus, it is important to conduct a systematic research to determine the acyl migration mechanism during an IE process catalyzed by sn-1,3 specific lipase in oil samples which in turns can provide a theoretical foundation for IE of TAG to obtain ideal food specialty fats.

The aforementioned methods are currently used to monitor the change in *sn*-position during IE for both the industry and scientific research. However, there are some disadvantages to the application of these methods. For instance, SFC and SMP determination methods are too empirical. Determination via measurement of TAG compositions are sometimes too complicated due to the composition of the raw materials as the measurement is based only on the total carbon number and there is no accurate method to determine all TAG species (including isomers). Calculation based on acyl-exchange has low accuracy as it is difficult to distinguish the total carbon number and molecular weight of short and medium fatty acids to the long-chain MAG or DAG present in the sample. The measurement of the *sn*-2 fatty acid can reflect the ID to a certain extent but it is also limited to the case of randomized IE.

The specificity of a *sn*-1, 3 lipase is reduced due to the occurrence of acyl migration which in turn, affects the properties of the interesterified product. Therefore, it is crucial to reveal the law of acyl migration that occurs during the *sn*-1,3 specific lipase catalyzed IE reaction in fats and oils. Insights acquired from the acyl migration law aids in

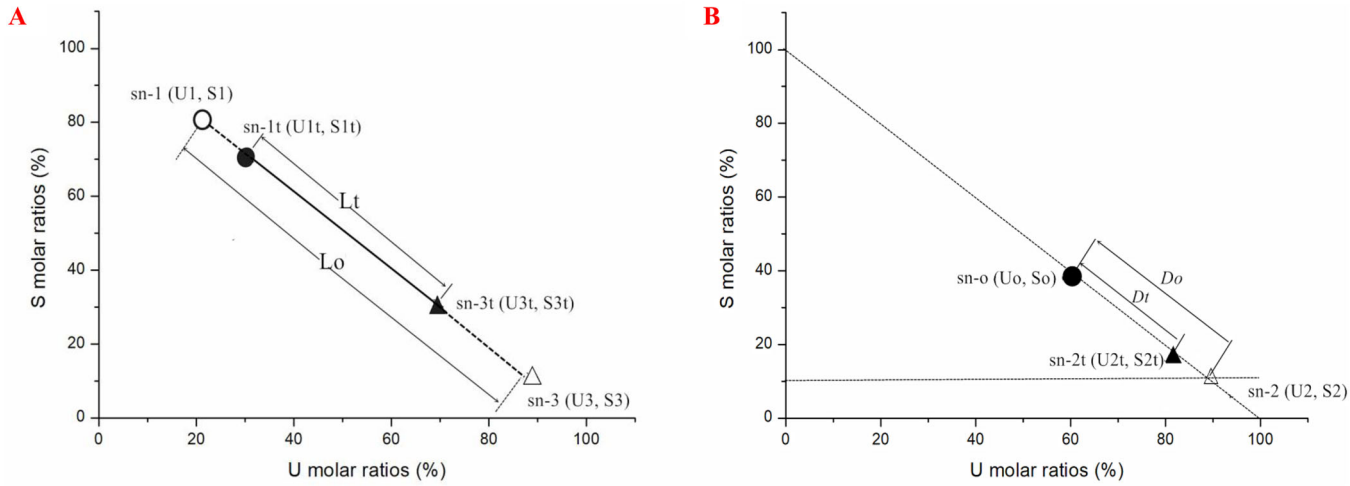


Figure 6. (a) Calculation model for IE degree (calculated based on the ratio of U and S in the *sn*-1 and *sn*-3 position), and (b) calculation model for the acyl transfer degree at the *sn*-2 position (calculated based on the ratio of U and S in the fatty acid at *sn*-2).

obtaining a breakthrough technology that is able to control the acyl migration and finally a proposition of a theory for IE to produce products with specific physical and nutritional properties (Pacheco, Crapiste, and Carrín 2015).

Namal et al. performed stereospecific analysis to determine the positional distribution of fatty acids in the TAG by using Grignard reaction combined with the stereospecific hydrolysis of synthetic phospholipids by phospholipase A2 (Namal Senanayake and Shahidi 2002). We propose a modification on this method by using phospholipase A1 to carry out the stereospecific hydrolysis step (Supplementary Figure 2). From this reaction, the fatty acid composition at *sn*-1 of TAG in modified oils could then be identified. Determination of the fatty acid composition at *sn-2* of the sample can be done according to the method as aforementioned. The regiospecific fatty acid distribution at the *sn*-1, *sn*-2, *sn*-3, could then be obtained from the following equation:

$$\mathrm{sn}(3)\% = 3 \times \mathrm{total}\% - (\mathrm{sn}(1)\% + \mathrm{sn}(2)\%) \tag{2}$$

Sn-1,3 position ID determination

For example, one of the main TAG in palm oil is in the form of POO (approximately 23%). During a *sn*-1,3 positional-specific EIE reaction, the TAG in the forms of POP and OOO, will be obtained. From Figure 6, new TAG is synthesized through the *sn*-1,3 positional-specific modification with significant alteration in the fatty acid distribution in the *sn*-1 and *sn*-3 positions. According to the theory of randomization, the type and molar amount of the fatty acid will equilibrate and the equilibrium point of the reaction is the end point of the ID. In order to establish the analysis and calculation method of ID in a more accurate manner, we can simplify the fatty acid composition of palm oil to S (saturated fatty acid which consist of 12:0 lauric acid + 14:0 myristic acid + 16:0 palmitic acid +18:0 stearic acid) and U (unsaturated fatty acids which consist of 18:1 oleic acid + 18:2 linoleic acid + 18:3 linolenic acid).

During the sn-1,3 specific lipase-catalyzed IE, the distribution of fatty acids at sn-1 and sn-3 changes according to the random distribution theory, whereby the types and molar ratios of the fatty acids tend to equilibrate at the end of the reaction. Similarly, the molar percentages of the fatty acyl group at sn-1 and sn-3 is altered during IE. An evaluation model for the ID can be established by assuming the molar percentage of (U) and (S) at sn-1 and sn-3 molar percentage is similar (Lt = 0) as the equilibrium end point of the IE process.

$$\begin{aligned}\mathrm{ID}\ (\%) &= \left(1 - \frac{Lt}{Lo}\right) \times 100\% \\ &= \left(1 - \frac{\sqrt{(\mathrm{U3}t - \mathrm{U1}t)^2 + (\mathrm{S3}t - \mathrm{S1}t)^2}}{\sqrt{(\mathrm{U3} - \mathrm{U1})^2 + (\mathrm{S3} - \mathrm{S1})^2}}\right) \times 100\%\end{aligned} \tag{3}$$

U1 and S1 are the initial molar percentages of U and S at sn-1; U1*t* and S1*t* are molar percentages of U and S at reaction time, *t*, at sn-1; U3 and S3 are the initial molar ratios of U and S at sn-3; U3*t* and S3*t* are molar percentages of U and S at reaction time, *t*, at sn-3. Lt is the distance between sn-1*t* and sn-3*t* at reaction time (t), and L_0 is the distance between sn-1 and sn-3 at the start of the reaction. In a sn-1,3-specific lipase-catalyzed IE, change in the molar ratios of U and S at sn-1 and sm-3 tends to be linear, the shorter is the distance of Lt, the higher is the ID. When U1*t*=U3*t* and S1*t*=S3*t*, *Lt* = 0, whereby the ID reaches 100%.

Sn-2 position acyl migration degree determination

Since acyl migration affects the specificity of IE at sn-1 and sn-3, fatty acid compositions and content at sn-2 will be significantly altered. Thus, acyl migration can be monitored via determining the change of sn-2 fatty acid under different reaction conditions. Due to the complexity of the fatty acid compositions in natural fats and oils, we found that the evaluation method based on the monitoring of the change in certain type of fatty acid is not universal and the calculation for each type of fatty acid are inconsistent. In order to

establish a more accurate ID calculation method, fatty acid compositions of the reaction substrates can be simplified into S and U as well. When there is an occurrence of acyl migration during the sn-1,3 specific lipase-catalyzed IE, molar ratios of U and S at sn-2 will be modified according to the total molar ratio of U and S in the substrate, which is also the equilibrium point of the reaction.

$$\text{Acyl migration degree } (\%) = \left(1 - \frac{Dt}{Do}\right) \times 100\% = \left(1 - \frac{\sqrt{(\mathrm{U2}t - \mathrm{U}o)^2 + (\mathrm{S2}t - \mathrm{S}o)^2}}{\sqrt{(\mathrm{U2} - \mathrm{U}o)^2 + (\mathrm{S2} - \mathrm{S}o)^2}}\right) \times 100\% \quad (4)$$

U2 and S2 are the initial molar ratios of U and S at sn-2 of the substrate; U2*t* and S2*t* are the molar ratios of U and S at sn-2 after reaction time, *t*; U*o* and S*o* are molar ratios of U and S of the initial total fatty acid, *Dt* is the distance between the sn-2t and sn-o after reaction time, t; *Do* is the distance between sn-2 to sn-o at the start of reaction. When *Dt*=*Do*, it can be proposed that the sn-1,3 specific lipase has the strongest catalytic specificity with the absence of acyl migration, resulting in IE only occurs at sn-1 and sn-3. When $Dt = 0$, the specificity of sn-1,3 specific lipase has the weakest catalytic specificity and the highest occurrence of acyl migration can be observed. The reaction achieves a completely randomized IE at sn-1, sn-2 and sn-3. Therefore, the molar ratios of U and S in the total fatty acid of the initial substrate (which is also in a completely randomized IE state), is taken as the equilibrium point of acyl migration, combined with the molar ratios of U and S in the sn-2 position of the interesterified sample in the actual reaction process. This change can be employed to quantify the degree of acyl migration.

sn-1,3 specific lipases for EIE reactions has been widely employed in the industry for the production of cocoa butter substitutes (*sn*-SUS-type), human milk fat substitutes (*sn*-USU-type) and etc (Xu 2000). Due to the underlying uncertainties during IE, research to confirm the specificity of commercially valuable lipases in different oil systems needs to be performed.

Conclusions

Through studying the EIE mechanism of a reaction that is catalyzed by *sn*-1,3 positional specific lipase, models for determining the ID and degree of acyl migration can be established. This model is useful for the development of key technologies in producing structured lipids and food-specialty fats. EIE performed in a traditional stirring tank reactor requires lower enzyme addition but the longer reaction time leads to the occurrence of acyl migration. This acyl migration can be controlled by adjusting the reaction conditions to promote acyl migration at the *sn*-2 position to prepare structured lipids which are rich in saturated fatty acids at the *sn*-2 position. With the use of a packed bed reactor (PBR), the reactions can be controlled by employing various types of specific lipases. In a PBR, the amount of oil in the enzyme reaction column is always in excess, the contact reaction time between the oil and the lipase can be reduced by increasing the flow rate. For example, by using a *sn*-1,3 specific lipase, the ID can be controlled and the rate of acyl migration can be reduced. This will result in a more uniform distribution of fatty acid at *sn*-1 and *sn*-3, leading to the production of a symmetrical TAG.

Disclosure statement

The authors have declared no conflict of interest.

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References

Abed, S. M., W. Wei, A. H. Ali, S. A. Korma, A. H. Mousa, H. M. Hassan, Q. Jin, and X. Wang. 2018. Synthesis of structured lipids enriched with medium-chain fatty acids via solvent-free acidolysis of microbial oil catalyzed by Rhizomucor miehei lipase. *LWT - Food Science and Technology* 93:306–15. doi: 10.1016/j.lwt.2018.03.057.

Adhikari, P., J. A. Shin, J. H. Lee, J. N. Hu, X. M. Zhu, C. C. Akoh, and K. T. Lee. 2010. Production of trans-free margarine stock by enzymatic interesterification of rice bran oil, palm stearin and coconut oil. *Journal of the Science of Food and Agriculture* 90 (4):703–11. doi: 10.1002/jsfa.3872.

Adhikari, P., J. A. Shin, J. H. Lee, H. R. Kim, I. H. Kim, S. T. Hong, and K. T. Lee. 2012. Crystallization, physicochemical properties, and oxidative stability of the interesterified hard fat from rice bran oil, fully hydrogenated soybean oil, and coconut oil through lipase-catalyzed reaction. *Food and Bioprocess Technology* 5 (6):2474–87. doi: 10.1007/s11947-011-0544-4.

Aktas, A. B., C. Alamprese, D. Fessas, and B. Ozen. 2019. IR spectroscopy and chemometrics for physical property prediction of structured lipids produced by interesterification of beef tallow. *LWT - Food Science and Technology* 110:25–31. doi: 10.1016/j.lwt.2019.04.057.

AOCS Official Method Cc 3-25. 1997. Slip melting point AOCS standard open tube melting point.

Berry, S. E. E. 2019. Triacylglycerol structure and interesterification of palmitic and stearic acid-rich fats: An overview and implications for cardiovascular disease. *Nutrition Research Reviews* 22 (1):3–17. doi: 10.1017/S0954422409369267.

Biswas, N., Y. L. Cheow, C. P. Tan, and L. F. Siow. 2018. Physicochemical properties of enzymatically produced palm-oil-based cocoa butter substitute (CBS) with cocoa butter mixture. *European Journal of Lipid Science and Technology* 120 (3):1700205. doi: 10.1002/ejlt.201700205.

Bogevik, A. S., H. Nygren, T. Balle, B. O. Haugsgjerd, and K. Kousoulaki. 2018. Enzymatic interesterification of heterotrophic microalgal oil with rapeseed oil to decrease the levels of tripalmitin. *European Journal of Lipid Science and Technology* 120 (7):1800063. doi: 10.1002/ejlt.201800063.

Bryś, J., L. F. V. Flores, A. Górska, E. Ostrowska-Ligęza, A. Bryś, T. Niemiec, and P. Koczoń. 2019. The synthesis followed by spectral and calorimetric evaluation of stability of human milk fat substitutes obtained from thistle milk and lard. *International Journal of Analytical Chemistry* 2019:5417962. doi: 10.1155/2019/5417962.

Cao, X., J. Mangas-Sánchez, F. Feng, and P. Adlercreutz. 2016. Acyl migration in enzymatic interesterification of triacylglycerols: Effects of lipases from Thermomyces lanuginosus and Rhizopus oryzae, support material, and water activity. *European Journal of Lipid Science and Technology* 118 (10):1579–87. doi: 10.1002/ejlt.201500485.

Chen, Y., X. Zhang, D. Li, H. Yi, T. Xu, S. Li, and L. Zhang. 2019. Fatty acid and triacylglycerol comparison of infant formulas on the Chinese market. *International Dairy Journal* 95:35–43. doi: 10.1016/j.idairyj.2019.02.017.

Danthine, S., E. Lefébure, H. N. Trinh, and C. Blecker. 2014. Effect of palm oil enzymatic interesterification on physicochemical and structural properties of mixed fat blends. *Journal of the American Oil Chemists' Society* 91 (9):1477–87. doi: 10.1007/s11746-014-2494-2.

de Paula, A. V., G. F. M. Nunes, H. F. de Castro, and J. C. dos Santos. 2018. Performance of packed bed reactor on the enzymatic interesterification of milk fat with soybean oil to yield structure lipids. *International Dairy Journal* 86:1–8. doi: 10.1016/j.idairyj.2018.06.014.

Dian, N. L. H. M., L. Y. Ying, N. M. A. N. Ibrahim, N. A. M. Hassim, S. M. Huey, T. C. Ping, and L. O. Ming. 2019. Low-temperature directed interesterification increases triunsaturated and trisaturated triacylglycerols of palm oil and affects its thermal, polymorphic and microstructural properties. *Journal of Oil Palm Research* 31 (1): 95–109. doi: 10.21894/jopr.2018.0046.

Dollah, S., S. M. Abdulkarim, S. H. Ahmad, A. Khoramnia, and H. M. Ghazali. 2015. Enzymatic interesterification on the physicochemical properties of Moringa oleifera seed oil blended with palm olein and virgin coconut oil. *Grasas y Aceites* 66 (2):e073. doi: 10.3989/gya.0695141.

Dollah, S., S. M. Abdulkarim, S. H. Ahmad, A. Khoramnia, and H. Mohd Ghazali. 2016. Physico-chemical properties of Moringa oleifera seed oil enzymatically interesterified with palm stearin and palm kernel oil and its potential application in food. *Journal of the Science of Food and Agriculture* 96 (10):3321–33. doi: 10.1002/jsfa.7510.

Ebrahimi, L., J. Farmani, and M. Bahmaei. 2017. Description of melting curves of enzymatically interesterified blends of fully hydrogenated palm olein and soybean oil by sigmoidal functions. *Food Bioscience* 17:29–34. doi: 10.1016/j.fbio.2016.12.003.

Farfán, M., M. J. Villalón, M. E. Ortíz, S. Nieto, and P. Bouchon. 2013. The effect of interesterification on the bioavailability of fatty acids in structured lipids. *Food Chemistry* 139 (1–4):571–7. doi: 10.1016/j.foodchem.2013.01.024.

Ghosh, M., A. Sengupta, D. K. Bhattacharyya, and M. Ghosh. 2016. Preparation of human milk fat analogue by enzymatic interesterification reaction using palm stearin and fish oil. *Journal of Food Science and Technology* 53 (4):2017–24. doi: 10.1007/s13197-016-2180-5.

Guedes, A. M. M., C. C. Ming, A. P. B. Ribeiro, R. C. Da Silva, L. A. Gioielli, and L. A. G. Gonçalves. 2014. Physicochemical properties of interesterified blends of fully hydrogenated crambe abyssinica oil and soybean oil. *Journal of the American Oil Chemists' Society* 91 (1):111–23. doi: 10.1007/s11746-013-2360-7.

Hu, P., X. Xu, and L. L. Yu. 2017a. Effect of fatty acid chain length on the crystallization behavior of trans-free margarine basestocks during storage. *Journal of Oleo Science* 66 (4):353–62. doi: 10.5650/jos.ess16210.

Hu, P., X. Xu, and L. L. Yu. 2017b. Interesterified trans-free fats rich in sn-2 nervonic acid prepared using Acer truncatum oil, palm stearin and palm kernel oil, and their physicochemical properties. *LWT - Food Science and Technology* 76:156–63. doi: 10.1016/j.lwt.2016.10.054.

Ilyasoglu, H. 2017. Production of structured lipid with a low omega-6/omega-3 fatty acids ratio by enzymatic interesterification. *Grasas y Aceites* 68 (2):191–11. doi: 10.3989/gya.0565161.

Jahurul, M. H. A., I. S. M. Zaidul, N. A. Nik Norulaini, F. Sahena, M. Z. Abedin, A. Mohamed, and A. K. Mohd Omar. 2014. Hard cocoa butter replacers from mango seed fat and palm stearin. *Food Chemistry* 154:323–9. doi: 10.1016/j.foodchem.2013.11.098.

Jin, J., C. C. Akoh, Q. Jin, and X. Wang. 2018. Preparation of mango kernel fat stearin-based hard chocolate fats via physical blending and enzymatic interesterification. *LWT - Food Science and Technology* 97:308–16. doi: 10.1016/j.lwt.2018.07.018.

Jin, J., Q. Jin, C. C. Akoh, and X. Wang. 2019. Mango kernel fat fractions as potential healthy food ingredients: A review. *Critical Reviews in Food Science and Nutrition* 59 (11):1794–801. doi: 10.1080/10408398.2018.1428527.

Kadivar, S., N. De Clercq, D. Van de Walle, and K. Dewettinck. 2014. Optimisation of enzymatic synthesis of cocoa butter equivalent from high oleic sunflower oil. *Journal of the Science of Food and Agriculture* 94 (7):1325–31. doi: 10.1002/jsfa.6414.

Karupaiah, T., and K. Sundram. 2007. Effects of stereospecific positioning of fatty acids in triacylglycerol structures in native and randomized fats: A review of their nutritional implications. *Nutrition & Metabolism* 4:16–7. doi: 10.1186/1743-7075-4-16.

Khodadadi, M., and S. Kermasha. 2014. Modeling lipase-catalyzed interesterification of flaxseed oil and tricaprylin for the synthesis of structured lipids. *Journal of Molecular Catalysis B: Enzymatic* 102: 33–40. doi: 10.1016/j.molcatb.2014.01.011.

Kok, W. M., C. H. Chuah, and S. F. Cheng. 2018. Enzymatic synthesis of structured lipids with behenic acid at the sn-1, 3 positions of triacylglycerols. *Food Science and Biotechnology* 27 (2):353–66. doi: 10.1007/s10068-017-0271-3.

Korma, S. A., X. Zou, A. H. Ali, S. M. Abed, Q. Jin, and X. Wang. 2018. Preparation of structured lipids enriched with medium- and long-chain triacylglycerols by enzymatic interesterification for infant formula. *Food and Bioproducts Processing* 107:121–30. doi: 10.1016/j.fbp.2017.11.006.

Kowalska, D., E. Gruczynska, and M. Kowalska. 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):507–17. doi: 10.1007/s10973-014-3869-1.

Kowalska, D., M. Kostecka, K. Tarnowska, and B. Kowalski. 2014. Oxidative stabilities of enzymatically interesterified goose fat and rapeseed oil blend by rancimat and PDSC. *Journal of Thermal Analysis and Calorimetry* 115 (3):2063–70. doi: 10.1007/s10973-013-3125-0.

Kowalska, M., M. Woźniak, A. Krzton-Maziopa, S. Tavernier, Ł. Pazdur, and A. Żbikowska. 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. doi: 10.1080/01932691.2018.1467328.

Kowalski, B., K. Tarnowska, E. Gruczynska, and W. Bekas. 2004. Chemical and enzymatic interesterification of a beef tallow and rapeseed oil equal-weight blend. *European Journal of Lipid Science and Technology* 106 (10):655–64. doi: 10.1002/ejlt.200400973.

Lee, J. H., J. M. Son, C. C. Akoh, M. R. Kim, and K. T. Lee. 2010. Optimized synthesis of 1,3-dioleoyl-2-palmitoylglycerol-rich triacylglycerol via interesterification catalyzed by a lipase from Thermomyces lanuginosus. *New Biotechnology* 27 (1):38–45. doi: 10.1016/j.nbt.2009.10.006.

Lee, N. K., S. W. Oh, D. Y. Kwon, and S. H. Yoon. 2015. Production of 1, 3-dioleoyl-2-palmitoyl glycerol as a human milk fat substitute using enzymatic interesterification of natural fats and oils. *Food Science and Biotechnology* 24 (2):433–7. doi: 10.1007/s10068-015-0057-4.

Li, D., P. Adhikari, J.-A. Shin, J.-H. Lee, Y.-J. Kim, X.-M. Zhu, J.-N. Hu, J. Jin, C. C. Akoh, and K.-T. Lee. 2010. Lipase-catalyzed interesterification of high oleic sunflower oil and fully hydrogenated soybean oil comparison of batch and continuous reactor for production of zero trans shortening fats. *LWT - Food Science and Technology* 43 (3):458–64. doi: 10.1016/j.lwt.2009.09.013.

Li, Y., J. Zhao, X. Xie, Z. Zhang, N. Zhang, and Y. Wang. 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* 255:405–13. doi: 10.1016/j.foodchem.2018.02.086.

Liu, S., X. Dong, F. Wei, X. Wang, X. Lv, L. Wu, S. Y. Quek, and H. Chen. 2017. Lipase catalyzed synthesis of ABA-type structured lipid from single cell oil and tripalmtin. *Journal of Food Processing and Preservation* 41 (2):e12843. doi: 10.1111/jfpp.12843.

Lopes, T. I. B., M. D. M. M. Ribeiro, C. C. Ming, R. Grimaldi, L. A. G. Gonçalves, and A. J. Marsaioli. 2016. Comparison of the regiospecific distribution from triacylglycerols after chemical and enzymatic interesterification of high oleic sunflower oil and fully hydrogenated high oleic sunflower oil blend by carbon-13 nuclear magnetic resonance. *Food Chemistry* 212:641–7. doi: 10.1016/j.foodchem.2016.06.024.

Lopez-Hernandez, A., C. Otero, E. Hernández-Martín, H. S. Garcia, and C. G. Hill. 2007. Interesterification in batch and continuous flow processes of sesame oil and fully hydrogenated fat catalyzed by immobilized lipase. *European Journal of Lipid Science and Technology* 109 (12):1147–59. doi: 10.1002/ejlt.200700112.

Mohd Hassim, N. A., N. H. Ismail, and N. L. H. Mat Dian. 2018. Enzymatic interesterification of palm fractions for the production of cocoa butter alternatives. *Journal of Oil Palm Research* 30:537–47. doi: 10.21894/jopr.2018.0038.

Moore, M. A., and C. C. Akoh. 2017. Enzymatic interesterification of coconut and high oleic sunflower oils for edible film application. *Journal of the American Oil Chemists' Society* 94 (4):567–76. doi: 10.1007/s11746-017-2969-z.

Morselli Ribeiro, M. D. M., C. C. Ming, I. M. Silvestre, R. Grimaldi, and L. Ap. G. Gonçalves. 2017. Comparison between enzymatic and chemical interesterification of high oleic sunflower oil and fully hydrogenated soybean oil. *European Journal of Lipid Science and Technology* 119 (2):1500473–9. doi: 10.1002/ejlt.201500473.

Namal Senanayake, S. P. J., and F. Shahidi. 2002. Positional distribution of FA in TAG of enzymatically modified borage and evening primrose oils. *Lipids* 37 (8):803–10. doi: 10.1007/s11745-002-0964-5.

Neeharika, T. S. V. R., R. Rallabandi, Y. Ragini, S. S. Kaki, K. N. P. Rani, and R. B. N. Prasad. 2015. Lipase catalyzed interesterification of rice bran oil with hydrogenated cottonseed oil to produce trans free fat. *Journal of Food Science and Technology* 52 (8):4905–14. doi: 10.1007/s13197-014-1563-8.

Norizzah, A. R., K. Nur Azimah, and O. Zaliha. 2018. Influence of enzymatic and chemical interesterification on crystallisation properties of refined, bleached and deodourised (RBD) palm oil and RBD palm kernel oil blends. *Food Research International (Ottawa, Ont.)* 106:982–91. doi: 10.1016/j.foodres.2018.02.001.

Nunes, G. F. M., A. V. de Paula, H. F. de Castro, and J. C. dos Santos. 2011. Compositional and textural properties of milkfat-soybean oil blends following enzymatic interesterification. *Food Chemistry* 125 (1):133–8. doi: 10.1016/j.foodchem.2010.08.049.

Oh, J.-E., K.-W. Lee, H.-K. Park, J.-Y. Kim, K.-I. Kwon, J.-W. Kim, H.-R. Kim, and I.-H. Kim. 2009. Lipase-catalyzed acidolysis of olive oil with capric acid: Effect of water activity on incorporation and acyl migration. *Journal of Agricultural and Food Chemistry* 57 (19): 9280–3. doi: 10.1021/jf9023245.

Oliveira, P. D., A. M. C. Rodrigues, C. V. Bezerra, and L. H. M. Silva. 2017. Chemical interesterification of blends with palm stearin and patawa oil. *Food Chemistry* 215:369–76. doi: 10.1016/j.foodchem.2016.07.165.

Pacheco, C., G. H. Crapiste, and M. E. Carrín. 2015. Study of acyl migration during enzymatic interesterification of liquid and fully hydrogenated soybean oil. *Journal of Molecular Catalysis B: Enzymatic* 122:117–24. doi: 10.1016/j.molcatb.2015.08.023.

Pang, M., Y. Ge, L. Cao, J. Cheng, and S. Jiang. 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):131–9. doi: 10.5650/jos.ess18201.

Paula, A. V., G. F. M. Nunes, H. F. De Castro, and J. C. Santos. 2015. Synthesis of structured lipids by enzymatic interesterification of milkfat and soybean oil in a basket-type stirred tank reactor. *Industrial & Engineering Chemistry Research* 54 (6):1731–7. doi: 10.1021/ie503189e.

Rao, R., K. U. Sankar, K. Sambaiah, and B. R. Lokesh. 2001. Differential scanning calorimetric studies on structured lipids from coconut oil triglycerides containing stearic acid. *European Food Research and Technology* 212 (3):334–43. doi: 10.1007/s002170000254.

Reena, M. B., S. R. Y. Reddy, and B. R. Lokesh. 2009. Changes in triacylglycerol molecular species and thermal properties of blended and interesterified mixtures of coconut oil or palm oil with rice bran oil or sesame oil. *European Journal of Lipid Science and Technology* 111 (4):346–57. doi: 10.1002/ejlt.200800065.

Ribeiro, M. D. M. M., C. C. Ming, T. I. B. Lopes, R. Grimaldi, A. J. Marsaioli, and L. A. G. Gonçalves. 2018. Enzymatic synthesis of structured lipids from liquid and fully hydrogenated high oleic sunflower oil. *International Journal of Food Properties* 21 (1):702–16. doi: 10.1080/10942912.2018.1454943.

Rohm, H., C. Schäper, and S. Zahn. 2018. Interesterified fats in chocolate and bakery products: A concise review. *LWT - Food Science and Technology* 87:379–84. doi: 10.1016/j.lwt.2017.08.076.

Rousseau, D., S. M. Ghazani, and A. G. Marangoni. 2017. 12: Chemical interesterification. In *Food lipids: Chemistry, nutrition, and biotechnology*, eds. C. C. Akoh and D. B. Min. 4th ed., 351–83. New York, NY: Marcel Dekker.

Ruan, X., X. M. Zhu, H. Xiong, S. Wang, C. Bai, and Q. Zhao. 2014. Characterisation of zero-trans margarine fats produced from camellia seed oil, palm stearin and coconut oil using enzymatic interesterification strategy. *International Journal of Food Science & Technology* 49 (1):91–7. doi: 10.1111/ijfs.12279.

Santoro, V., F. Dal Bello, R. Aigotti, D. Gastaldi, F. Romaniello, E. Forte, M. Magni, C. Baiocchi, and C. Medana. 2018. Characterization and determination of interesterification markers (triacylglycerol regioisomers) in confectionery oils by liquid chromatography-mass spectrometry. *Foods* 7 (2):23. doi: 10.3390/foods7020023.

Sharma, M., and B. R. Lokesh. 2012. Effect of enzymatic trans- and interesterification on the thermal properties of groundnut and linseed oils and their blends. *Journal of the American Oil Chemists' Society* 89 (5):805–13. doi: 10.1007/s11746-011-1985-7.

Shi, C., M. Chang, R. Liu, Q. Jin, and X. Wang. 2015. Trans-free shortenings through the interesterification of rice bran stearin, fully hydrogenated soybean oil and coconut oil. *International Journal of Food Engineering* 11 (4):467–77. doi: 10.1515/ijfe-2014-0279.

Shin, J. A., Y. J. Heo, and K. T. Lee. 2019. Physicochemical characteristics of fat blend from hydrogenated coconut oil and acyl migrated palm mid-fraction. *Food Chemistry* 275:739–45. doi: 10.1016/j.foodchem.2018.09.131.

Silva, R. C. d., F. A. S. D. M. Soares, M. Hazzan, I. R. Capacla, M. I. A. Gonçalves, and L. A. Gioielli. 2012. Continuous enzymatic interesterification of lard and soybean oil blend: Effects of different flow rates on physical properties and acyl migration. *Journal of Molecular Catalysis B: Enzymatic* 76:23–8. doi: 10.1016/j.molcatb.2011.11.021.

Sivakanthan, S., A. P. Jayasooriya, and T. Madhujith. 2019. Optimization of the production of structured lipid by enzymatic interesterification from coconut (Cocos nucifera) oil and sesame (*Sesamum indicum*) oil using Response Surface Methodology. *LWT - Food Science and Technology* 101:723–30. doi: 10.1016/j.lwt.2018.11.085.

Soekopitojo, S. 2009. Enzymatic interesterification of palm oil midfraction blends edit. *Asian Journal of Food and Agro-Industry* 2 (4): 807–16.

Speranza, P., K. M. M. Leão, T. S. Narciso Gomes, L. V. C. Reis, A. P. Rodrigues, J. Alves Macedo, A. P. B. Ribeiro, and G. Alves Macedo. 2018. Improving the chemical properties of Buriti oil (*Mauritia flexuosa* L.) by enzymatic interesterification. *Grasas y Aceites* 69 (4): 282–8. doi: 10.3989/gya.0229181.

Sun, C., W. Wei, X. Zou, J. Huang, Q. Jin, and X. Wang. 2018. Evaluation of triacylglycerol composition in commercial infant formulas on the Chinese market: A comparative study based on fat source and stage. *Food Chemistry* 252:154–62. doi: 10.1016/j.foodchem.2018.01.072.

Svensson, J., and P. Adlercreutz. 2011. Effect of acyl migration in Lipozyme TL IM-catalyzed interesterification using a triacylglycerol model system. *European Journal of Lipid Science and Technology* 113 (10):1258–65. doi: 10.1002/ejlt.201100097.

Viriato, R. L. S., M. d S. Queirós, M. A. S. da Gama, A. P. B. Ribeiro, and M. L. Gigante. 2018. Milk fat as a structuring agent of plastic lipid bases. *Food Research International* 111 (May):120–9. doi: 10.1016/j.foodres.2018.05.015.

Wang, X.-Y., D. Yang, L.-J. Gan, H. Zhang, J.-A. Shin, Y.-H. Lee, Y.-S. Jang, and K.-T. Lee. 2015. Effect of positional distribution of Linoleic acid on oxidative stability of triacylglycerol molecules determined by 1H NMR. *Journal of the American Oil Chemists' Society* 92 (2):157–65. doi: 10.1007/s11746-015-2590-y.

Wirkowska-Wojdyła, M., J. Bryś, A. Górska, and E. Ostrowska-Ligęza. 2016. Effect of enzymatic interesterification on physiochemical and thermal properties of fat used in cookies. *LWT - Food Science and Technology* 74:99–105. doi: 10.1016/j.lwt.2016.07.040.

Xie, W., and X. Zang. 2016. Immobilized lipase on core-shell structured Fe3O4-MCM-41 nanocomposites as a magnetically recyclable biocatalyst for interesterification of soybean oil and lard. *Food Chemistry* 194:1283–92. doi: 10.1016/j.foodchem.2015.09.009.

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. doi: 10.1002/(sici)1438-9312(200004)102:4<287::aid-ejlt287>3.3.co;2-h.

Xu, Y., X. Zhu, X. Ma, H. Xiong, Z. Zeng, H. Peng, and J. Hu. 2018. Enzymatic production of trans-free shortening from coix seed oil, fully hydrogenated palm oil and *Cinnamomum camphora* seed oil. *Food Bioscience* 22:1–8. doi: 10.1016/j.fbio.2017.12.010.

Yamoneka, J., P. Malumba, G. Lognay, F. Béra, C. Blecker, and S. Danthine. 2018. Enzymatic inter-esterification of binary blends containing Irvingia gabonensis seed fat to produce cocoa butter substitute. *European Journal of Lipid Science and Technology* 120 (4): 1700423–9. doi: 10.1002/ejlt.201700423.

Yang, H., Y. Mu, H. Chen, C. Su, T. Yang, and Z. Xiu. 2014. Sn-1,3-specific interesterification of soybean oil with medium-chain triacylglycerol catalyzed by lipozyme TL IM. *Chinese Journal of Chemical Engineering* 22 (9):1016–20. doi: 10.1016/j.cjche.2014.06.027.

Yu, D., X. Qi, Y. Jiang, D. Zou, L. Wang, L. Jiang, and L. Qin. 2018. Preparation of margarine stock rich in naturally bioactive components by enzymatic interesterification. *Journal of Oleo Science* 67 (1): 29–37. doi: 10.5650/jos.ess17076.

Zarringhalami, S., M. A. Sahari, M. Barzegar, and Z. Hamidi-Esfehani. 2010. Enzymatically modified tea seed oil as cocoa butter replacer in dark chocolate. *International Journal of Food Science & Technology* 45 (3):540–5. doi: 10.1111/j.1365-2621.2009.02162.x.

Zhang, H., P. Smith, and J. Adler-Nissen. 2004. Effects of degree of enzymatic interesterification on the physical properties of margarine fats: Solid fat content, crystallization behavior, crystal morphology, and crystal network. *Journal of Agricultural and Food Chemistry* 52 (14):4423–31. doi: 10.1021/jf035022u.

Zhang, Z., W. J. Lee, H. Zhou, and Y. Wang. 2019. Effects of chemical interesterification on the triacylglycerols, solid fat contents and crystallization kinetics of palm oil-based fats. *Food & Function* 10 (11): 7553–64. doi: 10.1039/c9fo01648a.

Zhang, Z., X. Ma, H. Huang, and Y. Wang. 2017. Shea olein based specialty fats: Preparation, characterization and potential application. *LWT - Food Science and Technology* 86:492–500. doi: 10.1016/j.lwt.2017.08.035.

Zhang, Z., Y. Y. Shim, X. Ma, H. Huang, and Y. Wang. 2018. Solid fat content and bakery characteristics of interesterified beef tallow-palm mid fraction based margarines. *RSC Advances* 8 (22):12390–9. doi: 10.1039/C8RA00769A.

Zhao, M. L., J. N. Hu, X. M. Zhu, H. Y. Li, J. Li, Y. W. Fan, and Z. Y. Deng. 2014. Enzymatic synthesis of medium- and long-chain triacylglycerols-enriched structured lipid from *Cinnamomum camphora* seed oil and camellia oil by Lipozyme RM IM. *International Journal of Food Science & Technology* 49 (2):453–9. doi: 10.1111/ijfs.12322.

Zhao, M.-L., L. Tang, X.-M. Zhu, J.-N. Hu, H.-Y. Li, L.-P. Luo, L. Lei, and Z.-Y. Deng. 2013. Enzymatic production of zero-trans plastic fat rich in α-linolenic acid and medium-chain fatty acids from highly hydrogenated soybean oil, *Cinnamomum camphora* seed oil, and perilla oil by lipozyme TL im. *Journal of Agricultural and Food Chemistry* 61 (6):1189–95. doi: 10.1021/jf305086j.

Zhao, S.-Q., J.-N. Hu, X.-M. Zhu, C.-Q. Bai, H.-L. Peng, H. Xiong, J.-W. Hu, and Q. Zhao. 2014. Characteristics and feasibility of trans-free plastic fats through lipozyme TL IM-catalyzed interesterification of palm stearin and akebia trifoliata variety Australis seed oil. *Journal of Agricultural and Food Chemistry* 62 (14):3293–300. doi: 10.1021/jf500267e.

Zhou, L., C. Zhang, F. Liu, Z. Qiu, and Y. He. 2019. Application of deep learning in food: A review. *Comprehensive Reviews in Food Science and Food Safety* 18 (6):1793–19. doi: 10.1111/1541-4337.12492.

Zhu, T., Q. Liu, H. Weng, B. Li, X. Zhang, M. Zong, and H. Wu. 2018. Effect of temperature on the crystallization behavior and physical properties of fast-frozen special fat during storage. *Journal of Food Engineering* 223:53–61. doi: 10.1016/j.jfoodeng.2017.12.002.

Zhu, T., H. Weng, X. Zhang, H. Wu, and B. Li. 2018. Mechanistic insight into the relationship between triacylglycerol and crystallization of lipase-catalyzed interesterified blend of palm stearin and vegetable oil. *Food Chemistry* 260:306–16. doi: 10.1016/j.foodchem.2018.03.147.

Zhu, T., Y. Zhao, M. Zong, B. Li, X. Zhang, and H. Wu. 2017. Improvement of physical properties of palm stearin and soybean oil blends by enzymatic interesterification and their application in fast frozen food. *RSC Advances* 7 (55):34435–41. doi: 10.1039/C7RA02829F.