

Modern Approaches to Oil and Fat Modification through Interesterification: A Critical and Comprehensive Review

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1. ABSTRACT

The modification of edible oils and fats through interesterification (IE) has emerged as a sustainable alternative for producing trans fat-free lipid systems with desirable functional properties. In this study, binary blends of vegetable oils and fats were subjected to both chemical and enzymatic interesterification, and the resulting products were evaluated for their physicochemical characteristics. Key quality parameters including free fatty acids (FFA), iodine value, peroxide value (PV), soap content, plastic range, slip melting point (SMP), solid fat index (SFI), oxidative stability, and triacylglycerol composition were systematically analysed and compared with commercially available margarine fats. The interesterified blends exhibited complete melting at body temperature, indicating their suitability for a diverse range of fat-based food applications. Chemical interesterification resulted in increased FFA and soap content, accompanied by reductions in PV, oxidative stability at 110 °C, SMP, and SFI, thereby shifting the plastic range toward lower temperatures. Conversely, enzymatically interesterified fats demonstrated lower SMP and SFI than their chemically interesterified counterparts, reflecting a more controlled structural rearrangement. Due to the non-specific catalytic nature of chemical IE, the potential for undesirable side reactions is greater compared to the highly specific enzymatic process. Overall, the findings highlight enzymatic interesterification as a greener, safer, and more effective approach for producing trans fat-free structured lipids suitable for industrial applications.

KEYWORDS: Interesterification (IE), Enzymatic lipase-catalyzed IE, Trans fat-free structured lipids, Binary vegetable fat blends, Thermal and physicochemical properties, Oxidative and melting characteristics

2. INTRODUCTION

Fat modification technologies, namely fractionation, hydrogenation, and interesterification, play a crucial role in expanding the industrial applications of edible oils and fats. Fractionation involves crystallization-driven mass transfer, whereas hydrogenation and interesterification are catalytic processes that rely on either chemical catalysts or enzymes. Hydrogenation can be partial or complete; however, partial hydrogenation promotes the formation of trans fatty acids (TFAs), which pose significant health risks and have consequently been banned worldwide [15,18].

Intesterification has gained prominence as a preferred fat modification method because it overcomes several limitations associated with simple blending. While blending can improve functionality to some extent, it often results in phase separation when oils with differing physicochemical properties are combined. In contrast, interesterification rearranges fatty acids on the glycerol backbone of triacylglycerols (TAGs), thereby producing trans fat-free, plastic fats with improved physicochemical and functional attributes.

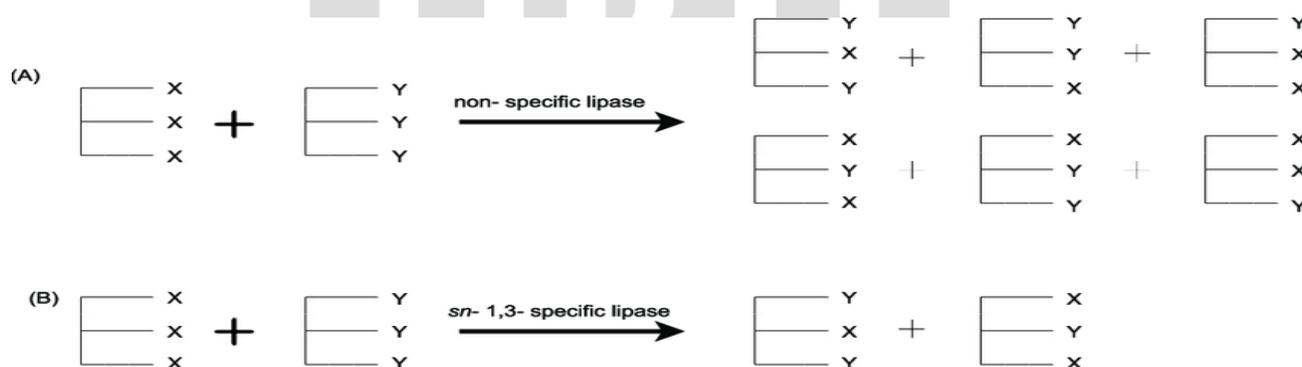
Intesterification may be catalyzed chemically (chemical interesterification, CIE) or enzymatically using lipases (enzymatic interesterification, EIE). EIE offers several advantages over CIE, including higher yields, lower processing losses, reduced cleaning requirements, and improved product quality. Lipase catalysts exhibit high positional and fatty acid specificity, enabling precise modification while minimizing the

formation of impurities or contaminants. Furthermore, EIE operates effectively at lower temperatures, allowing tighter control of reaction conditions and facilitating manipulation of thermodynamically reversible reactions [32]. In contrast, CIE may generate undesirable byproducts and toxic compounds that reduce oil recovery and negatively impact environmental sustainability [18].

Owing to its high selectivity, low environmental impact, and ability to produce zero-trans products, EIE is increasingly recognized as a green and emerging technology. Lipases catalyze hydrolysis of ester bonds as well as esterification and transesterification reactions (acidolysis, alcoholysis, and interesterification). Their catalytic behavior parallels that of sodium methoxide used in CIE, but with significantly greater specificity and reduced contaminant formation. These enzymes are highly effective in producing structured lipids (SLs) TAGs engineered to alter fatty acid composition and/or positional distribution making them valuable tools for functional lipid design [31].

This review evaluates advancements in chemical and enzymatic interesterification, with emphasis on the modification of binary and ternary fat blends, their characterization, and their potential applications in food industries such as margarine and shortening. Numerous blends have been studied, including Palm Stearin (PS) with Palm Kernel Olein, Soybean Oil with Walnut Oil, Palm Stearin with Palm Olein, Coconut Stearin with Palm Stearin, and Rice Bran Oil with Hydrogenated Cottonseed Oil. These blends were subjected to interesterification, characterized, and analyzed to determine the extent of TAG rearrangement and their suitability for industrial applications.

Additionally, emerging approaches such as genetic modification of lipid profiles through crossbreeding and clonal selection have demonstrated potential for developing oil-bearing crops with enhanced properties. In olives, such techniques have resulted in cultivars exhibiting improved disease resistance, pest tolerance, and resilience to abiotic stresses [7].



X and Y are two types of fatty acid

Fig: 1 Action of Lipase on Triglyceride molecule

S. No.	Characteristics	Chemical interesterification	Enzymatic interesterification
1	Catalyst	It involves a chemical catalyst (sodium methoxide)	It involves an enzyme as a catalyst (<i>Rhizomucor miehei</i> lipase)
2	Specificity	It is a random process (non-specific)	It is more specific (regio-, fatty- and stereo-specificity)
3	Reaction Temperature	The reaction requires a higher temperature (70-120°C) which further initiates side reaction	The reaction is done at milder temperatures (55°C-70°C)
5	Stability	Reduction in oxidative stability (loss of natural antioxidants)	Improvement in Oxidative stability (as low temperature retains the tocopherol)
6	Environmental concern	The chemical catalyst used are hazardous to the environment	It is environmental friendly
7	Treatments	It requires more pre and post-treatment	It requires fewer pre and post-treatments such as bleaching and deodorization
8	Effect on flavor	It can show flavor reversion	It does not show flavor reversion
9	By-product formation	Formation of by-products like sodium salts and high processing loss due to saponification	It generates fewer by-products
10	Cost	Chemical used is less expensive compared to enzyme	The enzyme used as a catalyst is expensive
11	Technical challenge	It is highly reproducible and easily operated in a batch configuration	It is a complicated process, and chances of cross-contamination increase due to continuous

Fig:2: Comparison for EIE & CIE

3. Preparation of Blends:

The oils used in the specific blends were procured from their respective sources and gently melted at controlled temperatures to enable uniform mixing in predetermined proportions appropriate for food industry applications.

3.1 Enzymatic Interesterification:

The prepared blends were combined with the enzyme catalyst and subjected to continuous stirring to ensure uniform interaction between the substrate and the enzyme. The reaction temperature was carefully maintained throughout the process to prevent substrate solidification and to sustain optimal enzymatic activity. Upon completion of the reaction, the mixture was filtered to recover the enzyme catalyst and to obtain an enzyme-free interesterified product.

3.2 Chemical Interesterification:

Initially, the oils were melted at their respective specified temperatures and blended in predetermined ratios. The resulting mixtures were subjected to vacuum drying at 100 °C to remove residual moisture. Following dehydration, the required amount of sodium methoxide was added to each blend, and the interesterification reaction was carried out under continuous monitoring. The process was continued until the slip melting point (SMP) reached a constant value, which was used as an indicator of reaction equilibrium. Upon attaining equilibrium, a 2% (w/v) aqueous citric acid solution was introduced to neutralize and inactivate the sodium methoxide catalyst. The reaction mixture was subsequently filtered to remove residual catalyst and citric acid, yielding the final interesterified product.

BINARY BLENDS: Enzymatic IE**Table 1: the ratio and reaction conditions of various binary blends using EIE**

Sr. no.	Blends	Ratio (w/w)					Enzymes used/ Conc. (by wt.)	Reaction conditions	Reference
1	Palm Stearin/ Palm Kernel Olein	30: 70	40: 60	50: 50	60: 40	70:3 0	Lipozyme (1%)	60°C (6 hours; at 2 hours interval)	19
2	Soybean oil/Walnut Oil	20: 80	40: 60	-	60: 40	-	Lipozyme TL IM (5%)	70±2°C, (1/2, 2, 4 hours)	27
3	Palm Stearin/ Palm Olein	35: 65	40: 60	-	60: 40	-	Rhizopus oryzae lipase (1000 IU)	50°C ;72 hours	18
4	Coconut Stearin/ Palm Stearin	30: 70	40: 60	50: 50	60: 40	70:3 0	Lipozyme TL IM (6%)	70°C, 30 min	10
5	Rice Bran Oil/ Hydrogenated Cottonseed Oil	-	-	-	60: 40	70:3 0	Novozym 435 (5%)	60°C, 6 hours	13
6	Palm Stearin and Soybean oil	-	-	-	-	70:3 0	Lipozyme TL IM (4%)	70°C, 30 min	14
7	Rapeseed oil & fully hydrogenated rapeseed oil	-	-	80: 20	60: 40	70:3 0	Lipozyme TL IM (4%)	70°C, 8 hours	15
8	Hard Palm Stearin/ Rice Bran Oil	30: 70	40: 60	50: 50	60: 40	70:3 0	Lipozyme TL IM (10%)	60°C, 6 hours	17
9	FHPOlein/ Soybean oil	25: 75	35: 65	45: 55	55: 65 65: 35	75:2 5 85:1 5	Lipozyme TL IM (6%)	70°C, 6 hours	12
10	Canola oil/ palm olein	58: 42	45: 55	30: 70	25: 75	-	Lipozyme TL IM (6%)	70°C, 4 hours	1
11	PO/SFO	80: 20	60: 40	50: 50	40: 60	20:8 0	Lipozyme TL 8%	70°C, 30 min	22
12	Hybrid PS/ PK oil	-	-	-	-	70:3 0	Lipozyme TL IM (10kg)	70°C	8

BINARY BLENDS: Chemical IE**Table 2: the ratio and reaction conditions of various binary blends using CIE**

S r · n o ·	Blends	Ratio w/w				Reaction condition s	Sodi m metho xide used (%)	Citric acid (%)	Refere nces
1	Palm stearin/ sunflower oil	1 0 : 9 0	3 0: 7 0	50:50	-	90°C, 1 h, 0.8 bar, 300 rpm	0.5	2	2
2	Soybean oil/ walnut oil	2 0 : 8 0	4 0: 6 0	6 0: 4 0	-	90±2 °C, for 10, 15, 60 min, 150 rpm	0.5	1.78 g per g of sodium metho xide	27
3	Palm stearin/ patawa oil	3 0 : 7 0	4 0: 6 0	5 0: 5 0	60 :4 0	70°C, 1 h, 400 rpm, 20-30 mm Hg	1	-	

TERNARY BLENDS: Enzymatic IE**Table 3: Enzymes used for the EIE of ternary blends**

Sr. no.	Blends	Enzymes used	References
1	Palm Stearin/ Palm Kernel Oil/ Soybean Oil	Lipozyme TL IM	25
2	Palm mid fraction/ Palm Stearin/Medium Chain Triglycerides	Lipase IM 60	21
3	PO/Palm Olein/ Soft PMF	Lipozyme TL IM	23

TERNARY BLENDS: Chemical IE**Table 4: the reaction conditions used for the CIE of ternary blends**

Sr. No.	Blends	Ratio w/w		Reaction conditions	Sodium methoxide used(%)	References
1	Refined olive oil/ tristearin/palm oil	75:15:10	85:10:5	100°C, 50 min	0.5	16

4. Characterization of properties of IE Blends

- a. **Iodine value:** It is the degree of unsaturation of oil or fat. It is defined as the grams of iodine absorbed by 100 grams of oil/fat.

Characterization of IV: It was calculated using the following equation.

$$\text{Iodine value} = (\% \text{ C18:1} \times 0.89) + (\% \text{ C18:2} \times 1.732) + (\% \text{ C18:3} \times 2.616)$$

- b. **Slip melting point (SMP):** The slip melting point of oil or fat is the temperature at which the sample of oil/fat in rises in an open capillary tube upon heating.

Characterization of SMP: The SMP of blends was examined following the AOCS Cc 3-25 open tube melting point method after being tempered at $6 \pm 1^\circ\text{C}$ for 16 hours (AOCS, 1996).

- c. **Oxidizability measurement by Rancimet:** It measures the degree of oxidation, up to what time the fat is resistant to oxidation.

Characterization of Oxidizability: Based on the contents of oleic acid (C18:1), linoleic acid (C18:2), and linolenic acid (C18:3), the oxidizability of fats was calculated according to equation

$$\text{Oxidizability} = [(\% \text{ C18:1} \times 0.02) + (\% \text{ C18:2}) + (\% \text{ C18:3} \times 2)] / 100$$

- d. **Solid Fat Content (SFC):** Solid Fat Content is the percentage of lipids that are solid at the selected temperatures. Because the SFC affects physical characteristics such as spreadability, hardness, mouthfeel, organoleptic properties and stability, SFC values are essential for defining the properties of plastic fats and are regarded as a qualitative measure of margarine texture. Since IE influences the physical properties of lipids such as Melting point, SFC is an indicator to monitor the degree of interesterification.

Characterization of SFC: The solid fat content of interesterified blends was measured using pulsed nuclear magnetic resonance (p-NMR) according to the AOCS Official Method Cd 16–81.

- e. **Polymorphism:** It is the ability of fat to have different crystalline forms. Since physical properties of fat such as melting point, rheology are important for texture of food, controlling of polymorphism of fat crystals is necessary for food industry.

Characterization of Polymorphism:

Different polymorphic forms of fat in the blends were determined by X- Ray Diffraction.

- f. **Texture:** Texture of fat is very important for value added products which are to be made from modified fat blends. It influences mouthfeel and sensory evaluation.

Characterization of Texture:

It is determined by a constant speed compression test by texture analyzer.

5. INDUSTRIAL APPLICATION

Historically, enzymatic interesterification (EIE) was considered an expensive and less commercially viable technology. Over time, advancements in catalytic systems led to the parallel development of both chemical interesterification (CIE) and EIE. Compared with hydrogenation, CIE produced fats of improved functionality but still carried the risk of generating trans fatty acids under certain conditions. In contrast, EIE greatly minimized the formation of trans fats and other undesirable by-products due to its high catalytic specificity and mild reaction conditions. As EIE technology has progressed, its cost efficiency and product versatility have significantly improved, enabling the production of a wide range of tailored lipids for food and oil industries.

EIE modifies the physical and functional attributes of edible fats and yields a diverse portfolio of lipid products, including confectionery fats, margarines, shortenings, human-milk-fat substitutes, structured lipids, fat replacers, and formulations aimed at reducing trans-fat content. Interesterification is therefore a valuable tool for producing trans fat-free fats suitable for margarine and shortening applications. The fat phase strongly influences the physical and functional characteristics of these products, affecting parameters such as consistency, spreadability, color, visual appeal, flavor release, mouthfeel, and—particularly in margarines emulsion stability. Since there is a direct relationship between solid fat content (SFC) and the structure, plasticity, and overall performance of the final product, SFC profiles of interesterified fats were evaluated and compared with standard SFC curves for margarines and shortenings reported in the literature.

Enzymatically interesterified blends containing 75–80% coconut oil (CO) and 20–25% fully hydrogenated soybean oil (FHSBO) was found to be suitable for soft tub margarine. Additional viable alternatives for soft tub margarine include chemical or enzymatically interesterified blends of 45% CO and 55% palm olein (POo), as well as an EIE-derived blend composed of 85% CO and 15% FHSBO. These formulations exhibit lower SFC values than stick margarines, enabling spreadability directly from refrigeration temperatures. Figure 1 shows the SFC profiles of selected interesterified blends compared with the standard SFC curve for soft tub margarine.

As shown in Figure 2, both chemically and enzymatically interesterified blends containing 70% CO and 30% FHSBO are suitable for the production of stick margarine. Although these blends have a slightly higher melting point relative to conventional stick margarines, their SFC values at 10 °C and 20 °C remain unchanged. Consequently, unlike high-trans stick margarines or butter, they are less prone to excessive hardening during refrigerated storage.

In baked goods, the use of margarine or shortening contributes essential functional properties, including improved texture and tenderness, aeration, desirable mouthfeel, heat transfer during baking, and enhanced shelf life. Figure 3 illustrates the SFC curves of selected interesterified blends in comparison with baker's margarine. Chemically or enzymatically interesterified blends of CO and FHSBO at a 65:35 ratio was identified as promising candidates for baker's margarine formulations.

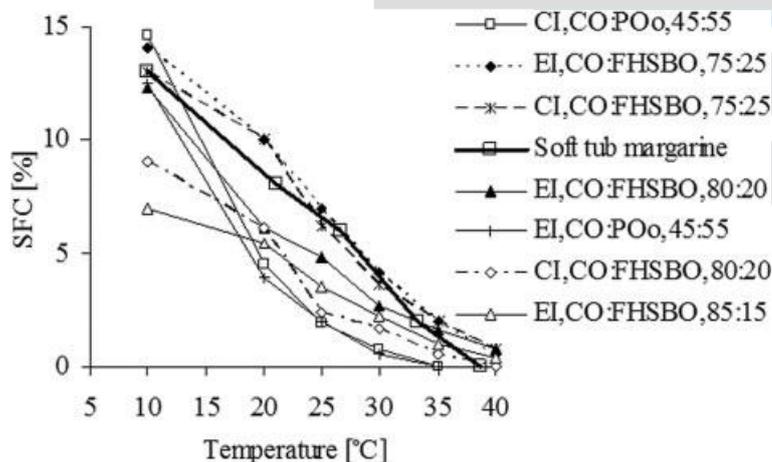


Fig 3: Comparison of SFC curve of interesterified fat blends with the typical SFC curve of soft tub margarine fat [31]. SFC, solid fat content; CI, chemically interesterified; EI, enzymatically interesterified; CO, canola oil; POo, palm olein; FHSBO, fully hydrogenated soybean oil.

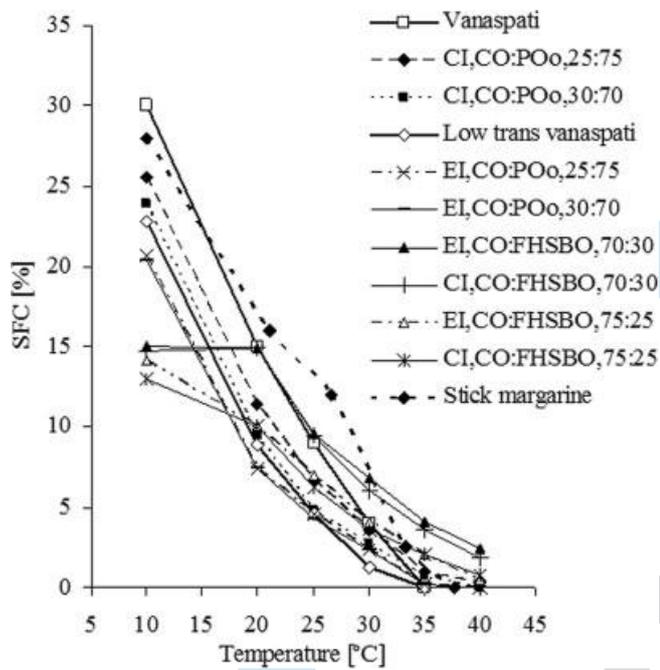


Figure 4. Comparison of SFC curve of interesterified fat blends with the typical SFC curve of stick margarine and commercial vanaspati fat.

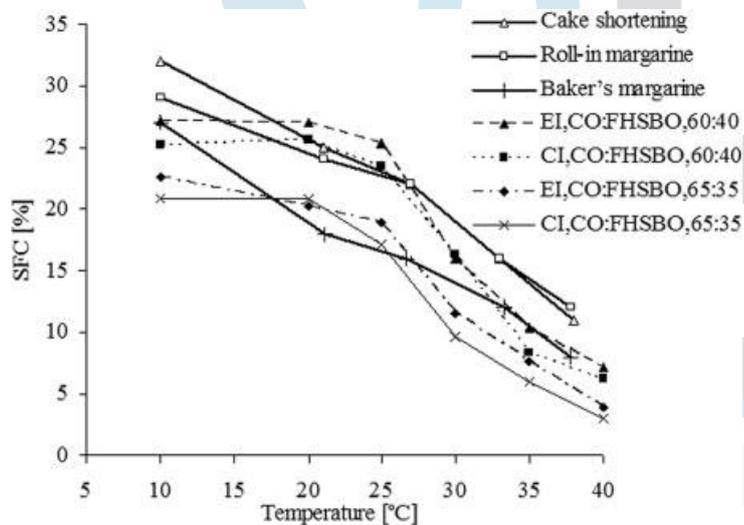


Figure 5. Comparison of SFC curve of interesterified fat blends with the typical SFC curve of cake shortening, roll in and baker's margarine fat

1. Substitution of Coating Materials

Polysaccharide-based coating materials, although widely used, exhibit hygroscopicity and tend to absorb moisture—an undesirable characteristic in many food applications. To overcome this limitation, hydrophobic lipids such as stearin and beeswax can be incorporated into the coating to reduce water absorption. Palm-based lipids, particularly palm stearin, serve as effective encapsulating agents due to their high melting point and ability to form a crystalline matrix. Additionally, film-forming agents such as xanthan gum have been utilized to enhance coating stability and mechanical integrity [31].

2. Margarine

The formation of trans fatty acids during fat hydrogenation is associated with increased health risks; therefore, blending and interesterification strategies have been adopted to develop trans fat-free margarine formulations. Interesterification redistributes fatty acids within triacylglycerol (TAG) molecules without altering the total saturated fatty acid content, thereby enhancing functionality while lowering trans-fat levels. According to Sellami et al., zero-trans margarine prepared via enzymatic transesterification of palm stearin and palm olein exhibits a modified TAG profile, improved rheological behaviour, and properties comparable to commercial margarines. Similar results have been achieved using blends of palm stearin, palm kernel oil, and soybean oil subjected to sodium-methoxide-catalysed interesterification.

The inclusion of soybean oil increases monounsaturated and polyunsaturated fatty acids, yielding a healthier margarine. The resulting microstructure typically consists of dispersed aqueous droplets embedded within a network of crystallized fats. Droplet size significantly influences melting behaviour and emulsion stability: larger droplets formed during storage reduce solid fat content below a critical level, compromising emulsion stability. Margarines produced from interesterified fats show crystal structures similar to those of commercial margarines. Furthermore, incorporating rice bran oil into palm stearin reduces crystal size and enhances product quality [31].

3. Bakery Shortenings

Shortenings are fat-based crystalline ingredients used to impart desirable texture, tenderness, air incorporation, and mouthfeel to baked goods. Conventional shortenings often rely on hydrogenated fats, which introduce trans fatty acids. As an alternative, blends of saturated fats such as palm stearin with fractions of coconut oil or palm kernel oil can be interesterified to achieve the desired functional properties without generating trans fats.

Bakery shortenings generally require a solid fat content (SFC) of 15–25% at 20 °C and a melting point above 38 °C. Palm oil naturally exhibits an SFC of approximately 22–25% at 20 °C, providing consistency comparable to plastic cake shortenings and making it inherently suitable as a shortening base [31].

4. Cocoa Butter Alternatives (CBAs)

Due to the high cost of cocoa butter, the confectionery industry increasingly employs more economical vegetable fat alternatives. Palm stearin is widely used for this purpose and is often blended with fats such as mango kernel fat and palm kernel oil, followed by interesterification to enhance physicochemical and melting characteristics. These blends are trans fat-free and form stable crystalline structures suitable for use as cocoa butter replacers.

Palm stearin naturally favours the formation of stable β' crystals, which contribute to desirable creaming properties. According to [31], interesterified palm stearin-based CBAs form small, needle-like crystals that are numerous and exhibit high stability. Crystal morphology is influenced by processing temperature, chemical composition, and polymorphism, as TAGs can crystallize into α , β , and β' forms. The β' structure is preferred in confectionery applications due to its fine texture and stability.

5. Pig Lard Replacers

Lard is valued in the food industry for its characteristic taste, texture, and high palmitic acid content (~25%), which contribute to its functional properties. However, concerns regarding excessive saturated fat intake and dietary restrictions have increased interest in vegetable-based lard substitutes. Interesterification of vegetable fats such as palm oil, cocoa butter, avocado butter, and mee fat has been explored as a means to mimic the functionality of pork fat. For lard replacement, blends of palm stearin, palm oil, soybean oil, cocoa butter, and mee fat have shown promising results.

Crystals with a β polymorphic structure yield superior textural properties by providing smooth mouthfeel and efficiently entrapping liquid oil. Naturally formed spherulitic β crystals make interesterified vegetable fat blends suitable substitutes for traditional lard [31].

6. Emulsions and Related Products

The demand for stable emulsions in the food industry has driven research into palm stearin-based emulsifying systems. Emulsifiers derived from palm stearin via glycerolysis followed by cold fractionation consist of approximately 91% monoacylglycerols and 9% diacylglycerols. These emulsifiers exhibit an emulsion capacity of 95.55% and demonstrate excellent stability.

A blend of palm stearin and rice bran oil at a 3:7 ratio produces a soft, creamy, and palatable mayonnaise. Palm stearin has also been employed in oleo gel formation, providing structured fat systems with desirable texture and stability [31].

7. Baker's Whipping Cream

Traditional dairy whipping cream lacks adequate stability under high-temperature conditions. A specially interesterified fat blend consisting of 34 parts fully hydrogenated palm kernel oil (iodine value 1.0) and 66 parts palm stearin (iodine value 19.0) has demonstrated superior performance in baker's cream formulations. When aerated to a density of 0.34 and stored between 25 °C and 40 °C, the cream remained fully stable for up to 4 hours at 37 °C and exhibited only minimal changes after 20 hours. Both firmness, measured using a cone penetrometer, and mouthfeel were rated satisfactory, indicating the suitability of this interesterified blend for high-temperature whipping cream applications.

Table 5: Solid fat content SFC (%) of palm stearin-palm kernel olein blend [11]

Temperature (°C)	Before interesterification	After interesterification
10	61	68
15	39	57.5
20	25	44
25	18	29
30	15	12
35	12	1.5
40	11	-
45	7.5	-

6. Necessity of Fat Replacement

The need for fat replacement arises from the dual role that dietary fats play in human health. On one side, fats contribute essential nutritional and functional benefits, including energy provision, structural integrity, and sensory enhancement of food products. On the other side, extensive epidemiological and clinical evidence links excessive consumption of certain dietary fats to increased risks of obesity, cardiovascular diseases, and diabetes. The persistent rise in such health conditions, partly driven by unhealthy dietary patterns, has encouraged researchers and the food industry to explore strategies for reducing fat intake without compromising product quality.

This challenge has led to the development of fat replacers—compounds designed to mimic the desirable attributes of fats while lowering overall caloric intake and improving public health outcomes. An ideal fat replacer should reduce the fat and energy content of the final product while preserving the chemical, physical, sensory, and shelf-life characteristics of traditional high-fat foods. Fat replacers include a wide range of bulking agents and functional ingredients capable of simulating the behaviour of fats within food matrices (Roller and Jones, 1996).

Fat replacers can be categorized based on their chemical structure and mode of action [5]:

(i) Carbohydrate-based fat replacers

These compounds primarily function as texture modifiers rather than direct fat mimetics. They enhance firmness, mouthfeel, viscosity, and moisture retention in food products (Lucca and Tepper, 1994). This category mainly includes polysaccharides such as cellulose, starches, fibres, gums, and resins.

(ii) Fat-based fat replacers

These ingredients resemble fats in appearance, texture, and thermal behaviour. They are typically divided into two groups: emulsifiers and lipid analogs. Mono- and diglycerides improve the dispersion of fat in aqueous systems, enabling partial reduction of fat content. Lipid analogs, which include low-calorie fats and true fat substitutes, are chemically engineered to provide similar functionality to conventional fats but with reduced caloric value.

(iii) Protein-based fat replacers

Protein-derived fat replacers are formulated to mimic the mouthfeel and structural attributes of fats (Napier, 1997). Although they cannot substitute fats on a gram-for-gram basis, they provide significant sensory benefits. Common examples include microparticulate proteins (marketed under names such as *Simplese* and *Trailblazer*) and modified whey protein products (e.g., *Dairy-Lo*).

7. Regiospecific Analysis of TAG by Hydrolysis

Regiospecific analysis of triacylglycerols (TAG) was performed via enzymatic hydrolysis using porcine pancreatic lipase. Briefly, 4 mL of Tris buffer (1 mol L⁻¹, pH 8.0), 1 mL of 2.2% calcium chloride, and 1 mL of 0.05% bile salts were added to each sample (20 mg). The mixture was equilibrated at 40 °C for 1 min, after which 10 mg of porcine pancreatic lipase was introduced. The reaction was allowed to proceed with continuous stirring for 5 min at the same temperature.

Following hydrolysis, the reaction mixture was extracted with diethyl ether and washed with water. The ether layer was dried over anhydrous sodium sulphate. Hydrolysed lipid fractions were separated using preparative thin-layer chromatography (TLC) developed with a solvent mixture of hexane, ethyl acetate, and acetic acid (70:30:2, v/v/v). The band corresponding to 2-monoacylglycerol (2-MAG) was scraped and extracted with chloroform. The isolated MAG fraction was trans methylated to fatty acid methyl esters (FAME) using 2% sodium methoxide in methanol (prepared by dissolving 2 g sodium methoxide in 100 mL methanol) and subsequently analysed by gas chromatography (GC) to determine the fatty acid distribution at the sn-2 position.

The distribution of free fatty acids and TAG was calculated as described by Christie (1982). The average fatty acid composition at positions sn-1 and sn-3 was determined from the intact TAG, while the composition at the sn-2 position was calculated according to Eq. (1):

$$\text{Positions 1 \& 3} = 3 * [\text{TAG}] - [\text{sn-2}] / 2 \quad (1)$$

Evaluation Methods for Degree of Interesterification

The degree of interesterification (DI) was evaluated using two independent approaches as described in [14]: (i) TAG composition analysis and (ii) SFC profiling.

(1) TAG-based method

DI was calculated by comparing the experimentally determined TAG composition before and after interesterification with the theoretical random TAG composition. The theoretical random TAG distribution (RAND) was computed from the fatty acid composition (mol%) by applying the law of probability, assuming complete randomization (Desmet Ballestra Internal Programme).

DI was calculated according to Eq. (2):

$$\text{DI (\%)} = 100 \times \frac{\binom{A}{B}_{\text{FDS}} - \binom{A}{B}_{\text{IE or EIE}}}{\binom{A}{B}_{\text{FDS}} - \binom{A}{B}_{\text{RAND}}} \quad (2)$$

where

- **DI** = degree of interesterification,
- **A** = TAG (or TAG group) that decreases most during IE,
- **B** = TAG (or TAG group) that increases most during IE,
- **FDS** = feedstock blend,
- **IE** = chemically interesterified blend,
- **EIE** = enzymatically interesterified blend,
- **RAND** = theoretical random TAG composition.

(2) SFC-based method

DI was also derived from changes in solid fat content (% SFC) at different temperatures using Eq. (3):

$$DI (\%) = 100 \times \frac{(SFC_0 - SFC_t)}{(SFC_0 - SFC_\infty)} \quad (3)$$

where

- **SFC₀** = SFC of the feedstock at time 0,
- **SFC_t** = SFC at reaction time *t*,
- **SFC_∞** = SFC at equilibrium (post-CIE).

Reaction rate determination

The reaction rate constant (*k*) for both TAG-based and SFC-based methods was calculated using Eq. (4):

$$DI (\%) = 100(1 - e^{-kt}) \quad (4)$$

All equations and methodologies follow the procedure described in reference [14].

8.RESULTS & DISCUSSION

The physicochemical parameters of the non-interesterified and interesterified fat blends were systematically compared. The results indicate that interesterification induces significant modifications in the functional and structural properties of the fats, leading to measurable differences across multiple analytical attributes.

Table 6: the values and ranges for the properties of blends of interesterified fats

BLENDS	SMP (°C)		Plastic range (°C)	
	NIE	IE	NIE	IE
1. PS/SFO				
10:90	34.1 ± 0.7	25.9 ± 0.9	NIR (<5)	NIR (<5)
30:70	40.6 ± 0.4	31.3 ± 1.1	10.7–16.0	7–11.0
50:50	45.2 ± 0.8a	36.7 ± 0.9b	25.2–34.5	19.0–28.3
2. Canola oil/palm olein				
58:42	3.5	23.4	-	-
45:55	7.1	25.7	-	-
30:70	11.1	21.2	-	-
25:75	14.6	29.5	-	-
3. Coconut stearin/PS				
30:70	46.3 ± 0.3	46.6 ± 0.2	-	-
40:60	44.6 ± 0.4	45.0 ± 0.4	-	-
50:50	44.1 ± 0.8	41.9 ± 0.6	-	-

60:40	41.5 ± 0.4	39.5 ± 1.3	-	-
70:30	34.3 ± 0.3	33.3 ± 0.4	-	-
4. RBO/Cottonseed oil				
60:40	-	43.8± 1.0	-	-
70:30	-	40.2±0.4	-	-
5. PS/PKOlein				
30:70	-	29.9	-	-
40:60	-	31.5	-	-
50:50	-	33	-	-
60:40	-	34.1	-	-
70:30	-	37.5	-	-
6. PO/SFO				
80:20	37.5	27	-	-
60:40	34	21	-	-
50:50	31.5	19.5	-	-
40:60	30	20.5	-	-
20:80	17	8	-	-

Table 7: The soap content of Chemically and Enzymatically interesterified fats

Soap content	Chemically IE	Enzymatically IE
Canola oil/palm olein	86.4–99.8	10.1 –13.8
PS/SFO (10:90, 30:70, 50:50)	19.43 ± 3.7	-
	22.83 ± 4.2	-
	19.78 ± 2.8	-

9.CONCLUSION

Our analysis demonstrated that interesterification was performed on multiple fat blends formulated in specific proportions using different oil types, employing both enzymatic and chemical pathways under controlled conditions of temperature, pressure, and catalyst or enzyme concentration. Comprehensive characterization of the resulting blends including iodine value, melting point, and solid fat content (SFC) was conducted. The corresponding SFC profiles are presented in the figures above, along with a comparative evaluation of chemical versus enzymatic interesterification.

A broad spectrum of industrial applications was identified, indicating that the interesterified blends possess functional attributes suitable for diverse sectors of the food industry. Importantly, this technique eliminates trans-fat formation typically associated with hydrogenation, thereby offering a healthier alternative. Comparative analysis revealed that enzymatic interesterification is more advantageous than chemical interesterification, primarily because the enzyme does not participate in or alter the final product. Overall, the study demonstrates that enzymatic interesterification is an efficient and versatile method for tailoring the physical and chemical properties of fats for industrial applications.

10.REFERENCES

1. Trans-free fats through interesterification of canola oil/palm olein or fully hydrogenated soybean oil blends, Jamshid Farmani, Mohammad Safari and Manouchehr Hamed, Eur. J. Lipid Sci. Technol. 2009, 111, 1212–1220

2. Production of Trans-free fats by chemical interesterified blends of palm stearin and sunflower oil, Davood Farajzadeh Alan, Mohammad Hossein Naeli, Mehdi Naderi, Seid Mahdi Jafari, Hamid Reza Tavakoli, Wiley Food and Science Journal
3. Enzymatic Interesterification of Coconut and Hemp Oil Mixtures to Obtain Modified Structured Lipids, Ranahansi Rangadharee Bandara, Chloé Louis-Gavet, Joanna Bry's, Diana Ma 'nko-Jurkowska, Agnieszka Górska, Rita Brzezi 'nska, Marta Siol, Sina Makouie, Bharani Kumar Palani, Marko Obranovi 'c and Piotr Koczo 'n, *Foods* 2024, 13, 2722
4. Triacylglycerol structure and interesterification of palmitic and stearic acid-rich fats: an overview and implications for cardiovascular disease, Sarah E. E. Berry, *Nutrition Research Reviews* (2009), 22, 3–17 © The Author 2009
5. Oils and fats in texture Modification, B. Emadzadeh, B. Ghorani, *Modifying Food Texture*, Volume 1, © 2015 Elsevier Ltd.
6. Physico-chemical properties of blends of palm olein with other vegetable oils, Bazlul Mobin Siddique, Anees Ahmad, Mohamad Hakimi Ibrahim, Sufia Hena, Mohd Rafatullahb and Mohd Omar A. K, *rasas y aceites*, 61 (4), Octubre- diciembre 423-429, 2010, issn: 0017-3495.
7. Genetic Improvements of Olives, Enzymatic Extraction & Interesterification of Olive oil, Fabiano Contesini, Camilo Teixeira, Paula Speranza, Danielle Lopes, ResearchGate Chapter -February 2012, DOI: 10.5772/29015.
8. Enzymatically interesterified hybrid palm stearin as an alternative to conventional palm stearin, Reyna Janin Flores Ruedas, Elena Dibildox-Alvarado , Jaime David Pérez, Martínez & Nayma Itandehuit Murillo Hernández, © 2019 The Author(s). Published with license by Taylor & Francis Group, LLC
9. Trans-Free Plastic Shortenings Prepared with Palm Stearin and Rice Bran Oil Structured Lipid, Brenda H. Jennings, Casimir C. Akoh, *J Am Oil Chem Soc* (2010) 87:411–417, DOI 10.1007/s11746-009-1516-y
10. Trans-free plastic shortenings from coconut stearin and palm stearin blends, T. Jeyarani, Mohd. Imtiyaj Khan, Sakina Khatoon, Elsevier, *Food Chemistry* 114 (2009) 270–275
11. Trans-Free Fats with the Products of the Oil Palm – a Selective Review, Kurt G. Berger, *Czech J. Food Sci.*, Vol. 25, No. 4: 174–181
12. An investigation on the physicochemical characterization of interesterified blends of fully hydrogenated palm olein and soybean oil, Raheleh Mahjoob, Abdorreza Mohammadi Nafchi, Elahe Omidbakhsh Amiri, Jamshid Farmani, *Food Sci Biotechnol*
13. Lipase catalyzed interesterification of rice bran oil with hydrogenated cottonseed oil to produce trans free fat, T. S. V. R. Neeharika & Ramya Rallabandi & Y. Ragini & Shiva Shanker Kaki & K. N. Prasanna Rani & R. B. N. Prasad, *J Food Sci Technol* (August 2015) 52(8):4905–4914, Association of Food Scientists & Technologists (India) 2014
14. Chemical and Enzymatic Interesterification of a Blend of Palm Stearin: Soybean Oil for Low trans-Margarine Formulation Raquel Costales-Rodríguez, Véronique Gibon, Roland Verhe', Wim De Greyt, *J Am Oil Chem Soc* (2009) 86:681–697
15. Enzymatic interesterification to produce zero-trans and dialkylketones-free fats from rapeseed oil, Sabine Danthine, Sébastien Closset, Jeroen Maes, Steven Mascrez, Christophe Blecker, Giorgia Purcaro and Véronique Gibon, *OCL* 2022, 29, 36
16. Development of fats based on olive oil by chemical interesterification and its utilization in cookie production d. K açar, h. Erinç and d. S ivri özay, *Acta Alimentaria*, Vol. 47 (2), pp. 154–161 (2018)

17. Trans-free Margarine Fat Produced Using Enzymatic Interesterification of Rice Bran Oil and Hard Palm Stearin Pimwalan Ornlai-ied, Sopark Sonwai, and Sawang Lertthirasuntorn, *Food Sci. Biotechnol.* 25(3): 673-680 (2016)
18. Enzymatic transesterification of palm stearin and olein blends to produce zero-trans margarine fat Mohamed Sellami, Hanen Ghamgui, Fakher Frikha, Youssef Gargouri and Nabil Miled, Sellami et al. *BMC Biotechnology* 2012
19. Enzymatic Interesterification of Palm Stearin and Palm Kernel Olein, Zaida Zainal and Mohd Suria Affandi Yusoff, *JAOCs*, Vol. 76, no. 9 (1999)
20. Use of palm stearin as a component of interesterified blends, JM deMan, © Society of chemical industry ISSN1353-114X, LPS119/2000
21. Enzymatic Interesterification of Palm Products for Producing Low Calorie Cocoa Butter Substitutes, Ros-Haniza Borhan, Mamot Said, Miskander mat sahri, *Journal of Applied Sciences* 11 (22):3750-3754, 2011
22. Improvement of palm oil and sunflower oil blends by enzymatic interesterification, Zahra Kouchak Yazdi & Iran Alemzadeh, *International Journal of Food Science and Technology* 2011, 46, 1093–1099
23. Enzymatic Interesterification of Palm Oil and Fractions: Monitoring the Degree of Interesterification using Different Methods, Nathalie De Clercq, Sabine Danthine, Mai Tuyet Nguyen, Veronique Gibon, Koen Dewettinck, *J Am Oil Chem Soc* (2012) 89:219–229
24. Enzymatic Interesterification of Palm Stearin and Coconut Oil by a Dual Lipase System, Nuzul A. Ibrahim & Zheng Guo & Xuebing Xu, *J Am Oil Chem Soc* (2008) 85:37–45
25. Effects of Enzymatic Interesterification on the Physicochemical, Polymorphism and Textural Properties of Palm Stearin, Palm Kernel Oil and Soybean Oil Blends, Siti Hazirah Mohamad Fauzi, Norizzah Abd Rashid, and Zaliha Omar
26. Enzymatic Interesterification of Vegetable Oil: A Review on Physicochemical and Functional Properties, and Its Health Effects, Priyanka Singh, Meenakshi Garg, Rajni Chopra, Aishwarya Dhiman, *Journal of Oleo Science*
27. Comparison of Chemical and Enzymatic Interesterification of Fully Hydrogenated Soybean Oil and Walnut Oil to Produce a Fat Base with Adequate Nutritional and Physical Characteristics, Mariel Farfán, Alfredo Álvarez, Alan Gárate and Pedro Bouchon, M. FARFÁN et al.: Interesterified Oil Blends for Fat Base Production, *Food Technol. Biotechnol.* 53 (3) 361–366 (2015)
28. Quality control and storage studies of virgin olive oil: Exploiting UV spectrophotometry potential, Despina Grigoriadou, Maria Z. Tsimidou, *Eur. J. Lipid Sci. Technol.* 108 (2006) 61–69
29. Effect of argan kernel storage conditions on argan oil quality, Harhar Hicham, Said Gharby, Zoubida Charrouf, *European Journal of Lipid Science and Technology* · August 2010
30. The Recent Application of Palm Stearin in Food Industry: A Review, Edy Subroto, Rizki Lutfiani Nurannisa, *INTERNATIONAL JOURNAL OF SCIENTIFIC & TECHNOLOGY RESEARCH VOLUME 9, ISSUE 02, FEBRUARY 2020*
31. High Oleic Enhancement of Palm Olein via Enzymatic Interesterification, Siew Wai Lin and Saw Mei Huey, *Journal of Oleo Science*
32. Effect of Enzymatic Interesterification on Melting Point of Palm Olein, ATIFA. A. YASSIN IBRAHIM, O. MOHAMED, MOHD, N. IBRAHIM AND MOHD, S. A. YUSOFF, *Applied Biochemistry and Biotechnology*, Vol. 110, 2003