Enzymatic Interesterification of Vegetable Oil: A Review on Physicochemical and Functional Properties, and Its Health Effects

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Abstract: In recent years, scientists and technologists have become increasingly interested in producing modified lipids with enhanced nutritional and functional properties. The application and functional properties of fats and oil depend on the composition and structure of triacylglycerols (TAG). As a result, lipid TAG changes can be used to synthesize tailored lipids with a broader range of applications. However, no natural edible oil is available with appropriate dietary and functional properties to meet the human recommended dietary allowances (RDA). On the other hand, the arising health concern is the transfat consumption produced during the chemical modification of vegetable oil through the partial hydrogenation process. Therefore, innovative technologies are shifting toward modifying fat and oil to improve their functionality. Enzymatic interesterification (EIE) is one of the emerging and novel technology to modify the technological traits of naturally available edible oil. It helps in modifying physicochemical, functional, oxidative, and nutritional characteristics of fats and oil due to the rearrangement of the fatty acid positions in the glycerol backbone after interesterification. Enzymatic interesterification utilizes lipase as a biocatalyst with specificity and selectivity to produce desired lipids. Alternation in the molecular structure of triacylglycerol results in changes in melting/dropping point, thermal properties, crystallization behavior, solid fat content, and oxidative stability. Because of its high acyl exchange reaction efficiency, simple reaction process, flexibility, eco-friendly, and generation of fewer by-products, (EIE) is gaining more attention as a substitute lipid modification approach. This review paper discusses the uses of EIE in developing modified fat with desirable physicochemical and nutritional properties. EIE is one of the potential techniques to modify vegetable oil's physicochemical, functional, and nutritional characteristics without producing any undesirable reaction products. EIE produces different modified lipids such as trans-fat-free margarine, plastic fat, bakery, confectionery fat, therapeutic oil, infant food, cocoa butter substitute, and equivalent.

Key words: enzymatic interesterification, structured lipids, modified fat, bakery fat, trans fat

Abbreviations: EIE; Enzymatic interesterification, CIE; Chemical interesterification, PUFA; Polyunsaturated fatty acid, MUFA; Monounsaturated fatty acid, SFA; Saturated fatty acid, RDA; Recommended dietary allowances, CAGR; Compound annual growth rate, CVD; Cardiovascular diseases, CBS; Cocoa butter substitute, CBE; Cocoa butter equivalent, MLCT; Medium long chain triacylglycerols, HDL; High density lipoproteins, LDL; Low-density lipoproteins, FFA; Free fatty acid, HMFS; Human milk fat substitute

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1 Introduction

Fats and oil are essential components of the human diet as they provide energy and essential fatty acids and act as a carrier for fat-soluble vitamins. Furthermore, they add flavor, texture, and satiety to our diets. Essential fatty acids in fat and oil are linoleic acid and linolenic acid. The fats and oils are composed of glycerol, and fatty acids linked by ester linkages and are chemically known as triglycerides (TAG) molecules. Depending on their physicochemical properties, fats and oils have different applications in food preparation. The functional, nutritional, and organoleptic properties of fats and oils vary depending on the fatty acid chain length, saturation level, and positional distribution of fatty acids in TAG species. The positional distribution of sn-1, sn-2, and sn-3 on the glycerol backbone also regulates fats and oil’s absorption and nutritional quality.

In order to broaden the applicability of vegetable oil, the physicochemical properties of fats and oils must be modified. Blending, hydrogenation, fractionation, interesterification, or a combination of these processes are some of the modification techniques used by the edible oil industry to improve physicochemical and functional properties. These modifications also help to enhance the oils’ oxidative stability and functional and textural characteristics. The most widely used technique by the food industry is hydrogenation which alters the vegetable oil from liquid to solid or semi-solid. The most widely used technique by the food industry is hydrogenation which alters the vegetable oil from liquid to solid or semi-solid. However, some cis fatty acids are converted to trans fats during partial hydrogenation, leading to cardiovascular diseases and other health implications. Another modification technique is fractionation, which is a physical process of separating fat into fractions based on melting and textural properties. However, it is only an expensive physical process using chemical agents and does not lead to any modification in the structure of triglycerides. Therefore, interesterification is the potential alternative for modifying oil and fats’ physicochemical and thermal properties. The global interesterified fats market value was around US$ 226 Mn in 2021. Interesterified fats sales increased a compound annual growth rate (CAGR) of around 3% over the past half-decade. As a result, Interesterified fats market revenue is expected to increase at a CAGR (compound annual growth rate) of around 5% during 2022-2032.

Blending is the process of combining two different oils to develop oil with a specific fatty acid composition and consistency. However, when the oils with different melting points are blended, it results in a phase separation problem, limiting the use of blended oil. In contrast, interesterification is a chemical/enzymatic process that employs a chemical or enzyme as a biocatalyst to rearrange fatty acids within and between the triacylglycerol, thus leading to the production of trans-free plastics fats.

Another recent technique is oleogelation, which is a process of converting liquid vegetable oil into solid gel-like viscoelastic material without changing its chemical structure. It has a functionality similar to bakery fat. EIE produces tailored lipid by incorporating specific fatty acids at the desired position of TAG and improving/balancing the fatty acid composition of the end product.

Also, EIE has wider applications such as frying oil, bakery, and confectionary, cocoa butter substitute and replacer, and human milk fat substitute whereas olegels have limited application in bakery and confectionery products.

Consumers focus more on the quality and quantity of fat and oils consumed, with an increasing emphasis on health and nutrition. People are aware that the excessive consumption of fats and oil will lead to numerous diseases like obesity, hypertension, and coronary heart disease. Also, agencies like WHO and FSSAI emphasize limiting the consumption of saturated fatty acid-rich diets and eliminating trans fats. Therefore, the population consumes non-hydrogenated, low cholesterol, and polyunsaturated fatty acid-enriched fats and oils. As a result of these health concerns, demand for modified and healthy fats and oil and its products has increased, due to which the researchers are focusing on developing novel fat without trans fat having a balanced fatty acid profile and better retention of fat-soluble vitamins.

2 Need for Modification of Oil

Three crucial parameters of healthy oils given by the World Health Organization (WHO) are the ratio of saturated to mono- to polyunsaturated fatty acids, the ratio of essential fatty acids, and the existence of antioxidants in the oil. The WHO balanced ratio of saturated: mono: polyunsaturated fatty acids and alpha-linolenic acid: linoleic acid is 1:1.5:1 and 1:5-10, respectively. In India, varieties of edible oil are available, but there is not a single oil that fulfills the WHO recommendation. The oil available has varied fatty acid profiles; some are rich in saturated fatty acid (SFA), whereas some are rich in polyunsaturated fatty acid (PUFA). Therefore, no individual vegetable oil has an ideal combination and rearrangement of fatty acids on their triglyceride moieties, fulfilling the fatty acid requirement. Thus, the industry is moving towards modifying fats and oil to fulfill this recommendation.

Most confectionery products such as margarine and shortening are made of hard fats, consisting of a high amount of saturated fats. Saturated fats can cause adverse health effects, such as an elevated risk of coronary heart disease and stroke. Enzymatic interesterification can be used to precisely modify fats and oils by interchanging the fatty acid type and location to those with the desired physi-
cochemical properties or by increasing or incorporating essential fatty acids such as PUFAs or short- and medium-chain fatty acids to produce modified lipids with improved nutritional value. Also, several researchers reported that EIE can be used to prepare cocoa butter substitute and transfat-free margarine with similar solid fat content (SFC) as compared to commercial cocoa butter and margarine.

Trans fat is generated by partial hydrogenation of fats, and its consumption causes an increase in low-density lipoprotein (LDL) cholesterol and a decrease in high-density lipoprotein (HDL) cholesterol. As a result, it’s important to replace trans fat with healthy fats and oils. Olive oil, linseed oil, perilla oil, and sesame seed oil are examples of edible oils with significant MUFA and PUFA content. However, since MUFA and PUFA are unstable fatty acids with poor oxidative and thermal stability, their usage as cooking oil is constrained. Therefore, it would help to improve their stability and balance the fatty acid ratio to mix or blend these oils with more stable oils like palm oil, coconut oil, and dairy fat (highly saturated).

3 Interesterification (IE)

Among the various oil modification technology, interesterification has received increasing attention in the food industry. Interesterification is the randomization of fats and oil triacylglycerols which refers to the reaction of fatty acid esters and react to new esters by exchanging fatty acid groups with other esters of fatty acids or alcohols. The interesterification process helps in modifying the physical properties of the fats and oils like desired melting point, viscosity, smoke point, color, odor, and application. The most crucial benefit of interesterification over partial hydrogenation is that there is no formation of trans fat since it only requires the rearrangement of fatty acid within the glycerol backbone. Rearrangement does not alter the structure of fatty acid in the interesterification process. Interesterification can be processed either by chemical interesterification (CIE) or enzymatic interesterification (EIE).

Acidolysis (fatty acid-TAG), Glycerolysis (Glycerol-TAG), and transesterification (TAG-TAG) are all examples of interesterification reactions. Acidolysis involves exchanging acylglycerol molecules with free carboxylic acid, whereas glycerolysis involves the exchange of glycerol with TAG. Transesterification involves the reaction between two esters. The process of transesterification is frequently employed to generate food lipids. During transesterification, the ester bonds that hold fatty acids to the glycerol backbone are broken. The free fatty acids are then moved around in a pool of fatty acids and re-esterified onto a new position, either on the same glycerol as in intresterification or on a different glycerol as in interesterification.

3.1 Methods of Interesterification

3.1.1 Chemical interesterification (CIE)

Chemical interesterification is a random fatty acid distribution on the backbone of glycerol. It is the mechanism in which a chemical catalyst such as sodium methoxide and randomization of the fatty acid on the glycerol backbone occurs. Factors influencing catalyst activity are the moisture content, the free fatty acid, and the oil’s peroxide value. The moisture decomposes the catalyst and leads to the formation of soaps, increases the amount of free fatty acids, and high peroxide value destroys the catalytic activity of the catalyst. Therefore, these three parameters, namely moisture content, FFA, and peroxide value, should be kept low to ensure the minimum by-products and high yield of end product. However, these processes have a few disadvantages, like undesirable side reactions and the formation of coloring compounds formed during side reactions. This process also produces a few by-products that are hard to eliminate. CIE also leads to the destruction of natural tocopherol due to a high temperature which further reduces oxidative stability.

3.1.2 Enzymatic interesterification (EIE)

EIE is the process in which the enzyme (lipase) is used as a biocatalyst. Therefore, the rate of reaction is controlled by various factors such as enzyme concentration, the temperature of the reaction, time of reaction, and substrate concentration such as oil (TAG) and fatty acid ester. It is possible to obtain a desirable TAG using a positional enzyme in the EIE reaction (Fig. 2). Also, there is a very low level of deterioration of the PUFA due to the mild conditions used during the process. The specificity of EIE depends on the type of lipases used. Lipases are categorized as random (non-regiospecificity), specific (1,3-regiospecificity), or fatty acid-specific. Candida rugosa lipase, Geotrichum candidum lipase are examples of random lipase and Mucor miehei lipase, Aspergillus niger lipase, pancreatic lipase are some of the examples of specific lipase. Figure 1 depicts the basic process of enzymatic interesterification.

EIE is employed to change the fatty acid composition of fatty acid distribution in the triacylglycerol molecule. The reaction can be carried out at random or specific depending upon the type of enzyme. If it is carried out using a non-specific lipase enzyme, the fatty acids are interchanged to the different positions of the triacylglycerol randomly. As a result, the triacylglycerol is randomly generated, which causes alterations to its physical characteristics. Theoretically, if it is done with a specialized enzyme, like sn-1,3 specific lipase, only the sn-1,3 position will hydrolyze, leaving the sn-2 position unaltered (Fig. 2). But in a real catalytic reaction, acyl migration caused the fatty acid at sn-2 to change. Acyl migration happens even during a regiospecific process, which causes randomization of the sn-2 fatty acid. The positively charged carboxyl
atom in TAG is attacked by the reaction catalyst to create a tetrahedral intermediate (DAG anion), and the DAG anion then attacks a second activated TAG ester bond to create an intermediate complex, which eventually dissociates the DAG anion and the newly formed TAG molecule before completing the interesterification.

Enzymatic interesterification reactions are typically carried out in a batch or a packed-bed reactor. An increase in the activity and stability of biocatalyst (enzymes) and the interesterification process improve the reaction efficiency of EIE. The factors affecting the efficiency of the EIE reaction are reaction parameters such as enzyme concentration, moisture content, reaction time, substrate ratio, temperature, agitation conditions, and pH. To preserve the integrity of the enzyme and increase the rate of esterification, the amount of water should be regulated, reducing the rate of hydrolysis.

Table 1 depicts the comparison of the different characteristics of CIE and EIE. EIE is advantageous to CIE in several ways, including controlled reaction conditions, reduced environmental impact, fewer by-products and less processing, improved nutrition, and a lower reaction temperature. Compared to CIE, which is random, the EIE response has more specificity, which aids in giving fats beneficial physical and nutritional properties. Low temperatures (55°C to 70°C) are suitable for EIE, which lowers
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The process of interesterification modifies the melting point and stability. Fat crystal usually has three polymorphic forms, and this phenomenon is known as polymorphism. It is analyzed using X-ray diffraction based on melting point and stability. Fat crystal usually has three different polymorphic fat crystals, mainly α, β', and β. The α is an unstable crystal with a low melting point. In contrast, the β' crystal is metastable, has intermediate melting points, and has a rough and sandy texture, or sandiness and graininess. The most stable is β crystal, with the highest melting point and a soft, smooth texture. It melts quickly when exposed to heat and is distinguished by smaller, finer crystals and uniform dispersion of crystals. Since the β crystal gives baking and confectionery items a smooth texture, fat with crystals is primarily preferred for margarine and shortenings.6,17

Several studies reported that EIE changes the melting profile of fats and oil due to the formation of a new TAG molecular structure. In a study by Cui et al.,18 enzymatically interesterified margarine was formulated using beef tallow and coconut oil. They reported that fat in EIE margarine has an abundance of β' and is also distributed evenly. The EIE margarine has a melting point ranging from 20-40°C, which does not produce a waxy mouthfeel and is preferable for use. In another study, it was found that there is a significant increase (p<0.05) in the melting point after EIE (24.1-39.2°C) when compared to the initial blends (21.2-26.2°C).19 The EIE of fats and oil provides a wide range of melting profiles because of the higher diversity of fatty acid and TAG composition, further facilitating the formation of specific polymorphisms.

EIE of fats and oil results in new melting peaks compared to the physical blend. For example, Zhou et al. (2021)20 reported that after EIE 2-3, new melting peaks

#### Table 1 Comparison of characteristics of chemical and enzymatic interesterification.

| S. No. | Characteristics | Chemical interesterification | Enzymatic interesterification |
|-------|----------------|-----------------------------|-------------------------------|
| 1     | Catalyst       | It involves a chemical catalyst (sodium methoxide) | It involves an enzyme as a catalyst (Rhizomucor miehei lipase) |
| 2     | Specificity    | It is a random process (non-specific) | It is more specific (regio-, fatty- and stereospecificity) |
| 3     | Reaction       | 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) |
| 4     | Temperature    | Reduction in oxidative stability (loss of natural antioxidants) | Improvement in Oxidative stability (as low temperature retains the tocopherol) |
| 5     | Stability      | The chemical catalyst used are hazardous to the environment | It is environmental friendly |
| 6     | Treatments     | It requires more pre and post-treatment | It requires fewer pre and post-treatments such as bleaching and deodorization |
| 7     | Effect on flavor | It can show flavor reversion | It does not show flavor reversion |
| 8     | By-product formation | Formation of by-products like sodium salts and high processing loss due to saponification | It generates fewer by-products |
| 9     | Cost           | Chemical used is less expensive compared to enzyme | The enzyme used as a catalyst is expensive |
| 10    | 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 process |

4 Effect of Enzymatic Interesterification on Physicochemical Properties of Fats and Oil

4.1 Melting and crystallization behavior

The melting range of fats and oil depends on the fatty acid and TAG structure. Each fat or oil contains triglycerides, melting at different temperatures depending on their fatty acid components. Differential scanning calorimetry measures fats and oil’s melting and crystallization behavior. The process of interesterification modifies the melting profile of fats and oil. Fat crystals exist in different polymorphic forms, and this phenomenon is known as polymorphism. It is analyzed using X-ray diffraction based on melting point and stability. Fat crystal usually has three different polymorphic fat crystals, mainly α, β', and β. The α is an unstable crystal with a low melting point. In contrast, the β' crystal is metastable, has intermediate melting points, and has a rough and sandy texture, or sandiness and graininess. The most stable is β crystal, with the highest melting point and a soft, smooth texture. It melts quickly when exposed to heat and is distinguished by smaller, finer crystals and uniform dispersion of crystals. Since the β crystal gives baking and confectionery items a smooth texture, fat with crystals is primarily preferred for margarine and shortenings.6,17

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appeared compared to the physical blend or pure oil due to
the formation of new TAG species. EIE of hydrogenated
soybean oil showed that fat crystals have changed from β
to β’ after interesterification. Most of the research shows
that EIE products have an abundance of β’ polymorphic
form, which is one of the valuable properties of fats des-
tined for applications in bakery and confectionery. The
change in the melting profile of EIE is mainly due to the
change in fatty acid regiodistribution and the formation of
new TAGs during EIE.
Solid fat content is also considered an essential criterion
for the physical characteristics of fats. For example, the
percentage of fat in the crystalline (solid) phase to total fat
across a temperature gradient is known as solid fat content
(SFC), and it is a vital characteristic to consider when de-
ciding if lipids are suitable for a given application.

In a recent study, at 35°C, EIE fats had a low SFC of
2.37%, indicating favorable melt-in-the-mouth qualities, as
opposed to non-interesterified fat (4.89%) and chemically
interesterified fat (3.75%), which had SFC that caused
waxy mouthfeel. It was evident that the mouthfeel charac-
teristics of the fats enhanced after the EIE reaction com-
pared to the non-interesterified fat. Zhou et al. (2021)
also reported that after EIE, the SFC value decreased due
to the increment in the trisaturate (SSS) TAG and reduction
in the SUU and SUS TAG content. Similarly, Ornla-ied et
al. (2021) reported that EIE lipids exhibited lower SFC
values than their physical blends with a sharper melting
characteristic. SFC curves of all EIE lipids were similar to
those of confectionery fats, notably CBS. Changes in SMP
and SFC values after EIE are ascribed to changes in the
type and concentration of TAG species, specifically SSS,
SU, and di-unsaturated (SUU) TAG, each of which has a
unique melting point. Furthermore, reduced SFC of
structured lipids compared to pure oil and non-interesteri-
ﬁed blends can be linked to the re-distribution of fatty
acids in TAG moieties.

Overall, the EIE of blends resulted in changes in the SFC
proﬁle and provided a wide range of plasticity that can be
used for plastic fats.

4.2 Color, smoke point, and viscosity
The color of vegetable oil has a crucial effect on market
value and consumer acceptability and depends on the pig-
mments present. The color of the oil depends upon the pig-
mments like chlorophyll and carotene present in edible oil.
Oils like palm, perilla, and mustard oil are dark, whereas
olive oil and coconut oil are lighter in color. Mixing a com-
bination of oil moderates the effect on the color of the
blended oil. The Lovibond spectrophotometric colorimeter
is mainly used to estimate the color of fats and oil. Frying
causes an accumulation of oxidized radicals, which darken
the color of oil used for frying. EIE rice bran and palm oil
vegetable oil were more resistant to thermal degradation
than the physical blend of oils.

The smoke point is the temperature at which heated fats
and oils start to emit smoke. According to a study, enzym-
atically interesterified mixes of MLCT and palm or
soybean oil raised their smoke points. The FFA content of
the blends changes when MLCT is added to them. The
MLCT’s oleic acid content raises the smoke point. Conse-
quently, EIE raises the smoke point of oil blends and en-
hances thermal stability.

Because of the interchange of fatty acids within and
between triglyceride molecular species, EIE causes
changes in oil viscosity. According to various researches,
EIE of fats and oil reduces viscosity compared to blended
and native oil. The intermolecular interactions among the
acylglycerol molecules may be responsible for the viscosity
reduction in EIE.

5 Effect of EIE on TAG and Fatty Acid Composition
Fat products’ physical and sensory characteristics, such
as hardness, spreadability, and flavor, are correlated with
TAG compositions. A slight modiﬁcation to the TAG struc-
ture will change the crystallization proﬁle and crystal poly-
morphism. Depending on the fatty acids attached to the
glycerol backbone, many TAG types exist. TAG appears in
a wide range of isomers and positional isomers and shares
almost all of the same physicochemical properties as TAG
found in synthetic fats and oils. As a result, the method for
separating TAG molecules has become crucial for deter-
mining the TAG ﬁngerprint spectrum in edible oil. Separat-
ing TAG in edible oils is commonly done using chromatog-
raphy and mass spectrometry (MS), both of which are
quick and effective. Gas chromatography (GC) and high-
performance liquid chromatography (HPLC) are two types
of chromatographic analysis. TAG is separated by GC using the carbon number of the TAG, whereas re-
versed-phase liquid chromatography uses the correspond-
ing carbon number (ECN). The difference between the
carbon number and the total amount of double bonds in
the TAG can be used to compute ECN. Table 2 shows the
applications of enzymatically interesterified fats and oil.

The oil type (liquid oil and hard fat) and their ratios im-
pacted the increased and decreased levels of TAG species
and fatty acid composition. After the EIE reaction process,
there were signiﬁcant increases in the concentration of
medium-chain TAGs and signiﬁcant decreases in the con-
centration of high melting TAGs following interesterifica-
tion from palm stearin and palm kernel olein. Additionally,
some unique TAGs were created during interesterification.

Most researchers reported that SU TAGs were the pre-
dominant TAGs present in all interesterified lipids following
interesteriﬁcation, followed by SU and U TAGs as com-
pared to the non-interesteriﬁed product demonstrated a
## Table 2  Current approaches and application of Enzymatic Interesterification used in the last 5 year.

| Application                  | Sources of Lipids                                      | Reaction Condition* | Source and type of enzyme | Reactor used                  | Health and functional benefits                                                                 | References                |
|------------------------------|--------------------------------------------------------|---------------------|---------------------------|-------------------------------|---------------------------------------------------------------------------------------------|---------------------------|
| Plastic fats                 | M. oleifera seed oil + palm kernel oil                  | T-60°C ; t-24h; R-70-30; S- 200rpm; E-10% | Lipozyme TL IM             | Magnetic stirrer              | Low $\Delta^\text{trans}$ fats with improved plasticity                                     | Dollah et al. (47)       |
|                             | soybean oil and fully hydrogenated palm oil             | T-60-100°C ; t-2-8h; R-1-2-5-2; S - 300rpm; E-1-5% | Lipozyme 435               | Magnetic stirrer              | Low $\Delta^\text{trans}$ fats analogue to beef tallow with improved spreadability          | Li et al. (26)           |
|                             | Palm oil + palm kernel oil                             | T-70°C ; t-6h; R- 25:75 to 75:25; E- 4% | Lipozyme TL IM             | Glass batch reactor           | Used as frying oil as its has high smoke point decreased SFC and lower SMP and alternative to partially hydrogenated oil for trans-free plastic fat. | Norizah et al. (26)      |
|                             | Beef tallow + palm stearin+ canelilla oil              | T-60, 70, 80°C ; t-3, 4, 5 h; R-6-4:4, 7:3:4, 8:2:4; S- 200rpm E-3.0%/ 3.5%, 4.0% | Lipozyme RM IM             | Shaking incubator with stirring |                                                                                             | Pang et al. (22)         |

| Human milk substitute        | Palm stearin fractionate (PSF)/ fish oil                | T-60°C ; t-8h, R-25;75; S- 200rpm; E-10% | Lipozyme TL IM             | Magnetic stirrer cuum hot plate | Rich in n-3 PUFA                                                                          | Ghosh et al. (45)        |
|                             | Palm oil + 2- MAG (2-monoacylglycerol)                  | T-37°C ; t-6h, S- 550rpm; E-30%         | Lipozyme RM IM             | Magnetic stirrer cuum hot plate | Obtained similar TAG as human milk with single base oil                                      | Shimane et al. (45)      |

| Low-calorie structural lipids| Soy oil + extra virgin olive oil + fully hydrogenated Crambe oil | T-60°C ; t-8h; S- 180rpm                | Lipozyme TL IM             | Shaking water bath               | Improvement in absorption of essential FA and can combat obesity                           | Moreira et al. (45)      |
|                             | Heterotrophic microalgal Oil+ rapeseed oil              | T-60/80°C t-8h, R-10-7: S- 200rpm       | Lipozyme RM IM & Candida Antarctica | Magnetic stirrer cuum hot plate | Low fats, nutritionally enriched with essential fatty acid                                | Bogevik et al. (35)      |
|                             | Palm stearin + rapeseed oil                            | T-60°C t-16h, R- 6-4; 7:3, E-10%       | Lipozyme TL IM & Lysozyme 435 | Fluidized-bed reactor            | Enriched with $\beta'$ crystal and efficiently used for preparation of fast frozen food   | Zhi et al. (46)          |
| Frozen food                  | Cinnamomum camphora seed oil + palm oil                | T-65°C ; t-8h, S- 200rpm, E- 10%       | Lipozyme RM IM             | Shaking water bath               | Low cost and low calorie chocoalte                                                        | Ma et al. (27)           |
|                             | Irvingiagabonensis seed fat + Dacryodesedulis pulp Oil | T-70°C ; t-16h; R-9:1; S- 200rpm        | Lipozyme TL IM             | Stirring reactor                 | Use of tropicls oil                                                                       | Yumoneka et al. (58)     |
|                             | Fully Hydrogenated Palm Kernel Oil + Coconut Oil + Fully Hydrogenated Palm Stearin | T-60°C; t-6h; R-4:1:1-8:1:1; S- 300rpm E-0% | lipase from Rhizopus oryzae | batch-type reactor                | Tempering is not required                                                                  | Ornl-led et al. (38)     |
|                             | Coconut oil + sesame Oil + Tween                      | T-45-64°C ; t-16-48h; R-50-70-50-30-0.5; S- 200rpm E-10% | Lipozyme RM IM             | Stirring reactor $\text{trans}$ free fats, decreased saturated fat |                                                                                             | Sivakanthan et al. (57) |
| Confectionary                | Hard palm mid Fraction + mango kernel Fats + cocoa butter | T-75°C ; t-5h; R-2-6-2:2; S- 200rpm | Lipozyme RM IM | Shaking rotor                   | polymorphisms and microstructures were close to those of CB                               | Jin et al. (56)          |
|                             | Algal oil + lauric acid                               | T-65°C ; t-2-5 h; R-1:8; S- 150mp E-12g/100g | Lipozyme RM IM & Lipozyme TL IM | Shaking rotor                   | DHA rich SLs                                                                              | Li et al. (57)           |
|                             | Microbial oil from Schizochytrium sp. + medium-chain triacylglycerols (MCT) | T-30-70°C ; t- 8 h; R-1:1; S- 600rpm E-4- 12% | NS40086, Lipozyme 435, Lipozyme TL IM and Lipozyme RM IM | Water bath with a magnetic stirrer | DHA-rich medium and long-chain structured lipids                                           | Zou et al. (55)          |
|                             | Amazonian Buriti oil and murumuru fat                 | T-40°C ; t- 24h; R-70:30; S- 150pm E-10% | Lipozyme RM IM and Lipozyme TL IM | Orbital shaking water bath        | Utilization of underutilized oil                                                            | Speranza et al. (54)     |

*T-temperature, t-time, R-substrate ratio, S-rotation per minute (speed), E-Enzyme concentration
much lower concentration of $S_2$ TAGs. From a health perspective, decreasing $S_3$ TAGs is beneficial for CVD. Also, the formation of $S_1U$ and $SU_2$ increases the applicability of the product formed as these TAGs improve the structure, plasticity, and low oxidative stability. Additionally, some unique TAGs were created during interesterification. Also, after the EIE reaction process, there were significant increases in the concentration of medium-chain TAGs and significant decreases in the concentration of high melting TAGs. Deb Nath et al. found that the EIE of rice bran oil with palm oil increased the content of monounsaturated and triunsaturated triacylglycerols. On the other hand, triunsaturated triacylglycerol decreased.

6 Effect of EIE on Oxidative Stability

To improve the physical and nutritional qualities of the designer or custom-made fats and oils through enzymatic interesterification, the finished product’s oxidative stability should be comparable to or better than the starting product. Several parameters like peroxide value and induction time may be used to determine the quality of edible oil. Rancimat, which measures the induction time, is used to determine oxidative stability. Longer induction times indicate more oxidation stability, while shorter induction times indicate low oxidative stability. Oils high in PUFA are more vulnerable to oxidation, which adds to the generation of free radicals and hydroperoxides, lowering the strength and stability of the oil.

The essential components that impact the oxidative stability of EIE blends are the different techniques of manufacture, purification techniques during interesterification, the nature of oil sources, or the presence of antioxidants throughout the manufacturing process. Furthermore, the molecular structure of TAG, such as fatty acid composition, positional distribution on the glycerol backbone, and the interplay of these components, are critical aspects that determine the stability of structured products. The majority of findings suggest that EIE leads to low oxidative stability compared to native/pure oil sources. An example showing this effect recently includes the preparation of human milk substitute (HMS) using lard and hemp seed oil. They observed that HMS prepared using different reaction conditions showed lower induction time ranging from 26.2 min to 28.5 min compared to lard (48 min). Also, this study found that the time used during the EIE reaction affects the peroxide value of the product. Another study by Wirkowska-Wojdylo reported that interesterified blends showed significantly lower induction time (18.2 min) than non-interesterified blends. Similar results were also reported by Aktas et al. After interesterification, especially for the EIE fats, the oxidative induction time of the samples was significantly reduced. Comparing the induction times of Chemically interesterified with enzymatically interesterified, for instance, EIE-induction time was 8.39 hours, whereas EIE was only 1.89 hours. It can be inferred that the induction time was correlated to the change in acylglycerol compositions in the product. Partial hydrolysis during EIE increased the product’s free fatty acid (FFA) and diacylglycerol (DAG) content. Hence, the EIE fats showed lower oxidative stability.

The low oxidative stability is due to the loss of antioxidants, natural phenolic compounds, and tocopherols during post and post-processing. The other important factor affecting oxidative stability is the spatial distribution of fatty acids on the glycerol backbone also affects oxidative stability. When comparing TAG with PUFA at the $sn$-2 position to TAG at the $sn$-1,3 positions, it was discovered that TAG with PUFA at the $sn$-2 position was the most stable against oxidation. In most studies, PUFA-rich oil is extensively used to prepare structural lipids or functional lipids to improve the fatty acid composition of EIE blends. Incorporating saturated fatty acids into the structure of the triacylglycerols results in improved induction time and improves oxidative stability.

7 Industrial Application

Over the last decade, enzymatic interesterification has become popular for high-value items, including confectionary fats. EIE’s main products are cocoa butter alternatives, low-calorie fats, human milk substitutes, shortenings, margarine, and functional lipids. As enzymatic interesterification improves the textural properties, the stability of the product modifies the melting characteristics, and a wide range of products can be manufactured.

7.1 Shortening and margarine

Margarine is plastic fat having a high content of fats and oils. Traditionally, shortening and margarine were produced mainly by the partial hydrogenation process, which reduces the degree of fatty acid unsaturation and the production of by-products like transfats. Because of the trans fat formation during hydrogenation, industries are replacing it with low-trans or trans-free plastic fats using lipase-catalyzed interesterification. The main characteristics of margarine are that it spreads easily and melts quickly in the mouth. It should crystallize as a β’ polymorph as this polymorphism improves the stability and granularity of the fat. Shortening and margarine prepared/produced using EIE have improved physicochemical properties in terms of melting behavior, polymorphism, and solid fat content.

Researchers have used a different combination of oil blends for the production of margarine using the EIE process. Oils high in lauric acids, such as coconut and palm kernel oil, which primarily contain medium-chain fatty
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acids (MCFAs), can be combined with hard fats that have a high melting point and liquid oils that have a low melting point to create margarine and shortenings. By changing the ratio of both fractions, it is possible to get the necessary melting point for the plastic fats. For example, a margarine stock has been produced by enzymatic interesterification by using Lipozyme TL IM. The enzymatically interesterified margarine produced with 40% CO had the best properties of margarine with evenly distributed β’ crystalline form. Also, margarine produced has diverse fatty acid composition, low melting TAG molecules, and absence of detectable β form polymorph, which is expected to provide resistance to developing a gritty mouthfeel. In a different study, EIE produced an analogue of beef tallow used to make low-trans margarine utilising Lipozyme R3 of soybean oil and fully hydrogenated palm oil in a 4:3 ratio. The generated analogue has a reduced melting point between 29.1-48.8°C compared to its non-interesterified counterpart (45.2-53.1°C). Additionally, compared to beef tallow and oil blend, interesterified oil demonstrated a reduction in crystallization rate, which is regarded preferable for the stability of margarine, and the development of a significant amount of small β’ form crystals.

Different varieties of margarine can be produced by varying the proportion of palm stearin and canola oil in the interesterification process. Fully hydrogenated palm oil with soybean oil was used to prepare trans-free margarine. In both the studies, small needle-like β’ crystals are formed which are desirable for margarine and had similar SFC and crystallization properties compared to commercial margarine. Therefore, the EIE process helps achieve desirable polymorphism and melting profiles to prepare trans-free margarine and bakery fat because of the formation of new TAG molecules after interesterification.

7.2 Cocoa butter substitute (CBS)

Cocoa butter (CB) is a natural lipid derived from cocoa plant seeds (Theobroma cacao). In the manufacturing of chocolate, it is widely utilized as a key fat element. CB is made up of three types of triacylglycerols (TAG): 1,3-dipalmitoyl-2-oleoyl-glycerol, also known as POP (18.2%), 1-palmitoyl-2-oleoyl-3-stearoyl-glycerol, also known as POSt (38.7%), and 1,3-distearoyl-2-oleoyl-glycerol, also known as StOSt (26.7%). Cocoa butter is crystalline and melts to give the desirable “mouth feel” between 25-35°C. Cocoa butter is an expensive and major ingredient in chocolate preparation. However, it can also produce less expensive replacements by combining interesterified lauric acid with other low-cost vegetable oils and fats. Enzymatic interesterification of different vegetable oil produces cocoa butter substitutes with similar physical and chemical characteristics to commercial cocoa butter. A CBS has been produced from fully hydrogenated palm kernel oil, coconut oil, and fully hydrogenated palm stearin using sn-1,3 specific lipase from Rhizopus oryzae. The produced SLs contained suitable TAG structure with a major proportion of SU and UUU type and suitable SMP content ranging from 33-42°C, a steeper SFC curve with major β’ polymorph.

In a study, palm oil, palm olein, and palm kernel oil were enzymatically interesterified using 1,3 specific lipase enzymes to prepare cocoa butter substitutes for chocolate manufacturing. It was reported that β’ form crystals were prominent, small, and dense in cocoa butter substitutes because of the suitable TAG formed during EIE. Compound chocolate made with fat has the requisite hardness, better texture, and fracturability. Therefore can be used in the chocolate industry. Similar results are also reported by Ornla-ied. Other oil mixtures, such palm oil and Cinnamomum camphora seed oil, were utilized as cocoa butter alternatives. Ma et al. created CBS utilising EIE by combining hydrogenated palm oil and cinnamomum camphora seed oil. The obtained fat primarily crystallised in the β’ structure and had a slip melting point close to a commercial CBS, indicating that tempering would not be necessary when used in confectionery products. Researchers have also explored tropical oil as a new source of CBS. Irvingiagabonensis seed fat and Dacryodes edulis pulp oil are enzymatically interesterified in formulating CBS. It was reported that the enzymatically interesterified CBS showed a melting profile and polymorphic behavior similar to commercial cocoa butter.

7.3 Cocoa Butter Equivalents (CBE)

Many fats and oils, including palm kernel oil, mango seed kernel fat, kokum butter, sal fat, shea butter, and illipe fat, can be combined or altered to develop cocoa butter equivalents because they have a similar fatty acid and triacylglycerol composition to cocoa butter and have a tendency to yield polymorph when subjected to controlled processing. CBE is made from lower-value fats and oils and has a triacylglycerol composition similar to cocoa butter. Different methods like fractionation, blending, and EIE produce CBE. By fractionating material fats based on the variable melting points of various triacylglycerols, components with different melting points can be produced. Through physical mixing, these components are transformed into CBE, which shares many characteristics with CB. However, it is difficult to produce CBE using blending and fractionation that closely resembles the triacylglycerol profiles of commercial CB. Desirable physical and thermal properties are produced when EIE 1,3-specific lipase incorporates fatty acids into the sn-1,3-positions while keeping the fatty acid residues in the sn-2-position unaltered. Lipoyzime Rhizopus oryzae was used to synthesize CBE from an illipe butter: Palm mid fraction blend with a different weight ratio using EIE. The CBE’s TAG profile was identical to...
that of a commercial CB, and it had the same crystallization and melting thermograms, polymorphic structure, and morphology as a commercial CB\textsuperscript{41}. A recent study by Huang \textit{et al.}\textsuperscript{42} used Lipzyme RM IM to make CBE from a 1:1 combination of palm mid fraction and stearic acid via EIE. The TAG composition, polymorphic structure, and SFC curve of the CBE were similar to those of the CB. Therefore, it could be combined with CB in any ratio without causing substantial eutectic effects.

7.4 Human milk-fat substitutes (HMFS)

For infants, human milk is natural and healthy food. To meet their nutritional needs in the early years, human milk contains the optimal composition and provides essential PUFAs to facilitate growth and development. Breast milk is the primary source of nutrients and energy for infants, and infant formulas are formulated to imitate the composition of breast milk fatty acids due to the limited breast milk supply. Therefore, numerous alternatives were required to fulfill the infant’s nutritional requirements. Most researchers have used EIE to formulate milk analogues\textsuperscript{31, 41–45}. EIE plays a vital application in infant formulas as by using \textit{sn}-1,3 specific lipase enzyme, the similar fatty acid and TAG composition of human milk can be prepared. Various researchers use the EIE method in infant milk formula or substitute for human milk. For example, Bryš \textit{et al.}\textsuperscript{31} formulated a human milk substitute from lard and hemp seed oil using EIE. It was found that the formula imitates fatty acid composition and acyl distribution on the glycerol backbone of human milk. In another study, milk fat is produced using soybean and canola oil (35\%) and milk fat (75\%) using CI & EIE. They showed a difference in the aroma of the chemical and enzymatic produced milk fat. Fat which is rich in palmitic acid at the \textit{sn}-2 position and also rich in unsaturated fatty acid at the \textit{sn}-1,2 position is suitable for the manufacturing of HMFS. A study manufactured HMFS using only single oil (palm oil) and showed that EIE improves the palmitic acid content at \textit{sn}-2 position\textsuperscript{43}.

Other oil, such as fish oil, a good source of EPA and DHA, is also used with other saturated fats to produce human milk fat analogue\textsuperscript{45}. To achieve similar physicochemical properties as human milk, varieties of oil rich in essential fatty acids in different proportions were used by researchers. Therefore, the mild reaction condition used in EIE helps protect PUFA and can be incorporated to produce a human milk fat substitute. Lipase enzyme (\textit{sn}-1,2 specific) is used during EIE due to its regiospecificity towards the \textit{sn}-2 position, which is beneficial for incorporating palmitic acid at the \textit{sn}-2 position of the oils and fats.

Because of EIE’s versatility and tailored properties, the food industry is also manufacturing a variety of confectionery and bakery products using this process. A variety of enzymatically interesterified oils and shortenings are available from The Archer Daniels Midland Company (ADM, Chicago, IL, USA), to offer ecologically friendly and healthful ingredients. A company Delta produces healthy oil rich in omega-3. Bunge Oils (St. Louis, MO, USA) manufactures shortening free of trans fats and optimize saturated fatty acid. The Abbott Nutrition for Healthcare and Professionals line seeks to improve particular fatty acid absorption while also offering other benefits. In the composition of their commercial product Vital AF Cal, SLs from interesterified marine oils are used. This therapeutic nutrition approach for oral or tube feeding aims to reduce inflammation and other signs and symptoms. A different oral or tube feeding solution, PediaSure Peptide Cal uses SLs from interesterified canola and MCTs to address the nutritional needs of kids aged of 1 and 13 years\textsuperscript{40, 46}. Commercial infant formula produced by EIE such as Betapol and InFat is produced by USA-based companies to mimic human milk fat. Therefore, the industries are growing and scaling up structural lipids for various applications. The industry also needs to explore unconventional omega-3-rich oil for EIE. There is no data related to the consumption and production of the SLs produced by companies. Also, the long-term effects of the SLs on health need to be studied.

8 Conclusion

Enzymatic interesterification can improve various physicochemical properties of fats and oil to produce a wide range of fat-based products. EIE of fats and oil leads to increased SMP profile, improved melting, and crystallization behavior. A newly identified base oil rich in omega-3 and natural antioxidants can be used as a substrate with other oils to improve the nutritional value of the products. Fat-based products such as cocoa butter substitutes, margarine, human milk substitutes, and bakery products are prepared using enzymatic interesterification. In the future, to understand the advantages and risks of SLs on human health after prolonged use, substantial research is required in this field. Due to the lower oxidative stability of SLs, efforts to increase oxidative stability require specific consideration.

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Conflict of Interest

There are no conflicts of interest to disclose.
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Author Contributions

"Conceptualization, R.C. and P.S.; methodology, P.S.; validation, A.D. and M.G.; formal analysis, P.S. and A.D.; investigation, A.D.; resources, A.D.; data curation, R.C.; writing-original draft preparation, P.S. and A.D.; writing-review and editing, R.C. and M.G.; visualization, M.G.; supervision, R.C.; funding acquisition, P.S. All authors have read and agreed to the published version of the manuscript."

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