



THE METHOD THAT MAKES OILS AND FATS HEALTHIER: INTERESTERIFICATION

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Abstract: Interesterification is a versatile modification technique with significant benefits for health, the environment, and the economy. It serves as an advantageous alternative to partial and full hydrogenation, preventing the formation of trans fatty acids and saturated fats. While more research is needed to fully understand its health impacts, existing studies suggest that the health effects may stem from end products rather than the process itself. This method is particularly valuable in producing alternatives to breast milk, enriching foods with omega-3 fatty acids, and contributing to food safety through applications like edible film production. From an environmental standpoint, enzymatic interesterification is especially advantageous due to its ability to reuse enzymes and minimize waste, thus reducing environmental impact. The process also offers energy savings and increased efficiency, which are both environmentally and economically beneficial. By preserving food quality, aiding in the production of trans fat free margarine, and reducing the need for hydrogenation, interesterification supports the food industry while indirectly contributing to lower health expenditures. Despite its advantages, interesterification is influenced by factors such as fatty acid composition, triacylglyceride stereochemistry, temperature, process duration, catalyst usage, and the condition of the oil mixture. Challenges such as acyl migration and residue formation can arise. While both chemical and enzymatic methods are utilized, enzymatic interesterification has become more popular due to its efficiency and environmental benefits. Future research should focus on enzymatic interesterification to optimize its applications. Given its widespread use, it is recommended that interesterification undergo thorough risk assessments by national and international authorities to ensure safety, particularly concerning its health effects.

Keywords: Interesterification, Margarine, Trans fatty acids, Hydrogenation

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1. Introduction

Fats and oils are indispensable parts of human life and are used for various purposes in food production. They primarily affect the organoleptic properties of food. They can improve the texture and mouth feel of food and its appearance and taste. Features such as the stability of air bubbles in products like cakes, absorbency to prevent the absorption of water from flour in bakery products like biscuits, extending shelf-life due to moisture retention, providing a shiny appearance when poured into hot foods and having an efficient heat transfer ability lead to the use of oils (Spessato et al., 2023).

Edible oils are a complex mixture of triacylglycerides, containing variety of saturated, unsaturated and trans fatty acids. They also have minor components such as phospholipids, pigments, tocopherols. Although fat components provide functionality to foods, saturated and trans fats can adversely affect human health (Oroian, 2024). Many important scientific authorities recommend that saturated fat and trans fat should be reduced. According to Harvard Health Publishing, saturated fat intake in diets should not exceed 10%. It states that there is no safe consumption limit for trans fat. It also

emphasizes that including monounsaturated and polyunsaturated fatty acids in the diet instead of saturated fat and trans fatty acids will positively affect human health (Harvard Health Publishing, 2022).

There are slight differences between the sequences of fatty acids. Fatty acids have hydrocarbon groups of different lengths and methyl and carboxylic groups at both ends. Depending on the double bond between the carbon groups in fatty acids, they are divided into saturated, monosaturated and polysaturated (Karageorgou et al., 2023). Saturated fats have not any double bonds in its carbon chain. In contrast, monounsaturated fats have one double bond, polyunsaturated fats have more than one double bond in their carbon chain (DiNicolantonio and O'Keefe, 2022). Mono and polyunsaturated fatty acids have cis or trans isomers. In cis isomers, H atoms are positioned at the same side of the carbon double bond. On the other hand, trans isomers have H atoms on the opposite side of the carbon double bond. Trans fatty acids provide better adherence and cell penetration. For this reason, no studies were conducted on removing trans fatty acids from oils for many years, and a method called partial



hydrogenation was used. However, in the 1990s, with the understanding of the negative effects of trans fatty acids on health, a method to remove trans fatty acids from oils was sought (Shah and Thadani, 2019).

Interesterification, one of the modification methods for reducing trans fatty acids in oils, has become increasingly widespread. Although its applications are increasing in the industry, there are significant gaps in the literature. According to the data of the National Library of Medicine (NIH), there are 321 articles using the term "interesterification" between 2004-2024; 89 of them (approximately 28%) belong to the years 2020-2024 (NIH, 2024). Moreover, in the Web of Science (WoS) database, there are 782 studies in the field of food science technologies that include the term "Interesterification" from the last 20 years, while there are 169 (almost 22%) studies from the last 5 years (WoS Database, 2024). The purpose of this review is to provide information about the application of the interesterification method, its effects on health, and legal regulations. The review will be a guide for future studies.

2. Modification Methods

Fats and oils that have not undergone any modification process do not always have the required properties. Fats and oils with the desired ideal properties are limited because they are expensive or raw materials that are available in limited quantities. Modification techniques offer a solution at this point. These techniques alter the physical and chemical properties of fats and oils and are effective in imparting desired properties to foods such as stability, melting point. They may also support consumer health by helping to regulate fatty acid ratios. In short, modification methods have an impact on fats and oils in terms of economy, technology and health. (Zbikowska et al., 2023).

2.1. Hydrogenation

The oldest modification technique used in oils is hydrogenation. This method, which started to be used in industry at the beginning of the 20th century, allowed the solidification of liquid oils. Hydrogenation is a process that involves saturating oil with hydrogen as a catalyst, typically Ni, and maintaining a temperature range of 160-200 °C. During hydrogenation, the double bonds of unsaturated fatty acids are saturated with hydrogen atoms, resulting in a product that has a relatively high content of saturated fatty acids. Hydrogenation extends shelf life by reducing the polyunsaturated fatty acid content and improving the functionality of oils for use in certain applications by converting them. The reaction is terminated when the product reaches the desired fat content and melting point. However, nickel catalysts cause the formation of trans fats and leave residues. This can pose a risk to both human health and product quality. Therefore, alternative catalysts to nickel can be used in hydrogenation. Palladium catalysts, although quite effective, tend to inactivate themselves, while platinum is less active and produces less trans fatty acids.

Hydrogenation is not only affected by the type of catalyst. Factors such as the initial oil composition, amount of catalyst, reaction temperature, pressure, time and reaction type also affect the reaction. There are three types of hydrogenation: selective, partial and full (Rasor and Duncan, 2014; Troncoso et al., 2022).

In selective hydrogenation, a small amount of hydrogenation is performed to increase the oil stability before it becomes a solid. In some cases, this technique has also been applied to soybean and canola oils, which include too much linolenic acid, to increase their stability. The cis- to trans-isomerization can be reduced by keeping the reaction temperature low and by a selective catalyst such as platinum, in contrast to the standard hydrogenation process (Rasor and Duncan, 2014).

Partial hydrogenation is defined as the process in which the unsaturated fatty acids in the oil are not completely removed. If the final iodine number, which is used as a measure of unsaturation, is greater than 4, it is interpreted as partial hydrogenation, and if it is small, it is interpreted as complete hydrogenation (Bhandari et al., 2020). During partial hydrogenation, temporary half-hydrogenated molecules are formed before the completion of the process, the loss of a hydrogen atom from the unsaturated fatty acid molecule and the conversion of the double bond to the trans configuration occur more easily than the addition of another hydrogen to the saturated form. Therefore, trans fatty acids are significantly formed under partial hydrogenation conditions (Zbikowska et al., 2019). The World Health Organization reported that consuming excessive amounts of trans fatty acids increases the risk of death for any reason by 34% and that of death from coronary heart disease by 28%. As a result, studies are being conducted worldwide to minimize trans fat consumption (WHO, 2018).

In recent years, the use of cold plasma for hydrogenation has attracted attention because it is both sustainable and reduces the amount of trans fat. Although the use of this technology creates secondary lipid products, it can be used as a new method in oil modification over time (Thirumdas, 2023).

2.2. Fractionation

Fractionation is a widely used process in the oil industry, which allows the separation of low and high melting triacylglycerols under controlled cooling conditions, and olein and stearin fractions with different chemical and physical properties are obtained. Fractionation is used in the modification of various oils, including margarine, frying oil and cocoa butter substitutes using palm oils. Because fractionation helps to obtain stearin and olein with the desired quality and distribution (Tong et al., 2021). Fractionation can be done through three different methods: dry fractionation, solvent fractionation, and detergent fractionation. Dry fractionation which is the most common fractionation method enables thermomechanical separation of a mixture of acylglycerols by crystallization of the melt followed by

separation of the fractions by vacuum filtration. It does not use any additives. The disadvantage of dry fractionation is that a greater amount of olein is usually trapped in the stearin. (Papchenko et al., 2021).

Solvent fractionation has high efficiency, effectively separates components at low temperatures and provides purer products than other fractionation methods. However, it has high operating costs. However, solvent fractionation produces high value-added products containing certain triacylglycerides (Hasibuan et al., 2021). In solvent fractionation, solvents such as acetone reduce the viscosity of the oil during the process and increase the heat transfer coefficient. This also provides benefits such as controlled triglyceride distribution and high purity. However, there is a health risk due to the possibility of solvent residue in solvent fractionation (Tong et al., 2021).

Detergent fractionation is done by adding a detergent such as sodium lauryl sulfate to facilitate the separation of the liquid fraction olein and the solid fraction stearin. This method offers the advantages of shortening the fractionation time and producing a harder stearin. Nevertheless, detergent fractionation is rarely used today because detergent residue is problem (Tong et al., 2021). Excessive use of detergent increases the possibility of residue as well as waste. Insufficient use slows down the separation of solid and liquid fractions, and may cause the surface of the crystals formed to be rough and narrow, reducing quality (Hasibuan et al., 2018).

2.3. Interesterification

The properties of fats and oils are determined by their fatty acid composition and the stereochemistry of triacylglycerides, which consist of three fatty acids and one glycerol. The physicochemical properties of triacylglycerides vary depending on factors such as fatty acid chain length, degree of saturation, and the position of fatty acids in the glycerol backbone. While their functionality is limited in the natural state, triglyceride esters are redistributed on the glycerol bond and become functional with interesterification. The interesterification process consists of two stages: first, hydrolysis, followed by esterification (Zhang et al., 2020). This change in the TAG molecular structure plays a role in thermal or physicochemical properties such as melting point, crystallization behavior, solid fat ratio, oxidative stability, while trans fatty acids are not produced and the degree of unsaturation of fatty acids is preserved. (Zhang et al., 2022). The interesterification method is divided into two: chemical and enzymatic interesterification.

2.3.1. Chemical interesterification

In chemical interesterification, fatty acid transfer is carried out in the presence of chemicals such as sodium methylate (CH_3ONa) in the range of 80-120 °C. This method has been used since the 1920s (AOCS, 2021a).

Since the sodium methylate used as a catalyst reacts with H_2O and free fatty acids, the oil mixture should be dry and neutral. To achieve this, neutralized components are used, an amount of KOH is added to the reaction vessel,

and any moisture present is evaporated by vacuum. Some color formation can be observed during interesterification. The bleaching process is subsequently applied; therefore, RBD oils should not be used as raw materials. The oil mixture to be interesterified was dried before the catalyst was added. An amount of 0.05% by weight is adequate for sodium methylate. Although the reaction is too fast, it is generally allowed to continue for up to thirty minutes. The catalyst is deactivated by the addition of water, which results in soap formation. The use of water, which includes some acid for inactivation, results in the formation of free fatty acids instead of soap. Both soap and free fatty acids cause loss of efficiency, which is detrimental for chemical interesterification. Using more catalysts will further increase this yield loss (AOCS, 2021a).

2.3.2. Enzymatic interesterification

Enzymatic interesterification is gaining popularity in industrial applications as an effective method for lipid modification due to advances in immobilized enzyme technologies. It offers several advantages, including higher specificity, reduced environmental pollution, and basic post-processing processes. In addition, immobilized enzyme can be reused multiple times in carefully optimized interesterification processes, reducing costs (Cui et al., 2022).

Enzymatic interesterification is done by using enzymes to alter the fatty acid composition and distribution within triacylglyceride molecules. This process can be random or specific depending on the enzyme used. When a nonspecific lipase enzyme is used, the fatty acids are randomly redistributed between positions in the triacylglyceride molecule, resulting in changes in its physical properties. In contrast, a specific enzyme, like a sn-1,3-specific lipase, is designed to hydrolyze only the sn-1 and sn-3 positions, ideally leaving the sn-2 position unchanged. However, during application, acyl migration can occur and alter the fatty acid at the sn-2 position. Acyl migration involves the positively charged carbonyl carbon in TAG being attracted by the reaction catalyst, forming a tetrahedral intermediate, the diacylglyceride anion (DAG). This DAG anion then attacks a second activated triacylglyceride ester bond, forming an intermediate complex. The complex eventually dissociates, forming the newly interesterified molecule but it can cause loss of specialty (Singh et al., 2022).

A vertical column is used in the enzymatic interesterification process. This column contains the immobilized lipase enzyme. The oil mixture to be esterified is pumped from the upper part of the column and the feeding continues from above during the process. The oil that has been made interesterifying is extracted from the base. This process was carried out continuously at 70°C. However, when using a single reactor oxidized oils or citric acid and mineral acid residues may decrease the activity of the enzyme. To prevent this, more than one reactor should be used in the process, refining should be applied to the oils before interesterification or the

amount of residue should be controlled before the process (AOCS, 2021b).

2.3.3. Uses of interesterification and regulations

The main use of interesterification is the production of margarine. Trans fat is formed because of partial hydrogenation, which is an old production method. Many countries, including Türkiye, have applied legal regulations in response to the negative health effects of trans fats to prevent the presence of trans fat in foods, with the discovery that trans fat causes cardiovascular diseases. At this point, interesterification has become an option for partial hydrogenation. The interesterification method allows the production of vegetable oils that are trans fat free, while the initial amount of unsaturated fatty acids is preserved (Sivakanthan and Terrence, 2020).

Another application area is breast milk substitutes. Breast milk fat is essential as a source of nutrition and energy for infants. Therefore, alternative oils are needed to meet these needs, such as the inability to breastfeed. However, the fatty acid composition of breast milk varies from person to person, depending on factors such as diet, and interesterification studies have been carried out on palmitic acid, which is the richest saturated fatty acid in breast milk (Wei et al., 2019).

Third, the addition of new fatty acids during interesterification results in the production of structured lipids with increased nutritional value and potential health benefits. For example, omega-3 fatty acids can be incorporated into oils in this way (AOCS, 2021b). Among nutrients, low-calorie structured lipids, which can be considered another derivative of structured lipids, reduce the caloric value of most energy-giving fats (9 kcal/g) to intervene in increasing obesity worldwide. The caloric value is reduced by relatively reducing the density of medium-chain fatty acids or by reducing the intestinal absorption of long-chain fatty acids (Zárate et al., 2017).

The area where interesterification has just begun to be used is edible films. Edible films include carbohydrates, fats and proteins. The animal or vegetable oils contained in these films provide moisture barrier properties. In addition, unsaturated fatty acids reduce surface tension. A high oleic acid content of lipids resulting from interesterification is also considered more suitable for edible film production (Moore and Akoh, 2017).

Owing to the cost of cocoa butter, which is characteristic of chocolate, the search for a substitute for cocoa butter or equivalent oil has begun. The aim of interesterification at this point is to obtain alternatives with the lowest cost, easy availability and fatty acid composition, such as cocoa butter

There is no direct regulation on the areas where interesterification can be used. The European Commission (EC), in its 'EC working paper' on food processes using food enzymes, emphasises that food enzymes can be used to rearrange the position of fatty acids in triacylglycerols, thereby changing their physical properties (melting point) without creating trans

isomers and/or altered sensory properties, typically using lipases, and that interesterification is operated as a continuous process with immobilised lipases (EFSA, 2023).

In United States Food and Drug Administration (FDA) regulations, it is mentioned that sucrose in sucrose oligoesters can be produced by interesterification with methyl esters of fatty acids derived from edible oils and fats (including hydrogenated oils and fats), lipase enzyme obtained from culture filtrate obtained from pure culture fermentation of a pathogenic and toxin-free strain of *Rhizopus niveus* can be used in interesterification of oils. (FDA, 2023; FDA, 2024a).

On the other hand, there are many regulations in the world regarding the reduction of trans fat provided by interesterification. The first regulation study on the elimination of trans fat was carried out in Denmark in 2003. In the regulation applied since January 1, 2004, the amount of trans fat per 100 grams of fat or oil could not exceed 2 grams (Bjoernsbo et al., 2022). In 2020, Bangladesh, India, Paraguay, the Philippines and Ukraine adopted industrial trans fat limits, followed by Brazil, Peru, Singapore, Türkiye, the United Kingdom and the European Union in 2021 (WHO, 2021). According to the regulation that came into force in the European Union, the amount of trans fat, other than trans fat naturally found in animal fat, has been determined as 2 grams per 100 grams of fat (European Union, 2019). In 2015, the FDA determined that partially hydrogenated oils (PHOs), the main source of artificial trans fats in the food supply, were no longer "Generally Recognized As Safe" (GRAS). In 2021, partially hydrogenated oils were removed from the U.S. market. Also, on nutrition labels, trans fatty acids must be listed as "Trans fat" or "Trans" on a separate line below the saturated fat list (FDA, 2024b).

3. Recent Studies

With the increasing prevalence of interesterification, studies on the development of technology are increasing, along with studies on health. Table 1. shows recent the studies carried out on this topic. Current studies show that both enzymatic and chemical interesterification methods can be used on different oil mixtures and are a versatile modification method. It is observed that enzymatic interesterification is generally carried out at lower temperatures and thus higher tocopherol content is preserved. This can be interpreted as a reason why the enzymatic method provides better nutritional value preservation under less harsh processing conditions and therefore is preferred more than chemical interesterification. Because chemical interesterification can generally result in unwanted side effects with higher temperatures and catalyst usage. It can lower the melting point of oils and lead to the formation of undesirable crystal structures. For example, in a study comparing chemical and enzymatic interesterification, it was concluded that the oil mixtures that did not undergo interesterification had higher peroxide values and were

less stable than those that were interesterified, compared to animal-vegetable oil mixtures used at different ratios. It was also observed that enzymatic interesterification had less trans fat content, more free fatty acids and melting points than chemical interesterification (Okcu and Aktas, 2024). In another study, investigating the effect of enzymatic interesterification on the physicochemical properties and rheological behavior of *Cinnamomum camphora* seed kernel oil, *Pangasius bocourti* stearin and perilla seed oil mixtures, differences

were observed on fatty acid distribution and physicochemical properties. As a result of interesterification, the crystal polymorphological form was transformed from $\beta > \beta'$ to $\beta < \beta'$ and a more stable structure was obtained. It also decreased the crystallization onset temperature and peak temperature of low melting triacylglycerides. It was observed that spreadability and mouthfeel were improved compared to noninteresterified oil mixtures (Tian et al., 2024).

Table 1. Current studies on interesterification

Study	TI	Used Blend	Process Conditions	Result	References
Modification of palm oil mixture by interesterification and investigation of physicochemical properties, crystallization behaviors, oxidative stability The impact of enzymatic interesterification on the nutritional and textural characteristics of butter containing milk fat.	Enzymatic and chemical	Palm olein (50%), palm kernel oil (30%), palm stearin (20%)	Enzymatic: Fat fed with 60°C/100 mL/min flow, presence of lipozyme Chemical: 105°C/30 min, presence of 0.3% sodium methoxy catalyst	Enzymatic interesterification has reduced the fat content, and spreadability has improved. Fats with enzymatic interesterification at a lower temperature than chemical interesterification, crystallized faster than oils with chemical interesterification, and their tocopherol content is preserved.	(Zhen et al., 2021)
Chemical intersterifications impact on the production of cocoa butter substitutes using palm kernel stearin, coconut oil, and fully hydrogenated palm stearin mixtures.	Enzymatic	Milk fat and palm olein mixture (100:0, 80:20, 60:40, 40:60, 20:80, 0:100)	Enzymatic: 70°C/6 hours in the presence of 5% lipase	Enzymatic interesterification has added spreadability to the oil containing probiotics. The butter containing milk fat were improved by interesterification, which rised the oil bases unsaturation degree.	(Santos et al., 2020)
Examination of Soybean Oil Interesterified with Propylene Glycol in Supercritical CO ₂ Using NMR Spectroscopy.	Chemical	Palm Kernel stearin, coconut oil, hydrogenated palm stearin (10:10:80, 20:10:70, 30:10:60, 40:10:50, 50:10:40, 60:10:30, 70:10:20, 80:10:10)	Chemical: 88±2 °C, 1 hour, in the presence of 0.3% sodium methoxy catalyst and bubbling N ₂	While the obtained oils have fine crystals in the desired β' polymorph, they have a lower solid fat value than cocoa butter. The melting behavior of oils originated from the interesterification of 60:10:30 and 70:10:20 mixtures showed a different melting characteristic and almost all melted at body temperature.	(Ornlaid et al., 2021)
Generating trans fatty acids free using enzyme-catalyzed interesterified rice bran oil and fully hydrogenated rice bran oil.	Enzymatic	17 g of soybean oil with an equal amount of not more than 0.5% water	Enzymatic: Under 276 bar supercritical CO ₂ , in the presence of 17 g 1-2 propanediol/oil, in the presence of 0.3% lipozyme, 70°C/15 min	The use of interesterified soybean oil containing 1,2-propanediol can cause more environmentally friendly fats with lower trans fat content compared to commercially available soybean oils.	(Vafaei et al., 2020)
	Enzymatic	Rice bran oil and fully hydrogenated rice bran oil (9 blends alternating at 10% intervals 10:90...)	Enzymatic: Incubation for 24 hours at 60°C, 200 rpm in the presence of 10% lipozyme.	Interesterification has produced new triacylglycerides with moderate degree of unsaturation and suitability. These alterations affected the thermal behavior of the products, decreasing their melting points and fat content.	(Campioni et al., 2021)

TI= type of interesterification

Table 1. Current studies on interesterification (continuing)

Study	TI	Used Blend	Process Conditions	Result	References
Synthesis and characterization of behenic acid-rich lipid by enzymatic interesterification	Enzymatic	Mixture containing olive oil, soybean oil and fully hydrogenated thyme oil in a percentage of 43:43:14 (w/w) in 500 g mixture	Enzymatic: In the presence of 10% lipozyme, continued for 0,1,2,3,4 hours at different times, 60°C, under vacuum, in a 350 rpm mixer	Processing conditions did not affect tocopherol and chlorophyll a level, but increased carotenoid losses. When only olive oil, soybean oil or fully hydrogenated thyme oil and the mixture of these raw materials were compared in terms of oxidative stability, it was observed that interesterification did not cause any difference.	(Moreira et al., 2020)
Preparing trans fat-free bakery products by using margarine containing palm stearin and rice bran oil.	Chemical	Palm stearin, rice bran oil composition (50:50, 70:30, 60:40, 40:60)	Chemical: 90°C/1 hour, 0.8 bar absolute pressure, mixer speed 300 rpm	Chemical interesterification decreased the peroxide value of the mixtures and increased the free fatty acid value. Noninteresterified margarine samples showed fat separation during storage.	(Sewwandi and Arampath, 2022)
Producing trans-free oil through lipase-catalyzed interesterification of rice bran oil with hydrogenated cottonseed oil	Enzymatic	Rice bran oil-hydrogenated cottonseed oil blend (60:40, 70:30, 80:20, 90:10)	Enzymatic: At 50-55-60°C for/6 hours, presence of lipase enzyme at different rates (2.5%, 5%, 7.5% and 10%)	It was observed that the solid fat content decreased with the increase of the lipase enzyme concentration. Higher fat content was obtained at lower reaction temperature.	(Neeharika et al., 2015)

TI= type of interesterification

The process conditions of interesterification (temperature, presence of catalyst, duration, etc.) can also affect the chemical and physical properties of oils. It has been observed that chemical interesterification at high temperatures reduces the solid fat content of oils and increases their spreadability. The behavior of different oil mixtures after interesterification is also important for different process conditions and plays a critical role in the production of products such as margarine. For example, it was observed that the chemical interesterification of solid oil, carried out at 80°C, in which the deep eutectic solvent potassium carbonate was pretreated with glycerol, was more efficient than in the noninteresterified and untreated states of glycerol, the melting point of the solid oil increased significantly, and finer and more regular snowflake-like spherical crystals were formed in the interesterified oil (Meng et al., 2023).

3.1. Health Effects of Interesterification

There are very few investigations on interesterification health effects. Studies on the health effects of interesterification are given in Table 2. Existing health studies on interesterified oils generally examine short-term effects and do not provide definitive information about long-term health outcomes. Interesterification does not significantly affect the digestion of fats, sugar balance and insulin resistance. However, some interesterified fats may negatively affect metabolism not because of the process but because of their fatty acid

content. They can particularly reduce insulin secretion and cause inflammation in white adipose tissue. Moreover, interesterification may alter the bioavailability of fats. The absorption of fats with high melting points may be delayed, which may lead to different processing of fats in the body.

Several human studies have shown that consuming interesterified oils for 3-6 weeks has no significant effect on blood lipids compared with noninteresterized oils. On the other hand, it can potentially contribute to reducing saturated fat intake to promote healthier dietary options. Nevertheless, reaching these bounds to the frequency and amount of consumption of products containing interesterified oil by different groups in the population. In addition, partial hydrogenation can produce trans fatty acids, while full hydrogenation can produce oils with a high saturated fat content. World Health Organization (WHO) recommends reducing trans fatty acid consumption and recommends that saturated fatty acid intake should not exceed 10% of the daily diet. WHO, which aims to decrease the consumption of trans fats that cause cardiovascular disease worldwide by 2023, recommends interesterified oils that do not contain trans fats as options for oils containing trans fatty acids (Berry et al., 2019; WHO, 2019). The effects of hydrogenation and interesterification on health make a significant difference in the content produced. It can be said that interesterification is a preferable fat modification method in terms of health because it prevents the production of

trans fat and reduces the saturated fat rate. Since fractionation is preferred as an intermediate stage, studies on health are insufficient to make a comprehensive assessment.

3.2. Effects of Interesterification on the Environment

When comparing the impacts of chemical and enzymatic interesterification on the environment, the energy consumption is greater in chemical interesterification,

which has more process steps and occurs at higher temperatures.

In addition, less loss occurs during production in enzymatic interesterification. Thus, enzymatic interesterification is considered more environmentally friendly because of the minimum loss of energy, the main saving source, and the catalysts reused during production (AOCS, 2021b).

Table 2. Studies on the effect of interesterification on health

Study	Materials and Method	Effects on Health	References
Comparing the effect of interesterified oil on in vivo postprandial and in vitro lipid metabolism compared to a nonequivalent oil with a reference oil	<p>Subject: Healthy adults aged 45-75 years (n=20)</p> <p>Tested oils: interesterified 80:20 palm stearin/palm kernel oil noninteresterified 80:20 palm stearin/palm kernel oil</p> <p>Experiment time: 8 hours</p> <p>Measurement: Triglyceride and lipoprotein values after consumption of 50 g fat for 4 weeks with 1-week intervals</p>	<p>The interesterification process does not affect the digestion of fats.</p>	(Mills et al., 2021)
Investigation of the effects of interesterified palm oil on blood sugar balance and livers of rats.	<p>Subject: Mice (n=72)</p> <p>Tested oils: palm oil, interesterified palm oil</p> <p>Interesterified palm oil, which is given at a level to meet 10% of the energy in the daily diet.</p> <p>Interesterified palm oil, which is given at a level to meet 60% of the energy in the daily diet.</p> <p>Measurement: For 8 weeks: Insulin tolerance (4-hour intervals), pyruvate level in liver, body weight (once a week).</p>	<p>Consumption of interesterified fats may have an adverse impact on metabolic properties and insulin secretion, and a high-fat diet can cause inflammation in white adipose tissues. It has been stated that these oils cause the same results in rodents and humans, but there is no clear result.</p>	(Miyamoto et al., 2020)
Investigation of the effects of diets rich in interesterified, noninteresterified and trans fats on the biochemical parameters and oxidative status of mice.	<p>Subject: Mice (n=24)</p> <p>Tested oils: Interesterified oil rich in palmitic acid (39%)</p> <p>Noninteresterified oil blend</p> <p>Partially hydrogenated vegetable oil (with trans fatty acid (20%) and linolenic acid (6%))</p> <p>Experiment period: 75 days</p> <p>Measurement: At the end of the 75. day, triglyceride and cholesterol values in the blood</p>	<p>To conclude the change in the triglyceride structure of the fats with interesterification, the cholesterol values in the blood of the mice were found to be higher than the partially hydrogenated vegetable oil.</p>	(de Lima et al., 2020)
Investigation of the effects of chemical interesterified oils rich in palmitic (C16:0) and stearic (C18:0) acids on insulin resistance using natural palm olein rich in C16:0.	<p>Subject: Overweight, healthy adults (n=85)</p> <p>Tested oils: interesterified palm oil rich in palmitic acid (C16:0)</p> <p>Interesterified palm oil rich in stearic acid (C18:0)</p> <p>Natural palm olein rich in palmitic acid (C16:0)</p> <p>Experiment duration: 8 weeks</p> <p>Measurement: Nutrition was carried out to take 35% of the daily diet from fat, and blood sugar and insulin resistance measurement at 0, 6 and 8 weeks</p>	<p>Interesterified oils do not have a significant effect on insulin resistance.</p>	(Ng et al., 2018)
Investigation of interesterification on the bioavailability of fatty acids in lipids (in flaxseed oil and palm stearin blends) using structured (sn-1,3 stereospecific lipase) effects	<p>Subject: Mice (n=48)</p> <p>Tested oils: Blends of interesterified and noninteresterified linseed oil and palm stearin (70-30, 60-40, 50-50 ratios)</p> <p>Experiment duration: After 1 time fasting for 18 hours.</p> <p>Measurement: Fat profile of plasma lipids at 0,1,5,3,4,5, 6 and 12 hours</p>	<p>Its high melting point delays lipid absorption. Interesterification changes the bioavailability of oils.</p>	(Farfán et al., 2013)

Table 2. Studies on the effect of interesterification on health (continuing)

Study	Materials and Method	Effects on Health	References
Impact of interesterification of palmitic acid-rich triglyceride on postprandial lipid	<p>Subject: Healthy people (n=38) Tested oils: Interesterified palm oil, crude palm oil, sunflower oil with high oleic acid Experiment Period: 3 days Measurement: Measurement of insulin, lipoprotein and triglyceride in the blood after meals (measurement in 1-hour intervals after the first 2 hours 30 minutes until the 6th hour)</p>	<p>Consumption of interesterified oils causes lower lipoprotein and triglyceride levels compared to crude palm oil and sunflower oil containing high oleic acid. Insulin concentration was not affected by oil.</p>	(Berry et al., 2007)
Impact of interesterification on acute metabolic risk factors for stearic acid-rich spreadable oil	<p>Subject: Health people (n=24) Tested oils: High oleic acid Sunflower oil – fully hydrogenated canola stearin (70-30%) oil is noninteresterified, chemically interesterified (0.3% sodium methoxy – 85°C) and enzymatic interesterified (5% Candida antarctica lipase – 60°C) Experiment time: 3 days Measurement: Individuals who consume 25% of the total energy in the diet with fat, 0-60. 15 min interval between min, 60-90. HDL cholesterol, total cholesterol, free fatty acid and glucose measurement in the blood with 30 minutes intervals between minutes and 60 minutes after 90 minutes</p>	No interesterification method has been found to affect glucose, insulin or free fatty acids and triglycerides.	(Robinson et al., 2009)
Effect of interesting palm olein on postprandial glucose-dependent response in type 2 diabetes	<p>Subject: People with type-2 diabetes (n=23) Tested oils: Palm olein, interesterified palm olein, sunflower oil with high oleic acid Experiment period: 1 year Measurement: Between 0-360. min after consumption of 50 g of oil per week. Measurement of lipid and glucose level in the blood</p>	Interesterified fat has no effect on glucose balance in type-2 diabetes subjects.	(Mo et al., 2019)
Effects of interesterified palm oil on inflammation of white adipose tissues and metabolic disturbances	<p>Subject: Mice (n=60) Tested oils: Soybean oil (4%), palm oil (23.8%), lard and interesterified palm oil Experiment period: 8 weeks Measurement: The control group (C) was fed a standard diet with 4% soybean oil, the high-fat group (HF) was fed 23.8% lard, the high palm oil fat group (HFP) was fed 23.8% palm oil, and the high interesterified palm oil fat group (HFI) was fed 23.8% interesterified palm oil.</p>	<p>The adverse effects of a high-fat diet do not differ whether interesterification in lipid sources is present or not. Adverse effects have been observed in mice regarding white adipose tissue hypertrophy, hepatic steatosis, and insulin resistance. However, human studies are needed. The long-term implications of these differences in postprandial markers of cardiometabolic health are unclear. However, commercial interesterified oil may have a neutral postprandial effect when compared with similar products without interesterified oil in healthy adults.</p>	(Martins et al., 2024)
Comparison of postprandial lipid and vascular responses of an interesterified palmitic acid-rich margarine to functionally equivalent noninteresterified margarine and butter.	<p>Subject: Healthy people (n= 50 (25 men, 25 women; 35–75 years)) Tested Oils: Interesterified, noninteresterified palm based fats, butter Experiment period: 1 week Measurement: Between 07:30-09:00 a consumption 50 g of oil a week. Measurement of blood samples.</p>	<p>An interesterified oil containing 17% palmitic acid causes similar postprandial lipemia to an oil containing 28% palmitic acid and spreadable butter.</p>	(Hall et al., 2024)

4. Conclusion

In conclusion, interesterification is a modification method that makes human life easier in terms of health, environment and economy, can be considered advantageous. Although more research is needed on the health effects, the health effects of trans fatty acids that may form in partial hydrogenation and saturated fatty acids that may form in full hydrogenation can be prevented by interesterification. Studies that especially show that the processing of oil does not affect health show that the effect may not be due to the process but to the end products that may be formed as a result of the process. In addition, products made as an alternative to breast milk, enriching foods with omega 3 fatty acids, and contributing to food safety by using them in edible film production also have a positive effect on health.

When examined from an environmental perspective, the fact that the enzyme can be reused and leaves little waste in enzymatic interesterification makes an important contribution. Process residues can negatively affect the environment. In addition, energy saving and efficiency also contribute to being environmentally friendly. Energy saving and reuse of the catalyst contribute not only environmentally but also economically. Moreover, it provides significant contributions to the food sector by preserving the quality of food, helping to obtain the desired product, and helping to produce trans fat-free margarine. It is also a good solution to partial hydrogenation, which the FDA has banned in order to limit trans fat. It also contributes to the reduction of health expenditures by indirectly minimizing the negative effects on health. Interesterification is affected by issues such as fatty acid composition and stereochemistry of triacylglycerides, temperature, process time, catalyst usage, and the condition of the oil mixture, and acyl migration and residue problems may be encountered. Although both interesterification methods are used, enzymatic interesterification has become more preferred in recent years. We recommended that researchers who will work on this subject in the future should work on enzymatic interesterification.

Although interesterification makes life easier in many ways, more studies are needed, especially in the health field. We recommend that such a widely used method be risk assessed by national and international authorities.

Author Contributions

The percentage of the author(s) contributions is presented below. All authors reviewed and approved the final version of the manuscript.

	B.İ.	F.A.
C	50	50
D	20	80
S		100
DCP	50	50
DAI	50	50
L	80	20
W	90	10
CR	20	80
SR	50	50
PM	20	80
FA	50	50

C= concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

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