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REVIEW

Oleochemical Industry Future through Biotechnology

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Abstract: Lipases are the most widely used class of enzymes in organic synthesis. Enzymatic processes have been implemented in a broad range of industries as they are specific, save raw materials, energy and chemicals, environmentally friendly and fast in action compared to conventional processes. The most notable benefit is the moderate process temperature and pressure with no unwanted side reactions. In the past two decades, intensive research was carried out towards enzymatic synthesis of oleochemicals. This review has a sharp focus on the current implemented enzymatic processes for producing different oleochemicals such as fatty acids, glycerin, biodiesel, biolubricant and different alkyl esters via different processes including hydrolysis, esterification, transesterification and intraesterification.

Key words: lipase, oleochemicals, esterification, transesterification, intraesterification

1 Introduction

Edible oils could be extracted by several methods such as solvent extraction, mechanical pressing and subcritical water technology[1-5]. Oleochemicals are chemicals derived from oils and fats in a way similar to petrochemicals which are derived from petroleum oils[6]. The world's fatty acids and fatty alcohols consumption in 2010 was 6 million tonnes for fatty acids and 2.5 million tonnes for fatty alchols. Such very large consumption comes from enormous applications including Personal care, home care, pharmaceuticals, food additives, paper, agriculture, animal feed, rubber, paints, coatings, plastics, polymers, textile, industrial chemicals, biofuels, detergents, lubricants,… etc.[7]. With the world's population increase, the need of fatty acids based products increased dramatically. The world's consumption of fatty acids and fatty alcohols is expected to reach 7.5 and 3.5 million tonnes respectively in year 2020[8].

Fatty acids, wax esters, fatty alcohols and glycerin are the most important oleochemicals. These products are produced by lipids Hydrolysis, esterification, transestrification and intraesterification processes. The capital cost required for producing these products is very high (including special towers, heavy duty industrial boilers and very complicated control systems). Also the running cost (including fuel, steam and electricity) is costly. Furthermore the process of production generates some wastes. A number of studies have been developed in the last twenty years to know whether using the enzymatic processes in the oleochemical industry lead to environmental improvement and assess whether they could play a role in moving toward economic and cleaner industrial production[9]. The environmental benefits of enzymatic processes compared to conventional processes in various industries have been discussed in several books, articles and reports over the past decade[10-15]. All agree that enzymatic process are favorable to the environment compared with the traditional one, as they are readily biodegradable and usually lead to reduced or no toxicity when they reach the environment after use in industrial production[15, 16]. These properties allow manufacturers to produce the same or sometimes even better quality products with less raw material, chemicals, water, energy and with less problematic waste generation than traditional processes[9]. However the economic benefits of replacing the chemical methods with the enzymatic one in doubt until now. As enzymes are expensive, it became necessary to find an economical routes for enzyme production as well as to find several systems that can keep the enzyme stability inside the process, to have high productivity with less amount of enzymes, in other words to cover the

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enzyme cost with the products profit margins.

The large scale enzymatic processes application is often hampered by a lack of long-term operational stability and difficult recovery and reuse of the enzyme. These drawbacks could often be overcome by enzyme immobilization. There are several reasons for using an enzyme in an immobilized form. In addition to more convenient handling of the enzyme, it provides for its facile separation from the product, there by minimizing or eliminating protein contamination of the product[15]. Immobilization also facilitates the efficient recovery and reuse of costly enzymes, and enables their use in continuous, fixed-bed operation[17]. Lipase has been immobilized by several methods, namely adsorption[18], covalent binding[19] and entrapment[20]. This review will discuss the current implemented enzymatic processes in the field of the oleochemical industry.

2 Oleochemicals Production via enzymatic processes

2.1 Fatty acids

Fatty acids currently are producing by very high capital and running cost processes. The currently used method of splitting of lipids to fatty acids and glycerol involves high temperature and pressure conditions for about 6 hours to achieve the desired 96-99% conversion. When these extreme conditions are employed, polymerization of fats and by-products formation take place result in dark fatty acids and colored aqueous glycerol solution[21]. To remove the color and the by-products, further purification by distillation is required. Both splitting and subsequent distillation of fatty acids and glycerol are energy intensive processes. The saving of energy and minimization of thermal degradation are probably the major attractions in replacing the current chemical technologies with biological ones. A comparison between both methods was demonstrated in Abdelmoez research[1] (Fig. 1).

Although many papers have been published about enzymatic production of fatty acids by fats splitting, however until know it didn't commercialized in large scale. Most of the currently published papers dealt with batch laboratory scale process. The following points will highlight some important issues explain the challenges associated with the industrial implementation of enzymatic fats hydrolysis.

2.1.1 Lipases inability to catalyze the hydrolysis process efficiently

During the past twenty years of research, a lot of efforts have been done in order to produce active lipases using different producing microorganisms. However when those lipases were used to catalyze the different reactions, it didn't show any promising results, which in turn had a significant effect on forcing the researchers to use the active enzymes produced by the famous lipases producers. Such behavior of the researchers is due to that the obtained lipases activities had no such activity that could catalyze the reaction efficiently. Abdelmoez[1] could produce *C.rugosa* lipase by submerged fermentation and the produced enzyme was directly used in sunflower oil hydrolysis, the highest conversion achieved was 39.5% of the original tested sunflower oil. Edwinoliver[2] could produce *Aspergillus niger* lipase using some agro-industrial residues by solid state fermentation, then the produced lipase was used directly in tallow hydrolysis which hydrolyzed to 52% which is not enough form the industrial point of view.

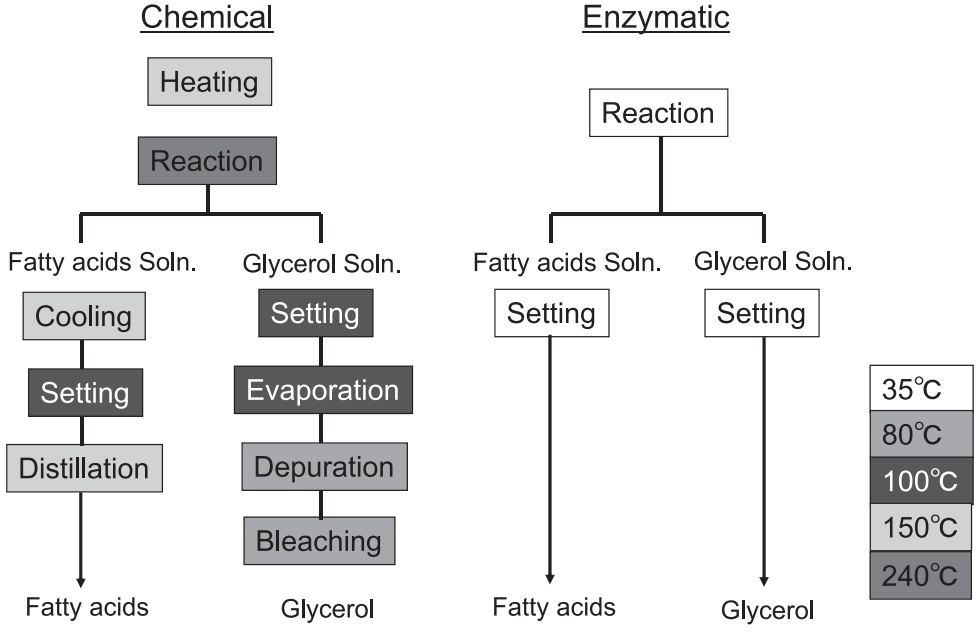

Fig. 1 Comparison between chemical and enzymatic fats hydrolysis[1].

In the other hand by utilizing the most widely used enzyme *C. Rugosa* lipase (Type VII, 746U/mg) which is very expensive, Serri[23] could catalyze the hydrolysis reaction of cooking palm oil to obtain a conversion of 97.12% with enzyme loading of 7.46 KLU/ml iso-octane with oil loading of 0.1 g/ml.

2.1.2 Lack of scientific papers dealing with enzyme production followed by using it in fats processing

Most of scientific papers lack of dealing with lipase production followed by using the produced lipase in oils and fats processing. In other words most of the researches now are divided into two categories, first deals with only lipase production without using the produced lipase in any application. Second using an immobilized lipase provided by common enzymes manufacturer to be used directly in the production of fatty acids. Accordingly the link between the two processes fermentation (lipase production) and subsequent fats processing (application) is missing. To close the loop between fermentation and hydrolysis, and to have a complete view, integrated industrial protocols are required. Abdelmoez[1] developed an integrated fermentation and industrial plant as shown in Fig. 2.

2.1.3 Biodiesel

Due to increasing environmental pressure on greenhouse gases coming from the fossil fuels, biodiesel is becoming the hot topic of every country's policy agenda[24]. Biodiesel has grabbed attention as a non-toxic, biodegradable and renewable source of energy and fuel with lower exhaust emissions of particulate materials and green-house gases such as CO, CO_2 and Sox. Conventional biodiesel is produced by transesterification of triglycerides from vegetable oils and an alcohol (methanol or ethanol) using an alkali catalyst. The process is carried out at high temperature and consumes heat, chemicals such as NaOH (catalyst) and sulfuric acid (neutralizing agent), while side reactions lead to soap formation[9].

2.1.4 Biodiesel production routs

Currently biodiesel feedstock are divided into three categories: (1) plant origin oil such as soybean oil[25], canola oil[26, 27], (2) animal fat such as tallow[28], (3) Waste cooking oil and industrial waste oil such as cooking palm oil[29]. Most of the existing biodiesel production plants are based on chemical methods. The catalysts used are acid catalyst, such as H_2SO_4, or alkaline catalysts, such as NaOH and sodium methoxide. The alkaline method is better than acid catalysis due to the high yield of biodiesel obtained and short reaction times. Generally, large molar ratio of alcohol to oil is needed for alkaline catalysis process to achieve high yield, and a distillation process will be needed for methanol recovery and biodiesel refining. Chemical methods give high yield of methyl esters (biodiesel) in relatively short times (4 hours to 10 hours). However, they have some disadvantages such as high energy consumption, difficulty in recovering the glycerol and significant

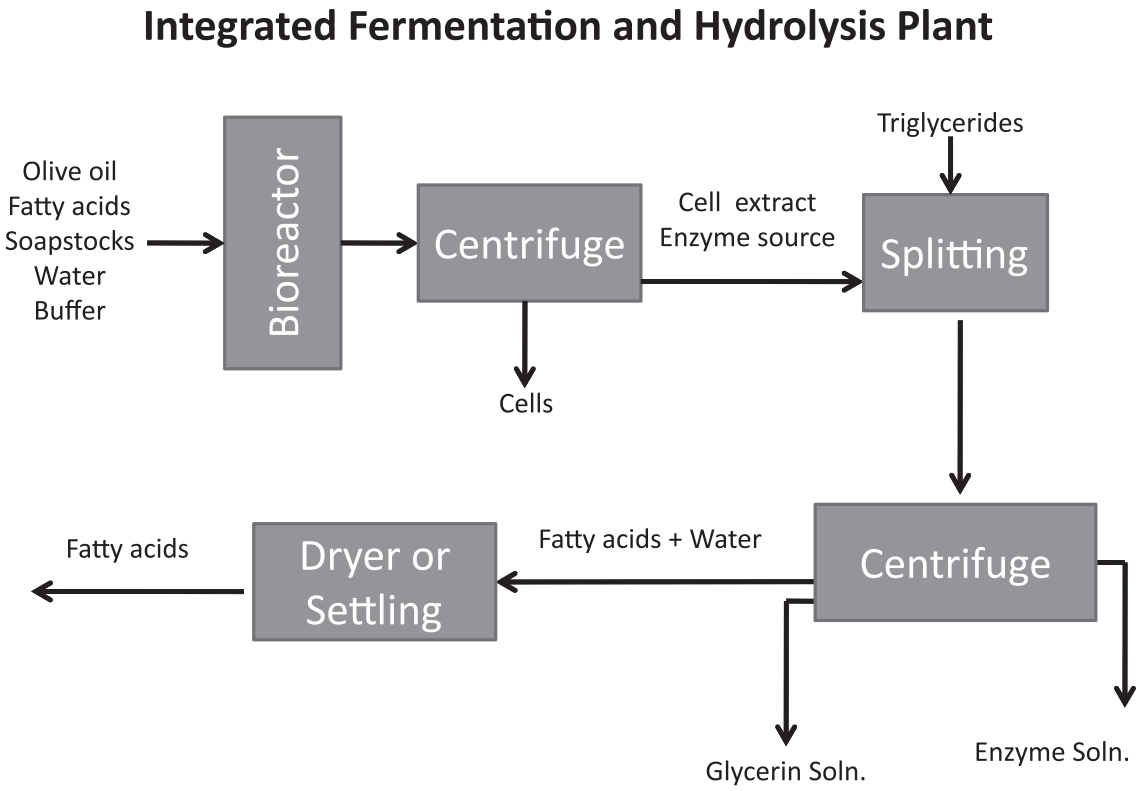

Fig.2 An integrated fermentation and enzymatic hydrolysis block flow diagram[1].

amount of alkaline waste water. Furthermore the formed fatty acid alkaline salts (soaps) have to be eliminated by washing with water. The chemical catalysis process is still the most popular method for industrial scale use. Lipases can be used to catalyze the reaction in very mild conditions[30, 31]. One of the bottlenecks for industrial implementation of enzymatic biodiesel production is the high cost of the biocatalyst. Immobilization methods have been introduced to improve lipase stability and permit for repeated utilization[32, 33].

Methanol shows a high degree of dispersion in the oil phase in a batch reactor. However, resulted shear stress from the stirring would disrupt the enzyme carrier by the physical agitation. So the immobilized lipase might not be reused for a long period of time. A packed bed reactor (PBR) is a promising regime as a continuous operation without separation of the catalyst from the reaction product. The by-product glycerol remains in the bottom of the PBR because of its high viscosity. The aggregated glycerol might deposit on the surface of the immobilized lipase, thus decreasing the catalytic efficiency. So glycerol must be eliminated in a timely manner during the process. Nie conducted a continuous transesterification process on PBR with lipase *Candida* sp. 99–125 and a hydrocyclone was set after PBR to separate glycerol[34]. The final conversion to FAME from plant oil and waste oil under the optimal condition was 90% and 92%, respectively. The life of the immobilized lipase was more than 10 days[34]. A comparison for different studies is shown in Table 1.

One of the very important factors that affect the enzymatic biodiesel production is the reactor configuration. One of the main scopes for this study is to highlight the usage of the packed bed reactor for biodiesel production. Many authors have been developed different configurations of packed bed reactors for optimizing the enzymatic process; this includes increasing the yield, lipase stability and finding different separating regimes for products. Shimada[31] used a flow rate of 6 ml/h for the three reaction steps, and 1/3 molar equivalent of methanol to oil was added at each step. The glycerol was separated by gravity sedimentation after each step. The conversion to FAMEs reached 90%, and the threes step reaction was continuously used for 100 days[31].

A Solvent-free methanolysis of canola oil in a packed-bed reactor with use of Novozym 435 plus loofa was conducted in Hagar research[27], who used a glass column packed bed. In this system water circulated through the jacketed glass column by use of water bath thus the system temperature could be regulated and kept constant as required. Loofa pieces cut into disc form having 1.5 cm diameter. The appropriate volume of methanol was added at three stages: at the beginning of the reaction and after 24 and 48 h. By passing 72 h from the methanolysis the esterified mixture was centrifuged at 5000 rpm for 10 min.

One of the most important researches in the field of biodiesel was developed by Hama[34] who developed a continuous formed glycerol separation system. A stainless steel pipe (length, 1.5 m; inner diameter, 15.7 mm; and volume, 290.2 ml) was packed with immobilized lipase at a volumetric packing ratio of 60% and used as a reaction column. The reaction mixture was supplied to the upper portion of the columns using a diaphragm metering pump and poly tetra fluoro ethylene (PTFE) tubing. To facilitate measurement of glycerol removal, a glycerol-separating tank was placed under the reaction column[36]. Based on density difference between the products, where in a given space in the glycerol-separating tank, the glycerol byproduct forms droplets and accumulates at the bottom of the separation tank because of its high density and hydrophilicity. Then, the outflowing liquid consisting mainly of fatty acid methyl esters and residual glycerides was collected as the effluent, into which methanol was further added until the comple-

Table 1 Comparison between different studies for biodiesel production.

Reactor	Oil/fat	Lipase	solvent	Yield (%)	Reference	other
PBR (in series)	Salad oil	Candida SP. Immobilized on a cotton membrane	Petroleum ether	92	[33]	Flow rate (15Lit./h) Productivity (100T/y)
Screw capped vial	Soybean	Novozym 435	glymes	95.5	[36]	
PBR	Waste cooking palm oil	Novozym 435	Tert-butanol	79	[28]	
PBR	canola	Novozym 435	free	97	[26]	The system was worked until 432 h
PBR	Canola	C. rugosa & R. oryzae (1:1)	free	84.25	[25]	
PBR	Rapeseed and soybean	Novozym 435	free	95	[28]	

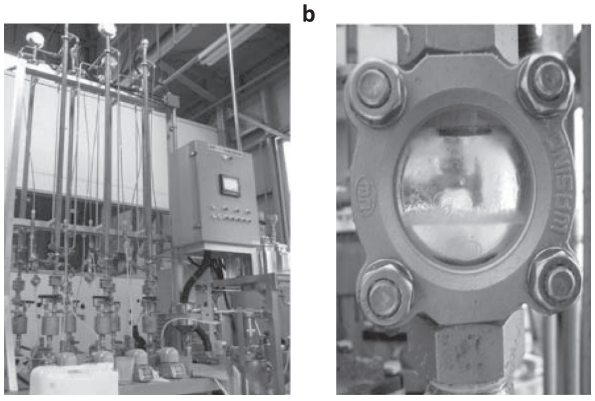

Fig. 3 (a) Photograph for constructed bench system. (b) Reprehensive picture showing two layers formed in glycerol separating tank[35].

tion of the reaction[36]. Figure 3(a, b) show a photograph for a system consisting of a reservoir tank and five reaction columns connected in series. By using a diaphragm metering pump, 10–20 ml of methanol per hour was continuously fed into each column. To facilitate mixing of oil and methanol, McMahon packings were set into the PTFE tubes. The reservoir tank and reaction columns were maintained at 30 C by circulating water[36].

In 2007, Lvming Co. Ltd. established an enzymatic production line with capacity of 10,000 tons in Shanghai, China. The factory employed the technique from Beijing University of Chemical Technology, with immobilized lipase Candida sp. 99–125 as catalyst. A high acid value waste cooking oil from Shanghai was used as raw material. The enzyme dosage was 0.4% to the weight of oil. The process was conducted in a stirred tank reactor, and a centrifuge was used to separate glycerol and water. Yields of FAMEs achieved 90% under optimal conditions. The cost of lipase was 200 CNY/t biodiesel. Another factory that conducted enzymatic catalysis in China was Hainabaichuan Co. Ltd., Hunan Province. The factory used the technology of Tsinghua University, with commercial Novozyme 435 used as the catalyst.

2.2 Major Challenges associated with enzymatic biodiesel production

2.2.1 Lipase inactivation caused by alcohols

For biodiesel synthesis, at least a stoichiometric amount of methanol is required for the complete conversion of triacylglycerols (TAGs) to their corresponding FAMEs. However, methanolysis is decreased significantly by adding N1/2 molar equivalent of methanol at the beginning of the enzymatic process. This inactivation caused by the polar short chain alcohols was the major obstacle for the enzymatic biodiesel production. The stepwise addition of methanol was the most common strategy in earlier studies. Methanolysis of vegetable oil for boidiesel production was conducted in Shimada research by using immobilized *Candida antarctica* lipase as catalyst[31]. The first step of the reaction was conducted at 30℃ for 10 h in a mixture of oil/ methanol (1:1, mol/mol) and 4% immobilized lipase with shaking at 130 oscillations/min. After more than 95% methanol was consumed in ester formation, a second molar equivalent of methanol was added and the reaction continued for 14 h. The third molar equivalent of methanol was finally added and the reaction continued for 24 h (total reaction time, 48 h). This three-step process converted 98.4% of the oil to its corresponding methyl esters[31].

Other important strategy is selecting an appropriate solvent to improve methanol solubility. T-butanol is a good solvent of the substrate methanol, since the immobilized lipase might be inactivated by the insoluble methanol in the system, and many previous studies are focused on this solvent. Recently glymes was used as a solvent with novozym 435 for catalyzing the transesterification of soybeanoil with methanol. It was found that soybean oil is fully miscible with glymes, which forms a homogeneous reaction mixture[36].

Although these solvents might solve the problem of lipase inactivation caused by methanol, difficulties in recovering the solvent would make these methods less competitive at industrial scale[29].

2.3 Biolubricant

Lubricants constitute an important market worldwide, their consumption being estimated at 37 million metric tons per year[37]. Biolubricants are environmentally alternatives for mineral oil based lubricants, exhibiting a combination of excellent technical performance with favorable ecological properties[38]. Such esters are thus highly suited as high performance lubricants for different industrial and automotive applications such as hydraulic fluids, metal working fluids, drilling oils, gear oils and lubricants for power saw chains[39].

Losses of hydraulic fluids are claimed to be as high as 70–80%, resulting in severe contamination of soil, groundwater and air[36]. Therefore, there has been an increasing demand for environmentally compatible lubricants or biolubricants, particularly in areas where they may come in contact with water, food or people. About 40,000 metric tons biolubricants are sold annually in the European Union and almost similar amount in the United States[41].

Production of one of the most important lubricants which is trimethylolpropane trioleate on industrial scale was achieved by reaction of trimethylolpropane with free fatty acids or esters catalyzed by a homogeneous or heterogeneous chemical catalyst such as acidic resins, acid oxides, and organic ion exchange resins[42–46] trimethylolpropane trioleate was produced in Akerman and Gaber research[42], using three different catalysts, included two heterogeneous acidic catalysts which are silica–sulphuric acid

Fig. 4 TMP- trioleate sample product obtained (A) silica- sulphuric acid (B). Amberlyst 15, and (c) Novozym 435. The Grander index for the samples was 5, 3-2 and 2 respectively[42].

and Amberlyst 15 as well as an immobilized lipase B from C. antarctica (CALB), commercially available as Novozym 435. All of these catalysts were catalyzed the estrification reaction efficiently. Novozym 435 showed the height esterification conversion of 96%, however with relatively low reaction rate compared to acid catalyst. In the reactions using acid silica as catalyst, the trimethylolpropane trioleate color obtained was turned dark brown (Gardner Index 5) as shown in Fig. 4[42]. In the lipase catalysed reaction the product exhibited Gardner index of 2 and looked similar to oleic acid.

2.4 Wax esters

The upgrading of plant oils through the production of fatty acid alkyl esters (FAAE) is thus a subject of great interest in many industries[47]. Wax esters can be defined as industrial esters that are produced in low volumes but are highly priced with high profit margins[48]. Wax esters are long chain esters consist of long chain fatty acids with different alcohols These compounds have been used in many potential applications such as excellent wetting behavior at interface[49] and non greasy feeling when applied on skin surface. Wax esters are the key ingredients in cosmetics, pharmaceuticals, lubricants, plasticizers and other chemical industries[50].

Chemical and enzymatic method can be used to synthesize wax esters from various raw material such as palm olein, palm stearin, palm kernel, oleic acid,…etc. with oleyl alcohol, iso propyl alcohol, iso butyl alcohol,…etc., over different catalyst. The homogenous chemical catalyst may lead to several problem such as corrosion of equipment, handle hazards of corrosive acids or base and high energy consumption. In the last few years, the research of alternative routes to the classical alkali or acid catalyzed synthesis of alkyl esters has demonstrated the ability of lipases to catalyze the transesterification and estrification of triacylglycerols[51].

The main drawback of enzyme-catalyzed wax esters production is the high cost of lipases. To decrease the operational costs of the biocatalyst, the process must be optimized using an immobilized enzyme in a stable and highly productive continuous reactor for months and even more[28].

In order to produce such value added products, there are several kinds of reactors can be used in a continuous operation such as continuous stirred-tank reactor (CSTR), packed-bed reactor (PBR), fluidized-bed reactor (FBR) or membrane reactor (MR). Among those kinds of reactors, PBR has traditionally been used for most large-scale catalytic reactions, because of its high efficiency, low cost and ease of construction, operation and maintenance.

Palm based wax esters are considered the most studied wax esters in the previous papers. Palm oil consists of triglycerides, a combination of glycerol and different fatty acids. Palm oil is rich in C_{16} and C_{18} fatty acids, while palm kernel oil is rich in C_{12} fatty acid[52].

Not much has been published on the large-scale synthesis of wax esters especially esters with long chain fatty acids[53]. Palm esters were synthesized in Keng research[54] through enzymatic transesterification of palm oil and palm kernel oil with oleyl alcohol using Lipozyme RM IM as a catalyst. Keng indicated that at the optimized alcoholysis reaction conditions, after 5 h reaction time, all palm oil exhibited a high percentage yields of esters (>80%). The main components of the obtained palm ester were oleyl palmitate and oleyl oleate while the obtained component using palm kernel oil were oleyl laurate and oleyl myristate.

The noteworthy scale up was achieved by the same author King in 2008 when the palm wax esters were synthesied by an alcoholysis reactin between palm oil with oleyl alcohol in presence of n-hexan in 75 L stirred tank reactor (50 L working volume) with agitator impellers of rushton turbine (RT) which gave the yield of 97.2% after 5 h of reaction time[55].

One of the most widely studied lipases for wax esters and fatty acids alkyl esters production is *Candida antarctica* lipase B, immobilized on a macroporous acrylic resin (commercially available as Novozym® 435). About 90% of the researchers who have been studied wax esters used this enzyme. Novozym 435 is immobilized on a support that is medium hydrophobic compared to the hydrophilic ones used for the other lipases[56].

2.5 Intraesterified fat

The recent interest of minimizing the *trans* fatty acid content of fat products especially in production of margarine has focused attention on palm oil products as a source of solid fat in placed of hydrogenated fats. Partially hydro-

genated products have been proven to be detrimental to human health due to the formation of *trans* fatty acids (trans fatty acids)[57]. The United States and part of Latin America implemented labeling regulation for TFA on food products in January and August 2006 respectively[58–62]. Those regulations have also prompted food industries to find alternatives in producing healthier foods and *trans*-free fats.

Interesterification can be carried out chemically or enzymatically. A chemical, such as sodium methoxide, is used as a catalyst in chemical interesterification which produces complete positional randomization of the acyl groups in the triacylglycerols. On the other hand, enzymatic interesterification uses microbial lipases as the catalyst. Each type of interesterification has its advantages and disadvantages. The advantages of chemical interesterification over the enzymatic reaction include cost recovery and low initial investment as the catalysts are much cheaper than lipases. The process has been around for a long time, with the industrial procedures and equipment available. Enzymatic interesterification is more specific, requires less severe reaction conditions and produces less waste than chemical interesterification[63]. Nowadays hydrogenation is being used less frequently due to *trans* fat generation.

It is clear that chemical interesterification needs more processing steps, uses higher temperatures and therefore more energy. When these extreme conditions are employed, polymerization of fats and by-products formation take place result in coloured product and therefore requires bleaching plus some other purification steps. On the other hand the enzymatic process is much simpler and carried out in a fixed-bed reactor under mild conditions. The end-product is ready to be deodorized without purification[64].

2.6 Cosmetics

The monoesters of glycerol and long fatty acids (monoglycerides, MG) are hydrophilic and hydrophobic molecules used widely in pharmaceutical, baking, and cosmetic industry as emulsifiers, Furthermore, they have a generally recognized as safe status[63] and some newly uncovered beneficial effects and nutritional properties had been reported, such as the antimicrobial activities of monolaurin, monomyristin, monolinolein, and monolinolenin[66].

Current processes for MAG and DAG production consist of the continuous chemical glycerolysis of fats and oils at high temperatures (220–250 C) employing inorganic alkaline catalysts under a nitrogen gas atmosphere and products are purified through high-vacuum distillation[67]. The major drawbacks of this process include high-energy consumption, low yield, and poor product quality[66]. The replacement of inorganic catalysts by lipases in the synthesis of partial glycerides has advantages of catalysis at lower temperatures which prevents the discoloration and avoids side product formation[68, 69], less polluting and energy consuming, moreover, it can produce glycerides with unsaturated fatty acids, that is commonly difficult by chemical methods.

The most widely used monoglycerids as food grade emulsifiers are glycerin monostearate, glycerin monopalmitate and glycerin monooleate. Ziobrowski[70] studied the enzymatic glycerolysis of stearic acid with glycerol in high polar organic solvent as well as a pervaporation unit was integrated in-line with the reaction system to remove the water formed during the reaction. The product yield obtained in Ziobrowski research was 90% after 6 h at 40C.

Glycerol and stearic acid are both soluble in organic solvents, for example hexane, toluene, chloroform, dioxane, acetone, etc., which in turn had a significant effect in dissolving these material but the main disadvantages of using such solvents that these media are toxic, carcinogen, flammable and not environmental friendly. For this reason alternative new media for the realization of the glycerolysis process was studied in Csanadi research[70], who used an ionic liquids and supercritical CO_2 for the reaction between glycerin and stearic acid in the presence of Novozym 435. The conversion reached 90% with minimum percentage of diglycerid.

Zhao[70] optimized the glycerolysis reaction of glycerol and oleic acid in a solvent free system using *Candida* sp 99-125 lipase. The maximum conversion obtained in this research was 81.4% of both mono and diglycerids.

Glyceryl palmitate in acetone was successfully synthesized in Kapoor research[70]. Conversion of more than 90% was obtained using *Candida antarctica* lipase in the presence of silica gel to control the water content.

3 Conclusion

Replacing the chemical process with the enzymatic one is thus a subject of great interest these days. However the economic benefits of replacing the chemical methods with the enzymatic one in doubt until now. As enzymes are expensive, it became necessary to find an economical routes for enzyme production and also to find several systems that can keep the enzyme stability inside the process to have high productivity with less amount of enzymes, in other words to cover the enzyme cost with the products profit margins. Enzymatic processes based oleochemicals are very promising issue especially after the continuous need of sustainable and green products by consumers.

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