Enzymatic preparation of mono- and diacylglycerols: A review

Jiawei Zheng [a], Yudong Liang [b], Jiaxi Li [b], Shuping Lin [a], Qiangyue Zhang [a], Kanghua Zuo [c], Nanjing Zhong [a,*], Xuebing Xu [c,*]

[a] School of Food Science, Guangdong Pharmaceutical University, Zhongshan 528458, China
[b] College of Food Science and Technology, Henan University of Technology, Zhengzhou 450001, China
[c] Wilmar (Shanghai) Biotechnology Research and Development Center Ltd., Pudong New District, Shanghai 200137, China

A B S T R A C T

Monoacylglycerols (MAGs) and diacylglycerols (DAGs) are partial glycerides widely used in food industry. They are safe and non-toxic food emulsifiers, especially for MAGs. MAGs account for approximately 75% of the total emulsifiers in food industry worldwide. DAGs are recognized as functional cooking oils, they can suppress body fat accumulation and postprandial serum triacylglycerols (TAGs) level. The traditional production of MAGs and DAGs is based on the chemical method, which requires high reaction temperature usually up to 200–260 °C. Such high temperature is not suitable for oil containing heat sensitive polyunsaturated fatty acids. Enzymatic approach has been received increasing attentions. Enzymatic production of partial glycerides to replace chemical processes has been in industry, particularly for DAGs production as the products have been claimed as a functional and nutritional oil. Enzyme technology for the processing of oils and fats has been moved to industry step by step and case by case during the last 20 years. More and more applications are particularly moving into bulky oils and fats processing. At the same time, the cost of enzymes as a commercial product is reducing steadily. This review summarized the recent 15 years advances on the the enzymatic preparation of MAGs and DAGs. The critical process parameters under different reaction routes were presented and emphasized. The reaction media not only increased the homogeneity of the reaction system, but also shifted the reaction equilibrium towards the target product generation, and this part was stated in detail. In addition, the patent evaluation was included, and the application of MAGs and DAGs was covered.

1. Introduction

Monoacylglycerols (MAGs) and diacylglycerols (DAGs) are nonionic molecules with both hydrophilic and hydrophobic portions. They occur in edible oils and fats in minor amounts. Based on the position of the ester linkage that covalently bonded to glycerol, MAGs can be divided into 1(3)-MAGs and 2-MAGs isomers, and DAGs consist of 1,2(2,3)-DAGs and 1,3-DAGs isomers (Fig. 1). For the MAGs, 1(3)-MAGs are the predominant isomers, they account for 92%–95% at low temperatures and approximately 70% at the very highest temperatures of the total MAGs [1]. While for the DAGs, 1,3-DAGs are more thermodynamically stable since the steric effect of the structure, and 1,2-DAGs in the edible oil will be largely converted to 1,3-DAGs to reach equilibrium at a ratio of (3–4):(7–6) between 1,2- and 1,3-DAGs [2]. In addition, the energy value and digestibility of DAGs is similar to that of triacylglycerols (TAGs). The energy value is 38.9 and 39.6 kJ/g

respectively for DAGs and TAGs. The digestibility of rats fed with DAGs resembles that of TAGs with digestibility value both at 96.3% (no significant difference) [3].

MAGs show excellent emulsifying properties, which are widely used in the food, cosmetic, pharmaceutical and chemical industries [4]. MAGs or their mixtures with DAGs account for approximately 75% of the world's annual emulsifier production [1]. DAGs are recognized as functional cooking oils, they are reported to be able to suppress body fat accumulation and postprandial serum TAGs level [5]. Yet the mentioned health benefits can only be observed with DAGs concentration not less than 40% [6], which is far greater than the DAGs present naturally in vegetable oil. The concentration levels of DAGs naturally in vegetable oils are less than 10%, in addition, MAGs occur naturally in various oils are less than 1% [5]. Therefore, production of MAGs and DAGs has been studied extensively over the years. MAGs and DAGs can be produced chemically or enzymatically. The chemical method offers a much
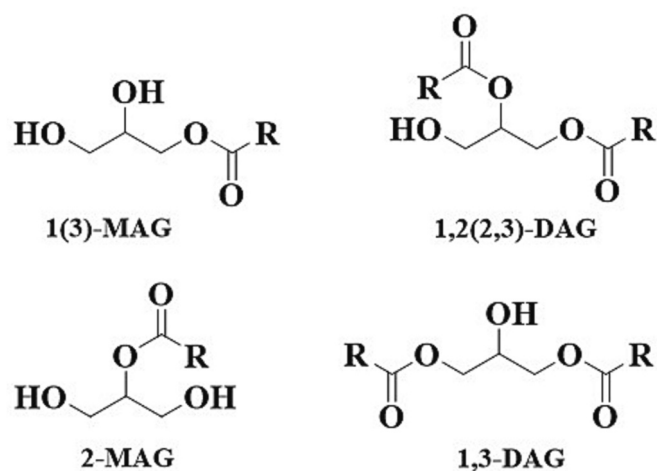
Fig. 1. Structures of MAG and DAG. MAG, monoacylglycerol; DAG, diacylglycerol.

cheaper alternative, yet this process is conducted at high temperature up to 220–260 °C, which is not suitable for oil containing heat sensitive polyunsaturated fatty acids; moreover, the high temperature will also burden extra operating cost. On the other hand, enzymatic approach is environmentally friendly, it is at mild conditions and enzyme catalyzed reactions are selective and specific. In this review, we summarized the advances in the recent 15 years on the the enzymatic preparation of MAGs and DAGs. The critical process parameters under different reaction routes were presented and emphasized. The effects of reaction media on the production of MAGs and DAGs are included, and the reaction media shifted the reaction equilibrium was emphasized. In addition, application of MAGs and DAGs, as well as the patent evaluation were also covered.

2. Properties and applications of MAGs and DAGs

2.1. Properties and applications of MAGs

MAGs consist of a hydrophilic head and a hydrophobic tail, therefore, MAGs display excellent emulsifying properties, particularly for oil and water combination. In food industry, MAGs are safe and non-toxic emulsifiers, they have been recognized as food-grade additives by the United States of Food and Drug Administration and the European Union. They are good emulsifiers for cake, bread and margarine products. And around 75% of the total emulsifiers in food industry worldwide are MAGs [7]. In addition, MAGs were also used in beverage clarifiers to help disperse the system [8].

Besides in food industry, MAGs also show biological activities. For example, monolaurin, monomyristin, monocaprin, monoolein and monolinolein were reported to display antimicrobial activities [9,10]. In a study from Cho et al. (2010), MAGs containing oleic acid were reported to have antioxidant, anti-diabetic and anti-atherosclerotic effects both *in vitro* and in cells [11]. In addition, MAGs containing medium-chain fatty acids and the fatty acids as pig feed additives are also beneficial for gut health improvement and feed pathogen mitigation [12]. Moreover, MAGs containing n-3 polyunsaturated fatty acids (PUFAs), like eicosapentaenoicacid (EPA) and docosahexaenoic acid (DHA), are beneficial for preventing different cardiovascular disorders. They are useful for human health based on their nutritive value, their regulation of inflammation, cholesterol metabolism, and brain functions, as well as their influence to erythrocyte fatty acid profiles and the expression levels of inflammatory circulating mediators [7]. Fish oil is the natural source of n-3 PUFAs. Most of the natural PUFAs exist in the form of TAGs, and only a small amount of PUFAs derived from krill oil exist in the form of phospholipids (PL) [13,14]. In functional oils, PUFAs

mainly exist in the form of ethyl ester (EE), 2-MAGs, structured TAGs and PL, and so on. Dyerberg et al. and Neubronner et al. found that n-3 PUFAs can be digested more effectively as TAGs than that as FAEE [15,16]. The position of PUFAs in TAGs also plays a role on their physiological activity [17]. However, high content of PUFAs in fish oil makes it easy to be oxidized [18]. Furthermore, MAGs are also used in synthetic organic chemistry as synthetic intermediates or as chiral building blocks [19].

MAGs are also used in textile, fiber processing and plastics production, because of their lubricating and plasticizing properties [1]. In addition, MAGs can be used as a binder in drug tablets, skin moisturizing agents in pharmaceutical industry. In the cosmetics industry, MAGs are helpful to improve the quality of creams and lotions [20].

2.2. Properties and applications of DAGs

DAGs are structured lipids, they are absorbed and metabolized differently from the common oils and fats (TAGs). During digestion in the small intestine, TAGs undergo hydrolysis to form 2-MAGs and fatty acids. These products are absorbed by the epithelial cells and they can be re-esterified to generate TAGs [21]. In addition, DAG has been shown to increase bone mineral density and improve bone microstructure [22]. Unlike that of the TAGs, DAGs are hydrolyzed into 1(3)-MAGs and fatty acids in the presence of pancreatic lipase. However, the generated 1(3)-MAGs and fatty acids can not be re-esterified to synthesis TAGs. Therefore, the ingestion of DAGs leads to increment in the β-oxidation of fatty acids [4]. Due to the such metabolic pathway, consumption of DAGs was indicated to be capable of suppressing visceral fat accumulation [23,24], lowering postprandial serm TAGs and cholesterol [25], as well as modulating serm glucose level [26,27].

The DAGs have therefore attracted global attention, and the DAGs-enriched oil was recognized as the functional cooking oil under the brand name of "Econa" and "Enova" in Japan and United States respectively. It was first developed by Kao Corporation in Japan and the DAGs content was around 80% (W/W) in the DAGs-enriched oil. However, the sales of DAGs-enriched oil were put to a halt since the presence of glycidyl fatty acid esters were confirmed, which was carcinogenic [5]. Although the technology was developed to eliminate the contamination from the DAG oil products, the market, especially in Japan, did not come back. Encouragingly, DAGs oil has been launched in China recently from a few companies under different brands as small pack retail products for cooking or other culinary applications. This new market wave raised the new consideration of the products and the revisit of the nutritional values of DAG oils in the current light life.

Besides as the functional cooking oils, the DAGs have extensive applications especially in food industry. They can be used as stabilizer for oil-in-water (O/W) and water-in-oil (W/O) emulsions [28–30]. In addition, they are potential anti-oxidants in oil-in-water emulsions [31]. Furthermore, they also can be used as new indices to measure quality of fresh oils [32]. Moreover, DAGs can be used as the frying medium in shortening [33]. In addition to food industry, DAGs can also be used as building blocks for organic products synthesis in pharmaceutical industry, such as phospholipids, glycolipids, pipoprotein, pro-drugs like 1,3-DAG conjugated chlorambucil and 1,2-DAG conjugated (S)-(3,4-dihydroxy-phenyl)alanine (L-Dopa) [34,35].

3. Reaction routes for MAGs and DAGs preparations

Compared with the chemical method, enzymatic preparations of MAGs and DAGs are more attractive, due to the mild reaction conditions and reaction specificity that enzymed afforded. The main reaction routes for MAGs and DAGs preparations are esterification, glycerolysis, partial hydrolysis. In addition, ethanolysis reaction was sometimes used to produce MAGs and DAGs.

3.1. Esterification

Enzymatic esterification refers to the dehydration of glycerol and fatty acids to form acyl glycerol under the catalysis of lipase (Fig. 2). The glycerol first reacts with the fatty acid to form MAG, the obtained MAG will further react with fatty acid to generate DAG and then the TAGs [36]. Therefore, the substrates molar ratio is key for desired product synthesis. From the reaction equilibrium point of view, molar ratio of glycerol to fatty acid at 1:1 would probably favor MAGs formation, and at 1:2 would the DAGs formation, more fatty acids would favor the TAGs formation. Nevertheless, it should be pointed out that, since the reaction is reversible, when the molar ratio of glycerol to fatty acid is 1:1 or 1:2 (or even other ratios), the reaction products are not the pure MAGs or the pure DAGs, but free fatty acid, MAGs, DAGs and TAGs are usually present in the reaction products. Table 1 and Table 2 [37–76] respectively presented the MAGs and DAGs preparation through enzymatic esterification. In the initial stage, some lipases need a small amount of water to maintain their activity. Interestingly, *Candida antarctica* lipase B (CALB) can catalyze the reaction in dry state [77]. In addition, Lipozyme RM IM and Lipozyme TL IM are also able to initiate the reaction without water addition.

Water will be generated as the esterification proceeds, therefore, removal of the generated water will shift the equilibrium towards the glycerides formation side [49]. The excess water could be removed by ventilation [45,49,56,59], vacuum pump [38,47,51–54,58], silica gel or molecular sieve desiccants [37,62,63,65–67,72]. As shown in Table 1 and Table 2, the maximum water content in enzymatic esterification is no more than 10%. Study indicated that vacuum can interestingly increase the amount of MAGs and inhibit the DAGs formation. However, too high vacuum would result in the lack of water to maintain the lipase activity [9]. Wang et al. explored the effect of water content (2%–14%, *W/W*) on the esterification catalyzed by Lecitase Ultra. It showed that the esterification efficiency (EE) of free fatty acids increased slowly with the increase of water content. However, EE decreased when the water content exceeded 8% [45].

Sometimes solvents were used as reaction media to increase the compatibility of glycerol and fatty acids and in turn accelerating the reaction. Salameh et al. investigated the effects of several organic solvents including isooctane, octane, toluene, acetonitrile on the enzymatic esterification. The results showed that isooctane was the most efficient solvent [48]. Actually as the esterification proceeds, the generated MAGs and DAGs would emulsify the reaction system and increase the contact of substrates. The compatibility of glycerol with fatty acids changes in real time with the advance of the reaction [61]. To our experience, it is unnecessary to introduce medium into the reaction system in most cases. In some cases, the organic solvent system may lead to acyl transfer and other side reactions [46]. Moreover, some lipases activity would be impaired in organic solvents. For example, *tert*-butanol is a amphiphilic solvent, it can combine oils and glycerol efficiently. However, most common commercial lipases (except for Novozym 435) exhibit lower esterification activity in *tert*-butanol system [46,66]. Therefore, solvent-free was more attractive for enzymatic esterification. While in the case of adding solvents can produce significantly higher industrial benefits, this reaction system with little solvent separation price will be acceptable and attractive.

3.2. Glycerolysis

Glycerolysis is the most widely used reaction route for MAGs and DAGs production, because of the easy access to the glycerol. In recent years, production of glycerol is increasing, especially from the biodiesel industry. It is therefore necessary to transform glycerol into valuable products, and glycerides are the such bioproducts. Table 3 and Table 4 [78–130] listed the enzymatic production of MAGs and DAGs through glycerolysis in recent 15 years.

Glycerolysis is a multi-step and reversible reaction, as shown in Fig. 3. DAGs are the intermediate products, as DAGs can also react with glycerol to form MAGs. More glycerol would help shift the reaction equilibrium towards MAGs generation. Therefore, molar ratio of glycerol to TAGs over 3, at 4 or even at 5, was usually used to obtain high content of MAGs. And MAGs content up to 70% [99–101] or even at 80% [83] and 90% [94] was observed from enzymatic glycerolysis.

However, the excess use of glycerol would cause immiscibility with oils in the reaction system. Moreover, lipases tend to be trapped by the glycerol, which prevents the contact of lipases with oils, and in turn the glycerolysis reaction difficult to proceed. To improve the miscibility and enhance the reaction, organic solvents, ionic liquids, surfactants were introduced into the reaction systems. Solvents such as *tert*-butanol, *tert*-pentanol, isopropanol, *n*-butane, hexane, et al. were introduced into the reaction system. Due to their amphiphilicity, *tert*-butanol, *tert*-pentanol, isopropanol were sufficient to mix oils and glycerol, and MAGs content up to 70% and even 90% could be obtained, as above-mentioned [83,94,99–101]. It is true that, the use of solvents would increase the production cost as well as safety issues in some cases, due to the added separation process and release of solvent into air. Yet such a high content of MAGs is still attractive and potential. Thus, a special section 4 is included to discuss the details of medium applications.

In addition to the organic solvents, surfactants, such as TritonX-100, Tween65, Tween40, Tween80, soy lecithin and sodium (bis-2-ethylhexyl) sulfosuccinate (Aerosol-OT or AOT), are also used to improve the compatibility of the system. They can form micellar systems and both hydrophilic and hydrophobic substances can be dissolved at high
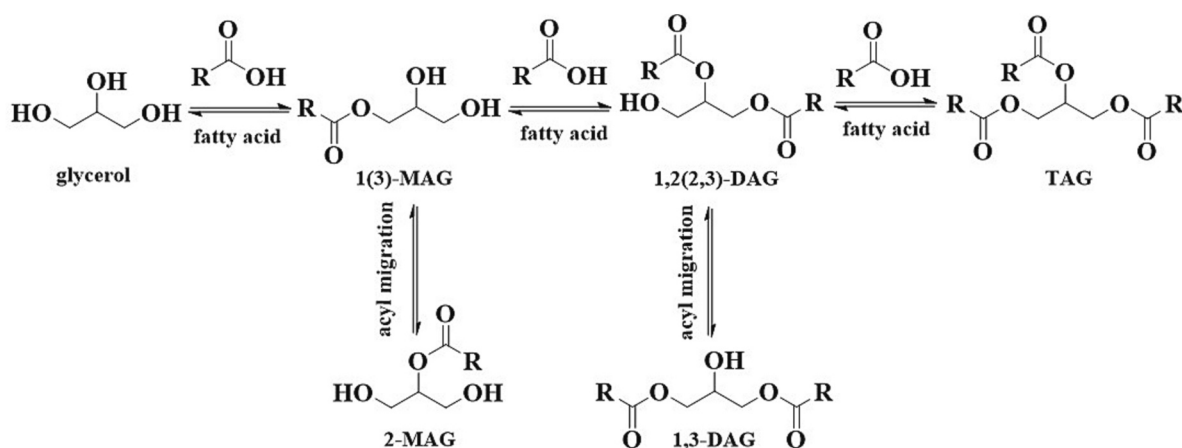

Fig. 2. Enzymatic esterification (of glycerol with fatty acid) reaction routes for glycerides preparation. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol.

Table 1

Enzymatic preparation of MAG through esterification.

Entry	Substrates	Reaction conditions	Lipases used	MAG content (%, W/W)	References
1	Palmitic acid and glycerol	Glycerol (0.65 g, 7.06 mmol), palmitic acid (0.46 g, 1.76 mmol), 20 mg molecular sieves (MS), 4 mL of acetone, 50 μL cross-linked protein coated microcrystals (CLPCMCs) of *Candida antarctica* lipase B, at 1% (V/V) water content, 50 °C and at 300 rpm, 24 h.	*Candida antarctica* lipase B	87	[37]
2	Glycerol and the fatty acid from pine nut oil	Step1: 20 °C, a molar ratio of 1:5 (fatty acid to glycerol), a water content of 2%, an enzyme loading of 600 units, a vacuum of 5 torr, and a reaction time of 24 h. Step2: −10 °C and 8 days.	*Penicillium camembertii* lipase	88.4	[38]
3	Palmitic acid and glycerol	Glycerol 0.11 g, glycerol/fatty acid molar ratio of 1.2:1, enzyme 23 mg-protein/g-glycerol, glycerol/KOH molar ratio of 1:0.05, in a solvent-free two-phase system, 65 °C, 800 rpm, 24 h.	Carboxylesterase derived from *Geobacillus thermodenitrificans* (EstGtA2)	97	[39]
4	Oleic acid and glycerol	Glycerol/oleic acid molar ratio 6:1, catalyst load 5%, reaction temperature 50 °C for MAG, and 5:1, 10%, 40 °C, β-cyclodextrin/lipase mass ratio 1.5 for DAG.	lipase *Candida* sp. 99–125	MAG: 49.6 DAG: 54.3 > 90 after distillation(DAG + MAG)	[40]
5	Oleic acid, corn oil and glycerol	Esterification combined with glycerolysis, reaction time 1.5 h, reaction temperature 75 °C, 3:3 mass ratio of oleic acid to corn oil and 1:7 molar ratio of fatty acids to glycerol.	Novozyme 435	MAG: 46.67 DAG: 35.56	[41]
6	Lauric- and caprylic acid and glycerol	The packed bed reactor (PBR) experiments for 100 g scale synthesis were carried out in an Omnifit® borosilicate glass column (13 cm in length × 1 cm diameter) containing 0.5 g CALB lipase (0.75% of used LA, W/W). It was connected to an in-house constructed stainless steel column (11 cm length × 2 cm diameter) containing 30 g of molecular sieves (activated overnight at 150 °C). A previously mixed 100 g substrate mixture (in a Schott Duran bottle) introduced into the reactor in downwards direction at a flow rate of 0.07 mL/min. The residence time in the packed bed for one pass was 10 min. Using a Watson Marlow 205S peristaltic pump.	CALB	55–70 MAG, 30–35 DAG and 1–10 TAG	[42]
7	(R, S)-1,2-oisopropylideneglycerol and stearic acid	Using *n*-heptane as solvent system, 48 h, 40 °C, 200 rpm, 50 mg of the immobilized lyophilized fungus, 10 mL of reaction medium containing 75 mM stearic acid and 150 mM (R, S)-1,2-Oisopropylideneglycerol (solketal).	*Sthemphyium licopersici*	78.00 ± 0. 06	[43]
8	Glyceryl ferulate (FG) and oleic acid	60 °C, enzyme loading 8.2%, substrate ratio 8.65:1 (oleic acid/(FG + glycerol), W/W), reaction time 1.8 h.	Novozym 435	87.6 ± 1.2	[44]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; CALB, *Candida antarctica* lipase B; FG, glyceryl ferulate.

concentrations. The increase of interface area afforded by the surfactants significantly promotes the preparation of glycerides catalyzed by interfacial lipases [79,81,84,85,93,104]. For example, study from Valerio et al. indicated that, the addition of TtitonX-100 increased the conversion rate, reduced the amount of the enzyme, and shorten the reaction time, during the enzymatic glycerolysis of olive oil [79].

If the desired products are DAGs, then the molar ratio of substrates is another scenario. When combine the reaction routes 1 and 3 in the Fig. 3, we can simply obtain route 5, that is, 1 mol glycerol with 2 mol of TAGs could be theoretically obtained 3 mol DAGs. Therefore, molar ratio of glycerol to TAGs usually at about 0.5 (1:2) was used for DAGs preparation. Unlike in the case of MAGs preparation through glycerolysis, in which molar ratio of glycerol to TAGs is usually over 3, the use of glycerol is quite less (molar ratio of glycerol to TAGs at about 0.5). In this case, the glycerolysis reaction can proceed without solvents or surfactants introduction at the enzymatic temperatures (usually 50 to 70 °C) under the vigorous stirring. Of course, the introduction of reaction media would enhance the reaction rate, however, it would add the production cost at the same time. To our experience, considerable content of DAGs from 50% to 60% could be obtained from solvent-free enzymatic glycerolysis. Actually it is unnecessary to introduce reaction media into the glycerolysis reaction system for DAGs preparation. Interestingly, Xu et al. found that ionic liquids can not only efficiently improve the miscibility of substrates but also increase the DAGs selectivity. DAGs content over 70% was firstly encouragingly observed from binary ionic liquid system of [TOMA]·[Tf$_2$N]/Ammoeng102 [112].

Another strategy to increase the contact of substrates in solvent-free enzymatic glycerolysis is with the aid of ultrasonic radiation, especially

for DAGs production. Since in DAGs preparation, the required glycerol amount is less and the glycerolysis could proceed under extensive stirring; while for MAGs preparation, more glycerol is required and the lipase tends to be trapped by the glycerol in this case, resulting in the glycerolysis reaction being difficult to proceed even under vigorous stirring. The cavitation collapse afforded by the ultrasonic radiation can increase the surface area and reduce the mass transfer resistance, and therefore speed up the reaction [110]. Results from Fiametti et al. indicated that, even under the weak radiation, the ultrasonic increased the reaction rate and reduced the enzyme usage, moreover, no obvious loss of enzyme activity was observed under the ultrasonic radiation [85].

3.3. Partial hydrolysis

Hydrolysis of oils would liberate fatty acids from TAGs. As showed in Fig. 4, MAGs and DAGs could be obtained under partial hydrolysis; while if totally hydrolysis, only free acids and glycerol were obtained. Therefore, it is key to control the hydrolysis degree for MAGs or DAGs preparation. Tables 5 and 6 [131–145] listed the recent advances for MAGs and DAGs preparation from partial hydrolysis. Water content is crucial in the hydrolysis reaction. It would affect the reaction equilibrium since it participates the hydrolysis reaction [132,137,139,140]. In addition, it also influences the enzyme structure and in turn the activity. In the initial stage, the oil-water interface is the main rate-limiting factor. Vigorous stirring, ultrasonic radiation or the introduction of surfactants, could help to increase the interface. Extensive stirring can keep the substrates in emulsion state, which is beneficial to speed up the

Table 2
Enzymatic preparation of DAG through esterification.

Entry	Substrates	Reaction conditions	Lipases used	DAG content (%, *W/W*)	References
1	Free fatty acids with glycerol	Water content 8% (*W/W*), temperature 40 °C, reaction time 6 h, molar ratio of GLY to FFA 2.0, enzyme load 80 U/g, reduce pressure 5 kpa, 200 rpm.	Phospholipase A1 (Lecitase Ultra)	74.8 and 83.10 after distillation of 170 °C	[45]
2	Free fatty acids (Lauric acid) with glycerol	Solvent-free synthesis, molecular sieves (4 Å, 10 g), glycerol vs. FFA 1: 2 (glycerol 50 mmol), MjL (in immobilized form, 1 g) 55 °C, 6 h, 200 rpm.	Immobilised MjL (Mucor javanicus lipase)	> 90	[46]
3	Oleic acid with glycerol	Solvent-free system; 65 °C, oleic acid/glycerol molar ratio 2:1, enzyme load 6%, reaction time 2 h, 0.01 MPa vacuum, 200 rpm stirring speed.	Lipozyme RM IM	62.6	[47]
4	1-Oleoyl-glycerol and lauric acid	Solvent systems: Isooctane, 100 mM fatty acids and 100 mM MAGs, 85 °C, 300 rpm, 100 h.	Alkalithermophilic LipA and LipB from *Thermosyntrophalipolytica*	62	[48]
5	Free fatty acids (rapeseed oil) and MAGs	60 °C, MAG to FFA ratio 8, enzyme content 4% (*W/W*), water was removed by nitrogen, 6 h.	immobilized lipase from *Rhizomucor miehei*	23.8	[49]
6	glycerol and oleic acids	Glycerol/oleic acids molar ratio 6:1, enzyme loading of 5% (*W/W*, with respect to total substrates), water addition of 5% (*W/W*, with respect to total substrates), temperature 30 °C, reaction time 36 h.	Immobilized SMG1-F278N lipase	49.64	[50]
7	High-purity MAG (≥95%) and caprylic acid	MAGs/caprylic acid mole ratio of 1:3, reaction temperature of 65 °C, reaction time of 30 min and enzyme load of 5% (*W/W*), under a vacuum condition (0.1 MPa), 200 rpm.	Novozym 435	44.8; 61.3 after molecular distillation; 86.6 after methanol crystallization at 0 °C	[51]
8	Oleic acid and glycerol	Solvent-free system; the molar ratio of oleic acid to glycerol 2.8:1 (glycerol 0.5 mol), vessel pressure 100 Pa, temperature 30 °C, the immobilized enzyme dosage was 8‰ (*W/W*), 400 rpm, 6 h.	Immobilized *Rhizopus oryzae*	> 76 and > 95 after purification	[52]
9	Glycerol and oleic acid	LA-FFA/glycerol with molar ratio 2.4, immobilized lipase (2%, *W/W*), at 60 °C under vacuum (0.1 MPa), 4 h.	Novozym 435	65.4 and 96.1 after purification	[53]
10	Linoleic acid and glycerol	FFA-C (16 g), organic solvent (4.8 g) and glycerol (2.18 g), mixture (6.5% of substrate, *W/W*) of the Lipozyme RM IM and Lipozyme TL IM lipases is 1:1; under a vacuum (0.1 MPa), 60 °C,1 h, Solvent systems: hexane.	Lipozyme TLIM/RM IM	60.4 and 94.7 after purification	[54]
11	Glycerol and oleic acid	Glycerol to oleic acids of 7:1, water (4% *W/W*), immobilized enzyme (5% by the total weight of substrates), 60 °C, 24 h, 200 rpm.	Immobilized *Malassezia globose* lipase (SMG1) M5D mutant	49.13	[55]
12	Free fatty acids and glycerol	Solvent Free System, glycerol with fatty acids, enzyme load of 5.0% (*W/W*), glycerol/FA molar ratio of 7.5, initial water content of 2.5% (*W/W*), reaction temperature 60 °C, 30 min and N$_2$ gas flow of 10.6 cm·min^{-1}.	Lipozyme 435	57.94 and 91.3 after distillation of 200 °C	[56]
13	Canola fatty acids and glycerol	Glycerol/canola FAs molar ratio 4:1, 30 °C, 8 h, in packed bed reactor.	Immobilized SMG1-F278N	50.87; and 99.7 after three-stage molecular distillation	[57]
14	Free fatty acids (from soy sauce by-product oil) with glycerol	Lipase loading 3%, 38 °C, glycerol/FFA and EE 21:40, 566 mmHg within the initial 10 h, 47 mmHg from the 10th to 14th hour, prepared by a combined glycerolysis and esterification.	Immobilized *Aspergillus niger* lipase (ANL-MARE)	66.76	[58]
15	Glycerol and oleic acid (from high acid soybean oil)	Solvent Free System, water was removed by nitrogen, glycerol 2.24 g, oleic acid 30 g, the Novozym 435 amount at 3%–5% (*W/W*), 500 rpm, 60 °C, 12 h.	Novozym 435	62.88 ± 0.37	[59]
16	*α*-Linolenic acid with glycerol	Solvent-free system, a-linolenic acid to glycerol 1:4, enzyme loading of 5% (*W/W*), 40 °C, 24 h, 200 rpm.	Immobilization of lipase PCL	54.49 and 99.28 after distillation	[60]
17	Oleic acid and glycerol	Solvent-free system, 65 °C, enzyme load 7.5%, substrate ratio 7.5:1 (OA/ (FG + glycerol), *W/W*), reaction time12 h.	Novozym 435	82.6	[61]
18	Purified MAG and DAG with CLA in its free fatty acid (FFA) form	Etahnolysis followed by esterification, molecular sieves to move water, 10% (*W/W*) biocatalyst based on total reactants, molar ratio of free conjugated linoleic acid (CLA) to hydroxyl groups in partial glycerides 5:1, 60 °C, 4.5 h.	Novozym 435	98.62	[62]
19	Oleic acid and glycerol	40 °C, 1.8 g Lipozyme TL IM, 3.5:1 molar ratio of oleic acid to glycerol; molecular sieve to remove water; 8.4 g toluene, 0.33 water activity, 200 rpm.	Lipozyme TL IM	50 (1,3-diolein)	[63]
20	Glycerol and capric acid	In n-heptane, adsorbed glycerol 250 mg, acid 110 mg, temperature 60 °C, and immobilized lipase 40 mg, 6 h.	lipase *Rhizomucor miehei*	dicaproylglycerol of 77	[64]
21	Oleic acid and glycerol	Oleic acid 10.0 g, glycerol 1.09 g, 80 U of immobilized PEL, 4 Å molecular sieves 6.4 g, 40 °C, 12 h.	Immobilized PEL	82.4	[65]

Table 2 (*continued*)

Entry	Substrates	Reaction conditions	Lipases used	DAG content (%, *W/W*)	References
22	Oleic acid and glycerol	Molecular sieves to move water, Novozym 435 0.75 g, substrate molar ratio (oleic acid/glycerol) 2.4, 4.8 g *t*-butanol, 62.4 °C,7 h.	Novozym 435	87.4	[66]
23	Monoolein and oleic acid	In *t*-butanol, molecular sieves to move water, molar ratio of oleic acid to monoolein 1.2:1, 60 °C, 3 h.	Novozym 435	93.7 (1,3-diolein)	[67]
24	Free fatty acids (enriched with linoleic acid) and glycerol	25 °C, FFA–CLA to glycerol and initial water content 1:3 (mol/mol), 3% of initial water content, 240 U/g LipaseSMG1, 400 rpm, 24 h.	Lipase from *Malassezia globosa*	53.4	[68]
25	Hydrolysates of soybean oil and glycerol	Hydrolysis followed by esterification, fatty acids/glycerol molar ratio of 1:4, lipase SMG1 at a concentration of 120 U/g (with respect to total reactants), initial water content 1% (*W/W*, with respect to total reactants), 30 °C,12 h.	Lipase SMG1	62.03	[69]
26	Duck oil fatty acid with glycerol	Glycerol-fatty acid ratio 2.0, reaction time 9 h, temperature50 °C, enzyme amount 1.5% (*W/W*, oil mass).	Novozym 435	94	[70]
27	The purified product from the first step (main compounds: TAG, DAG, MAG, and GLY) added with FFA	(1) Glycerolysis step: 57.7 °C, GLY: TAG 1:1 molar ratio, 4% enzyme; (2) esterification step 57.7 °C, FFA: TAG 1:1 M ratio, 4% enzyme. Both 48 h.	Novozym 435	62.05	[71]
28	Oleic acid and glycerol	Solvent-free system, molecular sieves to move water, temperature, 40 °C, 1.5 h, molar ratio of oleic acid to glycerol 1: 5, enzyme load 1.5% (*W/W*) of substrates, initial water content, 4% (*W/W*) of substrates, 200 rpm.	Lecitase Ultra	54.8 and 75 after distillation	[72]
29	The residue after purification of hydrolysate containing DAG MAG and FFA	Hydrolysis followed by esterification, enzyme loading of 80 U/g, reaction temperature of 40 °C, (Gly + MAG) to FFA is 6:1 or 5:1, 24 h.	Lipase PGL AOL-V269D	55.2 and 99.3 after distillation	[73]
30	Free fatty acids (high-acid SSR oil) with glycerol	Solvent-free system, glycerol to free fatty acid 2:3, Oil, lipase load of 2% (*W/W*, relative to total reactants), reaction temperature of 55 °C and reaction time of 8 h.	*Aspergillus niger* lipase (ANL)	58.46	[74]
31	Caprylic acid and glycerol	Esterification in the packed bed reactor ; caprylic acid (0.3% of total mass) at a space velocity of 34 h^{-1}, 65 °C in 7 h, molecular sieve, initial water (10% of glycerol mass).	Novozym 435	77.26	[75]
32	Lauric acid and glycerol	10 mmol glycerol, 20 mmol fatty acid, 3 h reaction at 50 °C, with 5% (*W/W*) of Lipozyme RM IM (based on reactants) amount, vacuum at 4 mmHg.	Lipozyme RM IM	80.3	[76]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; FFA, free fatty acid; CLA, conjugated linoleic acid; GLY, glycerol; ANL, *Aspergillus niger* lipase; OA, oleic acid.

reaction, however, high-speed shearing may destroy the enzyme structure and reduce the enzymatic activity [140,142]. The use of surfactants or ultrasonic radiation can reduce the substrates viscosity and increase the mass transfer, which improve the hydrolysis [137,142]. It is worth noting that, under the ultrasonic radiation, reaction temperature should be controlled, usually with the aid of circulating cooling water. Otherwise, high temperature caused by the ultrasonic would incur the enzyme inactivation [137].

3.4. Partial ethanolysis

Ethanolysis can also be used to prepare MAGs and DAGs. As shown in Fig. 5, partial ethanolysis would produce glycerides and fatty acid esters, while totally ethanolysis generates only fatty acid esters and glycerol. Like in the partial hydrolysis, ethanol content here is a key parameter. By controlling the reaction process and the ethanol amount, approximately 30%–50% of MAGs was observed (Table 7 [146–153]).

Overall, enzymatic glycerolysis was the most used reaction route for MAGs and DAGs production, due to its high time-space cost efficiency as well as the easy access of glycerol and TAGs. In addition, no or little glycidyl fatty acid ester would be generated at low temperatures. Since the immiscibility existence between TAGs and glycerol, the glycerolysis was difficult to proceed when more glycerol was added into the reaction system. And in this case, solvents were usually introduced to improve the miscibility and enhance the reaction.

Followed was the esterification, and it was the ideal route for pure

MAG or pure DAG synthesis, which used pure fatty acid as the substrate to esterify with glycerol. Besides, MAGs or DAGs containing mixed fatty acids were also sometimes obtained from esterification, especially for DAGs preparation (Table 2). In the esterification process, water would be generated, and it was necessary to remove the water to shift the reaction equilibrium towards the glycerides formation side.

As for the partial hydrolysis and the partial ethanolysis routes, they can be used for MAGs and DAGs preparation, but not widely. Partly because free fatty acids or fatty acid esters would be formed from these reaction routes and they should be removed from the products.

4. Reaction media are important in defining the processing technology

The corresponding reaction mechanisms of esterification, glycerolysis, hydrolysis and ethanolysis have been mentioned earlier. As shown in Fig. 6, the reaction equilibrium stage is reached in each reaction route, only for different reactions with different proportions of the various components (MAGs and DAGs specially as the targets). This gives us an opportunity to produce the desired substance in a rational way. If we need more DAGs or MAGs, it is time to add reaction media or adjust the ratio to interfere (glycerol/oil) to achieve our final desired purpose.

Table 3

Enzymatic preparation of MAG through glycerolysis.

Entry	Substrates	Reaction conditions	Lipases used	MAG content (%, *W/W*)	References
1	Cottonseed oil and glycerol	Cottonseed oil to glycerol 1:1 (molar ratio), deionized water at a concentration of 3.5% (*W/W*) relative to the glycerol, *C. antarctica* lipase B at 2% (*W/W*) relative to the oil, 65 °C, 48 h.	*Candida antarctica* lipase B	MAG: 31.3 DAG: 49.1	[78]
2	Olive oil and glycerol	16% (*W/W*) Triton X-100 concentration, glycerol to olive oil molar ratio 6:1, 50 °C, 240 min, 9% (*W/W*) enzyme concentrations, in solvent-free system.	Novozym 435	>35	[79]
3	Olive oil and glycerol	Glycerol-to-oil molar ratio 2:1, 70 °C, 2 h, 10 bar , 600 rpm, 7.5% (*W/W*) AOT, 7.5% (*W/W*) enzyme, *n*-butane-to-substrate mass ratio 4:1.	Novozym 435	71.9	[80]
4	Olive oil and glycerol	Glycerol to olive oil molar ratio 6:1, 70 °C, 2 h, stirring rate of 600 rpm, 16% (*W/W*) of surfactant Tween 65 and 9.0% (*W/W*) of Novozym 435 in solvent-free systems.	Novozym 435	MAG: 26 DAG: 17	[81]
5	Olive oil and glycerol	Glycerol to oil molar ratio of 3:1/6:1 in 8 h of reaction at 70 °C, 600 rpm and enzyme concentration of 10% (*W/W*) in *tert*-butanol system.	Novozym 435	MAG: 53 DAG: 50	[82]
6	Camellia oil and glycerol	Glycerol to camellia oil mole ratio 6:1, 50 °C, 10 h , Novozym 435 amount 4.5 g (45,000 PLU), 40% (*W/V*) of substrate concentration in *tert*-butyl alcohol.	Novozym 435	80.74	[83]
7	Olive oil and glycerol	Glycerol to olive oil molar ratio of 2:1, 7.5% (*W/W*) of Novozym 435, 600 rpm, 70 °C, 1 bar and 2 h, in *n*-butane or in solvent-free system, solvent to substrates 4:1 mass ratio (in low-pressure *n*-butane and at ambient pressure condition).	Novozym 435	58.3	[84]
8	Olive oil and glycerol	Glycerol to oil molar ratio of 2:1 in a solvent-free system, without the use of surfactant, at mild irradiation power supply (130 W) and low enzyme content (7.5%, *W/W*), 600 rpm, 65 °C, 2 h.	Novozym 435	MAG: 38.95 DAG: 14.54	[85]
9	Menhaden oil and glycerol	Glycerol-to-oil molar ratio of 1:1 and 5% (*W/W*) of lipase, in a solvent-free system, 70 °C and 24 h.	Novozym 435	MAG: 25.93 DAG: 41	[86]
10	Olive oil and glycerol	2:1 Glycerol/oil molar ratio in a solvent-free system and 3.5% (*W/W*) water content in glycerol, enzyme loading 0.015 g, 40 °C, 3 h.	*Candida antarctica* lipase B	26	[87]
11	Soybean/canola oils, glycerol	Molar ratio of glycerol/soybean oil 0.8:1, 70 °C, 900 rpm, 90/120 min of reaction time, 10% (*W/W*) of enzyme content, 40% of ultrasound intensity (52.8 W·cm^{-2}).	Novozym 435	Highest yields of DAG+MAG (75 and 65)	[88]
12	Olive oil and glycerol	50 °C, glycerol to oil 2:1 molar ratio, 0.02 g lipase, 6 h, 1:3 vol% isopropanol: *tert*-butanol, immobilized lipase concentration of 0.02 g/mL.	*C. antarctica* lipase and *C. rugosa* lipase immobilized on carboxylic acid-modified silica nanoparticles	66.5 and 72.8	[89]
14	Anchovy oil and glycerol	Gly/TAG molar ratio of 3/1, with 9% (*W/W*) Lipase PS-DI in a solvent-free system, stirred at 200 rpm, over 4 h, at either 45.8 or 54.7 °C.	*Burkholderia cepacian* lipase PS-DI	MAG: 24.58 or 28.34 DAG: 51.87 or 49.88	[90]
15	Palm stearin–olein blend and glycerol	In a batch stirred tank reactor (BSTR), palm stearin to palm olein at a ratio of 60:40 (*W/W*), *tert*-butanol to substrate ratio of 2:1 (*V/W*), glycerol to fat molar ratio of 1.5:1, 50 °C, 24 h, 15% enzyme concentration.	*C. antarctica* lipase B immobilized on the microporous hydrophobic matrix	MAG: 11.39 DAG: 27.24	[91]
16	Cocoa olein and glycerol	low pressure (2–3 mbar), 65 °C, 6–8 h, enzyme dose of 1%, a molar oil to glycerol ratio of 1:2, in a solvent free system.	*Candida antarctica* lipase B	MAG: 33.4 DAG: 44.2	[92]
17	Olive oil and glycerol	Glycerol to oil molar ratio of 2:1, stirring rate of 600 rpm, 10% (*W/W*) of AOT, 10% (*W/W*) of enzyme, mass ratio of pressurized propane to substrates 4:1, 30 °C, 30 bar, 1 h.	Novozym 435	Around 60	[93]
18	Tuna oil and glycerol	Glycerol/tuna oil 4:1 (mol/mol), *tert*-butano/tuna oil 2:1 (*W/W*), 15% (*W/W*) Novozym 435, and temperature 40 °C, 3 h.	Novozym 435	90.8	[94]
19	Olive oil and glycerol	Enzyme concentration of 7.5% (*W/W*) and glycerol:oil molar ratio of 0.8:1, 50 °C, 2 h, 132 W ultrasonic power, in solvent-free system.	Novozym 435	Around 30	[95]
20	Sardine oil and glycerol	Glycerol/oil molar ratio of 3:1, 5% Lipozyme 435, 50 °C, 2 h, *tert*-pentanol/ substrates 1.5:1(mol/mol).	Lipozyme 435	67 and 91 after distillation	[96]
21	Babassu oil and glycerol	In a continuous packed bed reactor with 7 g of immobilized lipase, babassu oil to glycerol 1:9 molar ratio, in a solvent-free system at 273.15 K, run at a fixed space time of 9.8 h.	*Burkholderia cepacia* lipase	31.5	[97]
22	The palm kernel olein (PKO) and palm kernel stearin (PKS) blend with glycerol	Enzyme concentrationof 10% (*W/W*), an oil-glycerol molar ratio of 1:4, a solvent–oil ratio of 2:1 (*V/W*), 40 °C, 600 rpm.	Lypozyme RM IM	31.64	[98]
23	Soybean oil and glycerol	50 °C , glycerol: oil 3:1, 24 h, 12% water (based based on glycerol content), 10% (*W/W*) of lipase loading (based on weight of reactants).	Lipozyme TL IM	70	[99]
24	Crude palm oil and glycerol	A mixture of *tert*-butanol and hexane (1:1) as the organic solvents, an immobilized lipase of 40%, a molar ratio of glycerol to CPO of 8:1, 4% of water content in the glycerol, an initial CPO concentration of 10%, 45 °C, 24 h, 300 rpm.	Lipase PS (*Pseudomonas* sp.)	74.3	[100]

Table 3 (*continued*)

Entry	Substrates	Reaction conditions	Lipases used	MAG content (%, *W/W*)	References
25	High oleic sunflower oil and glycerol	Enzyme load 3% of oil (*W/W*), substrate mole ratio of oil to glycerol 1:3, reaction temperature 50 °C and reaction period 8 h, *tert*-butanol and *tert*-pentanol ratio of 80/20 (*V/V*), initial water content 3% of glycerol (*W/W*), substrate: solvent 30% (*W/W*).	Novozyme 435	75.31 and 93.3 after purification	[101]
26	Olive oil and glycerol	35 °C, 7.5% (*V/V*) of Lipozyme TL 100, 2:1 molar ratio (glycerol:oil), 2 h.	Lipozyme TL 100 L	A mixture of MAG and DAG (yield 98.45)	[102]
27	Olive oil and glycerol	Initial glycerol to oil molar ratio of 1.2:1, initial enzyme content of 7.5% (*W/W*) related to the initial substrates mass (oil and glycerol), process time of 360 min, 70 °C, 10 bar, *n*-butane to substrate mass ratio 4:1, agitation of 600 rpm, AOT content of 7.5% (*W/W*).	Novozym 435	MAG: 49.3 ± 0.1	[103]
28	Olive oil and glycerol	60 °C , 600 rpm , a glycerol to oil molar ratio of 2:1 , enzyme 7.5% (*W/W*), AOT concentrations 20% (*W/W*).	Novozym 435	>70(1 h)	[104]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; PKO, palm kernel olein; PKS, palm kernel stearin; CPO, crude palm oil.

4.1. Solvent-free systems

The production of MAGs and DAGs is feasible in solvent-free systems [154–157]. As shown in Fig. 7, the whole system consists mainly of glycerol, oil (or fatty acids) and lipase, which are reacted by uninterrupted stirring in an intermittent reactor and finally reach equilibrium in principle. To further purify the products, distillation technologies can be used for further processing. Solvent-free systems are a very good choice for some systems because they do not require post-processing of the reaction medium compared to *tert*-butanol or ionic liquids [158]. However, the solvent-free system also has its disadvantages, with a relatively long reaction equilibrium time (about 10–24 h) [159], a relatively low yield of the resulting product, and a relatively poor miscibility due to the low homogeneity of this system [160].

From Table 8 [159,160], we observed that the yield of DAGs was around 45%, while the yield of MAGs was around 20%, and the yield of DAGs was more than two times higher than the amount of MAGs, which indicates that the preparation of DAGs is favored in the solvent-free system, while it is very inefficient for the production of MAGs since more glycerol in the system makes the system less homogenuous. If one wants to prepare MAGs, other methods should be chosen for the production of MAGs [161]. However, it was found from the table that the reaction equilibrium time required for the preparation was 24 h. The reaction time was long compared to the system with reaction medium, which may be due to the poor miscibility of hydrophilic glycerol and oil [160]. The conversion in solvent-free systems is also limited, but an increase in stirring speed will improve the conversion, while too high stirring speed can cause damage to the catalyst and also increase energy consumption [162], so proper reaction conditions will improve the conversion. Excessive glycerol can cause the formation of a glycerol layer around the enzyme pellet, which limits the contact with other substrates [163]. Therefore, some appropriate conditions such as glycerol ratio and temperature will increase the yield of DAGs in the solvent-free system and also facilitate the subsequent processing.

4.2. Tertiary alcohols

Although solvent-free systems are also a good system, to enhance the cleavage of glycerol, amphiphilic compounds are usually introduced into the system, which will increase the miscibility between glycerol and TAGs [164] and also enrich a product by breaking the reaction equilibrium, so as to speed up the reaction rate. Since glycerol has enzymatic hydrophilicity, it can bind to enzyme particles, while oil molecules become difficult to bind to the enzymes and mass transfer of glycerol is thus limited [160]. Moreover, high enzyme content may form aggregates, increasing mass transfer limitations and avoiding contact between the substrate and the active site [165]. Therefore, the addition of the reaction medium to the whole system may induce the formation of a more homogeneous and less viscous reaction mixture and also avoid acyl migration [166]. It can also increase the yield of the product and shorten the reaction time. While there are various types of reaction media involved in the reaction, the most commonly used reaction media are organic solvents.

The common organic solvent reaction medium in enzymatic reactions is *tert*-butanol (TB), a small amount of TB as a polar co-solvent can improve the miscibility and reduce the viscosity of the medium, increase the homogeneity of the solvent system and facilitate mass transfer [167], while mass transfer is one of the important factors affecting the rapidity of enzymatic reactions, an appropriate increase in temperature will also have a positive effect on the mass transfer process [168], a higher temperature can activate the substrate molecules, reduce the viscosity and accelerate the reaction rate [169]. No side reactions occur in biocatalytic reactions using TB as a solvent [170], as the reaction rate between TB and fatty acids is zero [171], proving that no reaction occurs between the two. After comparing the log *p* values of different reaction media [172], it was also found that TB is the most suitable solvent for efficient glycerol cleavage with high levels of MAGs yield (Table 9 [160,170,173–175]).

In the experiment of lipase cleavage of sunflower oil glycerol, as in Fig. 8, sunflower oil (20 g), glycerol, and TB were added to a capped beaker for mixing, and lipase was added to initiate this reaction. The reaction temperature was maintained at 40 °C with a glycerol/oil molar ratio of 4.5:1 and TB/oil of 2.2:1 and a lipase (Novozym435 derived from *Pseudomonas aeruginosa*, produced by deep fermentation of a transgenic *Aspergillus Oryza* [173,176], loading of 15% (*W/W*, oil base) proven in several experiments to be the most efficient enzyme for MAGs production [170,177,178]). Equilibrium is reached in about 2 h, and yields of up to 70% MAGs can be obtained, along with a DAG content of about 26% [160]. However, it was found that the sum of the obtained MAGs and DAGs yields did not change much by increasing the temperature up to 70 °C and changing the glycerol/sunflower oil ratio for glycerol cleavage, and the yields were both around 65%, so the too high temperature and glycerol/oil ratio did not significantly improve the yields [160]. The temperature of the reaction system should not be too high either, because the boiling point of TB is 83 °C [174], too high temperature can also have side effects on the reaction, so an appropriate temperature is necessary for the reaction. The yield of DAGs and MAGs production in solvent-free system was 50%–55%, but the reaction equilibrium time was as long as 24 h, which slowed down the reaction rate, but the solvent-free system is favorable for the formation of DAGs. The long reaction time and low conversion of DAGs and MAGs were mainly due to the poor miscibility of hydrophilic glycerol and lipophilic oil at low temperature, so the addition of reaction medium was very necessary [160].

Table 4
Enzymatic preparation of DAG through glycerolysis.

Entry	Substrates	Reaction conditions	Lipases used	DAG content (%, W/W)	References
1	Soybean oil with glycerol	Solvent-free system, glycerol/soybean oil mole ratio 10:1, initial water content 5% (W/W), enzyme loading 5% (W/W), 45 °C, 12 h.	immobilized lecitase ultra (IM-LU)	53.7	[105]
2	Sunflower oil with glycerol	Solvent-free system, under vacuum (756 mmHg), sunflower oil: glycerol is 2:1, TLIM 10% (W/W), 50 °C, 5 h.	TLIM	59.8	[106]
3	Rice bran oil and MAGs	Solvent-free system, MG/RBO 0.25, temperature 56 °C, enzyme load 4.77%, 5.75 h.	Lipozyme RM IM	27.61(DAG-enriched rice bran oil)	[107]
4	Silkworm pupa oil with glycerol	Organic solvent (tert-butanol), silkworm pupa oil/glycerol ratio of 1:3, lipase dosage 50 U/g, substrate concentration 40% (W/V), 40 °C, 6 h.	Immobilized lipase MAS1-H108A	MAG: 63.92; DAG: 30.6; And after molecular distillation, MAG: 99.18; DAG: 89.47	[108]
5	Fish oil with glycerol	Solvent-free system, 15% enzyme loading, 60 °C, 2:1 (oil/glycerol) substrates molar ratio, 200 rpm, 24 h.	Novozym 435	50	[109]
6	Lard oil with glycerol	Ultrasonic pretreatment, lard and glycerol in a molar ratio of 1:1, 5 min ultrasonic pretreatment time, 45 °C ultrasonic temperature, 250 W ultrasonic power, 4:100 (W/W) of Lipozyme RMIM to lard substrate, after ultrasonic pretreatment, 55 °C, 180 r/min, 4 h.	Lipozyme RMIM	46.91	[110]
7	Olive oil with glycerol	The organic solvent tert-butanol, glycerol to olive oil molar ratio 6:1(MAG)/ 0.5:1.5(DAG), enzyme concentrations (10%, W/W), 12 h, 55 °C.	Novozym 435	MAG: 65 DAG: 57	[111]
8	Triolein with glycerol	Glycerolysis in the binary IL system consisting of [TOMA]·[Tf₂N]/Ammoeng102, temperature between 55 and 60 °C, glycerol/oil amount between 1.8 and 2 mmol/ 2 mmol, [TOMA]·[Tf₂N] concentration less than 10%, 24 h, lipase amount 15% (W/W) based on oil mass.	Novozym 435	70	[112]
9	Soybean oil and glycerol	Solvent-free system, Soybean oil 3.52 g, glycerol 0.184 g, Immobilized TLL 0.2 g, 60 °C, 24 h, 200 rpm.	Immobilized TLL (R-SBA-15-TLL)	59.19	[113]
10	Microbial oil with glycerol	Solvent-free system, 8% (W/W) Novozym® 435, substrate ratio (DHA-rich microbial TAG/glycerol) of 1:1 mol/mol, 50 °C, 12 h.	Novozym 435	48.2	[114]
11	Palm olein with glycerol	100 g palm olein with 17 g glycerol anhydrous (1:1.5 molar ratio) in the presence of 10 g enzyme (10% of oil weight), 55 °C, 200 rpm, add molecular sieves, 22 h.	Novozym 435	50	[115]
12	Palm olein with glycerol	Solvent-free system, glycerol/palm oil molar ratio 7.5:1, initial substrate water content of 5%, enzyme load 2%, 40 °C, 8 h.	Phospholipase LU	59.5 and 88.1 after distillation	[116]
13	Lard oil with glycerol	Enzyme to lard substrate ratio 14:100 (W/W), lard to glycerol molar ratio 1:1, 500 rpm, first incubated at 65 °C for 2 h and then transferred to 45 °C for 8 h.	Lipozyme RM IM	61.76 and 82.03 after distillation	[117]
14	Soybean oil and glycerol	In tert-butanol system, mass ratio of soybean oil to glycerol 6.23:1, 40% (W/V) of substrate concentration in t-butanol, 50 °C	Novozym 435	48.5 and 96.46 after distillation	[118]
15	Palm olein with glycerol	1000 g palm olein, 170 g glycerol, 5% (W/W) of Lipozyme TLIM, 55 °C, 8 h, 200 rpm, molecular sieve to move water.	Lipozyme TLIM	34 and 89.9 after 2-step distillation	[119]
16	Corn oil with glycerol	Tert-pentanol system, 5% (W/W) of CALB on SBA-15, oil to glycerol 2:1, 50 °C, 12 h.	CALB on SBA-15	53.6	[120]
17	Vegetable oil with glycerol	Phytosterols/ALA/TAGs 1:3:2, CRL/TLL-PDA@Fe3O4 20 mg/mL, 40 °C, 8 h.	CRL/TLL-PDA@Fe3O4	39.29 ± 1.78	[121]
18	Soy oil and glycerol	In a solvent-free system, 24 h, 200 rpm, 60 °C, 3.52 g soybean oil, 0.184 g of glycerol, the immobilized CALB 0.2 g.	CALB on SBA-15	61.90 ± 2.38	[122]
19	Soybean oil and MAG	Refined soybean oil and distilled saturated MAG in a mass ratio of 70:30 (W/W), enzyme dosage (5%, W/W), 5 h, 60 °C.	Lipozyme TLIM	22.6	[123]
20	Soybean oil and glycerol	0.25 g CALA@NKA-9, soybean oil 3.52 g, glycerol 0.184 g, 12 h, 50 °C.	CALA@NKA-9	64.37	[124]
21	Soybean oil and glycerol	Water content of 10%, soybean oil 3.52 g, glycerol 0.184 g, immobilized CALA 0.15 g, 60 °C, 12 h, 200 rpm.	CALA@GMP/Fe	64.88	[125]
22	Soybean oil and glycerol	Soybean oil 3.52 g, glycerol 0.184 g, immobilized CALB amount 0.2 g, 60 °C, 12 h, 200 rpm.	CALB@SBA-15-CH₂CH₂(CF₂)₇CF₃	54.19 ± 1.91	[126]
23	Corn oil and glycerol	Corn oil 4.4 g, glycerol 0.23 g, catalysts amount 5% (W/W) of oil and glycerol, tert-pentanol 17 mL, 50 °C, 12 h.	IL-SBA-15-CALB	67.2	[127]
24	Soybean oil and glycerol	Soybean oil 3.52 g, glycerol 0.184 g, immobilized lipase 0.2 g, 60 °C, 24 h with 40 magnetic stirring, 200 rpm.	RML	59.03	[128]
25	Soybean oil and glycerol	Soybean oil 3.52 g, glycerol 0.184 g, immobilized TLL 0.2 g, 60 °C, 24 h, 200 rpm.	TLL	59.19 ± 1.10	[129]
26	Soybean oil and glycerol	Soybean oil 3.52 g, glycerol 0.184 g, SBA-15-LU 0.2 g, 60 °C, 24 h, 200 rpm, pH 6.	Lecitase®Ultra (LU)	56.33 ± 1.53	[130]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; CALB, *Candida antarctica* lipase B; CALA, *Candida antarctica* lipase A; DHA, docosahexaenic acid; CRL, *Candida rugosa* lipase; IL, ionic liquid; TLL, lipase from *Thermomyces lanuginosus*; LU, Lecitase®Ultra; ALA, α-linolenic acid.

In the enzymatic cleavage with camellia oil as raw material, 98.02% conversion was achieved and up to 82% MAGs content was obtained by adding 25 mL of TB and 0.5 g of lipase in the system under the conditions in Table 8 [170]. This is not significantly different from the yield obtained with MAGs produced from sunflower oil, indicating that the effect of different oils or fatty acids on the yield of MAGs and DAGs is not a major factor [160]. As the reaction medium is the key factor on the yield, the amount of reaction medium must have an effect on the yield obviously. Zeng et al. [170] used the same reaction substrates (camellia

oil and glycerol) in different volumes of TB to reach reaction equilibrium. It was concluded that a substrate concentration of 40% in TB was optimal to achieve 98.7% conversion with 82% MAGs content after 10 h. In the algal oil and olive oil cases with TB as a reaction medium [179,180], it was found that lower glycerol/oil ratios favored the formation of DAGs while the higher glycerol/oil ratios favored the formation of MAGs [165].

Among the tertiary alcohols, TB is often used as a reaction medium in addition to tert-pentanol (TP). TP is an intermediate polar solvent (log p
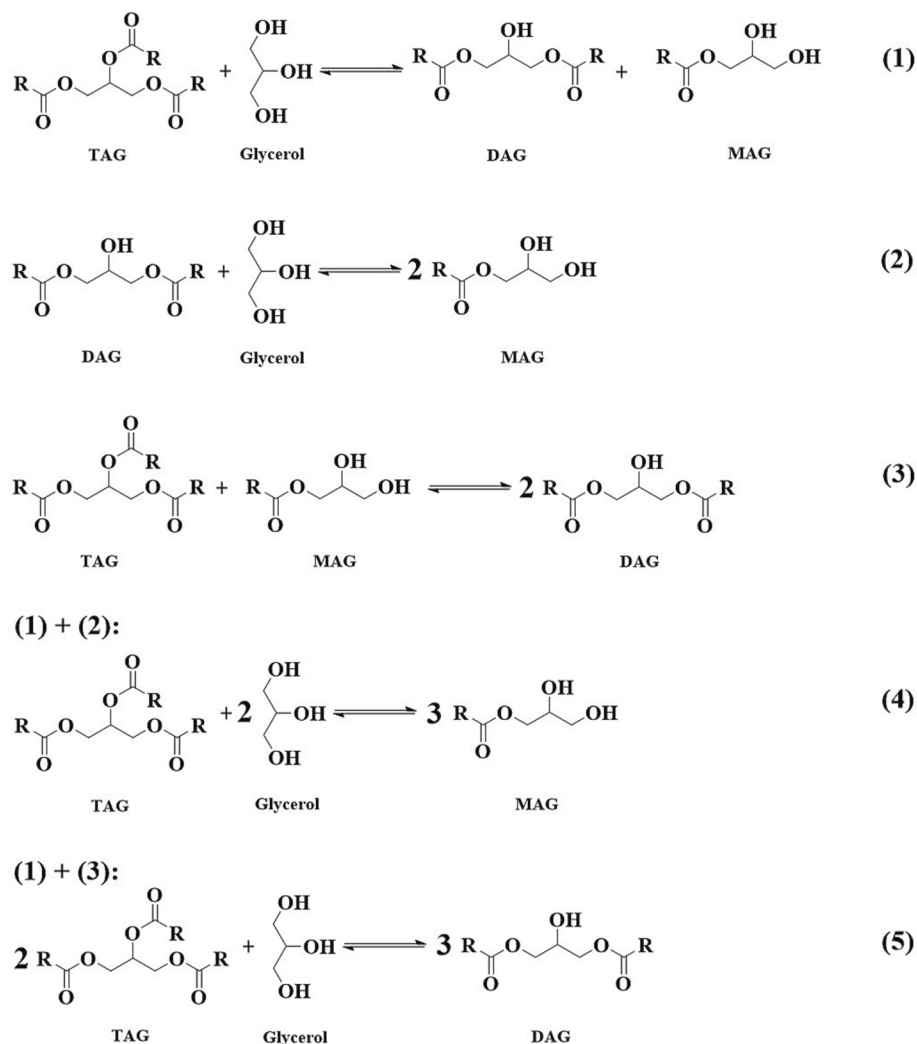
Fig. 3. Enzymatic glycerolysis reaction routes for glycerides preparation. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol.
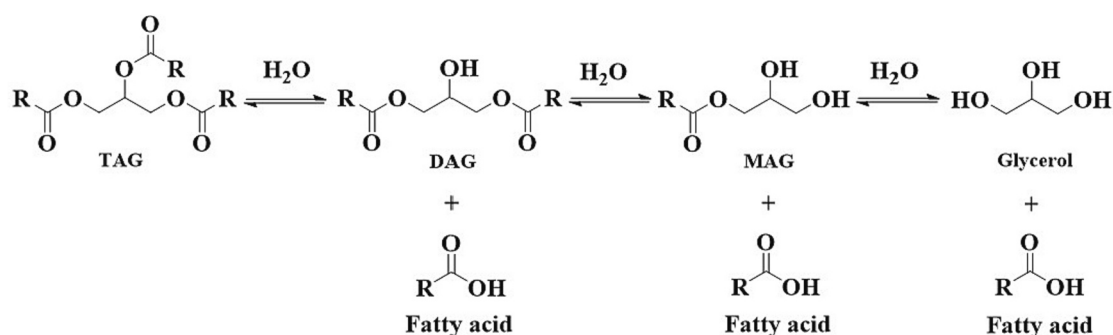

Fig. 4. Enzymatic hydrolysis of TAGs reaction routes for glycerides preparation. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol.

= 1.5) that maintains high lipase activity due to its large spatial site barrier effect [181]. Solaesa et al. (2016) [175] were able to produce MAGs rich in ω-3 polyunsaturated fatty acids (PUFA) by enzymatic glycerolysis of sardine oil using TP (99% TP purity) as a solvent followed by molecular distillation, achieving a yield of 67% and 91% purity. Tertiary alcohols were chosen as the reaction medium because they have low log p (0.35 and 0.89), exhibit better hydrophilicity and hydrophobicity, ensure the mixability of the reactants, and provide a mild reaction site for the reaction. It was found that TB has a higher selectivity and TP has a higher conversion rate, so some studies also choose a

mixture of TB and TP as the reaction medium [173]. Furthermore, it was also found that the price of TB was cheaper than TP, so a single TB or TB/TP (4:1) could be selected for an effective reaction system [182].

4.3. Ionic liquids

As a new type of reaction media, ionic liquids (ILs) possess many unique properties [183], such as near-zero vapor pressure, high chemical and thermal stability, and tunable physicochemical properties, made these compounds popular as a medium for chemical and

Table 5
Enzymatic preparation of MAG through partial hydrolysis.

Entry	Substrates	Reaction conditions	Lipases used	MAG content (%, *W/W*)	References
1	Oil-in-water emulsion of technical triolein	Oil/water emulsion with 20% oil, lipase calculated as 10% of the initial lipid mass, 180 rpm, pH 7.4 (0.01 M phosphate buffer), in a batch type reactor 50 °C/1.6 h (MAG), 60 °C/2 h (DAG).	Lipozyme RM IM	MAG: 19.2–21.4 DAG: 37.6–40.2	[131]
2	Sardine oil	Water activity at 0.3 (MAG)/0.6 (DAG), 300 rpm in a Unimax 1010 orbital shaker, 40 °C.	Lipozyme RM IM	MAG: 7.40 DAG: 33.16	[132]
3	Olive oil	5 mL of olive oil emulsion (0.5 mL of olive oil and 4.5 mL of Arabic gum at 10% (*V/V*)), 10 mL of distilled water, 50 μL of bovine serum albumin 12.5% (*W/V*), pH 7.0, 37 °C, 15 min.	*Rhizopus oryzae* lipase (ROL)	78	[133]
4	Hempseed oil	Substrate flow rate 0.092 mL/min, space velocity 0.027 L/min, 38 min.	The lipase from *Pseudomonas cepacia* (PCL)	A mixture of MAG and DAG (yield 40%, 620 mg)	[134]
5	Vegetable oils	Enzyme amount of 1425 IU, substrate molar ratio oil: hexane 1:4, pH 8.5, 55 °C, 200 rpm , 24 h.	Immobilized lipase from *Serratia* sp. w3	75% Degree of hydrolysis	[135]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; PCL, lipase from *Pseudomonas cepacia*; ROL, *Rhizopus oryzae* lipase.

biochemical reactions [184]. Some of the other tunable properties include polarity, hydrophobicity and sol-sol miscibility behavior by modifying the included cationic and anionic groups [185]. ILs are reported to have protective effects on enzymes for stability enhancement and to be recoverable and recyclable [186,187]. Perhaps the most attractive feature of ILs is the tunability of their properties for specific applications through the selection of suitable cations and anions [188,189]. The long-chain alkyl or acyl groups of the cationic portion of the ILs can significantly increase the solubility of the oils and fats and improve the initial reaction rate (manifested as a shortened induction period) [190]. Therefore, this is the reason why some of the enzymatic reaction experiments use ILs as a reaction medium to produce MAGs and DAGs.

There are also various types of ILs, such as the ionic liquid shown in Fig. 9 is a tetraammonium-based IL (CPMA.MS). Some of the ILs are able to form hydrogen bonds with hydroxyl groups on glycerol or ester groups, and the strong hydrogen bonding interactions of glycerol greatly reduce the thermodynamic activity of glycerol, corresponding to an improved TAGs conversion and products (more than 90% of MAGs) with strong tolerance [191]. Pure ILs are not as perfect as Newtonian liquids, and the viscosity of the mixture of ILs and substrate decreases significantly with increasing rate and shear time, where the behavior is somewhat similar to thixotropic liquids [192].

By testing many kinds of ILs, a comparison selected several of the most remarkable ones, such as [TOMA]·[Tf$_2$N], Ammoeng 120, and the reaction systems of Ammoeng 100 and 102 deserve further study [193]. [TOMA]·[Tf$_2$N] and Ammoeng 120 preferably promote the formation of DAGs because the three octyl groups in the cation increase the hydrophobicity of [TOMA]·[Tf$_2$N], which then increases the oil solubility of the reaction, as indicated in Table 10. The TAGs conversions under Ammoeng 100 and Ammoeng 102 were very high, and the glycerol in Ammoeng 102 was much faster and more reactive, reaching almost equilibrium at 4 h and up to 90% TAGs conversion after 8 h. Since it was found that a single IL system was not sufficient to achieve much higher yields of the desired products than conventional solvents, a binary IL reaction system was introduced, consisting of an IL with better selectivity for DAGs production and another IL capable of achieving high oil conversion rates [156,195]. The [TOMA]·[Tf$_2$N]/Ammoeng 100 system resulted in high DAGs yields and TAGs conversions with relatively low MAGs concentrations compared to the pure Ammoeng 100 system [196]. [BMIM]·[BF$_4$] and [BMIM]·[PF$_6$] are deeply studied ILs [194]. Lozano et al. (2016) [167] studied eight different ionic reaction media with lauric acid as substrate, using immobilized *Pseudomonas aeruginosa* lipase B at a temperature of 60 °C with a glycerol/oil ratio of 4:1. It was found that an equilibrium reaction in an IL reaction medium of [C$_{12}$mim]·[BF$_4$] (1-dodecyl-3-methylimidazolium tetrafluoroborate) for 8 h resulted in the formation of a mixture of 85% MAG and 15% DAG,

while 2 mmol of TB was used during this reaction to reduce the viscosity of the solution and speed up the reaction rate. COSMO-RS is a simulation program to integrate the main interactions between ionic liquid systems (electrostatic (polarity), hydrogen bonding and van der Waals (dispersion)), fully summarizing the multiple solventization interactions of ILs [197]. It could be used for the pre-screening of ionic liquids for a specific reaction system when all the reaction systems were clarified or defined [198].

Glycerol cleavage in CPMA.MS exhibited different characteristics, i. e. significantly high yield of MAGs and high oil conversion, which were not observed in conventional solvent and solvent-free systems [156]. As shown in Fig. 10, a comparison of the three systems, ILs, solvent-free and TB, showed the ability of ILs to promote MAGs ester formation and thus reach higher MAGs yields. This may stem from the fact that ILs not only have negligible volatile pressure, but also act very similarly to common solvents, dissolving both polar and non-polar compounds, even performing much better than common solvents in many cases [199,200]. However, so far, the use of ILs as reaction media is still rarely reported, which may result from the longer equilibration time of ILs due to the lower initial reaction rate. The regulations on ionic liquids for food ingredients processing are also highly needed.

The use of ILs as a reaction medium for the production of MAGs and DAGs has a high citation prospect, but the research on ILs is so limited in companies or industries that there are few ionic liquid reactions that can be referenced. It is highly needed to continue to explore the technology in terms of feasibility and practical applications as well as the ionic liquids development for food uses. Surely some of the research has put up the foundation for the screening and further optimization of ILs [186].

4.4. Other reaction media

In addition to tertiary alcohols and ionic liquids, there are many substances used as reaction media, such as alkanes, acetones [200], deep eutectic solvents (DES), etc. Alkanes such as *n*-hexane are also used as reaction media in some experiments because of their easy recovery properties [201]. However, the amount of glycerol dissolved in hexane alone is very small, so a polar organic solvent mixed with hexane is often used for glycerol cleavage to produce MAGs [202], where can also achieve high yields [203]. Some other studies have shown that conventional organic solvents are not favorable for the enzymatic synthesis of DAGs, while a deep eutectic solvent [204] was found having good aspects as a reaction medium [205]. Glycerol cleavage was then performed with soybean oil and the yield of DAGs was found to be in the range of 40%–60%, while the maximum yield of DAGs in the solvent-free system was around 68%, which is higher than the discussed system. However, the DES system is more selective than the solvent-free

Table 6

Enzymatic preparation of DAG through partial hydrolysis.

Entry	Substrates	Reaction conditions	Lipases used	DAG content (%, W/W)	References
1	Palm oil	11.20% (W/W) (water + oil mass) water content, 1.36% (W/W) (water + oil mass) enzyme loading, 12 h, 1.2 min and 200 W of exposure to ultrasound, irradiations were performed at 55.0 ± 0.1 °C.	Lipozyme RM IM	34.17	[136]
2	Palm oil	45.7 °C, 44% (W/W) water content, 8 h, enzyme loading 34 U/g.	Phospholipase A1	35.51 and 74.52 after distillation again	[137]
3	Soybean oil	45 °C, enzyme loading 30 U/g (of the oil mass), reaction time 8 h.	Phospholipase A1	42.64 and more than 80 after distillation	[138]
4	Soybean oil	200 r/min, pH 6.5, water content of 20%, enzyme at a concentration of 30 U/g, 40 °C, 6 h.	A lipase from *Rhizopus oryzae* (ROL)	32.09	[139]
5	Soybean oil	Solvent-free system, 35 °C, water content of 40%(W/W), 8 h, enzyme loading of 26 U/g.	Phospholipase A1	42.64; and 78.68 after distillation	[140]
6	Palm oil	In Packed Bed Reactor, water (5% oil mass, W/W), 10% enzyme, 60 °C, solvent (hexane), 0.5 mL/min flow rate, 6 min.	PS Amano IM	128 g in 24 h	[141]
7	Palm oil	water (5% oil mass, W/W), ultrasound irradiation, 1 h 30 min, 2% TL IM, 30 °C, 300 rpm.	TLIM lipases	39.77	[142]
8	Palm stearin	A water content of 30% (W/W, with respect to oil mass), T1 lipase at a concentration of 7.5 U/g (with respect to oil mass), 60 °C, 7 h.	Highly thermostable lipase (T1 lipase)	28.1 and 42.5 after distillation	[143]
9	Palm oil	In Packed Bed Reactor, solvent free, 500 rpm, 10 cm packed bed height, 3.8 mL/min flow rate, water (5% oil mass, W/W), 13.8% (W/W) Lipozyme RMIM, 55 °C, 6 h.	*Rhizomucor miehei* (Lipozyme RM IM)	29	[144]
10	Free fatty acids (Camellia Oil) with glycerol	First esterified and then hydrolyzed, glycerol/FFA (4:1, mol/mol), 2% water (W/W, with respect to the total mixture), 35 U/g (with respect to the total mixture), 35 °C, 24 h and enzyme loading 375 U/g, 45 °C, UL/buffer solution (10:10, W/W), pH 8, 48 h.	Lipase G50 and CBD-MGLP	49.0 after hydrolysis and 98 after distillation	[145]

Abbreviation: DAG, diacylglycerol; FFA, free fatty acid; ROL, lipase from *Rhizopus oryzae*.
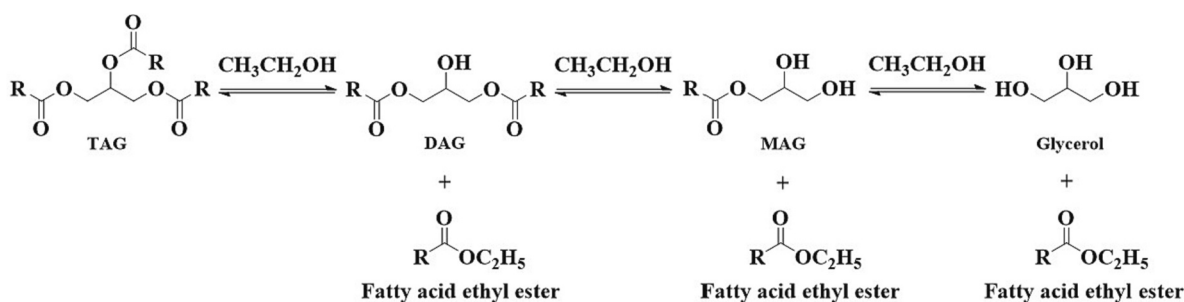
Fig. 5. Enzymatic ethanolysis reaction routes for glycerides preparation. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol.

Table 7

Enzymatic preparation of MAG through ethanolysis.

Entry	Substrates	Reaction conditions	Lipases used	MAG content (%, W/W)	References
1	Cod liver oil and ethanol	750 mg cod liver oil, 3 g 96% (V/V) ethanol, 375 mg lipase N-435, 35 °C, 3 h, 300 rpm.	Novozym 435	30.1 and 85 after silica gel chromatography or 77 after solvent extraction.	[146]
2	Oyster oil and ethanol	1.50 g of oyster oil, 6 g of ethanol (94%), 0.75 g of enzyme were placed in a 50-mL Erlenmeyer flask, in a shaking incubator maintained, 250 rpm, 37 °C, 3 h.	Lipozyme TLIM	31.75 ± 1.30	[147]
3	Tuna oil and ethanol	8% Enzyme loading (W/W, total reactants), tuna oil: ethanol weight ratio of 1:3 (W/W), at room temperature, 3 h.	Lipozyme 435	27 and 95 after liquid-liquid extraction	[148]
4	Fish oil or microalgae oil with erhanol	Ethanol/oil 3:1 (W/W), 5% water (W/W), 35 °C, 48 h, 10% CALA.	CALA	62.96	[149]
5	Atlantic salmon frame bone oil and glycerol	42.5 °C, 4.15 h, enzyme loading 42.81%, ethanol: oil molar ratio 49.82.	Lipozyme TLIM	41.81	[150]
6	Microalgae oil and ethanol	Lipase loading 10% (W/W, based on fish oil), 35 °C, weight ratio of ethanol to oil: 3:1, water content 5% (W/W, based on oil and ethanol), 12 h.	*Candida antarctica* lipase A (CAL-A)	34.58	[151]
7	Algal oil and ethanol	Algal oil to ethanol 1:60 (molar ratio), lipase load of 8% (W/W, relative to total reactants), 30 °C, 2 h, 300 rpm.	Lipozyme 435	26.50–30.95 and 67 after solvent extraction	[152]
8	Fish oils (cod liver oil and tuna oil)	In the STR operated in discontinuous mode, 750 mg oil, 3 g dry ethanol, 30 mg lipase, 35 °C, 300 rpm, TI 0.96−1 g·h·g⁻¹.	Novozym 435	65	[153]

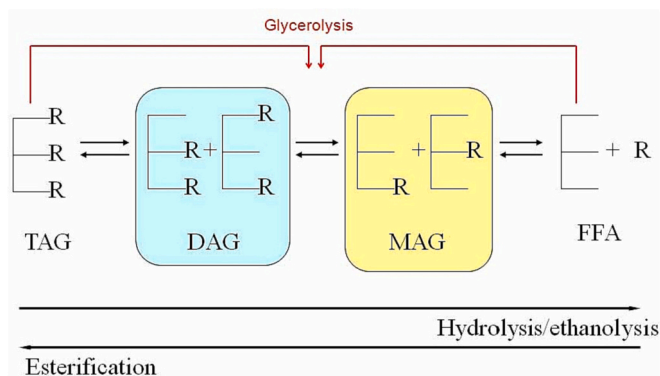Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; CALA, *Candida antarctica* lipase A.

Fig. 6. The substances contained in the reaction equilibrium system. TAG, triacylglycerol; DAG, diacylglycerol; MAG, monoacylglycerol; FFA, free fatty acid.
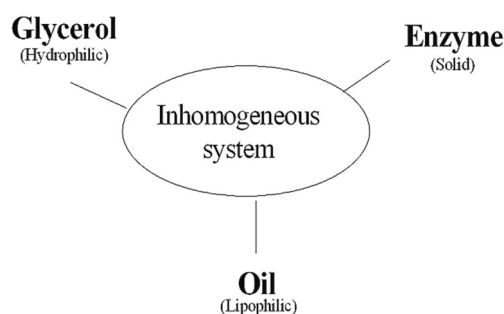

Fig. 7. Multi-phases exist in the solvent-free system.

quality/cost ratio, facility investment, as well as sustainability performance. The possibilities are there for your planning in terms of the optimal performance as targeted.

5. Patent evaluation

This evaluation was conducted using patent data for DAGs and MAGs extracted from the Orbit (www.orbit.com) database, which is one of the most trustworthy and comprehensive patent information providers worldwide. Firstly, MAGs related patents application was summarized in Table 11 [206–213]. Indeed, a relatively low number of inventions have been reported in this area (eight patents), mainly focusing on functional MAGs preparation, MAGs quality or yield improvement, and cost reduction.

On the other hand, a total of 55 patent families of DAGs published between 1986 and 2022 were retrieved. In the following, an overview of these patents in terms of invention year and regional distribution is given. Finally, a summary of the research interests, product forms, and process routes used is presented in the hope of providing a further insight into the innovation profile of DAGs.
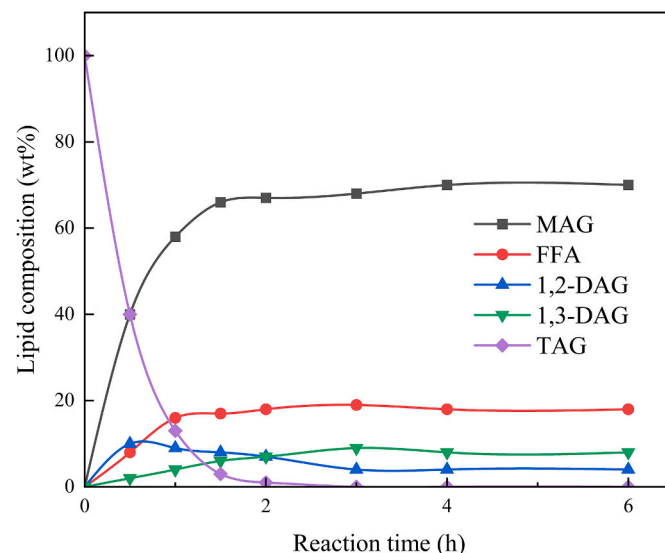

Fig. 8. Glycerolysis time courses of sunflower oil in *tert*-butyl alcohol. Adapted from [174]. MAG, monoacylglycerol; DAG, diacylglycerol; TAG, triacylglycerol; FFA, free fatty acid.
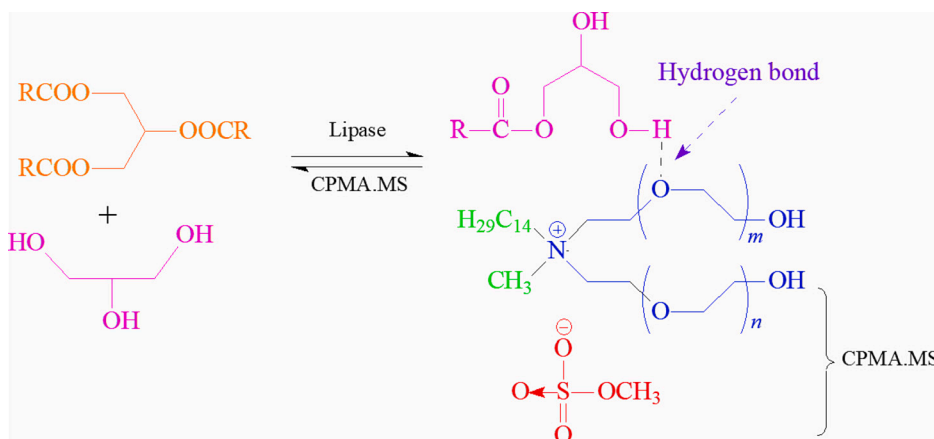
system, and the DES system decreases the activity of glycerol, leading to a decrease in the yield of MAGs, which in turn favors the selective formation of DAGs [205]. Therefore, the choice of media or not, or what type of reaction media to choose is based on the needs of the producers.

Thus, whether choose or not to choose a solvent system or which solvent system is chosen as well as what optimal solvent system or solvent-free system in rational designs are very much dependent on your target of the products, post-reaction purification or processing, product

Table 8
Reaction systems in solvent-free systems.

Reaction medium	Amount added (W/W)	Types of oil	Glycerin/oil (molar ratio)	Reaction parameters	Outputs and yields	Literature Sources
0	0	Soybean oil	8:1	70 °C, 24 h	21.7% MAG, 46.6% DAG	[159]
0	0	Sunflower oil	4.5:1	40 °C, 24 h	20% MAG, 45% DAG	[160]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol.

Table 9
Reaction systems in *tert* alcohol.

Reaction medium	Amount added	Types of oil	Glycerin/oil (molar ratio)	Reaction parameters	Outputs and yields	Literature Sources
TB	TB/oil 2:1(W/W)	Sunflower oil	4.5:1.0	40 °C, 2 h	70% MAG, 26% DAG	[160]
TB	25 mL	Camellia oil	6:1	50 °C, 10 h	82% MAG	[170]
TB	40 mL/10 g oil	Sunflower oil	4:1	50 °C, 1 h	83% MAG	[173]
TB	TB/oil 2.2:1 (W/W)	Sunflower oil	4.5:1.0	40 °C	70% MAG, 25% DAG	[174]
TP	40 mL/10 g oil	Sunflower oil	4:1	50 °C, 2 h	78% MAG, 20% DAG	[173]
TP	TP: Substrate 1.5:1 (W/W)	Sardine oil	3:1	55 °C, 2 h	90% MAG	[175]
TB/TP(1:1)	50 mL/10 g oil	Sunflower oil	5:1	50 °C, 2.5 h	75% MAG	[173]

Abbreviation: MAG, monoacylglycerol; DAG, diacylglycerol; TB, *tert*-butanol; TP, *tert*-pentanol.

Fig. 9. Proposed mechanism for the ionic liquid molecule inducing reaction equilibrium shifting. Adapted from [186,187].

Table 10
Reaction systems in Ionic liquids.

Reaction medium	Amount added	Types of oil	Glycerin/oil (molar ratio)	Reaction parameters	Outputs and yields	Literature Sources
[C$_{12}$mim]·[BF$_4$]	1 g	Lauric acid	4:1	60 °C, 8 h	85% MAG, 15% DAG	[167]
[CPMA]·[MS]	2 mL	Sunflower oil	5:1	45 °C, 10 h	90% MAG, 5% DAG	[194]
[TOMA]·[Tf$_2$N]/Ammoeng 102 (1:3)	1 g	Glycerol trioleate	1.75:2.00	75 °C, 8 h	90% DAG	[193]

Abbreviation: MAG, monoacylgycerol; DAG, diacylglycerol.
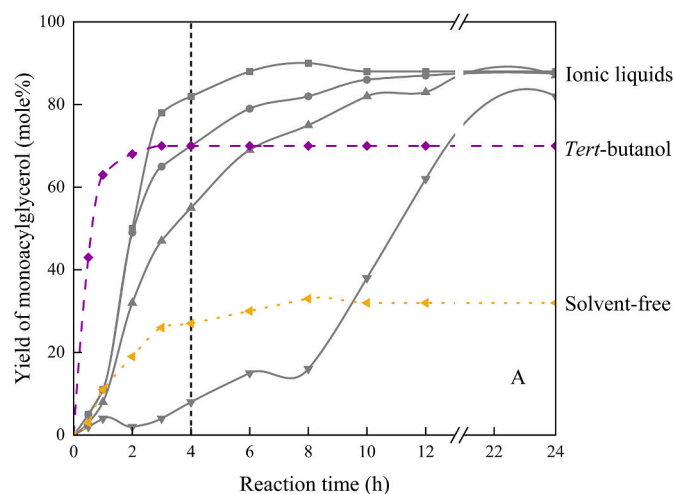
Fig. 10. Ionic liquids shift the reaction to monoglycerides beyond equilibrium. Adapted from [186].

Fig. 11 illustrates the annual application numbers of DAGs patents since 1986. Overall, the number of DAGs related patent applications is gradually growing over time. Particularly, the number of patents in 2019–2022 has increased by 100% compared to the total application number in 2015–2017. And there is a clear trend that the earliest technological inventions were mainly originated or protected in other regions, while, from 2003 on, Chinese companies have also started filing DAGs patent applications as well. Note that the inventions from other regions have gradually declined in recent years, and the current research has mainly originated or been filed in China. The geographic coverage of places where DAGs patent applications have been filed is shown in Fig. 12. As shown in Fig. 12, more than half of the patents were filed in China with 29 patents, followed by Japan with 16 patents, Korea with five patents and the United States and other regions with three patents and two patents, respectively. This gives a picture of the targeted geographic markets where the technology is currently most prominent

and commercially available, helping researchers to scan their filing strategy to ensure that their portfolios cover the top jurisdictions, and then make them an attractive acquisition target. Moreover, this may also help to identify the untapped markets for the technology. In summary, it can be inferred that the DAGs research area is currently a growing sector. And these data also point to an active market and a promising future for the DAGs industry in China. In addition, the extension of this technology into other regions may also be a potential opportunity.

In terms of the final form of products, apart from the conventional DAGs products (37 patents), the attentions in this filed were also paid to DAGs with different configurations as well as special functional characteristics, including 1,3-DAG, 1,2-DAG, DAG-poly unsaturated fatty acid (PUFA), and DAG-monounsaturated fatty acid (MUFA) (Fig. 13). It is well known that DAGs show a different metabolic pathway from normal TAGs in human body, thus suppress body fat accumulation and postprandial serum TAGs level [5]. This healthy effect could be further enhanced when their configuration or composition is changed, making the physiological functional advantages of the product more pronounced. From a data point of view, most of the research on functional DAGs has focused on 1,3-DAG with eight patents and DAG-PUFA with seven patents. On the other hand, only a small number of technologies targeted at 1,2-DAG (one patent) and DAG-MUFA (two patents) have been focused on or filed. This happens because some enzymatic methods (e.g., enzymatic hydrolysis) mainly produce 1,2-DAG rather than 1,3-DAG, whereas, in fact, 1,3-DAG exhibited higher physiological activities than 1,2-DAG [214,215]. Therefore, there are more research interested in preparing 1,3-DAG. On the other hand, DAG-PUFA have many attractive effects, including improving memory, reducing cholesterol, preventing cardiovascular system diseases and the like, while the high polyunsaturated fatty acid content also leads to poor stability and many side reactions, such as oxidation, polymerization, etc. [216]. That would certainly add to production requirements and cost concerns. Thus, a high-efficiency DAGs production process, especially for DAGs with particular functions or conformations, is needed to be found.

In fact, the performance of products and the impact of progress in production are the driving forces that promote the technological innovations. In the domain of DAGs invention, as shown in Fig. 14a, the

Table 11

Enzymatic preparation of MAG in patents.

Entry	Substrates	Reaction conditions	Lipases used	MAG content (%, W/W)	References
1	Kernel oil of geranium fruit	Kernel oil of geranium fruit: acetone/tertiary butanol molar ratio of 0.5:1–2:1, 35–45 °C, 6%–11% water content, 0.3%–0.7% lipase loaded (W/W total reactants).	Sn-1,3 specific lipase	MAG-capric acid: 8 MAG-laurate: 15	[206]
2	One or more of soybean oil, salad oil, olive oil, and castor oil, or glycerol and fatty acid	The molar ratio of glycerol to oil, (2–48):1 for glycerolysis or glycerol to fatty acid (1–10):1 for esterification, 1%–50% enzyme (W/W) of reaction substrates, 40–90 °C, 15–240 min, 1%–10% water content, 100–250 MPa pressure when using porcine pancreas lipase and 100–300 MPa when using wheat germ lipase.	Wheat germ lipase or porcine pancreas lipase	30.50 after molecular distillation	[207]
3	One or more of tea seed oil, high oleic acid sunflower oil, fish oil, algae oil (high arachidonic acid content), and algae oil (high DHA)	1%–15% Enzyme loading (W/W, total reactants), glyceride: ethanol 1:1–10 (W/W), 10–40 °C, 0.5–15 h.	One or more of immobilized enzyme Lipozyme RM IM, NS40086, Lipozyme TL IM, Novozym 435 and Lipozyme 435.	Sn-2 MAG: 88.15–93.26	[208]
4	Glycerol, low unsaturation degree fatty acids, with short or medium chain	Glycerol: fatty acid molar ratio of 1.0:0.2–1:1, 30–70 °C, 100–600 rpm, 1–12 h, 2%–8% 4Å molecular sieve (W/W, total reactants).	One or more of immobilized enzyme Lipozyme RM IM, Lipozyme TL IM, Novozym 435 and Lipozyme 435.	60.10	[209]
5	Glycerol, linoleic acid	Glycerol:fatty acid molar ratio of 1:1, 65 °C, 500 rpm.	Marine microbial-derived MAG lipase.	32.05	[210]
6	Glycerol and one oil selected from tung oil, castor oil, coconut oil, palm oil, and safflower seed oil	Glycerol:oil molar ratio of 1:0.2–1:1, 50–80 °C, 200–600 rpm, 6–12 h, 5%–10% lipase loaded (relative to total reactants).	Complex enzyme, comprises 20%–30% (W/W) of pancreatic lipase, 30%–35% (W/W) of porcine pancreatic enzyme, and 40%–45% (W/W) of lipozyme TL IM.	78.20–84.50	[211]
7	Glycerol and medium chain fatty acid	Glycerol:fatty acid molar ratio of 1:0.8–1:1.2, 50–80 °C and 100 rpm under reduced pressure at 5–15 kPa. Lipase loading of 3%–6% (relative to glycerol).	Lipozyme 435	55–65 medium chain MAG after extraction	[212]
8	PUFA-ethyl ester, glycerol	PUFA-ethyl ester:glycerol molar ratio of 3:1, 65 °C, 8 h, 300 Pa, Lipase loading of 2%–12% (relative to glycerol).	One or more lipase originated from *Candida antarctica, Penicillium camembertii, Burkholderiacepacia, Pseudomonas fluorescens.*	88.60	[213]

Abbreviation: PUFA, poly unsaturated fatty acids; MAG, monoacylglycerol; DHA, docosahexaenoic acid.
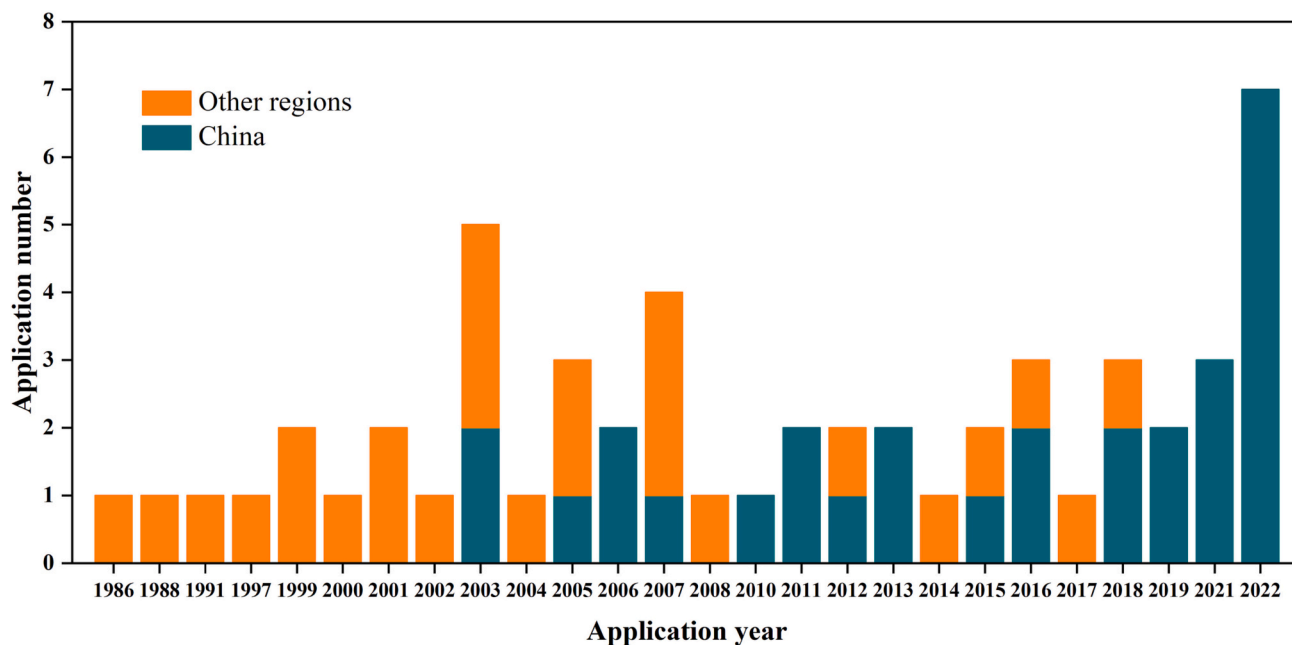
Fig. 11. The numbers of application patents between 1986 and 2022. (Update to Apr. 2, 2023).

area of most interests is the purity of the final product, followed by the efficiency and yield of the methods, the cost of production, and the functions of product. Other topics include co-production, flavor, color. In more detail, at an early stage, researchers mainly focused on finding ways to increase the overall yield and product purity, while avoiding discoloration of the DAGs at high temperatures. Subsequently, as basic production methods have matured, research interests have shifted towards the practical use of DAGs products, such as the flavor, functional properties, and capacity for co-production of the obtained products.

This fact also shapes the trajectory of the development of the process routes. Considering simplicity and cost, the earliest commercialized methods utilized in this field are hydrolysis, glycerolysis and
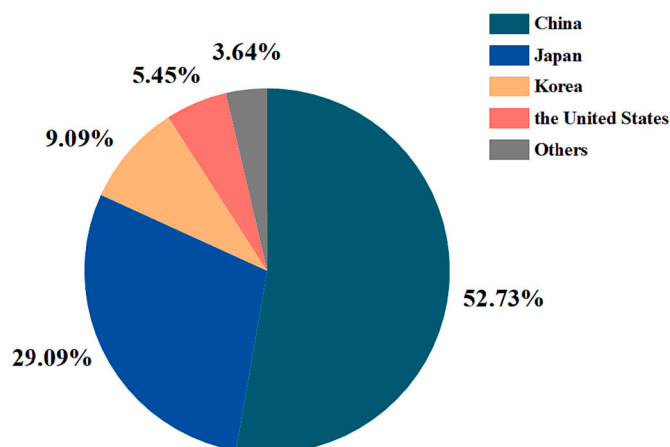
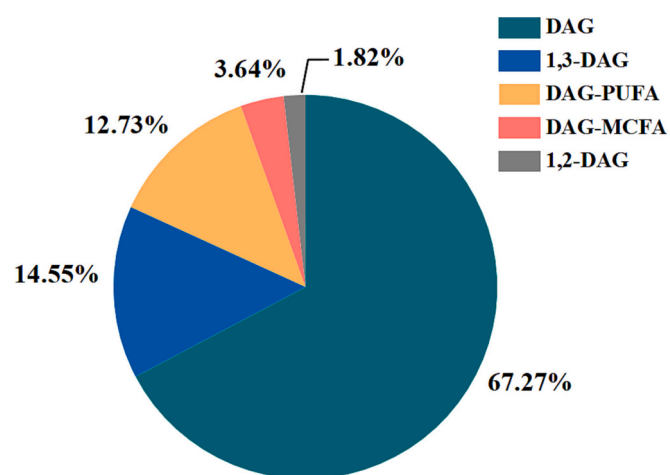**Fig. 12.** The number and distribution of patent application across the world. (Update to Apr. 2, 2023).

Fig. 13. The distribution of DAGs research interests in product form. (Update to Apr. 2, 2023). DAG, diacylglycerol; PUFA, polyunsaturated fatty acids; MCFA, medium chain fatty acids.

esterification (Fig. 14b). Since 2000, the use of esterification and glycerolysis has opened up new developments in the preparation of DAGs. Currently, esterification, hydrolysis and multi-step preparation of DAGs are now the three most widely used methods in this field. Wherein, the multi-step process method is mainly achieved by combining hydrolysis and esterification, acetylation and transesterification, or glycerolysis and transesterification. Besides, hydrolysis method provides a new possibility for natural oils to be converted into chemical raw materials through a simple process, which greatly shortens the preparation process [4]. Among these methods, esterification is the most concerned one for its high efficiency and yield, mild reaction conditions, environmental-friendly, etc. [217].

The importance of optimizing the production process has been emphasized repeatedly above. Finally, combining the current points of interest in this area, and the key words that are involved the most times in updating the techniques, it can be inferred that the improvement of purity is mainly achieved by optimizing reaction processes, solvents and enzymes (Fig. 14c). On the other hand, reaction processes also play a crucial role in increasing the overall yield. In addition, the substrate acts as a critical factor that largely determines the final functionality of the product and, moreover, is an important factor for increasing the efficiency of the reaction. At the same time, the efficiency also can be improved by a reasonable selection of enzymes.

6. Conclusion and outlook

MAGs and DAGs are partial glycerides which are derived from oils and fats in general. MAGs are typical food emulsifiers and DAGs are lately developed into a nutritional oil and fat product for edible uses. Various reactions can be applied for the synthesis or production of them including glycerolysis, esterification, hydrolysis, and ethanolysis. The reaction mechanisms are different and the choice of the reaction systems relates to many issues including the choice of fatty materials, product quality and specifications, product yields, medium selection, reactor designs, processing steps, purification technology, etc. Both esterification and glycerolysis have been used in industry.

Enzymatic production of acylglycerols to replace chemical processes has been in industry also particularly for DAGs production. MAGs are traditionally produced using chemical processes as food emulsifiers. In the case of polyunsaturated fatty acids such as DHA and EPA, the MAG products are not suitable with chemical processes for the production as the high temperature as well as the use of chemical catalysts is not friendly to the stability of the fatty acids. Furthermore, enzyme technology has many more advantages for common products such as the selectivity property leading to less by-products, mild temperature leading to higher quality and less energy consuming, less CO_2 production leading to better sustainability, etc.

Partial glycerides are part of reaction equilibrium as demonstrated in Fig. 6 in all the reaction routes. To increase either MAGs or DAGs portion in the end of the products, the first strategy is to create a system that can reach equilibrium. An efficient reaction system is required with a common incentive to make it homogeneous for the reactants and catalysts to meet freely for reactions to happen. In this case, media are important when a hydrophobic oil or fatty acids phase and a hydrophilic water, glycerol, or ethanol phase are in the reaction system. When an efficient system is possible, the yield of either MAGs or DAGs is quite dependent on the ratios of substrates. The ratios of the hydrophobic phase and hydrophilic phase is also influencing the homogeneity of the system. For the DAGs focusing case, the portion of hydrophilic phase is minor which can be dispersive in the oil phase so that a homogeneity can be reached. In an equilibrium containing MAGs and DAGs, higher yields could be also reached if the targeted products could be locked or separated from the system so that the reaction equilibrium is broken, and further reactions are continued beyond equilibrium. This is confirmed by the case using ionic liquids as a green solvent.

There is huge potentiality nowadays for the production of DAG oils with 1,3-DAGs as the main components as a nutritional oil product. For the current production of a few commercial products in the market, the cost seems high as the prices of the products are 3–10 times higher than normal oils and fats products. There should be space for development of a further efficient system with more effective cost terms of the technology. Besides the consideration of reaction routes, the media creation as well as the generation of industrial feasibility of breaking the reaction equilibria to lead a higher product turnover could be potential breakthrough of the technology, with the consideration of separation challenge of using a solvent medium in the reaction system. A smart medium with green solvents and no volatility could be an interesting target of development.

CRediT authorship contribution statement

Jiawei Zheng: Writing – original draft, Validation, Investigation, Conceptualization. **Yudong Liang:** Writing – original draft, Validation, Investigation. **Jiaxi Li:** Writing – original draft, Validation, Investigation. **Shuping Lin:** Validation, Investigation, Formal analysis. **Qiangyue Zhang:** Validation, Investigation, Formal analysis. **Kanghua Zuo:** Investigation, Formal analysis. **Nanjing Zhong:** Writing – review & editing, Writing – original draft, Supervision, Conceptualization. **Xuebing Xu:** Writing – review & editing, Supervision, Conceptualization.
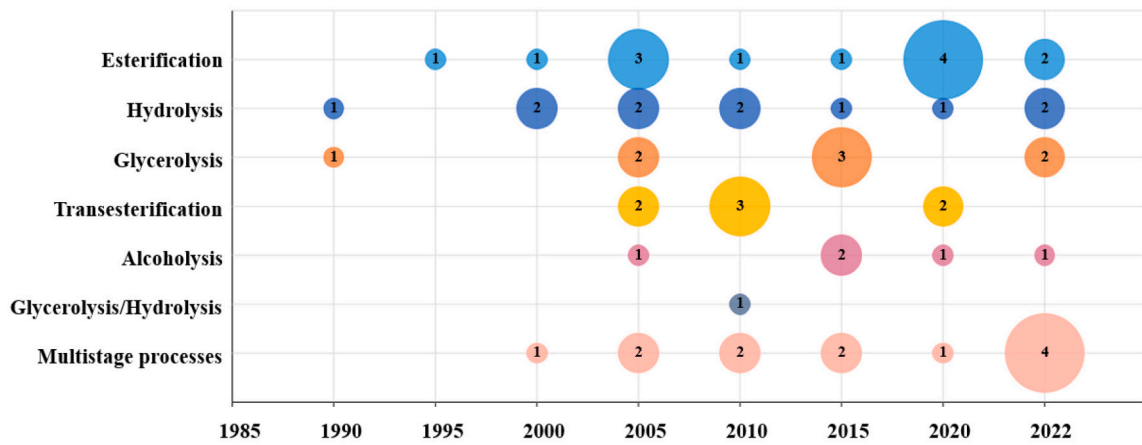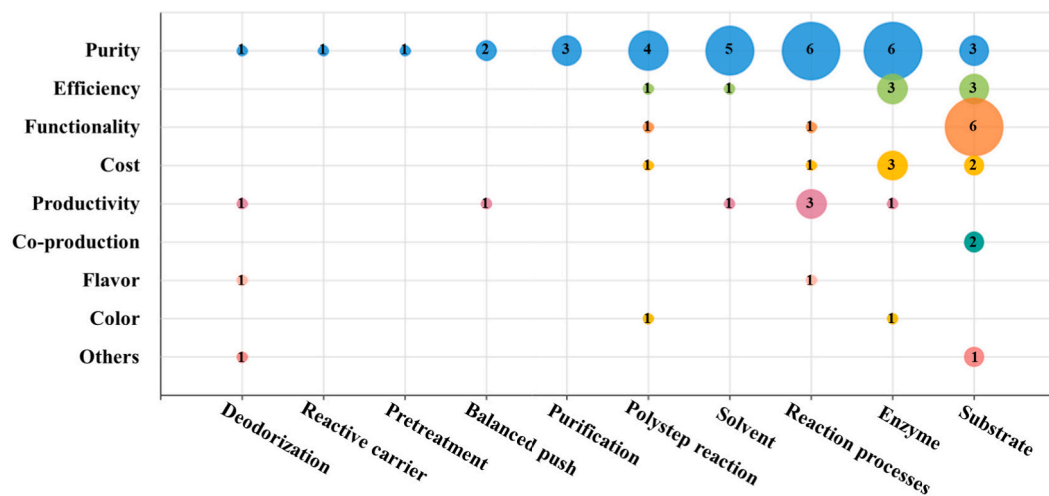
(a)
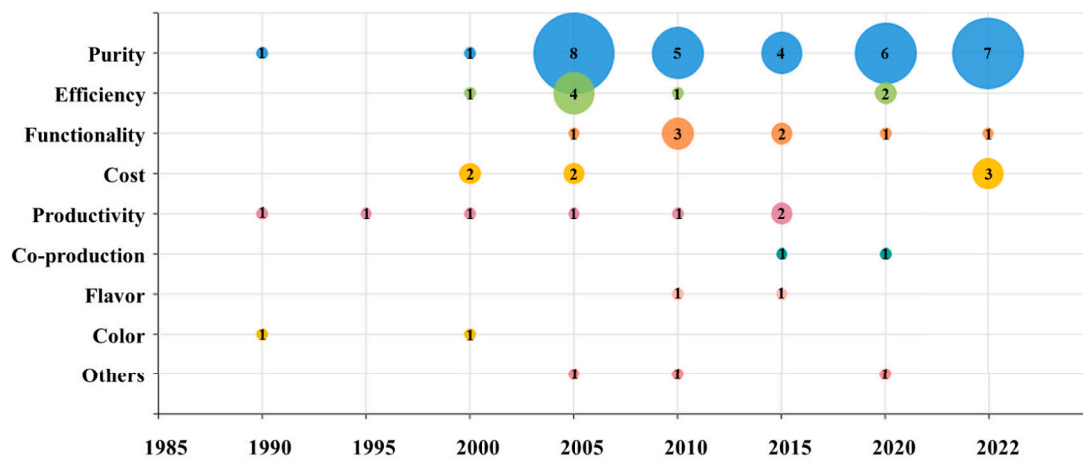
(b)

(c)

Fig. 14. The trend of (a) research interests (b) technical effects (c) key issues for process optimization of DAGs invention. (Update to Apr. 2, 2023). DAG, diacylglycerol.

Declaration of Competing Interest

The author Xuebing Xu is an Associate Editor for Grain & Oil Science and Technology and was not involved in the editorial review or the decision to publish this article. The authors declare that there are no conflicts of interest.

Acknowledgements

References

[1] N. Zhong, L.Z. Cheong, X. Xu, Strategies to obtain high content of monoacylglycerols, Eur. J. Lipid Sci. Technol. 116 (2014) 97–107.

[2] H. Takano, Y. Itabashi, Molecular species analysis of 1,3-diacylglycerols in edible oils by HPLC/ESI-MS, BunsekiKagaku 51 (2002) 437–442.

[3] H. Taguchi, T. Nagao, H. Watanabe, et al., Energy value and digestibility of dietary oil containing mainly 1, 3-diacylglycerol are similar to those of triacylglycerol, Lipids 36 (2001) 379–382.

[4] M.M.C. Feltes, D. de Oliveira, J.M. Block, et al., The production, benefits and applications of monoacylglycerols and diacylglycerols of nutritional interest, Food Bioprocess Technol. 6 (2013) 17–35.

[5] Y.Y. Lee, T.K. Tang, E.T. Phuah, et al., Production, safety, health effects and applications of diacylglycerol functional oil in food systems: a review, Crit. Rev. Food Sci. Nutr. 60 (2020) 2509–2525.

[6] B. Flickinger, N. Matsuo, Nutritional characteristics of DAG oil, Lipids 38 (2003) 129–132.

[7] F.O. Nitbani, P.J.P. Tjitda, B.A. Nurohmah, et al., Preparation of fatty acid and monoglyceride from vegetable oil, J. Oleo Sci. 69 (2020) 277–295.

[8] X. Xu, Biocatalysis for lipid modifications, in: N.T. Dunford, H.B. Dunford (Eds.), Nutritionally Enhanced Edible Oil and Oilseed Processing, AOCS Press, Champaign, 2004, pp. 239–263.

[9] C.P. Churchward, R.G. Alany, L.A.S. Snyder, Alternative antimicrobials: the properties of fatty acids and monoglycerides, Crit. Rev. Microbiol. 44 (2018) 561–570.

[10] B.K. Yoon, J.A. Jackman, E.R. Valle-González, et al., Antibacterial free fatty acids and monoglycerides: biological activities, experimental testing, and therapeutic applications, Int. J. Mol. Sci. 19 (2018) 1114.

[11] K.H. Cho, J.H. Hong, K.T. Lee, et al., Monoacylglycerol (MAG)-oleic acid has stronger antioxidant, anti-atherosclerotic, and protein glycation inhibitory activities than MAG-palmitic acid, J. Med. Food 13 (2010) 99–107.

[12] J.A. Jackman, R.D. Boyd, C.C. Elrod, Medium-chain fatty acids and monoglycerides as feed additives for pig production: towards gut health improvement and feed pathogen mitigation, J. Anim. Sci. Biotechnol. 11 (2020) 1–15.

[13] N. Castejón, F.J. Señoráns, Enzymatic modification to produce health-promoting lipids from fish oil, algae and other new omega-3 sources: A review, New Biotechnol. 57 (2020) 45–54.

[14] L. Burri, N. Hoem, S. Banni, et al., Marine omega-3 phospholipids: metabolism and biological activities, Int. J. Mol. Sci. 13 (2012) 15401–15419.

[15] J. Dyerberg, P. Madsen, J.M. Møller, et al., Bioavailability of marine n-3 fatty acid formulations, Prostaglandins Leukot. Essent. Fat. Acids 83 (2010) 137–141.

[16] J. Neubronner, J.P. Schuchardt, G. Kressel, et al., Enhanced increase of omega-3 index in response to long-term n-3 fatty acid supplementation from triacylglycerols versus ethyl esters, Eur. J. Clin. Nutr. 65 (2011) 247–254.

[17] Y. Zhang, Preparation and Purification of DHA and 2-DHA-MAG and their Regulation of Lipid METABOLISM in HepG2 Cells [dissertation], Jiangnan University, 2019.

[18] M.M.C. Feltes, D. de Oliveira, J.M. Block, et al., The production, benefits, and applications of monoacylglycerols and diacylglycerols of nutritional interest, Food Bioprocess Technol. 6 (2013) 17–35.

[19] R. Pawongrat, X. Xu, A.H. Kittikun, Synthesis of monoacylglycerol rich in polyunsaturated fatty acids from tuna oil with immobilized lipase AK, Food Chem. 104 (2007) 251–258.

[20] M.K. Naik, S.N. Naik, S. Mohanty, Enzymatic glycerolysis for conversion of sunflower oil to food based emulsifiers, Catal. Today 237 (2014) 145–149.

[21] H. Yanai, Y. Tomono, K. Ito, et al., Diacylglycerol oil for the metabolic syndrome, Nutr. J. 6 (2007) 1–6.

[22] H.S. Choi, S.J. Park, Z.H. Lee, et al., The effects of a high fat diet containing diacylglycerol on bone in C57BL/6J mice, Yonsei Med. J. 56 (2015) 951–960.

[23] S. Saito, I. Fukuhara, N. Osaki, et al., Consumption of alpha-linolenic acid-enriched diacylglycerol reduces visceral fat area in over weight and obese subjects: randomized, double-blind controlled, parallel-group designed trial, J. Oleo Sci. 65 (2016) 603–611.

[24] K.C. Maki, M.H. Davidson, R. Tsushima, et al., Consumption of diacylglycerol oil as part of are duced-energy diet enhances loss of body weight and fat in comparison with consumption of a triacylglycerol control oil, Am. J. Clin. Nutr. 76 (2002) 1230–1236.

[25] B.D. Flickinger, N. Matsuo, Nutritional characteristics of DAG oil, Lipids 38 (2003) 129–132.

[26] Y. Mori, H. Nakagiri, H. Kondo, et al., Dietary diacylglycerol reduces postprandial hyperlipidemia and ameliorates glucose intolerance in otsuka long-evans tokushima fatty (OLETF) rats, Nutrition 21 (2005) 933–939.

[27] J.S. Zheng, L. Wang, M. Lin, et al., BMI status influences the response of insulin sensitivity to diacylglycerol oil in Chinese type 2 diabetic patients, Asia Pac. J. Clin. Nutr. 24 (2015) 65–72.

[28] R. Ab Latip, Y.Y. Lee, T.K. Tang, et al., Physicochemical properties and crystallisation behaviour of bakery shortening produced from stearin fraction of palm-based diacylglycerol blended with various vegetable oils, Food Chem. 141 (2013) 3938–3946.

[29] L.Z. Cheong, C.P. Tan, K. Long, et al., Baking performance of palm diacylglycerol bakery fats and sensory evaluation of baked products, Eur. J. Lipid Sci. Technol. 113 (2011) 253–261.

[30] Z. Long, M. Zhao, N. Liu, et al., Physicochemical properties of peanut oil-based diacylglcyerol and their derived oil-in-water emulsions stabilised by sodium caseinate, Food Chem. 184 (2015) 105–113.

[31] T. Waraho, V. Cardenia, Y. Nishino, et al., Antioxidant effects of mono-and diacylglycerols in non-stripped and stripped soybean oil-in-water emulsions, Food Res. Int. 48 (2012) 353–358.

[32] F. Caponio, V.M. Paradiso, M.T. Bilancia, et al., Diacylglycerol isomers in extra virgin olive oil: effect of different storage conditions, Food Chem. 140 (2013) 772–776.

[33] N. Kudo, Y. Kameo, W. Mizuno, Oil-cooked or baked potatoes: US6896922, 2005-05-24.

[34] A. Garzon-Aburbeh, J.H. Poupaert, M. Cleasen, et al., A lymphotrophic prodrug of l-dopa: synthesis, pharmacological properties, and pharmacokinetics behaviour of 1,3-dihexadecanonyl-2-[(s)-2-amino-3-(3,4-dihydroxyphenyl) propanoyl] propane-1,2,3-triol, J. Med. Chem. 29 (1986) 687–691.

[35] J.C.S. Gonclaves, C. Razzouk, J.H. Poupaert, et al., High-performance liquid chromatography of chlorambucil prodrugs structurally related to lipids in rat plasma, J. Chromatogr. B 494 (1989) 389–396.

[36] W. Chen, L. Xu, N. Zhong, Encapsulation of CALB by nucleotide/metal ions coordination nanoparticles: highly selective catalysis of esterification while poor performance in glycerolysis reaction, J. Sci. Food Agric. 102 (2022) 1812–1822.

[37] M. Kapoor, M.N. Gupta, Obtaining monoglycerides by esterification of glycerol with palmitic acid using some high activity preparations of *Candida antarctica* lipase B, Process Biochem. 47 (2012) 503–508.

[38] Y.G. Pyo, S.I. Hong, Y. Kim, et al., Synthesis of monoacylglycerol containing pinolenic acid via stepwise esterification using a cold active lipase, Biotechnol. Prog. 28 (2012) 1218–1224.

[39] T. Osamura, K. Nonaka, F. Takahashi, et al., Efficient monoacylglycerol synthesis by carboxylesterase EstGtA2 from *Geobacillus thermodenitrificans* in a solvent-free two-phase system, J. Biosci. Bioeng. 134 (2022) 89–94.

[40] Y. Zhao, J. Liu, L. Deng, et al., Optimization of *Candida* sp. 99-125 lipase catalyzed esterification for synthesis of monoglyceride and diglyceride in solvent-free system, J. Mol. Catal. B Enzym. 72 (2011) 157–162.

[41] Z. Zhang, X. Ma, H. Huang, et al., Enzymatic production of highly unsaturated monoacylglycerols and diacylglycerols and their emulsifying effects on the storage stability of a palm oil based shortening system, J. Am. Oil Chem. Soc. 94 (2017) 1175–1188.

[42] Y. Satyawali, L. Cauwenberghs, M. Maesen, et al., Lipase catalyzed solvent free synthesis of monoacylglycerols in various reaction systems and coupling reaction with pervaporation for in situ water removal, Chem. Eng. Process. 166 (2021), 108475.

[43] K.S.C. Rocha, M.S.R. Queiroz, B.S. Gomes, et al., Lipases of endophytic fungi *Stemphylium lycopersici* and *Sordaria* sp.: application in the synthesis of solketal derived monoacylglycerols, Enzyme Microb. Tech. 142 (2020) 109664.

[44] S.D. Sun, S. Lan, Y.F. Liu, et al., Solvent-free enzymatic preparation of feruloylated monoacylglycerols optimized by response surface methodology, J. Agric. Food Chem. 56 (2008) 442–447.

[45] L. Wang, Y. Wang, C. Hu, et al., Preparation of diacylglycerol-enriched oil from free fatty acids using lecitase ultra-catalyzed esterification, J. Am. Oil Chem. Soc. 88 (2011) 1557–1565.

[46] X. Meng, G. Xu, Q.L. Zhou, et al., Highly efficient solvent-free synthesis of 1, 3-diacylglycerols by lipase immobilised on nano-sized magnetite particles, Food Chem. 143 (2014) 319–324.

[47] Z. Meng, S. Lu, W. Geng, et al., Preliminary study on acyl incorporation and migration in the production of 1, 3-diacylglycerol by immobilized lipozyme RM IM-catalyzed esterification, Food Sci. Technol. Res. 20 (2014) 175–182.

[48] M.A. Salameh, J. Wiegel, Synthesis of fatty acid esters and diacylglycerols at elevated temperatures by alkalithermophilic lipases from *Thermosyntropha lipolytica*, J. Ind. Microbiol. Biotechnol. 36 (2009) 1281–1287.

[49] D. von der Haar, A. Stäbler, R. Wichmann, et al., Enzyme-assisted process for DAG synthesis in edible oils, Food Chem. 176 (2015) 263–270.

[50] X. Li, D. Li, W. Wang, et al., Immobilization of SMG1-F278N lipase onto a novel epoxy resin: characterization and its application in synthesis of partial glycerides, J. Mol. Catal. B-enzym. 133 (2016) 154–160.

[51] G. Li, J. Chen, X. Ma, et al., Enzymatic preparation and facile purification of medium-chain, and medium-and long-chain fatty acid diacylglycerols, LWT-Food Sci. Technol. 92 (2018) 227–233.

[52] J. Zhao, J. Lin, L. Yang, et al., Preparation of high-purity 1, 3-diacylglycerol using performance-enhanced lipase immobilized on nanosized magnetite particles, Biotechnol. Bioproc. E. 24 (2019) 326–336.

[53] C. Huang, Z. Liu, W. Huang, et al., Fabrication, characterization, and purification of nutraceutical diacylglycerol components from Camellia oil, J. Food Sci. 87 (2022) 3856–3871.

[54] C. Huang, Z. Lin, Y. Zhang, et al., Structure-guided preparation of fuctional oil rich in 1, 3-diacylglycerols and linoleic acid from camellia oil by combi-lipase, J. Sci. Food Agric. 103 (2023) 108–117.

[55] L. Li, Y. Wang, R. Cui, et al., Engineering the thermostability of the mono-and diacylglycerol lipase SMG1 for the synthesis of diacylglycerols, Foods 11 (2022) 4069.

[56] M. Liu, J. Fu, Y. Teng, et al., Fast production of diacylglycerol in a solvent free system via lipase catalyzed esterification using a bubble column reactor, J. Am. Oil Chem. Soc. 93 (2016) 637–648.

[57] D. Li, X. Qin, B. Sun, et al., A feasible industrialized process for producing high purity diacylglycerols with no contaminants, Eur. J. Lipid Sci. Technol. 121 (2019) 1900039.

[58] K. Feng, H. Fang, G. Liu, et al., Enzymatic synthesis of diacylglycerol-enriched oil by two-step vacuum-mediated conversion of fatty acid ethyl ester and fatty acid from soy sauce by-product oil as lipid-lowering functional oil, Front. Nutr. 9 (2022), 884829.

[59] N. Zhong, M. Kou, F. Zhao, et al., Enzymatic production of diacylglycerols from high-acid soybean oil, J. Am. Oil Chem. Soc. 96 (2019) 967–974.

[60] N. Liu, D. Li, W. Wang, et al., Production and immobilization of lipase PCL and its application in synthesis of α-linolenic acid-rich diacylglycerol, J. Food Biochem. 42 (2018), e12574.

[61] S. Sun, L. Shan, Y. Liu, et al., Solvent-free enzymatic synthesis of feruloylated diacylglycerols and kinetic study, J. Mol. Catal. B Enzym. 57 (2009) 104–108.

[62] E. Hernández-Martín, C.G. Hill Jr., C. Otero, Selective synthesis of diacylglycerols of conjugated linoleic acid, J. Am. Oil Chem. Soc. 86 (2009) 427–435.

[63] Z. Wang, W. Du, L. Dai, et al., Study on lipozyme TL IM-catalyzed esterification of oleic acid and glycerol for 1, 3-diolein preparation, J. Mol. Catal. B Enzym. 127 (2016) 11–17.

[64] D.A. Sánchez, G.M. Tonetto, M.L. Ferreira, Enzymatic synthesis of 1, 3-dicap-royglycerol by esterification of glycerol with capric acid in an organic solvent system, J. Mol. Catal. B Enzym. 100 (2014) 7–18.

[65] Z.Q. Duan, X.L. Fang, Z.Y. Wang, et al., Sustainable process for 1, 3-diolein synthesis catalyzed by immobilized lipase from penicillium expansum, ACS Sustain. Chem. Eng. 3 (2015) 2804–2808.

[66] Z.Q. Duan, W. Du, D.H. Liu, Rational synthesis of 1, 3-diolein by enzymatic esterification, J. Biotechnol. 159 (2012) 44–49.

[67] Z.Q. Duan, W. Du, D.H. Liu, Improved synthesis of 1, 3-diolein by novozym 435-mediated esterification of monoolein with oleic acid, J. Mol. Catal. B Enzym. 89 (2013) 1–5.

[68] D. Xu, L. Sun, H. Chen, et al., Enzymatic synthesis of diacylglycerols enriched with conjugated linoleic acid by a novel lipase from *Malassezia globosa*, J. Am. Oil Chem. Soc. 89 (2012) 1259–1266.

[69] W. Wang, T. Li, X. Qin, et al., Production of lipase SMG1 and its application in synthesizing diacylglyecrol, J. Mol. Catal. B Enzym. 77 (2012) 87–91.

[70] B. Wang, M. Zhang, W. Ge, et al., Microencapsulated duck oil diacylglycerol: preparation and application as anti-obesity agent, LWT. 101 (2019) 646–652.

[71] J. Abd Awadallak, E.A. da Silva, C. da Silva, Production of linseed diacylglycerol-rich oil by combined glycerolysis and esterification, Ind. Crop. Prod. 145 (2020), 111937.

[72] N. Liu, Y. Wang, Q. Zhao, et al., Fast synthesis of 1, 3-DAG by lecitase® ultra-catalyzed esterification in solvent-free system, Eur. J. Lipid Sci. Technol. 113 (2011) 973–979.

[73] D. Li, X. Zhong, M. Faiza, et al., Simultaneous preparation of edible quality medium and high purity diacylglycerol by a novel combined approach, LWT. 150 (2021), 111949.

[74] K. Feng, Z. Huang, B. Peng, et al., Immobilization of *Aspergillus niger* lipase onto a novel macroporous acrylic resin: stable and recyclable biocatalysis for deacidification of high-acid soy sauce residue oil, Bioresour. Technol. 298 (2020), 122553.

[75] D.J. Hu, J.M. Chen, Y.M. Xia, A comparative study on production of middle chain diacylglycerol through enzymatic esterification and glycerolysis, J. Ind. Eng. Chem. 19 (2013) 1457–1463.

[76] N. Zhong, Z. Gui, L. Xu, et al., Solvent-free enzymatic synthesis of 1,3-diacyl-glycerols by direct esterification of glycerol with saturated fatty acids, Lipids Health Dis. 12 (2013) 1–7.

[77] W. Piyatheerawong, Y. Iwasaki, X. Xu, et al., Dependency of water concentration on ethanolysis of trioleoylglycerol by lipases, J. Mol. Catal. B Enzym. 28 (2004) 19–24.

[78] R.A. Nicholson, A.G. Marangoni, Enzymatic glycerolysis converts vegetable oils into structural fats with the potential to replace palm oil in food products, Nat. Food 1 (2020) 684–692.

[79] A. Valerio, R.L. Krüger, J. Ninow, et al., Kinetics of solvent-free lipase-catalyzed glycerolysis of olive oil in surfactant system, J. Agric. Food Chem. 57 (2009) 8350–8356.

[80] A. Valerio, K.G. Fiametti, S. Rovani, et al., Enzymatic production of mono-and diglycerides in compressed n-butane and AOT surfactant, J. Supercrit. Fluids 49 (2009) 216–220.

[81] A. Valério, S. Rovani, H. Treichel, et al., Optimization of mono and diacylglycerols production from enzymatic glycerolysis in solvent-free systems, Bioprocess Biosyst. Eng. 33 (2010) 805–812.

[82] R.L. Krüger, A. Valério, M. Balen, et al., Improvement of mono and diacylglycerol production via enzymatic glycerolysis in tert-butanol system, Eur. J. Lipid Sci. Technol. 112 (2010) 921–927.

[83] F. Zeng, B. Yang, Y. Wang, et al., Enzymatic production of monoacylglycerols with camellia oil by the glycerolysis reaction, J. Am. Oil Chem. Soc. 87 (2010) 531–537.

[84] A. Valério, K.G. Fiametti, S. Rovani, et al., Low-pressure lipase-catalyzed production of mono-and diglycerides with and without *n*-butane and AOT surfactant, Appl. Biochem. Biotech. 160 (2010) 1789–1796.

[85] K.G. Fiametti, M.M. Sychoski, A. de Cesaro, et al., Ultrasound irradiation promoted efficient solvent-free lipase-catalyzed production of mono-and diacylglycerols from olive oil, Ultrason. Sonochem. 18 (2011) 981–987.

[86] M.M.C. Feltes, P. Villeneuve, B. Baréa, et al., Enzymatic production of monoacylglycerols (MAG) and diacylglycerols (DAG) from fish oil in a solvent-free system, J. Am. Oil Chem. Soc. 89 (2012) 1057–1065.

[87] A.K. Singh, M. Mukhopadhyay, Olive oil glycerolysis with an immobilized lipase *Candida antarctica* in a solvent free system, Grasas Aceites 63 (2012) 202–208.

[88] D. Emonatto, C.M.T. Santin, A. Valério, et al., Lipase-Catalyzed glycerolysis of soybean and canola oils in a free organic solvent system assisted by ultrasound, Appl. Biochem. Biotechnol. 176 (2015) 850–862.

[89] A.K. Singh, M. Mukhopadhyay, Immobilization of lipase on carboxylic acid-modified silica nanoparticles for olive oil glycerolysis, Bioproc. Biosyst. Eng. 41 (2018) 115–127.

[90] D. Palacios, N. Ortega, N. Rubio-Rodríguez, et al., Lipase-catalyzed glycerolysis of anchovy oil in a solvent-free system: simultaneous optimization of monoacylglycerol synthesis and end-product oxidative stability, Food Chem. 271 (2019) 372–379.

[91] E. Subroto, T. Utami, C. Hidayat, Enzymatic glycerolysis–interesterification of palm stearin–olein blend for synthesis structured lipid containing high mono-and diacylglycerol, Food Sci. Biotechnol. 28 (2019) 511–517.

[92] L.S. Zamorano, P.C. Magaña, E.G. Cisneros, et al., Cocoa olein glycerolysis with lipase *Candida antarctica* in a solvent free system, Grasas Aceites 71 (2020) e383.

[93] À.F.A. Esmelindro, K.G. Fiametti, G. Ceni, et al., Lipase-catalyzed production of monoglycerides in compressed propane and AOT surfactant, J. Supercrit. Fluids 47 (2008) 64–69.

[94] R. Pawongrat, X. Xu, A. H-Kittikun, Physico-enzymatic production of monoacylglycerols enriched with very-long-chain polyunsaturated fatty acids, J. Sci. Food Agric. 88 (2008) 256–262.

[95] K.G. Fiametti, M.K. Ustra, D. de Oliveira, et al., Kinetics of ultrasound-assisted lipase-catalyzed glycerolysis of olive oil in solvent-free system, Ultrason. Sonochem. 19 (2012) 440–451.

[96] Á.G. Solaesa, M.T. Sanz, M. Falkeborg, et al., Production and concentration of monoacylglycerols rich in omega-3 polyunsaturated fatty acids by enzymatic glycerolysis and molecular distillation, Food Chem. 190 (2016) 960–967.

[97] R.N. Vilas Bôas, R. Lima, M.V.C. Silva, et al., Continuous production of monoacylglycerol via glycerolysis of babassu oil by immobilized *Burkholderia cepacia* lipase in a packed bed reactor, Bioproc. Biosyst. Eng. 44 (2021) 2205–2215.

[98] N. Ngatirah, C. Hidayat, E.S. Rahayu, et al., Enzymatic glycerolysis of palm kernel olein-stearin blend for monolaurin synthesis as an emulsifier and antibacterial, Foods 11 (2022) 2412.

[99] Á.G. Solaesa, M.T. Sanz, R. Melgosa, et al., Glycerolysis of sardine oil catalyzed by a water dependent lipase in different tert-alcohols as reaction medium, Grasas Aceites 66 (2015) e102.

[100] N. Majid, B. Cheirsilp, Optimal conditions for the production of monoacylglycerol from crude palm oil by an enzymatic glycerolysis reaction and recovery of carotenoids from the reaction product, Int. J. Food Sci. Technol. 47 (2012) 793–800.

[101] Q. Zhu, T. Li, Y. Wang, et al., A two-stage enzymatic process for synthesis of extremely pure high oleic glycerol monooleate, Enzyme Microb. Tech. 48 (2011) 143–147.

[102] J.V. Oliveira, G.F. Finco, K.G. Fiametti, et al., Kinetic study of liquid lipase-catalyzed glycerolysis from olive oil using lipozyme TL 100L, J. Am. Oil Chem. Soc. (2022), https://doi.org/10.21203/rs.3.rs-275118/v1.

[103] L. Koop, L.I. Soares, F. Augusto Pedersen Voll, et al., Dynamic optimization for the enzymatic production of acylglycerols, Chem. Eng. Commun. 207 (2020) 93–108.

[104] K.G. Fiametti, S. Rovani, D. de Oliveira, et al., Kinetics of solvent-free lipase-catalyzed production of monoacylglycerols from olive oil in aerosol-OT surfactant, Ind. Eng. Chem. Res. 48 (2009) 708–712.

[105] N. Liu, Y. Wang, Q. Zhao, et al., Immobilisation of lecitase® ultra for production of diacylglycerols by glycerolysis of soybean oil, Food Chem. 134 (2012) 301–307.

[106] R. Dhara, R.S. Singhal, Process optimization of enzyme catalyzed production of dietary diacylglycerol (DAG) using TLIM as biocatalyst, J. Oleo Sci. 63 (2014) 169–176.

[107] Z. Song, Y. Liu, Q. Jin, et al., Lipase-catalyzed preparation of diacylglycerol-enriched oil from high-acid rice bran oil in solvent-free system, Appl. Biochem. Biotechnol. 168 (2012) 364–374.

[108] H. Yue, R.M. Luo, X. Qin, et al., Synthesis of partial glycerides rich in α-linolenic acid efficiently from silkworm pupa oil with immobilized lipase MAS1-H108A, Food Sci. Technol. 42 (2021).

[109] K. Miranda, R. Baeza-Jiménez, J.A. Noriega-Rodríguez, et al., Optimization of structured diacylglycerols production containing ω-3 fatty acids via enzyme-catalysed glycerolysis of fish oil, Eur. Food Res. Technol. 236 (2013) 435–440.

[110] X. Zhao, Q. Sun, Z. Qin, et al., Ultrasonic pretreatment promotes diacylglycerol production from lard by lipase-catalysed glycerolysis and its physicochemical properties, Ultrason. Sonochem. 48 (2018) 11–18.

[111] F. Voll, R.L. Krüger, F. de Castilhos, et al., Kinetic modeling of lipase-catalyzed glycerolysis of olive oil, Biochem. Eng. J. 56 (2011) 107–115.

[112] D. Kahveci, Z. Guo, B. Özçelik, et al., Optimisation of enzymatic synthesis of diacylglycerols in binary medium systems containing ionic liquids, Food Chem. 119 (2010) 880–885.

[113] X. Zhao, F. Zhao, N. Zhong, Production of diacylglycerols through glycerolysis with SBA-15 supported *Thermomyces lanuginosus* lipase as catalyst, J. Sci. Food Agric. 100 (2020) 1426–1435.

[114] X. Zou, K. Nadege, I. Ninette, et al., Preparation of docosahexaenoic acid-rich diacylglycerol-rich oil by lipase-catalyzed glycerolysis of microbial oil from *Schizochytrium* sp. in a solvent-free system, J. Am. Oil Chem. Soc. 97 (2020) 263–270.

[115] C.M. Yeoh, T.S.Y. Choong, L.C. Abdullah, et al., Influence of silica gel in production of diacylglycerol via enzymatic glycerolysis of palm olein, Eur. J. Lipid Sci. Technol. 111 (2009) 599–606.

[116] N. Liu, Y. Wang, Q. Zhao, et al., Production of palm oil-based diacylglycerol using *Lecitase Ultra*-catalyzed glycerolysis and molecular distillation, Food Sci. Biotechnol. 23 (2014) 365–371.

[117] X. Diao, H. Guan, B. Kong, et al., Preparation of diacylglycerol from lard by enzymatic glycerolysis and its compositional characteristics, Korean J. Food Sci. An. 37 (2017) 813.

[118] W. Wang, T. Li, Z. Ning, et al., Production of extremely pure diacylglycerol from soybean oil by lipase-catalyzed glycerolysis, Enzyme Microb. Tech. 49 (2011) 192–196.

[119] C.M. Yeoh, E.T. Phuah, T.K. Tang, et al., Molecular distillation and characterization of diacylglycerol-enriched palm olein, Eur. J. Lipid Sci. Technol. 116 (2014) 1654–1663.

[120] C. Cai, Y. Gao, Y. Liu, et al., Immobilization of *Candida antarctica* lipase B onto SBA-15 and their application in glycerolysis for diacylglycerols synthesis, Food Chem. 212 (2016) 205–212.

[121] G. Yao, X. Wang, M. Yang, et al., Co-immobilization of bi-lipases on magnetic nanoparticles as an efficient catalyst for synthesis of functional oil rich in diacylglycerols, phytosterol esters and α-linolenic acid, LWT. 129 (2020), 109522.

[122] Y. Li, N. Zhong, L.Z. Cheong, et al., Immobilization of *Candida antarctica* Lipase B onto organically-modified SBA-15 for efficient production of soybean-based mono and diacylglycerols, Int. J. Biol. Macromol. 120 (2018) 886–895.

[123] Q. Chen, Y. Li, J. Fu, et al., Production of diacylglycerol-enriched oils by enzymatic interesterification and molecular distillation using soybean oil and distilled saturated monoacylglycerol, Eur. J. Lipid Sci. Technol. 119 (2017) 1600332.

[124] L. He, J. Zheng, S. Feng, et al., Immobilization of *Candida antarctica* Lipase A onto Macroporous Resin NKA-9: esterification and glycerolysis performance study, J. Oleo. Sci. 71 (2022) 1337–1348.

[125] W. Chen, L. He, W. Song, et al., Encapsulation of lipases by nucleotide/metal ion coordination polymers: enzymatic properties and their applications in glycerolysis and esterification studies, J. Sci. Food Agric. 102 (2022) 4012–4024.

[126] X. Wang, L. He, J. Huang, et al., Immobilization of lipases onto the halogen & haloalkanes modified SBA-15: enzymatic activity and glycerolysis performance study, Int. J. Biol. Macromol. 169 (2021) 239–250.

[127] N. Zhong, Y. Li, C. Cai, et al., Enhancing the catalytic performance of *Candida antarctica* lipase B by immobilization onto the ionic liquids modified SBA-15, Eur. J. Lipid Sci. Technol. 120 (2018) 1700357.

[128] N. Zhong, W. Chen, L. Liu, et al., Immobilization of *Rhizomucor miehei* lipase onto the organic functionalized SBA-15: their enzymatic properties and glycerolysis efficiencies for diacylglycerols production, Food Chem. 271 (2019) 739–746.

[129] X. Zhao, F. Zhao, N. Zhong, Production of diacylglycerols through glycerolysis with SBA-15 supported *Thermomyces lanuginosus* lipase as catalyst, J. Sci. Food Agric. 100 (2020) 1426–1435.

[130] M. Kou, S. Feng, N. Zhong, Immobilization of lecitase® ultra onto the amino-functionalized SBA-15 and their applications in glycerolysis, J. Oleo Sci. 69 (2020) 347–358.

[131] S.J. Hares Júnior, J.N.R. Ract, L.A. Gioielli, et al., Conversion of triolein into mono-and diacylglycerols by immobilized lipase, Arab. J. Sci. Eng. 43 (2018) 2247–2255.

[132] R. Morales-Medina, M. Munio, A. Guadix, et al., A lumped model of the lipase catalyzed hydrolysis of sardine oil to maximize polyunsaturated fatty acids content in acylglycerols, Food Chem. 240 (2018) 286–294.

[133] N. Ghattas, F. Abidi, S. Galai, et al., Monoolein production by triglycerides hydrolysis using immobilized *Rhizopus oryzae* lipase, Int. J. Biol. Macromol. 68 (2014) 1–6.

[134] T. Bavaro, I. Benucci, A. Pedrali, et al., Lipase-mediated hydrolysis of hempseed oil in a packed-bed reactor and in-line purification of PUFA as mono- and diacylglycerols, Food Bioprod. Process. 123 (2020) 345–353.

[135] Z. Zarai, A. Edahech, F. Rigano, et al., Monoacylglycerol and diacylglycerol production by hydrolysis of refined vegetable oil by-products using an immobilized lipase from *Serratia* sp. w3, J. Sep. Sci. 41 (2018) 4323–4330.

[136] J.A. Awadallak, F. Voll, M.C. Ribas, et al., Enzymatic catalyzed palm oil hydrolysis under ultrasound irradiation: diacylglycerol synthesis, Ultrason. Sonochem. 20 (2013) 1002–1007.

[137] Y. Wang, M. Zhao, S. Ou, et al., Preparation of diacylglycerol-enriched palm olein by phospholipase A1-catalyzed partial hydrolysis, Eur. J. Lipid Sci.Tech. 111 (2009) 652–662.

[138] Y. Wang, M. Zhao, K. Song, et al., Separation of diacylglycerols from enzymatically hydrolyzed soybean oil by molecular distillation, Sep. Purif. Technol. 75 (2010) 114–120.

[139] D. Li, X. Qin, J. Wang, et al., Hydrolysis of soybean oil to produce diacylglycerol by a lipase from *Rhizopus oryzae*, J. Mol. Catal. B-Enzym. 115 (2015) 43–50.

[140] Y. Wang, M. Zhao, S. Ou, et al., Partial hydrolysis of soybean oil by phospholipase A1 to produce diacylglycerol-enriched oil, J. Food Lipids 16 (2009) 113–132.

[141] L.M.C. Matos, I.C.R. Leal, R.O.M.A. de Souza, Diacylglycerol synthesis by lipase-catalyzed partial hydrolysis of palm oil under microwave irradiation and continuous flow conditions, J. Mol. Catal. B-Enzym. 72 (2011) 36–39.

[142] K.M. Gonçalves, F.K. Sutili, S.G.F. Leite, et al., Palm oil hydrolysis catalyzed by lipases under ultrasound irradiation–the use of experimental design as a tool for variables evaluation, Ultrason. Sonochem. 19 (2012) 232–236.

[143] Y. Xu, S. Guo, W. Wang, et al., Enzymatic hydrolysis of palm stearin to produce diacylglycerol with a highly thermostable lipase, Eur. J. Lipid Sci.Tech. 115 (2013) 564–570.

[144] E.T. Phuah, Y.Y. Lee, T.K. Tang, et al., Modeling and optimization of lipase-catalyzed partial hydrolysis for diacylglycerol production in packed bed reactor, Int. J. Food Eng. 12 (2016) 681–689.

[145] P. Zheng, Y. Xu, W. Wang, et al., Production of diacylglycerol-mixture of regioisomers with high purity by two-step enzymatic reactions combined with molecular distillation, J. Am. Oil Chem. Soc. 91 (2014) 251–259.

[146] M. del Mar Muñío, L. Esteban, A. Robles, et al., Synthesis of 2-monoacylglycerols rich in polyunsaturated fatty acids by ethanolysis of fish oil catalyzed by 1, 3 specific lipases, Process Biochem. 43 (2008) 1033–1039.

[147] H.J. Lee, M. Haq, P.S. Saravana, et al., Omega-3 fatty acids concentrate production by enzyme-catalyzed ethanolysis of supercritical CO₂ extracted oyster oil, Biotechnol. Bioprocess Eng. 22 (2017) 518–528.

[148] Y. Zhang, X. Wang, D. Xie, et al., Synthesis and concentration of 2-monoacyl-glycerols rich in polyunsaturated fatty acids, Food Chem. 250 (2018) 60–66.

[149] Y. He, J. Li, S. Kodali, et al., Rationale behind the near-ideal catalysis of *Candida antarctica* lipase A (CAL-A) for highly concentrating ω-3 polyunsaturated fatty acids into monoacylglycerols, Food Chem. 219 (2017) 230–239.

[150] M. Haq, P. Pendleton, B.S. Chun, Utilization of Atlantic salmon by-product oil for omega-3 fatty acids rich 2-monoacylglycerol production: optimization of enzymatic reaction parameters, Waste Biomass Valori. 11 (2020) 153–163.

[151] Y. He, J. Li, S. Kodali, et al., The near-ideal catalytic property of *Candida antarctica* lipase A to highly concentrate n-3 polyunsaturated fatty acids in monoacylglycerols via one-step ethanolysis of triacylglycerols, Bioresour. Technol. 219 (2016) 466–478.

[152] Y. Zhang, X. Wang, S. Zou, et al., Synthesis of 2-docosahexaenoylglycerol by enzymatic ethanolysis, Bioresour. Technol. 251 (2018) 334–340.

[153] L. Esteban, M. del Mar Muñío, A. Robles, et al., Synthesis of 2-monoacylglycerols (2-MAG) by enzymatic alcoholysis of fish oils using different reactor types, Biochem. Eng. J. 44 (2009) 271–279.

[154] H. Ghamgui, N. Miled, A. Rebaï, et al., Production of mono-olein by immobilized *Staphylococcus simulans* lipase in a solvent-free system: optimization by response surface methodology, Enzym. Microb. Technol. 39 (2006) 717–723.

[155] N. Weber, K.D. Mukherjee, Solvent-free lipase-catalyzed preparation of diacylglycerols, J. Agric. Food Chem. 52 (2004) 5347–5353.

[156] M. Tüter, B. Babalı, Ö. Köse, et al., Solvent-free glycerolysis of palm and palm kernel oils catalyzed by a 1,3-specific lipase and fatty acid composition of glycerolysis products, Biotechnol. Lett. 21 (1999) 245–248.

[157] M.A.P. Langone, M.E. De Abreu, M.J.C. Rezende, et al., Enzymatic synthesis of medium chain monoglycerides in a solvent-free system, Biotechnol. Appl. Biochem. 987-996 (2002).

[158] J.A. Arcos, C. Otero, Enzyme, medium, and reaction engineering to design a low-cost, selective production method for mono- and dioleoylglycerols, J. Am. Oil Chem. Soc. 73 (1996) 673–682.

[159] P.B.L. Fregolente, G.M.F. Pinto, M.R. Wolf-Maciel, et al., Monoglyceride and diglyceride production through lipase-catalyzed glycerolysis and molecular distillation, Appl. Biochem. Biotechnol. 160 (2010) 1879–1887.

[160] T. Yang, M. Rebsdorf, U. Engelrud, et al., Enzymatic production of monoacylglycerols containing polyunsaturated fatty acids through an efficient glycerolysis system, J. Agric. Food Chem. 53 (2005) 1475–1481.

[161] P.B.L. Fregolente, L.V. Fregolente, G.M.F. Pinto, et al., Monoglycerides and diglycerides synthesis in a solvent-free system by lipase-catalyzed glycerolysis, in: Biotechnology for Fuels and Chemicals: Proceedings of the Twenty-Ninth Symposium on Biotechnology for Fuels and Chemicals Held, Humana Press, in Denver, 2007, pp. 285–292.

[162] S.R. Bansode, V.K. Rathod, Ultrasound assisted lipase catalysed synthesis of isoamyl butyrate, Process Biochem. 49 (2014) 1297–1303.

[163] S. Ferreira-Dias, A.C. Correia, F.O. Baptista, et al., Contribution of response surface design to the development of glycerolysis systems catalyzed by commercial immobilized lipases, J. Mol. Catal. 11 (2001) 699–711.

[164] W. Chen, M. Kou, S. Lin, et al., Effects of solvents on the glycerolysis performance of the SBA-15 supported lipases, J. Oleo. Sci. 70 (2021) 385–395.

[165] L. Koop, L.I. Soares, F. Augusto Pedersen Voll, et al., Dynamic optimization for the enzymatic production of acylglycerols, Chem. Eng. Commun. 207 (2020) 93–108.

[166] M.M.C. Feltes, J. Vladimir Oliveira, H. Treichel, et al., Assessment of process parameters on the production of diglycerides rich in omega-3 fatty acids through the enzymatic glycerolysis of fish oil, Eur. Food Res. Technol. 231 (2010) 701–710.

[167] P. Lozano, C. Gomez, S. Nieto, et al., Highly selective biocatalytic synthesis of monoacylglycerides in sponge-like ionic liquids, Green Chem. 19 (2017) 390–396.

[168] N. Satriana, Y. Arpi, Lubis, et al, Diacylglycerol-enriched oil production using chemical glycerolysis, Eur. J. Lipid Sci. Technol. 118 (2016) 1880–1890.

[169] Y. Zheng, J. Quan, X. Ning, et al., Lipase-catalyzed transesterification of soybean oil for biodiesel production in tert-amyl alcohol, World J. Microbiol. Biotechnol. 25 (2009) 41–46.

[170] F. Zeng, B. Yang, Y. Wang, et al., Enzymatic production of monoacylglycerols with camellia oil by the glycerolysis reaction, J. Am. Oil Chem. Soc. 87 (2010) 531–537.

[171] G.A. Kovalenko, L.V. Perminova, A.B. Beklemishev, Catalytic properties of recombinant *Thermomyces lanuginosus* lipase immobilized by impregnation into mesoporous silica in the enzymatic esterification of saturated fatty acids with aliphatic alcohols, React. Kinet. Mech. Catal. 128 (2019) 479–491.

[172] W.S. He, J.J. Li, X.X. Pan, et al., Lipase-mediated synthesis of water-soluble plant stanol derivatives in tert-butanol, Bioresour. Technol. 114 (2012) 1–5.

[173] M.L. Damstrup, T. Jensen, F.V. Sparsø, et al., Solvent optimization for efficient enzymatic monoacylglycerol production based on a glycerolysis reaction, J. Am. Oil Chem. Soc. 82 (2005) 559–564.

[174] T. Yang, M. Rebsdorf, U. Engelrud, et al., Monoacylglycerol synthesis via enzymatic glycerolysis using a simple and efficient reaction system, J. Food Lipids 12 (2005) 299–312.

[175] A.G. Solaesa, M.T. Sanz, M. Falkeborg, et al., Production and concentration of monoacylglycerols rich in omega-3 polyunsaturated fatty acids by enzymatic glycerolysis and molecular distillation, Food Chem. 190 (2016) 960–967.

[176] J. Liu, J. Xu, F. Wang, et al., Studies on fermentation process for lipase production by Candida antarctica, J. Qingdao Univ. Sci. Technol. 26 (2005) 101–105.

[177] H. Keskin, D. Koçak Yanık, H.N. Mucuk, et al., Valorization of olive pomace oil with enzymatic synthesis of 2-monoacylglycerol, J. Food Sci. 81 (2016) C841–C848.

[178] S. Oh, C. Park, Enzymatic production of glycerol acetate from glycerol, Enzym. Microb. Technol. 69 (2015) 19–23.

[179] R.L. Krüger, A. Valério, M. Balen, et al., Improvement of mono and diacylglycerol production via enzymatic glycerolysis in tert-butanol system, Eur. J. Lipid Sci. Technol. 112 (2010) 921–927.

[180] F. Voll, R.L. Krüger, F. de Castilhos, et al., Kinetic modeling of lipase-catalyzed glycerolysis of olive oil, Biochem. Eng. J. 56 (2011) 107–115.

[181] E. Catoni, E. Cernia, C. Palocci, et al., Different aspects of 'solvent engineering' in lipase biocatalysed esterifications, J. Mol. Catal. A Chem. 105 (1996) 79–86.

[182] M.L. Damstrup, J. Abildskov, S. Kiil, et al., Evaluation of binary solvent mixtures for efficient monoacylglycerol production by continuous enzymatic glycerolysis, J. Agric. Food Chem. 54 (2006) 7113–7119.

[183] J. Dupont, R.F. De Souza, P.A.Z. Suarez, Ionic liquid (molten salt) phase organometallic catalysis, Chem. Rev. 102 (2002) 3667–3692.

[184] F. Van Rantwijk, R.A. Sheldon, Biocatalysis in Ionic Liquids, ChemInform 38 (2007).

[185] F. Van Rantwijk, R.A. Sheldon, Biocatalysis in Ionic Liquids, Chem. Rev. 107 (2007) 2757–2785.

[186] Z. Guo, X. Xu, Lipase-catalyzed glycerolysis of fats and oils in ionic liquids: a further study on the reaction system, Green Chem. 8 (2006) 54–62.

[187] Z. Guo, B. Chen, R.L. Murillo, et al., Functional dependency of structures of ionic liquids: do substituents govern the selectivity of enzymatic glycerolysis? Org. Biomol. Chem. 4 (2006) 2772–2776.

[188] D.M. Eike, J.F. Brennecke, E.J. Maginn, et al., Predicting melting points of quaternary ammonium ionic liquids, Green Chem. 5 (2003) 323–328.

[189] J.F. Brennecke, E.J. Maginn, Ionic liquids: innovative fluids for chemical processing, AICHE J. 47 (2001) 2384.

[190] B. Chen, Z. Guo, T. Tan, et al., Structures of ionic liquids dictate the conversion and selectivity of enzymatic glycerolysis: Theoretical characterization by COSMO-RS, Biotechnol. Bioeng. 99 (2008) 18–29.

[191] Z. Guo, X. Xu, New opportunity for enzymatic modification of fats and oils with industrial potentials, Org. Biomol. Chem. 3 (2005) 2615–2619.

[192] M.H. Oh, J.H. So, S.M. Yang, Rheological evidence for the silica-mediated gelation of xanthan gum, J. Colloid Interface Sci. 216 (1999) 320–328.

[193] Z. Guo, D. Kahveci, B. Özçelik, et al., Improving enzymatic production of diglycerides by engineering binary ionic liquid medium system, N. Biotechnol. 26 (2009) 37–43.

[194] Z. Guo, X. Xu, Lipase-catalyzed glycerolysis of fats and oils in ionic liquids: a further study on the reaction system, Green Chem. 8 (2006) 54–62.

[195] A. Coteron, M. Martinez, J. Aracil, Reactions of olive oil and glycerol over immobilized lipases, J. Am. Oil Chem. Soc. 75 (1998) 657–660.

[196] D. Kahveci, Z. Guo, B. Özçelik, et al., Optimisation of enzymatic synthesis of diacylglycerols in binary medium systems containing ionic liquids, Food Chem. 119 (2010) 880–885.

[197] H. Barbero, E. Masson, Design and recognition of cucurbituril-secured platinum-bound oligopeptides, Chem. Sci. 12 (2021) 9962–9968.

[198] Z. Guo, B.M. Lue, K. Thomasen, et al., Predictions of flavonoid solubility in ionic liquids by COSMO-RS: experimental verification, structural elucidation, and solvation characterization, Green Chem. 9 (2007) 1362–1373.

[199] J.S. Wilkes, Properties of ionic liquid solvents for catalysis, J. Mol. Catal. A Chem. 214 (2004) 11–17.

[200] M. Corzo-Martínez, L. Vázquez, P. Arranz-Martínez, et al., Production of a bioactive lipid-based delivery system from ratfish liver oil by enzymatic glycerolysis, Food Bioprod. Process. 100 (2016) 311–322.

[201] S.H. Duarte, G.L. del Peso Hernandez, A. Canet, et al., Enzymatic biodiesel synthesis from yeast oil using immobilized recombinant *Rhizopus oryzae* lipase, Bioresour. Technol. 183 (2015) 175–180.

[202] J.B. Monteiro, M.G. Nascimento, J.L. Ninow, Lipase-catalyzed synthesis of monoacylglycerol in a homogeneous system, Biotechnol. Lett. 25 (2003) 641–644.

[203] S. Miao, X. Li, Enzymatic esterification of lauric acid to give monolaurin in a microreactor, J. Chem. Res. 45 (2021) 660–667.

[204] E.L. Smith, A.P. Abbott, K.S. Ryder, Deep eutectic solvents (DESs) and their applications, Chem. Rev. 114 (2014) 11060–11082.

[205] S. Sun, Y. Lv, G. Wang, Enhanced surfactant production using glycerol-based deep eutectic solvent as a novel reaction medium for enzymatic glycerolysis of soybean oil, Ind. Crop. Prod. 151 (2020), 112470.

[206] J. Jiang, Z. Pu, W. Wang, et al, Method for preparing biodiesel, lauric acid, capric acid and monoglyceride thereof by taking lindera communis fruits as raw materials: CN101353588A, 2009-01-28.

[207] X. Xia, Y. Xia, X. Wen, et al, Method for catalytically synthesizing monoglyceride and diglyceride by using lipase under ultrahigh pressure: CN103074388A, 2013-05-01.

[208] X. Wang, J. Lu, Y. Wang, et al, Method for synthesizing sn-2-monoglyceride by enzyme method: CN105483170A, 2016-04-13.

[209] X. Zhou, H. Qian, J. Qing, et al, Method for synthesizing monoglyceride by enzyme method in ionic liquid system: CN106995827A, 2017-08-01.

[210] Y. Wang, M. Lan, H. Yuan, et al, Method for synthesizing monoglyceride by enzymatic catalysis: CN108531522A, 2018-09-14.

[211] J. Sun, Method for synthesizing monoglyceride by enzyme catalysis: CN110438174A, 2019-11-12.

[212] X. Zhou, High-purity medium-chain monoglyceride and preparation method and application thereof: CN112779298A, 2021-05-11.

[213] X. Wang, Y. Chen, Y. Wang, et al, Method for preparing n-3 polyunsaturated fatty acid glyceride by enzymatic method: CN113981018A, 2022-01-28.

[214] Z. Wang, Z. Guo, G. Yu, et al, Ester exchange synthesis method of diglyceride: CN112375789A, 2021-02-19.

[215] H. Li, W. Bao, X. Wang, Enzymatic production process of diglyceride: CN116042736A, 2023-05-02.

[216] Z. Wang, W. Bao, Y. Wang, et al, Method for preparing n-3polyunsaturated fatty acid diglyceride by enzyme method: CN116179622A, 2023-05-30.

[217] J. Cheng, The research on immobilized lipase preparation DAG technology of rice bran oil [dissertation], Heilongjiang Bayi Agricultural University, 2014.