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# Enzymatic Synthesis of Functional Structured Lipids from Glycerol and Naturally Phenolic Antioxidants

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## Abstract

Glycerol is a valuable by-product in biodiesel production by transesterification, hydrolysis reaction, and soap manufacturing by saponification. The conversion of glycerol into value-added products has attracted growing interest due to the dramatic growth of the biodiesel industry in recent years. Especially, phenolic structured lipids have been widely studied due to their influence on food quality, which have antioxidant properties for the lipid food preservation. Actually, they are triacylglycerols that have been modified with phenolic acids to change their positional distribution in glycerol backbone by enzymatically catalyzed reactions. Due to lipases' fatty acid selectivity and regiospecificity, lipase-catalyzed reactions have been promoted for offering the advantage of greater control over the positional distribution of fatty acids in glycerol backbone. Moreover, microreactors were applied in a wide range of enzymatic applications. Nowadays, phenolic structured lipids have attracted attention for their applications in cosmetic, pharmaceutical, and food industries, which definitely provide attributes that consumers will find valuable. Therefore, it is important that further research be conducted that will allow for better understanding and more control over the various esterification/transesterification processes and reduction in costs associated with large-scale production of the bioconversion of glycerol. The investigated approach is a promising and environmentally safe route for value-added products from glycerol.

**Keywords:** glycerol, phenolic antioxidant, phenolic structured lipids, microreactors, lipase

## 1. Introduction

Glycerol, also known as glycerin or propane-1,2,3-triol, is a chemical which has a multitude of uses in pharmaceutical, cosmetic, and food industries [1]. Currently the modifications of glycerol such as mono-, di-, and triglycerides are representing valuable products, as they have numerous applications such as modifying agents in food and pharmaceutical industries [2]. Especially, the triglycerides, which are also called structured lipids, have been widely concerned by researchers. Structured lipids are tailor-made fats and oils with special metabolic methods by incorporating

new fatty acids or changing the position of existing fatty acids on the glycerol backbone. By adding a special functional group to the glycerol skeleton, a certain functionality of the triglyceride can be imparted which is effective in delivering the desired fatty acids for maintaining healthy nutrition or treating specific diseases [3]. Lipid modification strategies for the production of functional fats and oils include chemically or lipase-catalyzed interesterification, acidolysis reactions, and genetic engineering of oilseed crops. Interesterification is used to produce fats with desirable functional and physical properties for food applications [4]. Since a physical blend of medium-chain triacylglycerols and long-chain triacylglycerols was used, with the medium-chain triacylglycerols being readily metabolized for quick energy [5], structured lipids were designed to provide simultaneous delivery of beneficial long-chain fatty acids at a slower rate and medium-chain fatty acids at a quicker rate [6]. Further, SL synthesis yields novel triacylglycerol (TAG) molecules and its derivatives, such as human milk fat substitutes (HMFS), which is used in infant formula to mimic the human milk fat. Although fat accounts for only 3–5% of human milk (TAGs > 98%), it provides more than half of the energy for the growth and development of infants [7]. In addition, adding 1,3-dioleoyl-2-palmitoylglycerol (OPO) into infant formula could improve the calcium deficiency, constipation, and even bowel infarction [8]. Moreover, many phenolic lipids have also been produced, such as cocoa butter equivalents, low calorie oil, acute energy supply, and structured phospholipids. Thus, numerous structured lipids were generated from glycerol which provided important environmental benefits to the new platform products.

Phenolic acids are natural antioxidants accounting for approximately one third of the phenolic compounds in our daily diet which are widely distributed in some agricultural products, beverages, and Chinese medicinal herbs [9]. Phenolic acids and its derivatives are used in several applications in food, pharmaceutical, and cosmetic industries due to their antioxidant effects [10]. Many synthetic phenolic acids have been used as antioxidants to control lipid oxidation in lipid-based foods, and synthetic phenolics such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), and tertiary butyl hydroquinone (TBHQ) are the commercially available antioxidants for many years. However, they have potential carcinogenic effects and toxicity. This has increased the utilization of natural phenolic acids to reduce the oxidation and render the health benefits [11, 12]. Nevertheless, the hydrophilic character of phenolic acids limits their effectiveness in stabilizing fats and oils as well as the applicability in food, cosmetics, and other fields. As a result of the hydrophilic nature, natural phenolic acids have been lipophilized to obtain modified amphiphilic molecules to increase their potential as antioxidants in oil-based products [13, 14]. Several reports employing chemical and enzymatic methods have been published where a number of phenolics were incorporated into different oils to lipophilize the phenolics [12, 15, 16]. With the increased interest in the phenolic lipid research, a novel phenolic lipid produced from glycerol is being pursued with improved antioxidant and biological activities.

Glycerol is a nontoxic, edible, and biodegradable compound and has over 2000 different applications [17], especially in pharmaceuticals, personal care, foods, and cosmetics. With a focus on recent developments in the conversion of glycerol into value-added chemicals, phenolic compounds conjugated with structured lipids would be interesting to study, which can expand the application of the phenolic acids and make full use of glycerol. Effective methods have been applied to produce modified phenolic acid compounds presently by lipase-catalyzed transesterification reactions in batch reactors. However, high enzyme amount, high reaction temperature, long reaction times, and low conversions often occurred. Furthermore, high vacuum was usually indispensable to remove the by-product including ethanol or water during the whole reaction course [18]. Recently, the concept of “miniaturizing

biocatalysis" (i.e., microfluidic biocatalysis) was proposed and recognized as one of the priority development directions in the field of chemical engineering. Due to its excellent mass transfer characteristic, the reaction process has been accelerated, which has attracted extensive attention. Therefore, new reaction systems as well as novel lipases with better catalytic properties should be explored.

This chapter covers a broad range of information concerning the production and applications of phenolic structured lipids produced from glycerol, including natural phenolic acids, production strategies, food and medical applications, and future prospects for research and development in this field.

## 2. Phenolic acid and its derivatives

In the traditional Chinese medicine industry, phenolic compounds have been used as antimicrobials, thickeners, and flavoring agents [19] or to maintain the color of red meats. In addition, they constitute potent preservatives as of their antioxidant activity [20]. In this regard, the structured lipids integrated with phenolics can help enhance their biological properties. Thus, the source and biological activities of phenolic acids will be covered in this sector.

### 2.1 Chemical structure

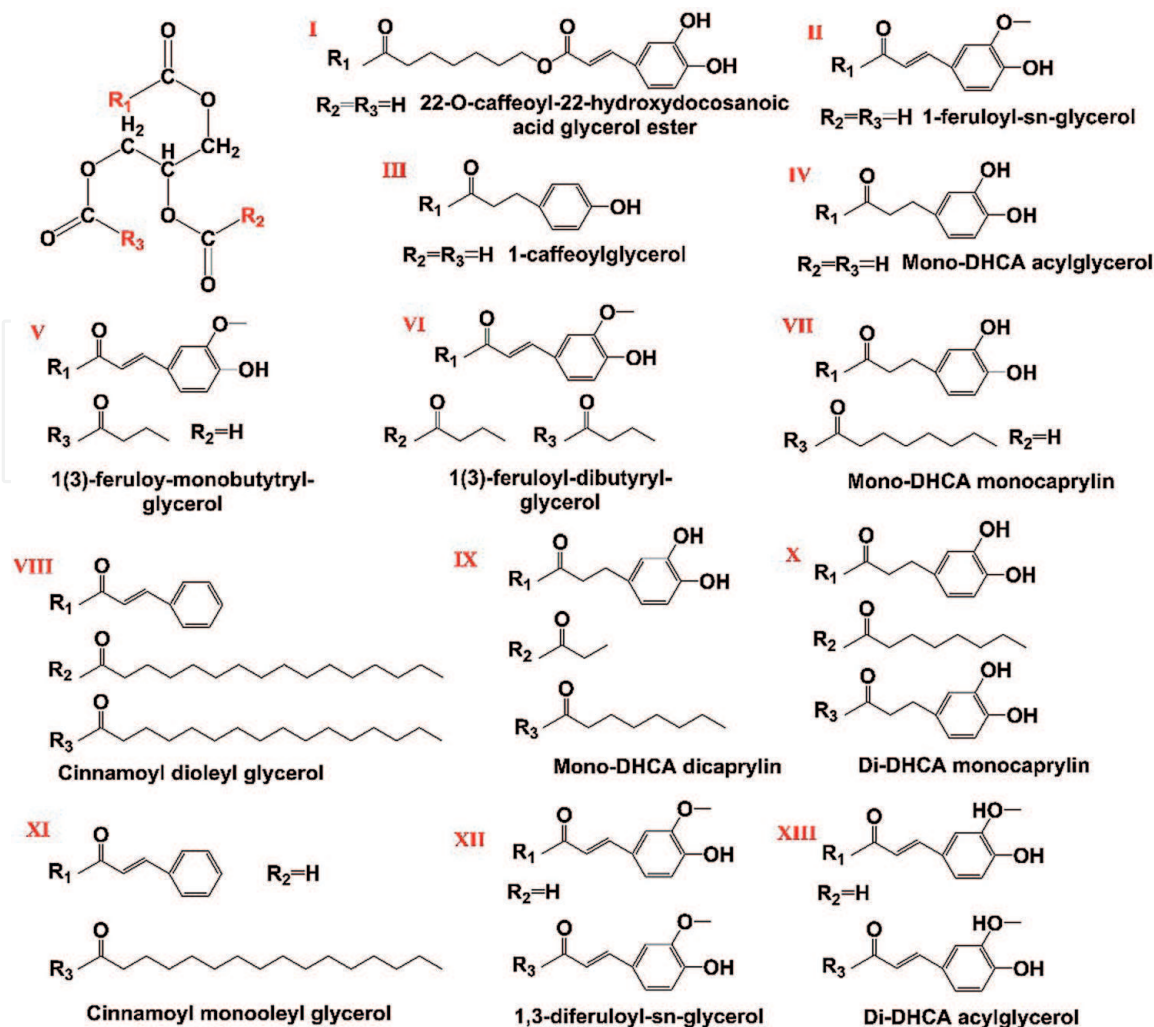
Natural phenolic acids are widely found in many medicinal plants, such as honeysuckle, pallet root, dandelion, angelica, and breviscapine of the honeysuckle family. **Figure 1** shows the structures of the reported phenolic lipids. **Table 1** shows the various phenolic structured lipids with mono- and bis-phenacyl groups.

Phenolic acid compounds are mainly composed of derivatives with benzoic acid ( $C_6-C_1$ ), phenylacetic acid ( $C_6-C_2$ ), and cinnamic acid ( $C_6-C_3$ ) as the parent nucleus and can be divided into two categories: simple phenolic acid compounds and polyphenols, including vanillic acid, *p*-hydroxybenzoic acid, salicylic acid, gallic acid, cinnamic acid, *p*-coumaric acid, ferulic acid, 3,4-dihydroxyphenylacetic acid, and protocatechuic acid [21]. Phenolic acids are resistant to oxidation mainly due to the conjugated action of the phenolic core structure and the side chain, which can easily form a stable conjugated structure [22]. Low-molecular-weight phenolic acid antioxidants mainly include (1)  $C_6-C_1$ -type of protocatechuic acid and ferulic acid, (2)  $C_6-C_2$  3-hydroxyphenylacetic acid, and (3)  $C_6-C_3$  tanshinu, caffeic acid, ferulic acid, *p*-coumaric acid, and erucic acid. Phenolic acid derivatives are also potential resources of natural medicines, which served as lead compounds for drug synthesis. Because the most important active site is *o*-hydroxyl group on the benzene ring, the structural modification of phenolic acids mainly focuses on the reaction of carboxyl groups, including the synthesis of amides, amine salts, and various ester compounds [23].

### 2.2 Prepare methods

Phenolic acids are abundant in the biomass feedstock that can be derived from the processing of lignin or other by-products from agro-industrial waste. Phenolic acid can be used directly in various applications, and their value can be significantly increased when they are further modified to high value-added compounds. Enzymatic reactions including esterification and decarboxylation are important for conversion of phenolic acids, which are stable and clean without toxic waste compared to the chemical methods. The products are useful for the pharmaceutical, cosmetic, food, fragrance, and polymer industries.





**Figure 1.**  
The structures of the reported phenolic lipids.

### 2.2.1 Extraction and separation

Phenolic acids are compounds that can be derived from plant biomass materials. They are the major components of lignin in lignocellulose. Depolymerization of kraft lignin using chemical, physicochemical, and biological processes can liberate substantial amounts of phenolic lipids [21, 24]. In addition to kraft lignin, depolymerization of organosolv lignin (lignin derived from environmentally friendly organic solvent-pretreated plant biomass) and extraction of lignin by aqueous formic acid can also generate many types of high-value phenolic acids [25]. By-products, residues, and wastes from fruit and vegetable industries such as from the fruit-based wine industry have significant amounts of bioactive phenolic acids which exhibit potent antioxidant activities [26]. Recently, it has been shown that derivatives of *p*-coumaric acid can be detected and isolated from palm oil waste and further processed to generate products of higher value [27].

### 2.2.2 Chemical and enzymatic synthesis

Many phenolic acid derivatives can be potentially used as bioactive ingredients in food, cosmetic, pharmaceutical, and perfumery industries, which are from plants such as benzoic acid, salicylic acid, gallic acid, cinnamic acid, *p*-coumaric acid, caffeic acid, and ferulic acid. However, the high polarity of these compounds limits their cellular uptake, which poses a challenge for their applications. The cellular

Type	Products	Phenolic hydroxyl position	Structural formula (Figure 1)	MW	Source	Source material	Ref.
Monophenol acyl	1(3)-Feruloyl-dibutyryl-glycerol	<i>sn</i> -1	VI	407	Enzymatic synthesis	Ethyl ferulate, tributyrin	[28]
	1(3)-Feruloyl-monobutyryl-glycerol	<i>sn</i> -1	V	338	Enzymatic synthesis	Ethyl ferulate, tributyrin	[28]
	1-Feruloyl- <i>sn</i> -glycerol	<i>sn</i> -1	II	265	Isolation	<i>Solanum tuberosum</i> (potato)	[29]
	Cinnamoyl dioleoyl glycerol	<i>sn</i> -1	VIII	698	Enzymatic synthesis	Ethyl cinnamate, triolein	[30]
	22-O-Caffeoyl-22-hydroxydocosanoic acid glycerol ester	<i>sn</i> -1	I	368	Isolation	Yellow cotton fiber pigment	[31]
	1-Caffeoylglycerol	<i>sn</i> -1	III	228	Enzymatic synthesis	Methyl caffeate, glycerol	[32]
	Chlorogenate fatty esters	<i>sn</i> -1	/	14 <i>n</i> + 264	Enzymatic synthesis	5-Caffeoylquinic acid, methyl chlorogenate	[33]
	Mono-DHCA dicaprylin	<i>sn</i> -1	IX	434	Two-step enzymatic synthesis	Octanol, dihydrocaffeic acid, triacylglycerols	[16]
	Mono-DHCA monocaprylin	<i>sn</i> -1	VII	378			
Mono-DHCA acylglycerol	<i>sn</i> -1	IV	252				
Bis-phenacyl	1,3-Diferuloyl- <i>sn</i> -glycerol	<i>sn</i> -1, 3	XII	444	Isolation	<i>Aegilops ovata</i> (wheat)	[34]
	Di-DHCA monocaprylin	<i>sn</i> -1, 3	X	543	Two-step enzymatic synthesis	Octanol, dihydrocaffeic acid, triacylglycerols	[16]
	Di-DHCA acylglycerol	<i>sn</i> -1, 3	XIII	417			

DHCA, dihydrocaffeic acid  
*n* = 3, 7, 11 or 15.

**Table 1.**  
 Various phenolic structured lipids with mono- and bis-phenacyl groups.

permeability of these compounds can be increased by the esterification in order to enhance their lipophilicity. Enzymatic synthesis is the priority for esterifying phenolic lipids compared to the chemical methods using base or acid catalysts with the disadvantages of high production costs. For instance, efficient esterification of BA with heptanol to form heptyl benzoate was conducted with immobilized Novozym 435 [35]. Esterification of salicylic acid with acetic acid can be catalyzed by lipase to synthesize aspirin [36]. Decarboxylation of plant-derived phenolic lipids such as hydroxycinnamic acids is a means for generating vinylic phenol, which can be modified for use in the synthesis of food-grade flavors [37]. Thus, the efficient enzymatic method provides an efficient and sustainable way to prepare specialty chemicals and precursors for the pharmaceutical, food, fragrance, and polymer industries.

### **2.3 Biological activities**

Phenolic acids in food industry have received considerable attention as powerful antioxidants to protect against the oxidative deterioration of such food components as polyunsaturated fatty acids (PUFAs) [38]. It has been proved that synthetic antioxidants have certain safety risks; the research on safe and efficient natural antioxidants has become a hot topic [39]. Additionally, phenolic acid and its derivatives have antiviral, anticancer, antioxidant, anti-inflammatory, antiaging, and other biological activities. This sector will cover the oxidation resistance, anticancer activity, and ultraviolet (UV) damage repair performance.

#### *2.3.1 Oxidation resistance*

At present, the most widely natural antioxidants studied are ferulic acid, cinnamic acid, coffee-acyl quinic acid, *p*-coumaric acid, and caffeoylquinic acid, mainly due to synthetic antioxidants such as butylated hydroxyanisole and butylated hydroxytoluene which may have potential carcinogenicity [40]. With the development of synthesis of novel structural lipids, phenolic compounds have been modified to the triglyceride backbone by chemical or enzymatic methods to improve its solubility in hydrophobic media. The phenolic acid derivative has a dihydroxyphenyl structure and an ortho-phenolic hydroxyl group (1–3) in its molecular structure. The former is a common free radical scavenging structure, and the latter is easily oxidized.

#### *2.3.2 Anticancer activity*

It is found that cinnamic acid derivatives have certain inhibitory effect on the proliferation of cancer cells and have certain application value in the field of anticancer. Cinnamic acid can effectively inhibit the proliferation of A-59 human lung adenocarcinoma cells [41]. Its derivatives play a significant role in inducing apoptosis of human hepatoma cells [42].

#### *2.3.3 Ultraviolet damage repair performance*

In recent years, with the development of industrial production, air pollution is becoming more and more serious, leading to serious damage to the ozone layer, which makes the intensity of ultraviolet radiation gradually increased, threatening the formation of skin diseases. Chemical sunscreens have problems such as poor light stability and oxidative deterioration, which can lead to skin allergies. Therefore, a new safe and efficient UV sunscreen with durable UV damage repair time and mild effect should be developed. It has been reported that ferulic acid protects against

oxidative damage and apoptosis of human keratinocytes (HaCaT cells) induced by UVB, and its mechanism may be involved in the enhancement of the antioxidant activity and reduction of oxygen free radicals [43].

### 3. Enzymatic production

The enzymatic reaction is mild and highly selective, and the process route is simplified, which is the priority for the production of phenolic structured lipids. Phenolic acid can readily be esterified with glycerol skeletons to form different structured lipids via enzymatic reaction, which greatly enhance their antioxidant and functional properties. The specific selectivity of lipase makes it possible to control the position of structural lipid fatty acids. Further, solvent-free bioprocess is the priority for the efficient and environment-friendly synthesis of phenolic structured lipids [44]. This sector will cover the role of lipase, the enzymatic reaction, and the separation and characterization of phenolic structured lipids.

#### 3.1 Lipase

Lipase (EC 3.1.1.3) is a general term for a class of enzymes that catalyze the hydrolysis of glycerides, which is a group of important multifunctional enzymes in the field of lipid biotechnology [45]. The most commonly used lipases in the production of phenolic structured lipids possess position or region specificity; they specifically hydrolyze the ester bonds of the triglyceride *sn*-1 and *sn*-3 and have no effect on the *sn*-2 ester bond due to steric hindrance effects. The specificity of lipase may be due to the source of lipase, the characteristic structure of the substrate, physicochemical factors at the surface, and differences in binding sites of the enzyme [46]. The transesterification of *sn*-1,3-specific lipase-catalyzed oil and triglyceride with high content of *sn*-2 unsaturated fatty acids can reduce oil saturation and increase the level of unsaturated fatty acids [47]. These kinds of lipase are mostly found in microorganisms, such as *Aspergillus niger*, *Rhizopus delemar*, *Mucor miehei*, and *Humicola lanuginosa* [48]. Lipases can be used to catalyze the resolution of optical isomers and the synthesis of chiral compounds due to their stereospecificity, which is a research hotspot in the field of enzyme engineering [49]. In addition to their high specificity and selectivity, they can remain stable under relatively high temperatures and conditions with organic solvents and no coenzymes involved, which would minimize the formation of side products and thus facilitate the subsequent separation and processing of the products [44] (Table 2). Thus, the use of lipases for specific lipid production is a technique that is used for many years with promising results, which allows the industry to meet the changing dietary requirement of consumers [44].

#### 3.2 Reaction type

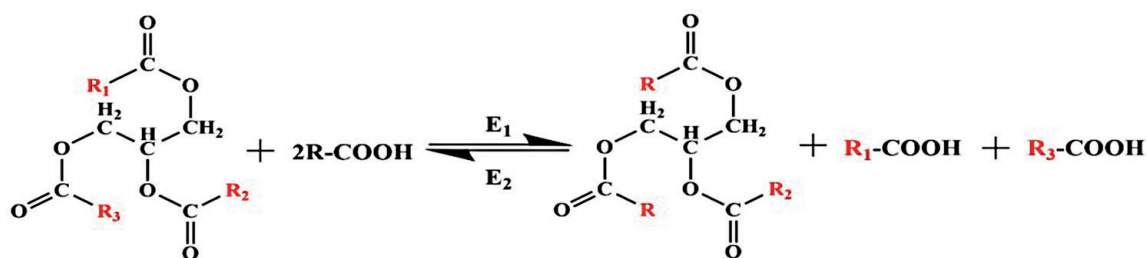
Glycerol serves as the feedstock for the production of phenolic lipids mediated by lipases through transesterification, acidolysis, alcoholysis, and interesterification [49]. Enzymatic esterification is an efficient, green and clean method for esterifying phenolic acid. The esterification of a phenolic acid compound is usually carried out by acylating a carboxyl or a hydroxyl group other than a phenolic hydroxyl one to retain its strong antioxidant capacity and enable the newly formed derivative to have good properties of fat solubility. The method for the production of structured triacylglycerols includes lipase-catalyzed esterification of fatty alcohols and phenolic acids and the transesterification of phenolic acids with acylglycerol models [44]. Figure 2 shows the transesterification of phenolic acids with acylglycerol. It has been



Products	Method	Substrate	Catalysts	Reactor	Reaction medium	Reaction time (h)	Substrate molar ratio	Reaction temperature (°C)	Agitation speed (rpm)	Water activity (a <sub>w</sub> )	Catalyst reuse ability	Refs
1(3)-Feruloyl-dibutyl-glycerol, 1(3)-feruloyl-monobutyl-glycerol	Transesterification	Ethyl ferulate, tributyrin	Novozym 435	Magnetic stirrer	Toluene	120	1:3	50	210	0.23	14	[28]
Oleyl cinnamate	Esterification	Cinnamic acid, oleyl alcohol	Novozym 435	Orbital shaker	Isopropanol/2-butanol	288	1:6	55	150	0.05	/	[50]
Chlorogenate fatty esters	Esterification Transesterification	5-Caffeoylquinic acid, methyl chlorogenate	<i>Candida antarctica</i> lipase B	Orbital shaker	Solvent-free	9	1:1	55	250	0.05	/	[33]
Structured phenolic lipids (cinnamic, ferulic, sinapic and dihydrocaffeic acid)	Transesterification	Flaxseed oil cinnamic, dihydrocaffeic, 3,4-dihydroxyphenylacetic, 3,4-dimethoxybenzoic, ferulic and sinapic acids	Novozym 435	Orbital incubator shaker	Solvent-free	126 240	/	55	150	/	/	[44]
1-Caffeoylglycerol	Transesterification	Alkyl caffeates, glycerol	Novozym 435	Shaken batch	Solvent-free	10	/	75	180	/	/	[51]
1-Feruloyl- <i>sn</i> -glycerol	Esterification	Glycerol + ethyl ferulate	Novozym 435	/	2-Methyl-2-butanol	168	1:1	55	/	/	/	[10]
1,3-Diferuloyl- <i>sn</i> -glycerol	Esterification	4-Hydroxy-3-methoxy cinnamic acid (ethyl ferulate), soybean oil	Novozym 435	Packed-bed column	/	50 g/h	1:5	60	/	/	/	[43]
1,3-Diferuloyl- <i>sn</i> -glycerol	Esterification	4-Hydroxy-3-methoxy cinnamic acid (ethyl ferulate), soybean oil	Novozym 435	Shaken batch	Solvent-free	144	5:9	60	125	/	/	[43]

Products	Method	Substrate	Catalysts	Reactor	Reaction medium	Reaction time (h)	Substrate molar ratio	Reaction temperature (°C)	Agitation speed (rpm)	Water activity (a <sub>w</sub> )	Catalyst reuse ability	Refs
Cinnamoyl monooleyl glycerol, cinnamoyl dioleoyl glycerol	Transesterification	Ethyl cinnamate, triolein	<i>Proteus vulgaris</i> K80 lipase (immobilized)	Incubator	<i>n</i> -Hexane/toluene (85:15, v/v)	72	1:6	35	210	/	/	[30]
Caffeoyl monoacylglycerols, caffeoyl diacylglycerols	Transesterification	Ethyl caffeate, castor oil	Novozym 435	Water baths with magnetic stirrers under 10 mmHg vacuum pressure	Solvent-free	46.5	1:3	90	/	/	/	[52]
Glyceryl monocaffeate	Esterification	Caffeic acid, glycerol	[BSO <sub>3</sub> HMIM] TS	Oil bath	/	2	1:10	90	250	/	/	[53]
1-Caffeoylglycerol	Transesterification	Methyl caffeate, glycerol	Novozym 435	Microreactor	Chloride-urea	/	/	65	/	/	20	[32]

**Table 2.**  
*Enzymatic reactions for the production of phenolic structured lipids.*



**Figure 2.**

The transesterification of phenolic acids with acylglycerol (R<sub>1</sub>, R<sub>2</sub>, R<sub>3</sub>, and R stand for the different fatty acids, respectively. E<sub>1</sub> and E<sub>2</sub> present the condition of the transesterification including the enzyme, temperature, and other reactions).

reported that ethyl ferulate can be transesterified with soybean oil to synthesize mono-ferulic acid triglyceride and di-ferulic acid triglyceride [54]. Kunduru et al. performed the chemo-enzymatic synthesis of four structured triacylglycerol bearing ferulic acids as a phenolic acid at *sn*-1,3 position and found the antioxidant potency of the phenolic structured lipids measured by the Rancimat method improved compared to ferulic acid [14]. Eliza et al. incorporated ascorbic into canola acylglycerols by enzymatic transesterification, significantly improving storage and frying performance compared to the control canola oil, which offered nutraceutical ingredients for food formulation [55]. Because of the extensive activities of phenolic acid, their incorporation into triacylglycerols could potentially result in novel structured phenolic lipids having the benefits of both functional and antioxidative properties [28].

### 3.3 Reaction conditions

The organic solvent system was commonly used for the enzymatic production of phenolic lipids. Nevertheless, the use of some organic solvents may limit the acceptability of nutraceuticals and food ingredients as well as the low volumetric productivity [56]. One of the most promising novel approaches consists of using solvent-free system (SFS), which may allow the use of a smaller reaction volume and higher substrate concentrations and avoid the process of solvent recovery [57]. Feruloylated structured lipids were produced by enzymatic transesterification in solvent-free system, which achieved relatively high conversion of ethyl ferulate, reaching  $98.3 \pm 1.1\%$  [15, 58]. To further improve the biological activity of phenolic lipids, ionic liquids (ILs) was also used in the enzymatic transesterification of ethyl ferulate with castor oil [59]. Castor oil-based caffeoyl structured lipids was successfully prepared which combined beneficial properties of both castor oil and caffeic acid [52]. Some other novel phenolic lipids were also produced with potential nutritional and functional benefits. Selected phenolic structured lipid synthesis was catalyzed by lipase via transesterification of 3,4-dihydroxyphenylacetic acid (DHPA) with flaxseed oil in solvent-free system. The reaction led to a significant increase in the relative proportion of linolenic acid (C<sub>18</sub>:3  $\omega$ -3) that is good for human health [60]. An efficient and solvent-free bioprocess for the synthesis of a phenolic ester of docosahexaenoic acid (DHA) was developed to expand its stability as the functional food ingredient [61].

Some other reaction variables such as reaction temperature and substrate ratio also play a crucial role in the phenolic lipid production. Temperature mainly affects the activity of lipase and the mass transfer rate in the enzymatic esterification. For instance, in the synthesis of caffeoyl structured lipids by enzymatic transesterification using monooleate as caffeoyl acceptors, with the increase of reaction temperature from 50 to 70°C, ethyl caffeate conversion reached  $97.5 \pm 1.9\%$  at 70°C. Moreover, high temperatures resulted in the decrease of the reaction system

viscosity, which favored the enzymatic synthesis [62]. However, temperatures above 100°C usually cause the enzyme deactivation. Similar effects of higher reaction temperature on enzyme activity were also found in other reports [15, 52]. Substrate ratio also has an impact on the enzyme activity of lipase. The high molar ratio of castor oil to ethyl ferulate from 1:1 to 1:5 led to the concomitant decrease of ethyl ferulate conversion. The reason was probably that excessive ethyl ferulate inhibits the enzyme by acidifying microaqueous phase surrounding the lipase [15, 63]. The production of such structured triacylglycerols, possessing various enrichment levels of selected fatty acids, has become an area of great interest because of their potential nutritional and functional benefits [64]; the enhancement of the solubility and miscibility properties of these novel biomolecules could increase their usefulness compared to their corresponding hydrophilic phenolic acids.

### 3.4 Separation and characterization

The separation and purification of phenolic lipids are commonly conducted by HPLC and thin-layer chromatography (TLC), further identified by FT-IR, GC-MS, atmospheric pressure chemical ionization-mass spectrometry (APCI-MS), and NMR quantitative analysis. In the lipase-catalyzed acidolysis of flaxseed oil with selected phenolic acids, the reaction components were monitored by HPLC. Cinnamic, 3,4-dihydroxyphenylacetic, and *p*-coumaric acids were monitored at 235 and 280 nm, which showed a UV-spectral scanning profile different from other phenolic acid components [65]. The transesterification reaction mixture of fish liver oil with dihydrocaffeic acid (DHCA) was analyzed qualitatively by TLC on silica gel 60 plates. The bands corresponding to the phenolic mono- and diacylglycerols visible under UV (265 nm) were recovered and separated. APCI-MS in the positive-ion mode was used to characterize the molecular structure of phenolic lipids for further analyses of the eluting peaks by HPLC [13]. The selectivity of *Candida antarctica* lipase B (CALB) was verified by elucidating the structure of the purified ester by NMR, indicating that the polyunsaturated chain was grafted on the vanillyl alcohol primary hydroxyl group, whereas the phenolic hydroxyl group remained unaffected [61].

## 4. Microreactor

Structured phenolic lipids were usually achieved through enzymatic derivatization by esterifying the carboxylic acid group with long-chain alcohols or glycerol, which could obtain an amphiphilic molecule without losing its original functional properties. Traditional methods for transesterification are simple and convenient to operate and widely used for industrial production. However, violent shaking of the mixture in the batch reactor may lead to the crack and collapse of lipase which could obviously reduce activity of lipase [66]. In addition, it is time-consuming which leads to oxidative deterioration of oil and limits the commercialization of products. Considering the violent collapse of enormous bubbles simultaneously, tremendous generation of heat and pressure occurs, which could be helpful to remove the by-product like ethanol without vacuum [67]. Thus, microreactor technology has been proposed to be beneficial for the effective production of phenolic structured lipids.

Microreactors are recognized as powerful tools for chemical synthesis. The specific surface area of microreactor is much larger than that of conventional reactor, which possesses strong heat exchange capacity and fast mass transfer rate. Moreover, the reaction time was greatly reduced, and the products and substrates can be easily separated. Therefore, microreactors are suitable for reactions with a severe reaction process or a high-temperature requirement.



## 4.1 Reactor type and characteristics

Propyl caffeate was achieved in the microreactor with 1-heptyl-methylimidazolium bis(trifluoromethylsulfonyl)imide [ $C_7\text{mim}$ ] [ $\text{Tf}_2\text{N}$ ] as a cosolvent. The yield of 99.50% was achieved, while the yield in the conventional reactor was 98.50%, and the reaction time was 9/10 shorter than that of the conventional reactor (24 h) [68]. Moreover, human milk fat-style structured triacylglycerols were produced from microalgal oil in a continuous microfluidic reactor packed with immobilized lipase, which obtained high conversion efficiency with reaction time being reduced by eight times [63]. The packed bed reactor was built in a stainless steel plate with lipozyme RM IM used as a biocatalyst, and *n*-hexane was employed as the medium (Figure 3). Further, the packed bed reactor was designed with polydimethylsiloxane which has a good chemical inertness with dimension  $10 \times 0.9 \times 75$  mm ( $W \times H \times L$ ), respectively. One inlet and one outlet were set in both ends of the microreactor to ensure the substrate was fed from one inlet and flow out of the reactor from an outlet which was on the other side of the reactor. The bottom of the groove was packed with Novozym 435; the homogeneous solutions of methyl caffeate, glycerin, and cosolvent were pumped into the reactor for the synthesis of 1-caffeoylglycerol (1-CG) [32]. Due to the advantages of strong heat exchange capacity and fast mass transfer rate in microreactors, the high yield of the 1-caffeoylglycerol was achieved with high reaction rate, low energy consumption, and short time and continuous production. Thus, microreactors represent a convenient and cost-saving method to produce phenolic lipids using microfluidic biocatalysis.

## 4.2 Reaction conditions

The reaction in microreactors is commonly affected by flow rate, temperature, and substrate concentration. With lower flow rate, the substrate will be fully mixed and collided with the enzyme which makes the substrate contact with the active site of the lipase sufficient, and a high yield of 1-caffeoylglycerol (1-CG) was achieved [32]. The viscosity at a low temperature is very high, due to the high boiling point of glycerin. It

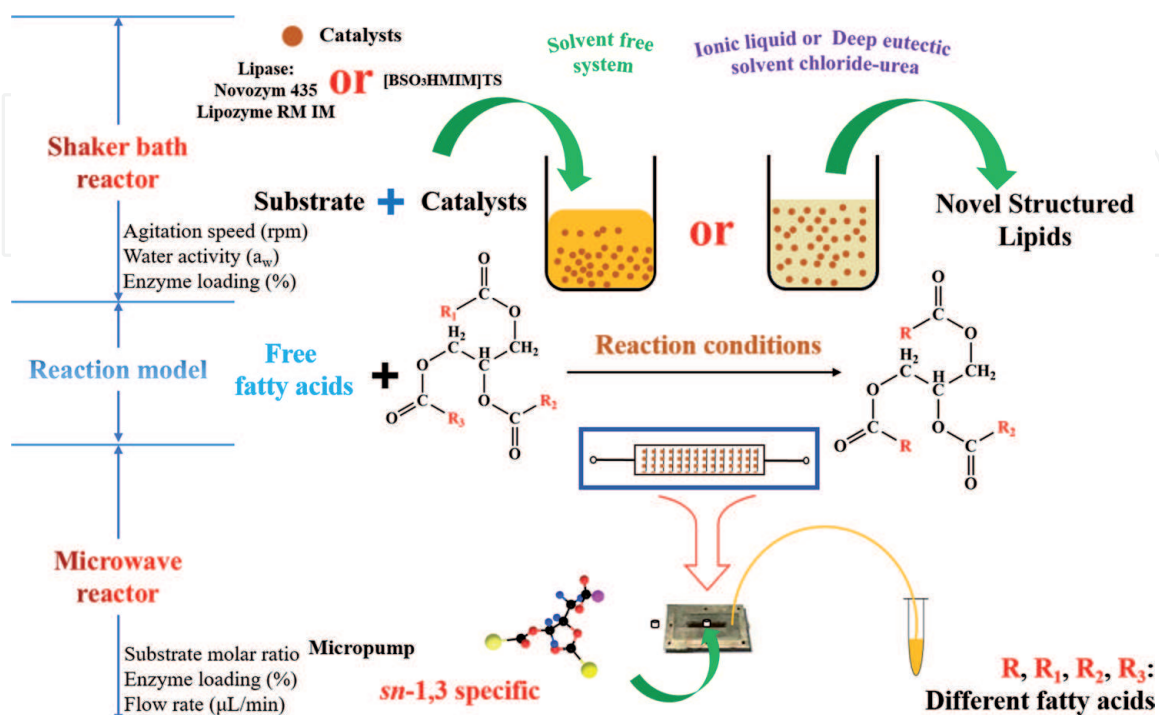


Figure 3. Synthesis of human milk fat-style structured lipids in a continuous microfluidic reactor.

is important to reduce the viscosity of the system and increase the mass transfer rate by raising the temperature in microreactors. Substrate concentration is expected to effect the incorporation of glycerol ester with fatty acid. Even though higher substrate concentrations can promote more incorporation of polyunsaturated fatty acids (PUFAs) to triacylglycerols at the initial reaction, the high level molar ratios of substrate may inhibit lipase activity and also complicate the downstream purification [69].

### 4.3 Kinetic analysis

Enzyme kinetics is an important means to evaluate different reactor performances. Kinetic modeling plays a role as an engineering practice in accelerating enzymatic reactions which indicates the behavior of substrate and enzyme [70]. In addition, enzyme kinetic modeling can explore pathways and reaction mechanisms of complex macromolecular substrates using many parameters prior to developing innovative process to ensure stability and desired efficiency [71]. Herein, the enzyme kinetic modeling was used to evaluate catalytic efficiency of enzyme and mass transfer in microreactors. In our previous study, the value of kinetic parameters ( $K_{m(\text{app})}$ ) which is relative to the flow rate was calculated for the aim of kinetic study in continuous-flow microreactors [72]. The effect of flow rate on enzyme kinetics is usually investigated using the Lilly-Hornby model [73]. In the production of human milk fat-style structured triglycerides, at the lower flow rate, a lower  $K_{m(\text{app})}$  value was obtained, which shows that the acyl donors and enzyme were in sufficient contact with one another [63]. In the enzymatic synthesis of 1-caffeoylglycerol (1-CG), ping-pong bi-bi model was used for the kinetic analysis. To further reveal the effect of internal mass on the reaction, the modeling of fluid flow in microchannels can be achieved by using a formulation in which a common flow field is shared by all of the phases. Numerical simulation liquid flow was applied in the microreactor with the COMSOL Reaction Engineering Lab 3.5a [32]. In summary, kinetic approach can analyze reaction parameters and explain the high efficiency of microreactor.

Thus, the microfluidic technology is promising for modified functional lipid production. However, there are still few reports about microfluidic bioconversion technology employed in the phenolic structured lipid production. More research on phenolic structured lipid production involving microfluidic technology stays explored to provide a cost-effective approach for producing high-value coproducts.

## 5. Applications

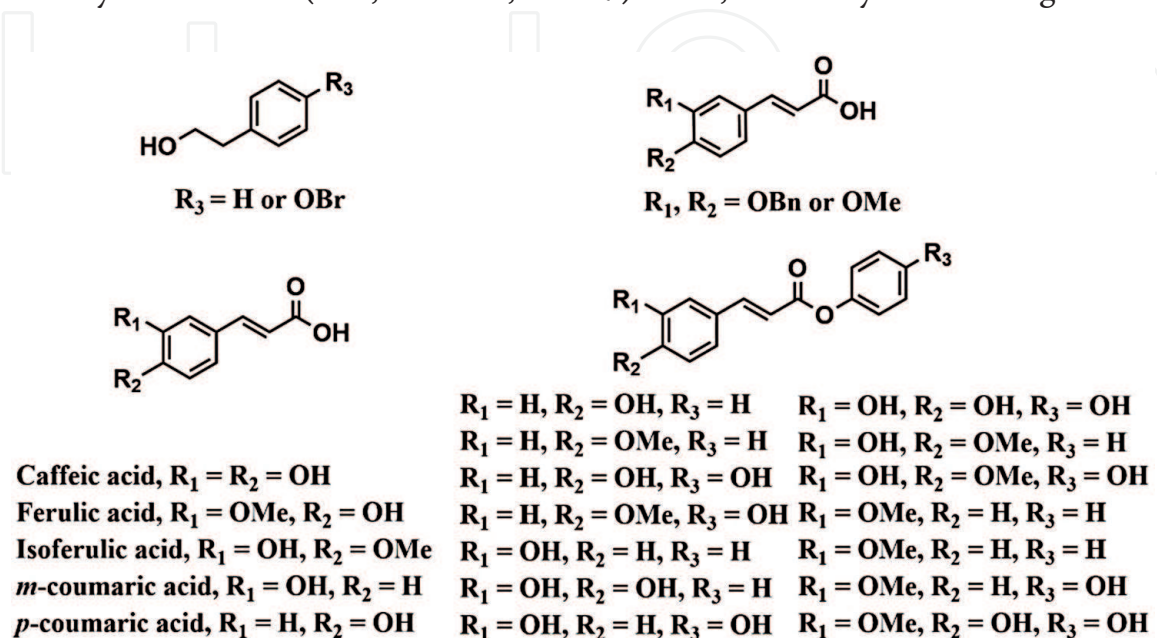
Glycerol is emerging as a versatile bio-feedstock for the production of a variety of chemicals, polymers, and fuels. New catalytic conversions of glycerol have been applied for the synthesis of products whose use ranges from everyday life to the fine-chemical industry. In addition, phenolic acids show a great potential ability of antitumor, antioxidant, antibacterial, and anti-ultraviolet activity due to the unique structure. Thus, in order to utilize potential ability of phenolic acids to meet the demand of cancer treatment and food antioxidants, artificial transformation of its structure using glycerol as feedstock is daily crucial and has been the mainstream research direction in recent years. However, there are few reports on application of the structure triglycerides containing phenolic acids; recent studies have focused on the antioxidant and anticancer aspects of mofetil. The research about repair ability of structure triglycerides on UV damage of cells has also been a hot topic. Therefore, this sector will focus on the application of phenolic acid structural lipid on the antioxidant and anticancer aspects of mofetil as well as the ultraviolet damage repair performance.

## 5.1 Antioxidants

Antioxidant capacity of phenolic acids ester is usually evaluated in the following three ways, such as 2,2-diphenyl-1-picrylhydrazyl free radical (DPPH) scavenging, antioxidant potency in lipid matrix using Rancimat, and the rate of inhibition of autoxidation of linoleic acid in micelles. Four compounds of structured phenolic lipids of varying chain lengths were synthesized to evaluate their antioxidant ability [14]. It was found that after the combination of ferulic acid and glyceride, the antioxidant capacity of ferulic acid glyceride was significantly improved. With ferulic acid as reference, the oxidation time was increased from 12.9 to 15.05 h. In addition, sinapic acid, which was considered as one of the dietary phenolic acids, also is evaluated in the study of Gaspar et al. Alkyl ester sinapates (linear alkyl esters) present almost the same antioxidant activity, albeit slightly lower, compared with the parent compound (sinapic acid) [74]. It was also found that the addition of an alkyl ester side chain shows positive effect on the utilization as an antioxidant in a more lipophilic medium via improving the partition coefficient. Furthermore, ester derivatives of ferulic acid also show the superior antioxidant to the parent ferulic acid [75]. In our previous study, we have synthesized 1-caffeoylglycerol (1-CG), and the ability of 1-CG to scavenge DPPH free radicals and repair UV damage of HaCaT cell was studied, which showed the most effective antioxidant function on DPPH and repair function on UV damage of HaCaT cells compared to methyl caffeate and caffeic acid. Thus, the combining phenolic acid with triglycerides helps phenolic acids to exert antioxidant functions in fat-soluble foods and pharmaceuticals.

## 5.2 Anticancer agents

Phenolic acids have been reported to have the ability of anticancer via inhibiting the growth of tumor cell or selecting inducers of cell death. The gray GM(0, N) approach was employed to analyze the structure activity relationship of phenolic acid phenethyl esters on oral and human breast cancers [76]. **Figure 4** shows the chemical structures of phenolic acid phenethyl esters, and among that structure  $R_1$  is the most important functional group which has a great influence on the cytotoxicity to tumor cell (SAS, OEC-M1, MCF-7). Thus, the ability of inhibiting the



**Figure 4.**  
Chemical structures of phenolic acid phenethyl esters.

growth of the tumor cell could be influenced by the variable of phenolic acid ester. In addition, phenolic acid ester can also induce the death of the cancer cell. It is reported that 13-D have the ability of selectively inducing apoptosis in white blood cancers [77]. These phenolic acid esters not only have selective osmotic effects but also block the cell cycle, and its target compounds are localized in the nucleus and cytoplasm. Furthermore, it is reported that the viability of Detroit 562 cells could be significantly influenced by caffeic acid phenethyl ester [77]. Thus, the phenolic acid ester presented the capability of inhibiting the growth and inducing the apoptotic response of cancer cells.

## **6. Conclusions**

New applications of glycerol as a low-cost feedstock for functional structured lipids have been found, which indicated converting glycerol into commercially valued products. In order to utilize potential ability of phenolic acids, they have been added to structured lipids produced from glycerol to impart even more functionality. Enzymatic esterification reaction was mostly used to enhance the liposolubility of phenolic acids. Microfluidic technology has also been an effective tool for the phenolic lipid production. Whether it is through improvement in functionality or physical properties of a food or the medicinal properties, phenolic structured lipids definitely provide attributes that consumers will find valuable. Therefore, it is important that further research is conducted that will allow for better understanding and more control over the various esterification processes and reduction in costs associated with large-scale production of phenolic structured lipids.

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## **Conflict of interest**

The authors have declared no conflicts of interest.



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