CHAPTER 1

INTRODUCTION

Introduction

Sugar esters (SE) or fatty acid sugar esters (FASE) are the ester of sugar or polyols with fatty acids. Formation of the ester bond is commonly called acylation. SE are non-ionic surfactants which have very good emulsifying, stabilizing or conditioning effects. Moreover, they are non-toxic, non-skin irritants, odorless, tasteless and giving normal food products after digestion (Maag *et al*., 1984). For theses reasons, they are used in many areas, environmental detoxification process, pharmaceuticals, detergents, cosmetics, agricultural and food industries (Ducret *et al*., 1995). For example, instance sucrose laurates with different degree of esterifications are currently being used in food and personal care formulation. In addition, sucrose laurates have anti-tumor and insecticidal properties thus indicating their great versatility (Bronscheuer, 2000).

There are two methods to synthesize SE. The chemical esterification method bases on high temperature and using alkaline catalysts. High temperature causes coloration of final products and cyclization in case of sugar alcohols (Ducret *et al*., 1995). Moreover, the catalysts (lead oxide and stannous soap) are toxic and noncompatible in food application (Akoh *et al*., 1994). Alternatively, SE can be synthesized enzymatically in organic solvent using lipases e.g. *Candida rugosa* lipase (Janssen *et al*., 1990), *Rhizomucor miehei* lipase (Schotterbeck *et al*., 1993), *Candida antarctica* lipase B (Arcos *et al*., 1998; Degn and Zimmermann, 2001 and Sakaki *et al*., 2006) or proteases e.g. α-chymotrypsin (Cameleyre *et al*., 1997) and subtilisin (Sin *et al*., 1998). Lipases and proteases can catalyze ester synthesis or the reverse of the hydrolysis reaction which occurs in very low water content conditions or in organic solvents. The yield of reversed hydrolysis reactions in organic solvents is strongly affected by type of solvent used, water content of reaction mixture, stability of the enzyme in the solvent and solubility of the substrate (Degn, 2000).

The oil palm (*Elaeis guineensis* Jacq.) is one of the important sources of edible oil. It is an economic plant grown widely in Southern Thailand. Palm oil is used mainly by food industry as an ingredient in formulated products, such as margarines or shortenings or directly used in food processing as cooking or frying fat. In the refining process of crude palm oil, palm fatty acid palm fatty acid distillates (PFAD) is main by-product, which is used as raw materials

for soap and candle manufacture (Rakmi *et al*., 1997). Although, PFAD is widely used in many industries but the product value is so low. Production of SE from PFAD is an approach, which possible increases high value of PFAD. The aim of this research is to investigate the synthesis of SE from PFAD and palm oil by immobilized lipases. Moreover, the screening of the microbial lipase and application for SE and fatty acid methyl esters (FAME) synthesis are also study.

Review of Literatures

1. Lipases

Lipases (triacylglycerol hydrolases, EC 3.1.1.3) are the hydrolytic enzymes that catalyze the hydrolysis of triacylglycerols (TAG) into monoacylglycerols (MAG), diacylglycerols (DAG), free fatty acids (FA) and glycerol. In addition, under low water condition, lipases can catalyze ester synthesis and tranesterification. The use of lipases is rapidly growing because of their enantio-selectivity, regio-selectivity and chemo-selectivity (Rathi *et al*., 2001). These characteristics have accounted for a marked increase in the industrial usage of lipases such as in food, chemical, pharmaceutical and detergent industries (Maia *et al*., 2001).

1.1 Lipases-catalyzed reaction

In aqueous media the main catalytic reaction of lipases is hydrolysis which involves an attack on the ester bond of triacylglycerol in the presence of water molecules to produce both alcohol functionality and carboxylic acid (Malcata *et al*., 1992). However, in organic media, lipases are capable of catalysis both ester synthesis and transesterification (Degn, 2000).

1.1.1 Mechanism of the lipases-catalyzed reaction

The reaction of lipases is catalyzed by a catalytic triad composed of Ser, His and Asp (sometimes Glu) similar to serine protease (EC 3.4.21.x) and carboxyl esterases (EC 3.1.1.1). The mechanisms for ester hydrolysis or formation are essentially the same for lipases o esterases and compose of four steps. Firstly, the substrate reacts with the active-site serine yielding a tetrahedral intermediate stabilized by the catalytic His- and Asp-residues. Next, the alcohol is released and a covalent acyl-enzyme complex is formed. Attack of a nucleophile (water in hydrolysis, alcohol in (trans-) esterification) forms again a tetrahedral intermediate, which callapses to yield the product (an acid or an ester) and free enzyme (Figure 1). Lipases are distinguished from esterases by their substrate specificities. Lipases accept long chain fatty acids in triacylglycerol as substrate where as esterases prefer short chain fatty acids.

More generally it can be stated that lipases readily accept water-insoluble substrates, esterases prefer water soluble compounds. The further differences are in the three dimension structures of these enzymes, lipases contain a hydrophobic oligopeptide (often called *lid* or *flap*) covering the entrance to the active site, which is not present in esterases. Lipases preferentially act at a water-organic solvent (or oil) interface, which presumably accounts for a movement of the *lid* making the active site accessible for the substrate. This phenomenon is referred to as interfacial activation. Further characteristic structural features of lipases is α, β-hydrolase folds (Figure 2.) (Ollis *et al*., 1992) and a consensus sequence around the active site

serine (Gly-X-Ser-X-Gly, where X denotes any amino acid). It can be showed that after removal of the *lid* by genetic engineering, the activity of a lipase is improved in solution, especially in detergent application (Schrag *et al*,, 1997).

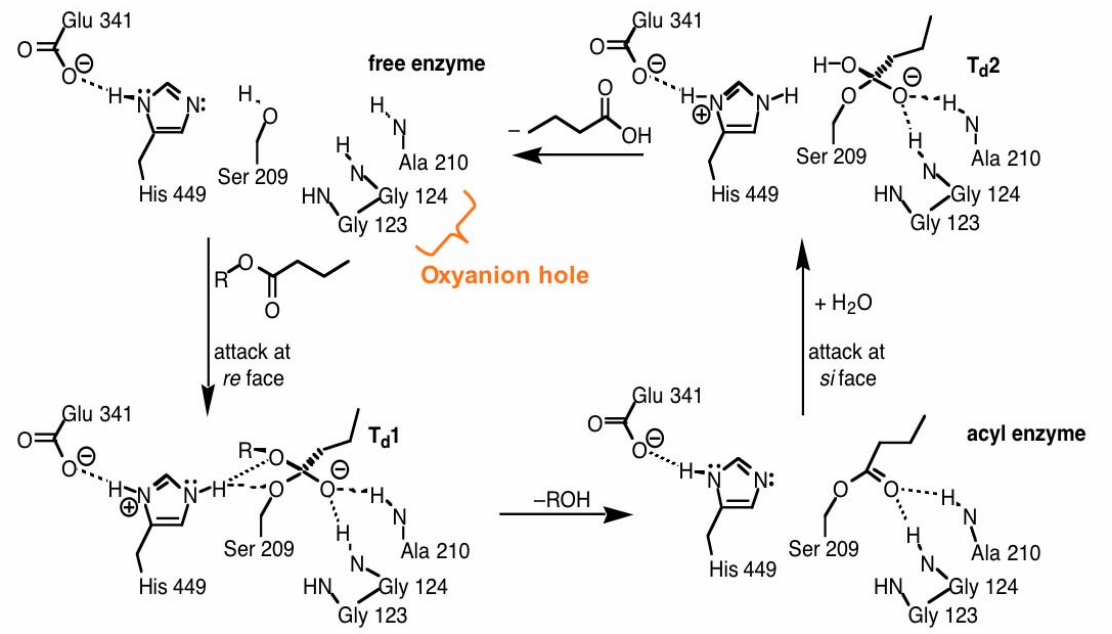


Figure 1. Mechanism of lipases-catalyzed ester hydrolysis of butyrate ester.

Numbering of amino acid residues is for lipase from *Candida rugosa* (CRL).

Source : Bornscheuer and Kazlauskas (1999)

1.1.2 Type of the lipases-catalyzed reactions

The lipase-catalyzed reactions can be categorized into three groups (Yamane, 1987).

(i) Hydrolysis reaction

Hydrolysis of ester refers to attack on the ester bond of glycerides in the presence of a water molecule to produce both an alcohol moiety and carboxylic moiety.

$$R\text{-}COOR' + H_2O \rightleftharpoons R\text{-}COOH + HOR'$$

(ii) Esterification or reverse hydrolysis reaction

Esterification of polyhydric alcohols and free fatty acids are the reverse of hydrolysis reaction. Formation of an ester bond is also commonly called acylation. The acid moiety, which participates in the ester formation, is called the acyl donor while the alcohol moiety is called the acyl acceptor. Generally, the relative rates of the forward and reverse reactions are controlled by the water content in the reaction mixture.

$$R\text{-}COOH + HOR' \rightleftharpoons R\text{-}COOR' + H_2O$$

(iii) Transesterification reaction

The term transesterification refers to the exchanges of the acyl residues of an ester with other molecules, such as carboxylic acid (acidolysis), alcohol (alcoholysis), amine (aminolysis) and another ester (interesterification).

1 Acidolysis

$R_1COOR' + R_2COOH \rightleftharpoons R_2COOR' + R_1COOH$

2 Alcoholysis

$R_1COOR' + HOR_2 \rightleftharpoons R_1COOR_2 + HOR'$

3 Aminolysis

$R_1COOR' + H_2NR_2 \rightleftharpoons R_1COOR_2 + H_2NR'$

4 Interesterification

$R_1COOR_1' + R_2COO\ R_2' \rightleftharpoons R_1COOR_2' + R_2COOR_1'$

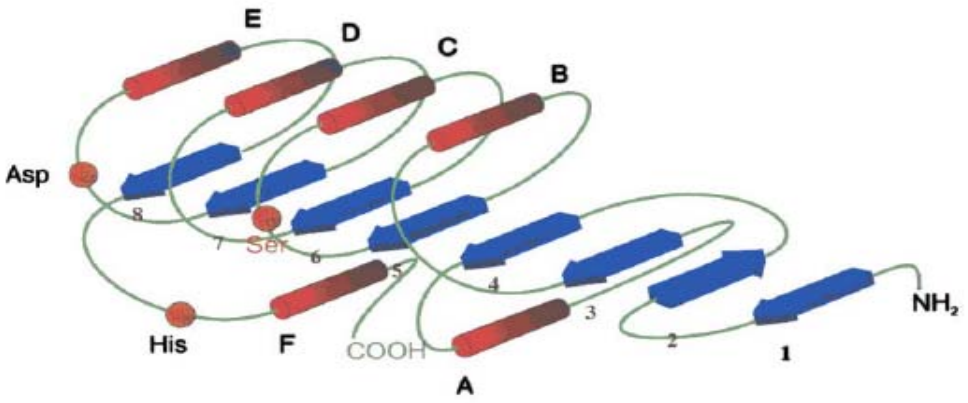


Figure 2. The α, β-hydrolase folds of lipase.
Source : Rusnak (2004)

1.2 Specificity of lipases

Lipases are specific to their substrates and operate at mild conditions. So they are preferred over chemical catalyst for the modification of lipids intended for modern consumers who demands more natural and less synthetic food products and additives (Akoh, 1996). There are three groups of lipases based on their specificities.

(i) Non-specific lipases or random lipases; the enzyme catalyzes reaction at all three positions on the TAGs e.g. lipases from *Candida cylindracea* and *Pseudomonas* sp.

(ii) Positional specific lipases or *sn*1,3-specific lipases; the enzyme catalyzes the release of fatty acids at preferential positions (primary or secondary ester) (Wong,

1995) or acts preferentially at the *sn*1 and *sn*3-positions of the acylglycerol molecules e.g. lipases from *Rhizomucor miehei* and *Rhizopus delemar*.

(iii) Substrate specific lipases; the enzyme shows specificity toward specific types of fatty acid e.g. lipases from *Geotrichum candidum* which acts specifically on oleic acid (C18:1, n-7) and lipase from *Fusarium oxysporum* acts specifically on saturated fatty acid only.

1.3 Microbial lipases

Lipases can be obtained from mammals, plants and microorganisms. Lipases produced by microorganisms are widely spread and have been increasingly interested due to higher stability in comparison to lipases from mammals and plants. Microbial lipases can be produced in large scale according to a rapid growth of microorganisms and it is easy to control the productivity and quality of the produced lipases (Malcata *et al*., 1992; Balcao *et al*., 1996). Lipases obtained from microorganisms such as yeast, fungi and bacteria show various properties according to types and production conditions. Moreover, the microbial lipases exhibit high stability in organic solvent, do not require cofactors, possess broad substrate specificity and exhibit high enantioselectivity (Jaeger and Reetz, 1998). There are numbers of commercial available microbial lipases (Table 1).

Most microbial lipases display maximum activity at pH value ranging from 5.6-8.5 and maximum stability in the neutral pH. With respect to temperature, most microbial lipases are optimally active between 30-40^{o}C (Malcata *el al*., 1992). The microbial lipases are used in detergent and food industries, biocatalysis of stereoselective transformation. The major target is application in the fat and oil industry. For example, the production of novel TAG by interesterification, improve nutritional value or alter physical properties of vegetable or fish oil, synthesis of MAG, DAG and SE, modification of phospholipids into biosurfactants and the production of high value specialty fats such as cocoa butter substitutes or hardened vegetable oils with butterfat properties (Jaeger and Reetz, 1998). The industrial applications of microbial lipases are shown in Table 2.

Table 1. Example of commercial lipases.

Source of lipase	Other name	Commercial source
Mammalian lipases		
Porcine pancreas		Amano, Sigma, Fluka, Boehringer Manheim
Pancreatic cholesterol esterase		Genzyme, Sigma
Fungal lipases		
Candia rugosa	*Candida cylindracea*	Altus Biologics, Amano, Boehringer Manheim
Candida antarctica A		Boehringer Manheim
Candida antarctica B		Novo Nordisk
Candida lipolytica		Boehringer Manheim, Novo Nordisk, Sigma
Geotrichum candidum		Amano
Humicola lunuginosa	*Thermomyces lanuginosa*	Boehringer Manheim, Novo Nordisk
Rhizomucor javanicus	*Mucor javanicus*	Amano
Rhizomucor miehei	*Mucor miehei*	Boehinger Manheim, Novo Nordisk, Amano, Fluka, Sigma
Rhizopus oryzae	*R. javanicus*, *R. delemar*, *R. niveus*	Amano, Fluka, Sigma, Seikagaku Kogyo
Penicillium roqueforti		Amano
Penicillium camembertii	*P. cyclopium*	Amano
Aspergillus niger		Amano
Bacterial lipases		
Pseudomonas cepacia	*Burkholderia cepacia*	Altus Biologics, Fluka, Sigma, Amano, Boehringer Manheim
Pseudomonas fluorescens		Amnao, Biocatalysts
Pseudomonas fragi		Wako Pure Chemical
Pseudomonas sp.		Amano
Alcaligenes sp.		Meito Sangyo

Table 1. (Continued).

Source of lipase	Other name	Commercial source
Chromobacterium viscosum	*Pseudomonas glumae*	Sigma, Genzyme, Asahi Chemnical, Biocatlysts Ltd.,
Bacillus thermocantenulatus		Boehringer Manheim

Soruce: Bornscheuer and Kazlauskas (1999)

1.4 Immobilization of lipases

Use of enzymes in industrial application has been limited because most enzymes are relatively unstable, the cost of enzyme isolation and purification are still high and it is technically expensive to recover active enzyme from the reaction mixture after completion of the catalytic process. These restrict the use of soluble enzymes to operate in batch condition. Moreover, the isolation of the products from the reaction mixture by pH or heat treatment causes inactivation of enzyme. To eliminate some of these deleterious effects, the immobilization of enzymes by attachment or entrapment of enzyme on/in water insoluble solid matrixes can immobilize of enzyme molecules and make them insoluble in aqueous media (Kenedy and Cabral, 1987).

Immobilization of enzyme may protect the enzyme from solvent denaturation. It helps in maintaining homogeneity of enzymes in the reaction media since it avoids aggregation of enzyme particles. In addition to ease of handling, immobilized enzymes are well suited for use in continuous packed-bed or fluidized-bed reactors (Yahya *et al*., 1998).

It has been shown that immobilized enzymes are cost-effective because they can be recovered and reused several times. Immobilized enzymes are more thermostable and can be stored over a longer period of time than non-immobilized enzyme (Akoh, 1996). However, immobilized enzyme may have a considerable effect on their kinetics. This may be due to structural change to the enzyme and the creation of a distinct microenvironmental around the enzymes (Chaplin and Bucke, 1990).

Lipases are soluble in aqueous solution but their substrates (i.e. fat and oils) are not. Though, use of organic solvents or emulsifiers help to overcome the problem of the contact between lipases and their substrates, the practical use of native lipases in such pseudohomogeneous reaction systems poses technological and economical difficulties. These problems lead to constrain on the product level because the final characteristics of the product depended on post-processing condition as storage time and temperature and the process level because the useful life of lipase is restricted to the space-time of the reactor. In both cases, part

of the overall potential enzymatic activity is lost. If the lipases are immobilized, they become an independent phase with in the reaction system, which may be easily retained in the reactor via mechanical means with concomitant advantages in preventing contamination of the products and extending their useful active life (Balcao *et al*., 1996).

Table 2. Industrial applications of microbial lipases.

Industry	Function	Product
Dairy	Hydrolysis of milk fat	Flavor agents
	Cheese ripening	Cheese
	Modification of butter fat	Butter
Bakery	Flavor improvement and lift life prolongation	Bakery products
Beverages	Improved aroma	Beverages
Food dressing	Quality improvement	Mayonnaise and dressings
Health food	Transesterification	Health foods
Meat and fish	Flavor development and fat removal	Meat and fish products
Fat and oils	Tranesterification	Cocoa butter, margarine
	Hydrolysis	Fatty acids, mono- and diacylglycerol
Chemical	Enantioselectivity	Chiral building blocks and chemicals
	Synthesis	Chemicals
Pharmaceutical	Tranesterification	Specialty lipids
	Hydrolysis	Digestive aids
Cosmetics	Synthesis	Emulsifiers and moisturing agents
Leather	Hydrolysis	Leather products
Paper	Hydrolysis	Paper products
Cleaning	Hydrolysis	Removal of cleaning agent e.g. surfactant

Source: Godtfredsen (1991)

1.4.1 Immobilization method

Immobilization of lipase requires an immobilizing agent which can be established between two enzyme molecules or between one enzyme molecule and another catalytically inert molecule, or between two catalytically inert molecules. To date, virtually every immobilization protocol employs for lipases encompasses immobilizing agents which comprises: 1) covalent forces as covalent attachment, cross-linking, containment with in porous membranes of polymeric nature and intermolecular cysteine bridges during precipitation; 2) ionic forces as in ion exchange, containment with in porous membranes of mineral nature and intermolecular salt bridges during precipitation in non-polar solvents; 3) hydrogen bonds as in intermolecular interaction between hydrogen atoms and electronegative atoms during precipitation in non-polar solvents; and 4) van der Waals forces as in hydrophobic adsorption, reversed micelles microencapsulation, containment within porous membranes of monomeric and hydrophobic nature and precipitation in polar solvents. The immobilizing agent may also include a material ligand. This is the case of multifunctional molecules in cross-linking and spacer molecules in covalent attachment following preliminary derivatization of the support (Balcao *et al.*, 1996).

Immobilization of lipases from several microbial and animal sources has been performed by different methods, including covalent attachment to activated supports, entrapment with photocrosslinkable resins and polyvinylchloride (PVC) or colloidion membranes and adsorption on the different materials e.g. ionic resin or waste animal bone (Montero *et al.*, 1993).

1.4.2 Support materials

Immobilization of enzyme can be achieved by the use of various support materials. For example, enzymes can be adsorbed on the insoluble materials, copolymerized with a reactive monomer, encapsulated in gels, cross-linked with a bifunctional reagent, covalently bound to an insoluble carrier or entrapped with in an insoluble gel matrix of natural or synthetic resin (Basri *et al.*, 1995).

The support materials (or the carriers) are the entities larger in size than the enzyme molecule which enzyme is directly bound or confined. The supports have the role of helping in the creation of an immobilized enzyme-rich phase. They may be a liquid as in reversed micelles or a solid as in the most commonly employed immobilization protocols. In the case of hydrophobic carriers, the immobilized enzyme is soaked in the organic liquid phase whereas in hydrophilic carriers the immobilized enzyme is soaked in water. The former situation is useful if one wants deactivation reaction of the enzyme to be maintained as slow as possible where as the latter situation should be used when hydrolysis reactions are desired (Balcao *et al.*, 1996).

The good support materials should contribute to distribution of substrate available to the enzyme in non-aqueous media. The presence of a small amount of water would allow the existence of free carboxylic acid ions of the substrates for lipase. The anion-exchange resin would tend to attract carboxylic acid due to its positive charge, resulting in high concentration of substrate available of the enzymes. This account for the high reaction rates for esterification reaction observed with lipases immobilized on anion-exchange resin (Yahya *et al*., 1998).

Hydrogels are polymeric materials made from hydrophilic and/or hydrophobic monomers, which can be a homopolymer or a copolymer. Their major characteristics are that they can imbibe larger quantities of water without dissolution of polymer network. This feature makes them interesting supports for immobilization of enzyme. In addition to providing the water needed for enzyme activity. The hydrogels also can absorb water produced during the esterification reaction thus increasing the products (Basri *et al*., 1995).

1.4.3 Application of immobilized lipases

The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications (Jaeger *et al*., 1999). Lipases have high potential for hydrolysis, glycerolysis and alcoholysis of bulk fats and oils because of their high specificity (Balcao *et al*., 1996).

Immobilized lipases are used for the hydrolysis and partial hydrolysis of fats and oils for the industrial production of fatty acid, MAG and DAG, which are widely used as emulsifiers in food and pharmaceutical industries. Examples of products obtained from lipase catalyzed esterification include partial acylglycerol, TAG, short chain esters and terpene esters which are use in flavor and fragrance. Sugar esters, which are used as emulsifiers and fat substitutes, could be also synthesized by this reaction (Balcao *et al*., 1996). Acidolysis and interesterification reactions have been extensively used for the production of partial acylglycerols, sugar esters and designer fats e.g. cocoa butter and other structure lipids (ST). Lipases are also used for modification of fatty acid composition of vegetable oils according to several functional, health and nutritional reasons. Lipases are currently used for production of optically active compounds for the fine chemicals and pharmaceutical industrial such as hydroxymethyl glutaryl coenzyme A reductase inhibitor, which is a potential anticholesterol drug (Akoh, 1996). Immobilized lipase B from *Candida antarctica* (CALB) could used for production of enantiopure (*S*)-indanofan, a novel herbicide used against grass weeds in paddy fields (Tanaka *et al*., 2002).

Immobilized lipase could be applied for production of new biopolymeric materials. Biopolymers including polyphenols, polysaccharides and polyesters show a considerable degree of diversity and complexity. Furthermore, these compounds are received

increasing attention because they are biodegradable and produced form renewable resources (Jaeger and Eggert, 2002).

Biodiesel fuel or fatty acid methyl esters (FAME) an alternative source of energy for public transport has been produced via transesterification of vegetable oil (i.e. palm oil and rapeseed oil) with methanol or ethanol catalyzed by immobilized lipase. Iso *et al.* (2001) reported the production of FAME catalyzed by immobilized lipase from *Pseudomonas fluorescens*. The enzyme was immobilized to increase the stability upon repeated use to produce FAME. Paripatanapairod (2003) also reported that FAME could be produced by methanolysis of palm stearin catalyzed by immobilized lipase PS on Accurel EP100 and FAME content reached to 92% after 24 h at 50°C.

2. Palm oil

2.1 Palm oil

Palm oil has a successful production exclusively in Southeast Asia (Berger, 2001). Two types of oils are obtained from palm fruits; palm oil from fruit mesocarp and palm kernel oil from nut. Palm oil contains almost equal amount of saturated and unsaturated fatty acids. Palmitic and oleic acids are the major fatty acids with small quaintities of linoleic and stearic acids. The fractionation of palm oil into palm olein and stearin fractions has significant influence on its fatty acid composition (Table 3). The olein fraction resembles other edible vegetable oils and contains a high proportion of unsaturated fatty acid, particularly oleic acid. The stearin fraction contains more saturated fatty acids, particularly palmitic acid and resembles other saturated fats (Salunkhe *et al.*, 1992).

Palm oil is solid at ambient temperature in temperate climates and fluid in tropical and subtropical climate with certain fraction held in crystalline form. Interesterification of palm oil using basic catalysts (e.g. NaOH, KOH), provides a method of transferring saturated fatty acid to predominated unsaturated glyceride. Fractionation of palm oil produces two fractions; one with a very low melting point (olein) and the other with a high melting point (stearin). The iodine value of palm oil is lower (44-58) than other vegetable oils because of a high proportion of saturated fatty acids but the saponification value of palm oil is higher (195-205) than other edible vegetable oils (Salunkhe *et al.*, 1992).

Table 3. Fatty acid composition of processed palm oil and palm oil fractions.

Palm oil or fraction	Fatty acid (%)								
	14:0	16:0	18:0	20:0	Total saturated	18:1	18:2	18:3	Total unsaturated
Neutralized	0.8	41.0	5.1	0.1	47.0	39.0	13.8	0.2	53.0
Interesterified	0.7	41.2	5.0	0.1	47.0	38.6	13.8	0.6	53.0
Olein	0.5	9.9	1.4	0	11.8	63.1	23.9	0.2	87.2
Stearin	1.0	66.0	8.4	0.5	75.9	18.6	5.4	0.1	24.1

Source: Salunkhe *et al*. (1992)

2.2 Palm kernel oil

Palm kernel oil is obtained as a minor product during processing of oil palm fruit. It is obtained from palm kernels after separation, drying and cracking of the shell or nut. The main fatty acid proportion of palm kernel oil is saturated fatty acid predominantly lauric acid and also contains some low molecular weight fatty acid that is not commonly found in other vegetable oils (Table 4). The fatty acid composition of palm kernel oil is closely to the coconut oil (Salunkhe *et al*., 1992).

Saturated TAG are the major triacylglycerol of palm kernel oil and constitute over 60% of the total TAG. Palm kernel oil is characterized as hard oil and it is nearly colorless and solid at ambient temperature. The melting point is 25-30°C and iodine value is very low (14-33). The saponification value of palm kernel oil is 245-255 (Salunkhe *et al*., 1992).

2.3 Palm fatty acid distillates (PFAD)

The physical refining of crude palm oil is carried out by the steps of degumming, bleaching, deodorization and cooling, respectively. The deodorization of palm oil uses high temperature (250-270°C) with stripping stream and under vacuum. Under these conditions, free fatty acid could evaporate and then condensed in the liquid by cooling. So they are termed palm fatty acid distillates or PFAD (Mohd Suria Affandi, 1994). PFAD about 4% is generated from the volume of palm oil refined (Rakmi *et al*., 1997). The characteristics of PFAD are shown in Table 5.

Table 4. Fatty acid composition of palm kernel oil.

Fatty acid	Content (%)
Caprylic acid (C8:0)	3-4
Capric acid (C10:0)	3-7
Lauric acid (C12:0)	46-52
Myristic acid (C14:0)	15-17
Palmitic acid (C16:0)	6-9
Stearic acid (C18:0)	1-3
Oleic acid (C18:1)	13-19
Linoleic (C18:2)	0.5-2.0

Source: Salunkhe *et al*. (1992)

2.4 Utilization of palm oil and palm fatty acid distillates

Palm oil is extensively used for edible purposes. The substantial quantity of palm oil is also used for the manufacture of soaps and candle and tin-plate industry. With the improvement in quality processing technology, using of palm oil for edible purposed is increasing as well as its nonfood usages, especially for FAME production. In food application, palm oil is used as the cooking and frying oil and in margarine, shortening and vanaspati manufacture. Moreover, it can be used as a fermentation substrate for the production of protein and antibiotics (Salunkhe *et al*., 1992).

Table 5. Standard specification for palm fatty acid distillates.

Composition	Content (%)
Saponification matter	95-97
Moisture and impurities (M&I)	< 1.0
Free fatty acid (as palmitic acid)	> 70

Source: Mohd Suria Affandi (1994)

Palm oil is also used as the substrate to produce value added products (i.e. ST, FAME). The well known ST is cocoa butter substitutes. Due to high value of cocoa butter and physical property, the commercial process using of *sn*1,3-specific lipase catalyzes interesterification of palm oil midfraction with stearic acid or ethyl stearate to produce cocoa butter substitutes. Moreover, the human milk fat replacers are also synthesized by *sn*1,3-specific

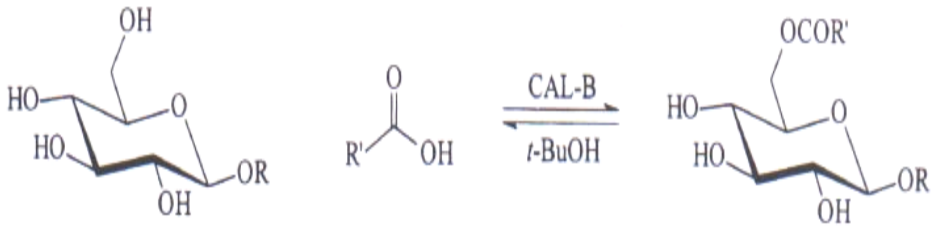
lipase, using tripalmitin, derived from palm oil with oleic acid or polyunsaturated fatty acid obtained from plant oil as the substrates (Mukherjee, 1998).

Monoacylglycerols (MAG) are the most widely used emulsifiers in food, pharmaceutical and cosmetic industries. The production of MAG from palm oil by glycerolysis with immobilized lipase from *Pseudomonas cepacia* (lipase PS) on Accurel EP100 could produce 55.8% MAG (Kaewthong *et al*., 2003).

The present utilization of PFAD is only used as a raw material for soap and candle manufacturing. Rakmi *et al*. (1997) reported the synthesis of fructose ester using PFAD catalyzed by Lipozyme IM in *tert*-butanol at 40°C. They found that fructose was esterified by PFAD to produced fructose monoester with yield 17.70 mg/mL solvent.

3. Sugar esters

Sugar esters (SE) are the carbohydrate ester that could be synthesized by chemical and enzymatic methods. The schematic of enzymatic SE synthesis is shown in Figure 3 (Otto *et al*., 1998).



Acyl acceptor Acyl donor Sugar ester

Figure 3. The schematic of sugar ester synthesis catalyzed by *Candida antarctica* lipase B (CALB) in *tert*-butanol.

Source : Otto *et al*. (1998)

3.1 Application of sugar esters

3.1.1 Detergents

Increasing of environment awareness among consumers has provided further stimulation for more serious consideration of biological surfactants as possible alternative detergents as important as their functional performance. SE have important application in household and industrial detergents because of their active properties and compositions. Furthermore, they are not harmful to environment because they are completely biodegradable

under both aerobic and anaerobic conditions (Ducret *et al*., 1995). SE and their derivatives are used as nonionic surfactants in modern industry (Table 6).

Table 6. Type of surfactants used in modern industry.

Surfactant type	Examples	Total production (%)	Major uses
Anionic	Carboxylates Sulphonates Sulphuric acid esters	66	Washing powders
Cationic	Amine oxides Monoamines Quaternary ammonium salts	9	Fabric softeners Shampoos
Nonionic	Carboxylic acid esters Glycerides Carbohydrate esters and their ethoxylated derivatives	24	Laundry co-surfactants Washing up liquids Personal-care products Foods

Source : Sarney and Vulfson (1995)

3.1.2 Foods

In food application, SE are applied as emulsifier in food such as low fat spreads, sauces, ice-cream and mayonnaise (Cao *et al*., 1996). Olestra or sucrose polyester (SPE) is the most useful sugar ester, which uses in food application. It is composted of a mixture of hexa-, hepta-, and octaester of sucrose formed with long chain fatty acid derived from edible oil as shown in Figure 3. Olestra is largely a nonnutritive fat substitute. It has taste and cooking characteristics similar to those of traditional fat and oil but it does not contribute any energy to the diet because it is not hydrolyzed by gastric lipases. Therefore, it is not absorbed from the gastrointestinal tract (Yankah and Akoh, 2001). The generally brand names and compositions of fat substitutes are shown in Table 7.

Table 7. Lipid-based fat substitutes.

Generic brand names	Composition
Sucrose polyester, Olestra	Sucrose polyester of 6-8 fatty acids
Sucrose fatty acid esters	Sucrose with 1-3 fatty acids
Trehalose ,raffinose, stachyose polyester	Carbohydrate with fatty acids
Sorbestrin	Sorbitol, sorbitol anhydrous with fatty acids
Alkyl glycoside polyester	Alkyl glycosides with fatty acids
Sorbitan monostearte	Sorbitol with fatty acids

Source : Yankah and Akoh (2001)

Furthermore, SE containing long chain unsaturated fatty acids (PUFA) such as arachidonic acid, are used in food because this fatty acid has important role in the structure and function of biological membrane and it is the precursor for a number of prostaglandins. Not only that, arachidonic acid is also important for health benefits due to their ability to reduce plasma cholesterol and the incidence of coronary heart disease (Ward *et al*., 1997; Akoh *et al*., 1994).

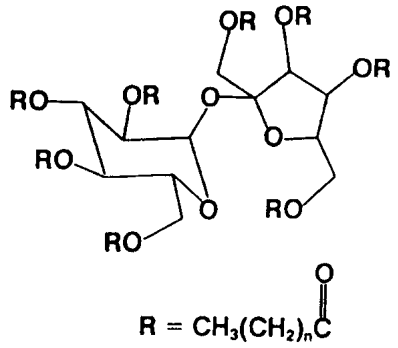


Figure 4. Structural of Olestra

Source : http://www.american.edu/TED/images3/fig12.gif

Ascorbic acid is one of sugar derivatives. Due to its antioxidant property, the ascorbic acid ester is used as a BHA and BHT substituent in food products to prevent lipids oxidation. Humeau *et al*. (1998) reported that 6-*O*-palmitoyl-L-ascorbic acid was synthesized using L-ascorbic acid and palmitic acid methyl ester catalyzed by *Candida antarctica* lipase B (Novozym 435) with yield of 19 g/L after reaction time of 5 h.

3.1.3 Cosmetics

SE containing linolenic acid (C18:2n-6) are used in cosmetic because this fatty acid is a major fatty acid in skin which maintains the integrity of epidermal water permeability barrier (Bousquet *et el*., 1999). Furthermore, the ester of α-butylglucoside with lactic acid or glycolic acid, which are alpha-hydroxy acids (AHA), is also used as exfoliating and moisturizing agent in cosmetic application. Bousquet *et al*. (1998) showed that the transesterification between α-butylglucoside and butyllactate using Novozym 435 was achieved with yield of 170 g/L in 30 h of reaction time.

3.2 Method of sugar ester synthesis

3.2.1 Chemical method

The chemical synthesis of SE is based on a high temperature esterification between sugar and fatty acid using the alkaline catalyst. High temperature causes coloration of the final products and dehydration and cyclization in case of sugar alcohols (Ducret *et al*, 1995). Furthermore, many of similar structures are obtained owing to the presence of numerous hydroxyl groups in carbohydrate substrates. An analysis of food grade sorbitan ester (i.e. SPAN-20) by gas chromatography showed the presence of at least 65 individual compounds which some are by products. Many of them are identified as various isomers of sorbitol, iso-sorbide and their mono-, di- and triesters and all of these by-products are allergenic and carcinogenic agents (Sarney and Vulfson, 1995). Moreover, using of alkaline catalyst e.g. lead oxide and stannous soap are not compatible in food application due to their toxicity (Akoh *et al*., 1994).

3.2.2 Enzymatic method

Although, SE can be synthesized by chemical method but it is very difficult to control and low productivity is obtained. However, the enzymatic method is the alternative approaches to synthesis these esters because it is easy to control, and only a few by-products are formed. To date, there are two main enzymatic methods based on solubility of substrates.

The first approach is based on the polarity reduction of sugar through the formation of a non-polar derivative e.g. sugar acetals, alkyl sugars and sugar boronate complexes. The sugar derivatives are easily dissolved in organic solvent. Alternatively, they can be melted and mixed in solvent-free conditions (Salis *et al*., 2004). Organoboronic acids (e.g. phenylboronic acid and butyl boronic acid), are known to dissolve sugars by forming a sugar boronate complexs by reversible condensation with sugar. The sugar-boronate complexes are soluble in non-polar organic solvents and readily hydrolyzed by small amounts of water after enzymatic step to obtain desirable products. However, the use of organoboronic acids results in the problem of biocompatibility on the final products (Salis *et al*., 2004).

Schlotterbeck *et al*. (1993) reported one step synthesis of fructose monostearate in *n*-hexane using phenylboronic acid as derivertizing agent. Phenylboronic was added directly to the reaction mixture of fructose, stearic acid and Lipozyme IM in *n*-hexane. The complex of fructose phenylboronate was occurred first then this complex was esterified with stearic acid catalyzed by lipase to form the complex of phenylboronic acid fructose ester. The conversion yield 40% of fructose monostearate was obtained after hydrolyzed the complex with acetone/water (2:3 v/v).

Sugar acetals such as 1,2-*O*-isopropylidene-D-xylofuranose, 1,2-*O*-isopropylidene-D-glucopyranose, maltose tri-acetal, lactose tetra-acetal and 1,2:3,4 di-*O*-isopropylidene-D-galactopyranose were used for synthesis of mono- and disaccharide fatty acid esters under solvent free condition. The products were obtained after mild hydrolysis of sugar acetal esters (Sarney *et al*., 1995). The esterification of 1,2-*O*-isopropylidene-D-xylofuranose with arachidonic acid under solvent free condition was achieved with the maximum conversion yield of 83-85% at the molar ratio of arachidonic acid to 1,2-*O*-isopropylidene-D-xylofuranose ratio of 1-2:1 (Ward *et al*., 1997).

The use of alkyl sugars as substrate for sugar ester synthesis has been also reported. The esterification of glucose with molten fatty acid, resulted of only 20% conversion while more than 90% conversion was obtained with ethyl-, *N*- and iso-propyl or butyl glucosides (Adehorst *et al*., 1990). The solvent free conditions are very difficult to operate because the substrate must be converted to the liquid form and elimination of substituted group before obtaining final products.

The second approach is based on the use of polar organic solvents and/or their mixtures and ionic liquid such as *tert*-butanol, acetone, pyridine, 2-methyl-2-butanol, which are suitable for the solubilization of both substrates (sugar and fatty acid). This method allows substrate dissolution while retaining enzymatic activity and offers the possibility of obtaining specific isomer in high yield. The use of lipase catalyzed esterification in organic solvent is highly dependent on the nature of solvent used. Thus, there are many different reaction systems and several aspects have to be studied, such as acyl donors (fatty acids), acyl acceptors, specificity of lipases, solvents and type of immobilized lipases (Degn and Zimmermann, 2001).

3.3 Factors affecting lipase catalyzed SE synthesis in organic solvent

The amount of literatures on lipase catalyzed the synthesis of SE using organic solvents are growing rapidly as shown in Table 8. There are many different factors affecting on enzymatic synthesis of SE in organic solvents. To obtain high reaction rate and conversion yield in the ester synthesis, these factors have to be investigated.

3.3.1 Type of lipases

The main reaction of lipases in aqueous media is hydrolysis. However, in organic solvent, lipases are able to catalyze both esterification and transesterification. Several lipases derived form a variety of sources including bacteria, yeasts, moulds and animals are screened for sugar ester synthesis (Degn, 2000). Early reports showed possibility of using of native lipase to synthesize SE. The native lipase from *Candida cylindracea* could synthesize the ester with relatively high yield of 60% conversion but the main problem of enzymatic esterification was lacking of enzyme stability in organic solvent (Senino and Uchibori, 1984).

Mutua and Akoh (1993) compared the activity of immobilized lipase from *Candida* sp. (SP382) with non-immobilized lipase from the same source. They found that the immobilized lipase gave higher conversion of alkyl glucoside with oleic acid of 76.5% while non-immobilized lipase gave only 34.2%.

The immobilized lipases are the most useful for sugar ester synthesis in organic solvent because of their stability, easy to recover and re-use. Different types of immobilized lipases are used in sugar ester synthesis (Table 8). The ester synthesis from fructose and palmitic acid catalyzed by immobilized *Candida antarctica* lipase B (CALB) and Lipozyme IM from *Rhizomucor miehei* in 2-methyl-2-butanol, was reported. The highest conversion yield of 53% was obtained with CALB while Lipozyme IM resulted only 30% of conversion yield at the same conditions (Sabeder *et al.*, 2006). The similar results were obtained from the synthesis of alkyl glucoside fatty acid ester catalyzed by CALB and immobilized *Candida rugosa* lipase AY in hexane. The product yield of 47 and 63% were obtained with immobilized lipase AY and CALB, respectively (Tsitsimpikou *et al.*, 1997).

Most reports have been used CALB for sugar ester synthesis (Table 8). The CALB shows little or no interfacial activity and hydrolyzes long chain fatty acid in triacylglycerol only slowly. For this reason, it may be better classified as esterase. It shows very high activity and high enantioselectivity toward a wide range of alcohols. It enantioselectivity is usually low toward carboxylic acids (Bornscheuer and Kazlauskas, 1999).

3.3.2 Solvents

Enzymes are generally not soluble in organic solvents. Hence, enzymatic reactions in organic solvent are carried out in two phases, liquid/solid system. The liquid phase consists of solvent which the substrates are solubilized and solid phase is the immobilized enzymes. The esterification reactions are occurred in organic solvent. When choosing an organic solvent for enzymatic synthesis of SE, there are three essential factors to be considered (Degn, 2000).

1) Is the enzyme stable in the solvent?

2) Is the enzyme catalytically active in the solvent?

3) Is the sugar soluble in the solvent?

The limitation of SE synthesis is low solubility of sugar in organic solvent but this problem can be solved by adding of solubilizing agent e.g. phenylboronic acid or using polar solvents e.g. acetone, *tert*-butanol (2-methyl-2-propanol), *tert*-pentanol (2-methyl-2-butanol), ethyl methylketone (EMK), dioxane dimethylsulfoxide (DMSO), tetrahydrofuran (THF) and pyridine.

Schotterbeck *et al.* (1993) reported that the synthesis of sugar ester in hexane was achieved when adding phenylboronic acid as solubilizing agent. The disadvantage of this method is the solubilizing agent which is very harmful for the final product for food, cosmetics and pharmaceutical application (Kim *et al.*, 1997).

The use of two organic solvent systems is promising because the mixture of solvents can increase solubility of sugar and stability of enzyme. Akoh *et al.* (1994) used the mixture of benzene/pyridine (2:1 v/v) to synthesize alkylglucoside catalyzed by *Candida cylindraceae* lipase. The highest conversion yield was 74%. The synthesis of 6-*O*-lauroylsucrose in 2-methyl-2-butanol/DMSO (4:1 v/v) provided the yield of 70% in 24 h with immobilized lipase from *Thermomyces lanuginosus* (Ferrer *et al.*, 1999). Recently, Castillo *et al.* (2003) reported the synthesis of xylitol oleic acid monoester in *tert*-butanol/DMSO (4:1 v/v) using Novozym 435 with the conversion yield of 73%. Although, the two solvent mixture systems are more interesting due to their ability to dissolve sugar but this system is not compatible for food application due to their high toxicity of organic solvents.

Alternatively, polar solvents, particularly, acetone, EMK and *tert*-butanol and 2-methyl-2-butanol are the suitable solvent because they are easy to eliminate and acceptable by the European Community for use in the food and food additive manufactures (Rakmi *et al.*, 1997; Arcos *et al.*, 1998 and Sabeder *et al.*, 2006). The best results for the synthesis of glucose palmitate were achieved in acetone with 86% conversion when used CALB as catalyst (Cao *et al.*, 1997). The synthesis of fructose dilaurate and glucose monolaurate was also

achieved in acetone catalyzed by CALB with conversion yield of 100 and 98%, respectively (Arcos *et al*., 1998).

Production of 6-*O*-acyl-α-D-glucopyranoside in *t*ert-butanol, was obtained using Novozym 435 and Lipozyme IM with the maximum catalytic activity of 3.9 and 0.45 μmol glucose converted/min (Degn *et al*., 1999). Sabeder *et al*. (2006) compared the influence of organic solvents on fructose ester synthesis and found that EMK provided the highest conversion yield of 82% while acetone, *tert*-butanol and 2-methyl-2-butanol gave only 69, 64 and 61%, respectively. The conversion yield of 79% was obtained in the synthesis of glucose stearate in the presence of small amount of EMK (Yan *et al*., 2001).

The most useful polar organic solvent for SE synthesis is 2-methyl-2-butanol (Table 8). The highest conversion yield of 70% was obtained with Novozym 435 (Flores *et al*., 2002). The fructose ester of saturated fatty acid was also synthesized by Novozym 435 in the presence of 2-methyl-2-butanol. The maximum yield of fructose palmtiate and fructose stearate reached 66 and 80%, respectively (Soultani *et al*., 2001; Sasaki *et al*., 2006).

3.3.3 Acyl acceptors

Various kinds of sugars are used in enzymatic sugar ester synthesis such as monosaccharides, disaccharides, trisaccharides, sugar alcohols and their derivatives. The main problem of sugar ester synthesis is low solubility of sugar in organic solvent. Therefore, the method to increase solubility of sugar has to be emphasis. The modified sugars, which have a substitute of hydroxyl group in sugar with alkyl group to from alkyl sugar such as methyl sugar and octyl sugar can increase solubility in organic solvent. As well as alkyl sugars, sugar acetals and organoboronic acid complex of sugar can be also increase solubility in organic solvent. The alkyl group, phenyl boronic acid and acetals can be removed under mild conditions after esterification. However, the modified sugars have more reaction steps than unmodified sugar, cause less feasible in an industry process and they are toxic for application with human products (Degn, 2000).

Monosaccharides which have only one primary hydroxyl group at C-6 position such as glucose, mannose and galactose, are usually acylated at C-6 by immobilized lipase. Cao *et al*. (1997) reported using of various monosaccharides with primary hydroxyl group at C-6 position to synthesize ester of palmitic acid catalyzed by Novozym 435. β-D(+)-Glucose and D(+)-mannose were the good substrates with conversion yield of 87 and 62%, respectively.

Fructose has two positions of primary hydroxyl groups at C-1 and C-6. The number of acylation are different in each method depended on the enzyme, temperature and solvent systems used. For example, Scheckermann *et al*. (1995) reported the monoacylation of fructose with various fatty acids in hexane catalyzed by immobilized lipase from *Rhizomucor miehei* liapse. The products were a mixture of C-1 and C-6 monoacylated fructose. In contrary,

the production of fructose monostearate in hexane using phenylboronic acid as solubilizing agent was reported that fructose could converted to the ester with yield of 40% after hydrolyzed of the complex of phenylboronic acid fructose ester with acetone/water (2:3 v/v) at 60°C and 12 h (Schlotterbeck *et al*., 1993). The effect of temperature on selection of primary hydroxyl group of fructose was also reported (Arcos *et al.*, 1998). The high temperature (60°C) effected on the formation of monoacylation of fructose while at low temperature (5-40°C) the diesters were formed.

Disaccharides such as sucrose, maltose, lactose and trehalose are also used in sugar ester synthesis. Oosterom *et al*. (1996) used various disaccharides, which were lactose, sucrose, matlulose, maltose, isomaltulose and trehalose to synthesize sugar ester with ethyl butanoate catalyzed by Novozym 435. The catalytic rates depended on the structure of disaccharide used. Trehalose, maltulose and isomaltulose afforded 66, 17 and 33% of conversion yield with equi-molar of mono- and diesters in 24 h. In contrary, maltose, lactose and sucrose afforded only monoester with conversion yield of 17, 2 and 1%, respectively.

Trisaccharide as maltotriose also showed the possibility for synthesis of sugar ester. The synthesis of maltotriose ester was achieved when used vinyl ester as acyl acceptor and catalyzed by immobilized lipase from *Thermomyces lanuginosus* on celite in the solvent mixture of 2-methyl-2-butanol/DMSO (4:1). The conversion yield of 21, 26, 27 and 28 % were obtained with vinyl laurate, vinyl myristate, vinyl stearate and vinyl palmitate, respectively (Ferrer *et al*., 2000).

Sugar alcohols e.g. sorbitol, mannitol, xylitol, inositol and glycerol are widely used for the ester synthesis. They usually give the mixture of mono-, di- and triester because of the hydroxyl group in their structures. The synthesis of biosurfactant from sugar alcohols, sorbitol and xylitol with oleic acid showed the same conversion yield of 98% in 2-methyl-2-butanol catalyzed by Novozym 435 (Ducret *et al*., 1995). The similar results were obtained with the acylation of sorbitol with palmitic acid in *tert*-butanol. The conversion yield of 97% was obtained while manitol showed low conversion of 35% and no product obtained with myo-inositol (Cao *et al*., 1996).

Table 8. Literature reviews on enzymatic synthesis of sugar esters.

Acyl acceptor	Acyl donor	Solvent	Lipase	Condition	Product	Reference
Sorbitol	Decanoic acid	Water/decanoic acid	CRL	30°C, 500 rpm	Mono-, di- and tri sorbitol ester	Janssen, 1990
Glucose, fructose	Stearic acid	*tert*-Butanol	MML, CALB	40°C, slow shaking	Glucose stearate, fructose stearate	Oguntimein, 1993
Fructose	Stearic acid	Hexane	MML	60°C, 120 rpm	1-Fructose stearate	Schotterbeck, 1993
Ascorbic acid	Palmitic acid methyl ester	2-Methyl-2-butanol	CALB	55°C, 250 rpm	Ascorbyl palmitate	Humeau, 1994
Glucose	Octanoic acid	Butanol	CALB	70°C, 200 rpm	Octanoyl glucose	Ljunger, 1994
Fructose	Fatty acids	Hexane, 2-methyl-2-butanol	CALB	45°C, 250 rpm	Monoalkyl fructose	Scheckemann, 1995
Fructose	Oleic acid	2-Methyl-2-butanol	CALB	40°C, 80 rpm	Fructose monoloeate	Coulon, 1997
Fructose, glucose	PFAD	*tert*-Butanol	MML	50°C, 250 rpm	Fructose ester, glucose ester	Rakmi, 1997
Fructose	Fatty acids	Acetone	CALB	5-60 °C, 200 rpm	1,6-Diacylfructose	Arcos, 1998[a]
Glucose	Fatty acids	Acetone	CALB	40 °C, 200 rpm	6-*O*-acylglucose	Arcos, 1998[b]
Sorbitol	Caprylic, lauric acid	Acetone, acetonitrile, dioxane	CALB	40 °C, 200 rpm	1,6-Diacyl-D-sorbitol	Arcos, 1998[c]

Table 8. (Continued).

Acyl acceptor	Acyl donor	Solvent	Lipase	Condition	Product	Reference
Various sugars	Fatty acids	Dioxane, acetone	CALB	60°C, 250 rpm	Monno- and diacylated sugars	Cao, 1999
Glucose	Fatty acids	*tert*-Butanol	MML, CALB	45°C, 250 rpm	6-*O*-acyl-α-D-glucopyranoside	Degn, 1999
n-Octyl, *n*-decyl β-D-glucoside	Lauric acid	Acetonitrile, acetone, *tert*-butanol	CALB	50°C	Lauryl sacharide	Watanabe, 2000
Glucose	Lauric acid, palmitic acid, stearic acid	Acetone	CALB	30-60°C, 80 rpm	Glucose ester	Arcos, 2001
Fructose	Palmitic acid	2-Methyl-2-butanol	CALB	60°C, 100 rpm	Fructose palmitate	Chamouleau, 2001
Fructose	Saturated fatty acid	2-Methyl-2-butanol	CALB	60°C, 200 rpm	Fructose ester	Soultani, 2001
Mannose	Lauric acid	Acetonitrile, acetone, 2-methyl-2-butanol, 2-methyl-2-propanol	CALB	50°C	6-*O*-Lauryl mannose	Watanabe, 2001
Glucose	Stearic acid	Ethyl methylketone	CALB	60°C, 250 rpm	6-*O*-Stearoyl-D-glucose	Yan, 2001

Table 8. (Continued).

Acyl acceptor	Acyl donor	Solvent	Lipase	Condition	Product	Reference
Glucose	Lauric acid	2-Methyl-2-butanol	CALB	60°C, 500 rpm	Glucose laurate	Flores, 2002
L-ascorbic acid	Oleic acid	2-Methyl-2-butanol	*Candida* sp. lipase	55°C, shaking	Oleoyl ester of L-ascorbic acid	Song, 2002
Xylitol	Oleic acid	2-Methyl-2-propanol:DMSO (80:20 v/v)	CALB	45°C, 200 rpm	Xylitol-oleic acid monoester	Castillo, 2003
Glucose	Octanoic acid	Acetonitrile	CALB	50°C, 2.7 rps	6-*O*-octanoyl-β-D-glucoside	Kobayashi, 2003
L-acorbic acid	Unsaturated fatty acids	Acetone	CALB	60°C, vigorous shaking	6-*O*-Unsaturated acyl-L-ascorbates	Kuwabara, 2003
Glucose	Fatty acid vinyl ester	Ionic liquid:*tert*-butanol (3:2 v/v)	CALB	60°C, 900 rpm	Glucose fatty acid ester	Ganske, 2005
Fructose	Palmitic acid	Ethyl methylketone, *tert*-butanol	CALB	60°C, 600 rpm	Fructose palmitate	Sabeder, 2006
Fructose, glucose	Palmitic acid	2-Methyl-2-butanol	CALB	40°C, 200 rpm	Glucose monopalmitate, fructose mono-and dipalmitate	Sakaki, 2006

The acylation of sugar derivatives such as ascorbic acid was also reported. The synthesis of oleoyl ascorbate and palmityl ascorbate by immobilized lipase in 2-methyl-2-butanol showed the conversion yield of 16.8 and 19 g/L, respectively (Humeau *et al*., 1998; Song and Wei, 2002).

The regioselctivity of lipase of the sugar on the synthesis of SE is the most important because the hydroxyl groups of sugars are different. In acylation reactions, the primary hydroxyl group will reacts first, followed by the secondary hydroxyl group. The relative reaction among the secondary hydroxyl group in either acylation or hydrolysis of the esters remains difficult to predict because it varies with the lipases, reaction conditions and structure of sugars. Not all reactions follow the generalization, for example, lipases sometimes acylate the secondary hydroxyl group in the presence of the primary hydroxyl group (Bronscheuer and Kazlauskas, 1999).

3.3.4 Acyl donors

The chemical nature of the acyl donor is important for the reaction set up. If an acid is used as acyl donor, a system to remove water must be used to obtain the high conversion yield. If an acyl ester is used as acyl donor (alcoholysis) water is no produced by the reaction (Degn, 2000). Lipases have specificity toward the acyl donor, which are different in the chain length of carbon. Many acyl donors such as saturated fatty acid, unsaturated fatty acid, fat/oil and fatty acid derivatives, are used in sugar ester synthesis. The chain length of acyl donor is very important because the surfactant property of SE is closely linked to their structure. The ester synthesized from short chain fatty acids are oil in water surfactant while with long chain fatty acids are water in oil surfactant (Coulon *et al*., 1997).

Cao *et al*., (1996) reported the effect of chain length of acyl donors on glucose ester synthesis in acetone catalyzed by CALB. The high conversions were obtained whit saturated long chain fatty acids ranging from C_{12} to C_{18}. The maximum conversion was obtained from stearic acid with yield of 92% after 72 h of reaction time while a monounsaturated fatty acid, oleic acid, yielded only 53%.

The medium chain fatty acid as caproic and caprylic acids gave relative low conversion yield of 45 and 51%, respectively. In contrast, the synthesis of fructose ester using fatty acid methyl esters with different chain length of fatty acid (C_{10}-C_{18}) as acyl donors in 2-methyl-2-butnaol catalyzed by CALB had no effect on the synthesis (Coulon *et al*., 1997). However, the effect of fatty acid chain length on maltose ester synthesis was also investigated that the initial rates and conversion yield of maltose ester synthesis catalyzed by CALB were decreased when increasing the chain length of fatty acid from C_4 to C_{12}. The highest conversion yield of 40.1 mol/mol with butyric acid at 144 h was obtained while lauric acid yielded only 3.6 mol/mol at the same conditions (Pedersen *et al*., 2002).

The fatty acid derivatives are also used as acyl donors for sugar ester synthesis. Ikeda *et al.* (1993) reported the use of fatty acid derivatives to synthesize glucose ester in anhydrous *tert*-butanol with phenylboronic acid as solubilizing agent catalyzed by *Pseudomonas* sp. lipoprotein lipase. They found that the highest conversions of 100% were obtained when used vinyl butyrate and trifluoroethyl butyrate as acyl donors. Recently, the influence of acyl donor chain length of vinyl ester of fatty acid between C_6 and C_{16} on glucose ester synthesis in solvent mixture of ionic liquid and *tert*-butanol catalyzed by CALB at 50°C was reported by Ganske and Bornscheuer (2005). They found that the maximum conversion of 60% was obtained with myristic acid vinyl ester while palmitic acid vinyl ester and short chain fatty acid vinyl ester as capric acid, caprylic and caproic acid gave low conversion (less than 30%).

Only few reports have been shown the possibility of using TAG as acyl donor for SE synthesis. Chopineau *et al.* (1987) reported the synthesis of sorbitol monoester using various type of edible oils e.g. corn, lard, olive, soybean and sunflower oil as acyl donor catalyzed by (porcine pancreas lipase) PPL in pyridine. They found that all of edible oil showed possibility to acylate with sorbitol. The highest conversion of 35% was obtained with corn oil. Similar results were reported by Ikeda *et al.* (1993) that synthesis of glucose ester with various triacylglycerols in anhydrous *tert*-butanol with phenylboronic acid as solubilizing agent catalyzed by *Pseudomonas* sp. lipoprotein lipase was achieved. The conversions yield of 54, 47, 42 and 41% were obtained with apricot seed, cotton seed, olive and corn oil. Though, TAG can be used as acyl donor in sugar ester synthesis but the system used is not compatible for food application due to using of very toxic organic solvent.

3.3.5 Molar ratio of acyl acceptor to acyl donor

The enzymatic production of SE is also affected by the molar ratio of sugar to fatty acid which seems to be a major role. Normally fatty acids will be acylated only at the primary hydroxyl group of sugar with molar ratio of 1:1. So the molar of fatty acid is depended on the sugar used. For example glucose has only one of primary hydroxyl group at C-6 position. Hence the suitable molar ratio of acyl donor to acyl acceptor might be 1:1. In contrast, fructose has 2 primary hydroxyl groups at C-1 and C-6 positions and other polyols e.g. sorbitol, mannitol and xylitol have more than 2 primary hydroxyl groups. So, more than 1 molecule of fatty acid can be esterified with fructose or sugar alcohols.

The effect of molar ratio on glucose ester synthesis in acetone catalyzed by Novozym 435 was reported. Increasing of the fatty acid concentration did not lead to increase in the productivity and conversion yield of glucose palmitate. The two fold excess of palmitic acid increased only 6% of the conversion yield (Cao *et al.*, 1996). Similar results were reported that only 3% of conversion yield increased when increased the molar ratio of glucose to palmitic acid

from 1:1 to 1:5 of the synthesis of glucose palmitate in 2-methyl-2-butanol catalyzed by CALB (Sakaki *et al*., 2006).

The effect of molar ratio on SE synthesis with fructose and polyols was reported. Effect of molar ratio of fructose to lauric acid on synthesis of fructose laurtate catalyzed by CALB in acetone had been reported that increasing of lauric acid concentration from equi-molar to 3:1 and 5:1 to fructose, the conversion yield was increased from 45 to 65 and 94%, respectively (Arcos *et al*., 1998). Similar results were obtained with the synthesis of fructose oleate catalyzed by CALB that increasing of oleic acid to fructose from equi-molar to 5:1 mol/mol increased the productivity of 44 g/L to 56 g/L and initial rate from 0.27 to 0.46 g/h.g, respectively (Coulon *et al*., 1999). The esterification of sorbitol with lauric acid in acetone catalyzed by CALB was studied by Arcos *et al*. (1998). They found that the conversion yield of sorbitol monolaurate did not increase when increased the molar ratio of lauric acid to sorbitol from 1:1 to 5:1 mol/mol. However, sorbitol dilaurate conversion yield dramatically increased from 45 to 85% at molar ratio of lauric acid to sorbitol 1:1 and 5:1 mol/mol, respectively.

3.3.6 Water content

Although the esterification reaction is carried out in organic solvent, it is essential that some water is present in the system. If a reaction system is completely depleted of water, the enzyme will lose its three dimensional structure and becomes catalytically inactive. On the other hand, the amount of water needed to maintain catalytic activity of enzyme, is depended on the type of enzymes (Degn, 2000). Water plays a very important role in enzymatic esterification reactions in organic solvents. The water concentration affects the productivity yield because esterification is an equilibrium reaction. Furthermore, the water content influences the enzyme conformational stability in organic solvent (Sakaki *et al*., 2006).

Several methods have been used for the removal of water produced during esterification in organic solvent such as addition of molecular sieves, azeotropic distillation, gas sparging and pervaporation. Among these techniques, addition of molecular sieves is more attractive because this method is easy to manage and reusing of molecular sieves. The molecular sieves are added to the reaction medium to reduce the effect of competition of hydrolysis reaction. However, the initial water content of the esterificaion reaction will be influenced by the water content of enzyme, substrate and solvent preparation. So the control of initial water of reaction mixture is necessary.

The initial water content of the substrate, enzyme and solvent will be adjusted before starting the reaction by using saturated salt solutions which have different water activity or a_w (Table 9). The effect of initial water on ascorbyl palmitate synthesis in 2-methyl-2-butanol catalyzed by CALB was studied by Humeau *et al*. (1998). The production yield of

ascorbyl palmitate of 5.7 g/L with initial a_w of 0.07 was obtained while a_w of 0.97 resulted only 1.0 g/L. It might be the competition of hydrolysis reaction was occurred with high water activity of reaction mixture.

Table 9. Water activity of saturated salt solution.

Saturated salt solution	Water activity (a_w)
LiBr	0.07
LiCl	0.11
CH_3COOK	0.25
$MgCl_2$	0.33
K_2CO_3	0.43
$Mg(NO_3)_2$	0.55
NaCl	0.75
$K_2Cr_2O_7$	0.97

Source : Humeau *et al.* (1998)

Chamouleau *et al.* (2001) reported the effect of initial water content of fructose monopalmitate synthesis in 2-methyl-2-butnaol catalyzed by CALB. The maximum conversion yield and initial rate of 16.5 g/L and 4.9 g/L.h were obtained with the initial water activity less than 0.07. They also reported the effect of molecular sieves adding, the conversion yield of fructose monopalmitate raised to 32.0 g/L as well as the initial rate raised to 10.1 g/L.h.

The effect of molecular sieves adding on the synthesis of L-ascorbyl oleate in 2-methyl-2-butanol catalyzed by immobilized lipase from *Candida* sp was reported by Song and Wei (2002). They found that the conversion yield of 18.5 g/L of L-ascorbyl oleate was obtained after 10 h of reaction with 50 g/L molecular sieves while only 14.0 g/L of the products was obtained with out molecular sieves adding.

3.3.7 Temperature

The reaction temperature has a great influence on sugar ester synthesis. There are two effects of reaction temperature, first the solubilization of sugar and product. Another is an increase of reaction rate constant of the ester synthesis (Arcos *et al.*, 1998). Though, increasing of temperature can activate the enzyme activity but the high temperature also can denature the enzyme. The effect of temperature on glucose ester synthesis in acetone catalyzed by CALB with the temperature range of 5-60°C was studied by Arcos *et al.* (1998). They found that the

conversion yield of 25, 80, 98 and 97% were obtained with the reaction temperature of 5, 20, 40 and 60°C, respectively.

Degn and Zimmermann (2001) reported the effect of temperature on enzyme activity and stability in the synthesis of glucose myristate catalyzed by CALB. They found that the synthetic activity of CALB increased from 14.1 to 20.2 μmol/min.g but the residual activity was 79 and 51% when increased temperature from 45 to 60°C, respectively.

Sabeder *et al.* (2006) reported the effect of temperature on fructose palmitate synthesis in 2-methyl-2-butanol catalyzed by CALB. The conversion yield after 72 h of reaction time increased to 78% at 60°C while 50 and 60% conversion yield were obtained at 30 and 40°C, respectively. However, at the higher temperature (70°C) showed very low conversion yield (1.3%) because the enzyme was thermal inactivation.

4. Production, purification and characterization of microbial lipases

4.1 Microorganism producing lipase

Lipases are produced by many microorganisms included bacterial yeasts, moulds and *Actinomycetes*. Most commercial lipases are from microbial sources. Some of lipases producing microorganisms are summarized in Table 10.

4.2 Isolation and screening of lipase-producing microorganisms

Lipase-producing microorganisms have been found in diverse habitats such as industrial wastes, vegetable oil diary and factories, oil-contaminated soil, oil seeds, decaying foods, compost heaps, coal tips and hot spring (Sharma *et al.*, 2001). The simple and reliable method for detecting lipase activity in microorganisms has been reported by Sierra (1957). This method used the surfactant (Tween 80) on agar medium supplemented with TAG to identification a lipolytic activity of lipase-producing microorganisms. Tributyrin was frequently used as a substrate and the formation of clear zone around colony was an indication of lipase producing by the microorganisms (Cardenas *et al.*, 2001).

Screening systems using of the indicator for detecting lipolytic activity have been described. Wang *et al.* (1995) used a modified Rhodamine B agar to screen lipase activity in a large number of microorganisms. The lipase-producing microorganisms showed clear zone around orange-pink colony. Nile blue and Cu^{2+} salts were also used as an indicator for detecting lipolytic activity (Sharma *et al.*, 2001).

4.3 Production media for lipase

Microbial lipases can be produced by submerged culture and solid state fermentation methods. Many of the studies have been studied on the optimization of culture and nutritional requirements for lipase production by submerged culture. Lipase production is influenced by physico-chemical conditions which are type and concentration of carbon sources, nitrogen sources, pH, cultivation temperature and dissolved oxygen. Lipidic carbon sources seem to be generally essential for obtain high lipase yield, however, a few authors have produced good yield in the absence of fat ands oils (Sharma *et al.* 2001).

4.3.1 Effect of carbon sources

Fats and oils are the traditional carbon source for lipase production. The production of lipase from *Pseudomonas fluorescens* S1K was reported. The specific activity (7,395 U/mg protein) with tricaprylin (C_8) was obtained when cultivation in a medium supplemented with emulsified olive oil (Lee *et al.*, 1993). Similarly, production of lipase from *Candida rugosa* in medium supplemented with 10% olive oil yielded 9.3 U/mL (Benjamin and Pandey, 1996).

Rhati *et al.* (2001) compared different vegetable oil (palm oil, coconut oil, mustard oil, linseed oil, jasmine oil and rose oil) for lipase production from *Burkholderia cepacia*. The highest activity (23.2 U/mL) was achieved using palm oil. However, when this strain was cultivated in the same medium and supplemented with 1.0% glucose, the highest activity (31.2 U/mL) was obtained.

Other carbon sources are also used for lipase production. Gordillo *et al.* (1995) reported the production of lipase from *C. rugosa* in batch culture was affected by initial concentration of oleic acid. The maximum lipase activity was obtained when cultivated in 2 g/L of oleic acid. The production of lipase from *Pseudomonas* sp. using *n*-alkane, oils and sugars showed that the maximum lipase activity and cell growth of 25.0 U/mL and 4.2 g/L were obtained with *n*-hexadecane (Kanwar *et al.*, 2002). When Tween 80 was used for lipase production the *Acinetobacter radioresistens* yielded lipase activity of 120 U/mL (Li *et al.*, 2001).

Table 10. Literature reviews of some lipase-producing microorganisms.

Source	Genus	Species	Reference
Gram-positive bacteria	*Bacillus*	*B. megaterium*	Godtfredsen, 1990
		B. brevis	Hou, 1994
		Bacillus sp. IHI-91	Becker *et al.*, 1997
		B. thermocatenulatus	Rua *et al.*, 1998
		B. stearothermophilus	Kim *et al.*, 1998
		Bacillus sp. THL027	Dharmsthiti and Luchai, 1999
		B. thermoleovorans ID-1	Lee *et al.*, 1999
		B. alcalophilus	Ghanem *et al.*, 2000
		B. sp. J33	Nawani and Kaur, 2000
	Staphylococcus	*S. aureus*	Lee and Yandolo, 1986
		S. hyicus	Meens *et al.*, 1997
		S. epidemidis	Simons *et al.*, 1998
	Lactobacillus	*Lactobacillus* sp.	Mayers *et al.*, 1996
	Streptococcus	*Streptococcus lactis*	Sztajer *et al.*, 1988
Gram-negative bacteria	*Pseudomonas*	*P. cepacia*	Terstappen *et al.*, 1992
		P. putida 3SK	Lee and Rhee, 1993
		P. fluorescens	Kojima *et al.*, 1994
		P. pseudoalcaligenes F-111	Lin *et al.*, 1996
		P. aeruginosa KKA-5	Sharon *et al.*, 1998
		Pseudomonas sp. Y0103	Kim *et al.*, 1997
		Pseudomonas sp. KWI56	Yang *et al.*, 2000
	Burkholderia	*Burkholderia* sp.	Yeo *et al.*, 1998
		B. glumae	El Khattabi *et al.*, 2000
		B. multivorans	Gupta *et al.*, 2005
	Chromobacterium	*Ch. viscosum*	Jaeger and Reetz, 1998
	Acinetobactor	*Aci. Radioresistens*	Chen *et al.*, 1999
Fungi	*Rhizopus*	*Rhizop. japonicus*	Nakashima *et al.*, 1988
		Rhizop. oryzae	Salleh *et al.*, 1993
		Rhizop. microsporus	Ghosh *et al.*, 1996
		Rhizop. chinensis	Ghosh *et al.*, 1996

Table 10. (Continued).

Source	Genus	Species	Reference
		Rhizop. delemar	Klein *et al.*, 1997
		Rhizop. niveus	Kohno *et al.*, 1999
		Rhizop. arrhizus	Elibo and Ozer, 2001
		A. oryzae	Ohishi *et al.*, 1994
		A. niger	Chen *et al.*, 1995
		A. flavus	Long *et al.*, 1996
	Aspergillus	*A. carneus*	Helisto and Korpela, 1998
		A. repens	Kaminishe *et al.*, 1999
		A. nidulans	Mayordomo *et al.*, 2000
		Pe. roqueforti	Petrovic *et al.*, 1990
		Pe. fumiculosum	Hou *et al.*, 1994
		Pe. camamberttii	Ghosh *et al.*, 1996
	Penicillium	*Pe. wortmanii*	Costa and Peralta, 1999
		Pe. cylopium	Chahinian *et al.*, 2000
	Mucor	*Mu. miehie*	Plou *et al.*, 1998
		Mu. racemosus	Ghosh *et al.*, 1996
Yeasts	*Geotrichum*	*Geotrichum* sp.	Macedo *et al.*, 1997
		G. candidum	Sugihara *et al.*, 1998
	Humicola	*H. lanuginosa*	Zhu *et al.*, 2001
	Rhizomucor	*R. miehei*	Jaeger and Reetz, 1998
	Candida	*C. rugosa*	Wang *et al.*, 1995
		C. antarctica	Jaeger and Reetz, 1998
		C. cylindracea	Helisto and Korpela, 1998
	Yarrowia	*Y. lipolytica*	Pignede *et al.*, 2000
	Pichia	*Pi. bispora*	Hou *et al.*, 1994
		Pi. sivicola	Sugihara *et al.*, 1995
		Pi. burtonii	Sugihar *et al.*, 1995
Actinomycetes	*Streptomyces*	*S. cinnamomeus*	Sommer *et al.*, 1997
		S. rimosus	Abramic *et al.*, 1999

4.3.2 Effect of nitrogen sources

The production of lipase is affected by type and concentration of nitrogen sources. The organic and inorganic nitrogen sources such as yeast extract, peptone, polypeptone, meat extract, beef extract, corn steep liquor, soybean meal, ammonium nitrate, ammonium chloride, ammonium hydrogenphosphate, ammonium sulphate, sodium nitrate and urea are reported to be used as nitrogen source for lipase production. The requirement of type of nitrogen source varied among microorganisms, some prefer inorganic form while others prefer organic nitrogen.

The use of many inorganic nitrogen sources for lipase production from *Pseudomonas* sp. using *n*-hexadecane as carbon source was reported. The highest lipase activity (25.0 U/mL) was obtained with ammonium nitrate 0.5% (w/v). Production of extracellular lipase from *Burkholderia cepacia* in the medium with organic or inorganic nitrogen sources (0.2% w/v) did not significantly affect productivity. The highest lipase activity of 35 U/mL was obtained with corn steep liquor and lowest of 32 U/mL was obtained with ammonium nitrate (Rathi *et al*., 2001). The production of lipase from *Bacillus coagulans* BTS-3 in the medium using refined mustard oil as carbon source showed that the mixture of peptone (0.5%) and yeast extract (0.5%) was the best nitrogen source. This strain produced highest lipase activity with 0.67 U/mL while only 0.12 and 0.43 U/mL were obtained when it was cultivated in the medium supplemented with urea and ammonium sulphate (Kumar *et al*., 2005).

4.3.3 Effect of pH and temperature

The initial pH and cultivation temperature also affect on lipase production. The optimum pH and temperature for cultivation of lipase are varied based on microorganism types. For example, the optimum pH and temperature for lipase production from fungus e.g. *Aspergillus terreus* were pH 9.0 and 37°C (Gulati *et al*., 1999), *Rhizopus oryzae* were pH 8.5 and 30°C (Essamri *et al*., 1998) and *Fusarium solani* were pH 8.0 and 40°C (Maia *et al*., 2001). While the thermophilic microorganisms showed broad optimum pH and temperature e.g. *Bacillus coagulans* BTS-3 were pH 8.5 and 55°C (Kumar *et al*., 2005), *B. thermoleovorans* CCR11 were pH 6.5 and 55°C, *Bacillus* sp. THL027 were pH 7.0 and 65°C (Darmsthiti and Luchai, 1999), *Bacillus* sp. RSJ-1 were pH 9.0 at 65°C (Sharma *et al*., 2002) and *Bacillus* sp J33 were pH 8.0 and 60°C (Nawani *et al*., 1998).

4.4 Purification and characterization of microbial lipases

Many lipases have been extensively purified and characterized in terms of their activity and stability profiles relative to pH, temperature and effect of metal ions and chelating agents. Purification methods used have generally depended on nonspecific techniques such as precipitation, hydrophobic interaction chromatography (HIC), gel filtration and ion-exchange

chromatography. Affinity chromatography has been used in some cases to reduced the number of individual purification step needed (Sharma *et al*., 2001).

4.4.1 Prepurification step

Most of the microbial lipases are extracellular and the fermentation process is usually followed by the removal of cells from the culture broth, either by centrifugation or by filtration. The cell-free culture broth is then concentration by ultrafiltration (UF) or ammonium sulphate precipitation or extraction with organic solvents. About of 80% of purification reports use precipitation method as a concentration method. Ammonium sulphate is commonly used for precipitation protein while using of ethanol or acetone is less. Precipitation is usually used as the first step for purification and is followed by chromatographic separations (Saxena *et al*., 2003).

4.4.2 Chromatographic steps

Most of the time, a single chromatographic step is not sufficient to get the required level of purity. Hence, a combination of chromatographic steps is required. Ion exchange chromatography is the most common chromatographic methods. The most frequently employed ion-exchanges are the diethylaminoethyl (DEAE) group in anion exchange and the carboxymethyl (CM) in cation exchange and strong ion exchangers based on triethylaminoethyl groups and Q-Sepharose are becoming more popular in lipase purification. Gel filtration is the second most frequently employed purification method schemes. Hydrophobic interaction chromatography has been used in 18% of the cases with the most popular hydrophobic adsorbents being octyl or phenyl functional groups (Saxena et. al., 2003).

Affinity chromatography has been used as a purification step in 27% of schemes. This method can be applied at the first step but as the materials are expensive, the less costly ion exchange and gel filtration are usually preferred after the precipitation step. Although gel filtration has lower capacity for loaded protein but it can be used at the early stage for fine polishing of the protocol (Saxena *et al*., 2003). Different procedures used in purification of lipase from various microorganisms have been presented in Table 11.

Table 11. Purification procedures for various microorganisms.

Microorganism	Purification steps	Recovery (%) and purification factor	Molecular mass (kDa)	Reference
Gram positive bacteria				
Bacillus sp. THL027	Ultrafiltraion and Sephadex G-100	27.2 and 2.5	69	Dharmsthiti and Luchai, 1999
Bacillus sp. H-257	Acetone precipitation, Qctyl-Sepharose CL-4B, Q-Sepharose and Superose 12	20 and 3,028	25	Imamura and Kitaura, 2000
Bacillus sp. J33	Ammonium sulphate precipitation and Phenyl sepharose	15.6 and 175	45	Nawani and Kaur, 2000
Bacillus sp. RSJ-1	Ultrafiltration, ammonium sulphate precipitation, Q-Sepharose and Sephacryl S-200	19. 7 and 201.4	37	Sharma *et al*., 2002
B. stearothermophilus MC7	Ultrafiltration, Sephadex G-100 and DEAE-cellulose	10.2 and 19.2	62.5	Kambourova *et al*., 2003
B. coagulans BTS-3	Ammonium sulphate precipitatin and DEAE-Sepharose	2.5 and 40	31	Kumar *et al*., 2005
B. coagulans MTCC-6375	DEAE-cellulose and Octyl Sepharose	- and 76.4	103	Kanwar *et al*., 2006

Table 11. (Continued).

Microorganism	Purification steps	Recovery (%) and purification factor	Molecular mass (kDa)	Reference
Gram negative bacteria				
Pseudomonas putida 3SK	DEAE-Sephadex A-50 and Sephadex G-100	21% and 5.3	45	Lee and Rhee, 1993
Burkholderia multivorans	Adsorption on Accurel and desorption by Triton X-100 and acetone precipitation	66 and 3.0	30	Gupta *et al*., 2005
P. aeruginosa san-ai	Ammonium precipitation, Butyl-Toyopearl and Toyopearl HW-55	16 and 12.5	54	Karadzic *et al*., 2006
Acinetobacter sp. RAG-1	Ultrafiltration, Mono Q and Butyl Sepharose	22.4 and 10.7	33	Snellman *et al*., 2002
Aeromonas sp. LPB 4	Acetone precipitation and QAE sephadex DEAE-cellulose, CM-cellulose,	7.5 and 53.5	50	Lee *et al*., 2003
Actinomycetes				
Streptomyces rimosus	Hydorxylapatite, Mono S and Sephadex G-75	12.5 and 51.5	27.5	Abramic *et al*., 1999

Table 11. (Continued).

Microorganism	Purification steps	Recovery (%) and purification factor	Molecular mass (kDa)	Reference
Basidiomycetes				
Antrodia cinnamomea BCRC 36396	Ammonium precipitation and Phenyl Sepharose	33.7 and 17.2	60	Shu *et al*., 2006
Yeast				
Cryptococcus sp. S-2	Ultrafiltration and SP-5PW	11.4 and 17.1	22	Kamini *et al*., 2000
Fungi				
Aspergillus terreus	Ammonium sulphate precipitation, acetone precipitation, Sephadex G-100	18.0 and 11.9	41	Yadav *et al*., 1998
Mucor hiemalis f. *hiemalis*	Ultrafiltration, ammonium sulphate precipitation, sephadex G-75, Q-Sepharose and Sephacryl S-200	18.1 and 2,200	49	Hiol *et al*., 1999
A. carneus	Ammonium sulphate precipitation and Octyl Sepharose	7.5 and 53.5 38.4 and 24.1	50 27	Lee *et al*., 2003 Saxena *et al*., 2003

Objectives

1. To study the synthesis of sugar esters (SE) from palm oil and palm fatty acid distillates (PFAD) catalyzed by immobilized lipases.
2. To study the production of bacterial lipase, purification, characterization and application for SE and fatty acid methyl esters (FAME) synthesis.

Scope of Research Work

1. Optimization of SE synthesis from PFAD by immobilized lipase.
2. Screening of lipase producing bacteria, optimization of lipase production, purification and characterization of bacterial lipase.
3. Optimization of SE synthesis by selected immobilized lipase.
4. Optimization of FAME synthesis by selected immobilized lipase.