Literature Review

1. Palm Olein

Palm oil gives a more valuable olein (an excellent frying oil) and a less valuable stearin (used in part as a replacement for tallow in the oleochemical industry). The oils and their fractions can be further modified by blending with other oils, by partial hydrogenation, or by interesterification. Further fractionation of the palm oil fractions yields an intermediate fraction (PMF, palm midfraction) that can be used as a cocoa butter extender (Gunstone, 1997). Palm oil is characterized by high levels of carotene and of tocopherols and tocotrienols (vitamin E), which can also be isolated or concentrated as products of considerable value. Palm oil fatty acid distillate (PFAD), a by-product of the principal refining procedure, is an important component of animal feed (Gunstone, 1997).

Palm oil is widely used as a food oil with limited non-food uses also. It differs from other commercially available oils in its fatty acid and triacylglycerol (TAG) composition. It contains almost equal amounts of saturated acids (mainly palmitic with some stearic) and unsaturated acids (mainly oleic with some linoleic acid). The proportion of palmitic and oleic acids in the major TAG leads to stability of the crystals. These are very desirable in the production of margarines and shortenings, especially those with high levels of unsaturated acids.

2. Lipases

Lipases or glycerol ester hydrolases (EC 3.1.1.3) were originally employed for the hydrolysis of ester bonds of TAG to produce free fatty acid (FFA), glycerol (G) and partial acylglycerols (monoacylglycerol, MAG and diacylglycerol, DAG). Lipases occur widely in animals, plants and microorganisms. Numerous mammalian tissues, organs and fluids, such as pancreas, kidney, adipose tissue, heart, brain, muscle and serum, have been known to contain lipases. Among animal lipases pancreatic lipase has been studied most extensively. The hog pancreatic lipase has been studied most extensively, presumably because of its high concentration (2.5% of the total protein in the pig pancreatic juice) and high turnover number. During recent years considerable attention has been devoted to lipases produced by microorganisms,

presumably because of their stability and their practical medical and industrial applications. Several microorganisms produce intracellular or extracellular lipases. Especially, extracellular microbial lipases have high potential for application and are appropriated for mass production. A variety of microorganisims produce lipases. These include the genera of *Candida* yeast; *Rhizopus, Penicillium, Aspergillus, Geotrichium* and *Mucor* molds; and *Pseudomonas, Achromobacter,* and *Staphylococcus* bacteria (Godtfredsen, 1993).

Lipases catalyze three types of reaction. The catalytic action of lipases is reversible. It catalyzes ester synthesis in a microaqeous system. However, in view of biotransformation of oleochemical industry yielding value-added products, the transesteritication action seems more worthwhile than hydrolysis and ester synthesis. The difference in free energy involved in TAG hydrolysis is quite small and the net free energy of transesterification is zero. Gandhi (1997) suggested that lipase catalyzed reaction has classically been divided into two main categories: (i) hydrolysis, and (ii) synthesis. Reactions under synthesis category can be further separated: (a) esterification, (b) interesterification, (c) alcoholysis and (d) acidolysis (Figure 1).

(i) Hydrolysis

$$RCOOR' + H_2O \longleftrightarrow RCOOH + R'OH$$

(ii) Synthesis

(a) Esterification

$$RCOOH + R'OH \longleftrightarrow RCOOR' + H_2O$$

(b) Interesterification

$$RCOOR' + R''COOR''' \longleftrightarrow RCOOR''' + R''COOR'$$

(c) Alcoholysis

$$RCOOR' + R''OH \longleftrightarrow RCOOR'' + R'OH$$

(d) Acidolysis

$$RCOOR' + R''COOH \longleftrightarrow R''COOR' + RCOOH$$

Figure 1 Types of reaction catalyzed by lipases.

Source : Gandhi (1997)

Lipases obtained from natural sources can be positionally nonspecific or display one of two kinds of positional specificity: *sn*-1,3 specific or *sn*-2 specific. Non-specific lipases hydrolyse all three ester bonds of triglycerides equally well. Nonspecificity has been observed for lipases from *Chromobacterium viscosum*, *Pseudomonas fluorescens*, *Candida cylindracea*, *Geotrichum candidum*, and *Penicillium cyclopium*, and also for hepatic lipase. Specificity of the *sn*-1,3 type is associated with the preferential release of fatty acid residues from the terminal positions of the glycerol backbone rather than from the central carbon atom, whereas *sn*-2 specificity refers to preferential release from the central carbon atom. The *sn*-1,3 type of specificity has been observed for pancreatic and adipose tissue lipases and lipase from microorganism such as *Rhizopus arrhizus*, *Aspergillus niger*, *Rhizopus delemar* and *Mucor miehei*. The *sn*-2 specificity is extremely rare, and it has been ascribed to a lipase from *Geotrichum candidum* which has a particular ability to

hydrolyse oleic and linoleic acids from the *sn*-2 location. A more general classification states that the positional specificity of lipases is not divided clearly into the above categories; instead it changes continuously from highly specific an-1,3 activity to a very weakly specific or completely nonspecific activity (Malcata *et al.*,1992).

Lipase can be employed in the production of pharmaceuticals, cosmetics, leather, detergents, foods, perfumery, medical diagnostics, and other organic synthetic materials (Gandhi, 1997). Moreover, lipases have found applications in various fields of biotransformations. These can be classified according to the nature of the substrates into three main categories: (i) modification of fats and oils, (ii) acylation/deacylation of carbohydrates and protecting/deprotecting of peptides and (iii) synthesis of chiral compounds. The major focus will be given to the first field. These can be classified depending on the targeted product into the synthesis of MAG, an important class of emulsifiers and the synthesis of structured triglycerides, which are used as, e.g., cocoa-butter equivalents or in nutrition. Furthermore, lipases have found some special applications such as in the selective enrichment of specific fatty acids.

3. Immobilized lipases

Lipases are normally used in an immobilized form in industry because reuse or continuous use of the immobilized lipase is made possible and the separation of the product from the enzyme is easy. The stability of lipase is often increased by immobilization. The advantages of the various types of available enzyme reactors can also be more readily exploited by using immobilized lipases, especially the use of packed-bed reactors. Brady *et al.* (1988) searched for adsorbents suitable as supports for lipases. Adsorbents, such as celite, cellulose, ethyl cellulose, silica gel, kieselguhr, clay, alumina, CPG-100, carbon, Accurel, Celgard 2500, Profax PP, Microthene HDPE, etc., were screened as possible immobilization supports. Most of hydrophilic materials were found to decrease tremendously the lipase activity upon immobilization. On the other hand, hydrophobic microporous materials such as Accurel and Celgard 2500 were found to provide better performances (Brady *et al.*, 1988).

4. Kinetics of reaction catalysed by immobilized lipases

For a simple enzymatic reaction in soluble, the maximum intrinsic rate of reaction is limited by the rate at which lipase and substrate come together in the proper orientation. For the case of hydrolysis reaction, the substrate is often part of the disperse phase of an emulsion, a micelle, or a monolayer which contacts water. These structures may be orders of magnitude larger in size than the supported enzyme for the case where the carrier exists in powdered form. Thus, the maximum attainable rate is limited by the amount of lipase which can interact with the substrate continuum. In the case of lipases immobilized on continuous supports, or on discrete supports larger in size than the individual droplets of substrate, the above reasoning remains valid provided that the spacing between neighboring molecules of immobilized lipase is still larger than the area of contact between the droplet and the lipase carrier. For this situation, in contrast to the classic Michaelis-Menten rate expression where the reaction rate increases linearly with the total enzyme concentration, a limiting rate is approached as the lipase concentration is increased. In the present case a balance on the total number of adsorption sites is more relevant than a balance on the total number of active sites. This approach leads one to the following rate expression :

$$\frac{-dC_S}{dt} = \frac{V_{max}\ C_E}{K_m + C_E}$$

where the constants are defined as $V_{max} = k_{cat}C_{S(tot)}$ and $K_m = k_{des} / k_{ads}$, and where the physical interpretations of the constants are as follows: V_{max} is the rate when the adsorption sites on the surface of the fat globule are saturated with lipase, and K_m is a pseudo-Michaelis-Menten constant for the above rate expression. When a lipase from *Candida rugosa* was immobilized by adsorption on cellulose, values for V_{max} and K_m changed from ca. 6.48 to 2.92 mol/min, and from ca. 3.88 to 0.54 mg/mL, respectively. The primary mechanistic distinction between this mechanism and the simple Michaelis-Menten mechanism is that in the present case adsorption of lipase at the fluid solid interface (i.e., contact with the substrate molecules) is independent of catalysis in the interfacial plane. Observed K_m values for lipases may thus reflect the extent of adsorption of the lipase at the lipid/water interface rather than the affinity between enzyme and substrate at the active site (Malcata *et al.*, 1992).

5. MAG production and application

MAG are the most widely used as emulsifiers in the food, pharmaceutical, and cosmetic industries (Thude *et al.*,1997). MAG and DAG are the most common food emulsifiers (McEvily and Zaks, 1991). They serve to stabilize emulsions in sauces and baked goods (Jackson and King, 1997). In cosmetic industries, they used as texturising agents in cosmetics (Stevenson *et al.*, 1993). Monopentadecanoylglycerol is used as a hair care additive (Bornscheuer, 1995). MAG of octanoic and decanoic acid can act as dyes and perfume bases in cosmetics, toiletries, and phamaceuticals. They are also known to dissolve gallstones in humans (Gandhi, 1997). Furthermore, oleyl monooleate is used in bath oils, cosmetic creams and lotions, hair preparations, makeup, skin preparations and pharmaceuticals, etc (Gandhi, 1997).

In the current method, MAG are manufactured on an industrial scale by continuous chemical glycerolysis of fats and oils at high temperature (220-250°C) employing inorganic alkaline catalysts under a nitrogen gas atmosphere (Sonntag, 1982). The product produced by this strategy has several drawbacks (Bornscheuer, 1995). A molar excess of glycerol is used and because the reaction temperature is greater than 220 °C, dark-colored by-products with an undesirable flavor are formed. Moreover, the yield of MAG is rather low (30-40%) (McNeill and Yamane, 1991). Molecular distillation is necessary because MAG need to be highly pure in the food industry, since they have better emulsifying properties than a mixture of different acylglycerols (Bornscheuer, 1995).

Unlike ordinary chemical catalysts, enzymes have ability to catalyze reactions under very mild conditions in aqueous solution at normal temperature and pressure reducing the possibility of damage to heat-sensitive substrates and also reducing the energy requirements and corrosion effects of the process. Enzymes have high substrate specificity leading to a low content of undesirable by-products in the reaction solution, thus decreasing not only material costs but also downstream environmental burdens (Kennedy and Cabral, 1987).

Three lipase-catalyzed routes to MAG have been described: (a) hydrolysis or alcoholysis of triglycerides, (b) glycerolysis of triglycerides and (c) esterification or transesterification of glycerol with fatty acids or esters. Method (a) yields an 2-MAG,

while methods (b) and (c) usually yield an equilibrium mixture of MAG, from which the predominant 1(3)-MAG can be isolated in good yield (Bornscheuer, 1995).

6. The influence of reaction condition on glycerolysis

6.1 Sources of lipases

Various lipases of commercial origin were tested with respect to their glycerolysis activities by Yamane *et al.* (1986). The amounts of enzyme giving the same hydrolytic activity were used in all of the experiments. They found that lipases produced by *Alcaligenes* sp., *Arthrobacter ureafaciens*, *Phycomyces nitens*, *Pseudomonas fluorescens* and *Rhizopus delemar* gave relatively high conversion, while lipases produced by *Aspergillus niger*, *Candida cylindracea*, *Mucor javanicus* and *Penicillium cyclopium* gave vary low conversions. Especially, *Chromobacterium viscosum* lipase gave the highest conversion.

6.2 Initial water content

To activate the lipase catalyst, it is essential to dissolve a trace amount of water in the glycerol phase (Li and Ward, 1993). A similar requirement has been previously reported for other lipase catalyzed reaction where a low water concentration was desired (Macrae, 1983). However, the moisture content of the glycerol phase must be maintained at low levels to avoid excessive production of FFA. As much as 12 % FFA is produced when greater than 8 % water is dissolved in the glycerol phase. Moreover, the yield of MAG is considerably reduced when 12 % water was used (McNeill *et al.*, 1990). For glycerolysis of hydrogenated beef tallow, FFA content at equilibrium depended on the water concentration in the glycerol phase. The initial rate of FFA formation was low and hardly affected by the moisture content between 0.5 and 4 %, but at higher water content (4-6.7 %), there was a small increase in the rate (Yamane *et al.*, 1994). Yang and Parkin (1994) found that yield of MAG formation by glycerolysis of butter oil using a gel-entrapped lipase increased with increasing water content up to 10 % in glycerol.

6.3 Glycerol/triacylglycerols molar ratio

Theoretically, 3 mol MAG were obtained when 1 mol TAG and 2 mol glycerol were used as substrates for glycerolysis. Bornscheuer and Yamane (1994) found that 96 % MAG was achieved when glycerol/triolein molar ratio of 2.7 was

used for glycerolysis of triolein in solid-phase system. A yield of 70 % MAG was obtained when glycerol/tallow mole ratio ranging from 1.5 to 2.5 was used in glycerolysis of tallow. The yield of MAG was independent of the glycerol/fat mole ratio at 5:1 or greater (McNeill *et al*, 1990). Furthermore, Yamane *et al.* (1994) reported that at low glycerol to tallow molar ratio (1:2), the main DAG product was obtained, Brady *et al.* (1988) and Yang and Rhee (1991) also reported that glycerol stabilized the lipase dramatically as the glycerol concentration was increased.