RESEARCH INAUGURAL LECTURE

Unlocking the Miracle of Lipases

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Contents

Abstract

Lipases or acylglycerol acylhydrolase (EC.3.1.1.3) have been defined as enzymes that hydrolyze esters of long-chain fatty acids from acylglycerol. Today, lipases stand amongst the most important biocatalyst carrying out novel reactions in both aqueous and non-aqueous media. Their ability to utilize a wide spectrum of substrates, and high stability towards extreme temperatures, pH and organic solvents are due to their capacity to work in both aqueous and non-aqueous conditions. Their power make them phenomenal and indispensable for bioconversion of lipid in nature. In addition to their biological significance, lipases hold tremendous potential for exploitation in biotechnology. The lipase selectivity is often crucial to its application for analytical and industrial purposes. Accordingly there is a need to look for new lipases with high activity, from less expensive sources, high selectivity towards fatty acids and high regioselectivity towards the *sn*-2 position of the acylglycerol.

Lipases find immense applications in various industries such as food, detergent, leather, textile, cosmetic, paper processing, oils and fats processing, nutrition/functional lipid and pharmaceutical. The last two applications are the most challenging and popular at the moment, as they can help improve human health and is a billion-dollar business. A few examples of functional lipids that are commercially available in the market are Betapol™ , Olestra, Salatrim, concentrating DHA and EPA, MCT oil and DAG oil.

In Malaysia, the potential applications of lipases have taken off to greater heights as we discovered newer applications of lipases. A technology for removing diacylglycerol (DAG) of crude palm olein has been filed for a Malaysia patent (PI No 20040900) by MARDI in 2004. This finding is related to a selective hydrolysis process for the removal of DAG from crude olein using selected lipases. The technology is expected to benefit existing oil palm refineries in Malaysia as it can increase the separation efficiencies

during the process of dry fractionation. This low DAG palm olein is also useful in the specialty fats industry as low DAG content hardens the fats and makes demoulding an easy task. In addition, this technology is expected to further expand both local and international markets for the Malaysian oil palm products.

Another significant finding is the production of DAG oil from palm oil as an anti-obesity functional oil. We have filed this technology in Malaysia, USA and Japan (Malaysia patent pending No PI 20056218, USA Patent Application Pending: Application Number 11/439,447, Japanese Patent Application Pending: Application Number 2006-170926). As obesity is now a serious global epidemic and has always been linked to life threatening diseases such as cardiovascular diseases, diabetes and hypertension, the development of healthful functional oils and fats such as DAG will surely benefit consumers. When consumed daily, DAG has been proven to reduce body fat and prevent obesity. Oil palm, being Malaysia's golden agriculture crop generates an annual output of 16 million metric tonnes of crude palm oil. Thus, it is timely to manufacture our very own Malaysian palm-based anti-obesity functional oil. This functional oil will command a high selling price in the international arena, hence enhancing the market value and providing a strong competitive advantage to palm oil.

Other major achievements include the production of value-added virgin coconut oil (VCO)/modified virgin coconut oil (MVCO) using selective lipases. MVCO is a novel product and thus has been filed for a local and an international patents (No PI 200557 and No PCT /MY2006/000028) covering 10 countries, namely, Malaysia, Indonesia, India, Philippines, Singapore, Japan, United Kingdom, USA, Germany and Ireland. This novel product contains an effective level of medium chain fatty acids and its corresponding monoacylglycerols, have the ability to kill gram-positive bacteria, gram-negative bacteria and fungus. Currently, this product offers several advantages over many synthetic antimicrobial/antifungal products that are available in the market. It is safe for long term application, easily absorbed into the skin, enriched with vitamin E and, most importantly, it is cheap. The technology is economically viable as the process conditions are simple. This new product is expected to revitalize the coconut industry that has been lagging behind other plantation-based crops in Malaysia.

Introduction to enzymes

Enzymes have been used by man throughout the ages, either in the form of vegetables rich in enzymes, or in the form of microorganisms used in brewing process, and in making tempe, tapai, yogurt, cheese etc. The history of modern enzyme technology really began in 1874 when a Danish chemist, Christian Hansen produced the first specimen of rennet by extracting dried calve stomachs with saline solution. Apparently this was the first enzyme preparation of a relatively high purity used for industrial purposes. In 1876, William Kuhne proposed that the name 'enzyme' be used as the term to refer to ferments isolated from viable organisms in which they were formed. The word itself means 'in yeast' and is derived from the Greek 'en' meaning 'in' and 'zyme' meaning 'yeast'.

In 1897, Eduard Buchner began to study the ability of yeast extracts to ferment sugar despite the absence of living yeast cells. In a series of experiments at the University of Berlin, he found that sugar was fermented even when there were no living yeast cells in the mixture. He named the enzyme that brought about the fermentation of sucrose as 'zymase'. In 1907, he received the Nobel Prize in Chemistry for his biochemical research and his discovery of cell-free fermentation. In 1965, a large scale and highly specific microbial fermentation was developed and huge quantities of bacterial amylases and proteinases were produced by submerged fermentation.

Enzymes are biocatalysts and most are protein consisting of long chains of amino acids held together by peptide bonds. They are present in all living cells, where they perform a vital function by controlling the metabolic processes. Enzymes bind temporarily to one or more of the reactants of the reaction they catalyse. In doing so, they lower the amount of activation energy needed and thus, speed up the reaction. Although they participate in the reaction, they themselves remained unchanged at the end of reaction.

Enzymes are very specific compared to inorganic catalysts such as acids, bases, metals and metal oxides. Enzymes exhibit a high specificity as they are able to discriminate the slight difference in substrate molecules. Furthermore, they have the ability to operate at moderate temperature, pressure and pH. These make them attractive catalyst for industrial use. *Ultimately enzymes are nature's gift to mankind and we have to discover and fully utilize them*. Presently, more than 3,000 different enzymes have been isolated and classified based on the nature of the reactions they catalyse.

These enzymes are classified into six major groups: oxidoreductases (Group 1), transferases (Group 2), hydrolases (Group 3), lyases (Group 4), isomerases (Group 5) and ligases (Group 6). Among these groups, hydrolases enzymes that catalyse a hydrolytic cleavage reaction are widely used for industrial purposes. More than 75% of industrial enzymes are hydrolases.

The enzyme market

Although world enzyme volumes have been consistently rising since 2002, the growth rate slowed somewhat in 2008, with CAGR of 1.3% (*Euromonitor International, Company Report, January 2009*). The growth in enzyme volumes is attributed to their role in lowering costs, increasing yield and their efficiency. Moreover new applications in baking and new development of functional food products have added to the volume growth.

Overall demand for enzymes will grow 7.6% through 2011 (*Euromonitor International Company Report, January 2009)*. A total of 12 major suppliers and 400 minor producers will meet the world demand for enzymes. Europe alone produces around 60% of the total world supply of industrial enzymes which are mainly hydrolytic enzymes, including proteases, carbohydrases (mainly amylases and cellulose) which account for the bulk volume for 2008. Lipases are only a small fraction of the 2008 volumes (21%) and are mainly used in detergent (*Euromonitor International Company Report, January 2009)*.

Data obtained from *Euromonitor International* showed that Turkey, China and USA were the top three consumers accounting for 56% of the top 10 volumes in 2008. The enzyme market is dominated by a few large players, such as Novozyme A/S and Genecor which are the main contributors (about 70% of market) and are major enzyme players for detergents. The Novozyme A/S markets its enzymes to approximately 130 countries including Malaysia and manufactures 75 types of enzyme with more than 500 products. The company is already targeting expansion in Asia, having established production, sales and R&D activities in the region. In 2007, Novozyme A/S got hold of the enzyme business of Biocon Ltd., the largest enzyme producer in India.

Microbial lipases

Lipases are placed only after proteases and carbohydrases in the world of enzyme market. However, they have the potential to increase market share due to a wide range of different applications discovered recently. Lipases are widely distributed in animals, plants and microorganisms. The demand for industrial enzymes, particularly of microbial origin, is ever increasing due to easy cultivation, stability and their applications in a wide variety of hydrolytic and synthetic reactions.

Microbial lipases are produced by fungi, yeast and bacteria. Some relatively smaller number of bacterial lipases have been well studied compared to fungal lipases (Sugiura 1984). Most of the bacterial lipases reported so far are constitutive and non-positional in their substrate specificity and a few bacterial lipases are thermostable. Among the bacterial lipases, the focus is usually on those from the genus *Pseudomonas* which are especially interesting for biotechnology because they exhibit the most versatility, reactivity and stability in catalysing reactions in a non-aqueous environment (Gao et al. 2000)

On the other hand, fungal and yeast lipases are widely exploited due to the low cost of extraction, thermal and pH stability, and positional specificity and activity in organic solvents. The main producers of commercial lipases mainly are from fungal and yeast likes *Aspergillus niger, Candida cylindracea, Humicola lanuginosa, Mucor miehei, Rhizopus arrhizus, Rhizopus delemer, Rhizopus japonica* and *Rhizopus oryzea* (Godfredson 1990). Lipases from *Geotrichum candidum, Rhizopus* and *Aspergillus* strains are attractive catalysts for lipid modification.

Geotrichum candidum lipases show preference for fatty acids containing a cis-9 double bond regardless of their positions in the triacylglycerol (Burkert et al. 2004; Loo et al. 2006). The lipases are used to obtain rapeseed oil enriched with polyunsaturated

fatty acids especially docosahexaenoic acid (DHA) (Shimada et al. 1995). Four species of *Aspergillus* lipase demonstrate high preference towards medium chain triacylglycerol and discriminate against triunsaturated triacylglycerol e.g. triolein (Long et al. 1998b). On the other hand, *Aspergillus flavus* Link shows a great substrate preference towards short chain triacylglycerol and less active towards triunsaturated triacylglycerol (Long et al. 1998a). This property has determined their biotechnological importance in industry as they can be used for the production of oil that contains high concentration of oleic acid.

Screening and selection of microbial production strains

In selecting the production strain, several aspects have to be considered. Ideally, the enzyme is secreted from the cell. This makes the recovery and purification process of the enzyme simpler compared to the production of intracellular enzymes. Secondly, the production host should have a GRAS-status (Generally Regarded as Safe). This is important when the lipase produced by the organism is used in food processing. Thirdly, the microbe should be able to produce high amount of the desired enzyme in a reasonable time frame. Most of the microorganisms used in the industries have been genetically modified to overproduce the desired lipase activity. The fermentation process and its optimization, which include media composition, cultivation type and process condition, have to be developed.

The cost of microbial lipase production depends on the cost of the fermentation to produce lipases, the cost of downstream processing (separation and purification) and the cost to stabilize the lipases. Lowering cost, increasing enzyme productivity and increasing enzyme stability will contribute to a more viable process. The enzyme production can be increased by selecting the best fermentation technique and optimization of culture conditions.

Submerged culture method has been the main cultivation technique used over the last two decades for the production of microbial enzymes. This submerged culture allows greater control of various parameters during fermentation such as temperature, pH, nitrogen composition, carbon, lipid and inorganic source and finally dissolved oxygen tension in culture. Lipase production is mostly growth-associated and occurs during the early exponential rate. High lipase yield is obtained when cell growth reaches stationary growth (Long et al. 1996b; Lin et al. 2001).

Production and purification of lipases

Lipase production involves many steps (*Figure 1*). The first step involves a fermentation process whereby the microbe will multiply and produce lipase to break down the lipid substance. The second step includes the separation of microorganism from the medium supernatant by either centrifugation (Schmidt-Dannert et al. 1994) or filtration or both (Abdou 2003). Enzymes are concentrated by precipitation from a large supernatant volume with ammonium sulphate (Abbas et al. 2002), acetone, ethanol and acids or by ultrafiltration (Snellman et al. 2002). These steps involve the treatment of large supernatant volumes and a precipitation technique that usually ends up with higher enzyme yield (Aries-Barros et al. 1994).

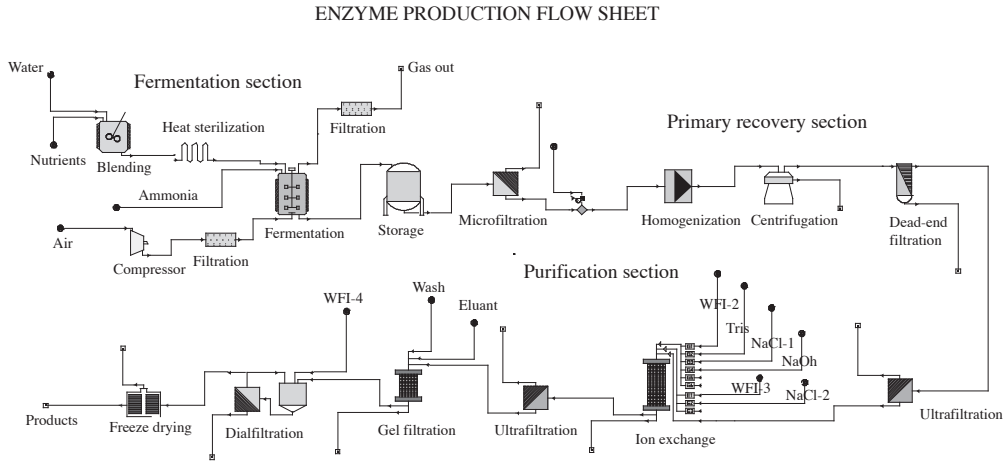


Figure 1. Process flow for the production of microbial enzyme (Source: Leisola et al. 2001)

The purification process goes through one or combination steps involving mainly chromatography procedures. Generally, purification will increase the specific activity of the enzyme and a part of it may be lost during the process. The use of liquid enzyme in industry faces several drawbacks and they are: a) water solubality which makes them hard to recover, b) feedback inhibition and c) inactivation by proteases. In many cases during submerged fermentation, lipases are produced together with protease (Henriette et al. 1993; Guillou et al. 1995; Long et al. 1996b). The protease is a metallo-protease which is very active and tends to degrade lipase rapidly even at 4 °C (Long et al. 1996b). Due to several problems with the use of liquid enzyme, efforts are made in the industry to immobilize the liquid enzyme to a carrier so that the enzyme can be reused.

Lipase and immobilization

Enzymes can be immobilized by fixing them to a solid surface. This has a number of commercial advantages i.e. the enzyme is easily removed, it can be packed into columns and used over a long period, speedy separation of products, feedback inhibition is reduced, thermal stability is increased allowing higher temperatures to be used and higher operating temperatures increase rate of reaction (Long 1997). As shown in *Figure 2a,* the free lipase activity has an optimum temperature of 30 °C. However, when bound lipase is used, the optimum temperature is shifted to 50 °C, which implies that upon extraction, *A. flavus* lipase becomes more sensitive to thermal inactivation (*Figures 2a–b*).

Although much has been claimed for the benefit of using immobilized enzyme in continuous processing either as stirred reaction or in fixed-bed column, there are still only a small number of industrial scale applications of lipases. This is due to the high price of the carrier materials or the high cost of the process involved in attaching the enzyme to the carrier. Alternatively, the use of a naturally-bound lipase can be cost effective because the biomass can be used directly, thus eliminating isolation, purification and immobilization procedures. Furthermore, this approach could prevent lipase from being

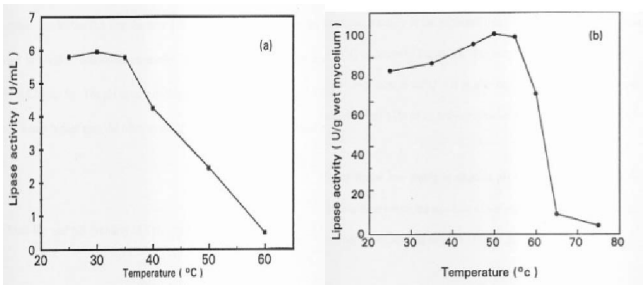


Figure 2. Temperature profile of (a) free lipases and (b) bound lipase of *Aspergillus flavus* Link

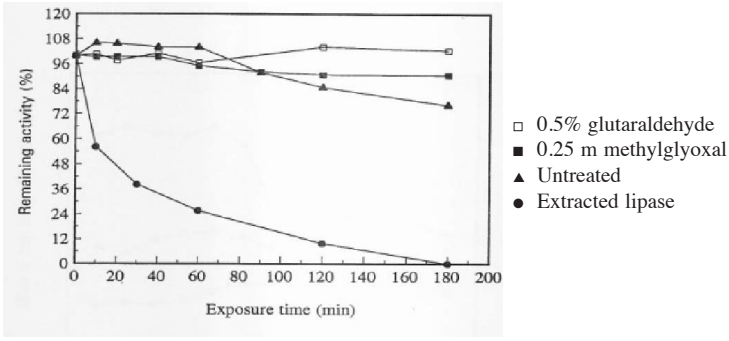


Figure 3. Thermal stability of free, untreated and treated with mycelium-bound lipase at 50 °C

easily denatured by the coexistence of proteases in the liquid fermentation. Besides, this naturally-bound lipase offer advantages such as increased stability to organic solvents, high temperature optimum (Long 1997) and extreme pH reaction (Patterson et al. 1979).

Long (1997) showed that a portion of the bound lipase is relatively loosely-linked to the mycelium and about 28% of its activity is removed during washing and de-fatting. To increase the stability and minimize the loss of lipase covalent binding of the enzyme to the cellular material, the use of bifunctional materials has been introduced (Abdul-Naby 1993; Carrara and Rubiolo 1994; Long et al. 1996a). The mycelium-bound lipase of *A. flavus* is stabilized by cross-linkage using glutaraldehyde and methylglyoxal (*Figure 3*). Cross linking with methylglyoxal increases the thermal stability of the lipase activity by 58% at 50 °C and in addition enhances lipase activity by up to 48% (Long et al. 1996a).

Dozens of lipases are now commercially available. Nevertheless those lipases employed in large-scale industrial processes and products are still limited. This is mainly due to the high price/low availability or non-optimal operational features of naturally available enzymes. In 2002, Novozyme A/S introduced a new patented immobilization method based on granulation technology. Silica is being introduced as a lipase carrier and this immobilized lipase has a commercial name, Lipozyme TL IM. This immobilization method is inexpensive but the immobilization enzyme can only be used in a non-aqueous system. Otherwise, the enzyme and the carrier material will simply disintegrate.

Recombinant DNA Technology

Perspectives in the use of lipases as industrial catalysis strongly rely on the production of recombinant enzymes with biochemical and catalytic features improved by protein engineering. Developments of strategies to apply these techniques at the industrial scale have allowed a significant decrease in lipase cost and consequently stimulate the development of more industrial applications. Currently, with a new and more-efficient expression system in combination with improvement of protein engineering technologies, the production costs of many lipases have been successfully reduced and consequently, has increased the market share.

Recombinant DNA technology is used to increase lipase production mainly in isoenzymes where the purification leads to very low yields. Generally, this technology can allow up to 40% decrease in the cost of raw material, water, steam and electricity compared to that of native enzyme production (Anonymous, Novozyme, http://www.novozyme.com). The main microbial expression systems are *A. oryzea*, *Saccharomyces cerevisiae* and *Pichia pastoris*. The first lipase produced by recombinant DNA technology was Lipolase® introduced in the market by Novozyme in 1988. This lipase was originally from *Thermomyces lanuginosus* and was expressed in *A. oryzea*. Novozyme A/S markets a range of enzymes for various industrial purposes. Many of these enzymes are produced by fermentation of genetically modified microorganisms (*Table 1*). Due to an efficient separation process, the final enzyme product does not contain any genetically modified microorganism.

Table 1. Commercial Novozyme's lipase produced by genetically modified organisms for food and other applications (Source: http://www.novozymes.com)

Brand name	Main aplication
Lecitase® Novo	Oils and fats industry
Lecitase® Ultra	Oils and fats industry
Lipopan®	Baking industry
Lipozyme®	Oils and fats industry
Noopazyme®	Pasta/Noodles
Palatase®	Dairy industry
Greasex®	Leather industry
Lipex®	Detergent industry
Lipolase®	Detergent industry
NovoCor® ADL	Leather industry
Novozym® 388	Biocatalysis
Novozym® 435	Biocatalysis
Novozym® 525 F	Biocatalysis
Novozym® 735	Textile industry
Novozym® 871	Pet food industry
Novozym® 51032	Paper industry
Resinase®	Paper industry

Lipase specificity

Lipases (triacylglycerol hydrolases E.C. 3.1.1.3) are enzymes that catalyse the hydrolysis of triacylglycerol to glycerol and fatty acids. The substrate specificity of lipases is often crucial to their application for analytical and industrial purposes. Substrate specificity of lipases is classified into fatty acid specificity, positional specificity, alcohol specificity, acylglycerol specificity and stero and chiral specificity.

Fatty acid specificity

Fatty acid specificity of lipases is affected by the carbon length of fatty acid, and the number and position of double bonds. Many lipases recognize C_8–C_{24} and react strongly but a group of lipases, such as *C. rugosa* and *G. candidum* lipases react weakly on C_{20} or greater carbon length of fatty acids. Their lipase activities are also affected by a number of double bonds. The activity of *C. rugusa* and *G. candidum* lipases on C_{18} fatty acids are in the order of stearic acid ($C_{18:0}$)< oleic acid ($C_{18:1}$) < linoleic acid ($C_{18:2}$) <α-linolenic ($C_{18:3}$).

In general, lipases act weakly on polyunsaturated fatty acids (PUFAs) such as arachidonic acid ($C_{20:6}$ n-6), eicosapentaenoic acid (EPA, $C_{20:5}$ n-3) and docosahexaenoic acid (DHA,$C_{22:6}$ n-3). *Aspergillus flavus* lipase shows greater preference for tricaprin than the triolein (Long et al. 1998a). Lipases from *C. rugusa*, *G. candidum*, *Rhizopus oryzea, R. mehei* react strongly on α-linolenic acid carrying double bond at Δ9, but weakly on γ-linolenic acid (GLA: $C_{18:3}$ n-6). Many lipases react more weakly on DHA than on EPA. On the contrary, *Pseudomonas* sp. lipase (lipase A.K, Amano Enzyme Co, Aichi, Japan) and *Alcaligenes* sp. lipases (Lipase QLM, Meito Sangyo Co., Aichi Japan) do not follow this rule and are more active towards EPA than DHA. *Mucor*

miehei lipase preferentially hydrolyses butyric acid from milk fat especially at low pH (Moskowitz et al. 1977).

Positional specificity

The second class of lipase specificity, which is most important for the modification of fats and oils, is regioselectivity. In terms of regioselectivity, lipases are usually considered either as 1, 3 positional specificity and non-positional specificity. A relatively large group of lipases, including the pancreatic lipase and most of fungal lipases show specificity for the 1- and 3-position of triacylglycerol (Adlercreutz 1994) including *R. miehei, R. oryzea, T. lanuginose, R. arrhizus* and *A. flavus* Link (Long et al. 2000). Examples of non-specific lipases include those from *C. cylindracea* (Macrae 1983), *Penicillium cyclopium* (Okumura et al. 1976), *C. rugusa* (Sonnet 1988) and *Humicola lanuginose* (Liu et al. 1972). A lipase with strong specificity for the 2-position of the triacylglycerol has not yet been discovered.

Alcohol specificity

Zaks and Klibanov (1988) showed that lipases can be active even in nearly anhydrous organic solvents and they have been used widely as catalysts in chemical reaction. Alcohol specificity is also important in the field of oleochemical reactions. Generally, primary alcohols are the best nucleophiles while secondary alcohols are less reactive and tertiary alcohol seldom react at all (Rangheard et al. 1992). The clearest example is the lipase from *R. meihei*, which accepts a very wide range of alcohols containing a variety of other functional groups (Miller et al. 1988).

Acylglycerol specificity

Lipids constitute triacylglycerol, diacylglycerol and monoacylglycerol. During hydrolysis some lipases hydrolyse mono- and diacylglycerol faster than the triacylglycerol e.g. lipase from *Penicillium camembertii* (Isobe et al. 1992) and *A. flavus* (Long 2006a).

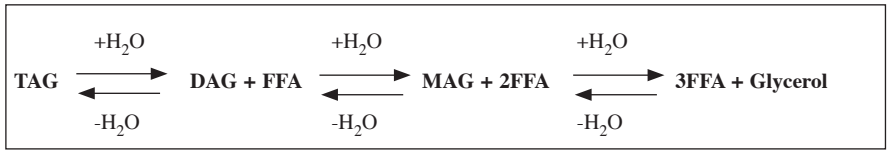
Stereo and chiral specificity

Stereo specificity is defined as the ability of lipases to distinguish between *sn*-1 and the *sn*-3 position of TAG. The stereo selectivity for the *sn*-3 and *sn*-1 positions by lipases has been widely used in the resolution of racemic mixtures of esters other than acylglycerol (Haraldson 1992). This ability has recently become very important in producing pure chiral isomer as intermediates for drug synthesis. Rogalska et al. (1993) observed that a change in stereo selectivity of a few lipases occur with different substrates e.g. steroselectivity for *sn*-3 position for trioctanoin changes to *sn*-1 position for triolein.

Lipase reaction mechanisms

Enzymes, including lipases have specific three-dimensional structures in aqueous environment with polar groups exposed and non-polar groups buried inside. Unlike the other enzymes, the nature of lipase catalysed reaction is very complex in which the lipid substrates are water insoluble. The need for water to maintain and activate lipase and the immiscibility of lipids in water makes the reaction media heterogeneous by forming a liquid-liquid interface. Biologically, both intra- and extracellular lipases are designed to catalyse hydrolytic reactions since the living cells are surrounded by water. Water plays an important role as a medium to disperse the enzyme molecule and participate as a co-substrate in hydrolysis. Reduction in water content may not affect the direction of hydrolysis as long as the water activity, (a_w) is maintained at 1. Reduction in water activity below 1 affects the equilibrium constant of the system and the direction of hydrolysis is changed to synthesis (*Figure 4*).

The application of water immiscible solvents such as *n*-hexane in lipid reaction serves two purposes: a) the ability to control the water content and b) the possibility to modify high-temperature melting lipids at low temperature. Other advantages of using water immiscible solvents in lipid reaction include: a) easy process control in large



TAG = Triacylglycerol; DAG = Diacylglycerol; MAG = Monoacylglycerol; FFA = Free Fatty Acid

Figure 4. Lipase catalysed reactions

scale production by reducing viscosity of the oil, b) the ability to keep the enzyme in insoluble form, c) easy recovery and reuse of enzyme, (d) easy recovery of products and (e) increased enzyme stability due to low water content and hence increased productivity.

Other than hydrolysis, there are many ways by which lipids can be modified. In synthetic reactions, a variety of lipid derivatives such as acids, esters and alcohols can be used. Lipases can be used to carry out a variety of synthesis reaction in microaqueous system such as direct esterification, transesterification, acidolysis, glycerolysis and alcoholysis (Macrae 1984).

Applications of lipase in food and lipid nutrition industry

Lipases can be used in food and non-food industries. In non-food industries, lipases are being applied in the detergent, pharmaceutical, leather, textile, cosmetic, paper and oleo-chemical industries. The most significant industrial applications of lipases have been found mainly in the food, detergent, and biopharmaceutical sectors. Food enzymes are expected to show the most promising growth prospects over the period of 2007–2012 (*Euromonitor International Company Report 2008*). This section highlights a number of lipase catalysed reaction applications in food and lipid nutrition industry.

Lipase in the dairy industry

Lipases are used extensively in the dairy industry for the hydrolysis of milk fat. Current applications include flavour enhancement of cheese, acceleration of cheese ripening, manufacture of cheese-like products, and lipolysis of butter fat and cream. Lipase primarily releases short-chain (C_4 and C_6) fatty acids that leads to the development of a sharp, tangy flavour and the release of a medium-chain fatty acid (C_{12}) that tends to impart a soapy taste to the product. More recently, a whole range of microbial lipase preparations has been developed for the cheese manufacturing industry, such as *R. miehei*, *A. niger* and *A. oryzea*. Palatase®, is a type of 1,3- specific lipases produced by *R. miehei* and demonstrates good results in Italian cheese making. The preference of Palatase® in hydrolysing short chain fatty acids results in optimal flavour formation.

Lipase in the production of cocoa butter equivalent and cocoa butter substitute

Cocoa butter is currently the fat of choice in chocolate and other confectionery industries based on its important organoleptic and physical properties in various chocolate and confectionery products. However, due to the degree of uncertainty in supply and the high

price of cocoa butter compared to that of other fats, these have driven the search for an alternative cocoa butter-like fat. Attempts have been made to prepare cocoa butter-like fat by enzymatic interesterification reaction using a cheap raw starting material.

Palm oil is important fat used in the production of cocoa-butter like fat. The enzymatic interesterification process was clearly recognized in the early 80s when Unilever (Coleman and Macrae 1980) and Fuji Oil Company (Matsuo et al. 1991) filed their patent applications. In both cases, the process relied on acidolysis of cheap raw materials for palm oil e.g. palm mid-fraction, which contains a significant amount of 1,3 dipalmitoyl-2-oleyl glycerol (POP), with tristearin or stearic acid to produce a cocoa butter equivalent.

Cocoa butter substitute can also be prepared using immobilized *sn* 1,3-positional specific lipase by interesterification of olive oil with palmitic acid using Lipozyme from *R. miehei* (Nielsen 1985). The main component of cocoa butter that gives a sharp melting property rely on its TAG with palmitic and stearic acid in the 1,3-position and oleic acid in the 2-position. The modified fat, 1, 3-stearoyl-2 oleyl glycerol, is produced by the exchange of fatty acid at the 1, 3-position of 2-oleyl TAG with stearic acid.

Lipase in varying physico-chemical properties and nutritional value of palm-based products

Naturally occurring fats and oils are very different from each other in terms of fatty acid composition, fatty acid distribution, ratio of saturated to unsaturated, melting point, crystallization behaviour, storage stability, nutritional value, caloric value and health-promoting effects. Different kinds of food preparation demand a range of physico-chemical characteristics from fats and oils, to achieve a particular functionality. Palm oil contains about 47% saturated fatty acids and the rest are unsaturated fatty acids. Palm oil can be separated into solid and liquid fractions because it is made up of a mixture of triacylglycerol (TAG) with a broad range of melting points.

The solid fraction contains a higher amount of saturated FAs (fatty acids) such as palmitic acid (49–68%) and has a melting point from 48 ºC to 50 ºC (Gunstone and Norris 1993). The percentage of solid fat content at room temperature (25 ºC) makes the stearin fraction useful in the manufacturing of edible fat products such as margarine, shortening, vanaspati and pastry. Nevertheless, due to its high saturation, this fraction cannot impart much plasticity and body to the end products (Pantzaris 2000). Thus, modification of the physico-chemical properties of stearin fraction is desirable to increase its nutritional value and market potential (Graille et al. 1977; Long et al. 2003a, b).

Palm oil has low levels of polyunsaturated fatty acids (PUFAs) especially omega-3 and enrichment of these fatty acids into palm-based products will increase their nutritional values. Enzymatic interesterification has been carried out to incorporate PUFA by utilizing the 1,3-positional specific lipases. Under the right conditions, 'tailor-made' glycerides with desired configurations and characteristics can be obtained. Long et al. (2003a) found that the use of commercial lipases such as Lipozyme IM and Novozyme 435 decreased the slip melting point (SMP) of the palm stearin/flaxseed oil mixture (*Table 2*).

The drop in SMP for the PS/FS mixtures could be due to the hydrolysis of the trisaturated TAG, triplamitin which is a known high-melting acylglycerol (Long et al.

Table 2. The amounts of DAG, TAG, Slip Melting Point (SMP), tripalmitin and 1,3-dipalmitoyl glycerol (area % by HPLC) in transesterified and nontransesterified palm stearin/flaxseed oil (PS/FS) and palm olein/flaxseed oil (PO/FS) mixtures

Types of samples	DAG (%)	TAG (%)	SMP (°C)	1,3-dipalmitoyl glycerol (%)	Tripalmitin (%)
Nontransesterified PS/FS	2.4	97.6	48.3	4.3	12.8
PS/FS transesterified	10.6	89.4	40.7	5.7	9.1
PS/FS transesterified with Novozyme 435	13.9	86.1	43.5	7.9	9.6

2003a). Enzymatic interesterification of palm olein with cod liver oil using *Pseudomonas cepacia* lipase increased the PUFA content of the mixture compared to that of a control (Chew et al. 1999).

Zainal and Yusoff (1999) used *R. mehei* lipase in enzymatic interesterification of palm stearin and palm kernel olein to achieve the physical properties of margarine fats. Lai et al. (1999) reported using non-specific *Pseudomonas* lipase and 1,3 specific *R. miehei* lipases for the transesterification of palm stearin and sunflower oil, resulting in melting properties appropriate for use as table margarine.

Lipase in functional foods and nutraceuticals

California-based Nutrition Business Journal (NBJ) of the United States defines a functional food as one which is fortified with added or concentrated ingredients and/or is marketed to emphasise 'functionality' to improve health or performance. The functions of food lipids are considered to originate from several characteristics of lipids. The nutritional and biological functions of food lipid are mainly dependent upon the chain length and degree of unsaturation of fatty acids. Accordingly, the position of the fatty acids in the acylglycerol molecule is also important especially for structure lipids products such as Betapol™ 45, Salatrim, Olestra, Caprenin etc.

Production of oil rich in polyunsaturated fatty acids (PUFAs) by selective hydrolysis

The biological roles of PUFAs in human body have been reported elsewhere and the consumption of PUFA is known to reduce the risk of coronary heart disease, lower triglyceride levels in blood, prevent irregular heartbeat, decrease high blood pressure, diminish blood clotting potential, improve neurological development in infants and has a mood stabilizing effect (Dziezak 1989). Docosahexaenoic acid (DHA)-rich oils have been produced by the traditional method of winterization, but the yield obtained was low. Therefore, attempts to produce an oil containing a higher concentration of DHA using lipase catalysed reaction are introduced.

Microbial lipases generally act weakly on PUFAs especially *Candida* and *Geotrichum* lipase. It will hydrolyse the preferred fatty acids and leave the non-hydrolysed oil fraction enriched with PUFA fatty acids. Selective hydrolysis of borage oil containing γ-linolenic acid (GLA) increased the content of GLA from 22% to 45% at 60% hydrolysis using *C. rugosa* lipase as catalyst (Rahmatullah et al. 1994). Another example of the enrichment of EPA and DHA from fish oil is transesterification reaction

of the oil using *Pseudomonas* sp. lipases in ethanol media. This process yielded about 46% EPA and DHA (Breivik et al. 1997). Oil containing high concentration of DHA has been on the market in Japan as a nutraceutical since 1994.

Production of human milk-fat substitute

Human milk fat contains 20–25% palmitic acid and about 70% of the palmitic acid is esterified to the 2-position of TAG (Breckenridge et al. 1969). Gastric and pancreatic lipases in infants hydrolyse the human milk fat to 2-MAG and fatty acids. It has been hypothesized that the high absorption efficiency of human milk fat is the result of specific positioning of palmitic acid of the TAG moiety (Innis et al. 1994). Absorption efficiency of palmitic acid is relatively low compared when it is esterified at the 2-second position of TAG.

Betapol™ 45 is designed to provide the total fat phase in an infant formula. It is a vegetable fat blend that closely mimics the physical and chemical structure of human milk-fat. As such, it makes a major contribution to the health of babies who, for whatever reason, are unable to receive breast milk. Betapol™ 45 is produced via the interesterification process between tripalmitin with oleic, linolenic or linolenic acid using 1,3-positional specific lipase. This baby infant formula has approximately 45% of the total palmitic acid which is esterified in the middle position of the glycerol molecule. Such high levels cannot be produced by simple blending of vegetable oils. Studies in animals and newborn babies have confirmed that this infant formula is well absorbed into the bloodstream. This greatly improves the absorption of dietary calcium and significantly reduces the amount of calcium lost in the stool (http://www.betapol.com).

Production of reduced calorie shortening for baked products

Salatrim is a reduced-calorie fat marketed by Cultor Food Science as a family product under the trade name Benefat™ . The word salatrim is an acronym derived from short and long-chain triacylglycerol. Salatrim is a mixture of TAG consisting of at least one long-chain (C_{16}–C_{22}) fatty acids and at least one short-chain fatty acid (C_2–C_4) per triacylglycerol molecule and is not as easily absorbed as other fats. Due to the unique molecular composition, the available calories derived from salatrim average 5 kcal per gram, or 55% of the caloric value of conventional fats (9 kcal/g) A shortening from a salatrim has been developed and is marketed in the United States. The shortening is a 1:1 replacement for fat in bakery application such as cookies, pies, crème fillings, cakes, brownies and muffins. Caprenin is another example of a low-calorie fat. It is a randomized triacylglycerol comprising caprylic ($C_{8:0}$), capric ($C_{10:0}$) and behenic acids ($C_{22:0}$). The usable energy value of caprenin was calculated to be 4.3 kcal/g.

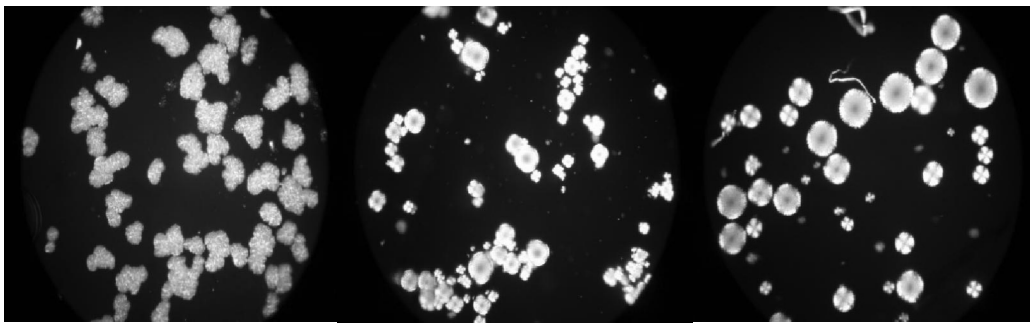
Current Malaysian inventions in fats and oils processing technology using lipase-catalysed reaction

The need to value add Malaysian natural resources are important in order to make Malaysian products more competitive in the world market. Currently there are three technologies developed using lipases-catalysed reactions that have great potentials in contributing to the development of the oils and fats industry in Malaysia. These technologies are related to the increase of the functionality properties of palm-based and coconut-based products and are being filed for patent.

Lipases in the production of low diacylglycerol (DAG) palm olein – Selective hydrolysis

Long (2004) reported a selective lipase that has preference to hydrolyse the DAG faster than the TAG of crude olein. Under specific conditions, lipase tends to hydrolyse DAG more rapidly than the TAG and leave the non-hydrolysed fraction enriched with TAG. Selectivity of lipases towards hydrolysing DAG depends on the water content of the mixture and their reaction times. Optimization of the process reaction leads to the reduction of DAG in crude palm olein by 82% (Long et al. 2005b).

The presence of DAG (4–7.5%) in palm oil during processing has several disadvantages. Long et al. (2005b) showed that the amount of DAG present in palm oil affect the formation of the size of fat crystals during dry fractionation. The fat crystals of oils with low DAG content tend to be solid and bigger (*Plate 1c*) compared with fat crystals of oils with high DAG content (*Plate 1a* and *b*). This is especially so with DAG content around 8.83% (*Plate 1*a) where fat crystals do not seem to have nuclei, are not solid and are in clumps. Treated oil with low DAG content tends to form fat crystals faster than the untreated oil (*Table 3*) probably because DAG may induce formation of



(a) (b) (c)

Plate 1. Fat crystals under 10x magnification (a) crude olein with 8.83% DAG content (b) crude olein with 5% DAG content and (c) crude olein with 0.86% DAG content
Source: Long et al. (2005b)

Table 3. Yield of liquid (olein) and solid (stearin) of treated and non-treated oil after passing the membrane filtration press

		Yield of liquid (olein)	Yield of solid (stearin)	Nucleation time (h)
1st fraction-RBD Olein	Non-treated with 4.95% DAG	84.2	15.8	12
	Treated with 0.85% DAG	62.7	37.3	5

Source: Long (2004)

Table 4. Physico-chemical characteristics of low melting fraction (obtained after 1st fractionation)

Analysis	Non-treated with DAG 4.9%	Treated with DAG 0.85%
Iodine Values (IV)	60.5	65.2
Slip Melting Points (°C)	16.9	14.6
TAG Composition (area by HPLC)		
S_3	1.08	0.98
S_2U	40.46	32.15
SU_2	50.82	57.34
U_3	7.64	9.53
Cloud point (°C)	4.1	1.3

S = Saturated fatty acids; U = Unsaturated fatty acids
Source: Long et al. (2005b)

fat nuclei easily. As a result, higher amount of solid (stearin) will be obtained from the process (*Table 3*).

The TAG profile of the low melting fraction obtained from the treated oil with low DAG showed a higher amount of the diunsaturated TAG i.e. dioleyl pamitoyl glycerol in the olein fraction (POO) (57.3%). This consequently resulted in olein fraction with better iodine value (IV 65), lower cloud point (1.2 ºC) and better clarity at cold temperature compared with oils with high DAG content (4–9%) (Long et al. 2005b) (*Table 4*).

It seems that this low DAG oil will benefit existing oil palm refineries in Malaysia as it can increase the separation efficiencies of both high and low melting glycerides during the process of dry fractionation. The oil will be more stable during storage at 28 °C and does not form cloud. The formation of cloud tends to influence a consumer's perception that the oil is of low quality. Swe et al. (1995) reported that DAG especially 1, 3 dipalmitoyl-glycerol, which is known as a high melting glyceride, has been found to cause the formation of cloud upon storage of olein at low temperature.

The presence of certain amount of DAG has also a detrimental effect on the cocoa butter equivalent fat (stearin) because it softens the fat considerably, making demoulding a very difficult task and its retention in the SUS fraction (saturated unsaturated-saturated) for speciality fat application would be disastrous (Okawachi and Sagi 1978). Long et al. (2005a) found that the low DAG hard PMF (obtained after 2nd fraction) has excellent properties for speciality fat applications. The low level of trisaturated TAG (i.e. tripalmitin) and DAG (0.61%) in the treated hard PMF caused no waxiness to the fat (*Figure 5*). The hard PMF can be used to partially substitute cocoa butter in confectionery applications or it can be blended with SUS rich fats such as illipe to make a true cocoa butter equivalent (CBE).

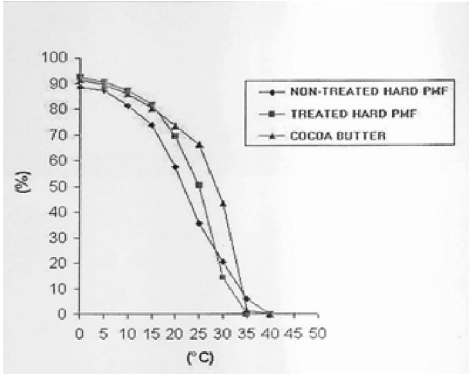


Figure 5. Solid fat content (SFC) of treated and non-treated hard palm mid-fraction (PMF) compared with true cocoa butter

This DAG cannot be removed during the retaining process while mutual separation of DAG from TAG is a very difficult task. At the moment, no effective industrial separation method for DAG has been reported.

This technology is expected to further expand both local and international markets for the Malaysian oil palm products. In spite of their apparent superiority, this process has

not yet attained a level of commercial exploitation commensurate with their advantages because the high cost of lipases remained prohibitive. The introduction of the second generation of this particular enzyme by Novozyme in the near future will hopefully make the process economically viable.

Lipases in the production of palm-based obesity functional oil

As obesity is now a serious global epidemic and has always been linked to life threatening diseases such as cardiovascular diseases, diabetes and hypertension, the development of healthful functional oils and fats such as DAG as well as medium- and long- chain TAG (MLCT) will surely benefit the consumers. DAG is a natural component to various edible oils and most oils contain approximately 2–10% relative content depending on the origin of the oil. When consumed daily, DAG has been proven to reduce body fat and prevent obesity (Nagano et al. 2000). In Japan, DAG oil was commercially marketed as a 'food for specified health uses', since 1999 (Econa Cooking Oil, Kao Corporation, Tokyo, Japan). This speciality oil is synthesized basically by esterification of glycerol with 2 moles of fatty acids using immobilized *sn*-1, 3-specific lipase (Hirota et al. 1988).

Another way to produce DAG oil is through lipase-catalysed partial hydrolysis of palm-based fraction *(*Lai et al. 2005*)*. Recently this process has been filed for a patent in Malaysia, USA and Japan (Lai et al. 2005). The present invention provides a partial hydrolytic process using 1,3-positional specificity lipase for the production of a high-purity DAG at a high yield in a short period of time (*Table 5*). The complex reaction mixtures containing un-reacted TAG, MAG, free fatty acid will be removed more to obtain a high-purity DAG (Cheong et al. 2007; Lo et al. 2007; Lo et al. 2009).

Table 5. Percentage of DAG purity at final stage

Partial hydrolysis	Diacylglycerol purity (%)	
	Before purification	After purification
Palm olein	41.56	88.70
Soya bean oil	43.68	90.83

Source: Lai et al. (2005)

The consumption of DAG oil is claimed to reduce postprandial serum TAG levels and is thus beneficial for the prevention and management of obesity. Matsui et al. (2001) described the DAG oil composition as being capable of reducing arteriosclerotic factor in the blood and thereby lowering the risks of arteriosclerotic and other degenerative diseases. A review on the properties, process and products of DAG oil is made by Lo et al. (2008).

DAG has a wide range of applications. It can be used as cooking oil, frying oil, salad dressing and mayonnaise, shortening and margarines, chocolates, ice cream fats, confectioner's fats and baked food products (Cheong et al. 2009).

Other than DAG oil, medium- and long-chain triacylglycerol (MLCT – generally consists of C_6–C_{10} fatty acids and long-chain fatty acids in the same TAG) oils produced using lipase-catalyzed synthesis reaction, have also been reported to have potential for

weight control (Matsuo et al. 2001; Kasai et al. 2003; Matsuo and Takeuchi 2004). This speciality oil can be used as a healthy cooking oil or salad oil (Koh et al. 2008a; Koh et al. 2008b) and shortening (Ariffin et al. 2009). In addition, the lipid emulsion based on the MLCT structure has been shown to provide efficient energy to patients requiring parenteral nutrition on a long-term basis (Matulka et al. 2006).

At the present growth rate of obese population throughout the world, it can be expected that the global market demand for DAG oil will be boosted in the near future.

Lipases in the production of enhanced/modified virgin coconut oil (MVCO) with a broad spectrum of antimicrobial activity

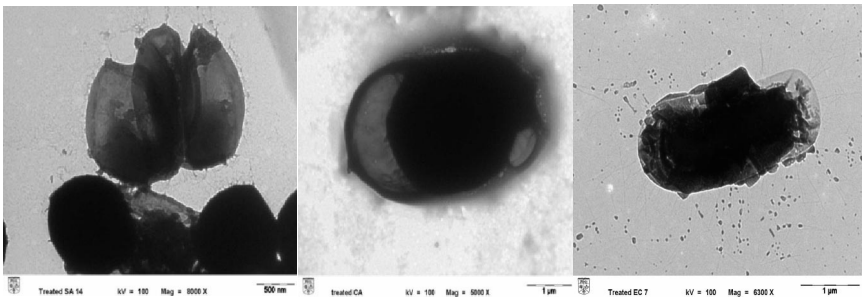
Lipases are being used to modify the lipid classes of VCO. This modification is aimed to increase the functionality of VCO and consequently will add extra value to it. This value-added VCO or modified virgin coconut oil (MVCO) contains free fatty acids (>9.4%), MAG (>1.3%), DAG (>22.8%) and TAG (>25%) and an in-vitro study has shown that this novel product has a broad spectrum of antimicrobial activities which can kill gram-positive bacteria, gram-negative bacteria and yeast. The products have been filed for a local and an international patents (Long 2006b) covering 10 countries namely, Malaysia, Indonesia, India, Philippines, Singapore, Japan, United Kingdom, USA, Germany, France and Ireland.

The present inventions involve the use of 1, 3-positional specific lipases to modify the virgin coconut oil under specific reaction conditions to obtain a sufficient level of saturated medium chain fatty acids and their respective MAG through partial hydrolysis or glycerolysis reaction. The enzyme used in the present invention is an enzyme with 1, 3-positional specificity i.e. Lipozyme TL IM. A series of MVCO is being developed and the most potent MVCO can kill the *Staphylococcus aureus* and *Candida albican* within a 10-minute incubation. In addition, these value-added VCOs also have the ability to kill or inhibit growth of gram-positive bacteria such as *Listeria monocytogenes, Streptococcus pyogene*, gram-negative bacteria i.e. *Vibrio cholerae, Echerchia coli* and yeast i.e., *C. albicans, Candida krusei* and *Pityrosporum ovale* (*Table 6*).

Previous studies showed that medium chain free fatty acids and their corresponding MAG formed a broad spectrum of anti microbial activity against enveloped viruses and various bacteria in vitro (Kabara 1978; Thormar et al. 1987; Isaacs et al. 1995), including human pathogens like herpes simplex virus (Kristmundsdóttir et al. 1999), *Nesseria gonorrhoeae* (Bergsson et al. 1999), *Candida albicans* (Bergsson et al. 2001), *Chlamydia trachomatis* (Bergsson et al. 1998), *Helicobacter pylori* (Bergsson et al. 2002) and *Staphylococcus aureus* (Kabara 1984). The mechanism by which these lipids kill bacteria is not known, but electron microscopy studies indicate that they disrupt the cell membrane permeability barrier (*Plate 2*).

Currently, this value-added VCO or MVCO offers several advantages over many synthetic antimicrobial/antifungal products that are available in the market. It is safe for long term application, easily absorbed into the skin, enriched with vitamin E and, most importantly, it is cheap. This MVCO also has the potential to be used as a preservative in food products, cosmetic products and can act as a disinfectant for household and hospital equipments. Finally, the technology is economically viable as the process conditions

are simple and this new product is expected to revitalize the coconut industry that has been lagging behind other plantation-based industries in Malaysia.



(a) (b) (c)

Plate 2. Electron microscope studies indicate that MVCO disrupt cell membrane permeability barrier of (a) *S. aureus*, (b) *C. albicans* (c) *E. coli*. Source: Ching (2008)

Table 6. Inhibition of pathogenic microorganisms by a series of modified virgin coconut oil (MVCO) evaluated by time-kill studies

Microbes	Modified Virgin Coconut Oil	Number of viable bacteria (log10 cfu/ml) at time interval (hours)						
		0	2	4	6	8	24	48
S. aureus	MVCO 1	5.69	5	3.6	3.11	2.62	0	0
	MVCO 2	6.32	0	0	0	0	0	0
	MVCO 3	6.27	0	0	0	0	0	0
	MVCO 4	6.04	4.25	0	0	0	0	0
	MVCO 5	6.08	3.96	0	0	0	0	0
	MVCO 6	5.87	0	0	0	0	0	0
L. monocytogenes	MVCO 1	5.71	5.02	3.62	3.15	2.66	0	0
	MVCO 2	6.28	0	0	0	0	0	0
	MVCO 3	6.15	0	0	0	0	0	0
	MVCO 4	6.06	4.25	0	0	0	0	0
	MVCO 5	6.08	3.76	0	0	0	0	0
	MVCO 6	5.34	0	0	0	0	0	0
C. albicans	MVCO 1	4.59	4.61	4.54	4.44	4.29	2.81	2.71
	MVCO 2	6.48	5.56	5.48	5.65	5.38	2.16	2
	MVCO 3	5.76	4.98	3.21	3.45	3.11	0	0
	MVCO 4	5.98	5.65	5.15	4.18	3.46	0	0
	MVCO 5	6.02	5.68	5.43	4.75	3.86	0	0
	MVCO 6	5.98	4.13	3.06	2.99	2.14	0	0
E. coli	MVCO 1	ND	ND	ND	ND	ND	ND	ND
	MVCO 2	6.32	6.48	7.52	8.34	9.16	10.27	11.16
	MVCO 3	6.21	6.54	5.82	5.96	4.32	2.58	2.69
	MVCO 4	6.01	6.26	5.64	5.28	5.04	2.96	2.71
	MVCO 5	6.02	6.15	6.01	5.78	5.44	2.84	2.56
	MVCO 6	6.14	5.28	5.35	6.02	4.67	2.64	2.63
S. pyogenes	MVCO 1	5.48	5.21	4.23	3.08	2.12	0	0
	MVCO 2	6.1	0	0	0	0	0	0
	MVCO 3	5.7	0	0	0	0	0	0
	MVCO 4	6.01	4.68	0	0	0	0	0
	MVCO 5	6.04	4.74	0	0	0	0	0
	MVCO 6	5.49	0	0	0	0	0	0
V. cholera	MVCO 2	6.34	0	0	0	0	0	0

Source: Ching (2008)

The way forward

The application of lipases especially in the food, fats and oils processing industries will continue to grow and get bigger. Development of new and more efficient expression system in combination with the improvement of protein engineering technologies should allow the industry to produce bulk lipases which in turn will make the process of manufacturing a cost-competitive process compared with chemical catalyst. There are many aspects of the Malaysian oils and fats industry that could benefit from lipases, apart from those already discussed in this paper. Additionally, in Malaysia there are a few areas of research that need to be continued and explored.

Bio-prospecting for novel enzymes from indigenous microbes

Malaysia is one of the world's hotspots of biodiversity and is rich with indigenous microbes which are as yet largely untapped. The tropics hold numerous extreme and interesting ecological niches. Bio-prospecting of microorganisms may lead to the discovery of new enzymes with added functionality. This work has to be continued and expanded. The use of the High-throughput screening (Wahler and Reymond 2002) procedure should accelerate the discovery of novel enzymes.

The need for novel lipases is obvious and the industry continues to look for new lipases with high activity from less expensive source, high selectivity towards fatty acids and high regioselectivity towards *sn*-2 position. Scientists worldwide are looking for lipases that have the ability to hydrolyze fatty acids at the second position of TAG. This novel lipase will be able to catalyze the production of a highly absorbable oil based on its ability to replace and substitute the fatty acid in the *sn*-2 position of the TAG molecules. As it is known, acids in this position are preferentially absorbed by the intestinal cells as the 2-MAG over the other two fatty acids on the TAG moiety.

With the new platform technologies such as protein engineering, gene shuffling, advances in functional genomics, proteomics, metabolomics and bioinformatics (Kuipers

2004), this area of research could be successfully implemented and achieved within a short time period.

Enzymatic modification of seed lipids for functional foods and nutraceuticals

Malaysia is well-endowed with natural resources in the area of seed oils other than palm i.e. coconut, cocoa beans, rice and tropical fruit seeds which have not been thoroughly studied. Therefore, research in Malaysia should be based on exploiting its own natural resources such as the extraction of high value added lipids like plant sterols (proven to have the ability to reduce serum cholesterol), plant phospholipids (plays important roles in human physiological functions) and antioxidants such as tocopherols and tocotrienols which exhibit vitamin E activity. This hopefully will lead to new and exciting findings and applications of functional lipids that will help to improve human health and consequently reduce increasing healthcare costs.

The enzyme industry is intensely competitive and players need constant product innovation to sustain growth, hence R&D investment is required to keep abreast with technology progress. A path for growth in this industry is to form smart partnerships with established companies like Novozyme, Genecor, Chr Hansen, and ADM. BioTec in Bangkok, Thailand has established a win/win collaboration with Novozyme. In this collaboration Biotec Bangkok has to collect, isolate, identify and finally screen the activity of the isolated microbes. Novozymes Denmark in turn, sponsors the research, initiates the technology transfer and gives royalties to Biotec Bangkok if the product is commercialized.

Conclusion

Lipases are the most versatile industrial biocatalysts compared with other hydrolytic enzymes as they can catalyze the hydrolytic and synthesis reaction under non-aqueous environment. The high production cost of lipases has become less of an issue these days with improved production techniques, such as the recombinant DNA technology, solid-state fermentation, immobilization, fed-batch culture and continuous culture. Additionally the application of genetic manipulation of microorganism is now accelerating the development of enzyme production by the transfer of productive genes. Currently there are many technologies or commercial products manufactured by the industries using lipases as catalysts as discussed in this paper. Most of the products can be commercially obtained in the market and are cost-efficient. At the moment the demand for functional lipids are getting bigger. However, the rate of growth is very much related to the prices of the products as they are expensive and limited to niche markets. Lipases are expected to play an increasingly important role in the production of foods containing oils and fats. *Discovering the unique properties of lipases and what they can do for mankind put them as one of the miracle gifts of nature*.

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Profil:

Dr. Kamariah Long

Born on 11 October 1959 in Johor Bahru

Education:

- BSc. (Hons.) Microbiology, Universiti Malaya (1984)
- MPhil. Microbial Enzyme Technology, Leeds University, England (1991)
- PhD. Microbial Enzyme Technology, University Putra Malaysia (UPM), and Westminster University of England (1997)

Work Experience and Achievements

Dr. Kamariah Long started working as a Research Officer in MARDI in 1984. She received a Research Grant during the Eighth Malaysia Plan (2000-2005) amounting to RM2.2 million to carry out a study on the transformation of fats and oils using enzymes. The study was aimed at value-adding products based on palm oil. The project had been a success whereby two technologies had been filed for copyrights ownership in Malaysia. One of these had been awarded the copyright MY 125435-A.

She was also part of the UPM research group that had filed a Malaysian patent and three other foreign patents for copyrights on the production technology of low calorie medium and long chained oils, diacylglycerol (DAG) and triacylglycerol (MLCT). The project cost of RM1.8 million has been fully funded by Sime Darby Bhd. and is now at the pre-commercial stage. The project has succeeded in obtaining a TechnoFund research grant of RM5.5 million from the Ministry of Science, Technology and Innovation (MOSTI).

Her latest finding is the modified virgin coconut oil, MVCO, which has broad spectrum anti-microbe characteristics. This product had been filed for a Malaysian patent in 2005. The product had also been filed for patents in nine countries: USA, Japan, Germany, Ireland, UK, India, Indonesia, Singapore and the Philippines, through the PCT in 2006. The project is currently running and has received a grant of RM4.5 million from MOSTI for pre-commercialization. The project now has two industrial sector partners, Wawasan Tebrau Sdn Bhd at PasirGudang, Johor, and Biotropics Malaysia Bhd.

She had been awarded the Malaysian Excellent Woman Scientist in 2006 by the International Federation Associations, Budapest (IFIA) in appreciation of her significant achievements in leading the agricultural innovations.

She is also involved in local universities and has been actively cooperating with them with a working linkage. Dr. Kamariah has also been appointed as an external examiner for students in the Masters programmes and has supervised more than 20 post graduate students.

She is now leading research as an Assistant Director of the Bio-Processing Programme at the Biotechnology Research Centre of MARDI.

Publications

Dr. Kamariah Long is the owner of 11 inventions, and has produced 85 scientific papers of which 45 are found in refereed national and international journals.

Awards

Dr. Kamariah Long has been acknowledged and is well known at the national and international levels for her creativity and innovativeness in research. She has received several awards in competitions at the MARDI Science and Technical Exhibition (MSTE), I-TEX, and the International Exhibition of Inventions, New Techniques and Products of Geneva, Switzerland. She received the Excellent Service award in MARDI for the years 1992, 2002 and 2006. Dr. Kamariah Long has also been the recipient of the MARDI Excellent Scientist award in 2007. The Excellence Award of the American Oil Chemists' Society (AOCS) for biotechnological papers was presented to her at its Annual Meeting and Exposition in 1997, in Seattle, USA.

Appendix:
Papers presented as Research Inaugural Lectures (*Syarahan Perdana Penyelidikan*)

No.	Date	Presenters	Title of papers
1	14 July 2009	Dr. Johari Jiken Abdullah	The Brakmas cattle – Potential beef breed for meat production in Malaysia
2	20 Aug. 2009	Dr. Azizan Ab. Rashi	Improving beef production in Malaysia
3	22 Oct. 2009	Dr. Kamariah Long	Unlocking the miracle of lipases
4	24 Nov. 2009	Latifah Mohd.Nor	Challenges of the fresh cut fruits industry in Malaysia
5	23 Feb. 2010	Ismail Abu Bakar	Strategies for mitigation of carbon emission in peatland
6	23 Feb. 2010	Wong Hee Kum	Manipulating nutrient composition in poultry
7	6 Apr. 2010	Fadelah Abdul Aziz	Blooming heritage: Wild orchid species to beautiful orchid hybrids
8	18 May 2010	Che Rohani Awang	Adding value to local aquatic resources
9	1 July 2010	Dr. Zamri Ishak	Elevating biosensor research in food and agriculture – Integrating the tools of biotechnology and nanotechnology
10	5 Aug. 2010	Dr. Mohamad Roff Mohd. Nor	The battle against virus diseases of chilli: How can we win?
11	5 Oct. 2010	Dr. Mohmad Mustafa	Livestock Entrepreneur Development- Is there a future for commercial Boer goat and Dorper sheep entrepreneurs in Malaysia?
12	20 Dec. 2010	Mohd. Zainal Ismail	Issues relating to traditional food mechanization