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INAUGURAL LECTURE

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Tapping the Power of Enzymes - Greening the Food Industry

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HASANAH MOHD GHAZALI

Prof. Hasanah, the eldest of 8 children of Tuan Hj. Mohd Ghazali bin Hj. Hassan and Hajjah Noriah Muhamad, was born on 28 October 1956 in Tampin, Negri Sembilan. She completed her primary and secondary education exclusively in schools in the state until 1975. She then pursued her tertiary education at Otago University, New Zealand, on a Colombo Plan Scholarship and returned to Malaysia in 1979 with a B.Sc (Hons.) degree in biochemistry. Her liking for microorganisms led her to double major in microbiology.

January 1980 saw the beginning of her career in academia, joining the then Universiti Pertanian Malaysia (UPM) as a tutor at the Department of Food Science and Technology. She left the same year for her M.Sc degree in Food Science at Reading University, United Kingdom, where she also had a brief stint at the Phillip Lyle Memorial Research Laboratory. UPM formally appointed her a lecturer in food chemistry and biochemistry at the Department of Food Science on January 1, 1982. Teaching has since then been her forte, while her passion in research began earlier during her undergraduate days and with the publication of her first refereed paper in 1980.

Her term as a lecturer in food chemistry and biochemistry was a rather brief one, as in 1986 she was made a lecturer at the Department of Biotechnology where she has remained since. She received her PhD degree in Enzyme Technology from UPM in 1990, and in April 1994, was promoted to an Associate Professor. She became a Professor in January 1999. She was appointed the Deputy Dean of the Faculty of Food Science and Biotechnology in 1996 and served the faculty in that capacity for 6 years. The National Biotechnology Directorate (BIOTEK) honoured her by making her the National Coordinator of Food Biotechnology Cooperative Centre since 1996.

Her academic background means that her teaching and research are largely focused on various aspects of food biotechnology, enzyme technology and food chemistry. Her vast experience in the teaching of the former two subjects has led to her appointment as a visiting lecturer with Universiti Malaysia Sabah and the 'ASIAN-EUROPEAN Masters Degree in Food Science and Technology' programme. To date she has published more than 200 papers of which more than 80 are in refereed journals. She was also a co-editor of two conference proceedings.

Porf. Hasanah sits on the editorial board of Pertanika Journal of Tropical Agricultural Science, and is an active reviewer for a number of international and national journals. She is a member of the Institute of Food Technologists' (USA), American Oil Chemists' Society (USA), and the Malaysian Oils Scientists' and Technologists' Society. She was an executive member of UPM Academic Officers' Association from 1998-2000.

In recognition of her contributions to the faculty, and university at large, UPM has awarded Prof. Hasanah twice, in 1995 and 1997, with the Excellent Service Award, and the Excellent Service Certificate every year since then. She was recently awarded the Cochran Fellowship (USA) to attend a course in Agricultural/Food Biotechnology.

This year marks the twenty second anniversary of her marriage to Zakaria bin Majid. Everyone deserves a miracle; the couple is blessed with five: Johari (20), Mohamad Kamal (17), Nurul Farihah (15), Maisarah (11) and Aiman Syukri (2).

TAPPING THE POWER OF ENZYMES - GREENING THE FOOD INDUSTRY

ABSTRACT

Stimulating pressures for better use of renewable resources and clamour for green technologies that will reduce damage to the environment have combined with substantial advances in biotechnology to significantly stimulate the growth of the markets and application areas for enzymes. The impact of genetic and protein engineering on production and modification of the enzyme molecule has been highly visible and this has resulted in a more intense study on tapping the power of enzymes for an even wider range of applications including in food processing.

Industrial enzymes are used widely in food processing and technical industries. The total market for them was estimated in 2000 to be in excess of US$ 1.3 billion, with applications as wide-ranging as biological detergents, high fructose corn syrups processing, and cosmetic additives. The manufacture of foods has rapidly changed from an art form to a highly specialised technology based on discoveries, increased availability and translation of knowledge from the basic and applied sciences. In the last 50 years the use of commercial enzymes in food processing has grown from one that is relative insignificant to a role that has become essential. It is such that nowadays, in some food industries, enzymes are used routinely to effect changes during processing that may be otherwise be very difficult to achieve. For some other processes, enzymes appear to be the only logical solution to food transformation and food ingredient production.

INTRODUCTION

Enzymes are the 'power machine' behind life processes, driving everything from bacteria to human beings. All living organisms produce enzymes but enzymes are not themselves living materials. They are protein molecules composed of amino acids. However, they are distinguishable from other proteins because of their catalytic activity. This means that they accelerate the rates of chemical reactions many times over by reducing the activation energy necessary to convert the reactants (substrates) into products. Although they participate in the reaction, they themselves remained unchanged at the end of the reaction. Enzymatic catalysis does not require extremes in temperature, energy or additional chemicals, and the formation of wasteful by-products rarely occurs. Enzymes are highly efficient biocatalysts and catalyse chemical reactions with great specificity compared to their chemical or metal counterparts. Enzymes also mediate the transformation of different forms of energy.

Currently over 4000 enzymes have been known (Swissprot Enzyme Nomenclature Database, 2004). Enzymes are named and grouped based the nature of the chemical reactions they catalysed. There are six classes of enzymes, as well as sub-classes and sub-sub classes. These are the oxidoreductases (Group 1), transferases (Group 2), hydrolases (Group 3), lyases (Group 4), isomerases (Group 5) and ligases (Group 6). Each enzyme is assigned two names, the second of which is based on a four-digit numeric classification system. For example, 1,4-alpha-D-glucan glucanohydrolase has the classification number EC 3.2.1.1, where EC stands for Enzyme Commission, and the numbers represent the class, sub-class, sub-subclass, and its arbitrarily assigned serial number in its sub-subclass, respectively. Simply, this enzyme is α-amylase.

Enzymes are involved in many aspects of metabolism. For instance, the enzyme N-acetylglucosamine kinase (Shephard *et al.*, 1980) is the first enzyme in the pathway for chitin synthesis in *Candida albicans* and its synthesis is induced during the invasive stage of the organism. Other enzymes like pectin methylesterase (Fayyaz *et al.*, 1993; 1994, 1995a-b) and polygalactronases are involved in the softening of many fruits such as guava (Ghazali and Leong, 1987) and starfruit (Ghazali *et al.*, 1989; Ghazali and Kwek, 1993), while polyphenol oxidases (Tengku Adnin *et al.*, 1985; Augustin *et al.*, 1985) cause cut surfaces of fruits and vegetables to undergo browning. Some enzymes like L-methionine-γ-lyase (Choo *et al.*, 2000) are potential chemotherapeutic enzymes. Others like fructose-6-phosphate phosphoketolase in bifidobacteria (Fandi *et al.*, 2001a, 2001b; Tee *et al.*, 1999) may be used as identification indicators. Carbohydrases such as amylases and β-mannosidase (Haris and Ghazali, 2002) are involved in mobilisation of food reserves in seeds.

Enzymes can also be used independently (ex-cells) to drive chemical reactions. The use of enzymes in the production of goods and services (i.e. enzyme technology) is recognised by the Organization for Economic Cooperation and Development (OECD) as an important

component of sustainable industrial development (OECD, 1998, 2001). As enzymes are biodegradable and environmentally friendly, they are exemplary agents of green technology. They can often replace chemicals or processes that present safety or environmental issues. Examples are replacement of acids, alkalis or oxidizing agents in fabric desizing, use of enzymes in tanneries to reduce the use of sulphide, replacement of pumice stones for ìstonewashingî denims, pre-digestion of animal feeds, and use in laundry products as a stain remover in place of phosphates.

The use of enzymes in food processing is one of the oldest applications of biotechnology. They have been safely used for thousands of years by communities who unknowingly employed microorganisms as sources of enzymes in the production of food and alcohol. Enzymes now have many applications in modern food processing. Their properties benefit both the food industry and the consumer. Their specificity offers food producers much finer product control, while their efficiency, requiring low energy inputs and mild conditions, has distinct environmental advantages. A striking example of the advantages of modern enzyme technology is the breakdown of starch to sugars. This process originally involved boiling the starch with acid, requiring large energy inputs and producing undesirable by-products. In contrast, the enzyme process takes place in mild conditions, saving energy and preventing pollution.

MARKET DEMAND OF INDUSTRIAL ENZYMES

The commercial use of enzymes has been steadily increasing on a global basis (Fig. 1). In 1994, the total global sales value was US$ 720 million. This figure nearly doubled by the end of the 1990s (Walsh, 2002) and is estimated at USS 1.7 billion by 2005. By 2009, this value is forecasted to reach US$ 2.25 billion (Godfrey, 2002). The strong and continued growth in enzymes may be attributed to both economic factors and to technical advances such as the use of genetic engineering and the development of new enzyme applications.

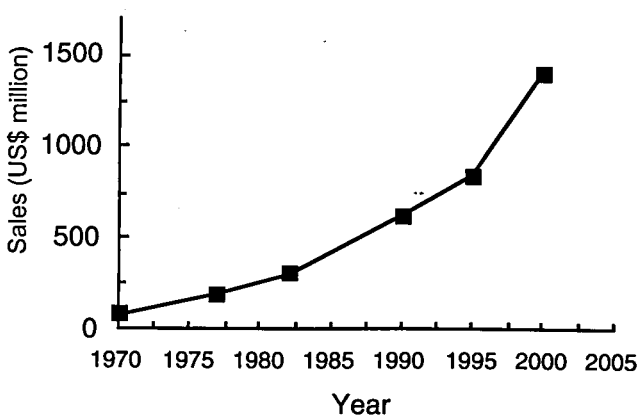

Fig. 1 : Growth of world industrial enzyme market

Fewer than 5% of all enzymes known to humankind have been commercially adapted for food use. Currently, the main users of bulk, commodity enzymes are the detergent, starch, textile and dairy industries (Fig. 2). However, the relative market share of the total enzyme market for these industries are likely to decrease in favour of industries categorised as 'Other' which include the baking, fats and oils, brewing, wine, fruits and vegetables, agriculture (e.g. animal feed), flavours, paper and pulp, waste treatments, leather, diagnostics and analytical, medical/ pharmaceutical/therapeutic and fine chemical industries (Godfrey, 2002). A recent report (Freedonia, 2002) suggests that the pharmaceutical industry, supported by the rapid growth of enzyme replacement therapies and heightened demand for chiral chemicals, and biotechnology research will become among the largest markets for speciality and industrial enzymes.

Of the industrial enzymes, proteases account for almost 50% (~US$ 700 million) of the market share, followed by the carbohydrases at US$ 555 million. Examples of the latter group of enzymes are α-amylases, cellulases, glucoamylases, glucose isomerases, pullulanases, lactases and pectinases. Although the carbohydrases will remain the single largest type of enzymes, the overall market share is likely to decline in the coming years (Freedonia, 2002). An enzyme with one of the biggest prospects will be the lipases due to continued penetration of the detergent market and chemical synthesis. The sales of enzymes new in the market such as enzymes used in enzyme-replacement therapies (e.g. glucocerebrosidase), phytase and cyclodextrin glucosyltransferase are also likely to grow rapidly in the near future (Walsh, 2002).

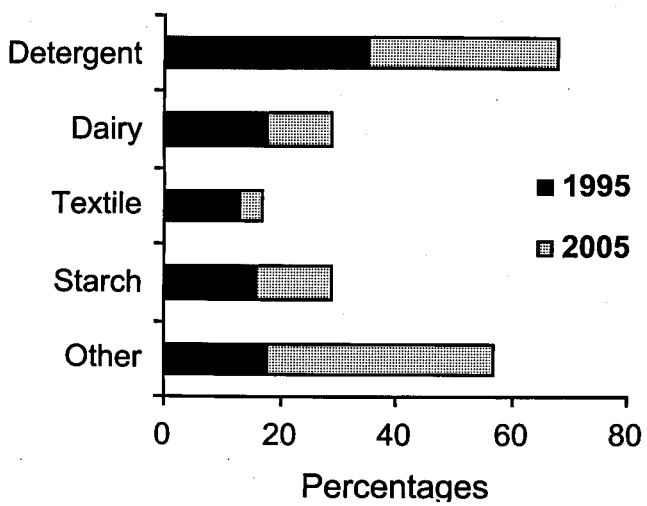

Fig. 2: Market share of enzymes for various sectors or enzyme application
Source: Godfrey and West (1996); Godfrey (2002)

There are now approximately 12 major producers of industrial enzymes (Godfrey and West, 1996; Walsh, 2002). Novozymes A/S is the world's largest supplier of industrial enzymes and they currently market some 600 different enzyme products used in nearly all industries utilising enzymes in their processes.

LEGAL STATUS AND SAFETY IMPLICATIONS OF ENZYMES USED IN FOOD PROCESSING

The majority of industrial enzymes are traditionally obtained from microorganisms; very few are produced by either plants or animals. Insofar as microorganisms are concerned, producer strains are usually members of a family of microbes classified as GRAS (Generally Recognised as Safe). Most notable producers are members of the genera *Bacillus* and *Aspergillus*. Other GRAS enzymes are derived from barley malt, *Papaya carica, Ananas comosus, A. bracteatus, Ficus* spp. and the stomach of calves.

The law regulating the use of commercial enzyme preparations in foods is generally controlled by national and international legislations and is highly varied throughout the world. In the United States, enzyme preparations are regulated either as secondary direct food additives or as GRAS substances (Cheeseman and Wallwork, 2002). GRAS enzymes do not require approval for their use in foods. However, enzymes that are considered as food additives require pre-market approval from the Food and Drug Administration (FDA). A partial list of enzyme preparations that are either GRAS or food additives has been posted by the Office of Food Additive Safety, FDA (2001). Table 1 shows some of these enzymes, their sources and applications. As an enzyme preparation may end up in the food that it has transformed, its safety must be assured. The burden of proof of safety is on the enzyme manufacturer/distributor. Most U.S. enzyme manufacturers use the decision tree of Pariza and Johnson (2001) when assessing safety of a new product. Safety concerns are mainly focusing on toxic properties of by-products and contaminants.

In the European Union (EU), the regulation of enzymes is not very clear. Generally, most of the enzyme preparations used for food processing are considered as processing aids since they are regarded as having no technical function in the final food. As such, their use in food is not currently covered by a community regulation, but this situation is being evaluated (Zeman, 2002). On the other hand, enzymes that do have a technical function in the final food are classified as food additives, and require pre-market approval. To date there are only two such enzymes.

Table 1. Sources and Applications of Industrial Enzymes

Enzyme	Source*	Food application
Bacterial α-amylase	*Bacillus subtilis; B. licheniformis*	Starch conversion
Fungal α-amylase	*Aspergillus oryzae*	Maltogenic saccharification
Glucoamylase	*Aspergillus niger*	Starch syrups, dextrose
Pululanase	*Klebsiella aerogenes*	Debranching starch
Neutral protease	*B. subtilis; A. oryzae*	Brewing/flavouring, Baking
Invertase	Yeast spp.	Confectionery industry
Pectinase	*A. niger*	Juice/wine processing
Glucose isomerase	*Streptomyces* spp.	High fructose syrups
Lipase	*Mucor* spp.	Dairy industry; fat modification
Lactase	*Kluveromyces lactis*	Diary industry
Glucose oxidase	*A. niger*	Analytical, food processing

* Other organisms have also been used to produce these enzymes

Enzymes produced using modern biotechnology (recombinant DNA technology or genetic engineering) often have additional regulations over those from traditional sources. The production of toxins resulting from unintended secondary effect is regarded as the main concern. Enzymes from genetically modified organisms (GMOs) are often evaluated on a case-by-case basis. The first food enzyme produced by a GMO is chymosin, the main enzyme in rennet produced by calf stomach, and was approved by the FDA in 1990. Chymosin is now used to make more than half of all cheese produced in the U.S. There are other GM enzymes but these are generally not sold as food enzymes. Many countries, including the EU, Japan, Australia, and New Zealand, are currently developing or reassessing their regulations for enzymes from GMOs (Zeman, 2002).

Apart from the law, an emerging and important consideration regarding the use of enzymes in foods is their acceptability in the eyes of Islam and the Jewish religion. Many enzyme producers have taken steps to ensure that their enzyme production methods and enzyme preparations comply with requirements for kosher and halal certifications. On 1 August 1998, the Council (Board of Trustees) of The Vegetarian Society of The United Kingdom made an exception to the use of GMOs as or in foods by endorsing vegetarian cheese produced using chymosin from GM yeast.

APPLICATIONS OF ENZYMES IN THE FOOD INDUSTRY

Enzymes are regarded either as problems or solutions, depending on their impact on food processing and product quality. For fresh-cut fruit and vegetable processors, endogenous enzymes from plant tissues are responsible for browning, adverse flavour changes and texture loss - changes that need to be avoided by heating, chilling or acidification. However, in many other food processing industries including the fruits

and vegetables industry, enzymes are viewed as valuable assets that make the job of turning out better products much easier. Therefore, many food product developers consider enzyme use innovative and, in some cases, the most elegant solutions in food processing and process design when creating new foods.

The applications of enzymes may be traced to the history of mankind. Traditional processes such as the production of alcoholic beverages and yeast-fermented dough in bread making, are displayed in Egyptian wall paintings (Fig. 3).

Fig. 3 : 3 Bread making in a court bakery of Ramses III.

The first recorded commercial use of an enzyme (Takadiasterase from 'kojií) in foods was in 1894 (Takamine, 1894). In 1907, Otto Rohm discovered the effectiveness of pancreatic proteases in bating of hides, and these enzymes help to revolutionise leather manufacturing by replaced more traditional sources of enzymes (e.g. dog excrements). These enzymes soon found their way into detergents as stain remover. From then on, the use of enzymes especially in the food industry, grew rapidly, and this phenomenon was spurred by progress made in enzyme immobilization, catalysis in nonaqueous media, and in fermentation processes. By the 1980s, modern biotechnology processes have begun to play an increasingly crucial role in modifying microorganisms such that they produce enzymes (e.g. chymosin) that they otherwise do not, and which allow enzymes to be tailored for specific applications.

Many types of enzymes are making considerable inroads into various sectors of the food industry. Among the food sectors that are deriving benefits from the use of enzymes are the starch, dairy, baking, fruits and vegetables, brewing and wine industries. A growing need for more friendly transformation processes in the fats and oils industry has now brought research in the area to the fore. Some of the enzymes used are the proteases, lipases and carbohydrases like amylase, pectinase and cellulase (Table 1) and non-traditional enzymes like sulphydryl oxidase and cyclodextrin glucosyl transferase.

In the following section, the roles enzymes play in these industries shall be highlighted. Special emphasis is given to the applications of enzymes in the modification of the properties of fats and oils as their use in the fats and oils industry is currently gaining momentum.

a. Starch industry

One of the largest users of food enzymes is the starch industry. Starch is a carbohydrate polymer composed of α-D-glucose and exists in two forms: linear amylose and branched amylopectin (Fig. 4). The ratio varies with the starch source but is typically 20:80 amyloses to amylopectin. The main sources of industrial starch are corn and wheat. Nearly half of the starch that is isolated annually in the US is enzymatically hydrolysed. About 6 million tons are used in the manufacture of high fructose syrups, the major sweetener used in the US food industry. The remainder is partially hydrolysed into dextrins and maltose syrups. These are used in many food applications.

Starch, in its native form, exists in relatively inert granular structures in which amyloase and amylopectin are found. These granules are insoluble in water and resistant to many chemicals and enzymes. Reactivity towards enzymes is enhanced when the granules are disrupted by heating in water (gelatinisation) (Fogarty and Kelly, 1990; Bentley and Williams, 1996). Thus, enzymatic diversification of starch begins when a suspension of starch is gelatinised. Addition of various amylolytic enzymes will hydrolyse the glycoside linkages of starch to produce a variety of products. The reaction involving hydrolysis of starch by α-amylase is known as liquefaction. α-Amylase, which hydrolyse the starch glucosidic bonds randomly, can partially digest starch into maltodextrins (Fig. 5) which are an excellent starting material for subsequent saccharification of starch. Maltodextrins are also used as an ingredient in chewy soft sweets, low fat foods (act as fat replacer), baby foods, hospital diets and instant soups. Prolonged liquefaction of starch with α-amylase produces dextrins and oligosaccharides.

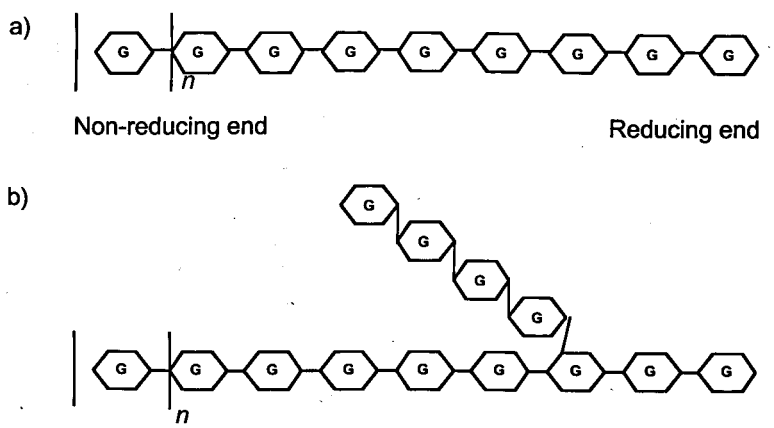

Fig. 4 : Structure of (a) amylose (linear) and (b) amylopectin (branched). 'G' denotes α-D-glucose.

Depending on the enzyme used, saccharification of starch can either yield glucose or maltose syrups (Fig. 5). Conversion of starch into glucose syrup requires the combined action of α-amylase, pullulanase and glucoamylase. A study conducted by Subhi and Ghazali (1986) shows that immobilized glucoamylase may be used to saccharify soluble dextrins obtained from α-amylase-digested-cassava starch.

Different saccharification conditions will result in the tailor-made generation of a wide range of glucose syrups for different applications. Crystalline glucose may be obtained from glucose syrup. Glucose syrup may be further converted into a much sweeter material - high fructose syrup - via isomerisation using glucose isomerase. High fructose syrups have found applications as a sweetening agent in cakes, confectionery, soft drinks, canned foods, jams, jellies and ketchup. Glucose syrups are also excellent feedstock in fermentation processes. Ghazali and Cheetham (1983) reported on the production of alcohol from dextrinised corn starch using immobilised glucoamylase co-immobilised with *Sacchromyces uvarum* in calcium alginate beads, while the study by Ho and Ghazali (1986) showed that when immobilised glucoamylase was co-immobilised with *Zymomonas mobilis*, a high concentration of alcohol may be produced from α-amylase liquefied cassava starch.

Maltose syrups, produced when starch is reacted with β-amylase, are characterised by low viscosity and hygroscopicity, good heat stability and mild sweetness. They are used as ingredients in various foods, confectionery, soft drinks and in ice cream. Maltose may

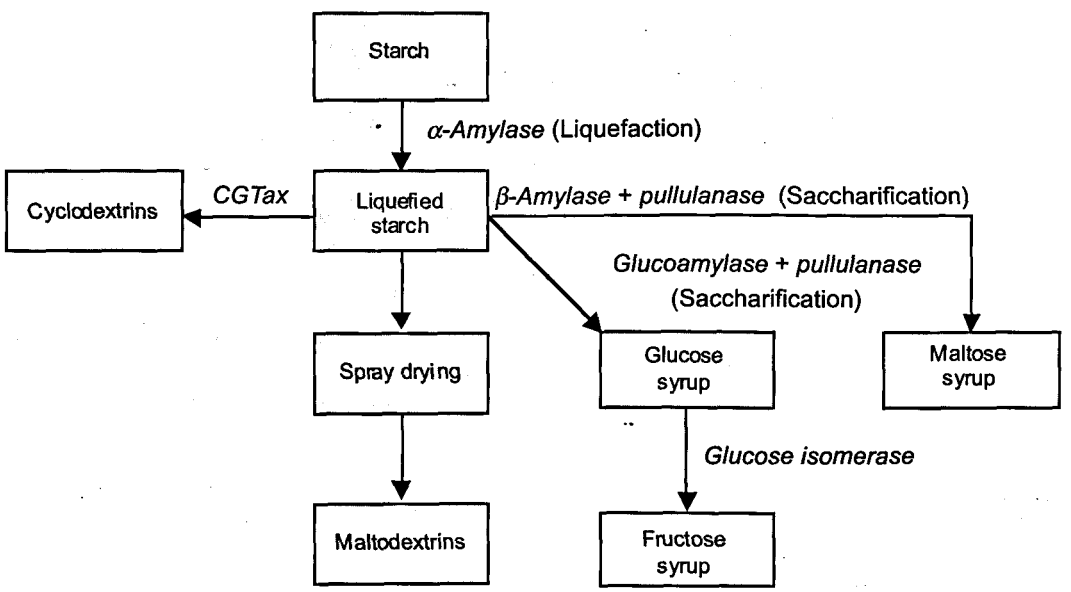

Fig. 5 : Hydrolytic degradation of starch yielding industrially important end products: maltodextrins, glucose syrup, maltose syrup, fructose syrup and cyclodextrins. *CGTase* is cyclodextrin glucosyltransferase

be converted into maltitol, a non-nutritive sweetener, by a reduction process. Starch may also be enzymatically converted into 5, 7 or 8-membered cyclodextrin rings. The enzyme used is cyclodextrin glucosyltransferase (CGTase). Cyclodextrins are used to encapsulate guest molecules such as vitamins, fragrances, flavour compounds or drugs.

The main starch source in Malaysia is sago. Sago starch has many traditional uses. Enzyme technology may be applied to enhance its applications. An example is the production of high amylose starch which is used in the food industry as ingredient for jelly-gum production and coating for deep fried foods. The industrial supply of high amylose comes from high amylose corn (Anon, 2002). High amylose starch may be produced by genetic More interesting products could be developed from different starches should amylolytic enzymes that act on native starch are available in bulk.

b. Dairy industry

The main activity of the dairy industry is, of course, cheese making. Cheese making has a very long history and is the most traditional method of preserving milk. The manufacture of most cheeses is initiated by the addition of starter cultures to curd obtained after milk has been coagulated with rennet which contains chymosin. The curd is then allowed to mature into cheese. The process involves a slow, controlled degradation of proteins, fats and carbohydrates of milk curd by enzymes produced by the starter cultures and residual milk lipases. Different starter cultures with their distinctive microflora produce a whole range of enzymes, and the milk lipases, turn bland immature cheese curds into the wide range of cheese flavours. Cheese ripening may be expedited through the addition of enzyme preparations containing proteases and lipases.

Enzyme Modified Cheese (EMC) is a natural concentrated cheese flavouring made from immature cheese treated deliberately with enzymes much like those produced by starter cultures. The main advantage of using young cheese is that it is much cheaper than mature cheese. Also, it only takes only 1-2 days to make EMC (InBrief.21, 2000a) whereas it may take 1-2 years to get cheese with an equally strong flavour. EMC is sold either as spray dried powder or paste and can be used in any application where a cheese flavour is needed including processed cheese, substitute cheese, dressings and dips, sauces, soups, pasta, convenience foods, biscuits and snack products, spreads and fillings.

Each year the cheese manufacturing industry produces large quantities of whey (Wigley, 1996). Whey is composed of two main components: lactose (70-75% whey solid) and whey protein (6% solid). Usually whey is released into the environment, and because lactose and whey proteins are harder to digest, causes severe pollution. Modern enzyme technology helps prevent waste by converting lactose in whey into a more soluble and sweet-tasting mixture of glucose and galactose. The product can be refined and concentrated into honey-like syrup that has a wide range of applications in the confectionery and soft drink industries.

There are other enzymes besides rennet, proteases and lipases that can be used in the dairy industry: β-galactosidase is used to produce low lactose milk for consumers who are lactose intolerant, and sulphydryl oxidase, which may be used to reduce the cooked flavour in HTST milk.

c. Fruits and Vegetables

Fruit processors rely heavily on enzymes to process a wide variety of fruits such as apples, pears, mango and berries (e.g. blackcurrant, grapes) into natural beverages (Uhlig, 1998; Alkorta *et al.*, 1998). In fact, juice clarification is one of the oldest applications of enzymes in the fruit and vegetable processing industry, and still is the largest user of enzymes.

The processes used in fruit juice extraction vary considerably depending on the type of fruit, its age and maturity. In general, extraction of fruit juice involves maceration followed by pressing or decanting to separate the juice from the solids. For some fruits like grapes and apples, pressing results in low juice yields and this is due to entrapment of juice inside cells by a gel-like pectin-hemicellulose network located at the cell walls of fruits. The cell wall is made more complex by hemicellulose being cross-linked to xylan, another cell wall polymer, and to the arabinogalactan side chains of the pectin. Thus, for efficient breakdown of the cell wall to release entrapped juice several enzymes including pectinases are usually used.

Sometimes, simple extraction alone is inadequate to obtain high yields of free-run juice from fruits that are too firm, pulpy and/or pectinaceous such as cucumber, pumpkin, papaya, mango, 'ciku' and banana. Extraction of juice from such fruits can be improved through the addition of a cocktail of enzymes that will catalyse the complete liquefaction of fruit cells. These enzymes not only increase juice yields, they also increase soluble solid content, improve colour and aroma, and increase health-promoting antioxidants in fruit and vegetable juices.

Studies conducted by the author and co-workers have shown that when enzymatically liquefied, higher volumes of free-run juice which were also more concentrated can be obtained from the pulp of starfruit (Ghazali *et al.*, 1999), 'ciku' (Nor Sulyana, 1999), roselle (Ghazali *et al.*, 1998) and honeydew melon (Ghazali *et al.*, 2003) compared to pressing alone. The juices obtained with these fruits were turbid but cleared rapidly when a further dose of enzymes was added. The end product is one that can be further diluted if required and has pleasant mouthfeel and flavour. Besides being marketed as clear fruit juices, clarified juices are sometimes carbonated and marketed as sparkling fruit juice.

In cases where fruit juice becomes turbid or hazy due to the presence of starch and/or arabinan, enzymes like amylases and arabinase help to clarify and stabilise juice by degrading these polymers.

Other uses of enzymes in the fruit and vegetable industry are:

- ❖ maceration of tissues into a suspension of individual intact cells for production of fruit nectars, 'pulpy' drinks, and as ingredients in the preparation of some baby foods, yogurts and puddings
- ❖ preparation of juice and fruit nectars with stable cloud
- ❖ production of citrus cloud from orange solid pulp residue after juice extraction
- ❖ extraction of essential oils from orange peel
- ❖ fruit peeling (Baker and Wicker, 1996)
- ❖ debittering of citrus juice particularly those that contain excessive amounts of naringin (Lea, 1995).
- ❖ maintaining firmness and shape of cut or whole fruit and vegetable pieces after undergoing heating or freezing. Such fruits are used in fruit-flavored yogurts, baked goods or dessert toppings.

d. Brewing Industry

Beer is one of the oldest and probably the most widely distributed alcoholic beverages in the world. Beer brewing involves the production of alcohol (ethanol) by allowing yeast such as *Saccharomyces cerevisiae* to act on plant materials like barley, maize and sorghum, in the presence of extracts from hops to provide a bitter taste. The yeast possesses a complementary of enzymes necessary in the anaerobic conversion of simple sugars like glucose into alcohol and carbon dioxide. The sugars are derived from the breakdown of starch by enzymes like α-amylase which are produced when the plant material (e.g. barley) used to make beer is malted or partially germinated

Enzymes have been proven to be useful when the process of malting becomes expensive and difficult to control especially when poor or variable quality malted grains are used (Uhlig, 1998). By adding enzymes such as α-amylase and glucoamylase to unmalted barley, starch conversion into simple sugars is more controlled and efficient and this makes the process simpler and less expensive. When the level of conversion is very high and fermentation is stopped early, a product called 'lite' beer is produced. This product contains fewer calories in the form of sugars and partially digested soluble polysaccharides, and a slightly lower alcohol content compared to 'regular' beer.

Newly fermented beer is often difficult to filter due to the presence of insoluble polysaccharides such as β-glucan and xylan. The combined action of enzymes like β-glucanases and xylanases to fermenting wort (a mixture of malted barley and adjuncts like hops) has been demonstrated to reduce the contents of the non-polysaccharides, and improve filtration (Biocatalysts Tech. Bull., 2001a). The use of the enzymes has also solved the problem of polysaccharide-induced haze in beer which often forms in finished beer during cool. This can easily be overcome by treatment of the beer with exogenous proteolytic enzymes (e.g. papain) in a process called chillproofing. After filtration, the beer is pasteurized where the added enzymes are denatured.

e. Wine industry

Enzymes have now become an integral part of oenologic methods along with the ancient knowledge of winemaking. Their activities begin during the ripening and harvesting of grapes, and continue through alcoholic and malolactic fermentation, clarification, and ageing. In recent years, winemakers often supplement naturally occurring grape enzymes with commercial enzymes to increase juice extraction yield, improve extraction of fermentable sugars and flavour/aroma components, reduce pressing time and improve clarity of wine. The result is an increased production capacity of clear and stable wines with enhanced body, flavor and bouquet (Grassin and Fauquembergue, 1996). A good extraction of pigments (colour) from the types of grapes used in red winemaking is especially important and this is often achieved through grape skin-contact treatment with pectinases that lack anthocyanase activity.

Some high quality wines, such as the *Sauternes*, are made from overripe grapes that are deliberately allowed to shrivel on the vine infected with the mold, *Botrytis cinerea* (noble rot). This organism produces a type of β-glucan which is not degraded by fermenting yeasts and remains in the final wine. Such wines are often difficult to clarify and filter. The enzyme, β-glucanase, speeds up clarification and filtration by hydrolyzing the β-glucan (Biocatalysts Tech. Bull., 2003).

Haze in wine is eliminated through the addition of acid proteases that clarify and stabilise some wines by reducing or removing naturally occurring and yeast synthesised, heat-labile proteins.

f. Baking Industry

Baking is one of the three oldest biotechnology industries. In a bakery operation, enzymes are viewed as valuable assets that make the job of turning out consistent bakery products a little easier. Historically, malt extracts - which are rich in native barley enzymes - were added to dough to get the benefit of those enzyme activities. Today, it is common to supplement native flour enzymes with exogenous enzymes produced by microorganisms, particularly amylases, proteases and xylanases. Some of the benefits of enzymes in bakery products are better dough handling, improved machinability, higher loaf volume, better control over crumb characteristics (texture and color), and longer shelf life by providing anti-staling properties. Different enzymes are often carefully blended so as not to over-treat the dough to the point that product quality or machinability is affected, and in baking, enzyme blending is as much an art as a science.

Baking enzymes are usually targeted for a particular flour (wheat, rye, oat) or a particular finished product such as bread or crackers. Most bread is made of wheat and when yeast is added to bread dough, carbon dioxide is produced from simple sugars which makes the bread rise. These sugars are produced from starch by native wheat enzymes but the amount often vary due to grain variety, harvesting conditions (e.g. maturity, the time of year it is picked, disparities in milling, and many other inconsistencies. Addition of

amylase and glucoamylase will convert damaged starch in flour into a continuous supply of fermentable sugars during dough development, thereby improving the leavening (loaf volume) and crumb structure of bread and rolls (InBrief.21, 2000b). Dough development time is also reduced.

Staling of bread is perceived as a loss of product freshness, manifested by a gradual increase in crumb firmness as soon as baking is completed. Bread becomes unacceptable and is discarded. In the US, bread staling is responsible for significant financial losses (Hebeda *et al.*, 1990). The shelf-life of bread may be increased between 38-75% through limited degradation of starch by using thermostable bacterial amylases (Hebeda *et al.*, 1991). However, over-dosing can cause continued hydrolysis of starch and crumbs can actually become gummy as the enzyme is still active in the finished baked product.

Another enzyme which may be added to wheat flour is protease. During dough preparation, gluten protein in wheat flour binds some water and expands forming a lattice-like structure (Uhlig, 1998). Proteases act on gluten and improve the elasticity of the dough. This can reduce mixing time and handling properties of the dough and gives bread with a good volume. Wheat flour that has lost its elasticity also benefit from the addition of protease. Proteases may be added to high protein flour used for biscuit manufacture where dough that is easy to roll out and does not rise much is required.

A recent innovation is the use of enzymes like glucose oxidase, sulphydryl oxidase, pentosanases and α-amylase, designed to replace chemical dough conditioners, such as potassium bromate (Inbrief.21, 2000b). The compound has been widely used to condition dough, age flour and stabilise its baking properties by acting as an oxidising agent (Popper, 1998). Although bromate was a cheap and effective dough strengthener, its degradation products were found to be possible human carcinogen (Kurokawa *et al.*, 1990). Bromate is banned in the EU except in exported wheat flour (Popper, 1998).

Another chemical compound that is being replaced with enzymes is sodium metabisulphite (Popper, 1998). Its sole used is in biscuit and cracker manufacture where low-protein flours are required, but which are not readily available in most countries. Metabisulphites are widely used to weaken the gluten structure of the protein, reducing its resistance to extension and making the resulting dough more plastic. However, metasulphites have undesirable side effects. They break down vitamin B2, inhibit browning reactions that are desirable in baked products and appear to evoke asthma attacks in affected individuals. Enzymes such as proteases, pentosanases and hemicellulases are now effective metabisulphite replacers. (*InBrief.*21, 2000b)

g. Other

There are many uses of enzymes other than those already described. These include applications in egg processing, protein (food flavour) hydrolysates production,

monosodium glutamate production, invert sugar production, meat tenderisation, fish sauce production and fats and oils modification. Of these applications, enzymes have the longest use in meat tenderisation.

i. Flavour hydrolysates

Flavourings can be produced by a number of technologies including cooking, compounding and enzymatic modifications. A well-known flavouring material for the food industry are the protein hydrolysates and they are used as flavouring ingredient in many types of meat and other savoury products including soups, sauces, snacks, pies, prepared meals and gravies. Examples of protein hydrolysates are isoelectric soluble soy protein, soluble wheat gluten, whey protein hydrolysate, casein hydrolysate, red blood cell hydrolysate and soluble meat hydrolysate. The latter two are by-products of the meat industry. Bones with residual meat and meat scraps are steeped in a solution of proteases (Uhlig, 1998) to produce meaty flavoured stock that can be added to sausages and pies during processing, and used in gravy for canned meat products. Red blood cell (RBC) hydrolysate is prepared from blood solids treated also with proteases following which, the hydrolysate is spray dried and used in some industrial food preparations.

Soybean can be processed chemically to make a meaty flavour called acid Hydrolysed Vegetable Protein (HVP). This is produced in bulk quantity by hydrolysing soya flour with strong hydrochloric acid at high temperatures and pressures. Acid HVP is increasingly seen to have many disadvantages, including unacceptable levels of the carcinogens, 3-MCPD (3-monochloropropane-1,2-diol) and 1,3-DCP (1,3-dichloropropanol) (IFST, 2003). The emerging alternative to acid HVP is enzyme HVP (eHVP). Meaty tasting soya hydrolysates produced with enzymes (proteases) are now being commercially produced.

Yeast extract is well known for its use as a food flavouring in many food products, e.g. soups, sauces, gravies, snack foods, and meat products. It is the main component of savoury spreads such as Vegemite® and Marmite®. It is produced by hydrolysing bakers' yeast with endogenous enzymes from within the yeast and also exogenous enzymes to accelerate this process (Biocatalysts Tech. Bull., 2002).

ii. Egg processing

Eggs are extremely useful food ingredients and have a variety of properties including foaming, gelation, emulsification and texturisation. The main components of egg are proteins and lipids and these are responsible for the functional attributes. Other components are present in small quantities.

Traditionally egg ingredients were supplied in the form of whole eggs. However, today's food processors can choose from a wide range of egg ingredients where various processes are used to produce liquid, frozen, dried whole eggs, whites or yolks. High pasteurisation

temperature can damage egg white. To lessen this damage, a combination of lower temperatures and hydrogen peroxide can be used (Biocatalysts Tech. Bull., 2001b). Residual peroxide is removed using the enzyme, catalase.

Another problem that is encountered during the heat treatment of eggs is browning caused by Maillard reaction. This occurs as a result of small amounts of glucose in the egg white reacting with amino acids. To minimise browning, enzymatic desugarisation is done. Other applications are the use of lipase to reduce contamination of egg white with egg yolk which interferes with the foaming capacity of egg white and improved emulsification and gelation properties of egg yolk using phospholipases.

iii. Invert sugar production

Invertase is used industrially to hydrolyse sucrose into an equal mixture of glucose and fructose, also known as invert sugar. Invert sugar is non-crystallising, and is therefore, used in the confectionery industry to form the liquefied filling present in the center of some soft-centred sweets. The enzyme is also used in the manufacture of artificial honey and invert sugar syrup. The latter is used in many branches of the food industry e.g. jam making.

APPLICATIONS OF ENZYMES IN FATS AND OILS MODIFICATION

An emerging technological application of enzymes is enzymatic modification of fats and oils or triacylglycerols (TG). It has only been recently introduced at industrial scale for TG processing for the enzymatic production of 1,3-diacylglycerol oil (Econa/Enova) developed by Kao/ADM. A recent industrial study conducted by Freedonia (2002) showed there is very positive indication that there will be a strong penetration of lipases in various industrial sectors including modification of fats and oils for use in food systems.

In the applications of enzymes discussed earlier and in many other applications, reactions take place in an aqueous environment. However, fats and oils are water-insoluble. When mixed with water in the presence of an emulsifier or a surface active agent (surfactant), they form stable oil-water interfaces (emulsions). Catalysis takes place at these interfaces, and is successful only if enzymes that are active at the oil-water interfaces are used. One such enzyme is lipase. In fact, it has become generally accepted that lipases preserve their catalytic activity even in organic solvents, biphasic systems and micellar solutions. The choice of solvent (solvent engineering) to be used is study in itself (Laane and Tramper, 1990).

Lipases are hydrolases and are classified as glycerol ester hydrolases (EC 3.1.1.3). These enzymes are ubiquitous in all living sources. The natural substrates of lipases are the medium to long chain fatty acid esters of glycerol (triacylglycerols/fats/oils), which may

be saturated or unsaturated (Brockerhoff and Jensen, 1974). They exhibit little or no reaction against soluble substrates in aqueous solutions. They become activated only at the water-oil interface via a process termed interfacial activation.

The biological function of lipases is the hydrolysis of the ester bonds of fats and oils yielding free fatty acids and glycerol (Fig. 6). Incomplete hydrolysis will release free fatty acids (FFA), diacylglycerols (DG), monoacylglycerols (MG) and glycerol.

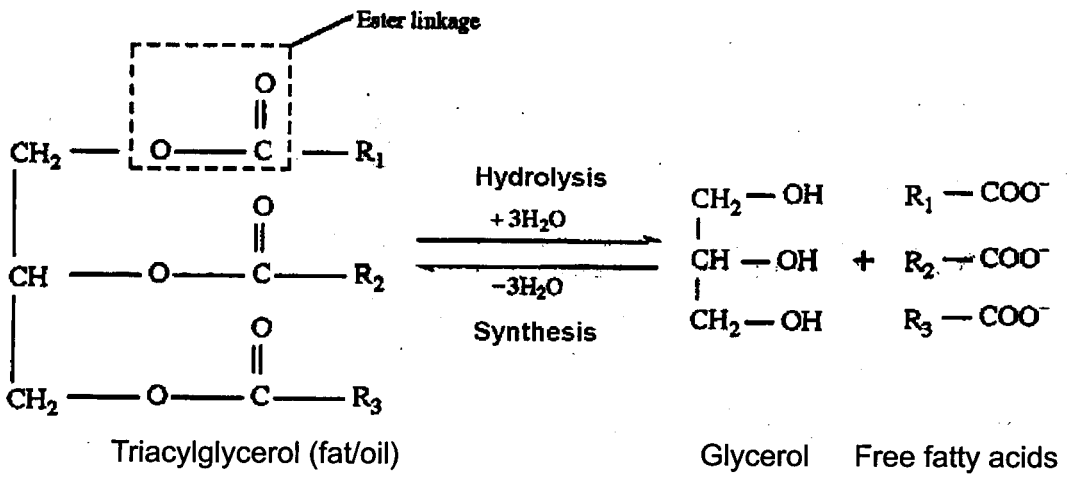

Fig. 6 : Reactions catalysed by lipases

Efficient hydrolysis of oil takes place when the total interfacial surface area between the oil and water is large. This may be obtained by forming a stable emulsion or by using reverse micellar systems (Martinek *et al.*, 1981) where the enzyme is contained in very tiny droplets of water surrounded by the oil dissolved in organic solvents such as isooctane. Stabilisation of the system is through the addition of a surfactant like sodium *bis*(2-hexylethyl) sulphosuccinate (Aerosol OT). Ghazali and Lai (1996) have shown that *Candida rugosa* lipase entrapped in reverse micelles was still catalytically active, hydrolysing palm olein and other oils to produce FFA.

Lipases can be classified into three groups based on their specificities: non-specific, 1,3-specific and fatty acid specific lipases (Macrae, 1983; Sonnet, 1988). Non-specific lipases release fatty acids from all three positions of the glycerol molecule and catalyse the complete breakdown of TG into FFA and glycerol. The second group of lipases catalyses the release fatty acids specifically from the outer 1- and 3-positions of TG producing FFA, 1,2- and 2,3-DG and 2-MG. Because the DG and 2-MG are chemically unstable, they undergo acyl migration to give 1,3-DG and 1- or 3-MG, respectively, and prolonged incubation of fats with 1,3-specific lipase will give complete breakdown of some of the TG. The last group of lipases catalyses the specific release of a particular type of fatty acid

from TG. A well known though rather rare example is the lipase produced by *Geotrichum candidum* which preferentially hydrolyse long chain fatty acids containing a *cis* double bond in the 9-position from TG. A study that is currently on-going utilises the cell-wall bound form of the enzyme produced by an indigenous strain to selectively hydrolyse such fatty acids from palm olein. The hydrolysate contains a significant quantity of oleic acid (Loo *et al.*, 2002a). The oleic acid in the hydrolysate can then be enriched through separation processes and can be used as a source of industrial oleic acid for the oleochemical industry.

The reaction catalysed by lipases is generally reversible and re-esterification (synthesis) can happen at the same time as hydrolysis (Fig. 6). All synthetic reactions catalysed by lipases are initiated by hydrolysis of TG into a FFA and DG (Foglia *et al.*, 1993). The synthesis of esters via esterification and interesterification occur under low moisture conditions (Sonntag, 1979) or even in solvent-free systems, which minimise hydrolysis.

Interesterification refers to the exchange of acyl radicals between an ester (e.g. TG) and an acid (e.g. fatty acid) (acidolysis), an ester and an alcohol (alcoholysis), or an ester and another ester (transesterifcation) to produce new interesterified products (Chaplin and Bucke, 1990). In alcoholysis, when the alcohol is glycerol, the reaction is called glycerolysis. The ability of lipases to modify fats and oils via interesterification reactions has been demonstrated countless times. Among the earliest studies using palm olein as substrate was by Ghazali *et al.* (1995a). In this study, several nonspecific and specific microbial lipases were used to mediate the transformation of palm olein in water-saturated hexane. Apart from the enzyme from *R. miehei* which was obtained already in the immobilized form (food grade), the rest of the lipases were immobilized onto Celite and dried by lyophilisation prior to transesterification. The catalytic performance of the enzyme was evaluated by determining changes in TG composition and formation of FFA. It was shown that optimum transesterification activity was obtained when drying was done for 4 hours, and this coincided with minimum hydrolytic activity (Fig. 7) (Ghazali *et al.*, 1995a; Lai *et al.*, 2000a).

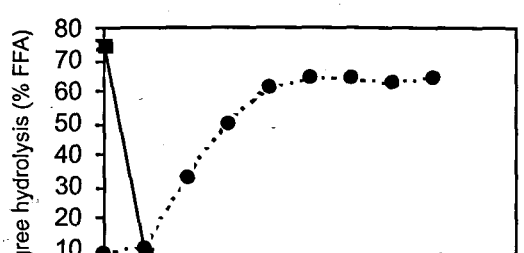

Fig. 7 : Effect of lyophilisation (drying) on hydrolytic (■) and transesterification (●) activities of *C. rugosa* lipase immobilised to celite (Ghazali *et al.*, 1995a).

The usual method of determining lipase activity is hydrolysis in aqueous medium. However, in the study by Ghazali *et al* (1995a), the activities of the immobilized enzymes used were assayed based on the rate of transesterification of palm olein at 30°C (Fig. 8). The most active lipase was from *Pseudomonas*, followed by the lipases from *R. miehei* and *A. niger*. Changes that occur in the TG profiles of unreacted palm olein and palm olein reacted for 24 hours with *R. miehei* and *Pseudomonas* lipases are shown in Fig. 9. It can be clearly observed that transesterification resulted in increases in the concentrations of some TG like trioleolylglycrol (OOO) and OOL, where O and L are oleic and linoleic acids, respectively. Tripalmitoylglycerol (PPP, where P is palmitic acid), which was identified based on a spiking experiment with standard (Fig. 10), was detected only in enzyme-treacted samples (Fig. 9). The best enzyme for the process was the nonspecific lipase from a *Pseudomonas* sp., followed by the 1,3-specific lipases from *R. miehei* and *A. niger* (Table 2) where there transesterification led to increases in the concentrations of saturated TG (PPP) and tri- and polyunsaturated TG with corresponding decreases in mono- and diunsaturated TG. Subsequent studies have shown that high melting TG present in reacted oils crystallised (Fig. 11) on standing at room temperature (Hamidah, 1995), giving an oil more fluid after removal of the solidified TG (Hazlina, 2002; Kerr, 2002).

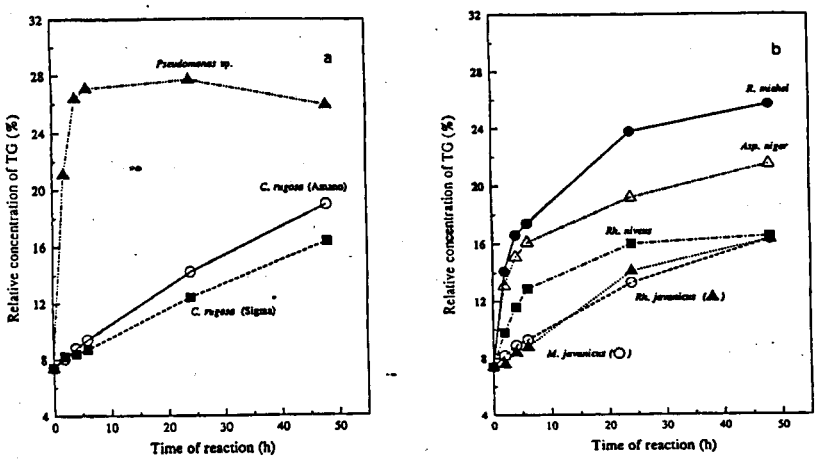

Fig. 8 : Transesterification activities of (a) non-specific and (b) specific lipases with time.

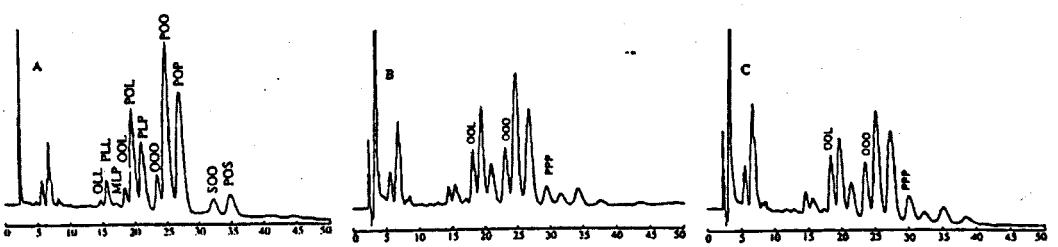

Fig. 9 : TG profiles of palm olein at the beginning 9A), and after 24 hours reaction with *R. miehei* (B) and *Pseudomonas* (C) lipases. P, palmitic; O, oleic; L, linoleic; S, stearic acid.

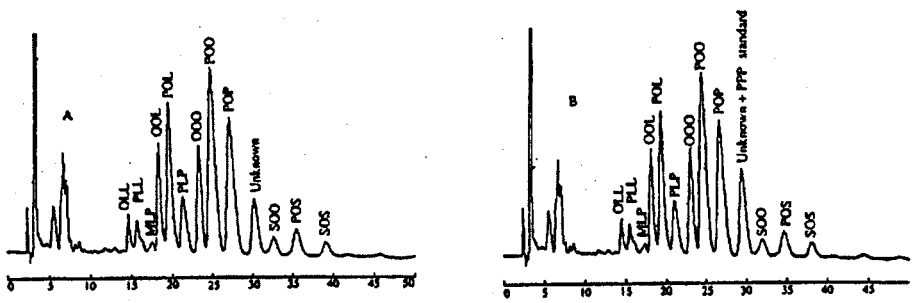

Figure 10 : Spiking experiment with PPP to determine the identity of unknown peak in unspiked (A) and spiked (B) transesterified palm olein. Source: Ghazali *et al.* (1995a)

Table 2. Rates of transesterification and concentrations of PPP and OOO with time of reaction. P and O are palmitic acid and oleic acid, respectively.

Source of lipase[b]	Specificity	Activity (% TG hydrolyzed)	PPP (% w/w)					OOO (% w/w)					Rate of trans. ($X h^{-1}$)
			2 h	4 h	6 h	24 h	48 h	2 h	4 h	6 h	24 h	48 h	
Candida rugosa (Sigma)	Random	8.4	0	0	0	1.6	3.0	4.0	3.3	3.1	3.5	4.2	3.3
C. rugosa (Amano)	Random	9.5	0	0	0	1.5	3.3	3.6	3.5	3.6	3.7	4.3	3.6
Pseudomonas sp. P	Random	8.0	4.8	5.4	6.0	6.3	6.2	6.3	7.0	6.3	5.8	6.0	59.4
Mucor javanicus M	1,3-Specificity	6.4	0	0	0	1.3	1.9	3.7	3.8	3.7	4.3	5.0	5.6
Rhizomucor miehei (Lipozyme IM 20)	1,3-Specificity	10.5	1.5	2.4	2.3	4.0	5.8	4.8	5.2	5.0	5.8	6.2	21.9
Aspergillus niger A	1,3-Specificity	11.8	0	1.4	1.8	1.9	3.3	4.6	4.8	4.8	5.2	5.3	16.3
Rhizopus javanicus F	1,3-Specificity	3.3	0	0	0	1.6	2.6	3.6	3.6	3.8	5.0	5.4	7.7
Rh. niveus N	1,3-Specificity	14.9	0	0	1.0	2.3	2.1	3.8	4.0	4.3	4.9	4.9	7.0

Source: Ghazali *et al.* (1995a.)

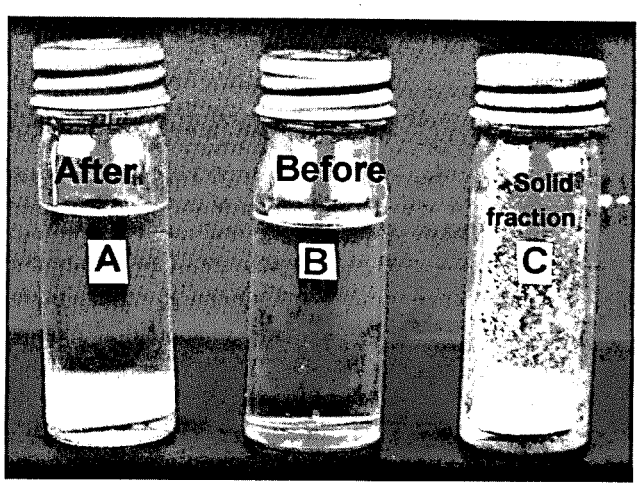

Figure 11 : Palm olein following enzymatic transesterification and storage at room temperature (A), before transesterification (B) and after removal of liquid fraction (C).

Besides using free lipases that are immobilised prior to reaction, lipases that are naturally immobilised to the cell-wall of the organisms producing them were also studied. Naturally immobilised lipase (NIL) was obtained by culturing the organism in the presence of oil, and harvesting the organism after maximum production of NIL has occurred. Two of these organisms, *A. flavus* (Long *et al.*, 1996a, 1996b) and *G. candidum* (Loo *et al.*, 2002b) were isolated from local sources while another, *R. miehei* (Liew *et al.*, 2000), was sourced from the American Type Culture Collection (ATCC). To enhance the stability of the cell-bound lipase from *A. flavus, in situ* cross-linking with was carried out using either gluteraldehyde or methylglyoxal (Long *et al.*, 1996c). Lipase activity was enhanced by up to 48% by treatment with the latter. Improvement in heat stability by 58% at 50°C was also observed with methyglyoxal-treated cell-wall bound lipase (Fig. 12). The physico-chemical properties (Long *et al.*, 2001) and substrate preference (Long *et al.*, 1998) of the bound lipase from *A. flavus* have been determined. The lipase prefers to hydrolyse shorter chain fatty acids from TG, as opposed to medium and short chain fatty acids. It was also shown that the enzyme is 1,3-specific (Long *et al.*, 2001).

The cell-bound lipases were used in a number of ways: hydrolysis of palm olein (Long *et al.*, 2000), acidolysis of several oils with selected fatty acids (Long *et al.*, 1997) and transesterification of palm kernel oil with anhydrous milk fat (Liew *et al.*, 2001a). In the study on acidolysis, added fatty acids which were incorporated into the oils modified the final products such that their TG profiles differed from the initial oils (Fig. 13). Incorporation was shown by an increase in the concentration of the added fatty relative to its initial concentration in the oil.

There are a number of potential applications for acidolysed fat products. The production of a specific TG of nutritional interest has been proposed by acidolysing medium chain TG (MCT) with linoleic acid. MCT are to improve their nutritional status of those who are unable to digest the conventional sources of fats and oils due to insufficient gastric lipase. Acidolysis of palm oil (especially palm mid fraction) with stearic acid has been studied successfully to produce cocoa butter equivalents (Bloomer *et al.*, 1990). The result

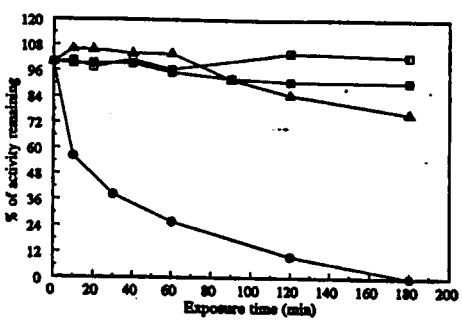

Fig. 12 : Thermal stability of extracted (●), untreated (▲), methylglyoxal- (■) and gluteraldehyde (□)-treated bound lipase.

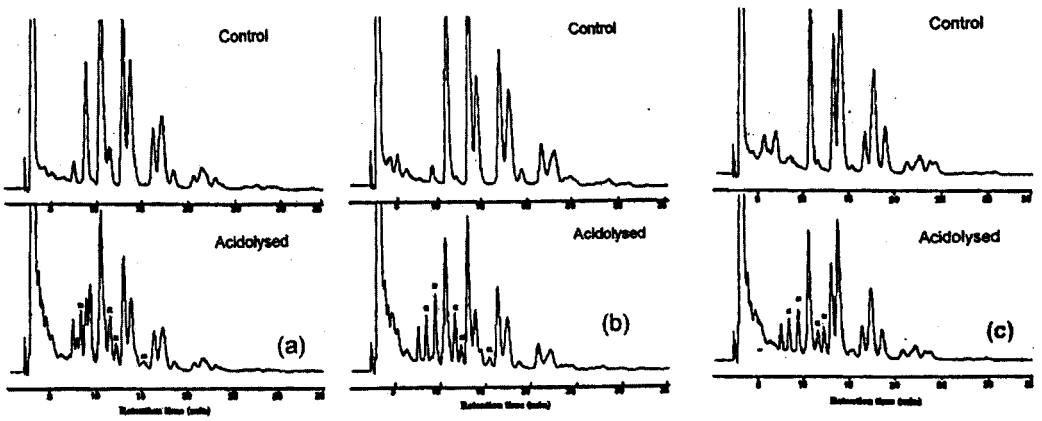

Fig. 13 : TG profiles of soybean oil (a), corn oil (b) and cottonseed oil (c) before and after acidolysis with lauric acid.

is a fat with a triacylglycerol composition resembling cocoa butter, which can be used as a cocoa butter equivalent in the chocolate and confectionary industry.

Lipase-mediated transesterification between TG provides a useful strategy for modifying the physico-chemical properties of fats and oils without the formation of *trans* fatty acid (TFA). TFAs are formed when unsaturated oils are hydrogenated to obtain harder products. The alternative is to interesterify a hard fat with an oil. The consumption of *trans* fatty acids, found largely in products like margarine and shortening produced by hydrogenation reaction (List *et al.*, 2000), have been shown to have an adverse effects such as increased plasma concentrations of low-density lipoprotein (LDL) cholesterol (Mensink and Katan, 1994) and reduce concentrations of high-density lipoprotein (HDL) cholesterol relative to the parent natural fat (Ascherio and Willett, 1997). It was recently suggested that TFA may also affect human fetal growth and infant development (Ayagari *et al.*, 1996). On July 11 2003, the FDA published the final rules mandating *trans* acid content to be included on food labels by January 1 2006, in accordance with the Nutrition Labeling Act of 2003.

During enzymatic transesterification, lipase interchanges the position of the fatty acid on a TG molecule either randomly or in a directed manner depending on the lipase used. A change in property can occur when the enzyme act on a single oil only (Ghazali *et al.*, 1995a) or two (Lai *et al.*, 1998a-c; Lai *et al.*, 2000a-b; Liew *et al.*, 2001a; Lim *et al.*, 2001, Chu *et al.*, 2000, 2002a-b). More changes are observed when two or more oils are mixed at different ratios and subjected to catalysis by lipase. Fig. 14 shows the TG profile of palm stearin interesterified with coconut oil at 1:1 while Fig. 15 shows the change in solid fat content of the modified mixture compared to the unmodified mixture. By varying the ratios of the reactants, the resultant interesterified mixtures can be tailor-made to suit different applications. For example, when the appropriate ratio of palm stearin and coconut oil was interesterified, the modified mixture could be used as pastry fat (Ghazali *et al.*, 1995b).

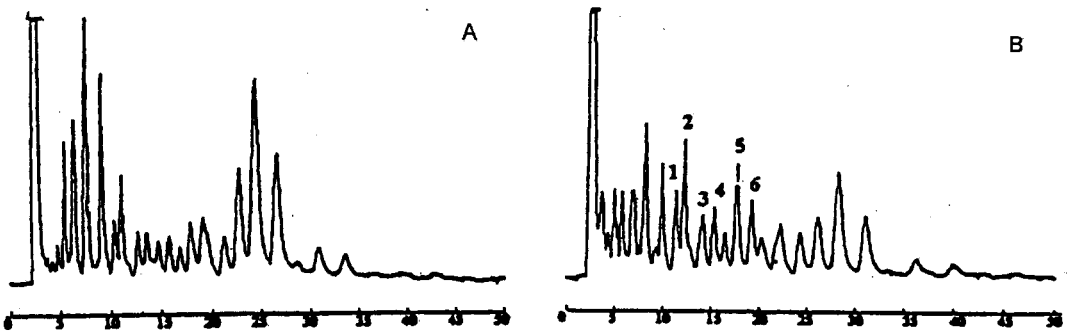

Fig. 14 : TG profile of a 1:1 mixture of palm stearin and coconut oil before (A) and after interestrification with Lipozyme IM 20 (commercial immobilized *R. miehei* lipase). Source: Ghazali *et al* (1995b).

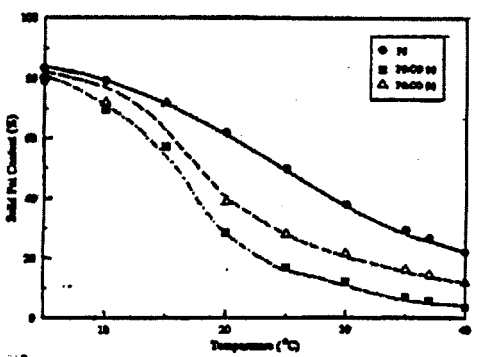

Fig. 15 : Solid fat content of palm stearin (PS), reacted PS:coconut oil [PS:CO (a)] and unreacted PS:CO (b) at 1:1 ratio. Source: As in Fig. 14.

There are numerous references in the literature pertaining to the potential uses of lipase-catalysed fats and oils in food formulations. Thus, a comprehensive review would not be practical. Instead, several examples will be given from the author's laboratory. Feedstock for *zero-trans* margarine production may be prepared from lipase interesterified palm stearin with sunflower oil (Lai *et al.*, 1999a-c) or with palm kernel olein (Lai *et al.*, 2000c). Frying shortening may be produced by interesterifying palm stearin with palm kernel olein (Chu *et al.*, 2001a-b; Tee, 2001). Liew *et al.* (2001b) reported on the rheological properties of ice cream emulsion prepared from lipase-catalysed interesterified palm kernel olein:anhydrous milk fat mixture. Not only that, fat feedstock comprising lipase interesterified palm kernel olein and anhydrous milk fat could be successfully used in the production of processed cheese (Ghazali *et al.*, 1996; Mariam, 1999). In this case, the transesterified mixture replaced pure anhydrous milk fat in the cheese, and sensory evaluation showed that the replacement could retain many of the important features of

commercial processed cheese. Palm stearin transesterified with coconut oil was used as lauric cocoa butter substitute in the preparation of chocolate (Ghazali *et al.*, 1997). Also, palm olein may be enriched with polyunsaturated fatty acids from fish oil, also via enzymatic interesterification (Chew, 2001).

An area of current interest in using lipases for fat modification is in the production of low calorie structured lipids (SL) (Xu, 2000). SL are TG containing mixtures of short-chain or medium-chain, or both, and long-chain fatty acids, preferably in the same glycerol molecule in order to exhibit their maximum potency (Akoh, 1998). Conventional fats and oils provide 9 kcal/g energy in the diet, as compared to the 4 kcal/g energy content of carbohydrates and proteins. SL would have a lower calorie value than conventional fats and oils especially if the longer chain fatty acids are found at the 1- and 3-positions of the fatís glycerol backbone. This is because, in the digestive tract, pancreatic lipase hydrolyses only fatty acids that are in those positions. The resulting monoglycerides are then absorbed by the body through the portal vein. Also, the longer chain fatty acids are poorly absorbed from the digestive tract into the portal vein compared to shorter or medium chain fatty acids. Combinations of these structural features have produced several new reduced calorie structured lipids such as salatrim, caprenin, captrin and bohenin (Auerbach *et al.* 2001).

R&D POTENTIALS

There are many aspects of the Malaysian food industry that could benefit from the applications of enzymes, apart from those already discussed above. As Malaysia has now placed greater emphasis on agriculture, it follows that there will also be a greater need to develop processes that will allow raw food materials derived from agricultural activities to be transformed into value-added and commercially competitive foods and food ingredients. A greater challenge would be to produce those that are accepted globally. Some R&D initiatives that are applicable are:

1. *Bioprospecting for food enzymes from local sources*

Malaysia is a rich and diverse source of food organisms, which are as yet largely untapped. These organisms would be natural sources of GRAS enzymes. Bioprospecting may lead to the discovery of known enzymes with novel features, or new enzymes with potentials as food processing. High-throughput screening (Wahler and Reymond, 2002) of enzymes should accelerate these discoveries. There is certainly room for more diverse generation of better food enzymes through protein engineering, gene shuffling technology and directed evolution (Farinas *et al.*, 2001), coupled with advances in functional genomics, transcriptomics, proteomiocs, metabolomics and bioinformatics (Kuipers, 2004).

2. *Bioextraction of edible oils*

There are a number of ways to extract plant oils from their sources. Besides physical pressing and solvent extraction, enzymes may also be used. The industrial potential for extraction of olive oil using an enzyme preparation has been reported (FAO, 1997). Enzyme-assisted oil extraction or bioextraction may be regarded as an ecofriendly process for oil extraction. The addition of appropriate enzymes during extraction enhances oil recovery by breaking down cell wall. Studies using enzymes to extract oil from the local sources namely seeds of *Moringa oleifera* (Ghazali, *et al.*, 2003) and *C. papaya* (Puangsri, 2003) have been reported. These oils are rich in oleic acid content (Mohammed *et al.*, 2002) and it should be exciting to explore the possibility of modifying these oils for food applications. Corbett (2003) has highlighted the increasing importance of high-oleic acid oils in health and food applications. Oils rich in monounsaturated fatty acids such as oleic acid are generally more stable to oxidative rancidity, stable as deep frying oils and are usually more healthy (lower risk of coronary heart disease).

3. *Development of biosensors for food analyte and contaminant detection and quantification*

The power of enzymes may be tapped further for the food industry by using them as analytical aid for the detection and quantification of food analytes and contaminants, and food process monitoring. Devices that may be used for this purpose are the biosensors. They are hybrid devices combining a biological sensing compartment with an analytical measuring element. The biological component is selective and typically reacts or binds to the analyte of interest to produce a response that can be quantified by an electronic, optical or mechanical transducer (Giese, 2002). For most biosensors, the biological component is an immobilised enzyme. Others are antibody, nucleic acid, microorganism, or cell. Although most biosensors have to date found application in a diagnostics/clinical setting, some are used for food analysis. Glucose biosensors dominate the market. Other biosensors include those for sucrose and lactose determination.

In spite intense research and numerous concepts, only a few biosensors have been successfully commercialised. The challenge for the Malaysian scientists would be to develop biosensors for detection and quantification of specific analytes present in indigenous foods or that is formed during postharvest handling or processing. Another potential area of research is the development of biosensors for rapid detection and identification pathogenic microorganisms from complex food materials. This will result in significant improvements in food safety, reducing acute and chronic health risks.

4. *Bioremediation/Waste treatment*

Starch and sugar residues represent large amounts of waste from the food and beverage industries. Large amounts of proteins in a variety of states ranging from edible to contaminated and fermenting suspensions, are generated from the slaughter, oil seed extraction, fish, gelatine and dairy industries. The Malaysian food industry produces

some of these wastes and discharge of such wastes into the environment is a matter of great concern.

There are several ways by which enzymes may be used to reduce wastes: processing of waste into useable form, recovery of useful materials from the waste and accelerated digestion of waste food polymers. Waste treatment may be accelerated by adding enzymes such as amylases, proteases and cellulases at the start of anaerobic digestion (Karam and Nicell, 1997). The action of these enzymes will increase the availability of digestible small molecules to microorganisms involved in the digestion process.

It may be worth considering the applications of enzymes for the treatment of wastes generated by the Malaysian food industry, and assessing the impact of enzyme-treated waste on the environment into which they are released. In addition, R&D should also be targeted at producing these enzymes in bulk and at cheaper costs.

CONCLUSION

The enzyme market and the number of competitive enzyme-based processes are growing rapidly, because of cheaper production, new applications fields and new enzymes. Their indispensability today as processing, analytical and even as beautification aids rests on fundamental discoveries that relate enzyme structure to function. It is without doubt that understanding of enzyme conformation, substrate specificity, thermostability, action especially at water-lipid interfaces and production using modern biotechnology methods will lead to a more rational design in the utilisation of enzymes, not only for food processing but also other technical areas of application. Thus, scientists and researchers with visions to tap the power of enzymes have brought to light many applications of enzymes, all for the common good. There are many more discoveries to be made, and the onus is on us as scientists and researchers to do so.

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