The Use of Lipase Enzymes in The Modification of Oils and Fats – A Review

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INTRODUCTION

Enzymatic reactions have been applied in industries where the end-products cannot be produced by chemical means or their subsequent separation is too expensive. The ability of microbial lipase to modify lipid structures has attracted keen interest in the use of enzymatic reactions to replace the conventional energy-intensive chemical processes as well as for the creation of novel products (Cheah and Augustine, 1987; Graille *et al.*, 1988).

Microbial lipase (glycerol ester hydrolases EC 3.1.1.3) which occurs naturally in some of the oil-bearing fruits such as the oil palm are enzymes that can hydrolyze fats and oils to produce partial glycerides and glycerol. These microbial lipases are also produced by a large number of micro-organisms, fungi and psychotropic bacteria. Since lipases hydrolyze only emulsified acyl lipids (insoluble in water), they are active at an oil-water interface. Most enzymes are effective in aqueous systems where both of the enzymes and their substrates are soluble. But the true lipases catalyze the hydrolysis water-insoluble substrates and are distinguished from the esterase which acts solely on water-soluble substrates. The esterase reaction is reversible and the enzyme catalyzes the synthesis of esters from free fatty acids and alcohol even when the water content of the reaction mixture is reduced (Macrea, 1983, Brockerhoff and Jensen, 1974; Paul and Stephen; 1993, Cheah and Augustine, 1987).

IMMOBILIZATION TECHNIQUE

Lipase can be studied in both free and immobilized forms. The process of immobilization of enzymes allows food manufacturers to develop a variety of process control previously unavailable. The main problem which normally occurs in reactors is the loss of the enzymes since they are soluble in the liquid food medium. The enzymes can however be retained in the bioreactor by immobilizing them on a support system or within small gel particles. Immobilization techniques fall into four major categories such as physical adsorption, carrier-binding, cross-linking, and entrapping as shown in *Figure 1* (Macrea, 1983; Neidleman, 1991; Poldermans and Maat, 1991; Boyce, 1986).

Immobilization techniques offer a number of advantages. They can be reused, hence reducing effluent disposal problems, they are flow through systems which offer ease of regulation, improved stability and continuous operations. Even though the primary objective of an immobilized enzyme is to reduce the cost of production, the process usually requires large capital which is due to the high cost of reagents and the support system. A

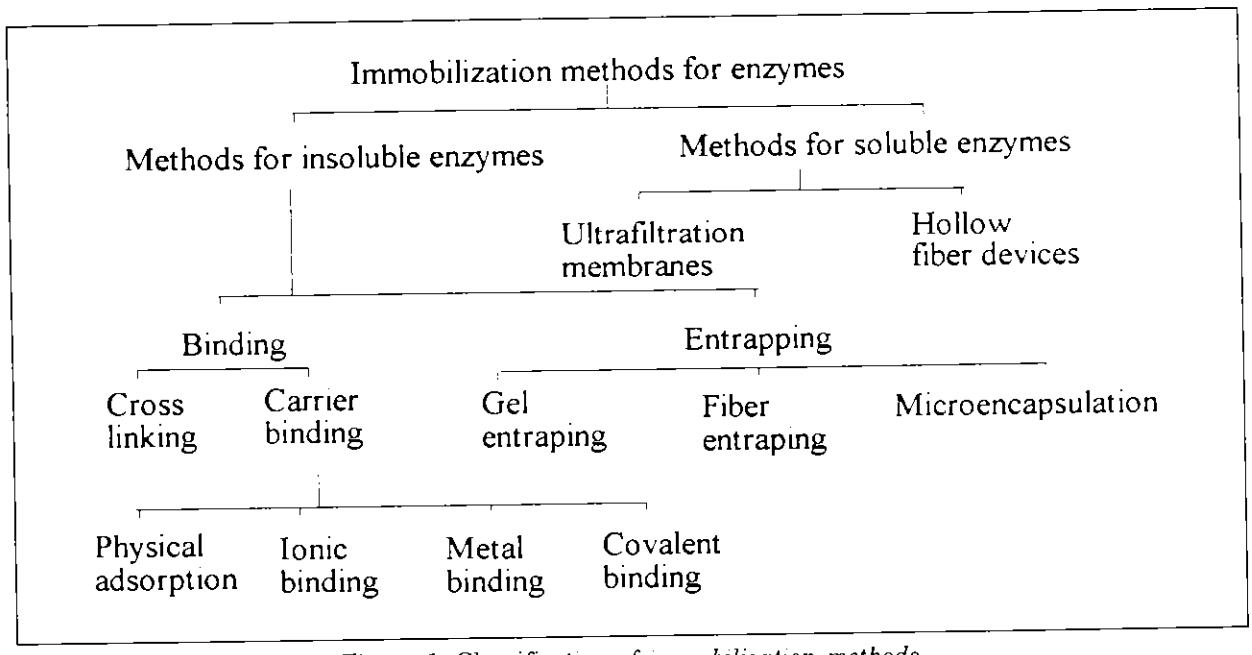
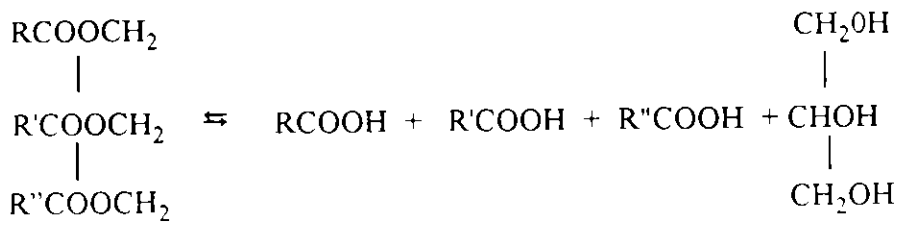
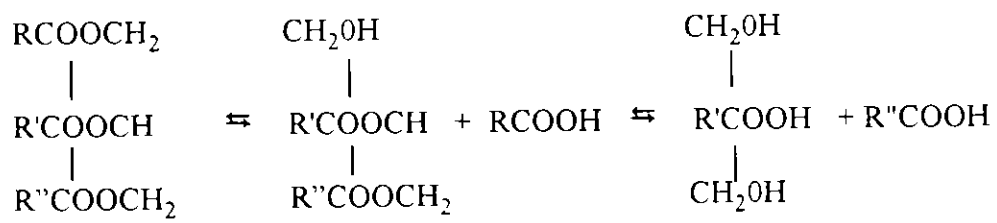


Figure 1. Classification of immobilization methods.

(I) Nonspecific lipase:



(II) 1,3-specific lipase:



(III) Fatty acid specific lipase:

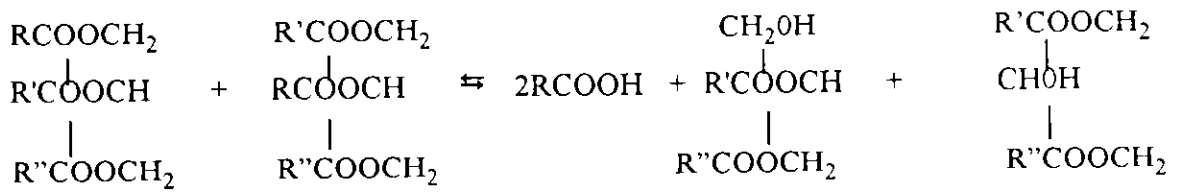


Figure 2. Products formed by lipase-catalyzed hydrolysis of triglycerides

large amount of the soluble enzyme is required to make one unit of immobilized enzyme, and in addition the production process is complicated (Lanning, 1988; Paul and Stephen, 1993; Macrea, 1983).

SUBSTRATE SPECIFICITY

Microbial lipases are widely used as biological catalysts because of their high specificity of action. Enzymic specificity is based on the original "Lock-and-Key" concept whereby the enzyme and the substrate must fit closely with one another before the reaction can take place. Generally, there are four types of substrate specificity, namely; absolute specificity, group specificity, reaction specificity and stereochemical specificity producing different effects (Laidler and Bunting, 1973; Gurr and James, 1980). On the other hand, microbial lipases which are used industrially have various specificities as well and they can be divided into three groups as shown in *Figure 2.*

In the past, extracellular lipases have found little industrial uses compared to the protease and carbohydrase although their properties have been studied over a number of years. However, recently, interesting new applications using microbial lipase have been developed particularly in the reactions of oils and fats particularly palm oil, involving hydrolysis, acidolysis, interesterification and ester synthesis. These processes will be discussed in this article.

HYDROLYSIS OF OILS AND FATS

Fat hydrolysis or fat splitting can be considered as a heterogenous reaction which occurs at the oil-water interface. This is the reason why the microbial lipase are the most suitable enzyme for fats and oils hydrolysis. The hydrolysis of fats and oils is the reaction between one mole of triglyceride with three moles of water to produce three moles of fatty acids and one mole of glycerol (Khor *et al.*, 1986; Graille *et al.*, 1988; Cheah and Augustine, 1987) as shown in *Figure 3.*

The intermediate products during the hydrolysis of fats and oils are the di- and monoglycerides as shown in *Figure 4.* The reaction is reversible and re-esterification can happen at the same time as hydrolysis. This reaction is regarded as fundamental to the oleochemical industry (Gunstone, 1958; Sonntag, 1979).

Depending on the type of substrates, microbial lipases usually have different rates of hydrolysis for fats of different origins. Laidler and Bunting (1973) reported that microbial lipase acts more rapidly on the esters of short-chain acids such as butyric acid compared to the long-chain acid, such as stearic acid.

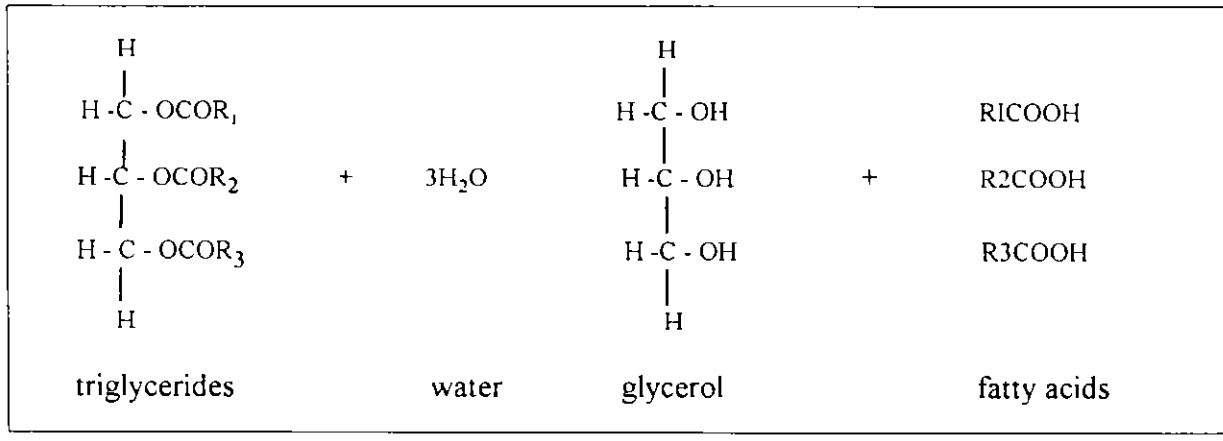


Figure 3. Hydrolysis of triglycerides yield a glycerol and fatty acids.

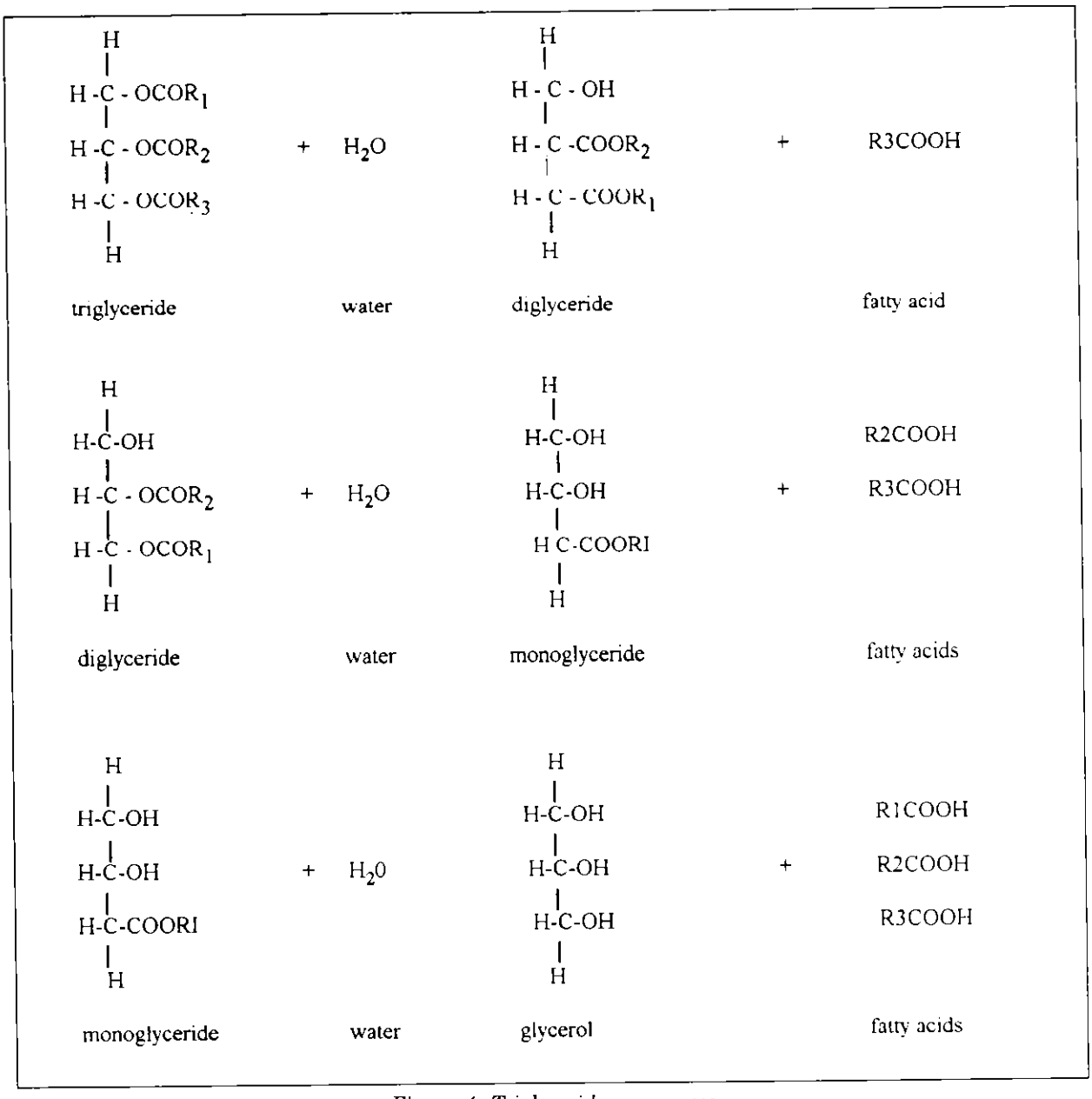


Figure 4. Triglycerides sequences

Chemically, the combination of high temperatures and pressures is more effective in a continuous hydrolysis reaction to produce fatty acids or other lipid derivatives required for the many food-grade applications. However, such an approach consumes considerable amounts of energy and is expensive since single or double distillation is needed to obtain the end products. Thus, the use of an enzymatic approach at 60°C compared to 250°C in a chemical process is preferred to produce a higher quality fatty acids.

According to Graille *et al.* (1988), the total hydrolysis of fats and oils by lipase in a continuous reactor gives a higher quality fatty acids than those obtained from traditional methods. Good quality fats and oils have to be used to obtain the maximum yield products otherwise the lipase are inactive.

Hydrolysis by lipase can offer several advantages. It leads to very little colour development in the product and a more concentrated glycerol solution is obtained.

Fatty acids produced at lower temperature are not only cheaper but they are also less corrosive and hence less corrosion-resistant reaction vessels can be used. Furthermore, the enzymic reaction can also be used to split fats with highly unsaturated fatty acids without fear of their polymerization and decomposition into ketone and hydrocarbons as would normally occur in the conventional process (Macrea, 1983; Graille *et al.*, 1988, Cheah and Augustine, 1987; Hammond and Glatz, 1988).

ESTERIFICATION

The reverse reaction of fat hydrolysis is an esterification process or ester synthesis. A low concentration of water is desirable in order for the reaction to take place; generally below 2%. Therefore, anhydrous or "dry" solvents typically hexane or other paraffins must be used in the esterification process (Graille *et al.*, 1988; Yusuff, 1988; Sonntag, 1979; Lanning, 1988). The reversibility of the lipase reaction enables the enzymes to be used as a catalyst in the formation of esters from alcohols and fatty acids. This can be shown by the incubation of fatty acids and glycerol-water mixtures with various microbial lipase to produce glycerides. Lipase-catalyzed direct esterification reaction is important for the production of novel esters for use as fragrances and flavours, which are typically terpene esters, due to the inherent mild conditions of the process (Boyce, 1986; Macrea, 1983; Cheah and Augustine, 1987). It is also useful for the synthesis of carbohydrate esters for applications such as emulsifiers in foods, medicines and cosmetics. Other products are partial glycerides, esters of short-chain alcohols, wax esters and ester oligomer (Graille *et al.*, 1988; Lanning, 1988; Hamilton and Bahti, 1980). The enzymatic route may prove to be the method of choice for the synthesis of monoglycerides, for example, 2-monoglycerides can be synthesized, enzymatically via partial hydrolysis of triglycerides, transesterification or direct esterification (Brockerhoff and Jensen, 1974; Paul and Stephen, 1993; Neidleman, 1991).

Even though, lipase-catalyzed esterification attracts much interest in industrial applications, commercialization is quite slow. Due to the high cost of enzymes, the biocatalystic process is not competitive enough against chemical synthesis. However, the main advantages of the lipase esterification process are improved colouring of the end-products and the absence of solvent toxicity that are related to chemical synthesis (Cheah and Augustine, 1987; Graille *et al.*, 1988). *Figure 5* shows the ester synthesis reaction.

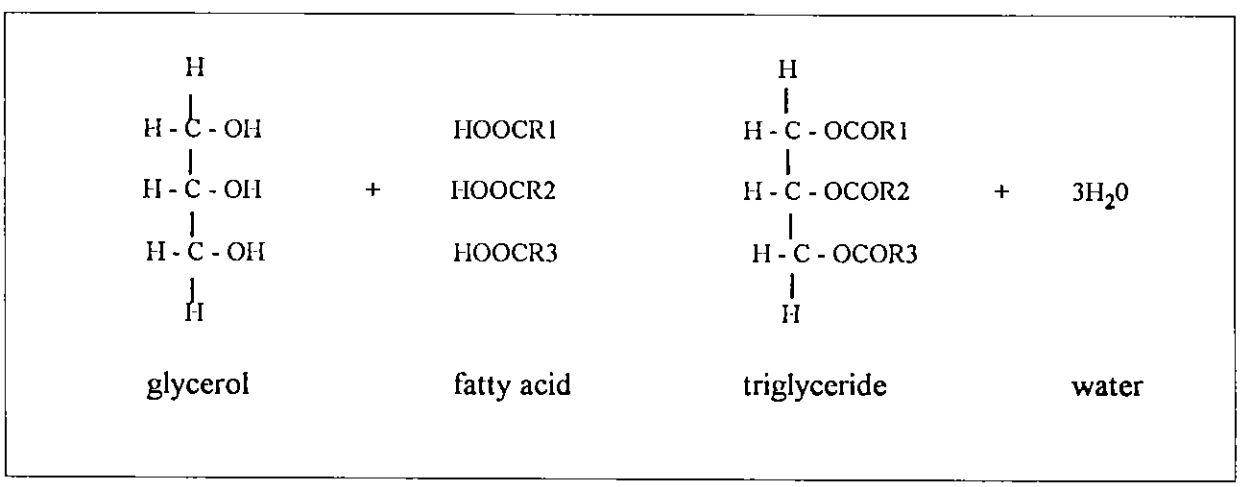


Figure 5. Condensation/esterification of glycerol with free fatty acids to yield water and triglyceride

INTERESTERIFICATION

Interesterification can be defined as a process of rearrangement of glyceride fatty acid moieties by random or directed manner. The process involves reaction of triglycerides with fatty acids, alcohols or other esters. The products produced using interesterification have a different physical behaviour compared to the original oils or fats due to the interchange in the fatty acid groups. Chemical interesterification is typically a random exchange process in which, random removal of fatty acids from glyceride molecules take place. Usually, this reaction is effective at 250°C or above without a catalyst or at lower temperatures in the presence of a catalyst (Yussuf, 1993; Lanning, 1988; Macrea, 1983). Alkaline catalyst commonly employed such as sodium methoxide in which the function of sodium methoxide is to promote acyl-migration between glyceride molecules. The process is normally applied to palm kernel, palm and coconut oils to produce a variety of products such as margarines and shortenings, whose melting point and crystallization characteristics are important (Hammond and Glatz, 1988; Yussuf, 1993; Macrea, 1983).

The other type of interesterification is directed interesterification or non random interesterification. This type of interesterification can be directed away from its usually random end products if the reaction mixture is allowed to crystallize during the reaction. By controlling the reaction temperatures to allow for selective crystallization, higher melting and more saturated triglycerides are obtained. This process requires catalysts which are active at low temperatures such as sodium alloy. However, a lipase enzyme can be used as a catalyst to modify the oils and fats by the directed interesterification process. Hydrolysis and resynthesis of glyceride occur when lipase is incubated with oils and fats. Since the lipase reaction is reversible, breakdown and resynthesis occur resulting in acyl migration between glyceride molecules to give the interesterified products. Similar to chemical interesterification, hydrolysis of the fat can be minimized if the quantity of water in the system is restricted so that the reaction equilibrium is in favour of the lipase-catalyzed interesterification (Lanning, 1988; Yussuf, 1993; Gunstone, 1958; Macrea, 1983; Boyce, 1986).

Interesterification by lipase enzymes alters specifically the outer 1- and 3-positions of the acylglycerols. It is also possible to change the acyl groups of triglycerides at precise positions on the glycerol molecule with an added free fatty acid (Paul and Stephen, 1993). This principle has been used in the production of specialty fats. For example, the glyceride 1,3-dipalmitoyl-2-monoleine (POP) in palm mid fraction (PMF) can be converted in the presence of free stearic acid to a mixture of triglycerides enriched in 1(3)-palmitoyl-3(1) stearoyl-2-monooleine (POSt) and 1,3-distearoyl-2-monooleine (StOSt), a cocoa butter like fat (Goh *et al.*, 1992; Geoff, 1991; Poldermans and Maat, 1991; Lanning, 1988) as shown in *Figure 6*. However, if a positional non-specific lipase is used to catalyze the interesterification of a triglyceride mixture, the triglycerides produced are similar to those produced by chemical interesterification. Lipase-catalyzed interesterification is also useful for the hardening of vegetable oils to produce fats like butter properties since the reaction system does not give rise to the *trans* fatty acids. Palm kernel oil can also be hardened by transesterifying the oil with stearic, lauric or myristic acids in the presence of a lipase specific for oleic acids. Increasing the polyunsaturation of the palm oil with oils rich in linoleic acid or other polyunsaturated fatty acid makes it more acceptable to some consumers in terms of health benefits. Lipase could also be used to incorporate high valued

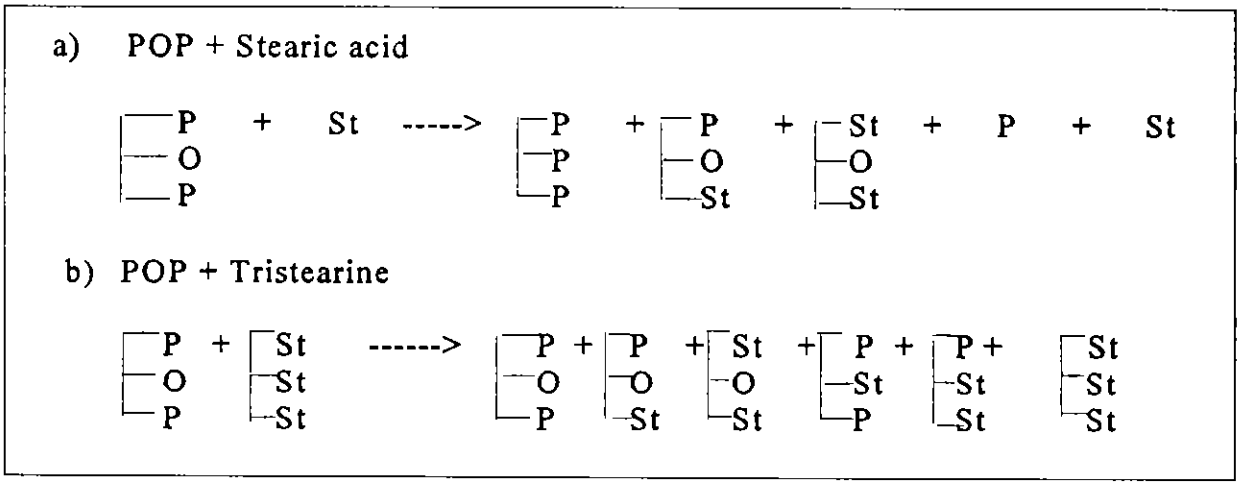


Figure 6. Products formed by interesterification of 1,3-dipalmitoyl-2-oleoyl-glycerol (POP) with either stearic acid or tri-istearin using a 1, 3-specific lipase as catalyst.

nutritional fatty acids into palm oils such as Docosahexaenoic Acid (DHA) and Eicosapentaenoic Acid (EPA) (Lanning, 1988; Paul and Stephen, 1993).

The action of lipases can be further improved by immobilizing the enzyme on an inert support. Recently, immobilized lipases which are available commercially have been used in directed interesterification processes. Examples of the sources of such 1,3-specific lipases are *Aspergillus niger, Mucor javanicus, Rhizopus delemar* and *Mucor miehei* (Macrea, 1983; Lanning, 1988; Geoff, 1991; Hammond and Glatz, 1988). Cocoa butter substitute is the most popular product produced by this lipase-catalyzed interesterification reaction even though the enzymes and downstream processing for product recovery can be much more expensive and difficult to handle compared to chemical processing (Geoff, 1991). However, on the other hand, this immobilized lipase may be used repeatedly given a good feedstock quality.

ACIDOLYSIS

Acidolysis is also an interesterification process. Interchange of acyl groups between fat and free fatty acids may only occur at specific sites in the triglyceride molecules (acidolysis) if a suitable lipase is present. Examples of acidolysis are reactions of cottonseed oil, peanut oil or mahua oil with lauric acid, coconut oil with stearic acid, and soybean oil with butyric oil (Lanning, 1988; Yussuf, 1987). The types of fatty acids present in triglycerides have an influence on the composition of the final product (Gunstone, 1958; Sonntag, 1979).

Any dietary fats with the desired specifications, can be produced if the suitable microbial lipase is present in the acidolysis process. The production of a specific triglyceride of nutritional interest has been proposed by blending medium-chain triglycerides (MCT) with linoleic acid allows for the exchange of fatty acids to take place (Lanning, 1988; Neidlemans, 1991). Medium chain triglycerides (MCT) are used by people who are unable to digest the conventional sources of fats and oils due to insufficient lipase and their combination with linoleic acid can help such people who are unable to absorb long chain fatty acids to improve their nutritional status. Furthermore, such type of glycerides supply fuel and energy to the body and has the ability to lower serum cholesterol (Babayan, 1991; Krishnamurthy, 1992).

CONCLUSION

Enzymic reaction must not be seen as a threat to the traditional means of production but rather as a partner in the process of creating novel products. It is now a commercial reality and it has become an important tool in the oil processing industry especially in the palm oil industry. Many opportunities lie ahead for the commercial applications of microbial lipase technology in the fats and oils industry. One highly visible area of a need for research is the biotechnological approaches for the production of cocoa butter equivalent fats. The traditionally high price of cocoa butter and its unstable supply makes it a good candidate for production by a biotechnology-based production process. Although the application of enzymic technology is now confined to high value products, it is hoped that further developments in biotechnology will bring the technology into the production of cheaper major consumer products. Other possible applications of commercial interest to the food industry include the lowering of the saturated fat content of several seed oils, the production of natural monoglycerides emulsifiers from palm oil, a reduction in the linoleic acid levels in soybean and canola oils, transesterification of palm oil with stearic acid and the development of novel glycerides containing linoleic acid and EPA. With the evolution of new applications, the role of lipases in the oils and fats industry is likely to further increase. ■

REFERENCES

BABAYAN, V (1991). "Medium Chain Triglycerides for Food Use". *Lipid Technology*, July-Sept. pp. 92-94.

BOYCE, C O L (1986). "Novo's Handbook of Practical Biotechnology". Novo Ltd. pp. 92-95.

BROCKERHOFF, H and JENSEN, R G (1974). "Lipolytic Enzymes". Academic Press, New York, pp. 25 - 35.

CHEAH, C and AUGUSTINE, O (1987). "Application of Enzyme Reaction to Oils and Fats". *Proceedings of 1987 International Oil Palm Conference*, Palm Oil Research Institute of Malaysia pp. 113-119.

GEOFF, T (1991). "A New Generation of Cocoa Butter Equivalents". *Confectionery Manufacture and Marketing* 7(4). pp. 30-34.

GOH, S H; YEONG, S K and WANG, C W (1992). "Cocoa Butter by Fungal Lipase". *JAOCS. 70 (6):* 567-570.

GRAILLE; PINA, J and MONTET, D (1988). "Biotechnology of Lipid: Some Possible Applications". *Oleagineux 43 (4):* 188-190.

GUNSTONE, F D (1958). "An Introduction of the Chemistry of Fats and Fatty Acids". Chapman and Hall Ltd. London. pp. 85-87.

GURR, M I and JAMES, A T (1980). "Lipid Biochemistry: An Introduction". Chapman and Fall Ltd. London, pp. 178-189.

HAMILTON, R and BAHTI, A (1980). "Fats and Oils: Chemistry and Technology". Applied Science Publisher Ltd. pp. 251-265.

HAMMOND, G and GLATZ, B (1988). "Biotechnology Applied to Fats and Oils". Food Biotechnology. King, R.D, (eds.) Vol. 2. Elsevier Applied Science Ltd. London, pp. 173-217.

KHOR, H T; TAN N H, CHUA, C L (1986). "Lipase catalyzed hydrolysis of palm oil". *JAOCS 63 (4)*: 32-35.

KRISHNAMURTHY, R E (1992). "Biotechnology Offers Route to New Fats and Oils". *INFORM 3 (4)*: 457-458.

LAIDLER, K J and BUNTING, P S (1973). "The Chemical Kinetics of Enzyme Action". Elsevier Applied Science Ltd. London. pp. 256-264.

LANNING, S (1988). "Fats, Oils, Fatty Acids and Oilseed Crops". Biotechnology and Food Ingredients. King, R.D. (eds) Elsevier Applied Science Ltd. London. pp. 173-217.

MACRAE, A R (1983). "Extracellular Microbial Lipase". Microbial Enzymes and Biotechnology.

MACREA, A R (1983). "Lipase-Catalyzed Interesterification of Oils and Fats". *JAOCS 60 (2):* 243.

NEIDLEMAN, S (1991). "Enzymes in the Food Industry". *Food Technology*. Vol. 5 (3): 88-89.

POLDERMANS, B and MAAT, J (1991). "Processing Aids for the Food industry: The State of the Art". Biotechnological Innovations in Food Processing. Butterworth-Heineman Ltd. pp. 7.

SONNTAG, M (1979). "Fat Splitting, Esterification and Interesterification". In Bailey's Industrial Oil and Fat Products. Fourth Edition. pp. 536-554.

WILLIAM, M. (eds) Applied Science Publishers. Ltd. pp. 225-247.

YUSSUF, M S A (1987). "Interesterification of Fats". Chemistry and Processing of Fats and Oils, PORIM. pp. 153-157.