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Review Article

Applications of Microbial Lipase

T. Sivakumar

PG and Research Department of Microbiology, Kanchi Shri Krishna Colege of Arts and Science, Kilambi, Krishnapuram, Kanchepuram -631 51, Tamil Nadu, India

*Corresponding author: *shiva.fungi@gmail.com*

Abstract

The use of enzymes in food preservation and processing predates modern civilization. Fermentation of common substrates such as fruits, vegetables, meat and ilk provide a diverse array of food in the human diet. Beer, wine, pickles, sausage, salami, yogurts, cheese and buttermilk are all fermented products. Irrespective of their origin, these fermented food products are, in fact, result of the enzymatic modification of constituents in the substrate. The use of enzymes in food industry also involves a range of effects including the production of food quality attributes such as flavors and fragrances and control o colour, texture, appearance besides affecting their nutritive value.

Keywords: Microbial enzymes, Lipase, Application.

Introduction

Lipases are widely distributed among yeast, fungi and bacteria. Short chain triglycerides are also very good substrates for lipases. Enzymes such as proteases and amylases have dominated the world market owing to their hydrolytic reactions for proteins and carbohydrates. However with the realization of the biocatalytic potential of microbial lipases in both aqueous and non-aqueous media in the last one and a half decades, industrial fronts have shifted towards utilizing these enzymes for a variety for reactions of immense importance. It is in the last decade that lipases have gained importance to a certain extent over proteases and amylases, especially in the area of organic synthesis. The regioselective nature of lipases have been utilized for the resolution of chiral drugs, fat modification, synthesize of cocoa butter constituents, biofuels and for synthesize of personal care products and flavor enhancers. Thus lipases are today the enzyme choice for organic chemist, pharmacists, biophysicists, biochemical and process engineers, biotechnologist, microbiologist and biochemist.

Industrial application of lipases

Lipases have wide applications as a good substitute for classical organic techniques in the selective transformation of complex molecules. The employment of lipases in the above reactions can reduce side reactions and easy the separation of molecules (Ashokpandey *et al*., 1999). In the present day industry, lipases have made their potential realized owing to their involvement in various industrial reactions either in aqueous or organic systems, depending on their specificity (John and Abraham, 1990; Kotting and Kibl, 1994).

Lipases in food industry

Lipases have become an integral part of the modern food industry. Now a days industrial enzymes especially lipases, are commonly used in the production of a variety of products, ranging from fruit juices, baked foods and vegetable fermentation to dairy enrichment (Mcgee, 1986; Zalacain *et al*; 1995). Microbial lipases have been used for the production of desirable flavors in cheese and other foods. Though microbial lipases are best-utilized food processing a few especially psychotropic bacteria of *Pseudomonas* sp and a few mould of *Rhizopus* sp and M*ucor* sp can be dairy products and soft fruits (Coenen *et al*., 1997, Stead, 1986). A beautiful strain of Pseudomonas fluorescence B52 that was identified as a major cause of food spoilage (Rodriguez and Dezfulian, 1997).

Lipases as biosensor

A promising new method involves the manipulation of microbial lipases as biosensors. Biosensors can be biochemical or electronic nature. Biochemical biosensors can have enzymes, antibodies, other proteins, large organs; cells or cell extracts, immobilized or linked to suitable (Krause *et al.,* 1990).

Lipases in biomedical application

Lipases have emerged as an important biocatalyst in biomedical applications. Recently (Pamer *et al.,* 1996) reviewed a variety of substrates accepted by hydrolytic enzymes, including lipases, to produce compounds in high enantiomeric excess, which can be used as a chiral building blocks for the synthesize for compounds of pharmaceutical interest. Lipases are also capable of catalyzing reactions, which lead to the production of life saving drugs and in the preparing of optically active homochiral intermediates for the synthesize of nikkomycin-B, non steroidal (Fernanadez *et al*., 1995 and Okamatu *et al*., 1995).

Lipases in leather industry

Leather processing involves the removal of subcutaneous fat, dehairing and stuffing. Tanning process were usually performed in an alkaline environment, so alkalophilic microbes ought to be better for exploration. Many *Bacillus* sp strains, which grow successfully under highly alkaline conditions, were found to be useful in leather processing.

Lipases in pesticide

Several pesticides, which are in current practices, are made up of variety of lipases. In the field of pesticide biotechnology much attention has been focused on the use of lipases as a selective biocatalyst in organic media (Ngooi *et al*., 1990).

Lipases in environmental management

Lipases have been used extensively in wastewater treatment (Dauber *et al*., Kurita, 1994). Dauber and Boehnke devised a technology to convert dewatered sludge in the factories to biogas in which an enzyme mixture including lipase was used. A method is suggested for the treatment of waste by the direct cultivation of lipophilic microbes into the waste, which controlled cost. The broadening use of lipases in bioremediation has achieved to the removal of biofilm deposits from cooling water systems.

Lipases in detergents

The usage of enzymes in washing powders still remains the single biggest market for industrial enzymes (Arbnige and Pitcher, 1989). The world wide trend towards lower laundering temperatures has led to much higher demand for house hold detergent formulation. Recent intensive screening programmes followed by genetic manipulations, have resulted in the introduction of several suitable preparations, for example, Novo nordisk's lipases (*Humicola* lipase expressed in *Aspergillus oryzae* (Hoq *et al*., 1985).

Lipases in 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 buffer fat, and cream (Falch, 1991). While the addition of lipases primarily releases short chain (C_4 and C_6) fatty acids that lead to the development of sharp, tangy flavour, the release of medium-chain (C_{12} and C_{14}) fatty acids tends to impart a soapy flavour, to the product. In addition, the free fatty acids take part in simple chemical reactions where they initiate the synthesize of other flavour ingredient's such as aceto - acetate, β- ketoacids, Methyl ketones, flavour esters and lactones (Vulflson, 1994).

More recently a whole range of microbial lipase preparations have been developed for the cheese manufacturing industry such as those of M*ucor miehei, Aspergillus niger and Aspergillus oryzae.* Using individual lipases or mixture of several preparations produced a range of cheese of food quality (Falch, 1991). Addition of lipases to cow's milk generates a flavour rather similar to that of ewe's or goat's milk. This is used for producing cheese or the so-called enzyme modified cheese (EMC). EMC is a cheese that has been incubated in the presence of enzyme at elevated temperatures in order to produce a concentrated flavour for use as an ingredient in other products such as dips, sauces, and soups (Falch, 1991).

Lipases in oleochemical industry

The scope for application of lipases in the oleo chemical industry is enormous as it saves energy and minimizes thermal degradation during hydrolysis, glycerolysis and alcoholysis (Arbige and Pitcher, 1989; Hog *et al.*, 1985; Buhler and Wandreus, 1985). Miyoshi oil and Fat Company in Japan, reported commercial use of *candida cylindracea* lipases in production of soap (Mc. Neill and Yamane, 1991). The introduction of the new generation of cheap and very thermo stable enzymes can change the economic balance in flavour of lipase use (Macrae and Hammond, 1985).

The current trend in the oleo chemical industry is a movement away from using organic solvents and emulsifiers (Ergan *et al*., 1988; Sonnatag, 1984). The various reactions involving hydrolysis, alcoholysis, and glycerolysis have been carried out directly on mixed substrates, using a range of immobilized lipases (Coleman and Macrae, 1980).

Lipases in synthesis of triglycerides

The commercial value of fats depends on the fatty acid composition within their structure. A typical example of a high value asymmetric triglyceride mixture is cocoa butter. The potential of 1,3-regiospecific lipases for the manufacture of cocoa- butter substitutes was clearly recognized by unilever (King *et al*., 1990) and Fuji oil (Jandacek *et al*., 1987). Comprehensive reviews on this technology, including the analysis of the product composition, are available (Macrae and Hammond, 1985; colemand and Macrae 1980). In principle, the same approach is applicable to the synthesize of many other structured triglcerides (Soumanou *et al.,* 1997) possessing valuable dietic or nutritional properties, for example, human milk fat. Acedolysis, catalyzed by 1, 3- specific lipases is used in the preparation of nutritionally important products, which generally contain medium-chain fatty acids (Eibl and Unger, 1990). Lipases are investigated extensively with regard to the modification of oils rich in high value polyunsaturated fatty acids such as arachidonic acid. Substantial enrichment in the polyunsaturated fatty acid content of mono-glyceride fraction has been achieved by lipases- catalyzed alcoholysis or hydrolysis (Fregapane *et al.,* 1991).

Lipases in synthesis of surfactants

Polyglycerol and carbohydrate fatty acid esters are widely used as industrial detergents and as emulsifiers in a variety of food formulations. (Low-fat spreads, sauces, ice creams). Enzymatic synthesize of functionally similar surfactants has been carried out at moderate temperature (60-80˚C) with excellent regioselectivity (Adelhorst *et al*., 1990) have carried solvent-free esterification of simple alkyl-glycosides using molten fatty acids and immobilized *Candida antarctica* lipase (Fregapane *et al*., 1991) obtained mono and diesters of monosaccharides in high yields using sugar acetals as starting materials. *Mucor miehei* lipase has been used for the transesterification of phospholipids, in a range of primary and secondary alcohols (Montet *et al*., 1990). Lipases may also be useful in the synthesize of a whole range of amphoteric biodegradable surfactants, namely amino acid based esters and amides (Hills *et al.,* 1990 Kloosterman *et al*., 1988).

Lipases in synthesize of ingredients for personal care products

Unichem international has recently launched the production of Isopropyl palmitate, and 2-ethyl hexyl palmitate (Young and Bratzler, 1990) for used as emollient in personal care products like skin, suntan creams and bath oils. Wax esters have similar application in personal care products and being manufactured enzymatically using *C. cylindracea* lipase in a batch bioreactor (Ho *et al*., 1985).

Lipases in pharmaceuticals and agro chemicals

The utility of lipases in the production of chiral synthons is well recognized and documented. Several processes have recently been commercialized which have been described by Sainz – Daiz et al., 1997 and Davis et al., 1990. The resolution of 2-halopropionic acids, the starting material for the synthesize of phenoxypropionate herbicides, is a process based on the selective esterification of (s)-isomers with butanol, which is catalyzed by porcine pancreatic lipase in anhydrous hexane (Kirchher *et al*., 1985). Another impressive example of the commercial application of lipases in the resolution of racemic mixture is the hydrolysis of epoxy ester alcohols (Margolin, 1990).

Lipases have applications as industrial catalysts for the resolution of racemic alcohols in the preparation of some prostaglandin's, steroids and carboxylic nucleoside analogues. Lipases from *Aspergillus terreus* show chemo and regiospecificity in the hydrolysis of per acetates of pharmaceutically important polyphenolic compounds (Parmar *et al.*, 1998).

Lipases in polymer synthesize

The stereo specificity of lipases is useful for the synthesize of optically active polymer (Margolin *et al*., 1991). These polymers are asymmetric reagents, and are used as absorbents. In the field of liquid crystals, suitable monomers can be prepared by lipase-catalyzed transesterification of alcohols, which with racemic alcohols may be accompanied by resolution (Margolin et al., 1990). The use of chiral glycidytosylates for the preparation of ferroelectric liquid crystals has also been reported (Margolin., 1990). Thus, this enzyme has diversified commercial use, both in terms of scale and processes. Lipases have been employed successfully in the food industry as well as in high tech production of fine chemicals and pharmaceuticals. Furthermore, this enzyme has potentials in newer fields, for example lipases have successfully been used in paper manufacturing apparently, the treatment of pulp with lipase leads to a higher quality product and reduced cleaning requirement (West, 1987).

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