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REVIEW ARTICLE

Application of Lipase in Industry

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ABSTRACT:
Lipase enzyme is a naturally occurring enzyme found in the stomach and pancreatic juice. They function is to digests fats and lipids, helping to maintain correct gall bladder function. As such, these constitute any of the fat-splitting or lipolytic enzymes, all of which cleave a fatty acid residue from the glycerol residue in a neutral fat or a phospholipid. The lipase enzyme controls the amount of fat being synthesized and that which is burned in the body, reducing adipose tissue. Lipases are one of the important groups of biocatalysts used in biotechnological applications. Lipases have been isolated from many species of plants, animals, bacteria, fungi and yeast. Lipases extracted from microorganisms are used in various industries such as dairy, food, detergents, textile, pharmaceutical, cosmetic and biodiesel industries. It is also used for synthesis of fine chemicals, agrochemicals and new polymeric materials. Research on microbial lipases, has increased due to their great commercial potential. Lipases are added to detergents such as household and industrial laundry and also in household dishwashers, where their function is removal of fatty residues and cleaning clogged drains. Their applications in our daily life are increasing day by day.

KEYWORDS: Lipase, lypolytic enzymes, industrial application, food industry

INTRODUCTION:

Enzymes are considered as nature's biocatalysts. Lipid constitutes a large part of the earth's biomass and lipolytic enzymes play an important role in the turnover of these water-insoluble compounds. Lipolytic enzymes are involved in the breakdown and mobilization of lipids within the cells of individual organisms as well as in the transfer of lipids from one organism to another (Beisson et al., 2000). Lipases are one of the important groups of biocatalysts used in biotechnological applications (Benjamin and Pandey, 1998). Lipases have been isolated from many species of plants, animals, bacteria, fungi and yeast. Lipases extracted from microorganisms are used in various industries suchas dairy, food, detergents, textile, pharmaceutical, cosmetic and biodiesel industries. It is also used for synthesis of fine chemicals, agrochemicals and new polymeric materials (Saxena etal., 1999; Jaeger and Eggert 2002). Research on microbiallipases, has increased due to their great commercial potential (Silva et al., 2005).

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Lipases are added to detergents such as household and industrial laundry (Kumar et al., 1998) and also inhouse hold dishwashers, where their function is removal of fatty residues and cleaning clogged drains (Vulfson, 1994). Microbial enzymes are often more useful than enzymes derived from plants or animals because of the great variety of catalytic activities, their high yields, ease of genetic manipulation, regular supply due to absence of seasonal fluctuations and rapid growth of microorganisms on inexpensive media. Microbial enzymes are also more stable than their corresponding plant and animal enzymes and their production is more convenient and safer (Wiseman 1995).

Lipolytic enzymes are currently attracting an enormous attention because of their biotechnological potential (Benjamin and Pandey 1998). Some of the industrially important chemicals manufactured from fats and oils by chemical processes could be produced by lipases with greater rapidity and better specificity under mild conditions (Sih and Wu 1989; Vulfson 1994). Lipases from a large number of bacterial, fungal and plant and animal sources have been purified to homogeneity (Saxena et al., 2003).Lipases isolated from different sources have a wide range of properties depending on their sources with respect to positional specificity, fatty acid specificity, thermostability, pH optimum, etc. (Huang 1984).

Applications of lipases:

Microbial lipases constitute an important group of biotechnologically valuable enzymes, mainly due to versatility of their applied properties and ease of mass production. Microbial lipases are widely diversified in their enzymatic properties and substrate specificity, which make them very attractive for industrial applications. In the industrial segment, lipases and cellulases are anticipated to post the best gains. It is expected that in the next few years lipases will benefit from their versatility and continued penetration into the detergent and cosmetics markets. Lipases and cellulases, like most specialty and industrial enzymes, will increasingly be produced via recombinant DNA technology. Lipases are used in two distinct fashions. They are used as biological catalysts to manufacture products such as food ingredients and by their application in making fine chemicals. Following proteases and carbohydrases, lipases are considered to be the third largest group based on total sales volume. The commercial use of lipases is a billion-dollar business that comprises a wide variety of different applications (Jaeger et al., 1999).

Fat and Oil Industry:

Fats and oil modification is one of the prime areas in food processing industry that demand novel economic and green technologies (Gupta et al., 2003). Fats and oils are important constituents of foods. Lipases allow us to modify the properties of lipids by altering the location of fatty acid chains in the glyceride and replacing one or more of these with new ones. In this way, a relatively inexpensive and less desirable lipid can be modified to a higher value fat (Sharma et al., 2001). Lipases catalyze the hydrolysis, esterification and inter-esterification of oils and fats. Among the lipolytic conversion of oils and fats, esterification and interesterification are used to obtain value added products, such as specialty fats and partial glycerides by using positional and fatty acid specific lipases, and have greater industrial potential than fatty acid production in bulk through hydrolysis. VenkataRao and Laxmanan (1991) constructed an immobilized lipase membrane reactor for fat and oil hydrolysis, which yielded products that require less downstream processing, thus reducing the overall processing cost. The removal of phospholipids in vegetable oils (de-gumming) using highly selective microbial phospholipases is also a recently developed environmental friendly process (Clausen 2001).

Lipases are part of the family of hydrolases that act on carboxylicester bonds. The physiologic role of lipases is to hydrolysetriglycerides into diglycerides, monoglycerides, fatty acids, and glycerol. In addition to their natural function of hydrolysing carboxylic ester bonds, lipases can catalyse esterification, interesterification, and transesterification reactions in nonaqueous media. This versatility makes lipases the enzymes of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries (Houde et al., 2004). Some fats are much more valuable than others because of their structure. Less valuable fats can be converted into more useful species using blending of chemical methods but these tend to give quite random products.

The majority of enzymes used in industry are for food processing, mainly for the modification and break down of biomaterials. A large number of fat clearing enzymatic lipases are produced on an industrial scale. Most of the commercial lipases produced are utilized for flavour development in dairy products and processing of other foods, such as meat, vegetables, fruit, baked foods, milk product and beer. Phospholipases have found industrial applications in egg yolk treatment for the production of mayonnaise and other emulsifiers, in lecithin modification, and for the oil-degumming step in there fining of vegetable oils. Introduction of a microbial phospholipase (Lecitase Nova) has significantly improved the economy of enzymatic degumming of vegetable oils. In this process, the phospholipids are hydrolyzed and rendered more water soluble, hence facilitating their washout (Pearce et al., 2002). The function of phospholipase in egg yolk treatment is to hydrolyze egg lecithin, iso-lecithin, which improves the emulsifying capacity and heat stability. The egg yolk thus produced can be useful in the processing of custard, mayonnaise, baby foods, dressings and in dough preparation. It is also applied in the processing of sauces, like hallandise, bernaise and cafe de Paris. Lipases have been successfully used as a catalyst for the synthesis of esters. The esters produced from short-chain fatty acids are used as flavouring agents in the food industry. Lipase immobilized on silica and microemulsion based organels were widely applied for ester synthesis (Sharma et al., 2001; Ghosh et al., 1996).

Dairy Industry:

Lipases are extensively used in the dairy industry for hydrolysis of milk fat. The dairy industry uses lipases to modify the fatty acid chain lengths, to enhance the flavours of various cheeses. Current applications also include the acceleration of cheese ripening and the lipolysis of butter, fat and cream (Sharma et al., 2001; Ghosh et al., 1996).The free fatty acids generated by the action of lipases on milk fat endow many dairy products, particularly soft cheeses with their specific flavor characteristics. The traditional sources of lipases for cheese flavour enhancement are animal tissues, especially pancreatic glands (bovine and porcine) and pre-gastric tissues of young ruminants (kid, lamb and calf). A whole range of microbial lipase preparation shave been developed for the cheese manufacturing industry from *M. miehei*, *A. niger, A. oryzae* and several others. Enzyme modified cheese is used when cheese is incubated in the presence of enzymes at elevated temperature in order to produce a concentrated flavour. The concentration of fat is 10 times higher in enzyme modified cheese to that of normal cheese (Sharma et al., 2001; Ghosh et al., 1996; Makhzoum et al., 1996).In 1976, Unilever filed a patent describing a mixed hydrolysis and synthesis reaction to produce a cocoa butter substitute using an immobilized lipase.

Gastric lipases have been used to accelerate ripening and flavour development of many cheese types, including cheddar, provolone and ras cheeses. Lipase addition enhances the rate of fatty acid liberation, which accelerates flavour development relative to control. These studies indicated that liberated fatty acid profiles of the accelerated process were identical to the control and the total quantities of short-chain liberated fatty acids (C4 to C6) were important for the development of typical cheddar cheese flavour during ripening. When a cock-tail of fungal protease and lipase were used, cheddar cheese developed a highly soluble proteins and free fatty acids and displayed better flavour within three months of ripening. The level of enzyme added to accelerate cheese ripening is also very important. High levels of enzyme during ripening may result in excessive enzymatic reactions that impart undesired characteristics and reduce the yield. Adaptation of liposome technology for accelerated cheese ripening reduces bitterness and losses in yield (Custry et al., 1987). Bacterial intracellular enzymes are released by celllysis and contribute to flavour through lipolysis and other enzymatic actions. Microcapsules of cell free extracts encapsulated in milk fat can be added to carryout milk clotting. Cheeses made with intact capsules contain substantially more enzymatic end products than the one obtained by direct enzyme addition. The capsule stability can be improved by encapsulating in a high melting fraction of fat (Custry et al., 1987).Inherent milk lipase in cheese, made from unpasturized milk, affects considerable lipolytic action. The cultures and secondary flora, such as the *P. roqueforti* and *P. camembertii*in Blue-vein and Camembert cheeses respectively, are lipolytic and produce lipases, which are responsible for lipolysis. In addition, lipases are usually added to Italian cheese, viz. paramesan, provolone, and romano, to intensify their flavour (Custry et al., 1987). During ripening, there is a steady increase in the concentration of liberated fatty acids and total soluble nitrogen. Lipases release the fatty acids from triglycerides, thereby triggering the development of cheese flavour (Maia et al., 1999).

The introduction of conjugated linoleic acid (CLA)in dairy foods has been made possible through the immobilization of lipases (Baianu et al., 2003). Lipases and proteases have been used to accelerate ripening both individually and as a "cocktail". The enzymes may be added as such or they may be encapsulated. During cheese ripening, a series of enzymatic reactions proceed very gradually, modifying the fresh, mechanically worked curd to the desired final ripe cheese texture and flavour. The enzymes, lipases, proteases and lactase hydrolyze lipids, proteins and lactose, respectively in order to raise the level of flavour moieties and/or flavour processors (Custry et al., 1987).

Lipases as Biosensors for Food Industry:

Immobilized lipases are fast, efficient, accurate and cost effective as sensors for the quantitative determination of triacylglycerol. This application is important in the food industry, especially in fats and oils, beverages, soft drinks, pharmaceutical industries and also in clinical diagnosis (Kynclova et al., 1995). The basic concept of using lipase as biosensors is to generate glycerol from the triacylglycerol in the analytical sample and to quantify the released glycerol by a chemical or enzymatic method (Pandey et al., 1999).Wei *et al* (1997) developed a method for the determination of organophosphorous pesticides with a surface acoustic wave impedance sensor by lipase hydrolysis. This method is also used to determine the dichlorvous residues in the root, stem and blade of Chinese cabbage. Lipases may be immobilized on to *p*H/oxygen electrodes in combination with glucoseoxidase, and these function as lipid biosensors and may be used in triglycerides and blood cholesterol determinations (Hasan et al., 2006).

Bakery Industry:

In baking industry, there is an increasing focus onlipolytic enzymes. Recent findings suggest that (phospho) lipases can be used to substitute or supplement traditional emulsifiers since the enzymes degrade polar wheat lipids to produce emulsifying lipids *in situ*(Kirk et al., 2002; Collar et al., 2000). Lipase was primarily used to enhance the flavour content of bakery products by liberating short-chain fatty acids through esterification. Along with flavour enhancement, it also prolonged the shelf-life of most of the bakery products. Texture and softness could be improved by lipase catalyzation (Loboret and Perraud 1999). An artificially expressed lipase in *A. oryzae* was used as processing aid in the baking industry (Greenough et al., 1996). All hydrolytic enzymes, including lipase, were found to be effective in reducing the initial firmness and increasing the specific volume of breads (Keskin et al., 2004).Yeast with bacterial lipase gene LIP A resulted in higher productivity of enzyme and found use in bread making as a technological additive (Sanchez et al., 2002).Increased butter flavour for baked goods was generated by hydrolysis of butterfat with suitable lipase (Uhling 1998).

Other Food Processing Industries:

In recent times, lipases have been commonly used in the production of a variety of products, ranging from fruit juices to vegetable fermentation (Pandey et al., 1999). Lipases facilitate the removal of fat from meat and fish products (Sharma et al., 2001). An interesting finding is the addition of lipase to noodles, resulting in significantly softer textural characteristics in noodles despite having the relatively low levels of the substrate acylglycerols present in the formulations (Undurraga et al., 2001).In confectionary, 1,3-regioselectivity of lipases was exploited in the process development of a fat production containing high concentration of1,3-disteraroyl-2-monoloein (Macrae 2000). This fat could be used as a substitute for sheastearine in the formulation of cocoa butter equivalents. Fats designed to inhibit bloom formation in chocolate products have also been produced by these types of enzyme esterification reactions (Macrae 2000).*C. rugosa*lipases have many applications in the food and flavour industry, in the production of ice cream and single cell protein, biocatalytic resolution of life saving pharmaceuticals, carbohydrate esters and amino acid derivatives not

obtainable by conventional chemical synthesis (Benjamin and Pandey 1998). Immobilized lipase from *C. antarctica* has been applied to perform the enzymatic esterification of bioactive compounds with fatty acids. Various bioactive compounds, like vitamins, secondary metabolites such as kojic acid from plants and microorganisms, can be acylated to generate products useful in the cosmetic, pharmaceutical, fine chemical, food and feed industries. A convenient scale able procedure for the downstream processing of the ester product comprises hexane-solid extraction of the unreacted lauric acid and water ethyl acetate extraction of the unreacted pyridoxine, yielding lauric acid-pyridoxine monoester as a white powder with more than 90% purity, which is soluble in vegetable oil (Zarevucka et al., 1995). Regio selective modification of polyfunctional organic compounds is yet another rapidly expand in garea of lipase application. The enzyme has also been used in conjugation with a microbial cocktail for the treatment of fat rich effluents from ice cream plants. This could also be utilized in waste processing of many food industries (Ghosh et al., 1996).

Lipases for Pharmaceutical Application:

Microbial lipases are used to enrich PUFAs from animal and plant lipids, and their mono and diacylglycerides are used to produce a variety of pharmaceuticals (Dong et al., 1999). PUFAs are increasingly used as food additives, pharmaceuticals and nutraceuticals because of their metabolic benefits. Many PUFAs are essential for normal synthesis of lipid membranes and prostaglandins. Microbial lipases are used to obtain PUFAs from animal and plant lipids, such as menhaden oil, tuna oil and borage oil. Free PUFAs and their mono and diacylglycerides are subsequently used to produce a variety of pharmaceuticals (Sharma et al., 2001). Liposomes are used in the medical field to optimize the action of drugs by transporting them to target areas, thus circumventing drug waste inactivation and anatomical barriers (Linko and Wu 1996).

Profens, a class of non-steroidal anti-inflammatory drugs, are active in the(s)-enantiomer form. Lee *et al*(1995) and Xie*et al* (1988)synthesized pure (s)-ibuprofen using lipase-catalyzed kinetic resolution via hydrolysis and esterification, respectively. In addition to racemization *in situ*, lipases are also capable of catalyzing synthetic reactions, which has led to the production of life saving drugs. Efficient kinetic resolution processes are available for the preparation of optically active homochiral intermediates for the synthesis of nikkomycin-B, non-steroid anti-inflammatory drugs (naproxen, ibuprofen, suprofen and ketoprox), the potential anti-viral agent lamivudine, and for the enantiospecific synthesis ofalkaloids, antibiotics, vitamins, and antiarteriosclerotic, anti-tumour and anti-allergic compounds (Pandey et al., 1999). Nutraceuticals are food components that have health benefits beyond traditional nutritional value. Novel biotechnology tools, like immobilization, have also been applied for the isolation and incorporation of such food components in ordinary foods. Successful synthesis of nutraceuticals has been reported by employing immobilized lipases, such as those from *C. Antarctica* and *Lactobacillus ruteri* (Linko and Wu 1996).

CONCLUSION:

The tremendous potential of lipases in food and allied technology applications shows the need to develop novel cost-effective technologies for increased production, scaling up and purification of this versatile enzyme. The large number of hydrolytic applications, like flavour development in dairy products (cheese, butter and margarine), alcoholic beverages, milk chocolate, etc., is a promising field of lipase enzyme. Production of diet control food stuff, meat technology and the processing of sausages are some areas in food industry with commercial potential. The applications of lipases are broadening rapidly and new applications are still to be explored in food industries. The properties of lipases are being improved by protein engineering and genetic engineering to widen their applications in extreme conditions. Various innovations in the immobilization of enzymes play a vital role in using this enzyme as an effective and efficient biocatalyst in food processing technology. Other than the food industry, lipases have been applied in the synthesis of fine chemicals, biodiesel production, the production of biopolymeric materials, the detergent industry, organic synthesis, the paper and pulp industry, the synthesis of ingredients for personal care products, the synthesis of surfactants and of structural triglycerides, the oleochemical industry, agrochemicals production, the pesticide industry, and in environmental management. The characterization and application of lipases to catalyze reactions with commercial potential will significantly broaden the spectrum of industrial biotechnology. To cater to the needs of these enzymes in industries, novel lipase genes have to be isolated and the existing lipases are to be engineered for desired properties. The engineered lipases can be evolved by directed evolution, ultra high-throughput screening system based on electrospray ionization masss pectrometry (ESI-MS) and by phase display techniques. The rapid boom in the future prospects of lipase technology is evident from the large number of patents, publications and research reports in the recent years and indications are that this growth will be sustained for many years.

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