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Lipase Catalyzed Ester Synthesis for Food Processing Industries

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ABSTRACT

Lipases are one of the most important industrial biocatalyst which catalyzes the hydrolysis of lipids. It can also reverse the reaction at minimum water activity. Because of this pliable nature, it is widely exploited to catalyze the diverse bioconversion reactions, such as hydrolysis, esterification, interesterification, alcoholysis, acidolysis and aminolysis. The property to synthesize the esters from the fatty acids and glycerol promotes its use in various ester synthesis. The esters synthesized by lipase finds applications in numerous fields such as biodiesel production, resolution of the recemic drugs, fat and lipid modification, flavour synthesis, synthesis of enantiopure pharmaceuticals and nutraceuticals. It plays a crucial role in the food processing industries since the process is unaffected by the unwanted side products. Lipase modifications such as the surfactant coating, molecular imprinting to suit for the non-aqueous ester synthesis have also been reported. This review deals with lipase catalyzed ester synthesis, esterification strategies, optimum conditions and their applications in food processing industries.

Key words: Lipase, food applications, ester, esterification, synthesis

INTRODUCTION

Lipases (triacylglycerol acylhydrolase, EC.3.1.1.3) belong to the super class of hydrolases that act on the carboxylic ester bonds. They hydrolyze the triglycerides into diglycerides, monoglycerides, fatty acids and glycerol. Lipases also catalyze the synthesis of the esters formed from glycerol and long chain fatty acids (Sharma et al., 2001). They bring about a wide range of bioconversion reactions such as the hydrolysis, interesterification, esterification, alcoholysis, acidolysis and aminolysis (Pandey et al., 1999). This versatility makes lipases as the enzyme of choice for potential applications in the food, detergent, pharmaceutical, leather, textile, cosmetic, and paper industries.

Lipases are serine hydrolases which do not require any cofactor. Due to this unique feature, they remain dissolved in oil water interface and under the natural conditions they hydrolyse the triacyl glycerols which have low solubility in the water. In the presence of traces of water, they reverse the reaction leads to esterification and formation of glycerides from the fatty acids and glycerols (Ghosh et al., 1996; Sharma et al., 2001).

The kinetics of lipase catalyzed lipolytic reaction cannot be described by Michaelis-Menten equation

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since it is valid only for the reactions that take place in the homogenous phase. The kinetic study of the lipolytic reactions reports a phenomenon of 'interfacial activation' which describes that the activity of lipases is enhanced towards the insoluble substrates that forms an emulsion (Jaeger et al., 1998). The 3-D structure studies provide the explanation of the interfacial activation. The active site of lipase was found to be covered by a surface loop, known as the lid (or flap). Upon binding to the interface, this lid moves away, turning the 'closed' form of the enzyme into 'open' form with the active site now accessible to the substrate which is present in the solvent (Jaeger et al., 1999). The catalytic moiety of lipases have $G–X_1–S–X_2–G$ as the consensus sequence, where G= glycine, S= serine, X_1= histidine and X_2= glutamine or aspartic acid (Svendsen, 1994). The substrate hydrolysis starts with a nucleophilic attack by the catalytic-site Ser-oxygen on the carbonyl carbon atom of the ester bond of the triglyceride (Jaeger et al., 1998). Thus, the studies on the structure-function relationships contributes for a better understanding of the kinetic mechanisms of lipase action on the hydrolysis, synthesis and group exchange of esters (Ghosh et al., 1996; Taipa et al., 1992).

Extensive studies have reported the applications of the animal, plant and microbial lipases for various industrial purposes. Current applications mostly involve the microbial lipases at greater length. (Macrae and Hammond, 1985). Microbial lipases which are regiospecific and fatty acid specific could be exploited for the esterification and transesterification reactions (Gupta et al., 2003). The esterification by the lipases appears to be an attractive alternative to the bulk chemical routes. Lipases are being developed to carryout the transformations without the extreme temperature and pressure conditions which are essential for the traditional industrial processes.

In the chemical synthesis, mineral acids are most commonly used to catalyze the esterification. Other agents such as tin salts, organo-titanates, silica gel, and cation-exchange resins are also employed. The classical acid catalysis may lead to unwanted side reactions. Although the metal salts minimize the side reactions, they require higher temperature (Kirk and Othmer, 1979). Normally, the fat and oil modifications carried out by the chemical interesterification are energy intensive and non-specific (Gupta et al., 2003). Lipases display a high degree of specificity and enantio selectivity for the esterification and transesterification reactions (Okahata et al., 1995), which makes them a principle biocatalyst in the trans- and interesterification reactions for the synthesis of several useful acylglycerols. The use of lipase to carryout the esterification alleviates the need for a wide variety of the complex post-reaction separation processes, which may lead to lower the overall operating costs. Lipase catalyzed reactions in organic solvents have become recent subject of interest. This review focuses on the lipase catalyzed esterification and their applications in the food processing industries.

Lipase Production, Purification And Properties

Generally fungi and bacteria are the choice for the commercial production of lipase (Macrae and Hammond, 1985). The extracellular lipase production depends upon several culture conditions such as nitrogen, carbon, lipid inducer, presence of inorganic salts, availability of dissolved oxygen and aeration. The environmental factors such as pH and temperature also show high influence on the growth and excretion of microbial lipase. Normally, lipase production is stimulated by the lipids. Lipase activity is strongly induced by a wide range of fatty acyl esters including the triglycerides, spans, Tweens, etc and is repressed by the long-chain fatty acids, including oleic acid. Nitrogen source in the medium determines the production levels of lipases. Soyabean meal extract in *Rhizopus oligosporus* culture medium supported the growth and lipase production (Gosh et al., 1996). The presence of Ca^{2+}, Mg^{2+}, Mn^{2+}, Na^{+}, Zn^{2+}, Fe^{2+}, Al^{3+}, Co^{2+}, Hg^{2+} sometimes stimulated the production and sometimes acted as inhibitors. In most of the studies, Ca^{2+} stimulated the production by aiding the hydrolysis of the triglyceride (Sharma et al., 2001).

The initial pH of the growth medium is important in lipase production. The optimum initial pH lies between 6.0 to 8.0. The optimum temperature for lipase production ranges between 20 – 40°C. The optimum temperature for lipase production corresponds with the growth temperature of the respective organism. Highly thermostable lipases can be produced by the organisms isolated from the extreme environmental conditions such as the hot springs. Moderate aeration resulted in high lipase production than vigorous aeration and is critical to determine and has variable effects on different micro organisms (Gosh et al., 1996).

A large number of microbes have been exploited for the production of lipase. Microbial lipase can be produced by the submerged and solid-state fermentation. However, submerged culture technique is most widely used. Effect of various carbon, nitrogen sources and the environmental conditions on lipase production has been extensively reviewed. (Sharma et al., 2001). Rivera et al. (1991) performed the solid-state fermentation with several filamentous fungi and obtained higher lipase yield with low protease activity. A comparison between the kinetics of the solid-state and submerged fermentation was performed using *Penicillium candidum* in which the solid-state fermentation gave the highest titers and a stable production.

Purification

Lipases from a large number of the bacterial, fungal and a few plant and animal sources have been purified to homogeneity. This enables the successful sequence determination and their 3-D structure prediction which leads to the better understanding of their unique structure-function relationships during various hydrolytic and synthetic reactions.

Most of the microbial lipases are extracellular and the fermentation process is usually followed by the removal of the cells from the culture broth, either by the centrifugation or by filtration. The cell free culture broth is then concentrated by the ultrafiltration, ammonium sulphate precipitation or extraction with the organic solvents. Usually, the chromatographic separation follows the precipitation step. A single chromatographic step is not sufficient to get the required level of purity. Hence, a combination of steps can be applied (Saxena et al., 2003).

Ion exchange chromatography is the most widely applied chromatographic separation technique. The most frequently employed ion exchangers are the diethylamino ethyl (DEAE) group in anion exchange (58%) and the carboxymethyl (CM) in cation exchange (20%) (Veeraragavan et al., 1990). Gel filtration and affinity chromatography can also be used for enzyme purification. To purify a protein to homogeneity, with an overall yield of 30% and a purification factor of 320, four or five purification steps are usually required. Nowadays, some novel purification technologies are employed, which include membrane processes, immunopurification, hydrophobic interaction chromatography employing epoxy-activated spacer arm as a ligand and polyethylene glycol-sepharose gel, poly (vinyl alcohol) polymers as column chromatography stationary phase and aqueous tow-phase systems (Sharon et al., 1998) reversed miceller process (Ghosh et al., 1996).

Properties of Lipase

Lipase acts on the substrate in a specific or non-specific manner. The hydrophobic patches on the surface of lipases are responsible for the strong interactions with the hydrophobic substrates at the interface. In the absence of the water, lipases are capable of reversing the reaction that leads to the esterification and interesterification (Macrae, 1983). Besides the lipolytic activity, lipases also possess esterolytic activity, thus, have a wide substrate range. The chemo-, regio-, and enantio-specific behaviour of these enzymes have attracted tremendous scientific and industrial research (Saxena et al., 2003).

Macrae and Hammond (1985) grouped lipase according to its regio-specificity in which the first group showed no regio-specificity and released fatty acids from all three positions. The second group of lipases released the fatty acids regio specifically from the outer 1 and 3 positions of acylglycerols. The specificity and stability of lipase production used in the biotechnological applications can be modulated by the culture conditions. The presence of Tween 80 and Tween 20 in *Candida rugosa* culture medium not only promoted the lipase productivity but also changed the production of the multiple forms of lipase (Chang et al., 1994).

Some lipases have shown considerable stability over a wide range of pH values. An alkaline lipase active at pH 11.0 has been isolated from *Pseudomons nitreducens*. Normally, they are active under the pH of 6.5 to 7.0 and considerable till 8.0. Due to their partial sterio specificity, lipases can be used to isolate the optically pure esters and alcohols (Ghosh et al., 1996).

Divalent cations such as Ca^{2+} stimulates the lipase activity, but Co^{2+}, Ni^{2+}, Hg^{2+} and Sn^{2+} drastically inhibits the activity. Zn^{2+}, Mg^{2+}, ethylene diamine tetraacetic acid (EDTA) and sodium dodecyl sulfate (SDS) have moderate inhibition effect on lipase activity. The thermophilic organisms obtained from Icelandic hot springs showed lipase activity even at 40- 60°C (Ghosh et al., 1996). The salts of the heavy metals such as Fe^{2+}, Zn^{2+}, Hg^{2+}, Fe^{3+} strongly inhibited the lipase, suggesting that

they were able to alter the enzyme conformation (Sharon et al., 1998).

Esterification

Ester synthesis reactions make use of the law of mass action to drive the equilibrium in the direction of the synthesis by removing the water generated during the reaction. Ester exchange reactions take place at low water activity (Macrae, 1983). The optimum yield in the esterification was obtained by determining the optimal substrate and enzyme concentration. The inhibiting effects of the acids were strongly attenuated by reducing the quantity of the acid and increasing the amount of enzyme in the media (Laboret et al., 1999). The esterification performance was dependent on the alcohol structure, with maximum activity occurring for the primary alcohol. Secondary and tertiary alcohols decreased the reaction rates by more than 40%. The ester synthesis can be maximized for the substrates containing the excess acyl donors (Bruno et al., 2004). Garcia et al. (2002) developed a methodology for the esterification of an acid with an epoxide using 2-chlorobutyric acid and 1,2-epoxy-5 hexen-catalyzed by a *Mucor miehei* - immobilized lipase. This could be applied to obtain 2-chloroesters. The esterification of cinnamic acid and oleyl alcohol in the organic solvent media by the immobilized lipase Novazym 435 was optimized with a bioconversion of 100% after 12 days of reaction. The electrospray ionization mass spectroscopy analysis confirmed that the major end-product of the esterification reaction was oleyl cinnamate. Lipase mediated ester synthesis can be performed at the room temperature, pressure and with neutral pH in reaction vessels operated either batchwise or continuously.

The effect of fatty acid chain length on *C. antarctica* lipase B (Novazym 435) catalyzed sugar esterification was studied in a mixture of 9:11 t Butanol and pyridine (v/v). α and β maltose 6'O-acyl esters in an anomeric molar ratio of 1.0:1.1 were synthesized independently of the chain length, but the initial specific reaction rate increased with the chain length, of the acyl donar. The highest initial reaction rate and yield were obtained with the shortest chain length of the acyl donar (Pedersen et al., 2002). The immobilized lipase from *C. antarctica* was applied to perform the enzymatic esterification of the bioactive compounds with the fatty acids pyridoxine condensate with lauric acid was studied as the model system and lauric acid pyridoxine mono esters with high retention time was obtained as the product. A convenient scalable original procedure for the downstream processing of the ester product was developed comprising hexane–solid extraction of the unreacted lauric acid and water ethyl acetate extraction of the unreacted pyrindoxine and yielding lauric acid-pyridoxine monoester as a white powder with more than 90% purity (Pelenc et al., 2003).

For the esterification between the hydroxyl group of lactic acid and the carboxyl group of organic acids, Kiran and Divakar (2001) employed lipozyme IM20 from *Rhizomucor miehei* and porcine pancreas. The reactions were carried out at both shake-flask and bench-scale levels. At the shake-flask level, maximum yields of 37.5 and 40% were observed in case of palmitoyl and stearoyl lactic acids, respectively, with Lipozyme IM20; at bench-scale level, the maximum yields were 85.1 and 99% respectively, when porcine pancreas lipase was employed. Moreover, this could be reused for three cycles with yield above 40%. The esters prepared were found to confirm the food chemical codex (FCC) specifications in terms of the acid value, ester value, sodium and lactic acid contents.

Lipase catalyzed formation of isoamyl bulyrate was investigated as model to study the kinetics of lipase –catalyzed esterification. The reaction rate was described in terms of the Michaelis-Menten equation with a Ping-Pong Bi-Bi Mechanism (Krishna et al., 2001). In this reaction, the increase in butyric acid concentration in microaqueous layer caused a pH drop in the enzyme microenvironment, leading to enzyme inactivation. Enantioselective resolution of trans-2-phenyl-1-cyclohexanol (TPCH) by a *C. rugosa* lipase was obtained by the esterification of the immobilized EP100 polypropylene powder using isooctane as the solvent and propionic acid as the esterifying agent. High value of conversion and enantioselectivity was observed in the continuous packed bed reactor. The TPCH, thus, produced has been used in the synthesis of wide variety of chiral molecules such as hydroxy aminoacids, diltiazen etc. due to its versality and a high level of stereo control (Sanchez et al., 2000). Langrand et al. (1988) checked thirteen commercial lipase preparations for their ability to catalyze the formation of flavour esters. Acetic acid esters were more difficult to obtain than isomyl, gernoyl acetate, propionate and butyrate.

Interesterification

Lipase is soluble in the water but the triglycerides and long chain carboxylic acid substrates are not soluble in the aqueous phase. Hence, most interesterification studies are carried out in the presence of little amount of water or in an emulsion (Holmberg et al., 1989). Lipase catalyzed interesterificaiton and hydrolysis reaction is shown in Figure 1. Lipase mediated transesterification are likely to occupy a prominent place in the oil industry for tailoring the structural lipids since the enzymatic modifications are specific and can be carried out at moderate reaction conditions. The tailored vegetable oils with nutritionally important structured triglycerides and altered physiochemical properties have huge potential in future (Gupta et al., 2003).

Considerable effort has been done by the European countries and in Japan to commercialize lipase-catalyzed interesterification for the production of relatively valuable triacylglycerol mixtures such as cocoa-butter equivalents (Macrae and Hammond, 1985). An immobilized lipase from *R. miehei* served as the biocatalyst for capric acid incorporation in the rice bran oil. The transesterification was performed in an organic solvent, hexane under the solvent–free condition. Pancreatic lipase catalyzed Sn-2 positional analysis and tocopherol analysis were performed before and after enzymatic modification. When the enzyme load, substrate mole ratio and incubation time increased, the mole percent of capric acid incorporation also increased (Jennings et al., 2000).

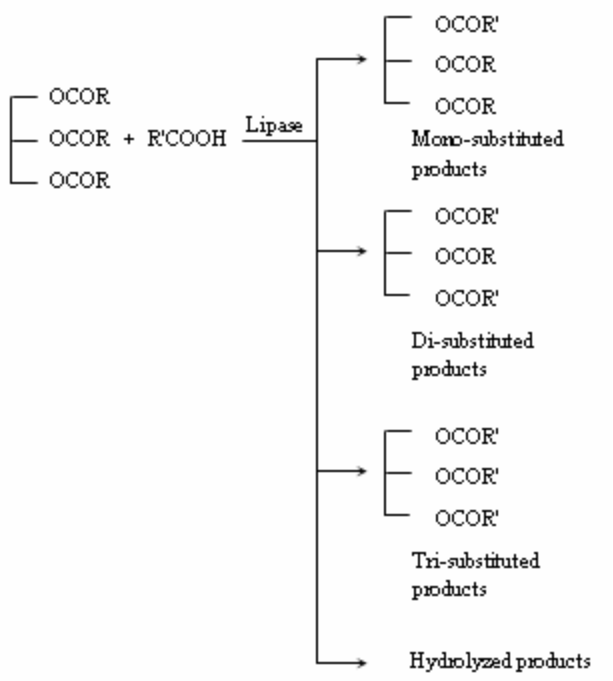

Figure 1 - Lipase induced interesterification and hydrolysis (Goto et al., 1995).

The esters can be synthesized by the transesterification. The main advantages of this compared to direct synthesis are (i) no water molecules are formed or consumed in the reaction (ii) the enzyme stability is usually higher under these conditions (Langrand et al., 1988). A mathematical basis for the design and operation of a continuous, packed-bed reactor for the interesterifiction of soybean oil was developed by Akimoto et al. (1999). Soybean oil containing 22.7% oleoyl and 54.3% linoleoyl moieties as the molar acyl moiety composition was interesterfied in hexane with oleic acid, using an immobilized Sn-1, 3-specific lipase (Lipozyme IM) from *M. miehei*. Based on the kinetics, the flow rate of the feed stream was stimulated to produce an oil of a fixed composition of oleoyl moiety in the continuous and packed bed reactors at 37°C. Unilever (1980) proved that lipases could catalyze

the interesterification by restricting the amount water in the system. The types of the triacylglycerol products obtained by the lipase-catalyzed interesterification depended on the specificity of lipase used (Macrae, 1983).

The production of a cocoa butter equivalent (CBE) through the enzymatic interesterification of the palm oil midfraction (POMF) with stearic acid in a solvent free system using Nova lipase Lipozyme as a catalyst was analyzed by Undurraga et al. (2001). The purpose of the interesterifiction was to incorporate selectively stearic acid residues into POMF tiglycerides at position 1 and 3 until a composition resembling CB composition was obtained. The highest specific productivity obtained in the shake flask was 0.0393 g/ Batch Interesterification Unit (BIU) h at a stearic acid–POMF ratio of 1.6 and enzyme–substrate ratio of 23 BIU/g. In the continuous packed bed reactor, the highest mass productivity observed was 1.54g/g.h using an enzymic load of 73 BIU/g. The thermogram of the obtained CBE was similar to that of cocoa butter. Pearce et al. (2002) used granulation, a new immobilization technology for lipase. It enabled the production of a food grade, cost effective immobilized 1, 3-regioselectie lipase (Lipozyme TL IM) targeted for the interesterification of commodity oils and fats for the production of frying fats, shortening and margarine components.

Modification of Lipase

Lipase can be prepared to act in the organic solvent. A new surfactant–enzyme complex was developed in which lipase was coated with the surfactant. This surfactant-coated lipase was insoluble in the water but soluble in most of the organic solvents since the hydrophobic tails of the surfactant solubilized the lipase in the organic media. This modified lipase could be widely applied in the esterification studies. The esterification by the surfactant–coated lipase resulted in a higher rate and yield than that in the direct dispersion or microemulsion system (Okahata and Ijiro, 1988; Okahata and Ijiro, 1992). Lipase absorbed on the surfactant were activated 1-9 to 150 fold compared to the crude lipase since the solubility was extremely low in the reaction media (Persson et al., 2002).

The enantioselectively of *Pseudomonas cepacia* and *C. cylindracea* was modulated by utilizing the surfactant-coating and molecular imprinting technique. The enantio selectivity of the surfactant coated *P. cepacia* was enhanced two folds in the formation of 2-octane in isooctane by the molecular imprinting effect in the presence of 2-octanol. The imprinting technique changed the enantio selectivity of *C. cylindracea* lipase (R) isomer with a preference for the (S) isomer. Thus, the selectivity of the native enzyme changed (Kamiya and Goto, 1998). This can be exploited in the synthesis and purification of enantio pure components in the non-aqueous media for various food and pharmaceutical applications.

C. rugosa lipases (semipurified and pure isoenzymes) were chemically modified using pNPCE –PEG. The lipases, thus, modified can be stored at 4°C for six months without losing the activity and they are stable even at 50°C in isooctane. The chemically modified lipases were used in the esterification of oleic acid and in the enantio selective esterification of (R,S) ibuprofen. Hernaiz et al. (1999) modulated the hydrophobicity of the biocatalyst by changing the degree of the modification of lipase which allowed the selection of the optimum biocatalyst for the esterification in different organic solvents to achieve the maximum yield.

Surfactant-coated enzymes have been prepared by coating lipases of various origins with a nonionic surfactant, glutamic acid dioleylester ribitol. The enzymatic interesterification of tripalmitin with oleic acid using the surfactant coated lipase was carried out in the organic media. The surfactant coated lipases could effectively catalyze the interesterification of the glycerides better than the powdered lipases (Goto et al., 1995).

Optimum Temperature for Esterification

In the food processing industry, lipase should possess high stability at room temperature. Generally, the thermal stability of the enzyme should be increased when the reaction has to be carried out in a hydrophobic organic solvent instead in an aqueous solution. The investigation of the temperature effect on the enantiomeric excess (ee) value is always recommended to select the optimum temperature for the particular process. By this, the yield and efficiency can also be improved. A study made by Garcia et al. (2002) revealed the temperature as the most important factor because it had a positive influence on the yield. The yield and isomeric excess of the ester catalyzed by *M. miehei* lipase showed a greater dependence on the temperature rather than the

catalyst concentration itself. The ester exchange reactions can take place in almost completely dry systems, even at temperatures upto 100°C (Macrae, 1983).

The thermostability of the procine pancreas lipase was studied by monitoring the esterification activity in the temperature range of 40-80°C. Native enzyme showed higher loss of activity at 60°C than at 40 °C and 80°C. Longer periods of incubation at 80°C for 100 days did not affect the activity. This indicated that the variation in the conformational changes in the process temperature was responsible for the loss in activity (Kiran et al., 2001). In an enzymatic acidoylsis incorporation of caprylic acid and the targeted di-incorporated structured triglyceride (ST) was increased by approximately 20% when the temperature increased from 40 to 70°C (Xu et al., 2000).

Influence of Hydrophobic Solvents and Water Activity

In order to obtain the esters of good quality and higher yield, the effect of the solvents and water activity were studied. In the transesterification with the immobilized lipase from *R. miehei* for incorporation of carpic acid in the rice bran oil, hexane and solvent free system were analyzed. The incorporation rate declined with the increasing amount of the water for both the conditions (Jennings and Akoh, 2000). The effect of fatty alcohol on the kinetics of lauric acid esterification with lipase catalyzation was studied by Shintre et al. (2002) Lowric acid has been esterified with some C_1-C_{18} aliphatic alcohols by a commercial lipase, Lipolase 100L, using isooctane as a solvent. First order kinetics was observed when lauric acid and fatty alcohols were taken in 1:1 mole ratio. The highest reaction rate was observed for n-butyl alcohol.

Optimal water activity (a_w) for the synthesis of structured triacylglycerol (STAG) containing eicosapentaenoic acid (EPA) at the Sn-1 (or 3) position using lipozyme in a solvent free system was investigated by Han and Yamane (1999). Vacuum was applied to shift the reaction equilibrium towards the synthesis reaction by removing the by-products. During the vacuum application, the water level of the reaction system was controlled at the optimal level by the addition of a suitable amount of the water at a predetermined interval. The intermittent periodic addition of a suitable amount of the water into the reaction mixture made the reaction rate faster than that without the water addition. The reactions carried out under the water activity control during the vacuum application yielded higher amount of the targeted structural triacyl glycerol, within shorter period.

The incorporation of caproic acid in Sn-1 position of phosphatidylcholine catalyzed by lipase from *R. oryzae* was investigated in a water activity–controlled organic medium. Under this condition it was carried out either as the esterification or transesterification. The comparison between these two reaction modes stated that the yield was the same under the identical condition. The highest yield was obtained at a water activity of 0.11. The reaction time was shorter in the esterification reaction than in the transesterification reaction (Adlercreutz et al., 2002).

The selectivity of a commercial immobilized lipase preparation was tested for the esterification reaction. The esterification reactions were carried out with different terpene alcohols and butyric acid. The alcohol structures had great influence on the performance of this enzyme preparation. The esterification degrees over 95% were attained for the primary alcohols such as citronellol, geroniol and nerol. Secondary (menthol) and tertiary (linallol) alcohols were not esterified under the tested conditions (Castro et al., 1997). The water content in the substrate has a mild influence on an enzymatic acidolysis carried out by immobilized 1,3-specific lipase, lipozyme IM, for the production of structured triacylglycerols (ST) from canola oil (Xu et al., 2000).

In developing the experimental reactor systems for the enzymatic interesterification, the reactants are generally been dissolved in an organic solvent to maintain the reactant or product stream in the liquid state and to limit the amount of water in the system, particularly water immiscible solvents appear to be preferable. Lipase catalyzed esterification activity was indetectable when 100% glycerol was employed (Macrae, 1983). In lipase catalyzed organic ester synthesis in non-aqueous media, ethyl ketone was found to be the best solvent for the shake flask reactions and chloroform best suited for the bench-scale level with higher yields (Kiran and Divakar, 2001).

Normally, the ester synthesis by the interesterification required a suitable mixture of the non-polar solvents, but in the food application process, solvent-free systems are preferable

(Undurraga et al., 2001). The enzymatic esterification of 2-(4- isobutyl phenyl) propionic acid (ibuprofen) was studied in different solvents at various water activities to measure the effects of these variables on the activity and selectivity of the enzyme. *C. antartica* lipase was employed for this study. A dependency of the enantio selectivity on the water activity was observed. The hydrophobic solvents yielded higher reaction rates than the hydrophilic ones even at constant water activity; however, better enantio selectivity was observed (Dueret et al., 1998). An aqueous environment is unsuitable for lipase catalyzed ester synthesis.

Water has a profound effect on the lipase behavior either by affecting the hydration of the enzyme or by changing the nature of the reaction media of the support material. The water content of the catalyst is more important in dictating the catalytic activity than the total water content in the system (Yahya et al., 1998). Apart from their activity in the organic media, the enzymes acquire remarkable properties such as the enhanced thermostability, radically altered substrate and enantiomeric specificities, molecular memory and the ability to catalyze the unusual reactions (Krishna and Karanth, 2001). The low solubility of enzymes in organic solvents decreases the amount of enzyme loss via desorption from the support material. This enables the use of the simple enzyme immobilization techniques such as the adsorption which covers the cost of enzyme preparation (Wehtje et al., 1993).

Food Industry Applications

Currently, the modification of the structure and composition of the oils and fats by the enzymatic interesterificaitn has become greater interest in the food industries (e.g; coco butter). The lipolytic reaction in the non-aqueous media has been indicated as a novel approach for the production of the nutrients, pharmaceuticals and several other basic materials which found enormous application in the industries (Zaks and Klibanov, 1988). Lipase was found to be fit in the organic solvent because of its rigid conformation and interfacial activation properties. Some unique properties of lipase such as their specificity, temperature, pH dependency and activity in organic solvents, non-toxic nature leads to their major contribution in the food processing industries. Ethyl, isobutyl, amyl and isoamyl acetates are widely used flavour esters.

A lipase gene from *Staphylococcus epidermidis* over-expressed in *Escherichia coli* produced a lipase with increased catalytic efficiency and altered the substrate specificity. It catalyzed the esterification in aqueous media which was more suitable in the food processing. The products obtained were considered to be natural and directly employed in the food industry. They found wide application in the flavour synthesis, wines, baked foods, emulsifier, pharmaceuticals and dairy products (Shaw, 2003). Sugar esters are nonionic surfactants and exhibit relevant properties such as structural diversity environmental and health safety. Their potential applications extend from human food to drug formulations and biochemical studies (Piccicuto et al., 2001).

The acylation of carbohydrates catalyzed by lipase found significant importance in the food industries (Nakamura, 1997). The enzyme catalyzed synthesis of sugar ester can provide regio and sterio selective products. Sucrose fatty acid esters are used as the non-ionic surfactants in the food processing and some other acylated carbohydrates are employed as the pharmaceuticals with the antimicrobial, antitumoural and/or antibiotic activity. Silica granulated *T. lanuginosa* lipase served as feasible biocatalyst for the synthesis of acylsucroses or other carbohydrates esters in the polar or slightly polar solvents (Ferrer et al., 2001; Ferrer et al., 2005). Lipases from *T. lanuginosus* and *C. antarctica* B were compared in terms of activity, regioselectivity and potentially reliable catalysts for sugar ester production. Lipase from *T. lanuginosus* was found to be convenient for the synthesis of 6-monoester and *C. antarctica* B for 6, 61 diesters and both lipases were similarly useful for the synthesis of maltose and glucose mono esters. The disaccharide monoesters exhibited better antimicrobial activity than the monosaccharide monoesters. Maltose esters, thus synthesized could be used as alternative food emulsifiers because in addition to surfactant properties, they exhibited antimicrobial properties comparable to sucrose esters. Most sugar fatty acid esters display significant activity against several food and clinical isolates (Ferrer et al, 2005). Natural methylthioesters of short-chain fatty acids (C_3-C_8) are of great interest in the flavour industry. Flavour substances such as S-methyl butanethioate and S-methyl 3-methyl butanethioate are important constituents of the dairy aromas, especially cheese aroma and of fruit aromas like strawberry.

In the processing of the dry cured ham, the intense action of lipase on the muscle and adipose tissue generated the free fatty acids which were transformed to volatiles as a result of the oxidation. Thus, lipase was utilized for the enhancement of the flavour quality (Toldra and Flores, 1998). Lipase catalyzed interesterification under the controlled condition was adapted to enrich the naturally occurring oils and fats which lacked well balanced fatty acids. Such blended oils are also enriched in the neutraceuticals.

Flavour Development

The main advantages of the enzyme catalyzed flavour generation are high selectivity or specificity of the reaction, high reaction rate even at low molar fractions, environmentally compatible and mild reaction conditions, and if viable cells are used there is a possibility to perform the multistep synthesis. Nowadays, this specific application meets the consumers demand for the natural products rather than synthetic products. Hexyl acetate, a short chain ester with fruity odor is a significant green non-flavour compound widely used in food industry (Shieh and Chang, 2001). Hexyl butyrate synthesized by the immobilized lipase (Lipozyme IM-77) from *Rhizomucor miehei* was employed as flavour and fragrance in the food, beverage and pharmaceutical industry. It was synthesized by lipase catalyzed mild tranesterification of hexanol and tirbutrin. It found enormous interest as the natural flavouring compound rather artificial or synthetic (Chang et al., 2003).

Low molecular weight esters have a potential interest for the food industry as the flavor compounds. Direct esterification of citronellol and geraniol with short-chain fatty acids were catalyzed by the free lipase from *M. miehie*. High yields were obtained in n-hexane. Excess substrate addition, i.e. calculated amounts of acid addition resulted in 10% yield enhancement and 100% pure terpenyl esters (Laboret and Perraud, 1999). *M. miehei* lipase immobilized on the magnetic polysiloxane – polyvinyl alcohol particles by the covalent binding was employed for the synthesis of flavour esters using heptane as solvent. High activity of lipase was recovered and the ester synthesis was maximized for the substrates containing excess acyl donar (Bruno et al., 2004).

Added Value Products

Cheap oils can be upgraded to synthesize the nutritionally important structural triglycerols, such as, low caloric triacylglycerols, PUFA-enriched and oleic acid enriched oils after which the physical properties of natural oils can be altered for converting them into margarines and hard butter with higher melting points or into special low caloric spreads with short or medium chain fatty acids (Gupta et al., 2003). Stearine fractions obtained from the tallow were blended and interesterified with the liquid oils, such as the sunflower, soybean, rice bran, etc by the microbial lipase. The olein fractions were blended with the sal (Shorea robusta) fat, sal olein, and acidolysed with karanja (*Pongamia glabra*) stearine. The products formed were suitable for shortening the margarine fat bases and vanaspati substitute. This was confirmed by their slip melting point and solid fat index. *Rizomucor miehei* lipase and lipozyme IM20 were used for this study (Bhattacharyya et al., 1999). The interesterification of the commodity oils and fats for the production of the frying fats, shortening and margarine components were carried out by the immobilized 1,3 regio selective lipase (Lipozyme TL IM). A new immobilization technology, granulation was employed to obtain this food grade lipase in cost effective manner (Pearce et al., 2002) and batch and fixed bed reactors were discussed.

The structural triacylglycerols (ST) from the canola oil were produced by enzymatic acidolysis in a packed bed bioreactor. A commercially immobilized 1,3 specific lipase, lipozyme IM, from *Rhizomucor miehei* was used as the biocatalyst and caprylic acid as the acyl donor. The study showed that all the parameters had effects on the yields of the expected di-incorporated (dicaprylic) ST products (Xu et al., 2000). The structured lipids (SLS) containing the palmitic, oleic, stearic and linoliec acids, resembling human milk fat (HMF) were synthesized by the enzymatic acidolysis reactions between the tripalmitin, hazelnut oil fatty acids and stearic acid, commercially immobilized sn-1, 3-specific lipase lipozyme RM IM was used as biocatalyst. The SLS, thus produced have potential applications in infant formula (Sahin et al., 2005).

The enzymatic esterification can be adopted for the acylation of various bioactive compounds (vitamins, secondary metabolites, from the plants and microorganisms like kojic acid), which are useful in the pharmaceutical, fine chemical and feed industries. They own both the functionalities of the starting bioactive compound (e.g; vitamins) and modified properties such as the molecular weight, solubility, diffusion properties, stability and metabolization rate. Thus, the bioavailability of the bioactive compounds can be enhanced by raising the solubility of compound in fatty phase or membranes or by slowing the metabolisms. Novozym catalyzed condensation of the pyridoxine with lauric acid was examined as a model and lowric acid pyridoxine monoester with high retention time (4.6 min) was obtained as the product which was soluble in the vegetable oil with new potential application as cell vitality enhancer and protectant (Pelenc et al., 2003).

Lipase catalyzed interesterification has been applied particularly in the production of cocoa butter-type triacylglycerols and these processes exploit the 1, 3 regio specific lipases from the microbial sources (Macrae and Hammond, 1985). The interesterification by 1,3-regio specific lipases have been used to enrich the cheap fats such as palm-oil fractions in to 1, (3) polmitoyl, 2-oleoyl, 3(1) stearoyl glycerol and 1(3) steraroyl, 2-oleoyl 3(1) stearoylglycerol, which found immense application as the confectionary fats (Unilever, 1980). To increase the oleic acid content in the soybean oil, lipase catalyzed acidolysis was carried out with oleic acid in the organic solvent and a minimal modification of Sn-2 position fatty acid composition was obtained as a product. The immobilized lipase from *R. miehei* was employed to carryout the acidolysis (Cossignani et al., 2004).

Other Food Processing Industries

Lipases are utilized in the dairy industry to develop the characteristic flavour of the cheese. The fatty acid esters of the hydroxy acids such as the lactic and citric acids and alkyl lactates constitute a very interesting group of the surfactants in the food industry. They are widely used in the manufacture of cereal products such as yeast-raised baked bread, cakes, doughnuts, noodles and puddings; dairy products such as ice cream, coffee whiteners, liquid and dry whipped toppings and also as the bactericidal and fungicidal agents (Kiran and Divakar, 2001). The ability of lipases to discriminate between the enatiomers of the racemic substrates or enantiotopic groups in the prochiral compounds makes them valuable assets in the preparation of optically pure compounds. The esters of the fatty acids serve as natural aroma components in the food industry. Ethyl butyrate and isoamyl acetate are respectively found in the aroma of strawberry and banana. Natural esters from the butter tirglycerides were obtained by the transesterification (Lagrand et al., 1988).

CONCLUSION

Lipases being a versatile enzyme are exploited in various industries, especially in the food processing industries. Lipase-mediated ester synthesis is the alternative technique to bulk chemical routes. Factors such as the nature of the solvent, water activity and temperature greatly influence the enzyme catalyzed synthesis. The enzyme-mediated production of the esters was found to be more cost effective than the chemical synthesis. Lipase finds wide application in the food processing industries. The esters, which could be obtained from lipase mediated biotransformation processes, better serve the food industry's needs. Although lipase catalyzed transesterification process has commercial significance, the utilization of the enzyme is not extensive because of its high cost. Increased understanding of the primary and 3D structure of lipase enzyme will pave the way for the variety of different applications. The future developments in low cost production and purification technologies would lower the cost of these enzymes for the increased applications. The immobilized lipase offers high chemical, mechanical, thermal stability, improved enantioselectivity and sterioselectivity. Various novel reactor designs with this immobilized enzyme would offer effective and efficient bioconversion using lipase. The development of lipases with novel properties by the directed evolution and molecular technologies, strain improvement by the site directed mutagenesis and medium optimization for lipase overproduction hold a major area of research in the future.

RESUMO

Lipases são catalizadores industriais dos mais importantes, os quais catalizam a hidrólise de lipídeos. Também podem reverter a reação a um mínimo de atividade de água. Devido sua natureza flexível, é amplamente explorada para catalizar uma diversidade de reações de bioconversão como hidrólise, esterificação, interesterificação, alcoólise, acidólise e aminólise. A propriedade de síntese de esteres a partir de ácidos graxos e glicerol promoveu seu uso em várias sínteses de esteres. Os esteres sintetizados por lipases encontram aplicação em numerosos campos como a produção de biodiesel, resolução de drogas racêmicas, modificação de gorduras e lipídios, sintese de aromas, síntese de produtos farmacêuticos enantiopuro e nutracêuticos. As lipases possuem um papel crucial nas indústrias de processamento de alimentos, pois os processos não são afetados por subprodutos indesejáveis. Modificações nas lipases como revestimento tensoativo, impressão molecular, para permitir a síntese de esteres não aquosos também são reportados. Esta revisão trata da síntese de éster catalizada por lipase, estratégia de esterificação, condições ótimas e suas aplicações em indústrias de processamento de alimento.

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