Industrial Application of Microbial Lipases: A Review

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ABSTRACT

Commercial microbial lipases have been used in dairy and other food processes, and lipases produced in situ by microorganisms are important in making foods palatable and acceptable. Microbial lipases have been used in detergents, pharmaceuticals, cosmetics, leather processing, production of aliphatic acids, and in the treatment of domestic and industrial wastes. Manufacturers offer lipolytic enzymes in powder form free of other enzymes, and sometimes they are microencapsulated for specialized applications.

INTRODUCTION

This review paper attempts to show the importance of microbial lipases produced in situ in certain food products, as well as the application of lipase preparations to food processing, in detergent compositions, in pharmaceuticals, and cosmetics, and in miscellaneous processes. Some of the applications cited may not involve a microbial lipase, as the sources were not disclosed, but have, nevertheless, been included because a process type or interesting approach was described. No attempt has been made to provide an exhaustive survey of patent and other literature, but it is hoped that the review will provide a glimpse of what has been accomplished and that it will provide examples of the practical significance of this relatively untapped source of an important class of enzymes.

MICROBIAL LIPASES PRODUCED IN SITU IN FOODS

Lipases are elaborated by microorganisms present or growing in raw materials used in the production of certain foods. Localized production of microbial lipases in food systems is necessary for flavor development and textural changes to make the food palatable and acceptable.

Cheese Ripening

According to Stadhouders (1), fat hydrolysis during the ripening of Dutch (pasteurized milk) cheese is largely dependent upon microbial lipases produced in situ, because no lipase was detected in Dutch rennet, and milk lipase(s) was more heat sensitive than bacterial lipases. Moreover, lipase activity of bacterial origin was found in young cheese (a few days) and was expected to affect cheese flavors. In related work, it was shown by Driessen and Stadhouders (2) that *Alcaligenes viscolactis* and its exocellular lipase, associated with raw milk used for cheese making, can survive a pasteurization, heat treatment of 74 C for 10 sec. A more severe heat treatment of 92 C for 10 sec almost destroyed the bacterium, while its lipase was inactivated by heating to 90 C for 10 sec. It was significant that only the cheese prepared from the milk receiving the lower heat treatment was found, after six weeks incubation, to contain a normal complement of acidity.

It is significant, from the standpoint of cheese flavor development, that the lipase of a raw milk organism, such as *Pseudomonas fragi* (Nashif and Nelson [3]) withstood a heat treatment of 62 or 72 C for 30 min (pasteurization). Complete inactivation of the lipase required heating at 66 C for 1 hr and 71 C for 10 min (Lu and Liska [4,5]).

Lipolytic microorganisms often present in raw milk (6-8) include *Achromobacter lipolyticum, Pseudomonas fragi, P. fluorescens,* and *Achromobacter lipidis.* Pasteurization of the milk for cheese making will not inactivate completely their respective lipases, which produce fatty acids essential to the flavor of cheese.

Swiss cheese has a higher free fatty acid (FFA) content (9) than do most other semihard cheeses like gouda and cheddar. The most important difference between swiss and other semihard cheeses is that only the former requires propionic acid forming bacteria (*Propionibacterium*) to carry on the ripening process. These bacteria have been shown by Oterholm, et al., (9) to contain intracellular lipase, but no extracellular lipase was found.

Studies by Hosono (10) on lipases in limburger cheese yeasts provided evidence that these enzymes are involved in the maturation of surface ripened cheese. He found that *Candida mycoderma* and *Debaryomyces kloeckeri* produced lipases which were most active at pH 4.5 and showed activity up to pH 7.0. The lipase from *C. mycoderma* produced different amounts of myristic, palmitic, palmitoleic, stearic, and oleic acids from milk fat than did the *D. kloeckeri* lipase.

All blue veined cheeses are dependent upon *Penicillium roqueforti* and related varieties of this mold for flavor development. Imamura and Kataoka (11) have isolated both exo- and endocellular lipases from this organism and showed them to be largely responsible for the development of flavor. Eitenmiller, et al., (12) studied the exocellular lipase and found that it had pH and temperature optima of 8.0 and 37 C, respectively. Butter oil inhibited lipase production, but a 5% butter oil emulsion as substrate resulted in maximum lipolytic activity.

The predominant bacterial flora in a rennet cheese, such as cheddar, are the lactic streptococci, added to the milk during cheese making, and adventitious lactobacilli. Studies by Oterholm, et al., (13) indicate that lactic starter culture bacteria, i.e. *Streptococcus lactis* and *S. cremoris*, and lactobacilli produced intracellar esterases and lipases. They showed that these lipases are important in cheese ripening.

Vegetable Fermentation

Vegetables, such as cabbage, cucumbers, brussels sprouts, etc., may be fermented by lactic acid bacteria. Studies by Vorbeck, et al., (14) showed that the amount of FFA increased considerably in fermented cabbage and brussels sprouts, due to the lipase activity of one or more species of lactic acid bacteria. The lipase(s) liberated from the vegetable substrates having optimal activity at neutral pH was not considered important, because the rapid increase in hydrogen ion concentration would effectively limit their activity.

In a subsequent study by Pederson, et al., (15) it was found that marked changes occur in all lipid fractions during lactic fermentation of cucumbers. A nearly fourfold increase in FFA occurred in the pickle forming process. Increased amounts of linoleic and linolenic acids were found; and, while no caproic, caprylic, or capric acids were found in fresh cucumbers, these acids were present in pickles. These results were explained on the basis of fat hydrolysis by lipases which were formed by lactic acid bacteria during the 22 day fermentation period.

Meat Product Curing

In the Italian method of preparing hams, dry sodium chloride is the only chemical used for curing. It was found by Giolitti, et al., (16) that hams in which a lactic fermentation had taken place contained increased levels of volatile flavor compounds, including fatty acids, such as butyric, propionic, and myristic. It was concluded that bacterial lipases were involved in flavor development and

curing through the production of these fatty acids.

In another study Cantoni, et al., (17) observed that, during the ripening of dry Italian sausages, lipolytic *Micrococcaceae* and *Lactobacilleae* play an important role in flavor development. Certain *Micrococcus* species were found to be especially lipolytic to pork fat and numerous volatile and nonvolatile fatty acids were detected in ripened sausages. Actual enzyme activity determinations during the ripening of Italian sausage were made by Caserio and Gervasini (18) over a 70 day period. Lipase activity detected during the ripening process was thought to be of microbial origin.

Fish Processing Using Lipolytic Fungi

A resourceful approach to removing excessive fat from menhaden fish was demonstrated by Burkholder, et al. (19). He found that two microorganisms, *Candida lipolytica* and *Geotrichum candidum,* reduced the lipid content of this fatty fish (Table I) and significantly increased the protein content of the final product. Both organisms elaborated lipases whose yield could be controlled by adjustment of growth conditions. This approach had the added advantage of providing a product having pleasant and appealing flavor characteristics.

TABLE I

Reduction of the Fat Content of Menhaden Fish by Cultivation of *Geotrichum candidum* on Whole Fish Medium[a]

Incubation time	Mature fish (percent fat)	Immature fish (percent fat)
0	41.8	9.9
24	37.4	8.8
48	36.8	8.0
72	36.5	6.9
96	33.7	6.5

[a]The growth medium (pH 7.0) contained ca. 6.25% whole ground lyophilized fish. Incubation was carried out at 30 C on a rotary shaker at 140 revolutions/min. This information was obtained from L. Burkholder, et al. (19).

MICROBIAL LIPASE PREPARATIONS APPLIED IN FOOD PROCESSING

As is already evident, lipases have played an important role in fermentation processes or other methods of preparing foods where microorganisms are not excluded. It was not until recently, however, that lipase preparations have gained commercial significance.

Lipase production on a commercial scale has been made possible by important advances in industrial microbiology which have overcome problems of strain selection and production scale-up. However, manufacturers have not disclosed details of their processes, and their mutant cultures are guarded carefully.

Lipase Preparations Used by the Dairy Industry

Traditionally most lipases applied to dairy manufacturing processes have been derived from animal, rather than microbial, sources. The classical example is the use, particularly in Italy, of lipase containing rennet paste, prepared from the stomachs of young suckling calves, kids, or lambs. This material is used in the manufacture of Romano, Mozzarella, and other Italian cheeses. During the last two decades, preparations of purified lipases from these sources have been made commercially available, and much work has been done in standardizing these preparations and studying their effects upon milk fat and other lipids (20,21).

Much research on the role of lipases of microbial origin on flavor production in dairy products, especially cheese, recently has been done (22). Working with an *Aspergillus* lipase preparation in 1957, Harper observed that this enzyme had greater specificity for fatty acid esters of chain length below C-12 than did pancreatic lipase. This lipase might, therefore, have value for the production of volatile flavor, inducing acids from milk fat.

Three years later, Shahani (12) observed that the lipase of a related mold, *Penicillium roqueforti,* was most specific for tributyrin, hydrolyzing tricaprylin, tricaproin, tripropionin, and triolein in decreasing order. Thus, the *Penicillium roqueforti* lipase preferentially hydrolyzed volatile, short chain fatty acids of importance to flavor.

Process for preparation of a cheese-like product: A process for the rapid manufacture of a cheese-like product was described in a U.S. patent (23) issued to the Pillsbury Co. A lipase preparation from *Penicillium roqueforti* was used to lipolyze butter fat, emulsified with a lactic fermented, condensed skim milk.

Process for preparation of a Japanese yogurt: A Japanese patent (24) describes a process in which lipase RH (10,000 u/g) prepared from *Rhizopus delemar* was added (0.005-0.5%) at the beginning of the fermentation for yogurt production. The yogurt product, fermented with *Lactobacillus acidophilus* in the presence of the lipase, assumed an improved odor devoid of a slight unclean or fishy smell sometimes associated with Japanese dairy products. In addition, the fermentation rate was accelerated by 33% if lipase was used. Also, the lipase gave the yogurt a more butter like aroma when *Lactobacillus bulgaricus* was used in the fermentation.

Microbial lipase produced flavors from milk fat: In a review paper, Higashi (25) described the utilization, production, purification, and properties of microbial lipases. He referred to microbial lipases used for lipolyzing milk fat for flavoring chocolate, ice cream, margarine, and other food products.

The Tanabe Seiyaku Co., which manufacturers the *Rhizopus delemar* lipase RH, claimed (26) that their enzyme has been used for enhancing flavors of dairy products, such as milk, butter, cheese, etc. For production of butter flavors, they suggest the addition of lipase RH to fresh milk or cream at concentrations of 0.05-0.2% (w/w) followed by incubation at 40 C for 2-5 hr. The reaction mixture then is heated to inactivate the enzyme and spray-dried.

Improvement of cheese flavor: A U.S. patent (27) which describes a process for preparing cheddar cheese from heated milk has been issued to the Kraftco Corp. Since lipase producing microorganisms and their lipases were destroyed by heat treatment, the milk was supplemented with a lipase (undisclosed source) at the rate of 0.259 g/1000 lb milk.

A fungal lipase, presumably lipase B, produced by the Rohm and Haas Co. has been used for improving the flavor of cheese (28). Similarly, Peters and Nelson (29) reported that the addition of *Candida lipolytica* lipase improved the quality of blue cheese made from unhomogenized, raw or pasteurized milk.

Lipase Preparation Used by Other Food Industries

Process for refinement of rice flavor: A Japanese patent (30) describes a process in which rice is treated with an aqueous-organic solvent solution of *Aspergillus* lipase AP. Following lipolysis the rice is treated with an oxidizing agent resulting in a rice product having improved odor and taste.

Process for modifying soybean milk: Lipase treatment of soybeans after cooking and mashing, but prior to other processing steps, was described in a Japanese patent (31). The enzyme prepared from an *Aspergillus* species apparently is not commercially available.

Process for preparation of smoked carp: A Japanese

patent (32) describes a process where carp is first steeped in a brine solution for ca. 2 weeks and then in a solution of lipase for 4-5 hr before the fish are smoked.

Process for flavor improvement of alcoholic beverage: Tanabe Seiyaku Co. (33) uses a lipase (0.001-0.5%) produced from *Rhizopus delemar* or a *Candida* species during fermentation in the preparation of an apple wine. This treatment improved the aroma and accelerated the alcoholic fermentation. If lipase was added 24 hr after inoculation with *Saccharomyces cerevisiae,* a yield of 9.10 g alcohol/100 ml wine was obtained compared to a value of 7.90 g/100 ml in the control batch without lipase.

Process for improvement of whipping quality of egg whites: Lipase RH (26) has been used for treating egg whites before drying to enhance whipping qualities. Minute amounts of lipids from traces of egg yolk have an adverse effect upon whipping properties and are removed effectively by the lipase treatment.

MICROBIAL AND OTHER LIPASE PREPARATIONS USED IN DETERGENTS

In the last decade the market for enzymes, such as proteases, amylases, and lipases in detergent products has increased greatly. However, this growth has been tempered, especially because of a mishap which occurred in a British detergent factory in 1969 (34) where several workers inhaled concentrated enzyme dust causing respiratory reactions.

Proteases, rather than lipases, have been considered most important in detergents; but, as should be evident from the following examples, lipase preparations are important.

Lipase-MY Containing Detergents

A detergent composition for removing proteins at 70-100 C has been described in a German patent (35) issued to Henkel and Cie, G.m.b.H. The composition contained alkalase (Novo Enzyme Corp.), protease (0-1.0%), *Candida cylindracea* (Lipase-My-Meito Sangyo Co.) (0.05%), Termozym (Novo Enzyme Corp.), α-bakterialamylase (0.1-1.0%), and the high temperature-resistant protease, thermolysin (0-1.0%), in various combinations. The total enzyme activity was 285-1275 proteolytic enzyme (PE)/g detergent composition.

A patent (36) recently issued to the Unilever Co. describes a cleaning composition in which a lipase of *Candida cylindracea* (0.5-5% by wt) is mixed with a condensate of 3-10 ethylene oxide and 1 mole nonylphenol, C_{11}-C_{15} secondary alcohol or a similar compound and builders, etc. The condensate has no adverse effect upon activity of the enzyme and increases its activity in many cases. Enzymes from microbial sources (*Candida lipolytica, Candida cylindracea,* and *Pseudomonas stutzeri* American Type Culture Collection 19154) are preferred over those obtainable from plant or animal sources, because they are more suitable for incorporation in the detergent system.

Lipase Containing Detergents and Washing Agents

A German patent (37) to Henkel and Cie, G.m.b.H., describes the use of a microbial lipase and other microbial enzymes, i.e. proteases and amylases, to increase general detergency of formulations based upon poly (sulfimide esters). It was suggested that this product could be used in general formulations for soaking or washing to replace or reduce the amounts of other surfactants. The enzyme preparations were derived from *Bacillus subtilis* or *Streptomyces griseus.*

A detergent product whose efficiency was increased if a lipase was added in sufficient concentration, i.e. 5-500 I units/g, was described in a German patent (38) to Henkel and Cie, G.m.b.H.

Enzyme Containing Cleaning Agents for Use in Dishwashing Machines

A French patent (39) describes a cleaning agent composition consisting of enzymes together with a nonfoaming surfactant to be added to the rinse water of dishwashing machines. Lipases and proteases were included to assist in removing fat and protein films, and amylases were added to aid in the removal of starch films. In a similar product to be used as a detergent for dishwashers, a French patent (40) disclosed that the composition included complex enzyme mixtures of microbial sources, such as yeast, higher fungi, and bacteria. The enzymes included amylases, proteases, and lipases; and those obtained from *Bacillus subtilis* were particularly advantageous because of their resistance to basic conditions and temperatures 45-70 C.

LIPASE PREPARATIONS USED IN PHARMACEUTICALS AND COSMETICS

Manufacturers of microbial lipases (Table II) have suggested that their enzymes may be used in pharmaceutical or cosmetic products. It would appear, therefore, that at least some of these products have been used in commercial products of this type, particularly in Japan.

Cosmetics Containing Lipase Preparations

A German patent (41) intended for use in combating local skin tissue inflammation, describes cosmetic and pharmaceutical preparations containing 1-5% by wt of lipase (source undisclosed), hyaluronidase, and thiomucase.

A German patent (44) describes a process in which a lipase was added to a permanent hair waving composition to promote penetration of the preparation into the hair.

Pharmaceuticals Containing Lipase Preparations

Creams for topical application to defat human skin have been described in a German patent (42). The compositions included 500 international units lipase (source undisclosed), 10 parts poly vinyl pyrrolidone, and 1 part leucylglycyl glycine as enzyme stabilizers, water, emulsifier, and ointment bases. Small amounts of bovine bile also helped to stabilize the enzyme.

The Kaken Chemical Co. of Japan produces a digestive preparation which includes a lipase. An encapsulated granular product was evaluated and found satisfactory as a digestive aid in an artificial gastrointestinal model (43).

The Trancoa Chemical Corp. was granted a French process patent (45) which describes a method for coating solid tablets or liquid containing capsules. The coating consists of a fat and a lipase which assists in rapid assimilation of the drug from the digestive tract.

LIPASE PREPARATIONS IN MISCELLANEOUS INDUSTRIAL PROCESSES

It seems probable that one of the first attempts to understand and utilize enzymes industrially was in the tanning of hides. Thus, it was shown by Wood and Law (46) that, in the treatment of hides during tanning, the process known as bating was made possible by the action of enzymes produced by bacteria. They discovered that lipase and other enzymes were developed by bacteria in the manure applied to the hides in the bating process to remove fats and protein debris not removable by liming.

Lipase Treatment of Hides

Lipase treatment still appears to be useful for leather processing, which is described in a Hungarian Teljes patent (47). In this process, sheep, lamb, and wild animal skins were treated with lipase and amylase in the presence of deoxycholic acid as catalyst to remove lipids and noncol-

TABLE II

Commercial Microbial Lipases

Manufacturer	Enzyme name	Microbial source	Properties	
			pH optimum	Temperature, C optimum
Amano Pharmaceutical	Lipase-AP	*Aspergillus* species	6.5	37
Amano Pharmaceutical	Lipase-MAP	*Mucor* species	7.0	37
Meito Sangyo	Lipase-MY	*Candida cylindracea*	6.5	37
Nagase	Lipase-Saiken	*Rhizopus* species	7.0	40
Rohm and Haas	Lipase B	*Aspergillus niger*	6.0	47.5
Toyo Jozo K.K.	Lipase Toyo	*Chromobacterium viscosum*, variation *paralipolylicum*		
Wallerstein	Lipase 3500	*Aspergillus oryzae*	6.25	30
Tanabe Seiyaku	Lipase RH	*Rhizopus delemar*	5.6	45
Osaka Saikin Kenkyu-Sho K.K.	Lipase-Saiken, Olipase	*Rhizopus* species		

lagenous proteins. No apparent damage was done to the skin.

Preparation of Aliphatic Acids from Dark and High Acids Value Oils and Fats

A Japanese patent (48) issued to the Nisson Oil Mills describes a process in which a lipase (undisclosed source) was used to hydrolyze oils and fats in the presence of a reducing agent to make high grade aliphatic acids.

Enzymes in Chewing Gum and Dentifrices

A U.S. patent (49) issued to the American Chicle Co. describes the use of fungal enzyme products at the rate of 0.1-6.0% in this type of product. A preparation by the Rohm and Haas Co. from an *Aspergillus* species, Rhozyme P-11, containing lipase, protease, and amylase activities, appeared to be effective in improving oral cleanliness.

Lipase Composition for Use in Sewage Treatment

The manufacturer of lipase-MY (50) has indicated that this *Candida* enzyme has been used in the U.S. in sewage disposal plants for treatment of sewage in large buildings and hotels and for cleaning sewer pipes.

FORMS IN WHICH LIPASE PREPARATIONS HAVE BEEN OFFERED FOR COMMERCIAL APPLICATIONS

A number of lipase products can be obtained as tan or whitish colored powders (Table II) free of proteolytic, amylolytic, or cellulytic activities. Enzyme activity assays often are carried out using olive oil emulsified and stabilized with polyvinyl alcohol as the substrate. The enzyme is then frequently standardized using lactose as a diluent. It is apparent from the literature, however, that not all applications call for, or can use, the powder form of the enzyme.

Encapsulation of Enzymes

The Fuji Photo Film Co. (51,52) has been issued two patents describing a process for the microencapsulation of porcine pancreatic and microbial lipases with a storage stability of 3 months. These patents disclosed a process using silicone dioxide or powdered dextran in a binder solution of ethylene maleic anhydride copolymer or dextrin. Thus, a solution of 20 g copolymer in 100 ml methanol-acetone was mixed with a dispersion of 100 g powdered lipase and 80 g powdered dextran in 800 ml acetone, atomized at a gauge pressure of one atmosphere and a temperature of 40-70 C with 1.3 m^3 hot air/min, to give 130 g capsules of 20-10 μ size.

The microbial lipase was supplied by the Toyo Brewing Co., which manufactures Lipase Toyo, type S, for industrial use and was found compatible for use in detergents when microencapsulated. The enzyme appears to have been derived from a special strain of *Chromobacterium viscosum*, variety *parolipolyticum*.

The effect of microencapsulation upon the enzyme activity of lipase and other enzymes was investigated by Kitajima, et al. (53). The enzymes were enclosed in small, semipermeable capsules of polystyrene, silicone derivatives, and ethyl cellulose. Microencapsulation of the enzymes was performed in an aqueous medium containing the lipase or another enzyme and a solution of synthetic polymer in benzene at 35-40 C. The capsules prepared under these conditions had diameters of several hundred μ.

It was found when encapsulated that the lipase did not attack large substrates, such as olive oil and Tween 20, presumably because of the limited permeability of such substrates through the capsule wall.

Lipase and Oxidase Containing Bran Product

A U.S. patent (54) describes a process for preparing an enzyme by cultivation of an *Aspergillus oryzae* on a bran medium fortified with vegetable oil sludge, mineral salts and H_2O_2, or peroxide salts. The lipase-rich product is simply dried at 30-40 C, finely ground, and added to condiments.

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