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Qureshi Mehar Afroz
Dairy Chemist, Dairy Chemistry, College of Dairy Science and Food Technology, C.G.K.V., Raipur, C.G., India

Khare Archana Khan
Prof. & Head, Dairy Chemistry, College of Dairy Science and Food Technology, C.G.K.V., Raipur, C.G., India

Pervez Ahmed
Ph. D Scholar, SoS Life Science, Pt. Ravishankar Shukla University, Raipur C.G., India

Uprit S
Dean, College of Dairy Science and Food Technology, C.G.K.V., Raipur, C.G., India

Correspondence
Qureshi Mehar Afroz
Dairy Chemist, Dairy Chemistry, College of Dairy Science and Food Technology, C.G.K.V., Raipur, C.G., India

Enzymes used in dairy industries

Qureshi Mehar Afroz, Khare Archana Khan, Pervez Ahmed, Uprit S

Abstract
An enzyme is a protein formed by the body that acts as a catalyst to cause a certain desried reaction. Enzymes are very specific. Each enzyme is designed to initiate a specific response with a specific result. There are many enzymes in the human body. In cystic fibrosis, the term enzyme generally refers to the enzymes created by the pancreas that are intended to initate a reaction that digests food. Dairy enzymes are enzymes used for the production of cheese and yoghurt as well as other milk products. The function of these enzymes varies widely from coagulants, which are used to make cheese, to bioprotective enzymes to enhance the shelf life and safety of dairy products. The application of enzymes (proteases, lipases, esterases, lactase, and catalase) in dairy technology is well established. Rennets (rennin, a mixture of chymosin and pepsin obtained mainly from animal and microbial sources) are used for coagulation of milk in the first stage of cheese production. Proteases of various kinds are used for acceleration of cheese ripening, for modification of functional properties and for modification of milk proteins to reduce the allergenic properties of cow milk products for infants. Lipases are used mainly in cheese ripening for development of lipolytic flavors. Lactase (-galactosidase, EC 3.2.1.23) is used to hydrolyze lactose to glucose and galactose as a digestive aid and to improve the solubility and sweetness in various dairy products.

Keywords: Enzymes, dairy, industries

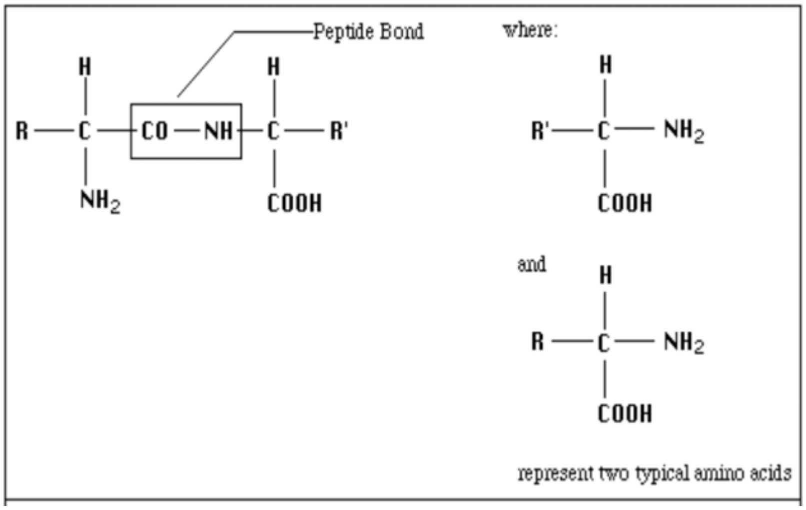
Introduction
Indians are known to be lovers of milk and its products. As a sequel to white revolution, India has surged ahead to become the largest milk producer in the world, the production figure touched to 110 million tons for the year 2010. With growing urbanization, demand for processed dairy foods has increased considerably, in particular demand for different cheese varieties, and low-lactose milk due to increasing intolerance of human beings to lactose in milk and other milk products. For improving the quality of milk and milk products, a number of different enzymes from microbial as well as from non-microbial sources have potential applications in dairy processing. India being the highest producer of milk in the world, and consequently the surplus availability of milk in our country has triggered the food and dairy industry to convert the liquid milk into value-added products using biochemical and enzymatic processes.

An enzyme is a protein formed by the body that acts as a catalyst to cause a certain desried reaction. Enzymes are very specific. Each enzyme is designed to initiate a specific response with a specific result. There are many enzymes in the human body. In cystic fibrosis, the term enzyme generally refers to the enzymes created by the pancreas that are intended to initate a reaction that digests food.

Natural proteins produced in tiny quantities by all living organisms (bacteria, plants, and animals) and functioning as highly selective biochemical catalysts in converting one molecule into another. Enzymes are essential to life because they speed up metabolic reactions to a very great extent, but do not undergo any change in themselves. In the industry they are used for degrading oil spills and wastes into harmless compounds, in cleaning fat (food) stains, and in fermentation processes to make alcoholic beverages. Enzymes are very sensitive to environmental conditions and function best only within a narrow range of temperature and acidity (pH) levels. In nutrition, enzymes are substances that break down carbohydrates, proteins and fats for digestion and absorption. Digestive enzymes include lipase that breaks down fats, amylase that breaks down sugars and carbohydrates, and protease that breaks down proteins.

Chemical Nature of Enzymes
All known enzymes are proteins. They are high molecular weight compounds made up principally of chains of amino acids linked together by peptide bonds. See Figure 1. Enzymes can be denatured and precipitated with salts, solvents and other reagents. They have molecular weights ranging from 10,000 to 2,000,000.
Many enzymes require the presence of other compounds - cofactors - before their catalytic activity can be exerted. This entire active complex is referred to as the holoenzyme; i.e., apoenzyme (protein portion) plus the cofactor (coenzyme, prosthetic group or metal-ion-activator) is called the holoenzyme.

Apoenzyme + Cofactor = Holoenzyme



(**Source:**www.worthingtonbiochem.com/introBiochem/introEnzymes.html)

Fig 1: Typical Protein Structure

Types of Enzymes
There are so many enzymes that it would be impossible to name them all. In fact, scientists have yet to discover many enzymes, or fully understand their structure and properties. On the other hand, many other enzymes have been successfully studied and applied to industrial and commercial uses. A few basic enzyme types are briefly described as follows

Type	Enzyme Function
Cellulase	Breaks down cellulose, a fibre found in the cell walls of all plants and trees. Cellulose is the basic raw material used to make products such as paper, cotton, and other textiles
Hemicellulase	Breaks down hemicellulose, another plant sugar that is not as complex as cellulose and is easier to break down
Xylanase	Breaks down xylan, a gummy sugar present in the cell walls of plants and trees. This enzyme type is used primarily in the wood and pulp industry
Amylase	Breaks down starches and other carbohydrates into basic sugars
Protease	Breaks down proteins
Lipases	Breaks down fats

(**Source:**http://www.bioportal.gc.ca/ENGLISH/BioPortalHome.asp?x=1)

The use of rennet in cheese manufacture was among the earliest applications of exogenous enzymes in food processing, dating back to approximately 6000 B C. The use of rennet, as an exogenous enzyme, in cheese manufacture is perhaps the largest single application of enzymes in food processing. In recent years, proteinases have found additional applications in dairy technology, for example in acceleration of cheese ripening, modification of functional properties and preparation of dietic products (Stroh, 1998) [16]. Principal among some enzymes that have important and growing applications are lipases and b-galactosidases. Enzymes with limited applications include glucose oxidase, superoxide dismutase, sulphydryl oxidase, etc.

Enzymes used in Dairy Industries
The application of enzymes (proteases, lipases, esterases, lactase, and catalase) in dairy technology is well established. Rennets (rennin, a mixture of chymosin and pepsin obtained mainly from animal and microbial sources) are used for coagulation of milk in the first stage of cheese production. Proteases of various kinds are used for acceleration of cheese ripening, for modification of functional properties and for modification of milk proteins to reduce the allergenic properties of cow milk products for infants. Lipases are used mainly in cheese ripening for development of lipolytic flavors. Lactase (-galactosidase, EC 3.2.1.23) is used to hydrolyze lactose to glucose and galactose as a digestive aid and to improve the solubility and sweetness in various dairy products. Many people do not have sufficient lactase to digest milk sugar. Lactose hydrolysis helps these lactose-intolerant people to drink milk and eat various dairy products. -Galactosidases from K. fragilis, A. niger, or A. oryzae are inhibited by galactose. Immobilized enzyme systems are used to overcome this inhibition problem and to lower the cost of lactase use. The cheese manufacturing industry produces large quantities of whey as a byproduct of which lactose represents 70–75% of the whey solids. The hydrolysis of lactose by lactase converts whey into more useful food ingredients. Lactases have also been used in the processing of dairy wastes and as a digestive aid taken by

humans in tablet form when consuming dairy products. Hydrogen peroxide is used as an effective chemical sterilant for the treatment of raw milk as an alternative to pasteurization with heat. Catalase (EC 1.11.1.6), which catalyzes the decomposition of hydrogen peroxide, is used at the end of the process to remove the remaining peroxide. Bitter off-flavors that develop in ripened cheese as it matures are due to the formation of bitter flavored peptides from milk proteins. Peptidases can break down the bitter peptides as they are formed and thus help maintain the traditional flavor of cheese (https://imtech.wikispaces.com).

Table 1: Sources of Industrially Important Enzymes

Enzyme	Source	Applications
Amylases	*Bacillus* and *Aspergillus* spp.	Starch liquefaction, baking, brewing, textiles, detergents, etc.
Beta-Glucanases	Bacillus spp.	Brewing and animal feedstuff
Bromelain	Pineapple	Meat tenderization, chill-proofing of beer
Cellulases	*Trichoderma* spp.	Textile biopolishing, pulp and paper, detergents
Chymosin	Calf stomach	Cheese manufacture
Ficin	Figs	Meat Tenderization
Glucose isojmerase	*Bacillus* and *Streptomyces* spp.	Glucose isomerization to fructose
Lipases	Pseudomonas spp.	Detergents, oils and fats, baking, leather, paper, etc.
Papain	Papaya latex	Meat tenderization, brewing
Pectinases	*Aspergillus* spp.	Pectin hydrolysis in fruit juice clarification
Proteases	*Bacillus* and *Aspergillus* spp.	Detergents, brewing, meat tenderization, baking, bone cleaning, hydrolyzed animal proteins, functional meat proteins, etc.
Pepsin	Stomach of slaughtered animals	Digestive aid
Transglutaminases	*Streptomyces* spp.	Protein cross-linking and gelation and meat binding.
Trypsin	Stomach of slaughtered animals	Digestive aid.

(Source:www.emc.maricopa.edu/facultyfarabee/biobk/BioBookEnzym.html)

Microbial rennets in dairy applications

Animal rennet (bovine chymosin) is conventionally used as a milk-clotting agent in dairy industry for the manufacture of quality cheeses with good flavour and texture. Owing to an increase in demand for cheese production worldwide coupled with reduced supply of calf rennet, has therefore led to a search for rennet substitutes, such as microbial rennets (Stanley, 1998) [15].

Rennin acts on the milk protein in two stages, by enzymatic and by non-enzymatic action, resulting in coagulation of milk. In the enzymatic phase, the resultant milk becomes a gel due to the influence of calcium ions and the temperature used in the process. Many microorganisms are known to produce rennet-like proteinases which can substitute the calf rennet. Microorganisms like Rhizomucor pusillus, R. miehei, Endothia parasitica, Aspergillus oryzae, and Irpex lactis are used extensively for rennet production in cheese manufacture.

Different strains of species of Mucor are often used for the production of microbial rennets. Whereas best yields of the milk-clotting protease from Rhizomucor pusillus are obtained from semisolid cultures containing 50% wheat bran, R. miehei and Endothia parasitica are well suited for submerged cultivation. Using the former, good yields of milk-clotting protease may be obtained in a medium containing 4% potato starch, 3% soybean meal, and 10% barley. During growth, lipase is secreted together with the protease. Therefore, the lipase activity has to be destroyed by reducing the pH, before the preparation can be used as cheese rennet.

Lactase enzyme

Popularly known as lactase, beta-galactosidases are enzymes classified as hydrolases. They catalyze the terminal residue of b-lactose galactopiranosil (Galb1 - 4Glc) and produce glucose and galactose (Carminatti, 2001). Lactose, the sugar found in milk and whey, and its corresponding hydrolase, lactase or b -galactosidase, have been extensively researched during the past decade. This is because of the enzyme immobilization technique which has given new and interesting possibilities for the utilization of this sugar. Because of intestinal enzyme insufficiency, some individuals even a population, show lactose intolerance and difficulty in consuming milk and dairy products. Hence, low-lactose or lactose-free food aid programme is essential for lactose-intolerant people to prevent severe tissue dehydration, diarrhea, and, at times, even death.

Another advantage of lactase-treated milk is the increased sweetness of the resultant milk, thereby avoiding the requirement for addition of sugars in the manufacture of flavoured milk drinks. Manufacturers of ice cream, yoghurt and frozen desserts use lactase to improve scoop and creaminess, sweetness, and digestibility, and to reduce sandiness due to crystallization of lactose in concentrated preparations. Cheese manufactured from hydrolysed milk ripens more quickly than the cheese manufactured from normal milk.

Technologically, lactose crystallizes easily which sets limits to certain processes in the dairy industry, and the use of lactase to overcome this problem has not reached its fullest potential because of the associated high costs. Moreover, the main problem associated with discharging large quantities of cheese whey is that it pollutes the environment. But, the discharged whey could be exploited as an alternate cheap source of lactose for the production of lactic acid by fermentation. The whey permeate, which is a by-product in the manufacture of whey protein concentrates, by ultrafiltration could be fermented efficiently by Lactobacillus bulgaricus.

Lactose can be obtained from various sources like plants, animal organs, bacteria, yeasts (intracellular enzyme), or molds. Some of these sources are used for commercial enzyme preparations. Lactase preparations from A. niger, A. oryzae, and Kluyveromyces lactis are considered safe because these sources already have a history of safe use and have been subjected to numerous safety tests. The most investigated E. coli lactase is not used in food processing because of its cost and toxicity problems. The remarkable interest in Aspergillus niger, a species of great commercial interest with a highly promising future and already widely applied in modern biotechnology, is due to its several and diverse reactions (Andersen *et al*, 2008) [2]. Moreover, A. niger not only produces various enzymes but it is one of the few species of the fungus kingdom classified as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA). The species is used in the production of enzymes, its cell mass is used as a component in animal feed and its fermentation produces organic acids and other compounds of high economic value (Couto and Sanroman, 2006) [4].

Microbial enzymes in accelerated cheese ripening

Cheese ripening is a complex process mediated by biochemical and biophysical changes during which a bland curd is developed into a mature cheese with characteristic flavour, texture, and aroma. The desirable attributes are produced by the partial and gradual breakdown of carbohydrates, lipids, and proteins during ripening, mediated by several agents, viz. (i) residual coagulants, (ii) starter bacteria and their enzymes, (iii) nonstarter bacteria and their enzymes, (iv) indegenous milk enzymes, especially proteinases, and (v) secondary inocula with their enzymes.

Proteolysis occurs in all the cheese varieties and is a prerequisite for characteristic flavour development that can be regulated by proper use of the above agents. Cheese ripening is essentially an enzymatic process which can be accelerated by augmenting activity of the key enzymes. This has the advantage of initiating more specific action for flavour development compared to use of elevated temperatures that can result in accelerating undesirable nonspecific reactions, and consequently off flavour development.

Enzymes may be added to develop specific flavours in cheeses, for example lipase addition for the development of Parmesan or Blue-type cheese flavours. Attempts to accelerate the multiple secondary flavour-forming-reactions, e.g. Strecker degradation, have been scarce. The pathways leading to the formation of flavour compounds are largely unknown, and therefore the use of exogenous enzymes to accelerate ripening is mostly an empirical process.

Proteolytic enzymes of lactic acid bacteria in fermented milk products

The proteolytic system of lactic acid bacteria is essential for their growth in milk, and contributes significantly to flavour development in fermented milk products. The proteolytic system is composed of proteinases which initially cleaves the milk protein to peptides; peptidases which cleave the peptides to small peptides and amino acids; and transport system responsible for cellular uptake of small peptides and amino acids. Lactic acid bacteria have a complex proteolytic system capable of converting milk casein to the free amino acids and peptides necessary for their growth. These proteinases include extracellular proteinases, endopeptidases, aminopeptidases, tripeptidases, and proline-specific peptidases, which are all serine proteases. Apart from lactic streptococcal proteinases, several other proteinases from nonlactostreptococcal origin have been reported. There are also serine type of proteinases, e.g. proteinases from *Lactobacillus acidophillus, L. plantarum, L. delbrueckii sp. bulgaricus, L. lactis, and L. helveticus*. Aminopeptidases are important for the development of flavour in fermented milk products, since they are capable of releasing single amino acid residues from oligopeptides formed by extracellular proteinase activity.

Other dairy enzymes

Other enzymes used for dairy food application include: Proteases to reduce allergic properties of cow milk products for infants, and lipases for development of lipolytic flavours in speciality cheeses.

The functional properties of milk proteins may be improved by limited proteolysis through the enzymatic modification of milk proteins. An acid-soluble casein, free of off flavour and suitable for incorporation into beverages and other acid foods, has been prepared by limited proteolysis. The antigenicity of casein is destroyed by proteolysis, and the hydrolysate is suitable for use in milk-protein-based foods for infants allergic to cow milk. Recently proteases represent 60% of industrial enzymes on the market, whereas microbial proteases, particularly fungal infections, are advantageous because they are easy to obtain and to recover. An enzyme extract (Neves-Souza, 2005), which coagulates milk and which is derived from the fungus Aspergillus niger var. awamori, is already produced industrially (Silva, 2007) [14].

Lipolysis makes an important contribution to swiss cheese flavours, due mainly to the lipolytic enzymes of the starter cultures. The characteristic peppery flavour of Blue cheese is due to short-chain fatty acids and methyl ketones. Most of the lipolysis in Blue cheese is catalysed by Penicillium roqueforti lipase, with a lesser contribution from indigenous milk lipase. Lipolytic enzymes such as lipases and esterases are an important group of enzymes associated with the metabolism of lipid degradation. Lipase-producing microorganisms such as Penicillium restrictum may be found in soil and various oil residues. The industries Novozymes, Amano and Gist Brocades already employ microbial lipases (Alkan *et al*., 2007) [1].

The NOVO process for production of enzyme modified cheese (EMC) uses medium-aged cheese which is emulsified, homogenized, and pasteurized, after which 'palatase' (a lipase from R. miehei) is added, with or without a proteinase, and the blend is ripened at a high temperature for one to four days. The mixture is reheated, a paste results which is suitable for inclusion in soups, dips, dressings, or snack foods. EMC technology has been developed to produce a range of characteristic cheese flavours and flavour intensities, for example swiss, blue, cheddar, provolo-nemor or romano, suitable for inclusion at low levels in many products[27]. The claims that exogenous enzymes are effective in accelerating ripening have not led to their wide-spread use, possibly due to their high cost, difficulties in distributing them uniformly in the curd, and the possible danger of over-ripening the cheese.

The other minor enzymes having limited applications in dairy processing include glucose oxidase, catalase, superoxide dismutase, sulphydryl oxidase, lactoperoxidase, and lysozymes. Glucose oxidase and catalase are often used together in selected foods for preservation. Superoxide dismutase is an antioxidant for foods and generates H_2O_2, but is more effective when catalase is present. Thermally induced generation of volatile sulphydryl groups is thought to be responsible for the cooked off-flavour in ultra-high temperature (UHT) processed milk. Use of sulphydryl oxidase under aseptic conditions can eliminate this defect. The natural inhibitory mechanism in raw milk is due to the presence of low levels of lactoperoxidase (LP), which can be activated by the external addition of traces of H_2O_2 and thiocyanate.

It has been reported that the potential of LP-system and its activation enhances the keeping quality of milk [28]. Cow milk can be provided with protective factors by the addition of lysozyme, making it suitable as an infant milk. Lysozyme acts as a preservative by reducing bacterial counts in milk without affecting the L. bifidus activity. The scope of application of minor enzymes to milk and milk products has been recently reviewed.

Conclusions

The global market for the production of microbial enzymes for use in dairy-products manufacture is considerably large, but is being dominated only by a limited number of enzyme producers. In India the microbial dairy enzymes requirement has been very limited till now. However, with the advent of technological processes for the manufacture of different varieties of milk products, such as cheeses by the State Dairy Federations, Co-operatives and Private Dairy Product Manufacturers like Amul, Vijaya, Verka, Dynamix, Nestle, Smith Kline Beechem, etc., the markets for the sale of such products in megacities and towns has been slowly growing for the past two to three years. Presently, many of these microbial enzymes, such as microbial rennets and other enzymes are being imported. Hence, there is a scope for the production of enzymes such as microbial rennet, lactase, proteinases, and lipases indigenously. In the near future, the requirement for these enzymes is bound to increase by leaps and bounds, basically due to requirement of value-added dairy products in the country.

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