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Scientific Report

Use of Enzymes in Dairy Industry: A Review of Current Progress

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ABSTRACT

This review paper aimed to provides precious information about the function and use of different enzymes in dairy food applications. An enzyme is called a protein and catalyzes a specific reaction. Every enzyme is intended to initiate a particular reaction with a specific outcome. Moreover, numerous enzymes are present in the human body. Dairy food applications include the use of different enzymes, such as protease, to lessen the allergic properties of bovine milk products and lipase to improve the flavor of the cheese. Caseins, which are acid-soluble, are free from a flavor and can be suitable for addition to beverages and acidy foods by the limitation of proteolysis. The hydrolysates of casein are better to use in foods based on milk proteins for newborn children with allergy to bovine milk. Lipolysis makes a significant role in the flavor of Swiss cheese. The peppery flavor of Blue cheese is produced by short-chain unsaturated fats and methyl ketones. Many minor enzymes with limited application in dairy processes are sulphydryl oxidase, lactoperoxidase, glucose oxidase, catalase, lysozyme, and superoxide dismutase. Both catalase and glucose oxidase are utilized in food preservation processes. The scope minor enzymes in milk products needed for better production of dairy products and for the future of dairy technology. The worldwide market for the production of microbial enzymes used in dairy products processing is impressively increasing; however, there are a limited number of enzyme-producing industries in the market. The production of proteinase, lactase, lipase, and microbial rennet is increasing in the laboratory and small scales. In near future, the need for these enzymes will be undoubtedly significantly increasing essentially due to the requirement of significant nutritional valuable dairy products in the country to overcome malnutrition and obesity and shift toward low-fat and healthy foods.

Keywords: Dairy industry, Enzymes, Dairy products, Dairy food technology

Les Progrès Actuels dans l'Utilisation des Enzymes en Industrie Laitière

Résumé: Cette étude visait à fournir des informations pertinentes sur la fonction et l'utilisation de différentes enzymes dans les produits laitiers. Les enzymes sont une classe spécialisée de protéines responsables de la catalyse des réactions chimiques. Chaque enzyme a pour vocation d'initier une réaction particulière menant à un résultat spécifique. De plus, de nombreuses enzymes sont présentes dans le corps humain. Les producteurs laitiers utilisent différentes enzymes, telles que la protéase pour réduire les propriétés allergiques des produits laitiers bovins et la lipase pour améliorer la saveur du fromage. Les caséines, qui sont solubles dans les acides, sont exemptes de saveur et peuvent intégrer la composition des boissons et aliments acides afin de limiter la protéolyse. Il est préférable d'utiliser les hydrolysats de caséine dans les aliments à base de protéines de lait pour les nouveau-nés allergiques au lait de vache. La lipolyse joue un rôle important dans la saveur du fromage suisse. La saveur poivrée du fromage bleu est produite par des graisses insaturées à chaîne courte et des méthylcétones. De nombreuses enzymes mineures ayant une application limitée dans les procédés laitiers sont la

sulfhydryl oxydase, la lactoperoxydase, la glucose oxydase, la catalase, le lysozyme et la superoxyde dismutase. La catalase et la glucose oxydase sont utilisées dans les processus de conservation des aliments. Les enzymes mineures dans les produits laitiers sont nécessaires pour une meilleure production des produits laitiers et pour l'avenir de la technologie laitière. Le marché mondial connaît une croissance impressionnante dans la production d'enzymes microbiennes utilisées dans la transformation des produits laitiers; cependant, il existe un nombre limité d'industries productrices d'enzymes sur le marché, même si la production de protéinase, de lactase, de lipase et de présure microbienne a augmenté en laboratoire et à petite échelle. Dans un avenir proche, la demande pour ces enzymes augmentera considérablement. Cela est dû à la demande grandissante en produits laitiers à forte valeur nutritive en Iran pour vaincre la malnutrition et l'obésité et passer à des aliments à faibles teneurs en gras et plus sains.
Mots-clés: Enzymes, Industrie laitière, Produits laitiers, Technologie laitière

INTRODUCTION

An enzyme is called a protein and catalyzes a specific reaction. Enzymes are precise in their action, and every enzyme is intended to initiate a particular reaction with a specific outcome (Farkye, 2004; Duruyurek et al., 2015; Sevindik et al., 2018). In addition, numerous enzymes are present in the human body (Erdemli et al., 2017; Salmas et al., 2017). The term enzyme for the most part in cystic fibrosis is referred to the enzyme made by the pancreas that is expected to start a reaction for the food digestion. Various indigenous enzymes play a significant role in the handling and nature of milk for utilization and cheese making. Apart from indigenous enzymes, exogenous enzymes can be included for specific objectives, namely changing milk coagulation, preparing cheese and aging, improving the shelf life of foods, providing safety, as well as cleaning and removing dairy wastewaters (Andersen et al., 2008). The present mini-review aimed to provide general information about the role and use of various enzymes in dairy food applications.

Enzymes in Dairy Industries. Dairy enzymes are utilized for processing cheddar, yogurt, milk, and milk products. The properties of these enzymes change broadly from coagulant, utilization in the making of cheese, bioprotective enzymes to improve shelf life aspects of dairy products processing. The utilization of enzymes (i.e., esterase, lactase, lipases, protease, and catalase) in dairy technology and food technology is well known. Rennet (also known as rennin, which is a blend of pepsin and chymosin extracted from animals and microbiological sources) is utilized for milk curdling as the primary phase of cheese processing technology (Merheb-Dini et al., 2010). Proteases of different types are utilized for speeding up cheese aging, as a functional property, and changing milk protein to decrease the allergic effects of cow milk products in infant foods (Fox, 2002). Lipase is mostly used in cheese maturing for the improvement of flavors. Lactase is usually applied to hydrolyze lactose to glucose and galactose sugars and increase the solubility and sweet flavor in different dairy items. The applications of enzymes are observed with their different sources available to use in food and dairy products (Table 1).

Rennet. Milk contains protein, particularly casein that preserves the liquid structure. When protease as an enzyme is added to cheese processing, it hydrolyzes casein, precisely kappa casein, which stabilizes the micelle function preventing from coagulation in milk. Rennet and rennin termed as enzymes are used to coagulate the milk. Actually, rennet is termed as the lining of a fourth part of the calf stomach. Chymosin is the widely recognized enzyme separated from rennet. Chymosin is easy to acquire from animals and microbial or vegetable sources; however indigenous microbial chymosin (i.e., fungi or microbial sources) can be insufficient for making cheddar and hard cheeses (Low et al., 2006). The restricted supply of calf rennet provokes the hereditary designing of microbial chymosin with cloning calf prochymosin genes into microorganisms. Bioengineered chymosin is associated with the creation of up to 70% of cheeses. Bioengineered enzyme saves the live of calve, it grants ethical issues for those people who are allergic to eat foods made with genetically engineered microorganisms (Fox, 2002).

Lactase. Lactase converts lactose into galactose and glucose sugars. For people suffer from lactose intolerant who have less creation of lactase in small intestine. Lactose brings about inconvenience (e.g., the looseness of the bowels, cramps, and gas) in the digestion of milk and milk products (Tanasupawat and Komagata, 2001). Lactase is utilized at a commercial level to develop products free from lactose for lactose-intolerant people. It is also utilized for frozen yogurt production to make creamy and better-tasting products. Lactase is typically obtained from *Aspergillus* species of fungi and *Kluyveromyces* species of yeasts (Wilkinson et al., 2003).

Catalase. Catalase has limited and specific usage in cheese processing. It is mostly used in Hydrogen peroxide, which is a potent oxidizer and dangerous to body cells. Therefore, it is utilized in making certain cheeses instead of pasteurization (e.g., Swiss cheese) in order to save regular milk proteins that provide benefits to finished product and flavor enhancement of the cheese (Fox, 2002). It is denatured by the higher temperature pasteurization; however, hydrogen peroxide residues hinder the bacterial culture that is compulsory in real cheese processing. Consequently, hydrogen peroxide residues should be removed before packing. Catalase is obtained from bovine liver or microbial sources. It is usually added to dissolve hydrogen peroxide to water and oxygen (Silva et al., 2007).

Lipases. Lipase is utilized to separate milk fat and gives desirable flavors to the cheese. Strong flavor cheeses (e.g., the Italian cheese Romano) are produced by utilizing the lipase. Free unsaturated fats provide a flavor that is produced by milk fat hydrolysis. Calf and sheep provide animal lipase; however, *Mucor miehei* as fungal species supply microbial lipase (Hasan et al., 2006). Microbial lipase is available for cheese processing and less specific to hydrolyze certain fats when animal-source enzymes hydrolyze short- and medium-length fats. Shorter length fats hydrolyze to favor in light of the fact that they give a better taste to numerous cheeses; nevertheless, longer chain unsaturated fats provide soapiness or no flavors after hydrolysis (Alkan et al., 2007).

Use of Transglutaminase in Dairy Industry. It was investigated that the use of transglutaminase (TGase) up to 0.5% was more effective in improving the functional properties of yogurt made from goat milk.

Table 1. Sources of 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	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 frut juice clarification
Proteases	Bacillus and Aspergillus spp.	Detergents, brewing, Meat tenderization, baking, cleaning, hydrolyze animals 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)

As a result of enzymatic cross-linking, gel consistency enhanced and whey separation reduced significantly. However, there were no significant differences observed in enzyme-treated yogurt samples and control samples (Farnsworth et al., 2006). Stirred yogurt was prepared through covalent cross-linking by the combined action of microbial TGase in inactivated state and glutathione, and there were no adverse effects on the fermentation of yogurt. There was a significant increase in apparent viscosity and protein polymerization, compared to yogurt, which was only manufactured by TGase (Bönisch et al., 2007). When TGase was used in higher concentrations, it reduced syneresis, along with an increase in the viscosity of yogurt. However, minor drawbacks were observed in the growth of LAB, which resulted in the low production of acid and acetaldehyde, compared to those reported for the control. Nevertheless, concentration up to 0.3 g L-1 was observed to be optimum, which was identified as a good alternative to stabilizers to be added in the production of fat-free yogurt (Ozer et al., 2007). The TGase appeared a more effective source in yogurt for the development of physical properties when its composition was altered by the addition of whey. In addition, it was observed that cross-linking caused by TGase has a direct influence on the rate of syneresis (Gauche et al., 2009). The effects of TGase were studied on Review of Literature 14 physiochemical and sensorial characteristics of yogurt at different incubation times and production steps. The results showed that enzyme addition caused no significant change in the chemical characteristic of yogurt. Nevertheless, after pasteurization, the addition of enzyme enhanced gel stability with the reduction of syneresis (Şanlı et al., 2011).

Other Related Dairy Enzymes and Their Uses. Dairy food applications include the use of different enzymes, such as protease, to lessen the allergic properties of bovine milk products and lipase for the improvement of flavor in the cheese. Caseins, which are acid-soluble, are free from a flavor and can be suitable for the addition into beverages and acidy foods by the limitation of proteolysis (Couto and Sanromán, 2006). The hydrolysates of casein are better to use in foods based on milk proteins for newborn babies with an allergy to bovine milk. Currently, protease presents 60% of the industrial enzymes; however, microbial proteases are valuable in light of the fact that they are easy to be obtained, used, and recovered. An enzyme extracted from *Aspergillus niger var. awamori* is currently produced at an industrial level (Silva et al., 2007). Lipolysis makes a significant role in the flavor of Swiss cheese. The peppery flavor of Blue cheese is produced by short-chain unsaturated fats and methyl ketones. The larger part of lipolysis in Blue cheese by Penicillium roqueforti lipase and smaller part come from milk lipase. Lipolytic enzymes, for example, esterases and lipases are related to the digestion of lipid degradation (Deeth, 2006). *Penicillium restrictum* is present in soil and different oil residual parts that produce lipase. Nowadays, industries, such as Amano, Gist-Brocades, and Novozymes, utilize the microbial lipases (Alkan et al., 2007). Medium-aged cheeses that are emulsified, homogenized, and pasteurized is NOVO procedure for generation of enzyme-modified cheese (EMC) which give 'palatase' (R. miehei which give this lipase) it is matured at higher temperatures for two to six days. The paste after heating is appropriate to use in dips, soups, snacks, and dressings. To deliver varied ranges of cheese flavors and flavor intensities in Blue, Cheddar, Swiss, Romano, and Provolo-Nemor, EMC technology has been developed for their inclusion in numerous dairy and food products at low levels. Exogenous enzymes are successful in increasing aging; however, there was no promotion in their wide-spread usage due to high costs and challenges to have uniform curd and over-ripening problem of the cheese (Manay and Shakuntala, 2001). Many minor enzymes with limited application in dairy processes are sulphydryl oxidase, lactoperoxidase, glucose oxidase, catalase, lysozyme, and superoxide dismutase. Both catalase and glucose oxidase are utilized in food preservation processes. Superoxide dismutase is an antioxidant for foods; nonetheless, it is more powerful under catalase availability and generates H_2O_2. Sulphydryl groups which is thermally induced generation believed the reason of cooked off flavor in Ultra High temperature treated milks. Aseptic conditions can overcome these defects (Wood, 2012). The low levels of lactoperoxidase (LP) in raw milk are due to the inhibitory mechanism initiated by H_2O_2 and thiocyanate. The capability of LP-system activation causes an increase in the preservation quality of milk with the addition of lysozyme, which is appropriate for newborn infants (Dajanta et al., 2008). Lysozyme diminishes bacteria in milk without influencing *bifidobacterium* in fermented milk types. The scope minor enzymes in milk products needed for better production of dairy products and for the future of dairy technology (Tamang and Fleet, 2009).

Conclusion

The worldwide market for the production of microbial enzymes used in dairy products processing is impressively increasing; however, there are a limited number of enzyme-producing industries in the market. Mostly, the use of microbial dairy enzymes has been extremely restricted up to now. Nevertheless, there have been new advancements in technological processes for the production of various types of dairy products, for example, the cheese by Nurpur Dairy Industry and Private Dairy Product Manufacturers, such as Nestle, Engro, Haleeb, and Adam's Cheese manufacturers. The business sectors for selling these valuable and nutritional products in big cities and towns are gradually developing for the past 4 or 6 years. Nowadays, a huge number of microbial enzymes, for example, microbial rennet and many different enzymes have been imported. Consequently, the production of proteinase, lactase, lipase, and microbial rennet is increasing in the laboratory and small scales. In near future, the need for these enzymes will be undoubtedly significantly increasing essentially due to the requirement of significant nutritional valuable dairy products in the country to overcome malnutrition and obesity and shift toward low-fat and healthy foods.

Ethics

We hereby declare all ethical standards have been respected in preparation of the submitted article.

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contribution

Study concept and design: Mir Khan U., Selamoglu Z.
Acquisition of data: Mir Khan U.
Analysis and interpretation of data: Selamoglu Z.
Drafting of the manuscript: Mir Khan U., Selamoglu Z.
Critical revision of the manuscript for important intellectual content:Mir Khan U., Selamoglu Z.
Statistical analysis: -
Administrative, technical, and material support: Mir Khan U., Selamoglu Z.

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