



# The Application of Novel Inputs and Advanced Technology in Dairy Product Processing a Review



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## Abstract

This review work summarizes the current advanced dairy processing technologies with a special focus on the application of novel enzymes, starter cultures, and technologies. Novel camel chymosin (CHY-MAX® M) a recent milk clotting enzyme that have application in cheese-making process shows more efficacy than other coagulant. It resulted better for camel milk coagulation.  $\beta$ -galactosidase enzymes commercially used to hydrolysis lactose in dairy products while microbial Transglutaminase is cross-linking enzyme with great role in dairy products processing through enhancing protein functional in yogurt, cheese, and ice cream. Phospholipases hydrolyze phospholipids and used in cheese and yogurt to increase cheese yield whereas a novel exogenous Lipases enzyme enhance cheese flavor and ripening. Novel Starter cultures will help to produce new commercial fermented products such as novel probiotic yogurt. Starters such as *Lactococcus lactis*, *Pediococcus acidilactici* and *Lactobacillus plantarum* were isolated as a potential lactic acid bacterium from spontaneously fermented camel milk. Many novel starter cultures and uses of advanced technologies such as membrane filtration in dairy industry for microorganism removal, cheese, yogurt, and whey processing whereas ultra-high-pressure homogenizer (UHPH) novel nonthermal processing technology were reviewed.

**Keywords:** Enzymes; Culture; Membrane; UHPH

## Introduction

Milk is a special complex fluid produced by the mammary gland of mammals. It is nutritionally complete food but perishable unless hygienically handled and processed to relatively shelf stable products. Processing of milk to different dairy product enhance shelf life, utilization of distance market, transportation, product diversification and improve quality [1,2]. The application of additives, inputs, ingredients, enzymes and technology mostly done to attain desired product characters [3-5]. In Ethiopia surplus milk is mainly processed traditional product such as butter, ghee, cottage cheese (*ayib*) and spontaneously fermented (*Ergo*) with traditional technologies and marketed informally. However; modern milk processing was expanding that can processes different dairy product like pasteurized milk, cheese like provolone, mozzarella, gouda type, white/feta, set yogurt, flavored yogurt, butter and cream [6-8]. Such development of Dairy industry will encourage application of different inputs and technologies. Therefore, this review work was done with the main objective of assessing available information on the application of novel enzymes, dairy cultures and advanced technology in dairy product processing to be available for intended local milk processing industries, researchers, consumers and ingredient suppliers.

## Application of Novel Enzymes in Dairy Product Processing

### Camel chymosin as Coagulating Enzymes

It has been reported that there are many kinds of enzymes applied by dairy processing from which, cheese making is former biotechnological application of enzymes rennet which is a crud extract obtained from the fourth stomach calf was applied. The action rennet on milk is cleavage k-casein and the precipitation of para-casein. Plant origin coagulant also reported by different authors for instance *Calotropis procera* (Sodom apple), *Cynara sp*, ginger, lemon [9,10]. *Moringa oleifera* flowers [11]. Increase in cheese production resulted increase demand for coagulant and locking alternative coagulant sources [12]. As indicated recently, novel camel has been obtained through heterologous expression in *Aspergillus Niger* from Camelus dromedaries (CC) [13].

It was a 2<sup>nd</sup> generation fermentation produced chymosin and commercially available as CHY-MAX M® from Chr. Hansen Denmark. CHY-MAX® M shows a seven-fold higher C/P ratio than bovine chymosin or 70%

higher clotting activity and only 25% of the general proteolytic activity [13]. Camel chymosin worked well for cow, goat, and

ewe milk cheeses and feasible application in donkey milk cheese [14]. CHY-MAX® M also results in better for camel milk coagulation for soft white cheese making. It exhibits a higher level of thermo stability than bovine chymosin and resulted lower level proteolysis, good flavor in cheddar cheese. Camel chymosin, contains two additional positive patches that favour interactions with the substrate [15]. It also has wide range of temperature tolerance that will ranging from 25 to 45 °C, with a maximum activity at 40 °C [15]. Cheeses by this coagulant resulted retained melt characteristics for a longer time and shelf-life [16].

### The use of Novel $\beta$ -galactosidase Enzymes

$\beta$ -Galactosidase is commonly known as lactase hydrolyses widely found naturally and has been isolated from animals, plants as well as microorganisms [17]. The commercially available  $\beta$ -galactosidase from microorganisms like fungi, bacterial, yeast that have great interest in industrial processes mainly depends on the hydrolysis reaction [18]. It helps to produce lactose free products for lactose-intolerant individuals, solve whey disposal issues on commercial scale and reduce the crystallization in ice creams and condensed milk which occurs due to high lactose concentration [19]. The cold-active  $\beta$ -galactosidase got biotechnological attention in food industrial for Lactose intolerance consumers since hydrolysis lactose of dairy products at lower temperature [20]. Production of  $\beta$ -galactosidase from Lactic acid bacteria shows that *Lactobacillus dulbrueckii* gives the highest enzyme activity around. While *Lactobacillus* strains isolated from yogurt and *Lactobacilli* from gastrointestinal tract produce lactase enzyme in dairy products [21].

### The Use and Application of Transglutaminase Enzyme

Microbial Transglutaminase (TG, EC 2.3.2.13) enzyme has a catalyzes transfer reaction between the acyl and  $\gamma$ -carboxamide of peptide-bound glutamine residues and primary amino groups in a variety of amino residues of proteins. Transglutaminase (TG) has been found in animal and plant tissue [22,23]. It has been reported that TG cross-linking enzyme commercially available and play great role in dairy products processing improving protein functionality. In yoghurts prevent syneresis and resulted firmer but soft texture [24]. According to the experiment of improves hardness, melting and stretch ability properties of high fat Mozzarella cheese by using TG level of 0.02% with best results organoleptic properties while low fat Mozzarella treated with 0.05% TG have acceptable meltability stretch ability, oiling of fat leakage and organoleptically. Its increased yield of soft cheese (17.38%) verses untreated (16.01%), less syneresis and longer shelf life, reduce bioavailability of nutrients for deteriorative microorganisms due to its cross-link property [25]. In ice cream it increased overrun, partial coalescence of fat globules, melting resistance, hardness, apparent viscosity and consistency. TG can be used as a partial replacement for fat in ice cream. TG gives a higher positive effect on yoghurt structure by increase viscosity due to protein binding effect. In stirred yoghurt best value for syneresis, firmness, and "flavor [26]. Also, in set and low-fat yoghurt stabilization against

syneresis and play role in goat milk yoghurt.

### The use and application of Phospholipases

Phospholipases (A1, A2, C and D) are a complex and crucially important group of enzymes that hydrolyze phospholipids. In dairy industry used in cheese and yogurt to increase cheese yield through interrupting milk fat and protein in curd [27]. Hydrolysis of milk phospholipids by phospholipases enzyme increases the yield of Mozzarella cheese, Pizza cheese, Chihuahua-type cheese [28]. The application of phospholipases increases cheese yield without affecting quality. It is a recent technology since it was introduced in 2005 by Novozymes A/S and Christia Hansen A/S with trade name YieldMAX™ from the filamentous fungi *Fusarium sp.* Yield MAX hydrolyzes the *sn*-1 ester bond of phospholipids, to lysophospholipids and fatty acids. The hydrolysis of cheese milk by phospholipase prior to renneting increase total cheese yield by 3.2% and DM yield in mozzarella cheese through moisture and fat retention, improved emulsification and water-holding capacity [29]. It can be applied up to 0.01-1% (w/w) of fat [30].

### The use and application of Lipases

Milk contains indigenous and exogenous enzymes like proteinases and lipases of microbial and animal origin. In Dairy industry Lipases used to hydrolysis of milk fat triglycerides (triacylglycerols), that can modify the fatty acid chain lengths and flavor formation in cheeses [31]. Lipases can be obtained from animal tissues such as pancreatic glands (bovine and porcine) and pre-gastric tissues of young kid, lamb and calf and from microbial *M. miehei*, *A. niger*, *A. oryzae* that mostly applied in cheese making [32]. Application of exogenous lipases in the dairy industry is enhancing cheese flavor and acceleration of cheese ripening throughout controlled hydrolysis of triglycerides [33]. Lipases have different kinds of selectivity toward their substrates and hydrolysis starts with the lipid binding and the catalytic serine attack on the carbonyl carbon atom of the susceptible ester bond [34]. Lipases could be added before starter and rennet in cheese milk.

### Application of Novel Starter cultures

Milk has been preserved by fermentation through the action of lactic acid bacteria, which convert lactose lactic acid and other organic acids [35]. Starter cultures were microbial preparation single or many strains and the lactic acid resulted from action of them form characteristic body and texture of the fermented milk products pays formation of overall flavor and enhances preservation [36,37]. Starter have additional advantages than fermentation termed functional starter cultures. The possibility of development of new commercial products using novel starter cultures were reported [38]. Indicative products like a novel probiotic yogurt with *Lactobacillus pentosus* KCA1 having both the genomic and functional capability [39]. There are Lactic acid bacteria stains that prevent lactose intolerance and accumulation of galactose.

On the other hand, form spontaneously fermented camel milk *Lactococcus lactis*, *Pediococcus acidilactici* and *Lactobacillus plantarum* were isolated as potential starter cultures bacteria resulted

good acidification and needed pH value [40]. Cheese ripening is a slow and expensive process therefore, attenuated starters can be used to accelerating cheese ripening and flavor since they produce less acid during fermentation but produces active starter enzyme [41]. Functional starters used as accelerating agents. *Lactobacillus casei*, *Lactobacillus plantarum*, *Lactobacillus rhamnosus* were adjunct starters cultures in cheese ripening [42]. Novel tarter culture that have potential as bacteriocins production and bio preservation *Lactococcus lactis ssp. lactis*, *Lactococcus lactis ssp. cremoris*, *Enterococcus spp. lactobacillus carvatus* *Lactobacillus sakei*, *Pediococcus acidilactici*, *Enterococcus faecium*, *Lactobacillus Plantarum*, *Lactobacillus ruteri* and *Streptococcus thermophiles*.

Exopolysaccharides producing lactic acid bacteria like *lactobacilli*, *streptococci* and *lactococci* give good body and texture in fermented dairy product while galactose fermenting *lactobacilli* and *streptococci* resulted low level galactose and low browning in mozzarella. Lactose negative *L.delbrueck ssp. bulgaricus* help to avoid over acidification in yoghurt resulted good body and texture of curd and syneresis prevention. Autolyzing lactic acid bacteria enhanced proleolytic and lipolytic and acceleration cheese ripping. While propionic bacteria improve vitamin content of fermented dairy product were reported as novel cultures.

### Application of Membrane Technology in Dairy Product Processing

Membrane filtration is a separation process of liquid like milk into “permeates” and “retentate” through semi-permeable membrane. Membrane filtration is applied in dairy industry since 1960s [43]. It was a novel non thermal environmentally friendly [44] suitable and economical alternative to centrifugation, lactification and evaporation, reducing cost of production as well generating new revenue resources [45-47]. Reverse Osmosis (RO), Nano filtration (NF), Ultrafiltration (UF) and Microfiltration (MF) were four membrane type that were commonly used in dairy industries [48]. Removal of microorganisms using heat treatment like Ultra high temperature induce irreversible modifications of milk component, change of flavor, cheese- making properties [49]. As the advance in technology using membrane separation technique become possible option for microbiological safety.

Microfiltration has larger pore size and used to remove bacteria and fat from milk and enable to produces bacteria free raw milk that can further be transformed into fluid milks, cheeses, powder or protein by products since it rejects fat and microorganisms while allowing other milk constituents to pass through the membrane [50]. MF is same time referred as non-thermal cold pasteurization as it doesn't affect sensory attributes. The other applications in cheese production help to improving the nutritive quality, reduce microbial load, adjust compositional and yield of cheese by increasing total solid content, utilization of whey, reducing dosage of rennet, starter culture and processing steps [51]. Cheese production application of all four membrane could be possible however; UF is most widely used while microfiltration is to less extent. In cheese production while MF is used in removal of bacterial from

milk, bacteria and fat from brine solution placed for longer time [52]. Ultrafiltration have three possibilities for cheese making for protein standardization, use of intermediate or medium concentrated retentates and use of liquid pre-cheeses. Different cheese variety such as Camembert, Cheddar, Mozzarella and many other cheese varieties be made with. Nanofiltration removal of minerals while Reves osmosis water from milk and whey [52].

UF can result good quality fresh and brine cheese with higher yield but same report indicates slower ripening rate due to decreased in proteolysis. Manufacture of most fermented milks is concentration of the milk base to increase solids content solids. Membrane techniques have been successfully applied for the preproduction of concentrated, set and stirred [53]. UF help to increase protein, fat content of the milk desired above the level present in raw milk and reduces lactose, some soluble protein fractions and minerals. Manufacturing of concentered yoghurt type such as Ymer traditional fermented dairy product in Denmark, hrikhand and chakka in Indian using membrane filtration [54]. The yogurt product that made by using UF is called ultrafiltered retentate concentrated yogurt [53]. UF technology in yoghurt manufacturers shorter gelation time, impruves physical quality of end product, extended shelf-life, reduce risk of syneresis and excessive acid development during post-fermentation due to reduced lactose content and consistency of the final product. The major benefits UF on yogurt is less whey separation, acceptability since assuming using UF have purity than other non-dairy stabilizers. Whey processing is main application of membrane technology with more than 75% of all membrane usages. Milk protein can be recovered by using MF, UF and NF processes. MF separates the casein micelles and whey proteins [55] whereas NF highly permeable to water and monovalent ions and used to concentrate whey and reduce mineral contents of the whey.

### Application of Ultra high-pressure Homogenizer (UHPH)

Conventional Homogenization carried out to reduce milk fat globule size to prevent creaming during storage as the milk is passed under moderate pressures up 18 - 20 MPa. [56]. UHPH has a similar principle as conventional homogenizers but it works at higher pressures up to 400 MPa. It is a non-thermal processing technology helps to develop novel products with longer shelf life , higher safety with better sensory and nutritional properties, less Maillard reaction, less whey protein denaturation, no lactose isomerization than in pasteurized milk, higher yield, longer shelf life and better textural of fresh cheese [57]. UHPH treated milk have equal or better microbial shelf life than high-pasteurized milk. Higher value of texture characteristics, viscosity, lower syneresis and higher water holding capacity in yogurt [58]. UHPH system is a dual function technology it has capacity to replaces functions of conventional separator equipment, homogenizer and pasteurizer. Even if not alone promise to sterilization and aseptic packaging application of UHPH is recent novel technological opportunity for dairy industry [57,59].

## Conclusion

The development of dairy processing and research resulted application of different inputs, new innovations and technologies. Novel enzymes such as camel chymosin (CHY-MAX® M),  $\beta$ -galactosidase, Phospholipases, Transglutaminase and lipases were indicative inputs that have a wider advantage in dairy processing industry. Similarly, novel starter culture that are multi-functional are also available. Membrane filtration is advanced technology that have been applied in dairy industries in fluid milk, whey processing, removal of microorganisms, cheeses and yogurt manufacturing. While ultrahigh pressure homogenizer a recent novel technological opportunity for dairy industry as it is replacing roles of conventional separator, homogenizer and pasteurizers equipment. Therefore, information on novel enzymes and cultures and technology were raised. However, more detailed study and assessment on consumer acceptability, availability, usage and if any negative effect on consumer should need to be further well investigated.

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