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A review of the use of enzymes in some dairy products

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Abstract

Enzymes are the most important group of proteins that carry out biochemical reactions and speed them up and for this reason, these compounds are called biocatalysts, which are also known as cellular catalysts. The thermal resistance of enzymes may be used to find out the efficiency or completeness of a given process. In the studies conducted, the transglutaminase enzyme has had the greatest effect in the processing of dairy products such as ice cream, cheese, doogh, milk and yogurt. It is believed that transglutaminase enzyme can bring the properties of low-fat ice cream closer to high-fat ice cream by creating a strong and homogeneous protein network. Increasing the amount of lipase, protease and peptidase enzymes into the cheese curd is an effective key in accelerating cheese ripening. But the non-uniform distribution and wastage of enzyme in cheese juice is one of the most important problems of using enzyme. The studies of the use of microbial transglutaminase, lipase and trypsin enzymes can be effective in the production of beneficial doogh with less microbial load and higher antioxidant and sensory properties. Also, microbial transglutaminase is an enzyme that, in addition to pH drop during fermentation, protein enrichment and heat treatment of yogurt milk, also affects its reaction.

Keywords- Enzyme, Ice cream, Cheese, Buttermilk, Milk, Yogurt

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1. Introduction

Enzymes are compounds that can increase the reaction rate by about 10^7 times. Enzyme, like an inorganic catalyst, accelerates the reaction rate by lowering the activation energy of the reaction. Catalysts remain unchanged in reactions, but enzymes, like other proteins, do not remain stable under different conditions. These materials change due to high heat and acids and alkalis. Catalysts do not affect the equilibrium of a reversible reaction, but only increase the rate of the reaction to reach equilibrium. Enzymes increase the rate of chemical reaction by reducing the activation energy.

The use of renin enzyme is the most important application of the enzyme in the dairy industry. In addition, other proteolytic enzymes such as neotrose (protease enzyme produced by Bacillus amylolycofacins) have also been widely used in the ripening process of hard and semi-hard cheeses. Today, the use of the combination of neotrose enzyme and streptococcus cell extract in the cheese ripening process has accelerated this process and improved the flavor of the produced cheese. Lactase is another widely used enzyme in the dairy industry that breaks down milk sugar into galactose and glucose units. Reports show that about 70% of the world's population has some intestinal lactase deficiency, and even in some people who lack this enzyme in the intestine, they face a disease (lactose intolerance).

Lactase enzyme is very useful in the production of low-lactose milk, which is widely used by consumers today. In addition to removing lactose, which is good for people with lactose intolerance, this enzyme increases the sweetness of this category of dairy products [1].

1.1. The use of enzymes as indicators of food processes

A specific example in this regard is checking the presence of alkaline phosphatase in pasteurized milk. The absence of this enzyme means that the pasteurization process is done well. Because when this enzyme is destroyed, the indicator is that the tuberculosis microbe, which is considered a dangerous and resistant microbe in milk, has also been destroyed.

Peroxidase and catalases are also used as indicators for the destruction of other enzymes. Because these enzymes have high thermal resistance and their destruction means the destruction of other enzymes. Peroxidases, especially plant peroxidases, are very resistant to heat. So that the temperature of 120 degrees Celsius for several minutes cannot completely deactivate the resistant peroxidases.

In the food industry, there are cases where the deactivated enzyme becomes active again and shows its destructive effects, Such a situation is observed in the case of peroxidases in milk and vegetables, catalase in vegetables and lipase in dairy products, and protein-degrading enzymes in citrus extracts. The reason for this

situation is that the process used was not sufficient and did not produce the necessary and sufficient changes on the active site of the enzyme. It means that despite the fact that no enzyme activity is observed after the end of a heat treatment, After some time, the active site part regains its original shape and the enzyme can show its bad effects on the food. Of course, this state of returning to the natural and primary structure in enzymes, unlike enzyme denaturation, occurs slowly and during the storage time of the food. Such a situation usually happens when enzyme removal is usually done quickly [2].

1.2. Esterases

Different ester bonds are attacked and decomposed into acid and alcohol. Lipases, which are a type of esterase, work at the interface between water and fat. The action of this enzyme is unfavorable in milk, but favorable in blue cheese.

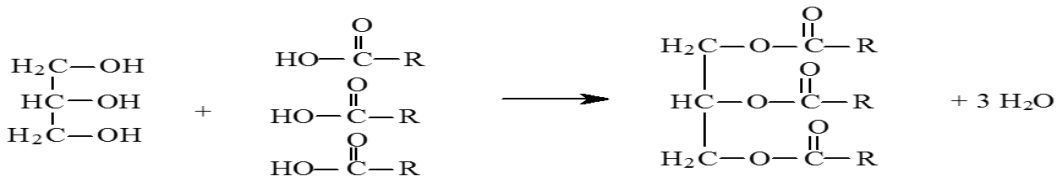

Figure 1. The effect of esterase enzyme on ester bonds

The most important esterases in the food industry are lipase enzymes, which hydrolyze glycerides and convert them into their constituent units, including glycerol and fatty acids. In the biological system, this enzyme leads to the breakdown and decomposition of glycerin as a compact and dense source of energy, which causes the absorption of fatty acids from the villi of the small intestine and their transfer to the cells. These fatty acids are broken down through a mechanism known as beta oxidation and finally lead to the production of energy in the form of ATP.

The activity of this enzyme in the unwanted and uncontrolled food system causes problems in some cases. such as an unfavorable change in flavor and taste in dairy products such as cream and butter, which is due to the release of short-chain fatty acids and the escape of butyric acid. Lipase in milk fat has a natural and microbial origin. The natural lipase of milk is destroyed by pasteurization temperature. The bacterial lipase of milk is the result of the activity of cold-loving bacterium Pseudomonas and it is resistant to pasteurization temperature and changes the taste and aroma during the storage period [3].

1.3. Proteases

Proteases attack specific links in the structure of proteins and cause hydrolysis of specific links of proteins.

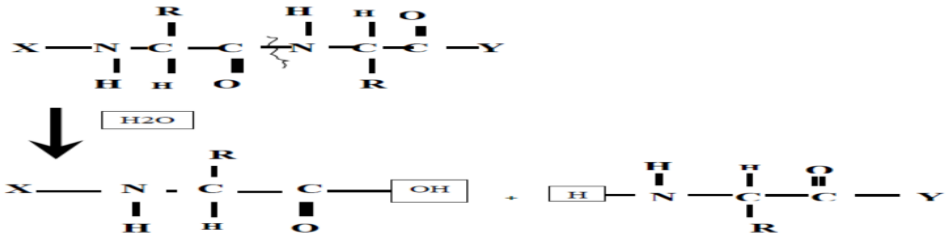

Figure 2. How to break the protein structure by the protease enzyme

The main difference between the enzymes of this group is the selectivity of R1 and R2 units. These enzymes are divided into endopeptidase and exopeptidase in terms of their hydrolysis action. Chymotrypsin enzyme

attacks only peptide bonds in which R1 is provided by the amino acid tyrosine or phenylalanine or tryptophan. Trypsin enzyme attacks peptide bonds whose R1 is arginine or lysine. Pepsin and carboxypeptidase enzymes attack peptide bonds whose R2 is phenylalanine. and they are divided into the groups of acid proteases, proteases with amino acid serine, sulfhydryl proteases and proteases with metal [4].

1.4. Renin

Renin is an enzyme found in rennet or whey. It is extracted from the stomach of an infant calf. Instead of renin, the stomach of a grass-fed calf secretes pepsin or its inactive form, i.e. prorenin. The conversion of prorenin to renin is accelerated by adding acid. The suitable pH for renin activity is 3.5, but its maximum stability is observed at 5, and cheese production takes place at pH = 5.5-6.5 [5].

1.5. Pepsin

It is produced in the mucous membrane of the surface of the stomach. It is inactively called pepsinogen, which is converted into active pepsin at low pH. It consists of 321 amino acids. It denatures at a pH greater than 5. It preferentially breaks the adjacent bonds of phenylalanine, tyrosine and tryptophan [6].

1.6. Trypsin and chymotrypsin

They are secreted from the pancreas into the intestine. Trypsin breaks the peptide bond formed by the carboxyl group of the amino acid lysine or arginine with other amino acids. Chymotrypsin includes several similar enzymes with alpha, beta, gamma, delta and pi prefixes, which breaks the adjacent bonds of amino acid tyrosine, phenylalanine and tryptophan. The suitable pH for its activity is 8 [7].

1.7. Cathepsins

They are located in cell lysosomes and do not play much role in the life of the animal. After death, they participate in the process of crisping the meat and rigor mortis. After death, they break down myofibrils and collagen by being released from the lysosome [8].

1.8. Papain, Ficin and Bromelain

It has plant roots and is obtained from the ripe fruit of the papaya tree, fig latex, and pineapple fruit and stem, respectively. The active site of these enzymes has a cysteine and a histidine. The suitable pH for its activity is 6-5.7. It hydrolyzes peptide, ester and amide bonds. The most important use of this group is to use them to crisp meat [9].

2. Application of some enzymes in dairy products

2.1. Ice cream

Ice cream is a complex foam-like system in which small bubbles of gas (air) are dispersed over a continuous phase that is partially frozen. In this phase, fat in the form of emulsion, thickeners and solids without fat in colloidal form, and sugars and salts form a true solution.

In recent years, the use of microbial transglutaminase enzyme has been developed in order to improve the sensory characteristics and texture of low-fat dairy products, including yogurt and cheese. Transglutaminase is an acyltransferase that can catalyze or accelerate reactions such as cross-linking, acyl transfer, and deamidation. When the amino acid lysine is an acyl acceptor, the acyl transfer reaction between the Y-carboxyamide group of the amino acid glutamine and the amine group of the first type in the amino acid lysine leads to the formation of a glutamine-lysine crosslink [10].

Rossa et al. (2011) based on the results of the electrophoresis test, showed that the enzymatic treatment of heated milk used for making ice cream with microbial transglutaminase leads to cross-linking of milk proteins with each other and the formation of structures with higher molecular weight, as a result, the viscosity and consistency of high-fat ice cream is improved and its pseudoplastic behavior is strengthened [11].

Jooyandeh et al. (2017) used microbial transglutaminase enzyme to produce semi-fat ice cream. The results showed that the addition of transglutaminase enzyme increased the consistency coefficient and the flow behavior index approached zero and as a result increased the loosening characteristic with shearing. The statistical findings of tissue analysis showed that transglutaminase treatment reduces the firmness and consistency and increases the stickiness of semi-fat ice cream [12].

Also, Faraji Kafshgari et al. (2017) used whey protein concentrate as a protein-based fat substitute, soy protein isolate and microbial transglutaminase enzyme in order to investigate the physical properties of low-fat vanilla ice cream. The results showed that the microbial transglutaminase enzyme in the ice cream samples compared to the control sample, caused a decrease in hardness and melted amount and according to the results, it can be found that the use of microbial transglutaminase enzyme in low-fat ice creams has more favorable effects than the use of these two types of protein [13].

2.2. Cheese

Cheese is known as one of the oldest dairy products produced by humans, which has a special place in people's nutrition, and by passing the ripening period, the desired characteristics of aroma, taste, texture and special therapeutic properties are created in the product. The ripening period of ripened cheeses in the world lasts from several weeks to several months and even years depending on the type of cheese and its different characteristics [14].

The process of reaching is a complicated, long and expensive process. Shortening the ripening period of cheese without harming its aroma, taste and texture is very important from an economic point of view [15].

In a research conducted in 2000 on the effect of bacterial and fungal protease enclosed in liposomes on the ripening of cheddar cheese, it was shown that tissue improvement and proteolysis in cheese occur faster than in the control sample. Observation of the electronic microstructure, which was done using a transmission electron microscope (TEM), showed that the microstructure of cheeses containing liposomes was less compressed and the liposomes were placed on the joint surface of fat-casein. The study of the pattern of peptides showed that bitter and sour peptides were accumulated in cheddar cheeses obtained from liposomes containing protease, and its amount depends on the type and concentration of the added enzyme. Examining the organoleptic properties during the ripening period of the cheese revealed that the aroma and taste of all the cheese samples improved, and no bitterness or bad taste (unless the enzyme concentration was high) was observed during the three-month ripening period [16].

The ripening process of cheese is slow, long and expensive. The methods that are considered to shorten the ripening time of cheese include: raising the storage temperature during ripening and using microorganisms and using enzymes [17].

Yazdanpanah et al. (2014) used Aspergillus niger encapsulated lipases based on the sol-gel method to compare the microbial properties of traditional cheese and ultra-refined feta cheese and the findings showed that on the 15th day of cheese ripening, significant changes were observed in the progress of lipolysis and chemical compounds, and the microbial population became negative after 15 days [18].

Beta-galactoside is used to hydrolyze lactose in dairy products such as milk and cheese. Different genera of Lactobacillus and Bifidobacterium and a small number of bacteria that are used as starters in dairy products often produce beta-galactosidase [19] [20].

Nowroozi et al. (2008) in a study determined that all lactobacilli in milk and cheese had beta-galactosidase enzyme, which can be used as a suitable probiotic in dairy products to treat lactose intolerance. In this study, except for one case, lactobacilli were not isolated from pasteurized milk, it seems that some lactobacilli are sensitive to pasteurization heat, therefore, it is recommended to use heat-resistant lactobacilli in probiotic products that sometimes require high heat [21].

Plasmin and chymosin enzymes are responsible for primary proteolysis and the production of large amounts of soluble nitrogen fractions at pH = 4.6, and they improve cheese texture by slow decomposition of αs1-casein

and to a lesser extent β-casein. Therefore, in many cheeses, the primary hydrolysis of casein is done by the remaining rennet and to a lesser extent by the main milk proteases (plasmin, cathepsin D and somatic cell proteases). This causes the formation of large and medium peptides. Finally, these peptides are decomposed by the available starter enzymes and turn into amino acids, and further, the catabolism of amino acids leads to the production of aromatic compounds [22].

Nezhad Razmjoui Akhgar et al. (2017) investigated the effect of adding a commercial enzyme derived from Bacillus polymyxa on Iranian ultra-refined feta cheese. No significant differences were observed between experimental and control cheese in terms of chemical composition. The pH values were significantly higher in the experimental cheese throughout the ripening period. Dissolved nitrogen at pH = 4.6 on the 45th and 60th days was significantly higher in the experimental treatment. On the 60th day, this index was 6.38 and 7.73% in the control and experimental treatments, respectively [23].

Moosavi-nasab et al. (2017) investigated the possibility of using date juice in the production of protease enzyme from Bacillus coagulans and also the use of this bacterium to produce analog probiotic cheese. The results confirmed the highest clot formation activity at 37 degrees Celsius and pH = 7-8 for Bacillus coagulans bacteria. In the following, different culture media were used to determine the most suitable media for the production of protease from Bacillus coagulans [24].

In another study, the effect of edible coating based on whey protein concentrate containing natamycin or lysozyme-xanthan enzyme conjugate on the microbial properties of ultra-refined white cheese was studied. The results showed that all treatments of edible coating significantly reduced the growth of Penicillium chrysogenum mold. Coatings containing natamycin were more effective in reducing the population of this mold than coatings containing lysozyme-xanthan. The coating with a concentration of 600 ppm reduced Escherichia coli by 2.09 logarithmic cycles compared to the samples without coating. Also, the growth of Staphylococcus aureus in all samples treated with lysozyme-xanthan enzyme was less than the control sample [25].

Transglutaminase is an acyltransferase that can catalyze reactions such as cross-linking, acyl transfer, and deamidation. The cross connections between the amino acids glutamine and lysine change the rheological characteristics of the scab and the coagulation process of the scab, preventing the intermingling of fat cells, remaining a larger amount of whey proteins in the curd and affecting the primary and secondary stages of coagulation in cheese. Based on this, by means of enzyme treatment, it is possible to modify the texture of cheese obtained by mixing whey protein concentrate. In fact, the transglutaminase enzyme, by intertwining milk proteins, leads to modifications in the functional characteristics of proteins and the formation of products with better sensory and rheological characteristics [26].

In a research by Torabi et al. (2019), microbial transglutaminase enzyme, a 34% solution of whey powder and inulin were used to produce synbiotic ultra-refined white cheese. The optimization results showed that by using 0.43 enzyme units per gram of protein, 8.24% whey powder solution and 0.71% inulin, synbiotic cheese with suitable physicochemical and sensory characteristics can be produced [27].

De Pierro et al. (2010) observed that transglutaminase is able to increase cheesemaking efficiency by maintaining moisture in the rind [28].

Fernandes De Sa and Bordignon-Luiz (2010) showed that the treatment of milk with transglutaminase seven minutes after adding rennet to it, during preparation from milk, is an efficient method to improve the physical characteristics of processed cheese prepared from those gels [29].

Hemati and Arianfar (2018) investigated the effect of microbial transglutaminase enzyme and gelatin on the rheological and sensory properties of low-fat cream cheese. Based on the results of optimization with the response surface method, transglutaminase enzyme at the level of 3.01% and gelatin at 0.38% were determined as the optimal level of the formulation for the production of cream cheese, which will lead to the production of a low-fat product with favorable rheological characteristics. Also, the results of sensory evaluation showed that the optimal sample has a much higher acceptability among consumers [30].

The effect of adding microbial transglutaminase enzyme at the same time as renin or after cutting the rind on the quality characteristics of low-fat Iranian white cheese was studied by Sayadi et al. (2012). In both cases, the addition of enzyme increased moisture content, cheesemaking efficiency, protein recovery, and moisture-to-protein ratio in cheese. The addition of transglutaminase after cutting the husk resulted in achieving a higher ratio of moisture to protein and a lower amount of tension at the breaking point and Young's modulus. The

simultaneous addition of transglutaminase and rennin resulted in the recovery of more protein in cheese. Transglutaminase enzyme created a network with porous microstructure and less compaction [31].

Due to the problems of long-term ripening of white cheeses in salt water, which causes additional costs, including storage in cold storage and on the other hand, due to achieving a pleasant and pleasant characteristic in the short term, we need to use methods to intensify the ripening process of white salt water cheeses. There are many ways to accelerate cheese and each one has its own advantages and limitations. Including increasing the temperature, adding lipase and protease enzymes, using auxiliary cultures, etc. One of the most desirable methods that has an important effect on the organoleptic properties of white salt water cheeses is the use of microbial lipase enzyme, which of course, by adding it to the cheese making milk, the excellent organoleptic properties of the popular white salt water cheeses can be achieved in a short period of time. This enzyme plays an important role in creating the specific taste of cheese by hydrolyzing the triglycerides in cheese and converting it into free fatty acids and glycerol, and finally changing and converting free fatty acids into other compounds that affect the taste, of course, considering that the milk used in the production of white cheeses is salted raw milk, it contains a percentage of lipase enzymes from the natural flora of milk, and by increasing its concentration, the ripening reactions of the cheese, especially lipolysis, can be intensified to some extent.

In a research conducted by Zarei and Shahab Lavasani (2020), the effect of three levels of somatic cells in raw milk and lipase enzyme on free fatty acids in white salted cheese and its sensory characteristics (taste and texture) were studied during a 70-day storage period. Most of the free fatty acids increased until the 35th day of the ripening period and then decreased until the end of the ripening period. The sensory evaluation of the treatments in terms of flavor and texture showed that the sensory scores of the treatments decreased during the ripening period of 70 days. According to the results, the control treatment as the superior treatment had the highest sensory acceptability, and the increase in the number of somatic cells in milk, in addition to the decrease in efficiency, also caused a decrease in the sensory quality of the final product [32].

2.3. Doogh

Doogh, an Iranian drink, is traditionally obtained by adding water to full-fat yogurt and stirring it in traditional bags called musk and extracting its fat, which is finally added to doogh with salt and aromatic herbs and is ready for consumption. Yogurt is made industrially from skimmed milk, then by adding water, salt and essential oils or herbal extracts, yogurt turns into doogh [33].

Today, transglutaminase enzyme is extracted and purified from an important species of bacteria called Streptoverticillium. The use of this enzyme in fermented milk products has increased the strength of acidic gels, increased viscosity, reduced water runoff and created a smoother texture in molded and stirred yogurts [34].

In a research conducted by Amini and Roufehgarinejad (2018) with the aim of improving the quality and stability of doogh using milk protein concentrate and microbial transglutaminase enzyme. The results showed that the use of milk protein concentrate and transglutaminase enzyme caused a significant increase in acidity content and a decrease in pH. Both variables caused a significant increase in viscosity and a decrease in the two-phase phase of doogh samples. Based on the final optimization results, the best concentration of milk protein concentrate and transglutaminase enzyme for the production of doogh with high stability and desirable sensory characteristics was obtained as 0.53% and 10 units per gram of protein, respectively [35].

Jokar and Yazdanpanah (2020) used microbial transglutaminase and lipase enzymes to produce beneficial doogh. The results showed that the treatment with the mentioned enzymes improved the stability. The amount of unsaturated fatty acids in the control sample is more than the treated sample. The results of the sensory evaluation did not show any significant difference between the treated and control samples, but the overall acceptance by the evaluators increased with increasing the dose of enzymes in doogh [36].

Also, Nateghi et al. (2021) investigated the effect of trypsin enzyme and probiotic bacteria on the physicochemical, microbial and sensory properties of doogh. The results showed that the use of probiotic bacteria along with trypsin enzyme and increasing its concentration caused increased viability of probiotic bacteria, antioxidant property, GABA, mold and coliform production rate after 60 days, pH and sensory evaluation score were maintained after 60 days. It also reduced mold and coliform in doogh. By adding bioactive peptides, overall acceptance increased significantly in all treatments. By using Lactobacillus

acidophilus and 3 mg/100 ml of trypsin enzyme, it is possible to produce beneficial doogh containing GABA with lower microbial load and higher antioxidant and sensory properties [37].

In another study, the effect of transglutaminase enzyme in the range of 0 to 5 units per gram of protein and sodium caseinate in the range of 0 to 1% on acidity, biphasing, viscosity and sensory characteristics of doogh was investigated. The results showed that the use of transglutaminase had a significant increase on acidity, while the effect of sodium caseinate was not significant. Transglutaminase and sodium caseinate had a significant increase on sensory characteristics and viscosity and a significant decrease on the amount of biphasing [38].

2.4. Milk

In the research conducted by Kouhestani et al. (2018), cow's milk and soy milk were used in equal proportions to produce a beneficial drink containing Lactobacillus casei. The highest number of Lactobacillus casei was related to the sample containing 0.5% whey protein and 150 ppm transglutaminase enzyme. Also, this sample obtained the highest overall acceptance score of sensory evaluation during storage time. During the storage time, Lactobacillus casei population and pH of the samples decreased, but the acidity and viscosity of the samples increased significantly [39].

Several attempts have been made to replace animal whey with other milk coagulant proteases due to production limitations and increasing prices. The fruit of the medicinal plant paneer bad (Withania coagulans) is a rich source of milk coagulating proteases, which has been used as a curd in the production of traditional cheeses in the south of Iran since the past. In the study by Beigomi et al. (2013), the enzyme extract of the fruit of Withania coagulans was extracted using 0.85% NaCl solution and the milk coagulation activity and the characteristics of the milk coagulant enzyme were investigated. The optimal temperature and pH for the enzyme were 70°C and 4, respectively. Partial purification showed that milk coagulation by enzyme purified with ammonium sulfate was 50% more than other purified components. The molecular weight of this component showed two bands (66 and 29 kDa) by SDS-PAGE. An important feature of the enzyme was its thermal stability. So that 74% of its activity was maintained at 60°C for 30 minutes [40].

2.5. Yogurt

In the last two decades, due to the negative impact of excess fat on human health, the desire to consume low-fat or fat-free dairy products, especially fat-free yogurt, has increased dramatically. However, consumers demand low-fat products with the same quality as high-fat products [41].

Jaros et al. (2006) stated that the polymerization of milk proteins by transglutaminase increases the water holding capacity and gel strength of yogurt [42].

Moayedzadeh et al. (2015) investigated the effect of microbial transglutaminase enzyme and sodium caseinate in fat-free probiotic yogurt during 19 days of storage at 5 ± 1 °C. The results of the statistical analysis of the data showed that the use of transglutaminase and sodium caseinate significantly increased the viscosity and percentage of the water holding capacity of the samples. But the enzyme treatment increased the brightness (*L) and decreased the yellow color spectrum (*b) of the samples, while the treatment with sodium caseinate, unlike the enzyme treatment, decreased the brightness and increased the yellow color spectrum of the samples [43].

Usually, compounds such as non-fat dry milk powder, whey protein concentrate and sodium caseinate are used to enrich yogurt milk protein in order to obtain yogurt with a favorable, viscous and mild structure. Among milk proteins, casein, especially sodium caseinate, is the best substrate for microbial transglutaminase [44].

Farnsworth et al. (2006) investigated the effect of transglutaminase on the viability of probiotics in yogurt made from goat's milk and showed that the enzymatic cross-linking of proteins has a positive role in the viability of probiotics [45].

Kuraishi et al. (2001) reported that the firmness of molded yogurt gel and the viscosity of transglutaminase-treated stirred yogurt had high sensory acceptability. By improving water retention properties, transglutaminase has increased the viscosity and improved the firmness of the gel [46].

Gauche et al. (2009) evaluated the effect of transglutaminase on the physical properties of yogurt prepared from a mixture of milk and whey and concluded that the consistency of yogurt increased with enzyme treatment [47].

The effect of microbial transglutaminase concentration, sodium caseinate amount and storage time on the survival of Lactobacillus casei in non-fat stirred yogurt and its physicochemical and sensory characteristics were investigated using the response surface method. The results of the statistical analysis showed that the survival of Lactobacillus casei and Lactobacillus bulgaricus significantly increased and the percentage of syneresis decreased with increasing enzyme concentration. By adding sodium caseinate, the acidity and moisture of the samples increased. Also, during the storage time, pH and humidity decreased and the acidity of the samples showed a significant increase. According to the results of the sensory evaluation, the color score significantly decreased with the increase in the amount of sodium caseinate, and the flavor score increased with the increase in the enzyme concentration [48].

Also, Soleymanpouri et al. (2014) investigated the formation of cross-links between milk proteins and soy proteins by the enzyme transglutaminase and its effect on the physical, chemical and microstructural characteristics of fat-free yogurt. At the beginning of the storage period, the pH of the enzyme-treated samples was lower than the control sample, while until the end of the storage period, the effect of soy protein isolate on pH changes was significant. Enrichment with soy protein isolate increased the acidity in the tested treatments compared to the control treatment, but the effect of the enzyme on the acidity was not significant. The presence of soy protein isolate led to the creation of a special sponge-like structure in enriched samples, and the enzymatic treatment of samples enriched with soy protein isolate created a structure with more uniform porosity and smaller particles [49].

Pavunc et al. (2011) investigated the effect of microencapsulation and transglutaminase on the viability of Lactobacillus heloticus and the consistency of molded yogurt and reported that the pretreatment of yogurt milk with transglutaminase increased the strength of the yogurt gel and reduced synergism and improved the appearance and consistency of the samples [50].

Sanli et al. (2011) produced the enzyme in different stages (after homogenization, after pasteurization and simultaneously with the initiator) and they added yogurt to the milk in two different greenhouse periods (10 minutes and 1 hour) and they showed that the addition of enzyme increased the firmness of the texture and decreased the synergism of molded yogurt [34].

Gauch et al. (2009) evaluated the effect of transglutaminase on the physical characteristics of yogurt prepared from a mixture of milk and whey and they concluded that the consistency of yogurt increases with enzyme treatment and according to textural and rheological results, the enzyme has compensated for the physical changes caused by adding whey to yogurt [47].

Moayedzadeh et al. (2015) investigated the effect of transglutaminase enzyme on proteolysis and rheological properties of fat-free yogurt. The results of the statistical analysis of the data showed that with the increase in the concentration of the enzyme, the viscosity and water holding capacity in the samples increased significantly and the amount of proteolysis decreased in them. By adding sodium caseinate, the viscosity and water holding capacity of the samples increased. Also, during storage, the water holding capacity decreased and the amount of proteolysis increased significantly. In optimal conditions, the enzyme concentration was 1.42 units per gram of milk protein, the amount of sodium caseinate was 0.47%, and the storage time was 15 days [51].

In another study, microbial transglutaminase enzyme was used as a substitute for a part of non-fat dry milk in spinach yogurt. The results showed that the addition of different concentrations of enzymes, while preventing specific changes in pH and acidity, increases the viscosity of yogurt and reduces the amount of water in yogurt. The concentration of 0.1 g/liter was able to create properties similar to the untreated sample, of course, the higher concentration caused better properties, but it was not economically justified, because the lower amount of enzyme was able to create yogurt similar to the control sample [52].

Mahmood and Sebo (2012) also showed that the transglutaminase enzyme can cause more consistency and reduce synergism in yogurt [53].

The purpose of a research by Mozaffarpour Nuri et al. (2018) was to investigate the effect of transglutaminase and beta-glucan enzymes on the survival of Lactobacillus acidophilus and the physicochemical, microbiological and sensory properties of probiotic low-fat yogurt. According to the results, the type of sample (transglutaminase enzyme and beta-glucan) and the storage time every week for 21 days had

a significant effect on the changes in pH and acidity of yogurt. Increasing the concentration of beta-glucan and transglutaminase enzyme significantly increased the survival of Lactobacillus acidophilus in low-fat yogurt. With the increase in the concentration of transglutamine enzyme, the water content of the tested yogurts decreased significantly, and with the increase in beta-glucan concentration, the water content increased significantly [54].

3. Conclusion

Enzymes increase the rate of chemical reaction by reducing the activation energy. A higher amount of enzyme can have a more favorable effect on the product, but considering economic issues in most studies and comparing treatments with control samples, enzyme addition to dairy products should be done with sufficient precision.

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