POTENTIAL OF THE TRANSGLUTAMINASE ENZYME IN DAIRY PROCESSING

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ABSTRACT

Enzymes play essential roles in industrial processes. The transglutaminase enzyme (TGase) is widely known to form isopeptide bonds inter- and intramolecular structures in various food proteins, through the cross-linking of amino acid residues of protein-bound glutamine (acyl donor) and lysine (acyl acceptor), forming high molar mass polymers. It can be used to, technologically, develop new dairy. In this review, the primary objective was to highlight the TGase from animal, vegetable and more specifically microbial transglutaminase (MTGase). The MTGase was correlated to emerging technologies, in order accord with to enhance the enzyme activity. The following platforms Web of Science, Scopus and PubMed were used to identify the scientific trends and gaps Based on the analysis of the articles that were collected from the bibliometric analysis, the TGase in the dairy sector represents a promising strategy applied to emerging technologies.

Keywords: Microbial transglutaminase enzyme. Crosslinking. Milk. Whey protein. Non-thermal technologies

1 INTRODUCTION

Enzymes have remarkable properties, including highly chemo-, regio-, and stereo-selective activities. They can act on complex molecules and thus induce specific chemical transformations. In this sense, the TGases are widely distributed in nature, being found in mammals, vertebrates, invertebrates, mollusks, plants and microorganisms. [1,2]

TGases belong to the family of transferase enzymes (EC 2.3.2.13) and play a notable role in covalent cross-linking between proteins, peptides, and other primary amines, it is an enzyme widely known to be able to form isopeptide bonds. The TGase from animal cells are dependent on Ca^{2+}, whereas microbial and plant TGases are Ca^{+2} independent. It is worth noting that the microbial TGases (MTGase) can be produced from different microorganisms, being mostly produced by submerged fermentation of *Streptoverticilium mobaraense*. [3,4]

Widely applied in the food sector, in particular to improve the texture of homogenized sausages made from pork, beef or poultry, to increase the hardness of fish and also to improve the quality of different dairy products. There is also research that is exploring the enzymatic reactions in various dairy products, impacting the technological and nutritional properties of yogurts, dairy drinks, ice cream, cheeses, among others. [5]

Currently, it is interesting both the enzymatic application and the unconventional and emerging technologies in the non-thermal concept that result in excellent quality and performance in food matrices, with the potential to be applied in dairy products and thus maintain quality using low temperatures associated with a short processing time.

According to the context presented here, this review aimed to discuss the enzyme-catalyzed reactions, active sites as well as the applications of MGTase in unconventional technologies relating the applicability in dairy products and modification of these protein structures.

2 MATERIAL & METHODS

For this review, a bibliometric analysis was carried out, a statistical tool that makes it possible to obtain compiled and consistent data on scientific literature on several specific topics, future trends and the evolution of scientific research. This research related the applicability of the microbial enzyme transglutaminase in dairy products, as well as new insights in the last 5 years, searching for words such as: enzyme transglutaminase, microbial transglutaminase, animal transglutaminase, vegetable transglutaminase, dairy products, milk, yogurts, polymerization, emerging technologies, ice cream, Dairy application , whey protein, milk protein structures, on online search platforms such as Scopus, Web of Science and PubMed that compiles the largest scientific data.

RESULTS & DISCUSSION

The Transglutaminases, have animal, plant, and microbial origins. They can be classified into taxonomic source groups, including microorganisms, plants, invertebrates, and mammals. These enzymes are found in both prokaryotes and eukaryotes. For instance, guinea pig TGase was first isolated from the liver of these animals in 1973. However, due to its high cost and dependence on calcium ions (Ca2+), this form of TGase did not attract much industrial attention and only entered the market in the late 1980s.[1,6]

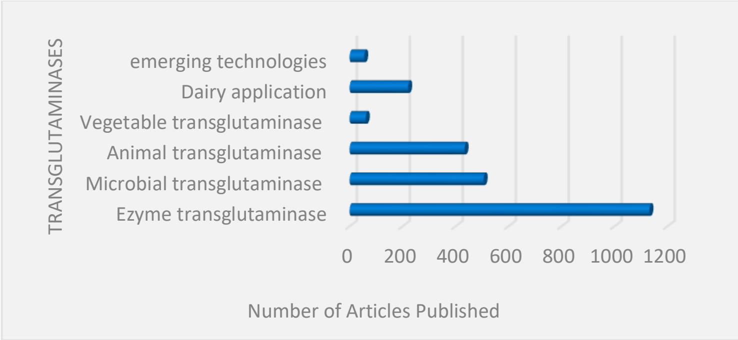

Figure 1 Bibliometric search of enzyme transglutaminase, base de dados Scopus, Web of science and Pub Med.

The enzyme for mammals, the TGases family is composed of nine enzymes: TG1 to TG7, factor XIII and band 4.2, in which, 7 out of 8 encode active enzymes, and the last one (band 4,2 of erythrocyte membrane proteins) has no enzymatic activity, however, although the overall primary structure of TGase enzymes appears to be different, they are all encoded by a family of closely related genes.[7]

Regarding plants, the first evidence of the presence of TGases was reported in 1987 with TGase activity reported in angiosperms being the most studied gene encoding a TGase protein in Arabidopsis (AtPNG1) that is expressed under undisturbed growth conditions ubiquitously during all stages of plant growth and can be induced by light conditions. However, research on plant TGases has faced challenges due to the difficulty in purifying these enzymes and the lack of significant amino acid sequence homologies between animal TGases and the polypeptides reported in the available plant databases. [8,9]

The discovery of MTGases occurred with an intensive screening of MTGase-producing microorganisms in the 1980s, and the results of these efforts were found to be *Streptomyces mobaraense,* which was isolated and evaluated with greater advantages over plant and animal enzymes. Among the advantages of using MTGase are the lower molecular weight (~ 38 KDa), since the lower mass facilitates its penetration into tissues and interaction with substrates, higher productivity and easier purification steps and in addition, it is recognized as Safe (GRAS) by the Food and Drug Administration (FDA).[10,11]

In dairy application, MTGase is more requested, as it promotes enzymatic modification in yogurts, cheeses, fermented milks, ice creams and milk protein structures, and has been suggested as a useful method exhibiting new physical and functional characteristics, which in turn beneficially affect sensory and nutritional characteristics, from reactions promoted by MTGase such as polymerizations. [5]. The reactions catalyzed by the enzyme can be cross-linking, deamination and acyl transference, which can be further potentiated with the emergent technologies in dairy products shown in Figure 2.

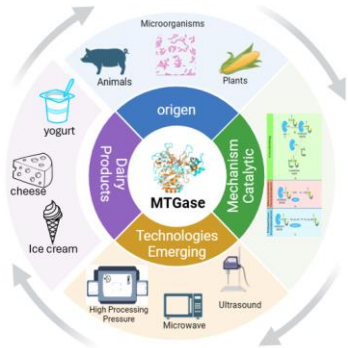

Figure 2 Reactions catalyzed by the enzyme transglutaminase.

However, from this reaction promoted by MTGase, the most successful polymerizations can occur at the level of exposure of the reactive sites found in the protein molecules, increasing the unfolding of the structure of these substrates when combined with the use of emerging technologies that have been increasingly tested to the detriment of conventional processes.

In the above context, the effect on the quality parameters of yogurts made from milk treated with high pressure (HP) and MTGase (alone or in combination) was investigated, demonstrating results with higher textural values and adhesion that were observed and maintained for yogurts made from milk treated with HP-TGase at 3.00%; 3.40% and 3.75% protein, respectively, exhibited a creamier perception than the heat-treated milk samples. Treatment with HP appears to improve protein structures in enzymatic action for cross-linking. [12]

There is an improvement in dairy quality to the use of high-pressure processing in conjunction with cross-linking mediated by the action of the enzyme MTGase in milk that may lead to an increase in the biofunctional properties of low-fat yogurt, which was reported in recent work, where researchers investigated the effect of high-pressure processing (HP) with MTGase treatment on bovine (cow) or sheep (sheep) milk. and reported an improvement in the textural attributes of yogurt, regardless of the type of milk, with a reduction in whey separation of values of 32.9% and 8.7% for bovine and sheep yogurt, and 333 g and 548 g for the firmness of bovine and sheep yogurt, respectively. [13]

The combined effects of MTGase with microwave irradiation for the polymerization of milk proteins were reported and the results showed to be more favorable to polymerize milk proteins with the formation of higher molecular weight components (>130 kDa), produced by casein crosslinking (α S-CN, β-CN and κ-CN).[14]

Ultrasound and pretreatment with microwave heating (M) were compared for the first time to investigate the influence on the structures and functional properties of MTGase-induced WPI. The researchers investigated the ultrasonic pretreatments (400 W, U) in combination with microwave heating (75 °C for 15 min) induced by MTGase. Among some results, the Zeta potential, emulsion stability and foaming stability of M−WPI−TGase were increased by 7.8%, 59.27% and 28.95%, and that microwave pretreatment had the strongest effect on the structure and functional characteristics of Tgase-induced WPI being effective in improving the functional characteristics of WPI.[15]

3 CONCLUSION

The great potential of MTGase has been addressed in this review. In the analysis of the bibliometric data, a sample of literary articles was obtained in a total of 1,134 documents in the last five years reporting the enzyme transglutaminase in its various animal, vegetable and microbial sources with 517, 75 and 584 documents obtained, respectively, which in observance was more expressive for MTGase in the food sector with 506 articles in the approach to dairy products. In the application of the enzyme in dairy, 221 documents were found, of which 13 are from the year 2024 depicting new discoveries and technological improvements. By modifying the properties of dairy products with MTGase using emerging technologies, 55 publications were obtained and some were reported in this review, but other unconventional technologies can be researched such as cold plasma, pulsed electric field and pressurized liquid extraction (PLE), and thus offer new insights. However, it is important to consider the possibility of denaturation of milk proteins during processing, but that such denaturation may bring benefits, such as the release of sulfhydryl groups that increase the resistance of milk and/or dairy products to oxidation, requiring further research. Thus, the combination of MTGase polymerization in milk proteins combined with non-thermal technologies provides good prospects and certainly a favorable proposal for the food industry.

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ACKNOWLEDGEMENTS

We thank the Postgraduate Program in Food Engineering - PPGEAL of the Federal University of Santa Catarina - UFSC, the Coordination for the Improvement of Higher Education Personnel - CAPES for the support provided.