

TRANSGLUTAMINASE AND ITS INDUSTRIAL APPLICATIONS – A REVIEW

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ABSTRACT

Transglutaminases are a class of catalytic enzymes that catalyze the creation of a covalent link between a free amine group of a protein- or acyl acceptor (lysine) and a gamma-carboxamide group of a protein- or acyl donor (glutamine). This causes the alteration of proteins through intramolecular or intermolecular pass-linking, increasing the protein's ability to function. Transglutaminase had a lot of attention in recent years due to its wide range of uses in industries like food science, pharmaceuticals, and biotechnology. Production of transglutaminase started to play a significant role. The production methods, significance, and uses of transglutaminase in numerous new sectors are outlined in this article. We also discuss how to effectively use fish waste meals in media optimization to boost the production of transglutaminase as well as the effects of fish waste on the environment.

Keywords: Transglutaminase. Catalytic enzymes. Cross-link. Importance. Application. Fish meal waste. Media optimization.

1. INTRODUCTION

One of the biocatalysts that facilitates the creation of isopeptide bonds between proteins is transglutaminase. It is frequently employed in the manufacturing of dairy products including cheese, fermented milk, yogurt, butter, etc., and has been utilized to alter the functional properties of proteins in the food processing sector. (Zhang et al. 2019) It benefits different foods and their by-products by improving their texture, water-holding capacity, and other crucial qualities. In many nations, it is also regarded as a processing aid.

One of the transferases catalyzes the acyl transition between glutamine residues is catalyzed by one of the transferases, a class of calcium-dependent enzymes. In the 1980s, Transglutaminase was also isolated by Clarke et al. from guinea-pig liver blood, the most important source of this enzyme. It plays a major role in enhancing the properties of final products (Chan et al. 2019). The various properties of transglutaminase are gelatine formation, high viscosity, withstand high thermal stability, the capacity for water retention, and holding stability. Its crosslinking property is used to improve food's physical and functional quality. It is heavily used in the food industry as a food additive, textile, and biopolymer, the interest in these enzymes is also focused on several biological processes such as curing membranes, blood clotting epidermal keratinization wound healing, and clinical applications such as neurodegenerative diseases and blood coagulation disorders, bone tissue healing processes and cell differentiation processes, tissue stabilization, and even in apoptosis (Li, X., & Fan, D. 2019). In addition, transglutaminase is considered responsible for growth regulation, differentiation, and cell proliferation and has an important role in allergy prevention. We optimize the medium using fishery waste as a nitrogen source instead of using favorable commercial nitrogen sources such as yeast extract, beef extract, peptone, and ammonium sulfate (Maktoof et al. 2020).

This review's objectives are to:

- Describe transglutaminase's function and enzymatic activity.
- Assess the Appropriate Microbial Source for the Production of Transglutaminase Enzymes.
- Explain how transglutaminase is used in the food and pharmaceutical industries as a processing help.
- Assess the fish waste-based medium optimization for Transglutaminase Production.

2. GENERAL PROPERTIES OF TRANSGLUTAMINASE

2.1. The Transglutaminase's Origin

The Streptovorticillium species was initially used to manufacture the transglutaminase enzyme. Cysteine, histidine, and either asparagine or aspartate residues are some of the necessary polypeptides found in their active center (Shimba et al. 2002) (Washizu et al., 1994). The pro-transglutaminase zymogen, which has a signal peptide and a 45-amino-acid pro-region, is the initial expression of the *S. mobaraensis* strain's transglutaminase synthesis (Ma et al. 2019). Then, transglutaminase is activated by a proteolytic process using a 331-amino-acid mature enzyme domain (Yokoyama et al. 2010). According to Lin et al. (2008), this Pro-peptide is necessary for transglutaminases to secrete effectively, fold correctly, and suppress their enzymatic activity.

2.2. The Transglutaminase's Structure

Transglutaminases have a folded 331 amino acid structure that is similar to other proteins seen in nature. The molecular weight and length of the human transglutaminase protein are 77197.64 Da and 687, respectively. According to Huang et al. (2016), transglutaminase has a theoretical isoelectric point of 5.11 (approximately). Several conserved domains, including a catalytic domain, an N-terminal domain, and a C-terminal domain, make up the fundamental structure of transglutaminases. A Rossman-fold found in the catalytic domain of transglutaminase is in charge of the binding of calcium ions and the enzyme's catalytic activity. Additionally, this domain is in charge of identifying the substrates and placing them appropriately for the creation of the isopeptide bond. The substrate-binding site is located in the N-terminal domain, which is also involved in substrate recognition. Since different transglutaminases have unique N-terminal domains that dictate their substrate specificity, this domain is in charge of the transglutaminase's specificity. The manipulation of substrate binding and the control of the catalytic activity are two aspects of how the C-terminal domain contributes to the regulation of enzyme activity. The C-terminal region of several transglutaminases also possesses an important domain in interacting with other proteins or membranes. Overall, transglutaminase's structure is essential to its function since it dictates its specificity, substrate recognition, and activity regulation. Our knowledge of the mechanisms underlying transglutaminase-catalyzed reactions and their potential applications in numerous domains has substantially improved as a result of our growing awareness of the structural specifics of transglutaminases. The following is the human tissue transglutaminase pictorial 3D representation.

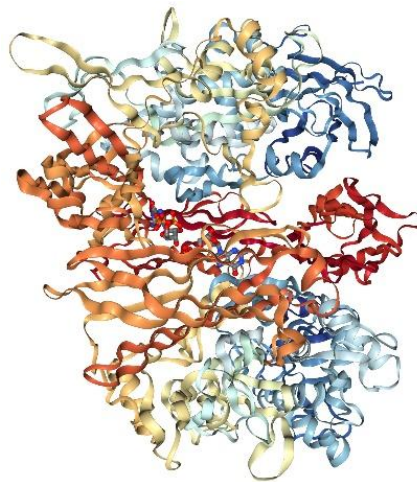


Figure 1. Liu, S., Cerione, R.A., Clardy, J. HUMAN TISSUE TRANSGLUTAMINASE IN GDP BOUND FORM, 2002-03-13

2.3. Enzymatic activity of transglutaminase.

According to Motoki et al. (1998), the pH range between 4 and 9 was determined to be the ideal range for transglutaminase activity. This biocatalyst's ideal temperature for enzymatic activity was 55°C (for 10 min at pH 6.0); 40°C for 10 min would yield its maximal activity, but 70°C would cause it to lose enzymatic activity quickly. It continues to be active at 10 °C, and some of its activity might even increase as we get closer to freezing. In terms of substrate specificity, albumin proteins such as fibrins, myosin, milk caseins, actins, -lactalbumin, and lactoglobulin could be cross-linked, as well as globulins, gluteins, wheat, egg yolks, legumes, and other albumins (Nonaka et al. 1997). Transglutaminase, which has important uses in the food and pharmaceutical industries, bio-catalyzes the transamidation processes. Due to their benefits in transglutaminase, which mediated the post-translational changes of proteins (Savoca et al. 2018) (Placido et al. 2008), deamidation and amine incorporation processes are also catalyzed. According to Pedersen et al. (1994), the transglutaminase reaction mechanism is the opposite of the proteolysis reaction catalyzed by thiol proteases.

2.4. Transglutaminase characteristics

Transglutaminase's physical characteristics, including its molecular weight and secondary structure, as well as its enzymatic characteristics, had already been established. Gel permeation chromatography and SDS-polyacrylamide electrophoresis (SDS-PAGE) measurements revealed that its molecular weight was close to 38,000 kDa and that its isoelectric point (pI) is 9. The Edman approach automates protein sequencing, and mass spectrometry revealed the 331 amino acids' basic structure of proteins (Kanaji et al. 1993). Similar outcomes are obtained when the complementary

DNA from the microbial source is sequenced (Washizu et al. 1994). With one cysteine residue and an estimated MW of 37,842 kDa, transglutaminase is very similar to the experimentally determined value of 38,000 kDa. Despite having two possible glycosylation sites, transglutaminase is a monomeric, straightforward protein like glycoprotein, lipoprotein, etc. (-Thr-Xxx-Asn-)

3. TRANSGLUTAMINASE PRODUCTION SOURCES:

Enzymes are made in a variety of methods, and the sources used to make them were more dependent on the needs and conditions of the products. Today, a variety of organisms, including plant, animal, fish, and microbial sources, are employed to synthesize transglutaminases. The following text provides a quick overview of these several sources for transglutaminase synthesis.

3.1. Plant source:

Transglutaminase is produced by plants, which is a naturally occurring supply. Utilizing the enzyme transglutaminase to create high biological value polypeptides from concentrates of rice, potato, pea, dry wheat gluten, and oat. In addition to being readily available throughout India, these plants are frequently employed as a source to yield plant transglutaminase (Shanthakumar et al. 2022).

3.2. Animal source:

Generally speaking, one of the finest sources for the creation of many enzymes is an animal. One of the animals used to produce transglutaminase is the pig. Using a phenylalanine Sepharose 4B affinity column, guinea pig liver is used in this instance as a bioreactor for the transglutaminase enzyme (Folk et al. 1966). As additional animal sources of transglutaminase, porcine skin, rabbit liver, rat brain, chicken gizzard, and frog liver are also used.

3.3. Fish source:

Sources of fish include Antarctic krill (*Euphausia superba*), Atka mackerel (*Pleurogrammus zones*), Bigeye snapper (*Priacanthus humor*), Botan prawns (*Pandalus nipponensis*), Carp (*Cyprinus carpio*), Cod (*Gadus morhua*), Crayfish (*Pacifastacus leniusculus*) are served as a bioreactor for transglutaminase production, we can also quickly extract this enzyme using a few bio separation approaches (Zhang et al. 2019).

3.4. Microbial source:

Transglutaminase is now produced using a wide range of modern techniques. The discovery of *Streptovercillium mobaraense* producing microbial transglutaminase was made by Ando et al (1989). The production process has been optimized using a variety of techniques, and new strains have been regularly chosen for greater enzyme yield (Zhu et al. 2008). Table 1 provides a summary of current advancements in the manufacture of transglutaminase using *Streptomyces* species and other microorganisms.

S.NO	STRAIN	Procedure	Enzyme activity (unit/ml)	References
1	<i>Streptomyces mobaraensis</i> CECT 3230	Low-cost culture media optimization	2.95	(Guerra et al. 2014)
2	Actinomycetes strains	Strain isolation and identification	0.04	(Mehdi et al. 2021)
3	<i>Streptomyces mobaraensis</i> TX	Purification and characterization of a high-salt-resistant MTGase	1.75	(Jin et al. 2016)
4	<i>Streptomyces</i> sp. D1	Strain isolation and culture media optimization	4.1	(Xavier et al. 2017)
5	<i>Streptomyces</i> sp. CBMAI 1617 (SB6)	Production optimization, enzyme characterization	6.07	(Berger et al. 2018)
6	<i>Bacillus nakamurai</i> B4	Isolation, screening, and optimization of bacterial strains	1.71	(Sorde et al. 2019)
7	<i>Bacillus subtilis</i> C2	Isolation, screening, and optimization of bacterial strains	1.61	(Sorde et al. 2019)

4. PRODUCTION METHODS FOR A TRANSGLUTAMINASE:

Transglutaminase enzymes can be produced in several different ways. The methods may change depending on the source that is chosen. These several sources, along with how they were compiled, are as follows:

4.1. Utilizing plant sources for transglutaminase biosynthesis:

The first stage is to gather acceptable plant tissues from the samples, after which the cold extraction procedure lasts for an overnight period or a whole day. The numerous filtering methods used to filter this overnight sample. The filtrate is then spun at 10,000 rpm for 30 minutes to centrifuge the remaining material. The pellet should be thrown away, and the supernatant should be considered. For purification methods such as gel layer or column chromatography, ion exchange chromatography, etc., this supernatant is acceptable. Finally, transglutaminase, the desired outcome, is obtained (Falcono et al. 1993). This method can also result in the illustration below.

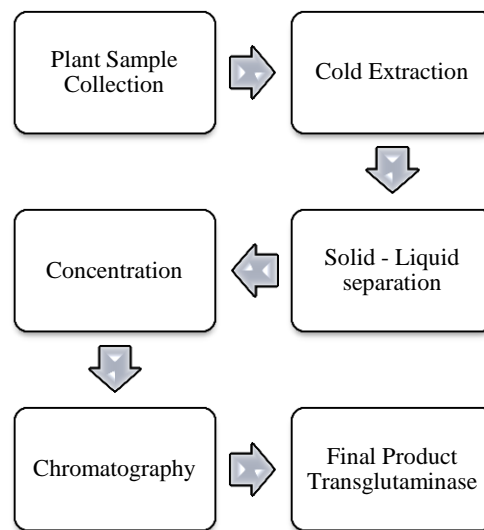


Figure 2, Flow chart for the biosynthesis of transglutaminase using plant source

4.2. Utilizing animal sources for transglutaminase biosynthesis

This procedure involves separating the respected animal tissue from the animal source before homogenizing it in sterile water. The procedure is then centrifuged for 30 minutes at 10,000 rpm. Discard the particle and transfer the supernatant to a fresh container. It passes through ultra-centrifugation once again for 30 minutes at 15,000 rpm. The filtration process then accounts for the supernatant. The filtrate is then exposed to QAE-Sephadex ion exchange in the following stage for further purification. After that, it receives hydroxyapatite adsorption treatment. The transglutaminase enzyme is then isolated after it has undergone ultra-purification using affinity chromatography (Brookhart et al. 1983). This method can also result in the illustration below.

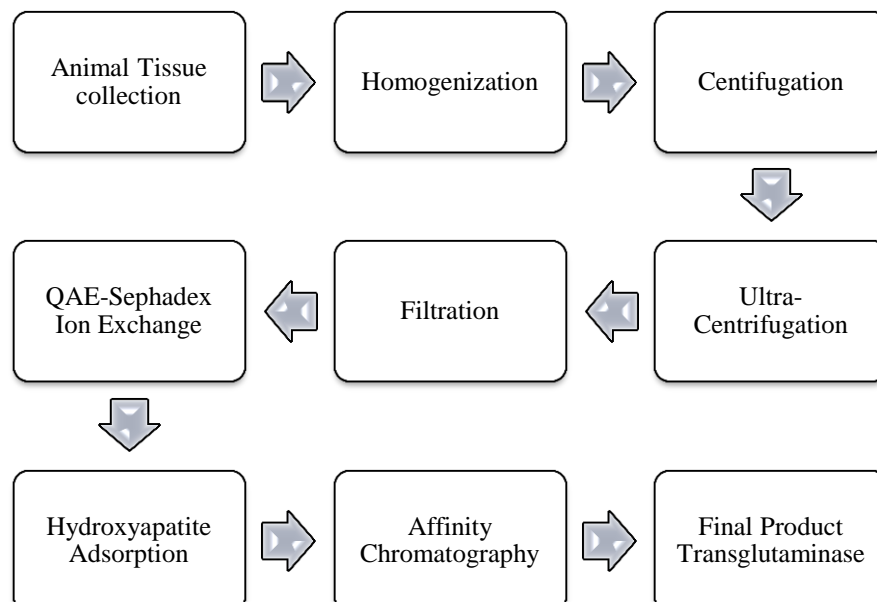


Figure 3, Flow Chart for the Biosynthesis of Transglutaminase using Animal Source

4.3. Using fish sources for transglutaminase biosynthesis

We used the same biosynthetic processes to produce transglutaminase from an animal source as we did from a fish source. Fish tissue is extracted from the specific fish source in the first phase, after which it undergoes a homogenization procedure in sterile water. The procedure is then centrifuged for 30 minutes at 10,000 rpm. Discard the particle and transfer the supernatant to a fresh container. The filtration process then accounts for the supernatant. The filtrate is then treated to column chromatography in the following step to purify the sample. After that, dialysis is used to ultra-purify it. The final two processes are once more performed for additional purification. To obtain the fish transglutaminase, it is finally subjected to ion exchange chromatography (Yasueda et al. 1994). This method can also result in the illustration below.

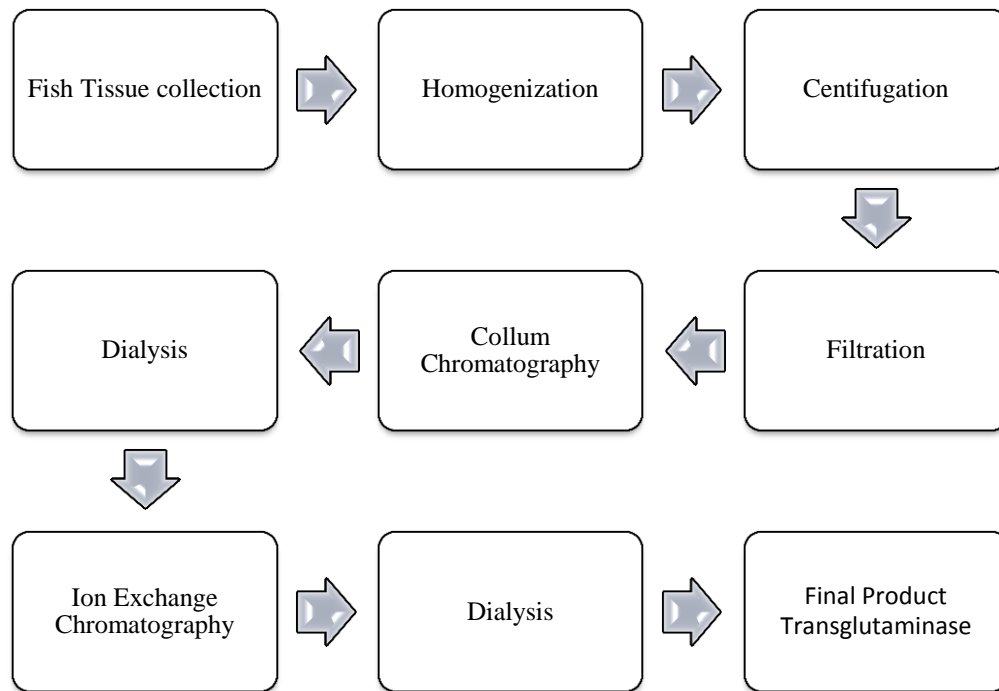


Figure 4. Flow Chart for the Biosynthesis of Transglutaminase using Fish Source

4.4. Utilizing microbial sources for transglutaminase biosynthesis:

The appropriate microbe is fermented in the sterile medium under the necessary circumstances for the microbial production of transglutaminase. The biomass is then removed from the fermenter and centrifuged for 30 minutes at 10,000 rpm. Based on the presence of the product, the procedures are split into two categories after the centrifugation process. The supernatant is considered if the substance is an extracellular product. To purify the transglutaminase enzyme, the supernatant is subjected to ion exchange chromatography (Gerber et al. 1994). If the product was intracellular, the supernatant should be discarded, and the pellet should undergo an ultra-filtration process after the cell disruption procedure utilizing mechanical and non-mechanical techniques. To get the transglutaminase enzyme, the filtrate is next subjected to ion exchange chromatography and gel filtration (Yasueda et al. 1994). Here is the illustration provided for your reference.

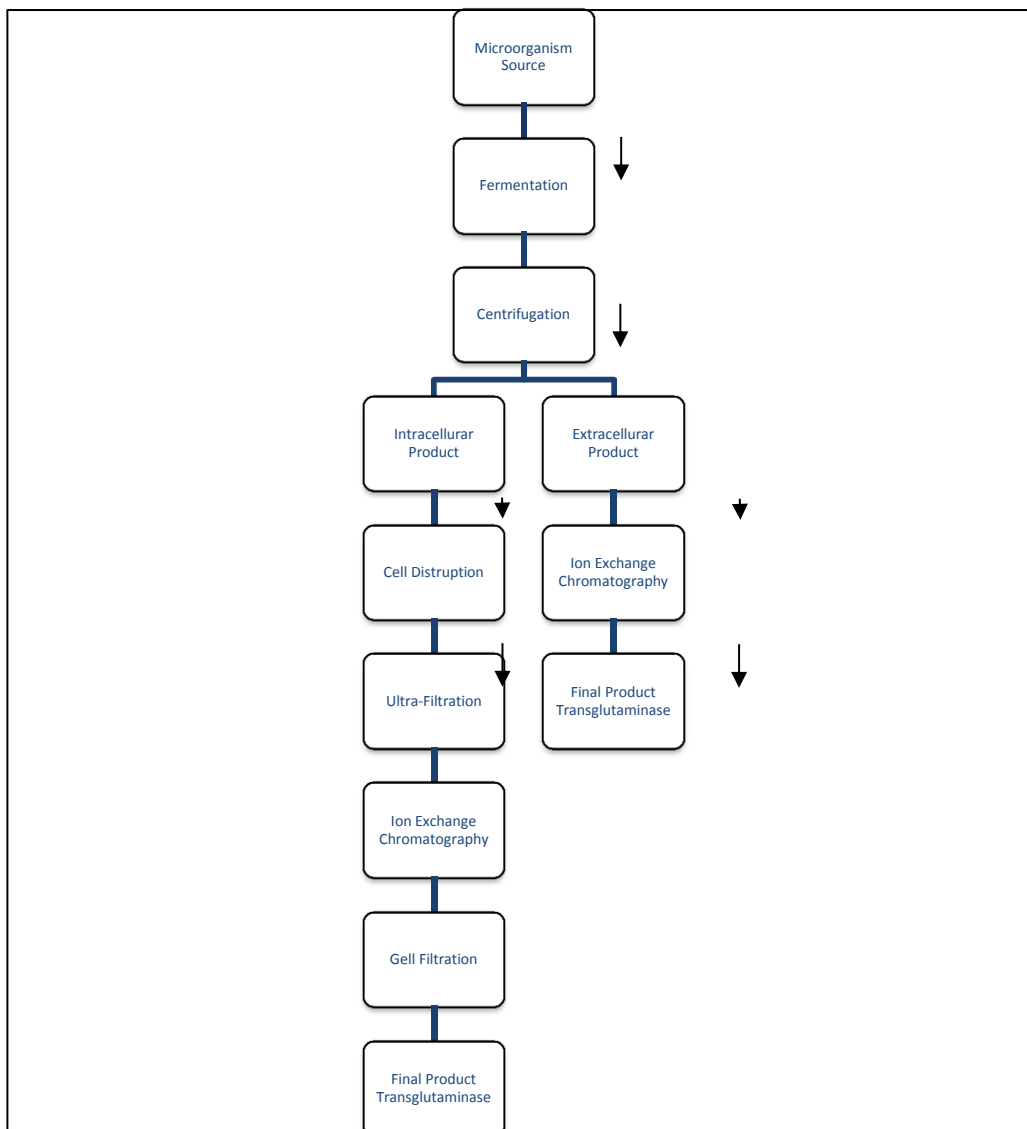


Figure 5, Flow Chart for the Biosynthesis of Transglutaminase using Microbial Sources

4.5. Media optimization by using fish waste:

Media optimization with fish waste is one of the best procedures used in the creation of bioprocesses. Media optimization is the process of improving a product where the media is a key factor. A simple modification in the nutritional content is considered to be media optimization. Media optimization is described as the process of standardizing the media when preparing the synthetic or specified media to raise the product's quality, quantity, and production cost. An expert or engineer will be present when generating biological products on a large scale to standardize or improve the production medium. Additionally, it draws more attention from researchers and scientists. Instead of using any commercial nutrient source, such as basal media, enriched media, selective media, Phenol red agar, tributyrin agar, starch agar, etc., we can use nutrient sources such as fish waste meal to produce transglutaminase enzyme. Numerous vital minerals, including carbohydrates, protein, and lipids, can be extracted from fish waste following serial examination and observation (Maktoof et al. 2020). Fish waste meals are used as growth substrates to manufacture a variety of commercial enzymes, including protease, lipase, chitinolytic, and ligninolytic enzymes (Faouzi et al. 2012).

5. APPLICATION OF TRANSGLUTAMINASE

The transglutaminase enzyme has excellent binding properties in bond formation between simple proteins, so it plays an important role in various food and pharmaceutical industries (Kieliszek et al. 2014). Most research experts strongly recommend the use of transglutaminase in various food processing industries to produce the products such as bread, cheese, meat products, and yogurt.

5.1. Role of transglutaminase in the food industry:

The transglutaminase enzyme contributes to the improvement of the gel strength and texture of meat products, including red meat and chicken meat. via creating Gln-Lys isopeptide linkages in the myofibrillar proteins myosin and actin. According to Herrero et al. (2008), this enzyme has assisted in enhancing some key qualities of meat products, including water binding, cooking loss, gelation, and emulsion stability. In yogurt, the transglutaminase enzyme catalyzes a protein bond formation reaction that results in transverse, cross-linking covalent connections, which have a stabilizing effect. Additionally, the development of a polypeptide chain loop or the cross-linking of neighboring chains also involves this enzyme. By creating a link between lysine and glutamine, this enzyme not only enhances the nutritional and functional qualities of yogurt but also lowers manufacturing costs by reducing the amount of skim milk powder, stabilizer, and even fat used in the formulation (Ziarno et al. 2019). Cheese that has been treated with transglutaminase enzyme forms intra- or intermolecular cross-links between Gln-Lys isopeptide bonds, shortens the coagulation time and reduces interspace volume, all of which increase the finished product's resistance to deformation forces (Metwally et al. 2014).

5.2. Role of transglutaminase in the pharmaceutical industry:

Transglutaminase's function in the pharmaceutical industry has been investigated as a potential pharmacological target for the treatment of several illnesses, including Alzheimer's disease, cancer, and neurological disorders. Transglutaminase has also been looked at for its usage in tissue engineering and drug delivery, where the enzyme can be utilized to crosslink proteins and peptides to create stable hydrogels for long-term drug delivery. PEGylation, the creation of antibody-drug conjugates, tissue engineering, regenerative medicine, and the manufacturing of microparticles for enteric distribution are all areas of investigation (Duarte et al. 2020). The transglutaminase enzyme also functions as a material of interest to the pharmaceutical business.

6. CONCLUSION

Transglutaminase is a versatile enzyme that has a wide range of uses in several industries. Transglutaminase is still a topic of ongoing research and development due to its potential to enhance food quality, advance medicine delivery, and facilitate protein engineering. Environmental and health issues also rise as a result of how seafood processing industries dispose of their trash. Therefore, the waste by-products obtained from fish processing can create better alternatives such as animal feedstock and also energy sources for the microbes that will utilize carbon and nitrogen sources for essential metabolites production. The food and drug industries both rely heavily on the transglutaminase enzyme, meanwhile producing this enzyme has a high production cost, the fish waste meal is one of the greatest alternative energy sources for making transglutaminase, which will eventually lead to the best way to recycle fish waste.

Compliance with ethical standards

Conflict of interest:

The writers state that they are not involved in any conflicts of interest.

Ethics-approved:

None of the writers of this publication conducted any research using human subjects for this article.

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Contribution of the Author:

The entire work has been created by all authors. DR: Concept, Method. MT: Introduction, Transglutaminase Properties. SM: Applications and Tables. GB: Editing and reviewing. The final manuscript was read and approved by each author.

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