# THE USE OF TRANSGLUTAMINASE IN FOOD PROCESSING

**Aleksandr Shleikin, Andrey Gorbatovsky, Nikolay Danilov**

*Saint-Petersburg State University of Low Temperatures and Food Technologies*
*Lomonosov Street 9, St.-Petersburg, 191002, Russia, e-mail: shleikin@yandex.ru*

## Abstract

Enzymes play an important role in the food producing of both traditional and novel products. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. But the traditional products like yoghurt and fermented beverages are performed by endogenous enzymes, that occur naturally in the plant and animal tissues or in the microorganism's cells. The idea of isolated, exogenous enzymes adding to improve existing reactions or to initiate new reactions, dates from the start since second part of past century in the USA led to the development of enzymes for the leather industry and started the commercial production of papain for use in the beer industry. Now there are many enzymes for food processing available that originate from different sources. Majority of applied in food industry enzymes are hydrolases such as glycosidases, and in part proteases used for the meat tenderizing. The new direction is the use of enzymes as a tool for modificaton of protein structure. For this aim are used microbial transglutaminase. It produces the both inter- and intra-molecular isopeptide cross-linking bonds in the proteins. We investigate its substrate specificity to attempt to develop the combined products consisting of the proteins from different sources.

**Key words:** fish, flour, processing, transglutaminase

## Introduction

Enzymes play an important role in the food industry in both traditional and novel products. The ancient processes of brewing and cheese-making rely on enzyme activity at various stages of manufacture. Traditional products like yoghurt and fermented beverages owe their character to enzyme reactions but these are performed by whole organisms rather than isolated enzymes. These changes in traditional products are due to enzymes that are endogenous; that is they occur naturally in the tissues of the plant or animal or in the micro-organism. The activity of endogenous enzymes can be manipulated by optimizing the conditions for enzyme activity (pH, temperature) or by altering the genetic control of enzyme expression. However, there are limitations to the degree of manipulation that can be achieved by these means. The idea of adding enzymes from other sources (exogenous enzymes), to improve existing reactions or to initiate new reactions, dates from the start of past century when was started the commercial production of papain for use in the beer industry. Now there are many enzymes for food processing available that originate from different sources.

When enzymes are considered for use in a food processing, it is essential to ensure that they will confer some commercial benefit. The availability of enzymes on a large scale, and at reasonable prices, the food industry reconsidered the use of enzymes in food processing. At present time, advances in biotechnology in the field of genetic manipulation created new perspectives and the new technology were made. Despite of these technological advances and the numbers of potential applications, the use of enzymes in the food industry is limited: about ten enzymes accounted for 65% of the total revenue and 20 others – for rest 35%. Usually used enzymes are hydrolytic that possessed with splitting action, such as proteases (46%) and carbohydrase (47%) being the most common. We are interested in a new enzyme of controversial action. It is microbial transglutaminase (protein-glutamine γ-glutamyltransferase, EC 2.3.2.13; TG) catalyzes acyl-transfer reactions introducing covalent cross-linkages between proteins, creating high molecular weight polymers.

In the early stages of TG research, attention was focused on the cross-linking of proteins involved in blood coagulation. In a later stage cross-linking of other proteins received more attention. Guinea pig liver TG was the most used enzyme for these cross-linking studies. In addition, partially purified transglutaminase was used from bovine blood (factor XIII) or human placenta. Guinea pig liver TG and factor XIII are calcium dependent, which plays a very important role in the conditions necessary for the cross-linking reactions. The discovery of a $Ca^{2+}$-independent TG isolated from *Streptoverticillium mobaraense* enhanced TG research and utilization in food products, because of the better availability of this enzyme.

In addition to plasma TG, mammal blood contains two other types of this enzyme. One of them is from blood platelets, which is very closely related to plasma TG. The other one is found in erythrocytes and because of that location is called erythrocyte TG. Purification of this TG has been performed from human erythrocytes. However, the relatively small quantities of human blood and its origin make this enzyme uninteresting for large scale applications in food. On the contrary, the large availability of bovine and pig blood from slaughterhouses would make it more practical to isolate TGs from these sources (Govardus de Jong *et al.*, 2001). Using the two types of animal blood TGs and bacterial TG, cross-linking experiments with seven proteins (α-lactalbumin, β-lactoglobulin, BSA, casein, hemoglobin, myosin, glycinin) these authors showed large differences in substrate recognition and in cross-linking rates.

Bacterial TG showed the lowest substrate specificity, as it was able to cross-link all seven proteins tested. However, cross-linking of BSA and α-lactoglobulin is observed only after reduction of the disulfide bridges by dithiothreitol (DTT), which will promote the unfolding of the protein. The unfolding of BSA and α-lactoglobulin will increase the accessibility of glutamine and lysine residues for the cross-linking reaction.

Erythrocyte TG was able to cross-link BSA, casein, and glycinin, indicating a higher substrate specificity for this enzyme. The presence of DTT was necessary for these cross-linking reactions, which was confirmed in the fluorescence assay. However, the latter assay uses monodansylcadaverine and dimethylcasein as substrates, so no disulfide bridges are present. Consequently, not only should the role of DTT be ascribed to that of an agent reducing disulfide bridges, but DTT plays a role in the reduction state of cysteine in the active site of erythrocyte TG. Plasma TG does not need DTT for its activity, as can be deduced from the observed cross-linking of casein, hemoglobin, and myosin in the absence of DTT. In some cases such as α-lactalbumin and glycinin, the presence of calcium in the cross-linking buffer caused a solubility problem. α-Lactalbumin and glycinin were much more quickly cross-linked by bacterial TG without calcium than in the presence of calcium. This means that cross-linking of these proteins by the two blood TGs can be inhibited by the decreased solubility in the presence of calcium. This study shows that the erythrocyte TG has the highest substrate specificity, whereas the bacterial enzyme has the lowest specificity. An intermediate specificity is exhibited by the plasma TG.

These differences between types of TGs should be ascribed to the roles of these enzymes in their concomitant natural processes. The fact that these enzymes are able to cross-link proteins different from their natural substrates means that they can be used in several applications for which enzymatic protein cross-linking is desired instead of chemical cross-linking. Applications may be aimed toward the development of protein polymers with modified functional properties but also to direct applications in complex systems, such as foods. Depending on the number and types of proteins in an application and the need for specific cross-linking of particular proteins in such an application, one can select the most suitable TG.

With respect to applications related to food products or protein ingredients, the cross-linking of the described substrates looks promising, especially as it is known that cross-linking of protein can have substantial effects on functional properties, for example, gelling capacity, emulsifying capacity, and solubility. The possibility of using different types of TGs for the desired effect can be interesting, especially because the rates and numbers of cross-links produced will differ depending on the type of enzyme used. The problems concerning the erythrocyte TG regarding self cross-linking, the necessity of using a reducing agent, and the difficulties in the purification process will narrow the possibilities of this enzyme. Plasma TG offers better possibilities, although purification to a homogeneous enzyme preparation may not be necessary. A good example of the use of a partially purified plasma TG is in the area of meat processing, for which plasma TG is used in combination with fibrinogen to form a system that enables cross-linking of meat parts. Bacterial TG shows the lowest substrate

specificity and offers the greatest possibilities in cross-linking of protein ingredients. Cross-linking of proteins with this enzyme is favored because of its independence from calcium, which can be beneficial when proteins are to be cross-linked because the solubility is negatively influenced by the addition of calcium.
The substrate specificity of TGs for primary amines was investigated to incorporate various functional groups into proteins and peptides. In next study, microbial and guinea pig liver TGs were used. For the primary amines to be incorporated into benzyloxycarbonyl-L-Gln-Gly (Z-Gln-Gly), they were required to have more than four carbon chains without side chains between the functional groups. These results suggest that with appropriate primary amines as spacers, various functional groups, carboxyl groups, phosphate groups, saccharides, and so on, can be incorporated into proteins by using TGs (Ohtsuka *et al.*, 2000).
Generally, protein substrates of TG are classified into four groups: (1) Gln-Lys-type, in which both Gln and Lys residues are available for crosslinking; (2) Gln-type, in which only the Gln residue is available for reaction; (3) Lys-type, in which only Lys residues are available; and (4) a nonreactive type, in which both Glu and Lys residues are unavailable for reaction (Ikura et al., 1984). This classification is mainly based on the accessibility of Lys and Gln residues located on the protein's surface. According to the above classification, a mixture of two Gln-Lys-type substrate proteins or a mixture of Gln-type and Lys-type substrate proteins should be able to form heteroconjugates in TG-catalyzed reaction. However, in addition to the availability of Lys and Gln residues, another factor that could potentially affect cross-linking of two different macromolecular protein substrates is the thermodynamic compatibility of mixing of the protein substrates at the enzyme's active site (Xiao-Qing Han and Srinivasan Damodaran, 1996).

**Materials and Methods**
As a fish raw material it has been used by a fillet and farce of bream, pikes, a pike perch and small fry and, as vegetable additives - a texturized flour of peas, rice, a buckwheat and corn. The range of a mass fraction of the brought vegetable component from 5% up to 15% was considered. The used enzyme was TG preparation “Activa EB " manufactured by firm Ajinomoto Co's, Japan. Processing by enzyme spent addition of a solution of TG preparation to fish farce at a stage of farce formulation (the mass fraction of enzyme made 0.2–0.5 %, therefore, for its careful distribution in a product the solution was used). As basis of a composition the fish farce received by crushing of a fish fillet or mechanical deboning of fish raw material used. Additives entered in various percentage parities to weight of fish farce. There were following kinds of products and control variants of each kind have been investigated. Samples of half-finished products with entering of 5%, 7.5%, 10%, 12.5% and 15% of a flour to weight of fish farce and samples with simultaneous entering flours and 0.2, 0.3, 0.4 or 0.5% of enzyme. The farce maturation was for 6 hours by +4 °C. Definition of the module of elasticity was made by means of automatic consistency gage. Action of this is based on measurement of a degree of compression (squeezing) of a punch on sample by constant loading (100 g) during certain time (5 s). Measurements were spent three times, thus the things in common of a punch with a fish each time were displaced. The final result was calculated as an average arithmetic of three significances. Calculation spent to within 0.1 mm.

**Results**
We have investigated the application of TG in the production of fish cutlets with vegetable flour. Experimental party of cutlets is got, each of which contained from 0.2 to 0.4% of TG preparation. Control samples, unlike proper experimental, did not contain TG. After bringing of all necessary additives both control and experimental samples were maintained at 0° C over day. Control samples had a friable pastelike consistence, and experimental - dense, elastic. Deformation of the investigated samples decreased proportionally to concentration of enzyme preparation: a maximum in 3.8 times. The best results have been received at use of a pea flour

as the vegetable component. The result no significantly depends on a kind of the used fish. The strongest influence of enzyme on samples with a pea flour speaks about more close affinity of peas amino acids to enzyme than of other flours. The optimum quantity in such compounds makes 0.25% of enzyme and 15% of a pea flour. In the lead experiments the tendency to increase in elasticity of a product and the general hardening of a consistence is traced at increase in TG mass fraction. The increase in TG mass fraction in a product promotes condensation of its structure, increase in percentage of fiber in a finished article and to increase in food value of a product. However, after achievement of the certain value of elasticity (in this case - at a mass fraction of TG equal 0.25%), the further increase of TG is inexpedient in view of significant growth of the price of a ready product. Considering all half-finished products with TG concerning the control sample, the increase food and sensor values of these products are noted. From all points of view, at observance of optimum quantity TG, a product more racks to deformation, the consistence and appearance is better, than at control samples. From the economic point of view, addition of TG slightly increases the cost price of finished products.

## Conclusion

One of the problems of meat and fish processing is utilization of crushed waste. Application of TG, which creates more large molecules from proteins by their cross-linking, allows to solve this problem. TG forms from the crushed meat a monolithic piece of the set form which after freezing and thermal processing gives the juicy product which is not differing on the parameters from a product, made from an integral piece of a beef or fish. The same enzyme preparation is used for reception of a “fillet” from fine scraps of a red fish. By means of TG is delivered also fermentative processing of proteins waste of an animal origin for giving cohesion and durability to a product, for example by manufacture of animal and vegetable row materials. By our results TG is more effective in connection of fish to pea proteins and it may be used in the next research work. Thus, TG application in food technology promotes improvement of their quality indicators, and also can serve for manufacturing of new kinds of food products. Use of TG in manufacture of fish products enables to receive from fish steady systems with the expressed elastic-plastic properties, to lower quantity of production wastes and to increase an yield of finished goods. Purposeful application TG in a complex with protein-containing additives, promoting the re-structuring of disperse food systems and output of food products with the set properties, is perspective and demands the further studying.

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