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Microbial Transglutaminase and Applications in Food Industry

*Marek Kieliszek** and *Stanisław Błażejak*

1. Introduction

Transglutaminase (TGase) belongs to the transferase class of enzymes (EC 2.3.2.13) and is the common name of protein-glutamine γ-glutamyltransferase (Ando et al., 1989). The term TGase was first introduced by Mycek et al. (1959) to differentiate the enzyme that catalyzes transfer reactions involving the glutamine carboxamide group from other enzymes catalyzing transfer or hydrolysis reactions of amide groups, wherein free glutamine, such as γ-glutamyltransferase participates.

Transglutaminase participates in the reaction that catalyzes the formation of isopeptide bond between glutamine residue of γ-carboxamide (donor) and primary ε-amine groups of various compounds, for example, proteins (acyl residue acceptor) (Fig. 1a). The result of this reaction is the transfer of acyl group from one substrate to the other, accompanied by the release of ammonia. This reaction enables attachment of hydrophobic or hydrophilic groups to a protein particle. When the acyl acceptor is lysine, the protein becomes enriched with this amino acid. Acyl transfer to the lysine residue bonded in a polypeptide chain results in cross-linking, that is, formation of inter- or intramolecular cross-links of ε-(γ-glutamylo) lysine (Fig. 1b) (Kieliszek and Misiewicz, 2014). The isopeptide bonds thus formed contribute to the formation of stable protein networks, which enable the formation of inter- and intramolecular cross-links. These inter-protein bonds lead to the formation of high-molecular-weight polymers, resulting in the occurrence of the process of aggregation or even gelation. Intramolecular bonds are characterized by a compact structure with reduced hydrodynamic radius (Djoullah et al., 2015).

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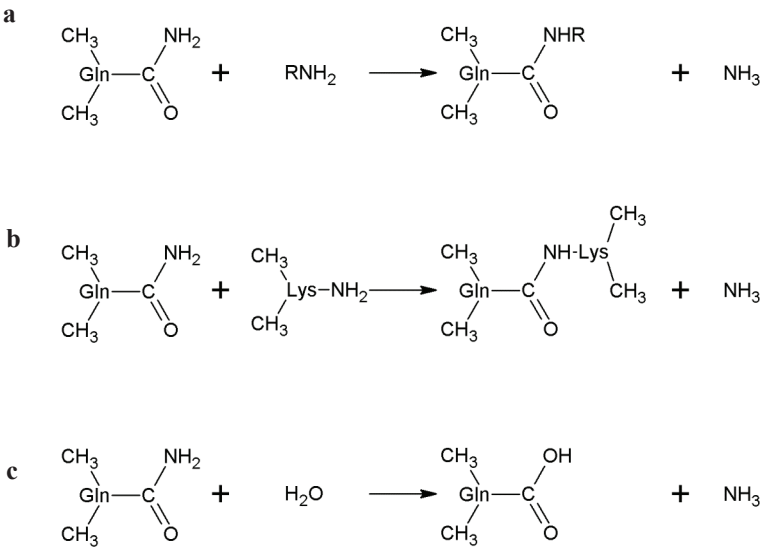

Figure 1. Reactions catalyzed by transglutaminase (TGase): (a) acyl-transfer reaction; (b) cross-linking reaction between Gln and Lys residues of proteins or peptides (c) deamidation.

When free amine groups are lacking, TGase participates in a deamination reaction (Fig. 1c) (Kuraishi et al., 2001). The reactions catalyzed by this enzyme bring about significant changes in the physical and chemical characteristics of proteins. These changes consist in the change of viscosity, thermal stability, elasticity and flexibility of a protein (Kieliszek and Misiewicz, 2014). Independent of the deamination or polymerization processes, the use of TGase affects the structure of protein systems. This causes changes in their functional properties during the process of production of different food products characterized by innovative textural properties (Gaspar and de Góes-Favoni, 2015).

2. Transglutaminase Characteristics

Transglutaminase (EC 2.3.2.13) is a natural enzyme commonly found in animal tissues and intercellular fluid (Fig. 2). Initially, it was presumed that a single enzyme is responsible for TGase activity; but today it is known that such activity is exhibited by a whole group of enzymes that show similarity in the type of the reactions catalyzed, but possibly differ in substrate specificity, expression method and physiological regulation. Microbial transglutaminase (mTGase) was isolated for the first time in 1989 from a strain of *Streptoverticillium* sp. The enzyme is a single-chain protein with molecular weight of approximately 38 kDa built of approximately 331 amino acids (Duran et al., 1998; Pasternack et al., 1998; Yokoyama et al., 2004). Active center of TGase is constituted by the residue of cysteine, histidine and aspartic acid or asparagine. Numerous factors affect the enzymatic activity of TGase. Maximum activity is exhibited at a temperature of approximately 50ºC and acidic pH of 5–6. Enzyme inactivation takes place after 5 min. at a temperature of 75ºC. The optimal

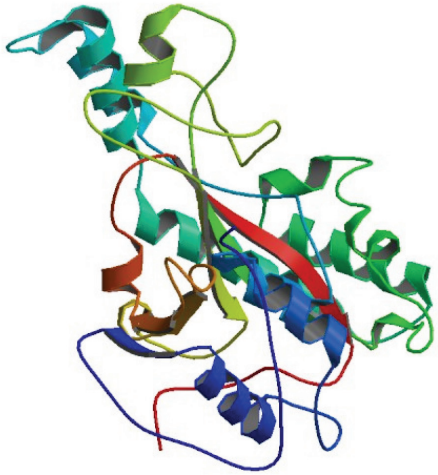

Figure 2. Structure of microbial transglutaminase from *Streptoverticillium mobaraense* strain (Kashiwagi et al., 2002; Kieliszek and Misiewicz, 2014; www.rcsb.org, with permission).

temperature favoring catalytic activity of TGase isolated from the *Streptoverticillium ladakanum* strain is 40°C (Ho et al., 2000). One of the exceptions is TGase isolated from *Streptomyces* sp., which acts most efficiently at slightly higher temperature of 45°C. At temperatures close to zero, the enzyme maintains its full activity (Kieliszek and Misiewicz, 2014; Yokoyama et al., 2004).

Presence of maltodextrin, sucrose, mannose, trehalose and reduced glutathione (GSH) in the media increases the thermal stability of the enzyme (Cui et al., 2006). Casein can protect TGase against degradation of extracellular proteolytic enzymes (Junqua et al., 1997). One activity unit of mTGase is defined as the amount of enzyme necessary for the formation of 1 μM hydroxylamine acid in 1 min. at a temperature of 37ºC.

Enzymes synthesized by bacteria are stable in a broad range of pH from 4,5 to 8,0. In addition, contrary to TGases of animal origin, mTGase does not need calcium ions for activation. This characteristic is particularly desirable in practical use for an enzymatic preparation. TGase activity is higher in the presence of Co^{2+}, Ba^{2+}, and K^{+} ions. Inhibitors of TGase activity include Zn^{2+}, Cu^{2+}, Hg^{2+}, and Pb^{2+} ions, which bind to the thiol group of cysteine found in the active center (Kieliszek and Misiewicz, 2014; Macedo et al., 2010).

Transglutaminase catalyzes the cross-linking reactions in proteins, leading to a stabilizing effect due to the formation of covalent bonds, which possess characteristics different to peptide bonds. Under the effect of this enzyme's activity, disulfide bridges stabilize the structure and improve the stiffness of a molecule. They also participate in cross-linking of neighboring chains or in the formation of polypeptide chain loop. This leads to changes in protein conformation; hence in modification of structure, gelation stability and water-binding ability, which, as a consequence, results in changes of rheological properties of protein products. These characteristics, along with the fact that the scientific community recognized the enzyme in 1998 as a safe substance

(it has the GRAS status—Generally Recognized as Safe) by the FDA (Food and Drugs Administration), makes it a very attractive product for the food industry (Gaspar and de Góes-Favoni, 2015).

3. Transglutaminase Production Technologies

Developments of biotechnology in recent years enabled biosynthesis, isolation and improvement of enzymes that have a large significance in different branches of the food industry. Solutions for many previous technological problems have been found and ways for exciting possibilities have been established. In the 1970s, in Guinea, a technology of TGase production from the liver of guinea pig was developed (from 1 kg of liver only 230 mg of pure enzyme was obtained) (Folk and Chung, 1973; Kuraishi et al., 2001). However, due to the source of the enzyme and relatively expensive isolation and purifying method of the enzyme, it constituted an obstacle for its wide use in the industry. Modern science provides a wide spectrum of possibilities in the areas of biotechnology, enzymology and enzyme use at industrial scale. Researchers (Liu et al., 2014; Yu et al., 2008) were searching for new sources of the enzyme, which would facilitate the process of production of various food products. Scientists hope to develop better and less expensive sources of the enzyme with the use of microorganisms that will help to utilize their biotechnological abilities in human economy.

Transglutaminases are commonly used in the modern food technology. Their properties can be used to the benefit of both the food industry as well as the consumer. Selectivity of their effect improves control of product formation, while high efficiency and low energy requirements are beneficiary for the environment.

Microbiological media used for *Streptoverticillium* cultivation are not attractive from the economic point of view due to the requirement of high amount of expensive nutritional components, such as yeast extract and peptone (Téllez-Luis et al., 2002, 2004). Glucose, sucrose, starch and glycerol in the role of carbon source can be used for TGase biosynthesis. Literature provides information on the possibility to use raw materials of plant origin as nitrogen source, such as NH_4NO_3, $(NH_4)_2SO_4$, urea, $NaNO_3$, NH_4Cl, soybean, corn, wheat or wheat flour, rice, bran, corn steep liquor, essential minerals, and trace elements like phosphate, magnesium, potassium, iron, copper, zinc, and vitamins (Zhu et al., 1995). Numerous studies (Herrera et al., 2003; Tellez-Luis et al., 2002) demonstrate the issues involved in the use of agricultural waste as carbon source in TGase production. Xylose is a hemicellulose sugar, which can be used as a potential carbon and energy source for the growth of microorganisms. The interest in the use of xylose as a source of carbon for bacteria proliferation can be even greater, if the media are prepared using inexpensive raw materials, such as hemicellulose hydrolysates like sorghum straw (Téllez-Luis et al., 2002; Kieliszek and Misiewicz, 2014).

Peptone, yeast extract and casein are common nitrogen sources used in TGase biosynthesis (Ando et al., 1989; Gerber et al., 1994; Zhu et al., 1995). Ammonium salts turned out to be less favorable sources of nitrogen (Zhu et al., 1995). Bourneow et al. (2012) demonstrated that peptone turned out to be the best nitrogen source for TGase

production from *Streptomyces* sp. P20 and *Streptoverticillium mobaraense* strains. Zhu et al. (1996), conducting a study on the optimization of medium composition, determined that introduction of additional compounds in the form of a proper set of amino acids to the medium containing peptone as the main nitrogen source caused a significant increase of TGase production by *Sv. mobaraense* strain (Kieliszek and Misiewicz, 2014).

Amino acids play an important role in the synthesis of mTGase. Precise mechanism of biosynthesis has not yet been entirely understood and this stage requires further study. Use of non-modified peptides limits synthesis of the enzyme by microorganisms (Zhu et al., 1995).

Microbial transglutaminase is an extracellular enzyme exhibiting the ability to dissolve in the culture medium. Methods commonly used in the process of enzyme purification are also used for the purification of mTGase from the medium. Examples include ethanol, acetone and isopropyl alcohol. Methods used for the purification of mTGase include ammonium sulfate, sodium chloride, dialysis processes, ultrafiltration, ion exchange chromatography, gel filtration, absorption and a method based on the isoelectric point analysis. Oftentimes, enzyme purification requires the use of more than one method and sometimes there is a need for combining some of the processes, which, as a consequence, may increase the mTGase recovery effect.

The process of expression and purification of TGase was performed using the following strains: *Streptomyces lividans* (Lin et al., 2004; Washizu et al., 1994), *Escherichia coli* (Yokoyama et al., 2000) and *Corynebacterium glutamicum* (Date et al., 2004). Currently, the enzyme is produced using *Streptomyces mobaraense* or *Bacillus subtilis* (Liu et al., 2014). Recently, a study was conducted on the production of recombinant TGase from the strain of *E. coli* (Yu et al., 2008). However, it should be emphasized that the processes of TGase translation as an active enzyme can have disastrous effects on microorganisms. Induction of mTGase in *E. coli* cells containing synthetic gene coding for the enzyme can influence inhibition of cell growth, followed by cell lysis. Cell death is apparently caused by the occurrence of cross-linking reactions between cytosol proteins in the presence of TGase (Pasternack et al., 1998).

The enzyme thus obtained can be bonded with enzyme stabilizers, such as various salts, sugars, proteins, lipids or surfactants (Zhu et al., 1995) in the subsequent stages. In the context of the above applications, it is justified to create more effective TGase production systems, which would be used in the food industry. The published literature does not provide many studies on elaboration of a commercial production process, improvement of the procedure, or efficiency of the whole enterprise related to the production of TGase.

In order to improve TGase production efficiency, studies were conducted on heterologous expression of recombinant genes responsible for mTGase production in the following microbes: *E. coli*, *S. lividans*, *C. glutamicum* and methylotrophic strains of yeast. mTGase isolated from the strain *Streptomyces* is naturally synthesized in the form of a zymogen (pro-TGase) which is then processed to obtain its active form by deleting its N-terminal peptide. Liu et al. (2015) conducted a study on the transformation of cloned gene from the strain *Streptomyces hygroscopicus* responsible for pro-TGase production to yeast strain *Yarrowia lipolytica* Po1h. The study demonstrated that the strain *Y. lipolytica* Po1h, which has vector pINA1297,

when cultivated on a medium with glycerol in a 3 L bioreactor, exhibited enzymatic activity of TGase (N355Q) of 35,3 U/mL, whereas mTGase activity of the enzyme obtained from the wild strain amounted to only 5,3 U/mL. This study provides new possibilities for the production of more efficient mTGase with increased enzymatic activity, which can be used in food processing.

Lin et al. (2007) elaborated a rapid and relatively simple system for purifying recombinant TGase from the strain *S. lividans* 25-2. Purification of TGase was conducted on a laboratory scale, resulting in the enzyme with a purity of 90–95 per cent and with the enzymatic activity of 61–65 U/mg. The technique followed for enzyme purification in this study ensured high recovery. Data presented by these authors proved that the recombinant mTGase activity was 3,3 times higher than that obtained from the strain *Streptomyces platensis* M5218 (Lin et al., 2006). In other strains, such as *S. lividans* 25-2 (78,2 mg/L), the obtained efficiency was significantly better than that observed for *S. lividans* 3131-TS (< 0,1 mg/L) (Kieliszek and Misiewicz, 2014; Washizu et al., 1994).

A study by Junqua et al. (1997) demonstrated that the addition of casein to the medium containing 38,4 g/L and 31,2 g/L of glycerol increased mTGase production by three times (0,331 ± 0,038 U/mL) by the strain *Streptomyces cinnamoneum* CBS 683.68. On the other hand, Itaya and Kikuchi (2008) demonstrated that strains of *Corynebacterium ammoniagenes* were able to synthesize more amounts of the enzyme TGase than the bacteria *C. glutamicum*, which are commonly used for the biosynthesis of the enzyme (Yokoyama and Kikuchi, 2004). These species are widely used in commercial production of amino acids, such as lysine and glutamate, which are used in the food industry.

Transglutaminases, due to their ability to modify physical and chemical properties of proteins, are used in a variety of ways in different branches of the industry. For large-scale production of TGases, the bacterial expression system of *S. mobaraense* is primarily used. However, the system has its disadvantages, that is, it has problems related to the post-translational protein modification (Griffin et al., 2002). In the context of the above limitations, studies on a considerably less expensive and more efficient system should be conducted to allow a reduction in the costs related to distribution, storage, extraction and purification of the recombinant proteins (Kieliszek and Misiewicz, 2014).

Microbial transglutaminase is used as a biological glue in many fields devoted to biomedicine and biotechnology. For continuous development in this field, continuous enrichment of the knowledge on future aspects of the use of the enzyme in other industrial branches is indispensable; most likely it is one of the most challenging areas of study in the field of biochemistry and biotechnology. Constant development of new technologies for biosynthesis of the enzyme and in the field of molecular biology can lead to increased TGase production, which eventually may lead to direct distribution of the enzyme through many food products. Such efforts enable adjustment and creation of the needs of the market and economy. Among a variety of factors that contribute to the development, a very important role is played by the consumer and his needs. By 2020, a further increase in TGase consumption is anticipated from 0,22 to 0,32 times a year, where daily mTGase dosage may amount to 15 mg. TGase use in the restructuring or dosage amounts to approximately 50–100 mg of the enzyme per one

kilogram of food (Lerner and Matthias, 2015). The great scale of interest in mTGase is reflected in the increasing number of patent applications on biosynthesis and use of the enzyme in various food products.

4. Use of Transglutaminase in the Food Industry

Transglutaminase preparations have potentially wide spectra of use (Fig. 3). They are very popular due to their use in the food industry for protein cross-linking (Buettner et al., 2012; Giosafatto et al., 2012; Kashiwagi et al., 2002; Pinterits and Arntfield, 2008; Zheng et al., 2002). The other field of use is represented by protein production, for example, casein films (Buettner et al., 2012; Dong et al., 2008).

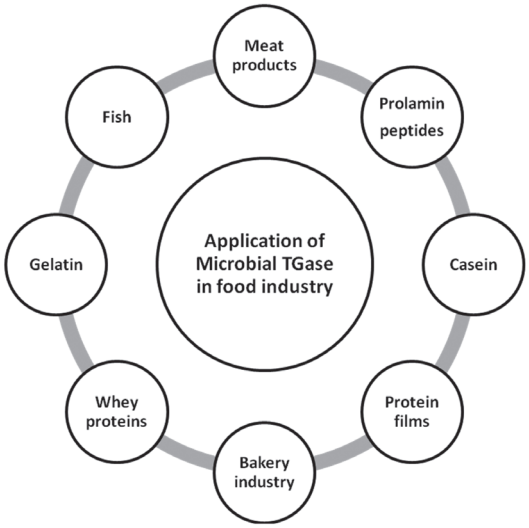

Figure 3. Transglutaminase and its use in food processing.

Transglutaminase can affect various food characteristics, such as texture, solubility, viscosity, gelation capacity and water retention capacity, which are reflected in various textural properties of the obtained food products (Table 1). TGase turned out to be very useful in this technology as it acts in a different direction than the majority of enzymes used in the food technology—it does not divide the media into smaller subunits but creates larger particles from the protein units *via* the cross-linking reaction and bond formation. Strong cross-linking in food products (i.e., pieces of meat) is maintained even during the process of cutting, freezing, or heating (Gaspar and Goes-Favoni, 2015; Lerner and Matthias, 2015).

The obtainment of products with characteristic quality is possible due to modifications of the food components, with both chemical as well as enzymatic methods. TGase of microbial origin (mTGase) belongs to the group of enzymes on which the food industry is currently focused. TGase forms larger particles from small

Table 1. Application of microbial transglutaminase in food processing.

Source	Product	Effect	Reference
Casein	Cross linked protein, mineral absorption promoters	Improved mineral absorption in intestine	Noguchi et al. (1992) Lauber et al. (2000) Ozer et al. (2007)
Milk	Cream, desserts, milk drinks, dressings	Improved quality and texture	Lauber et al. (2000) Şanlı et al. (2011)
Meat	Restructured meat, hamburger, meatballs dumplings canned meat frozen meat	Restructured meat texture, appearance, increased hardness	Zhu et al. 1995 Kuraishi et al. (1997) Motoki and Seguro (1998) Trespalacios and Pla (2007)
Wheat	Baked foods	Improved texture and high volume	Gerrard et al. (2001)
Gelatin	Sweet foods	Low calorie foods with good texture and elasticity	Giosafatto et al. (2012)
Fish	Fish paste restructured product	Increased hardness	Téllez-Luis et al. (2002)
Fat, oil	Solid fats	Pork fat substitute with good taste, texture and flavor	Zhu et al. (1995)
Soya bean	Mapo doufu tofu	Improved shelf-life and texture	Zhu et al. (1995) Kato et al. (1991)
Collagens	Shark-fin imitation	Imitation of delicious food	Zhu et al. (1995)
Plant proteins	Protein powders	Gel formation with good texture and taste	Zhu et al. (1995)

protein units *via* the reaction of cross-linking and bond formation. Use of TGase in the production of meat products influences the improvement of texture and color and some of the sensory quality factors of the food product.

4.1 Increasing shelf-life of fish products

For commercial purposes, the enzyme is produced through microbiological culturing of *S. mobaraensis*. mTGase is significantly cheaper, with activity comparable to that obtained from the animal origin. Examples of food products obtained with the use of mTGase are fish balls and fish fingers, surimi and products imitating crab meat and shark fins (Tani et al., 1990). Fish paste products are obtained from material containing primarily fish meat and 0,1–700 U TGase/g of fish proteins. The final product can be kamaboko, a Japanese fish paste, characterized by good texture and white color (Zhu et al., 1995).

One of the methods of improving shelf-life of the food product is by increasing the number of covalent bonds of the food matrix. In the case of products containing proteins, it can be obtained using TGase. This enzyme turned out to be useful in the production of various protein components and food products. Proteins are good substrates for TGase. In the production of surimi, stiff gels are produced by cross-linking of fish proteins (Benjakul et al., 2001). Gels can be produced from dissolved and dispersed proteins, colloidal systems, proteins covering interface surfaces, such as oil–water and air–water interfaces (Dłużewska and Florowska, 2014). Furthermore,

Kołodziejska et al. (2004) determined a positive effect of mTGase on gelating properties of gelatin from cod skin. The influence of mTGase on the durability of fish meat gel depends on the denaturation and degradation of the myofibrillar proteins (Jiang et al., 2000). Therefore, further studies on determining the efficiency of mTGase bonding in the products of fish muscles are necessary, an example of which can be Nile tilapia (*Oreochromis niloticus*) (Monteiro et al., 2015).

4.2 In production of tofu

The use of mTGase in the production of tofu is an example of practical use of the enzyme (Kato et al., 1991). Tofu is soybean curd produced in the process of soy milk coagulation. TGase added to soy milk with a coagulant (nigari) produces tofu with more compact texture and slows down the process of coagulation, facilitating its control. The ability to gelate tofu from old beans gets worsened by adding TGase. It is possible to obtain the same gel as with the use of young soy beans. It was determined that soy protein hydrolysates when subjected to enzymatic polymerization maintained their solubility in spite of being built of fractions with higher molecular mass as compared to the native protein. Furthermore, emulsifying and foaming properties of these proteins were improved with simultaneous elimination of sour flavor of the hydrolysate (Gaspar and de Góes-Favoni, 2015). Gels produced with the participation of TGase are more elastic and tougher than the thermally induced gels. Thus, a possibility exists to control their texture *via* the change of enzyme dozing method and production conditions. The addition of sodium chloride to soy protein isolate gels significantly decreases the value of breaking stress, whereas the addition of even 3 per cent salt to gels produced by TGase does not change their properties (Dłużewska and Florowska, 2014). Mapo-doufu (tofu, traditional meal of the Sichuan province in China) produced with the addition of mTGase exhibits good texture, taste and appearance even after six months of storage at 25°C and relative humidity of 60 per cent. Other methods to improve taste, texture, appearance and shelf-life were proposed by Nonaka et al. (1990).

In the food industry, endogenous animal TGase or TGase preparations of animal and microbial origin can be used. Enzymes of animal origin depend on the presence of metal ions, for example, calcium. In the absence of metal ions, the enzyme acts inefficiently or does not exhibit any activity at all. Metal ions not only influence the enzyme activity but also stabilize its structure; they may also be involved in the activation or inhibition of the activity. However, it is not feasible to add calcium salts to all the food products, particularly at concentrations required by the enzyme; mTGase does not require the presence of calcium ions. Therefore, production of commercial mTGase preparations significantly broadened the possibilities of its use in the food industry. Since 1989, TGase isolated from *Streptoverticillium* sp. has been used.

4.3 Production of restructured meat

Transglutaminase is used in the production of restructured meat (Monteiro et al., 2015). The use of TGase preparations—apart from positive effect on the texture of the finished product—enables strong binding of a meat block without the need for heat treatment and without the addition of common salt or phosphates (Kuraishi et

al., 1997). Technological creation of characteristic quality of meat products depends mainly on the type and amount of raw components used and on the addition of different functional substances, like, salts (Hong et al., 2014). Phosphates are responsible in increasing water absorption by the muscle tissue to minimize fat leakage and to improve the binding properties of the finished product. A decrease in salt or phosphate addition to sausages leads to obtaining a product with lower water absorption and decreased texture and consistency. Addition of TGase is an alternative for the use of these additives in the meat product technology. Regardless of favorable technological properties, the use of phosphates in meat processing may raise many health concerns. The main issue may be their effect on human calcium metabolism (Pyrcz et al., 2014). TGase enzyme preparations produce similar effects on phosphates, particularly in the area of improved binding properties and consistency in meat preparations. The addition of TGase, at lower concentrations, to sausages eliminates unfavorable changes because bonds formed by the enzyme are thermostable, which as a result leads to the improvement in texture of preserved food products.

Sakamoto and Soeda (1991) elaborated a method for manufacturing food products containing minced meat with TGase. Minced meat and other food components were mixed with TGase and then packed in pressure-resistant containers. Hamburgers, meat balls and dumplings can be the final products. Manufactured food exhibits improved elasticity, texture and more intense taste and smell. Similar methods of TGase use in meat products can be found in the literature (Seguro and Motoki, 1991; Zhu et al., 1995).

The use of TGase in the meat-processing industry allows improvement in the texture of the finished product, which is exhibited by improved hardness. The texture of poultry sausages with addition of TGase improved and was comparable to that of pork sausages (Cegiełka and Geryk, 2007). Spatial bonds formed as an effect of TGase action strengthen the meat protein structure, improve their water absorption and facilitate sausage slicing. The use of TGase in the meat industry has made possible the use of less valuable raw material, that is, collagen, blood proteins and mechanically separated meat, for the manufacture of meat products with increased nutritional value via enriching with the lack in the amino acids (e.g., exogenous lysine) (Kowalski and Pyrcz, 2009). These components can be subjected to enzymatic modifications; even other substances introduced into meat products can be subjected to similar processes. In order to give the texture typical to meat (fibrousness, juiciness), textured soy proteins were modified with the use of TGase (Gaspar and de Góes-Favoni, 2015; Weng and Zheng, 2015).

The use of mTGase created new technological possibilities on the production of fine and coarse minced sausages, wieners and smoked meats. To obtain these products, low-grade meat as well as additives, such as skimmed-milk powder, soy and wheat flour can be used. As a result of TGase effect on the proteins of these raw materials, products which do not differ from the analogous products manufactured from high-quality meat in their appearance, consistency, taste, smell and nutritional value (Kuraishi et al., 1998) are obtained. In order to improve meat value, salt extracts of muscle proteins or alkaline reagents are used, but heat treatment is necessary. An alternative can be found in the use of TGase, which does not require basic salting, freezing, or heating processes. TGase also contributes to maintaining the original taste and aroma of raw materials

with improved efficiency. A better texture of a product is obtained by simultaneous use of TGase and sodium caseinate (Mirzaei, 2011). However, the bonds formed were not strong enough to protect the raw, restructured meat from disintegration during heat treatment (Dłużewska and Florowska, 2014). Use of TGase allowed production of some sausage types with reduced fat content; for example, salami type. The products obtained with the addition of filler do not differ organoleptically from traditional meat products (Kieliszek and Misiewicz, 2014; Nielsen, 1995).

Transglutaminase has been used for the manufacture of a whole range of poultry meat products. Possible benefits related to this, apart from modification of the texture of meat, are increase of firmness, effect on water retention by the finished product and ability to change the appearance and taste qualities of products manufactured from the most valuable parts of poultry carcass, that is, the breast muscles. In order to use small meat pieces and to improve their commercial value, they may be restructured. The conventional method consists in binding meat by using salt extracts of the muscle proteins or alkaline reagents, but such preparations require heat treatment. Restructured poultry meat products manufactured with the use of TGase preparations can at the same time be marinated and then offered to the customers in various forms (de Almeida et al., 2015; Trespalacios and Pla, 2007; Tseng et al., 2000).

4.4 Improving functional characteristics of milk products

The study of Monogioudi et al. (2011) demonstrated that β-casein enzymatic cross-linking is more resistant to pepsin digestion as compared to noncross-linked β-casein form. Giosafatto et al. (2012) demonstrated that the obtained results may have a significant effect on the development of new food structures with better characteristics (Giosafatto et al., 2012). Polymerization of milk proteins *via* the effect of TGase may lead to the formation of protein film, which increases functional characteristics of milk products (Rossa et al., 2011). According to Hiller and Lorenzen (2009), cross-linking is a dominant process, which leads to the formation of ε-(γ-glutamyl)-lysine within and between isopeptide chains. The degree of cross-linking depends on the protein structure. More effective cross-linking takes place in proteins containing glutamine residues in the elastic areas of a protein. Thus casein constitutes a better substrate than ovalbumin and β-lactoglobulin. Furthermore, denaturation and adsorption on the oil-water interface increase the protein reactivity (Dłużewska and Florowska, 2014; Kieliszek and Misiewicz, 2014).

In the dairy industry, TGase has been used in a variety of products, for example, in yogurts, to prevent syneresis or improve structure (more firm with soft consistency) (Lorenzen et al., 2002). As a result of casein modification with TGase, it is possible to produce a range of new dairy products with their characteristic structure and consistency. TGase reactivity with casein decreases in the following order: κ-casein > α-casein > β-casein (Tang et al., 2005). However, it should be emphasized that the reactivity depends on the source of the enzyme. Cross-linked casein is characterized by better gelating and emulsifying properties and may be widely used as a functional additive in the food industry (de Kruif et al., 2002).

A recent study by Prakasan et al. (2015) showed that the addition of TGase to *paneer* (cottage cheese) results in improvement of textural properties and water

retention with a simultaneous minimization of heat treatment. *Paneer* is a traditional Indian dairy product that is popular among vegetarians. It is valued for its nutritional and health advantages. It primarily consists of solid milk substances. The study suggests that *paneer* exhibiting improved functional characteristics can be manufactured using enzymatic cross-linking method with mTGase.

The use of cross-linking enzymes allows for addition of proteins and polysaccharides or reduction of dry substances without changes in texture and the property of water retention. Yogurts obtained from milk incubated with TGase are manufactured using this method (Iličić et al., 2014; Ozer et al., 2007). They are characterized by a uniform, compact and very creamy consistency and smooth, dry surface of the coagulum. It is a result of the phenomenon of syneresis (Lorenzen et al., 2002). Yogurts manufactured by these methods are used in the preparation of creams, various frozen desserts, ice creams, milk drinks and dressings (Lauber et al., 2000; Şanlı et al., 2011). The enzyme can be added to milk before its heat treatment, which inactivates TGase, than adding a starter culture to initiate the fermentation process. The result of TGase action is increase in compactness and viscosity of a gel, improvement of water retention, and eventually, reduction of syneresis.

Currently, protein cross-linking processes by TGase are drawing a lot of attention. A study showed (Liu et al., 2014) that formation of ε-(γ-glutamyl)-lysine may improve water retention and stabilize the three-dimensional network of yogurt gel (viscosity), reducing separation of whey proteins. However, on the other hand, the pore size of the milk gel catalyzed by TGase is decreased and this may affect the rate and extent of the syneresis process and result in the release of liquid from it (Li et al., 2015). It should also be noted that whey proteins in their native structure are globular and less susceptible to cross-linking reaction, mainly due to the stabilization of globular conformation with disulfide bonds. Cross-linking of whey proteins can be improved by a prior denaturation step followed with heat treatment (Iličić et al., 2014).

Study by Mahmood et al. (2009) on the improvement of efficiency and properties of a soft cheese showed that TGase addition prior to the addition of rennet prevents milk coagulation. On the contrary, by adding the enzyme and rennet at the same time, the durability and hardness of cheese, as well as protein and fat level in whey, decrease considerably. Cheeses with TGase addition are characterized by a less dry structure. Furthermore, such cheese contains more whey proteins. Milk ice creams, particularly low caloric and sugar-free, modified with TGase have smoother consistency. The effect of the enzyme action is similar to that caused by the addition of ice-cream stabilizer—lower syneresis is observed, better water retention and lower susceptibility to excessive formation of ice crystals (Dłużewska and Florowska, 2014).

4.5 In baking industry

Transglutaminases are currently used in the bakery technology to create bonds between prolamin polypeptide chains. First observations of baking a dough with the use of TGase were performed by Gottmann et al. (1992). As a result of the study, it turned out that TGase affected the stability and volume of the dough while improving the quality of baking flours of poor quality, leading to the improvement of bread texture (Kieliszek and Misiewicz, 2014; Marco and Rosell, 2008). Losche (1995) noted

that TGase improve the rheological properties of the dough, ensuring proper size of pores and elasticity of bread after baking. Addition of TGase to dough improves the mechanical and water absorption properties and reduces the production costs. In fried cereal products, it is possible to obtain lower content in the finished product.

Addition of TGase strengthens the dough structure, which may be used in the production of frozen doughs, because the dough then is more resistant to degradation associated with the freezing process (Kim et al., 2008). In the bakery industry, TGase is used to improve the volume and texture of bread and durability of flours (Moore et al., 2006). The objective of TGase addition to gluten-free bread, with the participation of proteins other than gluten, is to obtain the spatial structure produced by this protein. Recently, numerous studies have been published on the effect of TGase on the quality of gluten-free bread (Dłużewska and Florowska, 2014). It was shown that the effect of this enzyme depends not only on its concentration in the dough, but also on the type of proteins added (Kuraishi et al., 2001). Various studies on modification of gluten proteins with the use of TGase, the objective of which is to reduce the allergenicity of gluten, were also conducted. As a result of TGase modification of wheat flour proteins, there was an improvement in the dough elasticity and flexibility. At the same time, a 14 per cent increase in the volume of the bread was comparable to the bread baked from the traditional dough (Gerrard et al., 2001). TGases are also used in enriching prolamins with lysine or other exogenous amino acids or fructooligosaccharides. From the nutritional viewpoint, rice flour contains many valuable nutrients, such as protein, dietary fiber and vitamins E and B. However, its use is limited to non-fermented bakery products. The study by Gujral and Rosell (2004) showed that the addition of TGase to rice flour improves the rheological characteristics of the dough. Changes in the amount of TGase added facilitated the control of pasta texture. The bonds formed as a result of cross-linking reactions in the presence of TGase are thermally stable, which in consequence leads to compactness and elasticity of pastas long after their cooking. Texture of such pastas does not change as a result of heat treatment (Yamazaki and Nishimura, 2002). In dough strengthened with TGase, starch remains to a greater extent on an expanded gluten network. Therefore, in the process of heat treatment, lesser amounts of dry mass components are released. As a result, the amount of starch lost is low, whereas the surface of pastas exhibits lower viscosity (Mariniello et al., 2008). In instant noodles, TGase addition may cause the product to absorb less fat during frying.

Kuraishi et al. (2001) studied the effect of TGase addition on fat absorption during donut frying. Donuts without the addition of TGase contained 18,2 per cent fat, whereas donuts with TGase contained 13,8 per cent. The value of fat absorption was reduced by 25 per cent. Similarly, use of TGase as a component of breadcrumbs may cause deep fried cutlets to absorb less fat and keep their energy value at a lower level. The addition of TGase strengthens dough structure, which may be used for production of doughs that are frozen prior to baking. This makes the dough less susceptible to freezing-related damage (Marapana and Jiang, 2004). The results presented by Li et al. (2015) demonstrated that recombinant TGase isolated from *Pichia pastoris* GS115 can be used in the food industry. It was determined that enzymatic cross-linking in the dairy proteins by the enzyme significantly improves the functional characteristics of fat-free yogurts. In addition, it was noted that properties of products with lower fat content can be modified with the use of the enzyme.

4.6 In fruits and vegetable processing

Transglutaminase enabled the creation of new products, for example, protein coatings used to coat fresh vegetables, fruits and processed food products in order to improve their shelf-life and freshness (Porta et al., 2015). One of the most important edible coating functions is the protection of fruits against moisture losses, improvement of shelf-life and reduction of losses related to transport, distribution and sale. Takagaki et al. (1991) described a method of coating vegetables and fruits with the addition of TGase. Freshness of vegetables and fruits could be preserved by coating with a film containing proteins and TGase. As an example, celery was treated with an aqueous solution containing TGase, gelatin and natural antibacterial substances, and then heated at a temperature of 50°C for 5 min. to form the coatings. Coated celery was stored at 20°C for three days. The obtained celery was characterized by a lower number of microbes than the product without coating (Zhu et al., 1995).

For the production of such coatings, whey protein modified with TGase is used. These coatings are edible and can be consumed with a product. In addition, depending on the technology used, they exhibit various properties such as water permeability, elasticity, flexibility, tensile strength, and resistance to mechanical damage (Yildirim and Hettiarachy, 1998). Natural coatings produced with the addition of TGase slow down the vital processes in plants, thus contributing to their longer shelf-life. They have protective functions, regulate gas exchange, protect against penetration of harmful substances from the environment (pathogens, contaminations) and protect the tissue against damage. Use of TGase in the production of coatings aims at reduction of technological losses, enrichment of products with protein (improving nutritional value) and ensuring the consistency of the products. When producing edible coatings with the use of various proteins, it is very important to understand the course of the gelating process and the composition and properties of the obtained gels, which may retain water molecules, lipids, or other substances favoring coating formation.

4.7 As protein modifier

In numerous branches of the food industry, TGase is used as a protein modifier to improve nutritional values of substandard proteins *via* embodying them with desired amino acids and peptides. Noguchi et al. (1992) elaborated a method for increasing the absorption of mineral components in humans. It is connected with the process of casein deamination *via* treatment with TGase. The obtained product increases absorption of mineral components in the intestines and can be used in the food industry or pharmaceutical industry for the production of medicines or mineral supplements for adults, children and infants. Casein structure can store mineral components or other chemical elements before they are transported to the intestine and dissolved. TGase is also used to block allergenic, proteolysis-resistant peptides of soy proteins (Babiker et al., 1998). Products of protein modifications formed as a result of the action of the enzyme are used in the cosmetic, pharmaceutical and leather industries (Nielsen, 1995; Zhu et al., 1995).

5. Conclusion

Discovery of a non-expensive TGase source in the form of microbes, which are able to biosynthesize the enzyme, and practical applications of the enzyme in many branches of the food industry are important for meeting customer expectations. The essential step in enzyme preparation is its production from the culture medium in a purified form. TGase acts in mild conditions and is considered an entirely safe food additive. TGase is listed as GRAS (Generally Recognized As Safe). On the background of the majority of enzymes, TGase is far from the standard due to its cross-linking properties. The present study on microbe modification and a constant development toward profitable production of the enzyme may result in the manufacture of more accessible products with a wider spectrum of applications. TGase use in the processes of food manufacture has numerous advantages: enables better raw material use, prevents unfavorable quality changes (color, taste, smell, or texture), improves sensory attractivity, increases manufacturing efficiency of the products, as well as allows for obtaining new products. Great application possibilities of mTGase encourage the search for new sources of this biocatalyst (of microbes), which are able to synthesize considerable amounts of the enzyme using the least expensive culture media.

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