Properties and Utilization of Transglutaminase in the Food Industry: A Review

El-Iraki, S.M., Taiseer, M. Abo Bakr, & Dalia, H. Eshra

Food Science and Technology Dept. Fac. of Agric., Alex. Univ, 21545 – El-Shatby, Alexandria, Egypt.

Received: 8 May 2011 ***Revised: 4 June 2011*** ***Accepted: 11 June 2011***

ABSTRACT

Transglutaminase (EC 2.3.2.13) is an enzyme that forms crosslinks between protein molecules. The enzyme is found in many different organisms where they have very specific roles. This crosslinkes have unique effects on protein properties such as viscosity, gelation, capability, thermal stability, solubility and water holding capacity. The basic reaction mechanism of transglutaminases is similar. It is able to modify proteins by catalyzing crosslinking reactions through an acyl transferase mechanism involving protein-bound glutaminyl residues and primary amines including the ε-amino groups of lysine residues in proteins.

Many patent applications and research papers in recent years reflect the potential of cross-linked proteins for developing novel foodstuffs and processing methodologies that allow the manufacture of products with high convenience, improved sensory and nutritional-physiological properties.

Transglutaminase is now widely used in meat products seafood surimi products, backed goods, noodles pasta and dairy products.

So, the overall applications of transglutaminase in the food industry are reviewed in this article.

Keywords: cross-linking, transglutaminase, food industry, dairy products, meat products, baked goods.

INTRODUCTION

Transglutaminase (TGase, Protein-glutamine: amine, γ-glutamyltransfer-ease, EC 2.3.2.13) is an enzyme that forms crosslinks between protein molecules. It is also known by other names such as Factor XIII or fibrinoligase. Factor XIII helps to stop bleeding by forming crosslinks of fibrin molecules and stabilizing fibrin polymers (Aeschlimann & Paulsson, 1994). It catalysis an acyl transfer reaction between the γ-carboxyamide group of peptide bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors). In the absence of primary amines, deamination of glutamine residues is the result of using water as acyl acceptor (Motoki & Seguro, 1998, Dube *et al*., 2007).

TGases are widely distributed in nature and has been found in various animal tissues, fish, plants (Folk, 1980, Icekson & Apelbam, 1987) and microorganisms (Ando *et al*., 1989, Klein *et al*., 1992).

The first characterized microbial transglutaminase (MTG) was that of the bacterium *Strpetomyces mobaraensis* (older synonym *Streptoverticillium mobaraense*). MTG does not require calcium for activity, shows broad substrate specificity and can be produced at relatively low cost. These properties are advantageous for industrial applications (Kanaji *et al*., 1993, Nielsen 1995, Date *et al*., 2004).

TGase is used in the food industry to modify a variety of protein-related functional properties, including stability to phase separation, the formation of heat and water resistant films, water holding capacity (WHC), protein solubility and rheological properties such as gelatin and viscosity characteristics (Zhu *et al*., 1995, Truong *et al*., 2004, Yokoyama *et al*., 2004).

Specific application have been reported for meat (Kuraishi *et al*., 1997, Motoki & Seguro, 1998), fish (Seguro *et al*., 1995, Jiang *et al*., 2000), dairy (Motoki & Seguro, 1998, Imm *et al*., 2000) bread and bakery products (Gerrad *et al*., 1998, 2001), and in soybean processing (Nonaka *et al*., 1996, Dube, 2007).

This review is intended as a background to transglutaminases. Also, focusing on its utilization in the food industry.

Reactions catalysed by transglutaminase (TG)

Cross-linking of proteins, resulting in the formation of high molecular weight polymers, seems to be the most dominant reaction in nature of TGs (Gerrad, 2002, de Jong & Koppelman, 2002). TGs form isopeptide bonds between glutamine and lysine residues in proteins, thus introducing both inter-and intramolecular covalent cross-links.

The general reaction of the cross-linking reaction between glutamine and lysine residues of protein is shown in Fig. (1).

However, two other important reactions can be catalyzed by TG. In the presence of primary amines, TGs catalyses an acyl-transfer between the γ-carboxylamide groups of peptide or protein bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the ε-amino group of lysine residues in certain proteins. In the absence of primary amines, deamination of glutamine residues is the result of using water as acyl acceptor (Nonaka *et al*., 1989, Zhu, 1995, Gerrad & Sulton, 2005). The reactions catalysed by TG are shown in Fig (2).

Sources of transglutaminase

Transglutaminases are widely distributed in nature having been found in various animal tissues, fish, plants and microorganisms (Folk, 1980, Icekson & Apelbaum, 1987, Lillely *et al*., 1998). It was found in animal tissues, such pig liver, rat liver, squid gill, Japanese oyster and from human blood (Gorman & Folk, 1980, Wong *et al*., 1990, de Jong *et al*., 2001).

The contribution of fish endogenous transglutaminase in the setting phenomenon of salted and ground fish flesh, so-called "Suwari" in Japan

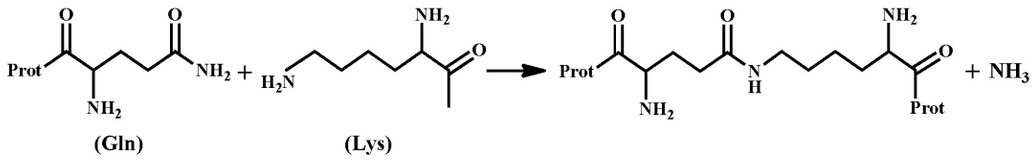

Fig. 1: Mechanism of the cross-linking reaction between glutamine (Gln) and lysine (Lys) residues of proteins (Prot) catalysed by TG
Source: Dube *et al.* (2007).

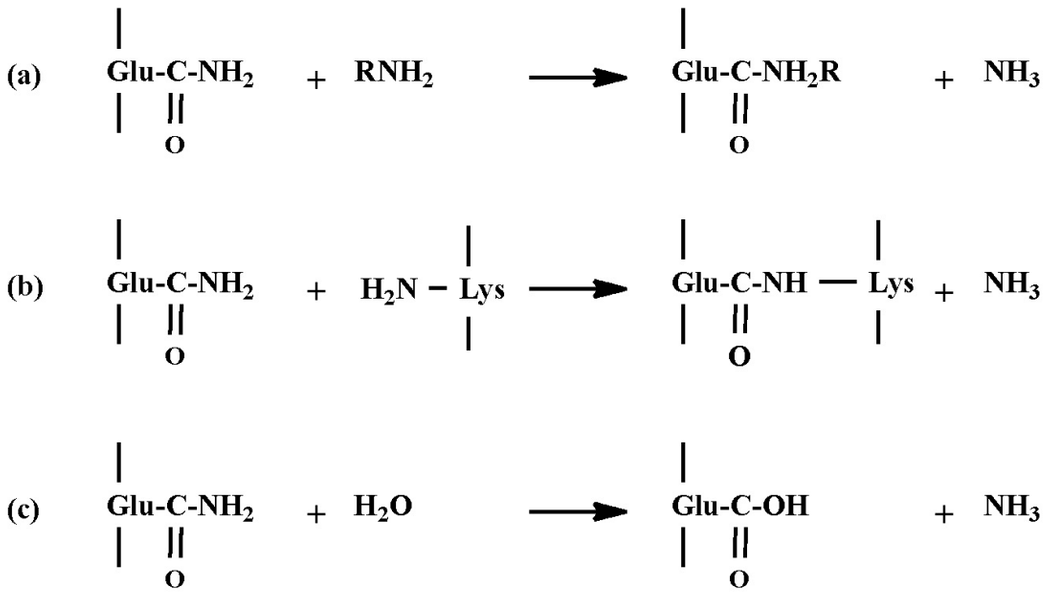

Fig. 2: Reactions catalyzed by transglutaminase (TGase). (a) Acyl transfer, (b) Crosslinking of Gln and Lys residues in proteins or peptides. The resulting bridge is called an ε-(γ-glutamyl) lysine (G-L) bond, (c) Deamidation
Source: Yokoyama, *et al.* (2004).

has been evidenced by many researchers (Tsukamasa *et al*., 1993, An *et al*. 1996, Motoki & Seguro, 1998).

However, application of TG in food industry was not feasible due to the relatively small quantities obtained and the extensive costs of separation and purification steps. Microbial TG was extracted from *Streptomyces mobaraensis* by Ando *et al*. (1989) and its purifications was rather easy and the production of MTG has been established commercially. Recently, many attempts to obtain MTG from other microorganisms by conventional fermentation (Junqua *et al*., 1997, Ho *et al*., 2000, de Barros Soares *et al*., 2003) or by means of genetics modification using host microorganisms are carried out (Yokoyama *et al*., 2000, Kikuchi *et al*., 2003).

Enzymatic characteristics of microbial transglutaminases

The characteristics of MTG obtained from various microorganisms vary even among strains (Table 1). There were differences in the enzymatic properties of MTG from *Streptomyces* sp. MTG from *Streptomyces libani* showed slightly lower optimum reaction temperature and thermal stability than from *Streptomyces mobaraensis* (Umezawa *et al*., 2002). The physicochemical properties of MTG such as molecular weight, and its enzymatic properties had been reported (Ando *et al*., 1989, Kanaji *et al*., 1993, Yokoyama *et al*., 2004). Its molecular weight (MW) was reported to be ranged between 29,000 and 45,000 Da, and its isoelectric point (PI) varied between 6.3 to 8.9 (Table 1). Protein sequencing by the automated Edman method, and mass spectrometry, revealed its primary structure to contain 331 amino acids (Kanaji *et al*., 1993).

The optimum temperature for enzymatic activity was 50°C. On the other hand, it lost activity within a few minutes on heating to 70°C. It still expressed activity at 10°C. The optimum pH for MTG was found to be between 5 and 8. However, MTG showed some activity at pH 4 or 9 (Ando *et al*., 1989), and was thus considered to be stable over a wide pH range.

MTG from a variant of *Streptooverticilium mobaraense* is totally independent of Ca^{2+}. So, this property is very useful in the modification of functional properties of food protein, because many food proteins, such as milk caseins, soybean globulins and myosins, are susceptible to Ca^{2+}. They are easily precipitated in the presence of Ca^{2+} and become less sensitive to MTG. MTGase are significantly inhibited by Cu^{2+}, Zn^{2+}, Pb^{2+} and Li^{+}. Since heavy metals such as Cu^{2+}, Zn^{2+} and Pb^{2+} bind the thiol group of the single cysteine residue, this strongly supports the idea that the cysteine residue could be part of the active site of MTGase.

With respect to substrate specificity, most food proteins, such as legume globulins, wheat glutens, egg yolk and albumin proteins, actins, myosins, fibrins, milk caseins, α-lactalbumins, and β-lactoglobulin, as well as many other albumins, could be crosslinked by MTGase (Nonaka *et al*., 1992, Seguro *et al*., 1995, Nonaka *et al*. 1997).

MTG is capable of gelling concentrated solutions of proteins such as soybean proteins, milk proteins, beef, pork, chicken and fish gelatin and myosins (Nonaka *et al*., 1992, Nielsen, 1995, Seguro *et al*., 1995, Zhu *et al*., 1995). Different proteins can be covalently linked by MTG to produce combinations with novel functionalities. For instance, conjugation of milk casein or soybean globulins to egg

Table 1: Characteristics of microbial transglutaminase (MTG) of different origin

Source	Molecular weight (Da)	Isoelectric point	pH optimum	Temp. optimum (°C)
Streptomyces mobaraensis S-8112	40.000	8.9	6-7	50
Streptomyces mobaraensis WSH-Z2	40,000	n.d	6.0	52
Streptomyces libani	37,900	6.4	5.0	53
Bacillus circulans	45,000	6.3	7.0	47
Bacillus subtilis	29,000	n.d	8.2	60
Streptomyces ladakanum	39,000	n.d	5.5	40
Streptomyces ladakanum	37,500	7.9	6.0	50

Source: Modified from Dube, *et al*., 2007.

ovomucin, one of the glycoprotein, improved the emulsifying activity as compared to both the starting proteins (Kato *et al*., 1991). Casein gelatin conjugation by MTG also yielded novel proteins that were highly soluble at acidic pH (Neilsen, 1995).

MTGase is capable of incorporating amino acids or peptides covalently into proteins. This reaction can improve nutritive values of food or feed proteins, because covalently incorporated amino acids or peptides behave like amino acid residues in a protein.

In practical application, all common amino acids, except lysine, should have their α-carboxyl group either amidated, esterified or decarboxylated to eliminate the negative charge on the α-carboxyl group. Lysine, whose ε-amino group is a primary amine, is a good substrate of MTGase. In such reactions, proteins act as acyl donors, while amino acids, including lysine, act as acyl acceptors (Yokoyama *et al*., 2004). Both lysine and glutamine containing peptides are capable of serving as substrates without modification. The lysine-containing peptides act as acyl acceptors, while the proteins act as acyl donors, whereas glutamine-containing peptides act as acyl donors, and the proteins as acyl acceptors.

Developing industrially useful TGase

In general, there are three approaches to developing industrially useful TGases. The first is to extract and purify the enzyme from the tissues or body fluids of animals, such as cattle, swine, and fish. The second approach is to obtain the enzyme by means of genetic manipulation using host microorganisms such as *Escherichia coli*, *Bacillus,* Yeast, or *Aspergillus*. Many researchers, have been working in an attempt to obtain a large amount of TGase at low price. Takehana *et al*. (1994) (*Streptoverticillium* TGase, in *E. coli*), and Yakoyama *et al*. (2000) (*Streptomyces* TGase in *E. coli*). However, none of these TGases has been commercialized due to factors such as food regulations and consumer acceptability. The third approach is to screen for TGase producing microorganisms. If an appropriate microorganism which produces TGase could be found, it would be possible to mass produce TGase by traditional fermentation technology.

Applications in food processing

Many of the possible applications in the industry have been proposed in detail in the scientific literature. Early work was carried out to modify food proteins using mammalian enzymes (Matheis & Whtaker, 1987), but this was impractical on a commercial scale. The situation changed in 1989 with the purification of the microbial TG from *Streptomyces mobaraensis* (Ando *et al*., 1989) which could be produced relatively cheaply by fermentation methods. Subsequently, the application of MTG in the food industry began to increase at a steady rate. The use of MTG in food processing has been covered in some reviews (Nielson, 1995, Motoki & Seguro, 1998, Kuraishi *et al*., 2001, Yokoyana *et al*., 2004).

Meat products

Muscle proteins, especially myosin, are polymerized by TG (Huang *et al*., 1992) and the resulting crosslinks strengthen the protein network in muscle-derived products. MTG can produce restructured meat by binding together small pieces of meat at temperatures below 10°C, overnight (Fig. 1). Kuraishi *et al*. (1997) developed a novel meat binding system using MTG and caseinate simultaneously.

Caseinate, when reacted with MTG, becomes viscous, and functions as a glue to bind different foodstuffs together (de Jong & Koppelman, 2002). MTG can be added during mixing as a dry powder, or in slurry with water.

In the case of sausages and other meat products the effect of TG addition is improved texture with higher breaking strength of firmness, and increased deformation and elasticity of the gel. This leads to an increase in manufacturers' profits due to reduced product loss during processing and slicing. Also, the possibility of utilizing by-products from the meat industry (e.g. mechanically deboned meat, greave protein, collagen/ gelatin) and improving the binding properties by TG treatment looks promising (Nielsen, 1995).

Meat pieces, including minced meat, can be also bound together without any need for sodium chloride or phosphates, yielding "healthy" meat products with improved water-holding capacity and texture (Motoki & Seguro, 1998, Yokoyama *et al*., 2004).

It should be expected also that protein hydrolysates with improved solubility could be utilized more widely in meat products.

Castro-Brions *et al*. (2009) reported that maximum mechanical properties (Texture profile analy-

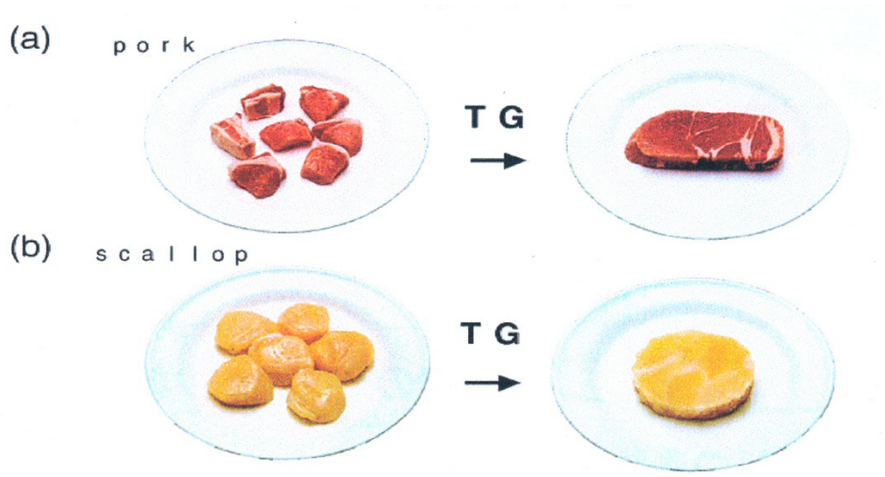

Fig (3): Application of microbial transglutaminase (MTGase): (a) restructured steak from small pork pieces, (b) new type of seafood

Source: Motoki & Seguro, 1998.

sis and puncture test) could be obtained by incubating beef pastes at 50°C for 30 min with minimal effect on colour and cooking loss when 0.3% of MTG was added.

Fish products

The addition of the TG during the setting process of surimi (fish paste products) results in a gel with greater breaking stress and improved elasticity (Sakamoto *et al*., 1995). Fish species with high proteolytic activity were traditionally not capable of being used for surimi production. In these under utilized fish species the addition of TG has been reported to improve the gel characteristics of fish paste and lizard fish mince and allowing their utilization in surimi production (Heajung *et al*., 1996, Benjakul, *et al*., 2008).

It is likely that the quality of frozen fish products also can be improved by injection/ tumbling with TG resulting in a reduction in loss during thawing of frozen seafood products, such as shrimps which must maintain their textural properties (Nielsen, 1995, Kuraishi *et al*., 2001, Yokoyama *et al*., 2004,).

Dairy products

Casein has been shown to be a very good substrate for TG, while the globular whey proteins have been shown to be poor substrates. It was found that a heat-resistant firm gel was formed from casein in the TG reaction (Motoki & Seguro, 1998, Kuraishi, *et al*., 2001, Yokoyama, *et al*., 2004). Yogurt, a milk gel formed by acidic fermentation with lactic starter, has the disadvantage of serum separation or syneresis upon change of temperature, or physical impact. As a gel formed with ε-(γ-Glu)lys bonds has improved water holing capacities, the set –type yogurt which is made from TG treated milk has a greater capacity for holding water. Also, it increases the firmness and viscosity, and whey syneresis is prevented (Kuraishi *et al*., 2001, Rodriguez-Nogales, 2006, Cancino *et al*., 2006, Yuksel & Kerdem, 2010).

In cheese manufacturing, TG treatment of milk results in an increase in curd yield, a less dry texture and reduced whey separation. Processed cheese products treated with the enzyme present higher heat stability reduced synersis index, maintaining viscosity when melting (Kuraishi *et al*., 2001, Cozzolino *et al*., 2003, Arrizubieta, 2007, Fernandes & Bordignon, 2010).

The TGase reaction also make it possible to produce another dairy products, such as cheese with low-fat contents, sugar-free ice cream which is softer, smoother and easier to scoop. This is probably because the growth of ice crystals is inhibited due to the structure of ice cream that can retain the water molecules more tightly (Motoki & Seguro, 1998, Kuraishi *et al*., 2001).

TG also has potential commercial application as a food grade additive capable of improving the heat stability of milk (Osullivan *et al*., 2002), thus conferring resistance to coagulation at sterilization temperatures or gelation during storage. TG has also potential applications in controlling the

stability of milk protein containing emulsions and foams (Dickinson & Yamamoto, 1996). Utilization of crosslinked whey proteins for formation edible films or coatings was studied by Mahmoud & Savello (1992, 1993).

Zhang & Zhong (2009) reported that when whey protein isolate (WPI) was cross-linked by transglutaminase within microemulsions could enhance the heat stability of WPI. This novel approach can be used to manufacture heat-stable protein ingredients for clear beverage applications.

Baked products

Several studies have proven that the use of TG improves baking properties and products quality. L-lysine was bound very efficiently to gluten by TG at reaction rates faster as compared to other substrates (Casein, soy protein) (Ikura *et al*., 1981, Iwami & Yasumoto, 1986). The action of the enzyme reinforces the protein network structure changing the viscoelastic properties of the dough (Larre *et al*., 2000). Many patent applications (Table 2) showed that TG greatly increased the crumb strength of baked loaves, reduced workload, and improved the water absorption of the dough. Each of these effects would lower processing costs for commercial baking (Gerrad *et al*. (1998). The usefullness of TG has been shown in other baked products, such as cakes, puff pastries, cookies and bread crumbs. By adding TG, the depression of sponge cake after baking is prevented. TG addition to dough also improved its stability and loaf volume, as well as, improved the lift of puff pastry and the volume of yeasted croissants (Gerrad *et al*., 2001). These effects are retained even after freezing offering a potential solution to the problem of dough deterioration during frozen storage. In addition, deep fried dough products such as doughnuts absorb 25% less oil when the dough has been treated with transglutaminase, with the concomitant health benefit for the consumer (Kuraishi *et al*., 2001).

The addition of MTG to such doughs with a low in wheat content, improved the quality of the dough and derived backed products. The stickiness of the dough was reduced, which facilitated mechanical processing. Due to the improved gas retention, bread volume increased. An enhancement of the fresh quality and inhibition of staling has been achieved in bakery products when MTG was added to α-amylase – supplement doughs. By combination of both enzymes, breads with softer and less chewy fresh crumbs were obtained due to a slower crumb staling kinetics (Collar & Bollain, 2005, Dube, *et al*., 2007).

Table 2: Patent review covering bread and bakery application

Patent number	Year of publication	Title
JP 02286031	1990	Wheat flour for crake and production of cake mix and cakes
JP 4360641	1992	Production of breads
US 5279839	1994	Bakery products and intermediates
JP 6078663	1994	Preparation of oil-fried processed food
JP 7184529	1995	Production of bakeries
JP 7250609	1995	Production of baked product
JP 9191820	1997	Method for preventing softening of cookie
EP 0760209	1997	Method of manufacturing baked products
JP 10028516	1998	Bread suitable for heating by microwave oven
EP 0847701	1998	Modified cereal flour and processed food using the same
JP 11155468	1999	Bread composition for half baking use
JP 11276056	1999	Frozen dough, bread from baking the same and oil and fat composition therefore
EP 0938845	1999	Method of producing breads (with transglutaminase and partial protein hydrolysates) and enzyme preparation
US 6517874	2003	Utilization of transglutamiases for the production of baked products with a low wheat content
DE 10346764	2004	Transglutaminase fermented pre-dough, useful in bakery products is obtained from cereal flour and/or non-cereal material optionally together with baker's yeast and/or lactic acid bacteria
JP 2004242647	2004	Manufacture of transglutaminase- and gliadin-containing bakery products with high volume, improved flavour, and soft and fine texture
US 20050202144	2005	Process for producing bread with extended shelf life, bread dough and bread improver composition for producing such bread

Source: Dube, *et al*., 2007.

Since cereal products, especially bread, are the basic components of the diets in many countries, there is a high demand for gluten-free breads by persons with celiac disease. Moore *et al*. (2006) tried to mimic the viscoelastic properties by utilization of MTG. They reported that it was possible to produce a stable network within a gluten free bread system, improving the loaf volume and crumb characteristics.

Seo *et al*. (2003) reported that the addition of MTG to wheat flour improved dough stability and chewiness of cooked noodles made thereform. Wu & Corke (2005) reported a drastic increase in both storage and loss modulus of fresh noodle sheets, even at a MTG level of 1g/Kg^{-1} of wheat flour, but the effect mostly decreased with higher levels of the enzyme. This could be related to the limited content of lysine in gluten, which would restrict the cross-linking reaction at high MTG dosages (5–20g/Kg^{-1} of flour). The addition of TG would also disturb the internal starch–protein interaction equilibrium, causing a decrease in viscosity. Despite the low content of lysine, the cross-linking reaction resulted in a positive change of elasticity and breaking strength of cooked noodles (Dube *et al*., 2007). MTG was generally added to the raw material (wheat flour) to obtain noodles with enhanced elasticity, texture and sensory properties (Table 3).

Also, it was found that the ability of MTG in the formation of heterologous polymers between soy protein isolate and durum wheat proteins to improve the quality of raw and cooked spaghetti (Aalami & Leelavathi, 2008). Min & Green (2008) suggested that the combination of 0.05% to 1% of MTG with 1.7% isolated soy protein was optimal for development of a low NaCl, phosphate-free patties using minced catfish belly flap meat.

Gujral & Rosell (2004) showed that the addition of TG to rice flour improved the dynamic rheological properties of rice flours dough, resulting in a progressive increase in its viscosity and elasticity with increase in TG concentration. So, it was possible to obtain rice bread with an increased specific volume and softer crumb at 1% TG level in the presence of 2% hydroxypropylmethyl cellulose. Analysis showed that rice proteins are polymerized through the TG reaction, providing a protein network necessary for holding the gas produced in fermentation.

Marcoa & Rosell (2008) evaluated the effect of the addition of different protein isolates (pea, soybean and whey proteins) and transglutaminase on the viscosimetric and rheological properties of the rice flour dough. It was found that the use of protein isolates and TG broads the applications of rice flour in the bakery industry with nutritional improvement of the resulting products.

Table 3: Patent review covering noodle application

Patent number	Year of publication	Title
JP 2286054	1990	Preparation of noodles
JP 5244887	1993	Production of noodles distributable at normal temperature
JP 6014733	1994	Noodles
JP 6225717	1994	Production of a multilayer noodle
JP 7322843	1995	Production of raw type packaged noodle
JP 8051944	1996	Manufacture of noodle with transglutaminase
JP 8256715	1996	Production of raw type packaged noodle
JP 9028334	1997	Production of noodles
JP 9154512	1997	Production of new noodle
JP 10179066	1998	Chinese noodles
EP 0870434	1998	Method for producing noodles
JP 11225695	1999	Extrafine frozen boiled noodle for cold noodle
EP 0948905	1999	Enzyme preparation comprising transglutaminase and process for producing noodles
JP 2000253841	2000	Production of boiled noodle or the like
JP 2002262794	2002	Noodle and method for producing the same
JP 2004187546	2004	Cereal-based noodle materials containing soy milk and transglutaminase and/or glucono-δ-lactone
JP 2004275056	2004	Raw Chinese noodles and their manufacture

Source: Dube, *et al*., 2007.

Soy products

Soybeans constitute the most important vegetable source of protein ingredients for food formulations. This is mainly due to their high protein content, a well-developed processing technology and beneficial effects on nutrition and health (Friedmann & Brandon, 2001). So, there has been some interest in the modification of characteristics of soy proteins. It was found that TG cross-link soy globulins resulting in modification of the gelation and textural characteristics of soybean products.

Tofu, a typical soybean curd product, is prepared through coagulation of soybean proteins. The use of MTG in tofu manufacture has claimed by several patent applications (Table 4). Due to the high moisture content (~90%), traditional tofu generally has a shelf life of 2-4 days, even when kept at temperatures below 10°C. When stable tofu is produced from sterilized soybean milk, larger amount of coagulants (Ca^{2+}, Mg^{2+} and/or glucono-σ-lactone) are required (Nonaka *et al*., 1996).

It is very difficult to produce long-life tofu, since the soft, smooth texture of tofu is easily destroyed by retort sterilization. However, the addition of TG improve the texture of tofu because it increases its water holding properties which results in tofu with a smooth and firm texture, which was stable for 6 months or longer. In addition, treatment with the enzyme allows for better control of the coagulation reaction and a reduction in weight loss during retort cooking (Nonaka *et al*., 1994, 1996, Motoki & Seguro, 1998, Kuraishi *et al*., 2001, Tang *et al*., 2007).

Also, Change *et al*. (2011) investigated the ef-

Table 4: Patent review covering soybean protein application

Patent number	Year of publication	Title
JP 2100647	1990	Production of fried bean curd
US 5055310	1991	Process of preparing shelf-stable "tofu" at normal temperature for long term
JP 3168059	1991	Production of retorted "Mabo-Dofu" preservable at ordinary temperature for long period
JP 6217729	1994	Production of bean curd having freeze resistance
JP 6269257	1994	Preparation of frozen bean curd
JP 8112071	1996	Preparation of new bean curd food
JP 10000067	1998	Production of fried bean curd
JP 11221039	1999	Production of filled bean curd
US 6042851	2000	Process for producing packed tofu
JP 2001352911	2001	Method for producing vegetable sausage substitute
JP 2001046003	2001	Bean curd having freezing resistance and its production
US 6342256	2002	Tofu products excellent in freeze resistance and process for producing the same
JP 2002281928	2002	Method for producing packed soybean curd
US 6582739	2003	Processes for producing functional okara milks and functional tofus
JP 2003052326	2003	Frozen tofu (soybean-curd) and method for producing the same
JP 2003023990	2003	Method for producing powder soybean curd from soybean powder as raw material, and powder soybean curd
JP 2003023988	2003	Method for producing powder soybean milk from soybean powder as raw material, powder soybean milk and milk product obtained by processing powder soybean milk
JP 2004097044	2004	Method for producing bean curd steak
JP 2004261107	2004	Sterilized soybean milk and soybean milk product containing sterilized soybean milk
US 2004180128	2004	Production method of soymilk curd
JP 2004222618	2004	Method for producing reconstituted tofu and ingredient material-containing reconstituted tofu
WO 2005087019	2005	Manufcture of isoflavone-high soy milk and tofu
JP 2005204660	2005	Soybean milk with good body, its manufacture and soybean milk products
JP 2005021070	2005	Manufacture of frozen tofu containing starch, trehalose, transglutaminase and bittern

Source: Dube, *et al*., 2007.

fect of MTG on the rheological and textural characteristics of black soybean packed tofu containing agar as the coagulant. Results showed that the addition of MTG increased the gelation temperature of soymilk, and produced a firmer and more elastic packed tofu with low cooking loss.

Other applications

Gharst *et al*. (2007) studied the effect of transglutaminase cross-linking on the rheological characteristics of heated peanut flour (PE) dispersions. They found that high molecular polymers were formed in TGase-treated peanut flour. These data suggested potential applications of polymerized PE dispersions in peanut-based food products, including protein bars, shakes and value added baked goods (Clare *et al*., 2007, 2008).

Shand *et al*. (2008) studied the rheological properties of heat-induced pea protein isolate (PPI) gels with added MTG. Enhancement of shear strain or gel elasticity of heat-induced PPI gels with MTG were found and provides opportunities for extending the properties of pea proteins when developing new food products.

Bioavailability of cross-linked proteins

The wide application of microbial TG in food production has raised concerns about the potential effects of the cross-linked proteins on consumer's health and the bioavailability of the cross-linked residues. Glutamine-lysine isopeptide bonds are widely distributed in natural products and food stuffs (with the exception of milk) and cooking itself results in glutamine-lysine isopeptide bond formation (Motoki & Seguro, 1998). Mammalian digestive enzymes do not cleave this isopeptide bond. However, two kinds of enzymes have been found in kidney and in intestinal brush border membranes that degrade the bond in vivo (Seguro *et al*., 1995, Yokoyana *et al*., 2004). Furthermore, nutritional studies have indicated that the glutamine-lysine moiety in cross-linked proteins can be metabolized and that the lysine is incorporated into proteins (Seguro *et al*., 1996). Thus, TG catalysed cross-linking does not reduce the nutritional value of the proteins. Pedersen *et al*. (2004) evaluated the allergenic potential of added MTG in food. They reported that the enzyme was fully degraded after 5 min of pepsin treatment and showed no homology with known allergens. Thus, they concluded that no safety concerns with regard to the allergenic potential of MTG have been identified (Pedersen *et al*., 2004).

Gerrad & Sutton (2005) suggested that using TG in baked products may act upon gliadin proteins in dough to generate the epitope associated with the coeliac response. Further work is urgently required to assess this possibility because they do not recommend the use of TG in baked products.

REFERENCES

Aalami, M. & Leelavathi, K. **2008**. Effect of microbial transglutaminase on spaghetti quality. Journal of Food Science, **73**: C306-C312.

Aeschlimann, D. & Paulsson, M. **1994**. Transglutaminase: Protein cross-linking enzyme in tissues and body fluids. Thrombosis and Haemostasis, **71**: 402-415.

An, H., Peters, M.Y. & Seymour, T.A. **1996**. Roles of endogenous enzymes in surimi gelation 1996. Trends in Food Science and Technology, **7**: 321-327.

Ando, H., Adachi, M., Umeda, K., Matsuura, A., Nonaka, M., Uchio, R., Tanaka, H. & Motoki, M. **1989**. Purification and characteristics of a novel transglutaminase derived from micro-organisms. Agricultural and Biological Chemistry, **53**: 2613-2617.

Arrizubieta, M.J. **2007**. Transglutaminases. In: Industrial Enzymes, Structure, Function and Applications. Polaina, J. and Mac Cobe, A.P. (Eds.). Springer, pp. 567-581.

Benjakul, S., Phatcharat, S., Tammatinna, A., Visessanguan, W. & Kishimura, H. **2008**. Improvement of gelling properties of Lizardfish mince as influenced by microbial transglutaminase and fish freshness. Journal of Food Science, **73**: S239-S246.

Cancino, B., Fuentes, P., Kulozik, U. & Bonisch, M. **2006**. Effect of the protein addition on the structure of set style and stirred yoghurt with and without the use of transglutaminase. Desalination, **200**: 531-532.

Castro-Briones, M., Calderon, G.N., Velazques, G.M., Rubio, S., Vazquez, M. & Ramires, J.A. **2009**. Effect of setting conditions using microbial transglutaminase during obtention of beef gels. Journal of Food Process Engineering, **32**: 221-234.

Chang, Y.H.O., Shiau, S.Y., Chen, F.U.B. & Lin, F.R.U. **2011**. Effect of microbial transglutaminase on the rheological and textural char-

acteristics of black soybean packed tofu coagulating with Agar. LWT-Food Science and Technology, **44**: 1107-1112.

Clare, D.A., Gharst, G. & Sanders, T.H. **2007**. Transglutaminase polymerization of peanut proteins. Journal of Agricultural and Food Chemistry, **55**: 432-438.

Clare, D.A., Gharst, G. Maleki, S.J. & Sanders, T.H. **2008**. Effects of transglutaminase catalysis on the functional and immunoglobulin binding properties of peanut flour dispersions containing casein. Journal of Agricultural and Food Chemistry, **56**: 10913-10921.

Collar, C. & Bollán, C. **2005**. Impact of microbial transglutaminase on the staling behaviour of enzyme-supplemented pan breads. European Food Research and Technology, **221**: 298-304.

Cozzolino, A., Di Pierro, P., Manniello, L., Sorrentine, Masi, P. & Porta, R. **2003**. Incorporation of whey proteins into cheese curd by using transglutaminase. Biotechnology and Applied Biochemistry, **38**: 289-295.

Date, M., Yokoyama, K., Umezawa, Y., Matasui, H. & Kikuchi, Y. **2004**. High Level of *Streptomyces mobaraensis* transglutaminase in *Corynebacterium glutamicum* using a chimeric pro-region from *Streptomyces cinnamoneus* transglutaminase. Journal of Biotochnology, **110**: 219-226.

de Barros Soares, L.H., Assmann, F. & Záchia Ayub, M.A. **2003**. Purification and properties of a transglutaminase produced by a *Bacillus circulans* strain isolated from the Amazon environment. Biotechnology and Applied Biochemistry, **37**: 295-299.

de Jong, G.A.H., Wijngaards, G., Boumans, H., Koppelman, S.J. & Hessing, M. **2001**. Purification and substrate specificity of transglutaminase from blood and *Streptoverticillium mobaraense*. Journal of Agricultural and Food Chemistry, **49**: 3389-3393.

de Jong, G.A.H. & Koppelman, S.J. **2002**. Transglutaminase catalyzed reactions: Impact on Food Applications. Journal of Food Science, **67**: 2798-2806.

Dickinson, E. & Yamamoto, Y. **1996**. Rheology of milk protein gels and protein-stabilized emulsion gels cross-linked with transgutaminase. Journal of Agricultural and Food Chemistry, **44**: 1371-1377.

Dube, M., Schäfer, C., Neidhart, S. & Carle, R. **2007**. Texturisation and modification of vegetable proteins for food applications using microbial transglutaminase. European Food Research and Technology, **225**: 287-299.

Fernandes, E.M. & Bordignon-Luiz, M. T. **2010**. The effect of transgluta-minase on the properties of milk gels and processed cheese. International Journal of Dairy Technology, **63**: 243-251.

Folk, J.L. **1980**. Transglutaminases. Annual Review Biochemistry, **49**: 517-531.

Friedmann, M. & Brandon, D.L. **2001**. Nutritional and health benefits of soy proteins. Journal of Agricultural and Food Chemistry, **49**: 1069-1086.

Gerrard. J.A. Fayle, S.E., Wilson, A.J. Newberry, M.P., Ross, M. & Kavale, S. **1998**. Dough properties and crumb strength of white pan bread as affected by microbial transglutaminase. Journal of Food Science, **63**: 472-475.

Gerrard, J.A., Fayle, S.E., Brown, P.A., Sutton, K.H., Simmons, L. & Rasiah, I. **2001**. Effect of microbial transglutaminase on the wheat proteins of bread and croissant dough. Journal of Food Science, **66**: 782-786.

Gerrad, J.A. **2002**. Protein-protein crosslinking in food: methods, consequences, applications. Trends in Food Science and Technology, **13**: 391-399.

Gerrard, J.A. & Sutton, K.H. **2005**. Addition of transglutaminase to cereal products may generate epitope responsible for coeliac disease. Trends in Food Science and Technology, **16**: 510-512.

Gharst, G., Clare, D.A., Davis, J.P. & Sanders, T.H. **2007**. The effect of Transglutaminse crosslinking on the rheological characteristics of heated peanut flour dispersions. Journal of Food Science, **72**: C369-C375.

Gorman, J.J. & Folk, J.E. **1980**. Structural features of glutamine substrates for human plasma factor XIII a (activated blood coagulation factor XIII). Journal of Biological Chemistry, **225**: 419-427.

Gujral, H.S. & Rosell, C.M. **2004**. Functionality of rice flour modified with a microbial transglutaminase. Journal of Cereal Science, **39**: 225-230.

Haejung, A., Peters, M.Y. & Seymour, T.A. **1996**. Roles of endogenous enzymes in surimi gelation. Trends in Food Science and Technology, **7**: 321-327.

Ho, M.L., Leu, S.Z., Hsieh, J.F. & Jiang, S.T. **2000**. Technical approach to simplify the purification method and characterization of microbial transglutamianse produced from *Streptoverticillium ladakanum*. Journal of Food Science, **65**: 76-80.

Huang, Y.P., Seguro, K., Motoki, M. & Tawada, K. **1992**. Cross-linking of contractile proteins from skeletal muscle by treatment with microbial transglutaminase. Journal of Biochemistry (Tokyo), **112**: 229-234.

Icekson, I. & Apelbaum, A. **1987**. Evidence for transglutaminase activity in plant tissue. Plant Physiology, **84**: 972-974.

Ikura, K., Yoshikawa, M., Sasaki, R. & Chiba, H. **1981**. Incorporation of amino acids into food proteins by transglutaminase. Agriculture and Biological Chemistry, **45**: 2587–2592.

Imm, J.Y., Lian, P. & Lee, C.M. **2000**. Gelation and water binding properties of transglutaminase- treated skim milk powder. Journal of Food Science, **65**: 200-205.

Iwami, K. & Yasumota, K. **1986**. Amine-binding capacities of food proteins in transglutaminase reaction and digestability of wheat gliadin with ε-attached lysine. Journal of the Science of Food and Agriculture, **37**: 495-503.

Jiang, S.T., Hsieh, J.F., Ho, M.L. & Chung, Y.C. **2000**. Microbial transglutaminase affects gel properties of golden Threadfin – bream and Pollack surimi. Journal of Food Science, **65**: 694-699.

Junqua, M., Duran, R., Gancet, C. & Goulas, P. **1997**. Optimization of microbial transglutaminase production using experimental designs. Applied Microbiology and Biotechnology, **48**: 730-734.

Kanaji, T., Ozaki, H., Takao, T., Kawajiri, H., Ide, H., Motoki, M. & Shimonishi, Y. **1993**. Primary structure of microbial transglutaminase from *Streptoverticillium* sp. Strain s-8112. Journal of Biological Chemistry, **268**: 11565-11576.

Kato, A., Wada, T., Kobayashi, K., Seguro, & Motoki, M. **1991**. Ovomucin-food protein conjugates prepared through the transglutaminase reaction. Agricultural and Biological Chemistry, **55**: 1027-1031.

Kikuchi, K., Date, M., Yokoyama, K., Umezawa, Y. & Matsui, H. **2003**. Secretion of active form *Streptoverticillium mobaraense* transglutaminase by *Corynebacterium glutamicum*: Processing of the pro-transglutaminase by a cosecreted subtilisin-like protease from *Streptomyces albogriseolus*. Applied and Environemental Microbiology, **69**: 358-366.

Klein, J.D., Guzman, E. & Kuehn, G.D. **1992**. Purification and partial characterization of transglutaminase from *Physarum polycephalum*. Journal of Bacteriology, **174**: 2599-2605.

Kuraishi, C., Sakamoto, J., Yamazaki, K., Susa, Y., Kuhara, C. & Soeda, T. **1997**. Production of restructured meat using microbial transglutaminase without salt or cooking. Journal of Food Science, **62**: 488-490.

Kuraishi, C., Yamazaki, K. & Susa, Y. **2001**. Transglutaminase: its utilization in the food industry. Food Reviews International, **17**: 221-246.

Larre, C., Denery Papini, S., Popineau, Y., Deshayes, C., Desserme, C. & Lefebvre, J. **2000**. Biochemical analysis and rheological properties of gluten modified by transglutaminase. Cereal Chemistry. **77**: 121-127.

Lilley, G.R., Skill, J., Griffin, M. & Bonner, P.L.R. **1998**. Detection of Ca^{2+}- dependent transglutaminase activity in root and leaf tissue of monocotyledonous and dicotyledonous plants. Plant Physiology, **117**: 1115-1123.

Mahmoud, R. & Savello, P.A. **1992**. Mechanical properties and water vapor transferability through whey protein films. Journal of Dairy Science, **75**: 942-946.

Mahamoud, R. & Savello, P.A. **1993**. Solubility and hydrolyzability of films produced by transglutaminase catalytic crosslinking of whey protein. Journal of Dairy Science, **76**: 29-35.

Marcoa, C. & Rosell, C.M. **2008**. Effect of different protein isolates and transglutaminase on rice flour properties. Journal of Food Engineering, **84**: 132-139.

Matheis, G. & Whitaker, J.R. **1987**. A review: enzymatic cross-linking of proteins applicable to foods. Journal of Food Biochemistry. **11**: 309-327.

Min, B. & Green, B.W. **2008**. Use of microbial transglutaminase and nonmeat proteins to improve functional properties of low NaCl, phosphate-free patties made from Channel Catfish (*Ictalurus punctatus*) belly flap meat. Journal of Food Science, **73**: E218-E226.

Moore, M.M., Heinbockel, M., Dockery, P., Ulmer, H.M. & Arendt, E.K, **2006**. Network formation in gluten-free bread with application of transglutaminase. Cereal Chemistry, **83**: 28-36.

Motoki, M. & Seguro, K. **1998**. Thansglutaminase and its use for food processing. Trends in Food Science and Technology, **9**: 204-210.

Nielsen, P. **1995**. Reactions and potential industrial applications of transglutaminase. Review of literature and patents. Food Biotechnology, **9**: 119-156.

Nonaka, M., Tanaka, H., Okiyama, A., Motoki, M., Ando, H., Umeda, K. & Matsura, A. **1989**. Polymerisation of several proteins by Ca^{2+}, independent transglutaminase derived from micro-organisms. Agricultural and Biological Chemistry, **53**: 2619-2623.

Nonaka, M., Sakamoto, H., Kawajiri, H., Soeda, T. & Motoki, M. **1992**. Sodium caseinate and skim milk gels formed by incubation with microbial transglutaminase. Journal of Food Science, **57**: 1214-1218.

Nonaka, M., Toiguchi, S., Sakamto, H., Kawajiri, H., Soeda, T. & Motoki, M. **1994**. Changes caused by microbial transglutaminase on physical properties of thermally induced soy protein gels. Food Hydrocolloids, **8**: 1-8.

Nonaka, M., Sakamoto, H., Toiguchi, S., Yamagiwa, K., Soeda, T., & Motoki, M. **1996**. Retort resistant tofu prepared by incubation with microbial transglutaminase. Food Hydrocolloids, **10:** 41-44.

Nonaka, M., Matsuura, Y., Nakano, K. & Motoki, M. **1997**. Improvement of the pH-solubility profile of sodium caseinate by using Ca^{2+} independent microbial transglutaminase with gelatin. Food Hydrocolloids, **11**: 347-349.

Osullivan, M., Kelly, A. & Fox, P. **2002**. Effect of transglutaminse on the heat stability of milk: a possible mechanism. Journal of Dairy Science, **85**: 1-7.

Pedersen, M.H., Hansen, T.K., Sten, E., Seguro, K., Ohtsuka, T., Morita, A., Bindslev-Jensen, C. & Poulsen, L.K. **2004**. Evaluation of the potential allergenicity of the enzyme microbial transglutaminase using the 2001 FAO/WHO Decision Tree. Molecular Nutrition and Food Research, **48**: 434-440.

Rodriguez-Nogales, J.M. **2006**. Effect of preheat treatment on the transglutaminase catalyzed cross-linking on goat milk protein. Process Biochemistry, **41**: 430-437.

Sakamoto, H., Kamazawa, Y., Toiguchi, S., Seguro, K., Soeda, T. & Motoki, M. **1995**. Gel strength enhancement by addition of microbial transglutaminase during onshore surimi manufacture. Journal of Food Science, **60**: 300-304.

Seguro, K., Kumazawa, Y., Ohtsuka, T., Toiguchi, S. & Motoki, M. **1995**. Microbial transglutaminase and ε-(γ-glutamyl)lysine crosslink effects on elastic properties of Kamaboko gels. Journal of Food Science, **60**: 305-311.

Seguro, K., Kumazawa, Y., Kuraishi, C., Sakamoto, H. & Motoki, M. **1996**. The epsilon-(gamma-glutamyl)lysine moiety in crosslinked casein is an available source of lysine for rats. Journal of Nutrition, **126**: 2557-2562.

Seo, H. Shin, W., Yoon, S & Lee, S. **2003**. Effect of microbial transglutaminase on physical and textural properties of noodles made with Korean wheat flour (Geurumil). Food Science and Biotechnology, **12**: 1-8.

Shand, P.J., Pietrasik, H.Ya. & Wanasundara, P.K.J.P.D. **2008**. Transglutaminase treatment of pea proteins: Effect on physicochemical and rheological properties of heat-induced protein gels. Food Chemistry, **107**: 692-699.

Takehana, S., Washizu, K., Ando, K., Koikeda, S., Takeuchi, K., Matsui, H., Motoki, M. & Takagi, H. **1994**. Chemical synthesis of the gene for microbial transglutaminase from *Streptoverlicllium* and its expression in *Escherichia coli*. Bioscience of Biotechnology and Biochemistry, **58**: 88-92.

Tang, C. He., Lin, L.I., Wang, J.L.I. & Yang, X.Q. **2007**. Formation and rheological properties of "cold-set" tofu induced by microbial transglutaminase. LWT– Food Science and Technology, **40**: 579-586.

Truong, V.D., Clare, D.A., Catignani, G.I. & Swais-

good, H.E. **2004**. Cross-linking and rheological changes of whey proteins treated with microbial transglutaminase. Journal of Agriculture and Food Chemistry, **52**: 1170-1176.

Tsukamasa, Y., Sato, K., Shimizu, Y., Lami, C., Sugiyama, M., Minegishi, Y. & Kawabata, M. **1993**. ε-(γ-Glutamyl)lysine crosslink formation in sardine myofibrils sol during setting at 25°C. Journal of Food Science, **58**: 785-787.

Umezawa, Y., Ohtsuka, T., Yokoyama, K. & Nio, N. **2002**. Comparison of enzymatic properties of microbial transglutaminase from *Streptomyces* sp. Food Science and Technology Research, **8**: 113-118.

Wong, W.S.D., Batt, C. & Kinsella, J.E. **1990**. Purification and characterization of rat liver transglutaminase. International Journal of Biochemistry, **22**: 53-59.

Wu, J. & Corke, H. **2005**. Quality of dried white salted noodles affected by microbial transglutaminase. Journal of the Science of Food and Agriculture, **85**: 2587–2594.

Yokoyama, K.I., Nakamura, N., Seguro, K. & Kubota, K. **2000**. Overproduction of microbial transglutaminase in *Escherichia coli*, *in vitro* refolding and characterization of the refolded form. Bioscience of Biotochnology and Biochemistry, **64**: 1263-1270.

Yokoyama, K., Nio, N. & Kikuchi, Y. **2004**. Properties and applications of microbial tranglutaminase. Applied of Microbiology and Biotechnology, **64**: 447-454.

Yuksel, Z. & Kerdem, Y. **2010**. The influence of transglutaminase treatment on functional properties of set yoghurt. International Journal of Dairy Technology, **63**: 86-97.

Zhang, W. & Zhong, Q. **2009**. Microemulsions as nanoreactors to produce whey protein nanoparticles with enhanced heat stability by sequential enzymatic cross-linking and thermal pretreatments. Journal of Agricultural and Food Chemistry, **57**: 9181-9189.

Zhu, Y., Rinzema, A., Tramper, J. & Bol, J. **1995**. Microbial transglutaminase- a review of its production and application in food processing. Applied of Microbiological and Biotechnology, **44**: 277-282.

خصائص واستخدامات إنزيم الترانس جلوتاميناز في التصنيع الغذائي: استعراض مرجعي

سمير محمود العراقي، تيسير محمود أبو بكر، داليا حسن عشره

قسم علوم وتكنولوجيا الأغذية، كلية الزراعة، جامعة الإسكندرية، الرقم البريدي (٢١٥٤٥) الشاطبي، الإسكندرية، ج.م.ع.

يحفز إنزيم الترانس جلوتاميناز (EC 2.3.2.13) تفاعل تكوين روابط عرضية داخل جزيئات البروتينات. ويؤدي هذا الربط العرضي إلى تأثيرات مختلفة في خواص البروتينات الناتجة مثل اللزوجة، خاصية تكوين الجيل، الثبات الحراري، الذوبان وقدرة البروتين على مسك الماء.

يتواجد الإنزيم في كائنات حية مختلفة ولكن ميكانيكية التفاعلات الخاصة به متشابهه فيها جميعاً، حيث يتم ذلك من خلال ميكانيكية نقل مجموعة الأسيل وربط متبقيات الجلوتامنيل مع الأمينات الأولية التي تشمل الـ ε-amino groups للحمض الأميني الليسين في البروتينات.

ويوضح كثير من براءات الاختراع والبحوث المنشورة في السنين الأخيرة أهمية الارتباط العرض للبروتينات في تطوير طرق تصنيع المنتجات الغذائية التي تتميز بالجودة المرتفعة والقيمة الغذائية العالية. حيث يستخدم حالياً إنزيم الترانس جلوتاميناز في تطوير وتحسين منتجات اللحوم، الأسماك، منتجات الخبيز والعجائن الغذائية ومنتجات الألبان المختلفة.

وفي هذا الاستعراض المرجعي تم إلقاء الضوء على استخدامات الإنزيم في التصنيع الغذائي.