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Protein cross-linking in food by microbial transglutaminase (MTGase) and its application & usefulness in food industry

R.A.U.J. Marapana and *Bo Jiang.
School of Food Science and Technology, Southern Yangtze University, Wuxi, Jiangsu, P. R. China. 214036

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ABSTRACT

Microbial transglutaminase catalyses in vitro cross-linking in soybean globulins, wheat proteins, whey proteins, casein, gluten, gelatin, egg protein, myosin B, actin, myosin and actomyosin. This cross linkage has unique effects on protein properties such as thermal stability, water holding capacity etc. It has great potential to improve the physico chemical and rheological properties of foods through the enzyme reaction. However, unlike many other transglutaminases, MTGase is calcium-independent and has a relatively low molecular weight.

Key words: Microbial Transglutaminase, Cross-linking

INTRODUCTION

Proteins are one of the main classes of molecules available to confer textural attributes, and the cross-linking and aggregation of protein molecules has been cited as one of the most important mechanisms for engineering food structures with desirable mechanical properties (Dickinson 1997).

Protein cross linking refers to the formation of covalent bonds between polypeptide chains within a protein (intramolecular cross links) or between proteins (intermolecular cross links) (Feeney & Whitaker, 1988). Protein-protein cross links play an important role in determining the functional properties of food proteins. Manipulation of the number and nature of such protein cross links during food processing (involves high temperatures, extremes in pH, particularly alkaline, and exposure to oxidizing conditions and uncontrolled enzyme chemistry) offers a means by which the food industry can manipulate the functional properties of food, often without damaging the nutritional quality (Gerrard 2002).

As related to food proteins many kinds of bonds or cross links are important for texture development. Such bonds or cross links might result in important changes in textural properties , as well as chemical and nutritional properties. Hydrogen bonds, electrostatic and hydrophobic interactions and disulfide bonds generally are responsible for construction and maintenance of protein structure. In complicated systems such as meat, however, a variety of cross links, including ε-(γ-glutamyl)lysine cross links and cross links through aldol condensation, are believed to be closely related to texture (Sakamoto *et al.*,1995). Furthermore, Sakamoto *et al.*, cited that the possibility of such cross links in proteins might be used to fabricate texturized products.

Many attempts to improve textural properties of foods through formation of ε-(γ-glu)lys cross links by catalytic action of transglutaminase has been reported(Motoki and Nio 1983). Transglutaminase (R-glutaminyl-peptide: amine γ-glutamyltransferase, E.C.2.3.2.13) is an enzyme (Sato *et al*,. 1992, Nonaka *et al*.,1989, Han & Damodaran 1996, Worratao & Yongsawatdigul 2003, Iwami & Yasumota 1986) which is widely distributed in nature and has been found in various animal tissues, fish, plants and microorganisms and is involved in biological processes such as blood clotting and wound healing (Gerrard 2002).

Transglutaminase catalyzes an acyl transfer reaction (Fig 1a) in which C-carboxyamide of peptide or protein-bound glutaminyl residues are the acyl donors. When transglutaminase acts on protein molecules ε -(γ-glutamyl)lysine [ε(γ-glu)lys] cross links are formed and lysine residues in proteins act as acyl acceptor (Ohtsuka *et al.*,2000, Jiang *et al.*, 1998, Sakamoto *et al.*1994, Ramirez-Suarez *et al*,.2001, Ruiz-Carrascal and Regenstein 2002).

In 1989, it was reported that a microbial

*Corresponding author

transglutaminase (MT Gase) was isolated from Streptoverticillium sp. (Ando *et al.*,1989). The MT Gase is active over a wide range of temperatures and stable between pH 5 and 9 and characterized by a calcium-independent activity. It is able to react without additional calcium ions, making it easier to handle and more practical to use in food processing. Since the isolation of transglutaminase from Streptoverticillium sp., others have reported microbial transglutaminases derived from other microorganisms. Some of them are reported as calcium dependent enzymes.

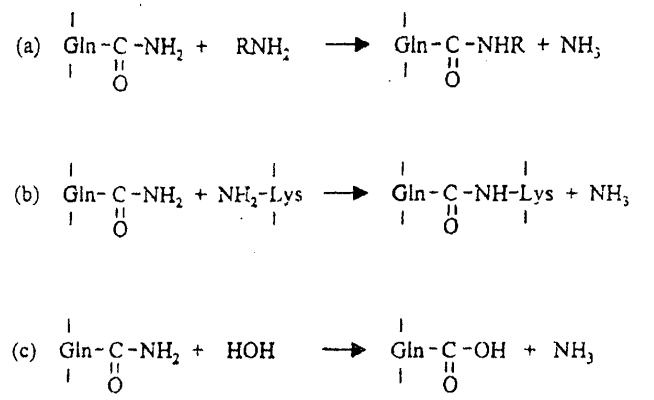


Fig.1.General reactions catalysed by transglutaminase: (a)acyl-transfer reaction; (b) Cross linking reaction (c) deamidation (Kuraishi *et al.*,2001)

Food treated with MTGase appeared to have an improved flavor, appearance and texture. In addition, this enzyme can increase shelf-life and reduce allergenicity of certain foods (Zhu *et al.*,1995). Therefore, MTGase is now widely used in sea food, surimi products, baked goods, meat products, noodles/pasta, dairy products and so on. It has great potential to improve the firmness, elasticity, viscosity, heat stability and water-holding capacity of prepared foods through the mild enzyme reaction (Kuraishi *et al.*,2001).

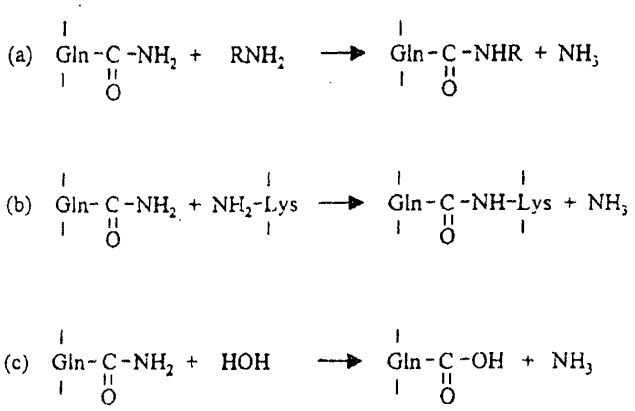
The use of this enzyme to modify the functional properties of foods is an area which has attracted considerable interest, since consumers perceive enzymes to be more 'natural' than chemicals. Enzymes are also favored as they require milder conditions, have high specificity, are only required in catalytic quantities, and are less likely to produce toxic products. Thus enzymes are becoming common place in many industries for improving the functional properties of food proteins (Gerrard 2002).

Production of Microbial Transglutaminase

The production of transglutaminase derived from microorganisms was not reported until the late 1980's. Ando *et al.*,1989 explored the possibility of producing transglutaminase from microorganism. He screened about 5000 strains isolated from soil collected from a variety of locations. Among these strains, Streptoverticillium S-8112 was found to have the capability of producing transglutaminase. Zhu *et al.*, 1995 cited that other Streptoverticillium strains, such as *S.griseocarneum*, *S. cinnamoneum* Sub sp. Cinnamoneum and *S. mobaraense*, also have the ability to produce transglutaminase. Transglutaminase activity has also been found in a culture of Streptomyces sp. (Ando *et al.*,1989).

The fermentation procedure for the production of transglutaminase is in principle the same for the various microorganisms mentioned (Ando *et al.*,1989). Glucose sucrose starch glycerine and dextrin can be used as carbon source. Inorganic as well as organic nitrogen sources can be used, for instance, NH_4NO_3, $(NH_4)_2SO_4$, urea, $NaNO_3$, NH_4Cl, soya rice, maize, wheat or wheat flour, bran, defatted soya bean, maize steep liquid, peptone, meat extract, casein, amino acids and yeast extract. Necessary minerals and trace elements are phosphate, magnesium, potassium, iron, copper, zinc, and vitamins, non ion surfactant and antifoam can be added if necessary. The culture is an aerobic fermentation so that aeration and agitation are necessary. The temperature for growth and product formation is between 25°C and 35°C, and the fermentation time is dependent on the culture conditions and determined by the highest transglutaminase activity that can be achieved, normally 2-4 days. MTGase is an extra cellular enzyme dissolved in the fermentation broth so that it can be recovered through separation of the solid material from the broth. The methods normally used in enzyme purification can be used for microbial transglutaminase. For instance, ethanol, acetone, isopropyl alcohol and other organic solvents can be used in down-stream processing. Salting out with ammonium sulphate and sodium chloride, dialysis, ultra filtration, ion-exchange chromatography, absorption chromatography, gel filtration, absorption and isoelectonic point methods can all be used to purify the enzyme. A good combination of the methods can increase efficiency and recovery. The enzyme obtained

transglutaminase (MT Gase) was isolated from Streptoverticillium sp. (Ando *et al.*,1989). The MT Gase is active over a wide range of temperatures and stable between pH 5 and 9 and characterized by a calcium-independent activity. It is able to react without additional calcium ions, making it easier to handle and more practical to use in food processing. Since the isolation of transglutaminase from Streptoverticillium sp., others have reported microbial transglutaminases derived from other microorganisms. Some of them are reported as calcium dependent enzymes.



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can then be mixed with enzyme stabilizers such as various salts, sugars, proteins, lipids and surfactants.

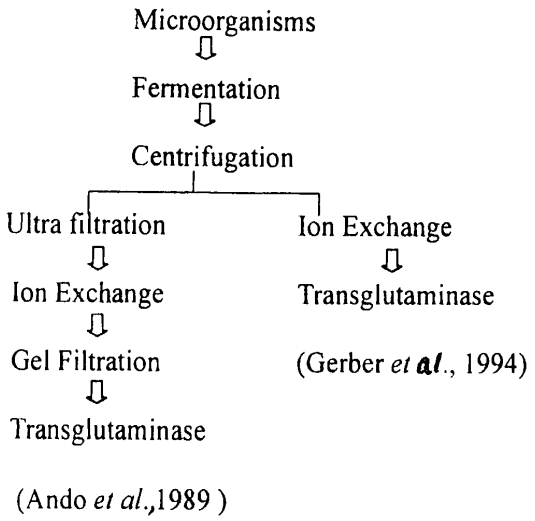

Fig 2. Process chart of transglutaminase production

Table 1: Application of MTGase on Food Processing

Source	Product	Effect	Reference
Meat	Hamburger, Meat Balls, Stuffed Dumplings, Shao- Mai	Improved elasticity , texture, taste and flavour	Sakamoto and Soeda 1991
	Canned meat	Good texture and appearance	Seguro and Motoki 1991
	Ham & Sausage	Improved sliceability Elasticity and firmness improved	Kuraishi 2001
	Restructured pork	Increased hardness and chewiness	Pietrasik and Li-Chan 2002
		Increased cohesion, hardness and elasticity	Nielsen et al., 1995
	Restructured beef	Improved water holding capacity and textural parameter	Pietrasik and Li-Chan 2002
	Restructured Chicken	Improved stability of emulsion	Ruiz-Carrascal and Regenstein 2002
Fish	Fish paste	Improved texture and appearance	Ichihara et al., 1990
	Gelatin	Improved elasticity and cohesiveness	Gomez-Guillen et al 2001
	Restructured product	Improved mechanical properties	Ramirez et al., 2002
	Surimi gel	Improved (glutamyl)lysine cross links	Sakamoto et al., 1995
	Fish Surimi	Improved quality	Juan et al 2000
Milk	Spreads, deserts, dressings	Improved texture	Dickinson and Yamamoto 1996
Casein	Cross linked protein	Allergenicity reduction	Yamauchi et al., 1991
Wheat	Baked foods	Improved texture and high volume	Ashikawa et al., 1990
Soybean	Mapuo doufu	Improved shelf life	Kuto et al 1991
	Tofu	Improved shelf life	Nonaka et al., 1990
Fruits and Vegetables	Celery	Food preservation	Takagaki et al., 1991
Gelatin	Sweet foods	Low calorie foods with good texture, firmness and elasticity	Yamanaka and Sakai 1992

MTGase in Muscle Foods

In meat processing it is of great interest to maximize the yield of marketable products. This includes development of methods for re-structuring low-value cuts and trimming to improve their appearance, flavour and texture and to enhance market value. Re-structuring treatment usually involves size reduction, reforming and binding (Kim *et al.*,1993). In such a treatment MTGase can have a very Important function. Sakamoto and Soeda 1991 developed a method for producing minced meat products containing transglutaminase. Minced meat and other food ingredients are mixed with transglutaminase, shaped, packed in pressure resistant containers and retorted to manufacture meat products such as hamburgers, meat balls, stuffed dumpling and shao-mai (a typical Chinese food). The foods show improved elasticity, texture, taste and flavour. Minced beef and pork, flour, onion, skim milk powder and condiments were mixed with water and MTGase, packed with sauce in bags and retorted to make raw hamburgers.

MTGase can also react with meat proteins. Myosin B, myosin and actin were reportedly polymerized by the addition of MTGase. In both heat induced gels of myosin B and raw sausage meat, a finer network structure was observed with MTGase than without by electron microscopy. The changes in ultra microstructure of the heat induced gels corresponded well with the changes in gel strength. It is considered that a strengthened network structure improves the physical properties of prepared meat.

In ham manufacturing, the MTGase reaction basically occurs during the cooking process, that is during drying, smoking, and boiling (steaming) and also proceed during the tumbling to some extent. After the cooking, the internal temperature is raised above 70°C and the MTGase is inactivated. The cross links that are formed by MTGase strengthen the protein network structure in prepared meat products such as sausage & ham and the physical properties of the product, such as elasticity and firmness, are greatly improved. The breaking stress, or firmness, increase with the amount of MTGase, as does the deformation, the elasticity or pliability of gel. When an excess of MTGase is used, however, the texture becomes too firm and less pliable, which is generally unfavorable (Kuraishi 2001).

In the case of hams, the effect of MTGase is seen in the improved slice ability, not only in improved textural quality. Slice-loss, the ratio of broken slices when hams are thinly sliced, is decreased when transglutaminase is used. The improved slice ability is profitable to ham manufacturers.

Another application is in health food products. In the processing of prepared foods, especially meat products, salt and/or some phosphates are usually added to improve or increase the water-holding capacity, binding, consistency, and over all texture. As the demands for health foods increases, more prepared foods with reduced salts or phosphates are being distributed on the market. The texture and physical properties of these " healthy" foods, however, are of low standard. By using MTGase, reduced salt/phosphate products with improved texture can be obtained. Even when the sodium content was reduced to almost one third that of normal sausage elasticity was maintained through the use of MTGase. The loss of cohesiveness and water-holding capacity in nonphosphate meat products is also expected to be recovered by using MTGase.

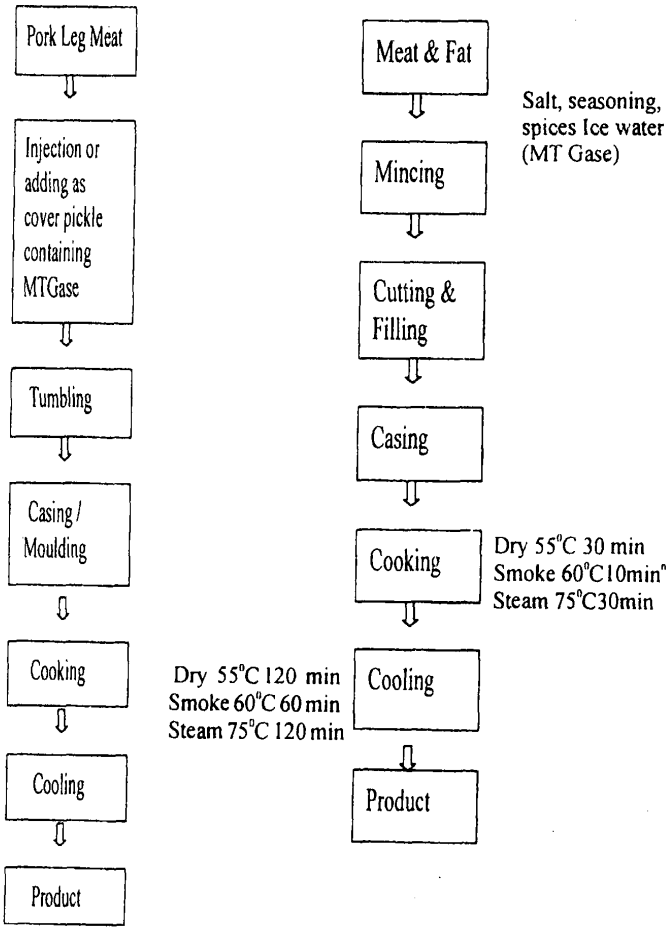

Fig. 3(a) Production flow of ham

(b)Production flow of sausage

Though MTGase can improve the physical properties of phosphate-free product to some degree, it is rather difficult to entirely replace the phosphate functions in meat products (Kuraishi *et. al.*,2001). Kuraishi *et al.*, 1997 reported that more convenient and practical is the production of restructured meat using MTGase and sodium caseinate. The binding system has great potential in the development of high quality restructured foods. There is no loss of taste and flavour, and strong binding is maintained even in slicing, freezing and cooking. Pork meat restructured using MTGase alone had slightly greater binding strength than the control. However, the binding was not strong enough to produce raw structured meat that would not break down during cooking. In an attempt to obtain more binding strength, the use of transglutaminase with various food proteins was studied. Further, Author reported that, satisfactory results were obtained with sodium caseinate, but not with soy protein, gelatin or whey protein. Caseinate treated with MTGase becomes more viscous, and it is thought that viscous caseinate acts as a stable glue that can be used to hold together different food stuffs. Increasing levels of caseinate increased binding strength for restructured meat when 0.1% MTGase was used. No binding was obtained with only caseinate, even at a 5% addition level. A binding period, or reaction time, is necessary for the MTGase /Caseinate binding system; the standard is at least 2hr at 5°C for restructuring raw meat pieces (Kuraishi *et al.*, 2001).

MTGase in Restructured Pork

Pietrasik and Li-Chan 2002 demonstrated that, MTGase addition favourably reduced the cooking loss and increased hardness and chewiness of pork gels, but was not to improve these parameters in low salt products to the same levels as high-salt products. Heating temperature was found to have relatively minor effect, primarily through its interaction with salt level and in a quadratic term affecting the elasticity and springiness of the gels. Sodium caseinate either alone or in combination with MTGase generally has been found to be interior to j-carrgeenan for functionality in comminuted pork meat. Although MTGase had no effect on binding properties it was found to increase

methodology. Further, they reported that, an increased addition of both j-carrgeenan and plasma protein favourably affected thermal stability of pork batters, but the effects were attenuated by increased BP and CGN addition respectively. MTGase had little effect on colour and water binding properties although it was found that its addition improved cohesiveness and elastic properties of meat gels processed with BP.

Nielsen *et al.*, 1995 has used recombinant protein (transglutaminase FXIIIa) produced by fermentation of *Saccharomyces cereviciae* for his experiments and shown that FXIIIa increased cohesion, hardness and elasticity for a time-temperature heat treatment of 37°C and 90 min in raw minced pork meat, while processing at 10°C for 23h caused only minor texture changes. Salt and phosphate addition together with FXIIIa resulted in a remarkable increase in binding properties. Thus, the texture parameters increased particularly at salt levels between 2 and 4% and phosphate level of 0.2 %. Binding of meat pieces containing 0.2% phosphate, 1% salt and FXIIIa as 0.4% active enzyme to substrate showed significant effect on the tensile strength compared to the samples without FXIIIa ; however, colour deterioration of the product was observed when adding FXIIIa.

Kuraishi *et al.*, 1997 demonstrated that, fresh pork meat cubes in combination with MTGase and sodium caseinate showed acceptable bind, and sodium caseinate appeared to be a superior substrate for the cross linking to meat proteins than soy protein, whey protein, or gelatin. Tsao *et al.*, (2002) have shown that the tensile strength and cooking yield of restructured pork sticks made with bisulfite-treated soy protein and MTGase were much higher than those of using salt, suggesting the high potential of using soy protein and MTGase as binders in products where NaCl reduction is desired.

MTGase in Restructured Beef

Pietrasik and Li-Chan (2002) investigated the combined influence of j-carrageenan (0.5%) and egg albumin or collagen isolate non- muscle proteins (NMP, 2%) on quality characteristics of beef gels processed without or with 0.5% MTGase. They found that, MTGase improved water holding capacity and textural parameters, it could not restore texture of NMP substituted gels to that of non-substituted meat gels. But no significant influence of MTGase on gel colour parameters was observed. Pietrasik (2003) has shown that egg albumin either alone or in combination with MTGase is generally inferior to j-carrageenan for functionality in comminuted meat systems.

Restructured beef steaks with added walnuts (0,10 and 20%) and salts (2% NaCl and 0.3% sodium trypolyphosphate), were prepared using microbial transglutaminase (0.7%)/ sodium caseinate (3%) as cold set binders, and stored in chilling conditions (6 days at 3°C). with MTGase, the restructured beef steaks presented suitable mechanical characteristics (meat particle binding) for handling in the raw state (Colmenero *et al.*, 2004). Ramirez-Suarez *et al.*, (2001) prepared myofibrillar protein concentrate (MPC) from beef heart muscle (3 or 5% protein), and the sols were incubated with 0.5 unit/mL transglutaminase at 5 and 15°C for 0, 0.5,2, 5, 10 and 15hr to determine the enzyme effect on MPC gelation. After each incubation time, dynamic gelling tests, differential scanning calorimetry, and electrophoresis (for sols and gels) were performed on all samples. MPC samples with MTGase had greater ($p<0.05$) gelling ability, regardless of pre incubation (time and temperature), showing increases in storage modulus from 600-900Pa (control) to 1700-2000(treated) at 70°C. The result suggest that the gelation enhancing effect by MTGase was attributed to cross-linking of myosin, and that, MTGase can be used to improve the binding ability of beef heart myofibrillar proteins in restructured meats. Kim *et al.*, (1993) have shown that, polymerization of beef actomyosin was induced by addition of transglutaminase resulted in gelation of the actomyosin that was visualized by confocal laser scanning microscopy.

MTGase in Restructured Chicken

Using response surface methodology, the effect of microbial transglutaminase concentration and incubation time after emulsification, but before creaming on the functional properties of chicken meat proteins were examined by Ruiz-Carrascal and Regenstein (2002). Further, they concluded that, MTGase improved the stability of emulsions made with a high-salt chicken meat protein extract, although MTGase did not significantly affect the amount of protein incorporated in to

the emulsion cream layer. MTGase increased the water uptake ability of both chicken muscle and chicken muscle protein extract incubated at 40° C and 4° C. Similarly, MTGase increased the protein and water content of the pellet formed in the water uptake ability test. MTGase therefore appears to be a useful method to enhance the stability and yield of meat batters.

A study was conducted by Ramirez-Suarez & Xiong (2003) to determine the effect of MTGase as emulsifying properties and rheology of emulsion gels mixed myofibrillar and whey or soy protein isolates in aqueous solutions with or without 0.6M NaCl at pH6.5. The enzyme treatment had a minor effect on protein emulsifying activity, but tended to decrease emulsion stability because of promoting flocculation. But MTGase treatment in a higher gel elasticity for all emulsion samples. The results suggest that MTGase influenced the rheological properties of mixed muscle/non muscle protein emulsion gels mainly through affecting the interaction or cross-linking of oil droplets and not the physical characteristics of the fat globule membrane per se.

MTGase catalyzed interactions of whey (WPI)/ Myofibrillar (MPI) protein isolates were investigated by Ramirez-Suarez and Xiong (2002) under five conditions such as ionic strength, calcium/ethylene diamine tetra acetic acid (EDTA), enzyme : substrate ratio, WPI : MPI ratio and preheating of WPI (80°C). MTGase treatments of MPI in distilled water converted myosin heavy chain and actin in to lower molecular weight polypeptides. The reaction, accelerated by the presence of WPI but diminished by NaCl, was completely reversed upon extended incubation. MTGase did not alter the melting pattern of WPI/MPI mixtures, but markedly enhanced their thermal gelling ability. The authors investigated another similar experiment and reported that, the enzyme treatment slightly increased the thermal transition (denaturation) temperatures of MPI/SPI but greatly enhanced ($P<0.05$) the elasticity of the mixed protein gels when compared with untreated samples, independent of incubation time.

The effect of MTGase and sodium caseinate on the quality of chicken doner kebab was investigated by Kilic (2003). The author described that the addition of MTGase with or Without sodium caseinate created cross-linking between meat proteins. Texture measurement indicated that the effect of the enzyme on binding properties of chicken meat is more effective if it is used with sodium caseinate ($P<0.05$).

MTGase in Fish Products

Zhu *et al.*, 1995 cited that fish paste products are manufactured from material containing fish meat as the main ingredient and 0.1-700u transglutaminase/g fish meat protein.Myosin polymerization and formation of ε-(γ-glutamyl) lysine linkages were quantified in Alaska Pollock surimi gels which contained no additive (control), or a commercial MTGase (Lee *et al.*, 1997). As pre incubation ("setting") time at 25°C was increased, the gel strength of control and 0.2% MTGase- added samples increased, with greater increases at higher MTGase levels. SDS-PAGE and HPLC analyses showed increasing non disulfide polymerization and ε (γ-glutamyl)lysine dipeptide correlated with gel strength (shear stress) and shear modulus at failure for these gels. Higher stresses were measured in samples containing 0.2% MTGase than in control at corresponding level of ε-(γ-glutamyl)lysine dipeptide indicating that rate of myosin polymerization may affect ultimate gel strength.

Gomez-Guillen *et al.*,(2001) have examined the effect of a microbial transglutaminase on the gelling and viscoelastic properties of a gelatin from megrim (*Lepidorhombus boscii*) skins. They observed MTGase extended the setting time of fish gelatin but melting temperature, gel strength and viscosity in solution at 60°C were considerably increased by the covalent cross-linking action of the enzyme, as observed by SDS-PAGE and scanning electron microscopy. Increasing concentrations of MTGase increased the elasticity and cohesiveness of gelatin gels but reduced gel strength and hardness.

Restructured products were obtained from fish paste of silver carp (*Hypophthalmichthys molitrix*) by massaging and cooking in ham presses (Ramirez *et al.*,2002). Changes on solubility of pastes mechanical properties, water holding capacity and SDS-PAGE of the cooked products were evaluated by 3 levels of salt (0, 1% or 2%) and 3 levels of MTGase. Further, they showed that MTGase needed the addition of NaCl to improve the mechanical properties of these

restructured products.Surimi from Alaska Pollock flesh was manufactured on shore with MTGase. Effect of MTGase was investigated by evaluating breaking strength and deformation of gels from MTGase treated surimi with or without setting at 30°C. Quantitative analysis of å-(ã-glutamyl)lysine (GL) cross link was also carried out to monitor the MTGase reaction. In set gels, breaking strength and GL cross links increased and myosin heavy chain decreased correspondingly with MTGase concentration. Results suggest that surimi gel could be improved through the formation of GL cross links by added MTGase in surimi (Sakamoto *et al.*, 1995).

The properties of surimi gels from threadfin-bream and Pollack surimi set at 30°C or 45°C with MTGase from *Streptoverticillium ladakanum* were determined by Jiang et.al., 2000. The optimal amounts of MTGase and setting conditions were 0.3unit/g surimi either at 30°C for 90 min or at 45°C for 20 min for threadfin-bream, and 0.2 unit/g surimi at 30°C for 60 min for Pollack. The strength of golden threadfin bream surimi gels with 0.35 unit MTGase set at 30°C for 90 min or 45°C for 20 min, almost 3-fold of the control. SDS-PAGE analyses indicated that inter-and/or intramolecular cross linking formed in the myosin heavy chain of MTGase- containing surimi gels.

Jiang *et al.*, 2000 used MTGase, reducing agent and protease inhibitor to improve the quality of under utilized fish surimi. SDS-PAGE indicated that cross-linking of myosin heavy chain occurred in MTGase contained samples, while myosin heavy chain of samples without MTGase disappeared after 120 min incubation at 45°C. Although the gel-forming ability increased with MTGase added up to 0.6units/g, it was still too low to be commercially acceptable. The results suggested the combined use of reducing agent. MTGase and protease inhibitor seemed to be a better way to improve gel-forming ability of hairtail surimi.

High pressure effects on the strength (Stress) and elasticity/ deformability (strain) of surimi and turkey breast meat gels containing MTGase were evaluated by Ashie & Lanier (1999). Pressurization of muscle proteins at 4°C prior to incubation at 25°C or 40°C (setting) increased gel strength 2-3 fold in uncooked surimi gels, but not in uncooked turkey gels. SDS-PAGE confirmed that myosin cross-linking occurred due to MTGase activity during the setting treatment, which had survived prior pressure treatment. High pressure rendered protein substrates more accessible to MTGase thereby enhancing intermolecular cross-link formation and gel strength. Shark-fin is considered to be a delicious and healthy (functional) food in South East Asia. An imitation of shark-fin is prepared by cross-linking gelations, collagens or a mixture thereof with transglutaminase and making a gel from the product.

MTGase in Dairy Products

Casein has been shown to be a very good substrate for transglutaminase, while the globular whey proteins have been shown to be poor substrates. Whey proteins become more susceptible to cross linking by MTGase in the presence of reducing agents such as dithiothreitol. Several investigations of MTGase-treated milk proteins have been conducted not only on the gelling properties, but also on the emulsifying properties. Dickinson and Yamamoto (1996) have shown that the milk protein-stabilized emulsion gels cross linked with MTGase. They proposed that the use of MTGase during the processing of emulsion gel products offered new opportunities for developing improved textures in protein-based spreads, deserts, and dressings. It has been also reported that enzymatic cross linking is a potential way of controlling the stability of protein-containing emulsions and foams by measuring surface shear viscosity and interfacial dilatational moduli at the oil-water interface (Faergemand, *et al.*, 1997).

Imm, *et al.*, 2002 demonstrated that, gelation and water binding properties of MTGase-treated skim milk powder resulted in significant increases in hardness and water holding capacity of heat-induced gels. A marked increase in storage modulus of MTGase treated skim milk powder upon heating suggests that MTGase treated skim milk powder has a greater gelling ability than control-skim milk powder prepared with pre denaturated MTGase.

The modification of whey proteins to improve their functional properties in specific food systems has been shown by Wilcox and Swaisgood 2002. The solutions of 8% whey

protein isolate were incubated with MTGase beads, resulting in limited cross-linking of whey proteins. As incubation time increased, intrinsic viscosity increased, gelation temperature decreased, and stronger, more brittle gels were formed upon heating.

When transglutaminase is utilized in yogurt production, milk is treated with the enzyme followed by heat treatment for inactivation of the enzyme, and then the fermentation process started with the addition of starter culture. MTGase can also be added to milk at the same time as starter culture. In this case, the enzyme reaction proceeds during fermentation. Major effects of MTGase when used in manufacturing are an increase in firmness and viscosity and improved water-holding capacity that results in reduced syneresis (Ishii *et al.*,1994). MTGase is used in the manufacturing of cheese, too. Curd yield is increased by using MTGase in the manufacturing process. Three patterns for producing natural cheese with MTGase are proposed.(a) adding MTGase to milk, heating the milk for pasteurization and deactivation of the enzyme, and then adding rennet to the milk. (b) adding rennet to milk, and then adding MTGase (c) adding MTGase to milk at the same time as rennet(Kuraishi *et al.*,1997, 1999).

Ice cream treated with MTGase is smoother and easier to scoop, especially in the case of low-calorie, non sugar ice cream, where "icy" texture often damages the quality (Okada *et al.*,1993). Stabilizers are often used during the manufacture of dairy products to enhance and maintain certain characteristics. The effect of MTGase in preventing syneresis, improving water holding capacity, improving viscosity, and preventing ice crystal growth can replace a stabilizer.

MTGase in Soy Products

Soy proteins are widely used in prepared foods such as sausages, ham, and kamaboko (surimi gel) because the gelation of soy proteins in food processing provides various textural properties. Both soy 7S and 11S globulins were polymerized by transglutaminase, and the protein solutions (50mg/ml) turned into self-supporting gels on the addition of transglutaminase. Chanyongvorakul *et al.*, 1995 reported that MTGase induced gels from 11S globulins are more rigid elastic than thermally induced gels. This rigid physical property may be due to excess cross linkage. Heat treated soybean glycinins are more Susceptible to enzyme polymerization than native glycinins, because the surface lysine and glutamine residues of glycinin increase with heating. The addition of NaCl to the soy protein isolate gel caused a decrease in breaking strength in heat induced gels without transglutaminase. However, even when 3% salt is added, transglutaminase-treated sample retains a breaking strength close to that of the control gel (without NaCl and without transglutaminase). This effect may be advantageous in several processed foods containing soy protein isolate, such as sausage, kamaboko and other surimi-based products.

Transglutaminase is used together with nigari coagulant (magnesium chlorides) to make tofu with a smoother, firmer texture. Another advantage of using transglutaminse in tofu manufacturing is easier handling during the coagulation process with nigari. Tofu with MTGase has an increased water-holding capacity, good consistency, and a silky and firmer texture. Because the cross links formed by transglutaminase are thermo stable, many of the physical properties are retained even after retort treatment. (Nonaka *et al.*, 1996) The transglutaminase treated tofu also shrank, but the weight loss was greatly decreased. The results suggested that tofu treated with MTGase, which forms more stable covalent cross links, is able to hold more water in spite of temperature changes. Transglutaminase offers a means to produce versatile sao protein products.

MTGase in Baked Products

Transglutaminase also acts on wheat proteins. In the case of gluten, the main component of wheat protein, transglutaminase induced the formation of high molecular weight polymers, despite the low lysine content in gluten proteins. The formation of ε-(γ-glutamyl)lysine cross links reinforced the network structure and modified the viscoelasticity of the gluten. Larre *et al.*, (2000) have studied the rheological behaviour of gluten treated with transglutaminase. Among the constitutive proteins of gluten, the high molecular weight glutenin sub units were most

effected in the cross linking reaction. They reported that glutens modified with transglutaminase are less sensitive to thermal processing than unmodified glutens. It was concluded that the enzymic treatment caused a considerable reinforcement of the network. In another interesting study on wheat proteins, Babiker *et al.*, 1996 found the functional properties of gluten to be improved by protease digestion or acid hydrolysis followed by transglutaminase treatment.

The dough stability and loaf volume were improved by adding transglutaminase during the mixing process. An enzyme preparation has since been introduced as a dough improver (Kuraishi *et al.*, 2001). Transglutaminase greatly increased the crumb strength of baked loaves, reduced work load, and improved the water absorption of the dough. Each of these effects would lower processing costs for commercial baking (Gerrard *et al.*, 1998).

The usefulness of transglutaminase has been shown in not only yeast-raised bread products, but also other baked products, such as cakes, puff pastries, cookies, and bread crumbs. By adding transglutaminase, the depression of sponge cake after baking is prevented, too. Puff pastries made with transglutaminase were larger than those without (Kuraishi *et al.*, 2001). It is possible to form a well-defined laminate in products such as puff pastries with the result that the dough layers rise more after baking. The increment in volume was 30% larger when transglutaminase was used in the puff pastry dough. The crispness of baked products such as puff pastries and cookies is strengthened and the crispness tends to last longer.

Kuraishi *et al.*, 2001 reported that oil absorption of doughnuts is reduced when transglutaminase is used. Doughnuts without transglutaminase contained 18.2% fat, while those with MTGase (0.1units/g flour) contained 13.8% fat. The amount of oil absorption was reduced 25% by MTGase.

MTGase was used to create new covalent intermolecular cross-links between proteins in gluten. This modification induced drastic changes in its physicochemical properties as well as in its rheological behaviour. The enzymatic treatment caused a considerable reinforcement of the network. The modified glutens were also less sensitive to thermal processing than unmodified glutens (Larre *et al*, 2000).

Modification of viscoelasticity of wheat flour dough by MTGase has been shown Tseng and Lai, 2002. MTGase has the ability to modify wheat protein effectively by .forming e(c-glutamyl) lysine bonds as a result of the modification of some important physical properties of wheat flour dough, including stickiness, extensibility and maximum resistance to extension. Results of SDS-PAGE indicate that the modification of MTGase on the viscoelastic properties of the dough may be due to polymerization effects of gluten.

Oat globulin was polymerized by a microbial transglutaminase, and some physicochemical and functional properties of polymers were studied by Siu *et al.*, 2002. Reversed-phase HPLC revealed that the number of e-(c-glutamyl) lysine isopeptide bonds formed after 4h of enzyme incubation was 2.21 imol/g of protein. Differential scanning calorimetry showed that both the denaturation temperature and denaturation enthalpy were decreased after MTGase treatment. MTGase incubation led to progressive changes in flow properties of oat globulin dispersions, indicating enhanced pseudo plasticity and increased viscosity and yield stress.

MTGase in Noodles and Pasta

Little has so far been reported about the use of enzymes in the production of pasta, though various enzymes have long been used in industrial baking. Transglutaminase, however, is utilized widely in the production of noodles and pasta in Japan.

Transglutminase is added when flour and other ingredients are mixed. With the addition of transglutaminase, the texture of various noodles, Chinese noodles, udon (Japanese noodle made from wheat flour), soba (made from buckwheat), and pasta are improved. In the case of Chinese noodles, the breaking energy increases with the amount of transglutaminase added. These effects of transglutaminase can be obtained in various kinds of noodles and pasta, but the degree of change in the physical properties varies with the sorts of products, that is, whether they are dried, fresh or cooked, etc.. By adjusting the amount of transglutaminase, the textural qualities of noodles/pasta can be

controlled.

Since the cross links introduced by transglutaminase are heat-stable, the firmness and elasticity of noodles are retained longer even after cooking. Noodles treated with transglutaminase maintain a firm texture in hot soup. As the structure of dough is strengthened by transglutaminase, starches in dough are better held in the gluten network. The solid contents released in to the boiling water were reduced by using transglutaminase. Because of this reduced starch loss, the surface of pasta/noodles is less sticky, reducing bulkiness, which benefits both manufacturers and consumers. Improved cooking yield is also expected. Noodles with transglutaminase, maintain their firmness and elasticity even after acid or heat treatment.

MTGase in Miscellaneous Application

There are several other possible applications for transglutaminase. Making modified proteins with improved thermal stability or gelation ability is one. Gelation of gelatin is a phenomenon dependent on thermal changes, and the gels are thermo reversible. The gelatin gel is stabilized by hydrophobic bonds, so if it is heated to certain temperatures, it changes to a solution or solution state. If a gelatin gel is treated with transglutaminase, the e-(c-glutamyl) lysine bonds are introduced in to its structure, and its thermo stability is significantly improved (Kuraishi *et al.*, 2001). Jelly products stable at high temperature can be produced. Gelatin gel treated with transglutaminase at 10 units/g protein and 50°C for 10 min kept its shape for 2h at 28°C, but the control gel without transglutaminase melted (Tani and Motoki 1990).

Takagaki *et al.*, 1991 have reported a method for coating vegetables and fruits with transglutaminase and proteins for preservation. Freshness of vegetables and fruits is maintained by coating with a membrane containing transglutaminase and proteins. Cut celery was treated with an aqueous solution containing transglutaminase, proteins, gelatins and partner-S (natural bactericide from spices) and then heated at 50°C for 5 min to form coating membranes. The coated celery was kept at 20°C for 3 days showing up to 300 bacterial cells/g, compared to 2×10^6 without treatment.

Yamauchi *et al.*, 1991 have reported a method for reducing the allergenicity of some food proteins and/or peptides.a_{s1}-casein (23 KDa) was treated with transglutaminase at 25°C for 20h in water to manufacture cross-linked casein (approx. 90 KDa) which was less allergenic.

REFERENCES

Ando H, Adachi M, Umeda K, Matsuura A, Nonaka M, Uchio R, Tanaka H and Motoki M 1989 Purification and characteristics of a novel transglutaminase derived from microorganisms. *Agric. Biol. Chem.* 53, 2613-2617

Ashie INA and Lanier TC 1999 High pressure effects on gelation of surimi and turkey breast muscle enhanced by microbial transglutaminase. J. Food Sci. 64(4) 704-708

Ashikawa N, Fukui H, Toiguchi S and Motoki M 1990 Transglutaminase- containing wheat and premix for cake, and manufacture of cake using them. Jpn Kokai Tokkyo Koho JP 02286031

Babiker E, Fujisawa N, Matsudomi N and Kato A 1996 Improvement in the functional properties of gluten by protease digestion or acid hydrolysis followed by microbial transglutaminase treatment. J.Agric. Food Chem. 44, 3746-3750

Chanyongvorakul Y, Matsumura Y, Nonaka M, Motoki M and Mori T(1995) Physical properties of soy bean and broad bean 11S globulin gels formed by transglutaminase reaction J.Food Sci. 60, 483-489, 493

Colmenero FJ, Serrano A and Cofrades S 2004 Transglutaminase as binding agent in fresh restructured beef steak with added walnuts. Food Chem. 85, 423-429

Dickinson E and Yamamoto Y 1996 Rheology of milk protein gels and protein stabilized emulsion gels cross-linked with transglutaminase J. Agric. Food Chem. 44, 1371-1377

Dickinson 1997 Enzymic crosslinkings as a tool for food colloid rheology control and interfacial stabilization. Trends in Food Science & Technology. 8, 334-339

Faergemand M, Murray BS and Dickinson E

bread as affected by microbial transglutaminase. J. Food Sci. 63, 472-475

Gerrard JA 2002 Protein- protein cross linking in food: methods, consequences, application. Trends in Food Science & Technology. 13, 391-399

Gomez-Guillen MC, Sarabia AI, Solas MT and Montero P 2001 Effect of microbial transglutaminase on the functional properties of megrim (Lepidorhombus *boscii*) skin gelatin. J. Sci. Food Agric. 81 665-673

Han X and Damodaran S 1996 Thermodynamic Compatibility of Substrate Proteins affects their Cross-linking by Transglutaminase. J.Agric. Food Chem. 44, 1211-1217

Ichihara Y, Wakameda A and Motoki M 1990 fish meat paste products containing transglutaminase and their manufacture. Jpn Kokai Tokkyo Koho JP 02186961

Imm JY, Lian P and Lee CM 2000 Gelation and water binding properties of transglutaminase- treated skim milk powder. J. Food Sci. 65(2) 200-205

Ishii C, Soeda T and Yamazaki K Method for the production of yoghurt, Europe Patent 0,610,649, 1994

Iwami K and Yasumoto K 1986 Amine-binding capacities of food proteins in transglutaminase reaction and digestibility of wheat gliadin with e-attached lysine. J. Sci. Food Agric. 7, 495-503

Jarmoluk A and Pietrasik Z 2003 Response surface methodology study on the effects of blood plasma, microbial transglutaminase and k-carrageenan on pork batter gel properties. Journal of Food Engineering 60, 327-334

Jiang ST, Hsieh J-F, Ho M-L and Chung Y-C (2000) Combination effects of microbial transglutaminase reducing agent and protease inhibitor on the quality of hairtail surimi. J. Food Sci. 65(2) 241-245

Jiang S-T, Hsiem J-F, Ho M-L and Chung Y-C 2000 Microbial transglutaminase affects gel properties of golden threadfin-bream and Pollock surimi. J. Food Sci. 65(4) 694-699

Jiang ST, Leu, SZ and Tsai GJ 1998 Cross-Linking of mackerel surimi actomyosin by Microbial transglutaminase and ultraviolet irradiation. J.Agric. Food Chem. 46, 5278-5282

Kashiwagi T, Yokoyama K, Ishikawa K, Ono K, Ejima D, Matsui H and Suzuki E 2002 Crystal structure of Microbial Transglutaminase from *Strptoverticillium mobaraense* J. Biol. Chem. 277 (46) 44252-44260

Kato T, Tomimatsu K and Toba S 1991 Manufacture of storage stable retort mapuo-doufu Jpn Kokai Tokkyo Koho JP 03168059

Kim S-M, Carpenter JA. Canier TC and Wicker L 1993 Polymerization of beef actomyosin induced by transglutaminase. J. Food Sci. 58(3) 473-474,491

Kuraishi C, Sakamoto J and Soeda T Process for producing cheese using transglutaminase Europe patent 0, 711,504, 1999

Kuraishi, C., Sakamoto, J., Soeda, T. Process for producing cheese using transglutaminase US patent 5,681,598,1997

Kuraishi C, Sakamoto J, Yamazaki K, Susa Y, Kuhara C and Soeda T1997 Production of restructured meat using microbial transglutaminase without salt or cooking. J. Food Sci. 62(3) 488-490, 515

Kuraishi C, Yamazaki K, and Susa Y 2001 Transglutaminase : Its utilization in the food industry. Food Reviews International 17(2) 221-246

Larre C, Denery-Papini S, Popineau Y, Deshayes G, Desserme C and Lefebvre J 2000 Biochemical analysis and rheological properties of gluten modified by transglutaminase. Cereal Chem. 77(2) 121-127

Lee.HG, Lanier TC, Hamann DD and Knopp JA 1997 Transglutaminase effects on low temperature gelation of fish protein sols. J. Food Sci. 62(1) 20-24

Motoki M and Nio N 1983 Cross linking between different food proteins by transglutaminase. J. Food Sci. 48, 561-566

Nielson GS, Petersen BR and Moller AJ 1995 Impact of Salt, Phosphate and Temperature on the effect of a Transglutaminase (FXIIIa) on the texture of Restructured Meat. Meat Science. 41(3) 293-299

Nonaka M, Sakamoto H, Toiguchi S, Yamagiwa K, Soeda T and Motoki M 1996 Retort resistant tofu prepared by incubation with microbial transglutaminase. Food

hydrocolloids 10, 41-44

Nonaka M, Soeda T, Yamagiwa K, Kobata H, 4 Motoki M and Toiguchi S 1990 Tofu for Long-term storage and its manufacture using a novel enzyme Jpn Kokai Tokkyo KohoJP 0269155

Nonaka M, Tanaka H, Okiyama A, Motoki M, Ando H, Umeda and Matsura A 1989 Polymerization of several proteins by Ca2+ independent transglutaminase derived from Microorganisms. Agric. Biol. Chem. 53(10) 2619-2623

Oh S, Catignani L and Swaisgood HE1993 Characteristics of an Immobilized form of Transglutaminase: A possible increase in substrate specificity by selective Interaction with a protein spacer. J.Agric. Food Chem. 41, 1337-1342

Ohtsuka T, Sawa A, Kawabata R, Nio N and Motoki M 2000 Substrate Specificities of Microbial transglutaminase for Primary Amines. J.Agric. Food Chem. 48, 6230-6233

Okada y, Murase K and Soeda T Low calorie ice cream. Japan Patent 5,918,40,1993

Pietrasik Z 2003 Binding and textural properties of beef gels processed with k-carrageenan, egg albumin and microbial transglutaminase. Meat Science 63, 317-324

Pietrasik Z and Jarmoluk A 2003 Effect of sodium caseinate and k-carrageenan on binding and textural properties of pork muscle gels enhanced by microbial t ransglutaminase addition. Food Research International 36, 285-294

Pietrasik Z and Li-Chan ECY 2002 Binding and textural properties of beef gels as affected by protein, k-carrageenan and microbial transglutaminase addition. Food Research International 35, 91-98

Pietrasik Z and Li-Chan ECY2002 Response surface methodology study on the effects of salt, microbial transglutaminase and heating temperature on pork batter gel properties. Food Research International 35, 387-396

Ramirez J, Uresti R, Tellez S and Vazques M 2002 Using salt and microbial transglutaminase as binding agents in restructured fish products resembling hams. J. Food Sci. 67(5) 1778-1784

Ramirez-Suarez JC and Xiong YL 2003 Effect of transglutaminase induced cross linking on gelation of myofibrillar/ soy protein mixtures. Meat Science 65, 899-907

Ramirez-Suarez JC and Xiong YL 2003 Rheological properties of mixed muscle/ non muscle protein emulsions treated with transglutaminase at two ionic strengths. International Journal of Food Science and Technology. 38, 777-785

Ramirez-Suarez JC and Xiong YL 2002 Transglutaminase cross-linking of whey/ myofibrillar proteins and the effect on protein gelation. J. Food Sci. 67(8) 2885-2891

Ramirez-Suarez JC, Xiong YL and Wang B 2001 Transglutaminase cross linking of bovine cardiac myofibrillar proteins and its effect on protein gelation. Journal of Muscle Foods 12, 85-96

Ruiz-Carrascal J and Regenstein J 2002 Emulsion Stability and water uptake ability of chicken breast muscle proteins as affected by Microbial Transglutaminase. J. Food Sci.. 67(2(734-739

Sakamoto H, Kumasawa Y and Motoki M 1994 Strength of protein gels prepared with Microbial Transglutaminase as related to reaction conditions. J. Food Sci. 59(4) 866-871

Sakamoto H, Kumazawa Y, Kawajiri H and Motoki M 1995 ε-(γ-glutamyl)lysine cross links distribution in foods as determined by improved method. J. Food Sci. 60 (2) 416-419

Sakamoto H, Kumazawa Y, Toiguchi S, SeguroK, Soeda T and Motoki M 1995 Gel strength enhancement by addition of microbial transglutaminase during onshore surimi manufacture. J. Food Sci. 60(2) 300-304

Sakamoto H and Soeda T 1991 Minced meat products containing transglutaminase Jpn Kokai Tokkyo Koho JP 03175929

Sata K, Tsukamasa Y, Imai C, Ohtsuki K, Shimizu Y and Kawabata M 1992 Improved method for identification and determination of ε-(γ-glutamyl)lysine cross-link in protein using proteolytic digestion and derivatization with phenyl Isothiocyanate followed by High-Performance Liquid Chromatography Seperation. J.Agric. Food Chem. 40, 806-810

Seguro K and Motoki M 1991 Manufacture of canned meats containing transglutaminase Jpn Kokai Tokkyo Koho JP 03210144

Siu N-C, Ma C-Y and Mine Y 2002 Physicochemical and structural properties of oat globulin polymers formed by a microbial transglutaminase. J. Agric. Food Chem. 50, 2660-2665

Takagaki Y, Narakawa K and Uchio R 1991 Coating of vegetables and fruits with transglutaminase and proteins for preservation Jpn Kokai Tokkyo Koho JP 03272639

Tani, T., Motoki, M. A method for producing highly polymerized gelatin. Japan Patent 2,867,43 1990

Tsao CY, Kao YC, Hsieh JF and Jiang ST 2002 use of soy protein and microbial transglutaminase as a binder in low-sodium restructured meats, J. Food Sci. 67(9) 3502-3506

Tseng C-S and Lai H-M 2002 Physicochemi--cal properties of wheat flour dough modified by microbial transglutaminase. J. Food Sci.. 67(2) 750-755

Wilcox CP and Swaisgood HE 2002 Modification of the rheological properties of whey protein isolate through the use of an immobilized microbial transglutaminase. J.Agric. Food Chem. 50, 5546-5551

Worratao A and Yongsawatdigul J 2003 Cross-tinking of Actomysin by Crude Tilapia (*Oreochromis niloticus*) Transglutaminase journal of Food Biochemistry 27, 35-51

Yamanaka F and Sakai K (1992) low-calorie sweet foods containing transglutaminase treated proteins. Jpn Kokai Tokkyo Koho JP 04144643

Yamauchi K, Uenikawa S, Enomoto A, Tanimoto H, Oohata K and Motoki M 1991 Transglutaminase for reducing allergenicity of food proteins and/or peptides and method of reducing their allergenicity. Jpn Kokai Tokkyo Koho JP 0327253.