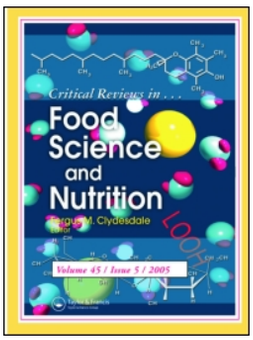

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Application of microbial transglutaminase in meat foods: A review

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ABSTRACT

Microbial transglutaminase (MTG) is an enzyme isolated from a variant of *Streptomyces mobaraensis* that forms covalent cross-links between protein molecules. Studies are being conducted since last two decades on utilization of MTG in meat foods to improve their characteristics, such as gelation, water-binding, emulsion stability, purge loss, cooking loss, etc. MTG is one of the important topics of interest in meat processing industry due to its advantages in practical utilization and commercial exploitation. This review will discuss about the overall applications of MTG in manipulating the functional properties of meat and meat products by means of various processes such as restructuring, value addition, etc.

KEYWORDS

Microbial transglutaminase; meat products; meat proteins; salt; phosphate

Introduction

Value addition of meat in various aspects is gaining a commercial focus in the meat processing industry. Consumer demands for a quality product at a moderate price could be met by effective utilization of the carcass through value addition of the low value meat. In meat industry, preparation of restructured meat products from low value meat pieces and trimmings can enhance their value. In addition, consumers are now more health conscious and the producers are in a pressure to develop new health oriented products such as low salt/low fat meat products enriched with a variety of non-meat ingredients. Various enzymes are used commercially for the structural engineering of restructured meat to improve their texture, of which microbial transglutaminase (MTG), a protein cross-linking enzyme takes a lead role. MTG enhances the texture, rigidity, and gel strength of meat products by forming a bond between glutamine and lysine of meat proteins without undesirable attributes, such as stickiness, high viscosity, and excessive meat adhesiveness (Ahhmed et al., 2009a). Many researchers recommended the usage of MTG in various meat products in improving their functional and textural properties with or without non-meat ingredients (Ahhmed et al., 2007; Pietrasik et al., 2007; Sun and Arntfield, 2012; Uran et al., 2013; Canto et al., 2014).

Transglutaminase (protein-glutamine:amine γ-glutamyl-transferase, EC 2.3.2.13) catalyses an acyl-transfer reaction between the -carboxyamide group of peptide bound glutamine residues (acyl donors) and a variety of primary amines (acyl acceptors), including the -amino group of lysine residues in certain proteins (Fig. 1a-b). In the absence of amine substrates, transglutaminase (TG) catalyses the deamidation of glutamine residues during which water molecules are used as acyl acceptors (Fig. 1c) (Motoki et al., 1986). Sarkar et al. (1957), Clarke et al. (1959) and Mycek et al. (1959) gave earlier reports on TG as a Ca^{++} activated enzyme derived from the soluble fraction of guinea pig liver which catalyzed the incorporation of primary amines into certain proteins and polypeptides. Folk and Cole (1965) first established the catalytic actions of TG in the glutamine residues and naturally occurring peptides as substrates. Various researches characterized Ca^{++} dependent factor XIII TG occurring as a zymogen from different tissues, such as liver, placenta, plasma, and platelets (Grundmann et al., 1986; Ichinose et al., 1986; Takahashi et al., 1986; Ikura et al., 1988; Tseng et al., 2002) which catalyzed the conversion of soluble proteins to insoluble high molecular polymers through formation of covalent cross-links (Traore and Meunier, 1992).

TG could modify functional properties of food proteins by means of amine incorporation, cross-linking, and deamidation (Motoki and Seguro, 1998) and adhering to the bonding surfaces of foods, such as meat, fish, eggs, and vegetables as a thin layer exhibiting strong adhesion in small amounts (Marques et al., 2010). In protein-containing food systems, the cross-linking reaction proceeds prior to the other reactions (Kuraishi et al., 2001; Abd-Rabo et al., 2010). Cohen et al. (1979) and Kahn and Cohen (1981) reported plasma TG catalyzed formation of glutamyl-lysine (G-L) bonds in myosin, myosin and actin, myosin and fibronection, fibrin and actin and fibrin and fibronectin. Substantiating the utilization of TG in modifying the functional and rheological properties of meat proteins, Tseng et al. (2002) demonstrated the reaction between myosin heavy chain and actin of spent hens catalyzed by TG purified from pig. Though the potential of TG in foods was explored scientifically, its limited source and complicated separation procedures from animal tissues increased its cost of production, thus restricting its commercial use in food processing.

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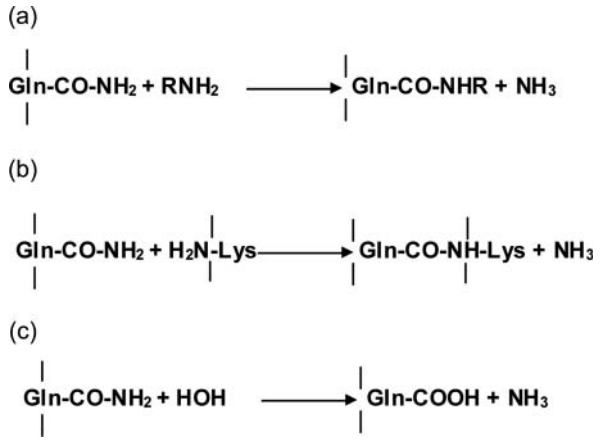

Figure 1. (a—c) Reactions catalyzed by transglutaminase (TGase). (a) Acyl transfer, (b) Crosslinking of Gln and Lys residues in proteins or peptides. The resulting bridge is called an ε-(γ-glutamyl)lysine (GL) bond. (c) Deamidation.

This resulted in finding of MTG from *Streptoverticillium sp.*, which could be produced in large scale by microbial fermentation using cheap cultures (Jiang and Yin, 2001).

Microbial transglutaminase (MTG)

MTG, a calcium independent and low molecular weight enzyme derived from a variant of *Streptomyces mobaraensis* (formerly classified as *Streptoverticillium mobaraense*), cross-linked most food proteins including the meat proteins through G-L bond (Nonaka et al., 1989 and Yokoyama et al., 2004). As per the reports of Ando et al. (1989), MTG was a calcium independent enzyme with a molecular weight of 40,000, isoelectric point 8.9 and optimal pH 6 to 7 with the reaction time of 10 min at 37°C. This was an important finding for the utilization of MTG, a low cost and easy to purify enzyme in food processing. Kanaji et al. (1993) established the complete amino acid sequence and chemical molecular weight of MTG from *Streptoverticillium* sp. strain S-8112 and found that the amino acid sequence was different from that of mammalian type I and II TG. Washizu et al. (1994) identified MTG producing strain S-8112 as a variant of *Streptoverticillium mobaraense*. In a study to optimize the fermentative medium and improve the MTG biosynthesis ability of the *Streptomyces* strains, Bahrim et al. (2010) found that the production of MTG increased 2.0 fold by *Streptomyces* sp. strain coded MIUG 13P with glucose and peptone (at 1.5% level each) as components of the medium.

The ability of MTG to incorporate amino acids or peptides covalently into substrate proteins could improve the nutritional value of food or feed proteins since covalently incorporated amino acids or peptides behave like endogenous amino acids (Yokoyama et al., 2004). Intrinsic TG in tissues and organs would catalyse the G-L bonds in proteins during cooking (Yokoyama et al., 2004). During slow heating of meat foods, the condition of the substrate would be conducive for the action of tissue TG (Ando et al., 1989) and may remain active for a while catalyzing the formation of G-L bonds. Since G-L bonds form by normal cooking catalyzed by intrinsic TG, the question of safety of the consumption of G-L bonds need not arise (Yokoyama et al., 2004). When Sakamoto et al. (1995) measured the naturally occurring G-L bonds in various raw meats and processed meat products, they found that G-L bonds were relatively higher in processed foods than in raw meat especially in fish and meat, except dairy products.

Advantages of using MTG in various types of meat products had been documented very well (Hammer, 1998; Ahhmed et al., 2007 and Canto et al., 2014). Motoki and Seguro (1998) listed the characteristics of gelation procedure and the gels formed in food protein substrates by MTG as follows;

- proteins not gelled by heating could be gelled
- gels that normally melt at higher temperatures no longer melt after the MTG gelation;
- protein in oil-in-water emulsions could be gelled even in the presence of sugars and/or sodium chloride;
- gel firmness would increase after heating;
- the gels could no longer be solubilized by detergents or denaturants.

Currently multifaceted researches are continuing for apt use of MTG in different meat systems and feasible methods that could be followed technologically at low cost and for use in commercial scale.

Cross-linkage of meat proteins by transglutaminase

Partial reaction of MTG occurred with water-soluble protein solution and major reaction in the myosin heavy chain proteins by protein–protein cross-linking (Ahhmed et al., 2009a and Kilic, 2003). In a notable study on interaction of myosin B with MTG in chicken and beef, Ahhmed et al. (2009b) observed that the gel strength of myosin B was improved in both species due to the newly formed protein mesh but was significantly higher in beef with higher protein viscosity and ε(c-glutamyl)lysine content. They suggested that the myosin B molecules in beef differ in complexity and figuration from those in chicken with presence of crucial proteins in chicken acting as inhibitors of MTG. It has been established that the action of MTG was different in chicken and beef where the elasticity of chicken was lower than beef, which was due to the presence of aminopeptidase H in chicken meat acting against the substrate of MTG (Ahhmed et al., 2009a,b). Though beef is considered as a non-setting protein, it was shown that the mechanical properties of restructured beef gels with 0.3% MTG could be improved by incubating at 50°C for 30 min and cooking at 90°C for 15 min

(Castro-Briones et al., 2009). Erwanto et al. (2003) confirmed collagen as a substrate for MTG where the mechanical properties of porcine collagen gel improved by cross-linkage through the G-L bonds with MTG as a catalyst and suggested development of collagen gels with modified functional properties for use in foods.

Nio et al. (1986) established that gelation of emulsions prepared with proteins and soybean oil occurred when incubated with TG prepared from guinea pig liver at 25°C for 10 min and the formed gel had a good heat stability. They also confirmed that the gels were formed by covalent cross-linking and not by hydrogen bonds and/or hydrophobic interaction. Sun and Arntfield (2011) explored the potential of MTG as a gelling agent in chicken myofibrillar protein isolate (MPI) to widen the utilization of chicken meat and reported that addition of appropriate amounts of MTG increased the gel stiffness and strength of myofibrillar proteins by cross-linking among MPI molecules through G-L interactions. They showed that the gel strength of MPI was highest at 95°C indicating the exposure of myosin active groups enabling the cross-linking of myosin heavy chain molecules. They recommended 0.9 M NaCl concentration at pH 6 in the MPI concentration range of 0.5–5.0% with a MTG concentration of up to 15 U/g of protein for improved gelation. Since NaCl concentration was a little higher than the recommended dietary intake of salt, they suggested a lower salt concentration of 0.6 M NaCl. Hong and Xiong (2012) demonstrated that the TG catalysed porcine myofibrillar protein (MP) deamidation and cross-linking depend on the pH, ionic strength, and protein surface charges where TG reactions were likely to be mediated by electrostatic interactions between the enzyme (TG) and the substrate (MP), possibly regulated by pH and salt concentrations.

Effect of MTG on meat product characteristics

MTG acts as a beneficial protein-binding agent due to its functional properties that improved the texture and gelation of mechanically treated meat products such as sausages where it enhanced the texture of beef and chicken sausages with improved breaking strength (Ahhmed et al., 2007). Hammer (1998) reported that addition of 0.2% of MTG in finely comminuted sausages increased their hardness and firmness, suggesting occurrence of meat protein linking during comminution of raw batter resulting in finer protein network structure after cooking. MTG effectively enhanced the texture of chicken breast patties reducing the cooking loss (Uran et al., 2013) and improved the water holding capacity in beef gels (Pietrasik and Li-Chan, 2002; Pietrasik, 2003). Inclusion of 0.3 g MTG/100 g proteins and a setting time of 60 min at 35°C were optimum to improve gel hydration and aggregation properties of the myofibrillar protein concentrate obtained from beef heart but the emulsion stability of cooked gel was lesser in MTG set gel compared to the sample without MTG (Ionescu et al., 2008).

In porcine myofibrillar protein, at all pH, MTG improved the emulsification activity index (EAI) and decreased the creaming index resulting in improved emulsion stability and it was suggested that MTG could act as an emulsifier and emulsion stabilizer improving the long-term stability of MP-stabilized emulsions, especially at pH values above 6.0. (Hong et al., 2012). Bak et al. (2012) prepared minced cured restructured ham using TG as binding agent with high-pressure (600 MPa) treatment without affecting the physicochemical characteristics of the ham, especially colour. Romero de Ávila et al. (2010) recommended the use of MTG in liquid or powder form to manufacture restructured dry-cured ham from deboned pork leg on a practical scale since it provided sufficient stable cross-links during salting and drying, and suggested initial application of salt over the meat surface followed by binding with MTG.

Canto et al. (2014) observed that addition of MTG to restructured caiman steaks improved the cooking yield and textural properties without affecting the color and flavo-r and advised a combination of MTG, KCl, and $MgCl_2$ for a suitable salt reduction strategy in restructured caiman steaks without compromising sensory attributes.

Salt and phosphate reduced meat products with addition of MTG

Studies had shown that a reduction in salt/phosphate in meat products would alter their sensory properties, juiciness, texture, and shelf life necessitating other measures such as technological optimization or the substitution of salt with other functional ingredients (Trespalacios and Pla, 2007a; Aaslyng et al., 2014). Various researchers explored the advantages of MTG in salt/phosphate reduced meat products. Nielsen et al. (1995) demonstrated the advantage of using TG in restructured meat by reducing the salt and phosphate content of the product without affecting the texture. Tseng et al. (2000) found that in low-fat chicken meat balls with 1% salt level, 1.0% of crude pig plasma TG improved the texture, juiciness, and overall acceptability with the highest gel strength. Dimitrakopoulou et al. (2005) formulated phosphate-free low salt (1%) restructured cooked pork shoulder with TG at 0.15% level recommending a processing at 72°C for 65 min which improved the consistency and the juiciness. Fulladosa et al. (2009) prepared restructured low-salt dry-cured hams with the addition of potassium lactate as NaCl substitute and addition of 2 g of TG per kg of raw muscle during vacuum massaging which had good binding without affecting the colo-r, flavo-r, and texture. Lennon et al. (2006) found that reformed beef quarter muscles injected with brine (to give a concentration of 0.5% salt) and 0.3% sodium tripolyphosphate (STPP) was at par with the whole muscle with regard to binding quality, overall acceptability and cooking loss and suggested that reformed meat with TG could be compatible with injection enhancement.

Effect of MTG in meat products with non-meat ingredients

Various studies had been conducted to evaluate the efficiency of MTG in improving the interaction of meat proteins and non-meat proteins. Ramırez-Suárez and Xiong (2003) established that MTG effectively cross-linked soy and muscle proteins producing a rigid mixed protein gel at a reduced myofibrillar protein concentration eliminating the adverse effects of soy protein and recommended MTG as a vital means for producing an adhesive mixed protein gel structure with a lesser quantity of extracted myofibrillar proteins. Sun and Arntfield (2012) showed that with addition of appropriate amounts of MTG, the gel strength of myofibrillar/pea proteins mixture (3:1) greatly increased indicating that some G-L cross-linking occurred between muscle and pea proteins.

Kilic (2003) noticed that the binding effect of MTG in chicken doner kebab enhanced when used with sodium caseinate. Sodium caseinate was a better non-meat protein substrate for MTG due to formation superior covalent cross-links with meat protein molecules than any other non-meat protein studied (Kuraishi et al., 1997; Pietrasik et al., 2007). Incorporation of TG in combination with sodium caseinate, KCl and wheat fibre (with 94.5% insoluble dietary fibre and 2.5% soluble dietary fibre) in low-sodium pork frankfurter with added walnut resulted in a firmer gel network with better water binding and fat binding properties than the use of TG alone (Colmenero et al., 2005). Martínez et al. (2011) formulated novel beef patties enriched with polyunsaturated n-3 fatty acids and dietary fiber with optimal texture as well as minimal effect on color and cooking loss and improved values of expressible water by a pretreatment with TG at 40°C for 17 min without the need for sodium caseinate addition. Muguruma et al. (2003) produced chicken sausages incorporated with various biopolymers prepared from soybean protein, casein, whey protein isolate (WPI), mixtures of soybean protein and casein, and soybean protein and WPI. They showed that cross-linking soy protein isolate and casein or WPI with MTG provided biopolymers with improved heat stability and emulsifying properties with improved texture even in the presence of 0.05% sodium tripolyphosphate. A combination of MTG (0.7%) and sodium caseinate (3%) as a cold-set binder in fresh restructured beef steak with added walnuts decreased the purge loss and cooking loss with acceptable sensory characteristics. However, water binding properties were inadequate in fresh and cooked products though the products were mechanically suitable (meat particle binding) for handling in the raw state (Serrano et al., 2004).

In chicken gels formulated with 1.5% salt and 0.3% sodium tripolyphosphate, Trespalacios and Pla (2007b) noticed cross-linking of myofibrillar proteins with globular proteins of egg catalyzed by MTG in combination with high pressure treatment (HPT) at 500 MPa for 30 min at 40°C which improved the binding properties, textural parameters, microstructure and colour. In another similar study with application of MTG and HPT of 700 and 900 MPa with 1.0% salt and eliminating phosphate, Trespalacios and Pla (2007a) observed a decrease in ionic strength and water holding capacity and favourable decrease in expressible moisture in MTG gels. Cutting force, hardness, and chewiness of gels was improved due to the combination of MTG with HPT (700–900 MPa) than HPT alone suggesting heterologous complex of meat and egg proteins due to combination treatment. Since a pressure above 700 MPa was denaturing as heat treatment, they recommended 700 MPa for synergistic effect of combining enzymatic cross-linking with high-pressure treatment, which would be of interest in food processing for producing high quality products with beneficial properties, such as low-fat, low-sodium content, phosphate-free and enhanced sensory attributes, while assuring the nutritive value and microbiological safety.

Hong and Chin (2010) suggested that the combination of MTG with sodium alginate could improve water-binding ability and produce cold-set myofibrillar protein gelation at a lower salt level than MTG alone. Cofrades et al. (2011) found that the addition of Sea Spaghetti seaweed with MTG improved the binding properties of low-salt restructured poultry steaks. Among various starches, potato starch was most suitable to be incorporated in surimi-beef gels at 3% level with 0.5% MTG with good gel strength and lowest cooking loss (Zhang et al., 2013). Inclusion of konjac flour in TG treated porcine myofibrillar protein containing sodium caseinate improved the texture and water holding capacity (Chin et al., 2009).

Moreno et al. (2010a) observed an increase in oxidation of restructured trout (with a high content of omega-3 fatty acids) and hake mince with inclusion of MTG compared to that without MTG during chilled storage with a significantly higher peroxide value for trout than hake with or without added cod liver oil. They opined that MTG might trigger oxidation depending on the antioxidative status of the fish used and not exactly on the amount of fat present. Though the activity of MTG was impaired in the presence of oil due to extended gel formation time to reach maximum hardness, they recommended use of MTG in hake gels with 5% added cod liver oil to produce gels with good physico-chemical properties and with good oxidative stability. In another similar study, Moreno et al. (2010b) found that the protein network that formed in the gel of restructured fish models using cold gelation was better in samples containing MTG than those with sodium alginate. Though the gel strength was enhanced in the course of frozen storage both by MTG and sodium alginate, lipid oxidation was more in the former.

Conclusion

Microbial transglutaminase had been proved as a "protein-maker" catalysing cross-linking reactions between protein and peptide molecules of meat foods with improved functional properties. From the above discussion, it is obvious that MTG could be used successfully in various kinds of meats to develop new type of products with improved characteristics. Studies are being carried out to effectively utilize MTG in meat processing on commercial scale eliminating the disadvantages. It can be concluded that MTG will take an important role in future in development of restructured and value added meat products.

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