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REVIEW ARTICLE

Texturisation and modification of vegetable proteins for food applications using microbial transglutaminase

Mark Dube · Christian Schäfer · Sybille Neidhart · Reinhold Carle

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Abstract Microbial transglutaminase (MTG) isolated from *Streptomyces mobaraensis* has been available on a commercial scale for several years. MTG generates inter- and intramolecular cross-links between γ-carboxylamide groups of glutamine residues and ε-amino groups of lysine residues in proteins. Due to its great potential to improve various functional properties of proteins, MTG is mainly used to enhance texture, stability, and water binding. Application of MTG for the production of plant protein-based foodstuffs such as tofu, noodles, bread and bakery products, is still limited to raw materials from soybean and wheat. However, with the increasing demand for vegetarian foods, the utilisation of novel proteins as functional ingredients, e.g. from peas, lupins, sesame, and sunflower, seems promising. To open new horizons for MTG application, this review aims at demonstrating the actual potential of MTG in processing foodstuffs based on vegetable proteins. Particular focus was laid on novel plant protein sources suitable for cross-linking with MTG. Furthermore, strategies for improving texture and nutritive value of the proteins are discussed.

Keywords Cross-linking · Microbial transglutaminase · Protein modification · Texturisation · Vegetable proteins

M. Dube · S. Neidhart (✉) · R. Carle
Institute of Food Technology, Plant Foodstuff Technology,
Hohenheim University,
August-von-Hartmann-Strasse 3,
70599 Stuttgart, Germany
e-mail: neidhasy@uni-hohenheim.de
Tel.: +49-711-459-2317
Fax: +49-711-459-4110

C. Schäfer
Fraunhofer Institute for Process Engineering and Packaging,
Giggenhauser Strasse 35,
85354 Freising, Germany

Present address:
C. Schäfer
DSM Nutritional Products Ltd.,
4002 Basel, Switzerland

Introduction

The demand for high-quality food proteins has increased over the past two decades. Particularly regarding consumers' acceptance and preferences, methods for modifying the techno-functional properties of proteins are of increasing interest in order to develop convenience foods and health foods [1]. The enzymatic cross-linking of proteins using transglutaminase (TG, protein-glutamine γ-glutamyl-transferase, EC 2.3.2.13) is suitable for improving the techno-functional properties of proteins [2]. Since the isolation of a microbial transglutaminase (MTG) from *Streptomyces mobaraensis* (older synonym *Streptoverticillium mobaraense*) in 1989 [3], its purification from *Streptomyces* sp. [4–6], and structure analysis [7], commercialisation of MTG for food applications has been started. The various effects of MTG on the techno-functional properties of proteins such as improvement of texture, stability (regarding temperature, syneresis and emulsification), increased water binding, and its utilisation in food industry have been described previously [8–15]. Specific applications have been reported for meat [16–18], fish [19–22], dairy [23–25], egg [26], bread and bakery products [27–29], and in soybean processing [30, 31]. A patent search from 1990 to date highlighted 165 relevant patent applications covering general applications of MTG (34), processing of dairy (24), fish (18), meat (15), bread and bakery (17), noodles and pasta (17), soybean (24), and other vegetable or animal foods (14). Whereas most of the inventions

dealt with animal foodstuffs, MTG has rarely been used in texturisation of plant proteins, with the exception of tofu production from soybean, bread making, and noodle production from wheat. However, there is an increasing demand for vegetarian foods in many branches of the food industry. Aside from the competitive price of plant proteins and their high security of supply as well as easy storage, derived products are well accepted by consumers [1]. Therefore, this review focuses on actual applications of MTG in processing foodstuffs based on plant proteins and deals with the suitability of various plant proteins for cross-linking with MTG.

Reactions catalysed by transglutaminase

TG catalyses an acyl-transfer between the γ-carboxylamide groups of peptide- or protein-bound glutamine residues and primary amines. In the absence of primary amines, deamidation of glutamine residues is the result of using water as acyl acceptor. Both nature and conformation of the amino acids in the vicinity of the glutamine residue are factors affecting the reactivity of the acyl donor. In the first step of the reaction, a complex between the glutamine residue (acyl donor) and the reactive centre of TG is formed [32]. Because of the formation of a sterically determined binary complex between enzyme and substrate, the glutamine content and the bonding environment of this amino acid are considered the limiting parameters of the cross-linking reaction. Formation of the intermediate acyl-enzyme complex that involves the thiol group of cysteine in the active centre of TG occurs under release of ammonia [33, 34]. When ε-amino groups of protein-bound lysine residues act as acyl acceptors, ε-(γ-glutamyl)-lysine cross-links are produced. Resulting isopeptide bonds create a stable protein network via inter- and intramolecular interactions. The hydrolytic deamidation is much slower than the linkage to primary amines and the formation of cross-links in the presence of accessible ε-amino side-chains from protein-bound lysine, respectively [35, 36]. Among the three possible reactions, only the cross-linking is of interest in modification of the techno-functional properties of proteins (Fig. 1). Furthermore, the reaction between the γ-carboxylamide groups and primary amines is a promising tool to improve the nutritional value of vegetable proteins by fortification with deficient amino acids.

Sources of transglutaminases

Occurrence of TG is widespread in eukaryotic organisms and has been found in various tissues of animal and plant origin [37–44]. The enzymatic characteristics of TG and the effects on the functional properties of proteins were investigated using a Ca^{2+}-dependent TG derived from guinea pig liver [45, 46], other animal tissues, such as squid gill, rat liver, Japanese oyster or porcine blood [47–50], and from human blood [51]. However, an application of TG in food industry was not feasible due to the relatively small quantities obtained, the extensive separation and purification steps required, and the costs involved. In 1989, a Ca^{2+}-independent microbial TG from *Streptomyces mobaraensis* was extracted by Ando et al. [3]. Purification was rather easy, and the production of MTG has been established commercially. However, for an industrial utilisation, a more efficient system for MTG production would have to be implemented, and variations of MTG have been considered a prerequisite to give preferable properties to foods. Therefore, attempts to obtain MTG from other microorganisms by conventional fermentation [52–57] or by means of genetic modification using host microorganisms [58–64] are in progress. Washizu et al. [58] expressed the TG gene of *Streptomyces mobaraensis* in *Streptomyces lividans* using a tyrosinase promoter, but the productivity was quite low, with recombinant TG amounting to 0.1 mg L^{-1} of culture supernatant. As shown by Takehana et al. [61], a chemically synthesized *Streptomyces* TG gene was expressed in *E. coli*. But again, the level of TG gene expression into the periplasmatic space (5 mg L^{-1} of culture supernatant) was not satisfactory. The authors assumed that direct expression of TG in *E. coli* cells in its active form did not enhance production levels, because the typical activity of TG, that is, formation of cross-links between proteins, might have a harmful influence on the cells, leading to their death. A method for expression of an inactive MTG as inclusion bodies in *E. coli* (200–300 mg L^{-1}) was described by Yokoyama et al. [59]. The refolded MTG, purified by ion-exchange chromatography, showed an activity equivalent to that of native MTG. Kikuchi et al. [60] succeeded in achieving an efficient secretion of active-form MTG by using *Corynebacterium glutamicum* as a host. The maximum yield accumulated was 142 mg L^{-1} of culture supernatant.

At present, MTG isolated from *Streptomyces mobaraensis* is still the only one available on a commercial scale. Due to food regulations and lack of acceptability by consumers,

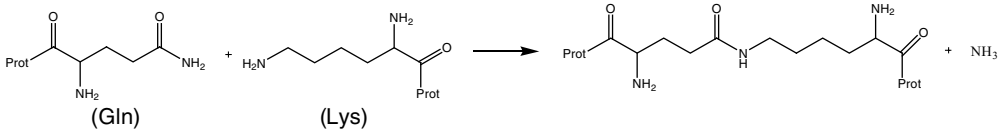

Fig. 1 Mechanism of the cross-linking reaction between glutamine (Gln) and lysine (Lys) residues of proteins catalysed by TG (Prot, protein)

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

Source	Molecular weight (Da)	Isoelectric point IEP	pH optimum	pH stability (EA 90–100%)	Temp. optimum (°C)
Streptomyces mobaraensis S-8112 [3]	40,000	8.9	6–7	5–9	50
Streptomyces mobaraensis WSH-Z2 [67]	40,000	n.d	6.0	4.5–6.5	52
Streptomyces libani [68]	37,900	6.4	5.0	5–7	53
Bacillus circulans [52]	45,000	6.3	7.0	6–8.5	47
Bacillus subtilis [57]	29,000	n.d	8.2	7.5–8.5	60
Bacillus subtilis [55]	23,000	n.d	8.0	7.4–8.2	50
Streptomyces ladakanum [56]	39,000	n.d	5.5	4.8–6.2	40
Streptomyces ladakanum [69]	37,500	7.9	6.0	4.6–8	50

n.d.: not determined; EA: enzyme activity.

production of MTG by means of genetic modification as described above appears to be less favourable [9]. Pedersen et al. [65] evaluated the allergenic potential of MTG, using a decision scheme of the FAO/WHO. Since no IgE-mediated allergy to any bacterial proteins has been reported, allergenicity of MTG was thought unlikely. The enzyme was fully degraded after 5 min of pepsin treatment and showed no homology with known allergens. Thus, the authors concluded that no safety concerns as regards the allergenic potential of MTG were identified. However, Gerrard and Sutton [66] recently discussed the risk that the addition of MTG to cereal products may generate the epitope responsible for celiac disease, which is a chronic inflammatory disorder of the intestines, induced by ingestion of gluten-containing grains. Indication of this risk was based on the observation that deamidation of a crucial sequence in gliadin proteins by tissue TG during the transport through the epithelial barrier of the gut resulted in an epitope that activated the T-cells deemed to be involved in the autoimmune response in celiac suffers. Although there has been no experimental evidence to suggest that cereal grain foodstuffs baked in the presence of MTG may trigger symptoms of celiac disease, the authors recommended to cease from using MTG in such products until a proof of safety.

Enzymatic characteristics of microbial transglutaminases

As shown in Table 1, the characteristics of MTG obtained from various microorganisms vary even among strains. Umezawa et al. [68] reported differences in the enzymatic properties of MTG from *Streptomyces* sp. MTG from *Streptomyces libani* showed slightly lower optimum reaction temperature and thermal stability than that from *Streptomyces mobaraensis*. Thus, deactivation of MTG from *Streptomyces libani* can be achieved at lower temperatures. A distinct difference was found in their gelation activities, as shown by the use of a sodium caseinate solution for cross-linking. Storage and loss modulus of a sodium caseinate solution increased much slower when MTG from *Streptomyces libani* was used instead of that from *Streptomyces mobaraensis*. The optimum pH of MTG from *Streptomyces mobaraensis* was between pH 5 and 9 [3], whereas that derived from *Bacillus subtilis* was within a much smaller range from pH 7.5 to 8.5 [57]. The optimum temperature of MTG from *Bacillus subtilis* (60 °C) was found to be considerably higher than that of MTG from *Streptomyces mobaraensis* (50 °C). Menéndez et al. [70] determined the activity of MTG from *Streptomyces mobaraensis* during thermal and high-pressure treatments. Enzyme inactivation at 0.1, 200, 400, and 600 MPa and 40 °C followed first-order kinetics. A residual activity of 50% occurred after 12 min at 600 MPa and 40 °C. This remarkable stability of the enzyme could be explained by the stability of its active site, which is located within an extended β-strand region. These structures were nearly incompressible and more stable than the α-helix structures against high hydrostatic pressure. Formation of protein cross-links, which was observed for samples treated for 30 min at 600 MPa and 40 °C or for 2 min at atmospheric pressure and 80 °C, was attributed to the formation of intermolecular disulfide bonds.

Thus, cross-linking with MTG of different origin should be thoroughly investigated in order to provide the necessary knowledge about the optimal processing parameters.

Application of microbial transglutaminase in food processing

The following examples illustrate the potential of MTG application in the processing of foodstuffs based on plant proteins, especially from soybean and wheat.

Soybean products

The use of MTG in tofu manufacture has been claimed by several patent applications (Table 2). MTG is primarily used to improve texture, sensory properties, and shelf life of tofu. Due to its high moisture content ($\sim$90%), traditional tofu

Table 2 Patent review covering soybean protein applications

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	Manufacture 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

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, a larger amount of coagulants, such as magnesium chloride, is required. This results in non-uniform coagulation, affecting particularly the manufacture of packed tofu. At present, slow-acting glucono-δ-lactone is used as the preferred coagulant [30]. Matsuura et al. [71] disclosed a process for producing tofu, in which MTG (0.1–5 U g^{-1} of soybean milk protein) was added either simultaneously or after the addition of glucono-δ-lactone to sterilized soybean milk. The packed tofu was evaluated as excellent in both hardness and flavour. Whereas sterilised soybean curds (e.g., 100–125 °C; 10–30 min) were generally too hard and dry, the MTG-treatment generated a softer and smoother product, which was stable for 6 months or longer at 25 °C [72].

MTG was responsible for a higher resistance to heat exposure. Tofu without MTG lost more than 35% of its weight through retort cooking for 30 min because of structural changes during heating and release of water enclosed in the microstructure. Tofu treated with MTG (10 U g^{-1} of solids) also shrank, but the weight loss only amounted to 15%. In contrast, enzymatically prepared tofu gels reached both a breaking strength and strain close to those of control tofu (before thermal preservation) [30]. Kwan et al. [73] confirmed these results and compared the physical properties of tofu treated with MTG or low levels of glucose. Oomura et al. [74] described a process for industrially produced tofu products with excellent taste and freeze resistance. Again the application of MTG (0.1% by weight of soybean milk) allowed easier control of the coagulation reaction and produced a smooth texture. The improvement of freeze resistance was not only achieved by adding MTG, but also by heating the soybean milk extracted from uncooked soybean slurry to obtain concentrated milk with a high content of solids (10–16%), but low viscosity. In a conventional process, extraction of soybean milk from cooked soybean slurry increases viscosity of the milk, which adversely affects the subsequent coagulation reaction.

An alternative method referred to the effective utilisation of soybean curd lees, which are discharged during the production of soybean milk and curd. Initially, the plant fibre of pulverised curd lees, mixed with water, was macerated by different tissue disintegrating enzymes, such as pectinases and cellulases. In an optional secondary enzyme reaction, MTG was added (0.1–2%) to the soybean curd lees slurry to produce a functional curd lees "milk" with solid particle sizes of approximately 50–100 μm [75].

Bread and bakery products

Several studies have proven that the use of MTG improves baking properties and product quality. As shown by Basmann et al. [76], the effects of MTG on the rheological properties of doughs from wheat flours and bread quality depended on the amount of MTG. Farinograph analyses revealed decreasing flour-water absorption with increasing enzyme levels (0–0.5%, w/w). Dough development time and stability values initially increased with rising MTG doses, but decreased at higher MTG levels (1.0–1.5%, w/w). Low levels of MTG improved the bread quality, such as crust and crumb characteristics, while higher amounts (1.0–1.5%, w/w) had detrimental effects.

Another study of Basmann et al. [77] dealt with the influence of MTG on the admixture of barley or soy flour to wheat flour. Bread from MTG-treated (0.25%, w/w) soft wheat flour with addition of barley flour up to 30% had higher loaf volumes as well as better crumb and crust characteristics, and were apparently softer than those from non-treated samples. MTG treatment of hard flour was associated with slight decreases in the loaf volume at all levels of barley flour supplementation. High proportions of fibres like bran or rye interfered with the balanced ratio of starch, gluten, and pentosans in the dough, thus reducing baking capacity. Addition of MTG to mixed flour systems with rye flour and/or fibre components resulted in a higher homogeneity and improved the stability of mixed doughs [78]. Due to insufficient formation of the gluten network during dough processing, a lack of elasticity and consequently poor product quality are known to occur during the manufacture of bread with low wheat content. The use of enzymes such as α-amylase and pentosanase to improve the properties of rye dough resulted in softer dough and noticeably flatter loaves [79]. The addition of MTG (3 U kg^{-1} of flour) to such doughs low in wheat significantly improved the quality of the dough and derived baked products. The stickiness of the dough was reduced, which facilitated mechanical processing. Due to the improved gas retention, bread volume increased. The dough stability and the loaf shape also improved even with a rye flour proportion of 80% [80].

An et al. [81] investigated the effect of MTG on the physicochemical properties of resistant starch-added wheat flour dough and baguettes. MTG increased the tensile strength of the dough after fermentation and the bread volume due to a well-developed gluten network. The sensory evaluation showed that yeast flavour, hardness, and chewiness decreased significantly, whereas the roasted flavour, the volume and the overall eating quality of the MTG-treated baguettes were higher than those of the controls without MTG. When added to doughs containing hydroxypropylmethylcellulose (HPMC), high-ester pectin and/or an emulsifier (diacetyl tartaric acid ester of mono-diglycerids, DATEM), MTG also induced synergistic effects on mixing parameters, resulting in increased water absorption, development time and stability [82]. Highly cohesive doughs with improved water-holding capacity and gluten strength during mixing and fermentation, and suitable pasting behaviour during cooking were achieved using MTG/pectin/DATEM mixtures, mainly associated with suitable interactions of the pairs MTG/DATEM and DATEM/pectin.

An enhancement of the fresh quality and inhibition of staling has been achieved in bakery products by the use of starch-degrading and non-starch-degrading enzymes. Synergistic beneficial effects were induced when MTG was added to α-amylase-supplemented doughs. By combination of both enzymes, breads with softer and less chewy fresh crumbs were obtained due to a slower crumb staling kinetics [83]. Furthermore, MTG (5 g kg^{-1} of flour) improved the lift of puff pastry and the volume of croissants. After baking, all the MTG-treated products had an excellent texture, mouthfeeling, and layer. Compared to the control samples, the main difference was an increase in size. These effects persisted after the pastry and croissant doughs had been stored frozen for periods of up to 90 days [29]. The addition of MTG to a croissant dough resulted in a diminished amount of protein in the albumin and globulin fraction, whereas the gliadin fraction was increased. Furthermore, specific high-molecular weight glutenin subunits appeared to be cross-linked by MTG [28]. MTG has also been used to counteract the negative effects of proteases in insect-damaged wheat flours. Some heteropteran insects attack developing wheat grain and inoculate it with salivary proteolytic enzymes. The resulting flour is uniformly contaminated with proteases causing hydrolysis of gluten proteins, which leads to a significant weakening effect in the doughs. Alveograph parameters, such as tenacity and dough extensibility, showed that bread-making quality of damaged flours was improved by the addition of MTG (0.5–1.0 U g^{-1} of flour). Dynamic rheometric tests demonstrated that the functional behaviour of damaged wheat flours could be recovered through MTG treatment enabling properties comparable to that of undamaged wheat [84].

To modify wheat flour, MTG is usually added to the flour or the dough prior to the baking process, which has sometimes caused problems due to overdosage and non-uniform mixing. Therefore, Yamazaki and Soeda [85] (Table 3) developed a method to modify wheat flour with application of MTG at grinding and/or the preceding tempering process. In the latter case, an aqueous solution of MTG (5–10 U g^{-1} of protein) was added to the cleaned wheat grain. Subsequently, tempering was performed preferably at 10–30 °C for 16–50 h to enable MTG permeation from the surface of the wheat grain into the endosperm to promote cross-linking of gluten. Sensory evaluation of the baked loaves indicated an enhancement in surface colour and quality as well as crumb and texture, when compared with the control.

Table 3 Patent review covering bread and bakery applications

Patent number	Year of publication	Title
JP 02286031	1990	Wheat flour for cake 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	Utilisation of transglutaminases 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

Rosell et al. [86] reported an alternative method for improving wheat gluten by the addition of MTG to the tempering solution, confirming the results of Yamazaki and Soeda [85].

Foodstuffs containing high amounts of water through creams and fruits filled into pastry or tart base frequently become soggy. MTG-treated products were found to be more crispy, flaky, and well-protected against dough-softening. Hardness and crispiness of a tart base as well as the texture of the whole product (tart with custard cream) were slightly more favourable when compared to the control [87].

Since cereal products, especially bread, are the basic components of the diets in many countries, there is a high demand for gluten-free breads from persons with celiac disease. In view of the fact that gluten is the major structure-forming protein, Moore et al. [88] tried to mimic the viscoelastic properties by utilisation of MTG (0.1–10 U g^{-1} of protein). The breads mainly consisted of white rice flour, potato starch, corn flour, and minor ingredients. The individual protein sources for each recipe were soy flour, skim milk powder, and egg powder. The crumb hardness was monitored over a storage period of 5 days. Distinct changes in the textural and crumb grain characteristics of all breads were observed over the whole storage period. The MTG-treated (10 U g^{-1} of protein) bread with skim milk powder revealed significantly higher crumb hardness than the other breads with lower MTG doses. The former type of bread was also firmer than those with soy flour and egg powder, respectively, throughout storage. No significant differences were found between the breads with soy flour at all enzyme levels. In conclusion, it was possible to produce a stable network within a gluten-free bread system, improving the loaf volume and crumb characteristics.

Noodles

As reported by Seo et al. [89], the addition of MTG (3–7 mg kg^{-1} of flour) to wheat flour improved dough stability and chewiness of cooked noodles made therefrom. Since the addition of 10 mg kg^{-1} had no impact on the dough stability, the authors assumed that excessive cross-links within the protein at high doses of MTG prevented the formation of a regular network. Wu and Corke [90] reported a drastic increase in both storage and loss modulus of fresh noodle sheets, even at a MTG level of 1 g kg^{-1} of wheat flour, but the effect mostly decreased with higher levels of enzyme. This could be related to the limited content of lysine in gluten, which would restrict the cross-linking reaction at high MTG dosages of 5–20 g kg^{-1} of flour. The addition of MTG 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 (boiling water, 5.5 min).

As described in several patent applications (Table 4), MTG was generally added to the raw material (wheat flour) to obtain noodles with enhanced elasticity, texture and sensory properties, which were maintained post-boiling. The main problem was a loss of the glutinous taste of processed

Table 4 Patent review covering noodle applications

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 noodles modified in texture
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 preparations comprising transglutaminase and process for producing noodles
JP 2000253841	2000	Production of boiled noodle or the like
JP 2002262794	2002	Noodles 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

noodles caused by heat treatments, freezing or drying. Therefore, attempts to improve the properties by adding starch, egg white, gliadin, glutenin or MTG were described. Yamazaki and Nishimura [91] pointed out that these techniques were not satisfying the demand for elastic and glutinous noodles. In the production of Japanese noodles, a treatment with TG (5 U g^{-1} of wheat flour protein) led to elastic, but poorly glutinous noodles after boiling for 12 min and storage for 30 min. The glutinous effect and also the elasticity could be improved by a combination of TG cross-linking (5 U g^{-1} of wheat flour proteins) and the addition of gliadin (2.0% by weight of wheat flour).

Suitability of vegetable proteins for cross-linking with microbial transglutaminase

As demonstrated by industrial applications of MTG, mostly proteins from soybean and wheat have been subjected to enzymatic cross-linking. In recent years, other crops have also been suggested as potential raw materials for the production of proteinous ingredients, e.g. isolates from pea [92, 93], lupine [94, 95], rice [96, 97], and sunflower [98, 99]. To evaluate the suitability of plant proteins for cross-linking with MTG, specific characteristics of the substrates, such as their amino acid composition, nutritive value and utilisation in human nutrition, have to be considered.

Soy proteins

Besides their major use for feed, 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 such as lowering of plasma cholesterol and osteoporosis [100]. To study basic properties of soy protein treated with MTG, rheological characteristics of MTG-treated protein gels from soy protein isolate (SPI) and concentrates (SPC), respectively, were compared [101]. Breaking strength and deformation of MTG-treated gels increased at an addition of 0.5 and 1.0 U g^{-1} of protein, while gels derived from SPI were more rigid and 1.5 times more elastic than gels obtained from SPC. This difference was attributed to the presence of higher carbohydrate contents in SPC. Deformation, breaking strength and cohesiveness decreased at a MTG dose of 5.0 U g^{-1} of protein, but still ranged above those of the control. Similar to the physical parameters, amounts of ε-(γ-glutamyl)-lysine cross-links increased with rising enzyme dosage. Compared to thermally induced glycinin gels, networks of MTG-induced gels were more resistant to large deformation and had a higher rigidity. The association of protein molecules through the isopeptide bond was considered more regularly than in thermally induced gels. Furthermore, the irreversible formation of covalent bonds was supposed to contribute to stronger protein–protein interactions [102]. Kang et al. [103] concluded that gel properties of soybean glycinin were not only controlled by the amount of cross-links formed but also by the nature of surface lysine and glutamine residues of glycinin molecules. A heat treatment of a glycinin solution prior to the MTG reaction resulted in a higher content of ε-(γ-glutamyl)-lysine cross-links with increasing heating time (30–120 s at 100 °C) combined with an increase of surface glutamine and lysine residues.

Wheat proteins

Wheat gluten is considered a protein of inferior quality as regards its nutritive value, primarily due to insufficient amounts of the essential amino acids lysine and threonine [104]. As to the MTG reaction, gluten is assumed to serve as a better acceptor than other food proteins because of its higher glutamine content, although its low lysine content limits its value as an amine donor substrate [105]. Porta et al. [106] reported that the reactivity of wheat glutenins and gliadins were superior to prolamines from oats, maize and rice. Among the constitutive proteins of gluten, the high-molecular weight glutenin subunits were the most affected ones during MTG reaction [107, 108]. When native and MTG-treated gluten were compared, viscoelastic properties of the cross-linked protein appeared to be less sensitive to heat treatment [109]. Despite its low lysine content, MTG-catalysed cross-linking of gluten resulted in gels with considerably enhanced elasticity and a reinforced structured network [110]. As shown above, numerous studies have dealt with the application of MTG in the manufacture of bakery products. Both positive and negative effects of MTG utilisation were reported. Hence, considerable expansion in the number of applications is difficult, as customised optimum enzyme levels are required for any individual protein in order to obtain maximum improvement of the techno-functional properties [76].

Rice proteins

Even though annual production of rice is nearly as high as wheat [111], reports about enzymatic cross-linking of proteins from rice in research papers and patent applications are scarce. Additionally, commercial availability of rice bran concentrates and isolates is limited, possibly due to the complex nature of rice bran proteins and their high content in phytate (1.7%) and fibre (12%). Both components were shown to bind with proteins [112]. In addition, the crude protein content of rice ranging from 5 to 10% is lower than the level found in most other cereals [113]. In contrast, the lysine content of rice proteins exceeds that of wheat by more than 50% [100]. The incorporation of hydrocolloids such as hydroxypropylmethylcellulose (HPMC) into dough formulations has enabled the production of bread from rice flour. Rice proteins lack the ability to form the necessary network for holding the gas produced during the fermentation process [114]. According to Gujral and Rosell [115], applications of rice flour could be broadened if rice proteins would be cross-linked to produce a stable network. By MTG application, a partial substitution of HPMC in baking of bread was possible. Cross-linking with MTG resulted in doughs with improved viscoelastic behaviour, as detected by dynamic rheometry. Due to the fact that rice constitutes a major food source for a large portion of the world population, extensive efforts have been made for the production of rice protein products and improvement of their functional and nutritive properties by the use of MTG.

Pea proteins

Peas (*Pisum sativum* L.) are an important grain legume, but more than 90% of the pea production in Europe is used for animal feed [116]. However, peas and other legumes contain a variety of antinutritive compounds also affecting protein quality. The interaction of phytic acid with proteins has been studied mainly in soybean. Processes for dephytinised proteins have been developed for both soy protein [117–119] and pea protein [120, 121]. The action of MTG on the constitutive polypeptides of pea legumin was studied by Larré et al. [122]. As indicated by the fact that only the α-polypeptides were polymerized in the native globular conformation, the β-polypeptides were inaccessible to MTG. Among the three types of α-polypeptides, the two heavy α-types were more involved in the reaction than the light type. Despite the relatively high content of glutamine residues, legumin was a rather poor substrate for MTG. The low affinity was related to the compact globular structure of these storage proteins, restricting the access to the reactive residues. As regards modification of the conformation, acylation was shown to be a useful technique for converting 11S seed globulins into suitable substrates for MTG [123]. The disposition of pea legumin to enzymatic cross-linking is clearly affected by the substrate conformation. The influence of phytate–protein interactions seems to be equally interesting for further research in MTG application to pea proteins.

Lupin proteins

Lupin seeds (*Lupinus* sp.) combine high protein content with high nutritive value. They even grow on poor soils that are unsuitable for soybeans [124]. A variety of cultivars from *Lupinus albus* L. and *Lupinus angustifolius* L., so-called sweet lupins, have found their way into the human food chain due to their very low alkaloid contents. The use of bitter lupins in human foods is restricted due to their high contents of toxic quinolizidinic alkaloids in the seeds. Therefore, the production of lupin isolates may overcome this problem because the water-soluble alkaloids are removed during the production of protein isolates [125, 126]. Pollard et al. [127] emphasised the potential of lupin flour as a bread additive. Substitution of up to 5% provided optimal bread quality, whereas detrimental effects at higher levels appeared to be associated with the non-protein components of the lupin flour. Dervas et al. [128] equally reported that lupin flour may replace wheat flour at levels of up to 5%. After defatting and concentration, substitution was enabled up to an elevated level of 10%. Due to their high nutritive value and high lysine contents of lupin

proteins [129], a considerable potential for food applications is evident on the basis of MTG-catalysed cross-linking. The suitability of protein isolates from pea and lupin for enzymatic cross-linking was recently reported by Schäfer et al. [130]. MTG-induced formation of ε-(γ-glutamyl)-lysine cross-links was determined via HPLC-MS after proteolytic digestion of the cross-linked protein. Amounts of generated isopeptide bonds correlated with increasing gel strengths due to the formation of protein networks and gel structures.

Sunflower proteins

Whereas soybean, wheat and sesame storage proteins display a considerable allergenic potential, sensitisation by sunflower (*Helianthus annuus* L.) proteins has rarely been observed. In contrast to leguminous proteins, sunflower is devoid of lipoxygenase-induced green and beany flavours. However, sunflower flour was found to contain approximately 4% phenolic compounds, mainly chlorogenic acid [131]. The discoloration of these polyphenols still represents the major hindrance in the use of sunflower protein in food products. The reactions of amino acids and peptides with *o*-quinones produced by enzymatic and non-enzymatic oxidation of chlorogenic acid and caffeic acid have been studied by Pierpoint [132]. Amino acids, except lysine and cysteine, primarily react through their α-amino groups to give red or brown products. The initial reactions are followed by secondary reactions that may absorb oxygen, resulting in black or green coloured products. The ε-amino group of lysine reacts with the *o*-quinones in a Michael addition. Consequently, several processes have been suggested to provide sunflower proteins with low chlorogenic acid levels [98, 99, 133, 134]. The utilisation of sunflower proteins low in phenolic compounds in bread baking led to improved rheological properties. Doughs made with sunflower protein concentrate and texturised soy protein were stronger and less elastic, with an increased water-binding capacity [135]. In contrast, the addition of succinylated sunflower protein concentrate and isolate to bread had a detrimental effect on loaf characteristics. Flour blends containing native sunflower protein concentrate or isolate at levels of up to 5% resulted in acceptable loaves [136]. Rossi [137] described the potential of textured sunflower protein for the use as a meat extender. Ground-beef patties, prepared by replacing 15–45% of their meat content with sunflower protein, showed higher juice retention and enhanced chewiness. Nevertheless, use of sunflower protein isolates in the food industry is so far limited due to their particular gelation behaviour. Gelation of sunflower proteins cannot be carried out by controlled acidification or heating [138]. The aggregation behaviour of helianthinin, the main storage protein of sunflowers, at different pH and ionic strengths was similar to other 11S globulins, except that it remained in a trimeric form at pH 11. Its thermal stability exceeded that of other 11S globulins [139]. Only after partial protein hydrolysis with trypsin, gels were obtained that showed the unusual property of becoming notably weaker when cooled below 80 °C [140]. The treatment with MTG may be a promising tool for achieving gelation without protein hydrolysis, but in the presence of phenolic compounds its efficiency may be affected due to enzyme inhibition.

Sesame proteins

Proteins obtained from sesame seeds (*Sesamum indicum* L.) have been used in bread making [141]. Sesame protein isolate added to wheat flour up to a protein level of 18% and the admixture of concentrates or flours up to a protein level of 16% were possible without observing detrimental effects on bread sensory properties. The water absorption was increased and dough development time lowered as the protein level increased in all blends. In contrast, dough stability decreased. The work of López et al. [142] dealt with the use of a commercially available sesame protein isolate as protein source in a liquid nutritional supplement. Functional properties such as the emulsion stability of an experimental formulation were similar to those of commercial beverages. A sensory test indicated that the product with sesame protein was preferred to one prepared with a soybean isolate and a commercial brand containing dairy proteins. Although sesame protein appears to be suitable for cross-linking with MTG, broader utilisation seems to be unlikely due to the low content of the limiting amino acid lysine [143].

As altogether shown by the cited examples, suitability of vegetable proteins for cross-linking with MTG does not only depend on protein quality, nutritive value, sensory acceptance, and chemical properties such as amino acid composition and structural conformation, but also on widespread commercial availability, healthiness and ethnic familiarity based on traditional usage.

Strategies for improving disposition to enzymatic cross-linking

Studies with modified proteins have shown that processes like enzymatic and/or physical treatments present a promising strategy to improve the suitability of proteinous substrates for cross-linking with MTG.

When partial proteolysis until a degree of hydrolysis (DH) of 0.5–2% was combined with enzymatic cross-linking of the hydrolysates, modified soy protein isolates (SPI) of considerably improved solubility in the low pH range (pH 3–5) were generated, irrespective of a pre- or post-hydrolysis application of MTG [144]. Even without proteolysis, the nitrogen

solubility profile of SPI was altered after incubation with MTG, since cross-linking resulted in a significant increase in the nitrogen solubility index (NSI 60%) at pH 3.0 relative to the unmodified control (NSI 10%). However, there was no significant change in solubility between pH 4.0 and 4.5, where SPI presented minimum solubility. Partially hydrolysed SPI (Alcalase, 1% DH, hydrolysis post- and pre-cross-linking) displayed a significant increase in nitrogen solubility at pH 4.0 (NSI 50%) when compared to the control (NSI 6%). Combination of enzymatic hydrolysis and cross-linking even showed synergistic effects, particularly at a DH of 2%. Similarly, hydrolysates of sodium caseinate incubated with MTG pre- or post-hydrolysis (Protamex, 1.3% DH) generally showed significant higher nitrogen solubility between pH 4.0 and 5.0 than controls incubated with Protamex or MTG alone [145]. Cross-linking after hydrolysis until a DH of 0.5% generated a product that displayed 1433% foam expansion at pH 6.0 in comparison with 735% for the control [146].

At high pH values far away from the isoelectric point (pI) and at low ionic strength, thermal denaturation of egg white proteins is possible without forming coagula. Proteins in this state are assumed to be more susceptible to TG, because they are partially unfolded and more flexible. As shown by Lim et al. [147], a high pH alone would not induce sufficient conformational changes to promote cross-linking by TG, but thermal treatments (60 and 80 °C, 20 min) proved effective in enhancing the susceptibility of egg white proteins to the cross-linking reaction.

These observations suggested that enzymatic and physical treatments, applied pre- or post-cross-linking, support the MTG-catalysed reaction in a positive manner and additionally improve the functional properties of proteins.

Improving the nutritive value through amine incorporation

The amino acid composition of plant proteins widely varies due to plant diversity, cultivation area, different function of proteins in the organism (e.g. storage proteins), purification (isolates, concentrates), and process technology (isoelectric precipitation, membrane filtration). Plant proteins frequently lack one or more amino acids that are required for fulfilment of the WHO/FAO standard [148] for a well-balanced amino acid composition in human nutrition. Therefore, methods for improving the nutritive value of food proteins other than blending of different protein sources and amino acid supplementation are desirable. Ikura et al. [149] investigated the feasibility of the incorporation of amino acids into food proteins through TG-catalysed acyl-transfer between the γ-carboxylamide groups of peptide-bound glutamine residues and primary amino groups. Free amino acids, with the exception of D- and L-lysine, did not serve as amine substrates, but most of the methyl or ethyl esters and amides of L-amino acids were suitable amine substrates. The incorporation of methionine ethyl ester into bovine casein components (α_{s1}-casein, β-casein) and soybean proteins (7S- and 11S-globulins) using TG resulted in an increase in the methionine content ranging from 50% (α_{s1}-casein) to 250% (11S-globulin) when compared to the starting material. Incorporation of lysine into wheat gluten was also successful [149]. Since esters and amides of amino acids have rarely been found in nature, Ikura et al. [150] also examined the suitability of different lysyldipeptides, containing various amino acid residues, for the TG-mediated transfer. The competitive generation of intra- and intermolecular ε-(γ-glutamyl)-lysine cross-links of the proteins lowered their binding affinity. A reversible blocking of the amino groups in the substrate protein suppressed cross-link formation [151]. Nonaka et al. [152] demonstrated successful incorporation of lysine into citraconylated α_{s1}-casein using MTG, whereas TG originating from guinea pig liver was found to be less effective.

MTG-catalysed incorporation of suitable dipeptides or enriched fractions containing the limiting amino acids may be a promising strategy for improving the nutritive value of food proteins. At present, feasibility of MTG application for this purpose seems to be questionable, since the carboxyl group of all common amino acids, except lysine, should either be amidated, esterified or decarboxylated in order to eliminate the negative charge at the reactive site. Moreover, a method for suppressing competitive cross-linking has to be established.

Conclusions and perspectives

The formation of ε-(γ-glutamyl)-lysine linkages catalysed by MTG presents an effective method for improving various functional properties of plant 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. The suitability of plant proteins for cross-linking with MTG mainly depends on the protein source, its nutritive value, the conformational structure of the protein, and the accessible amount of lysine and glutamine residues. At present, most applications of MTG for the production of plant protein-based foodstuffs are limited to raw materials from soybean and wheat. Limitation of plant sources is not solely due to their suitability as substrates for the enzymatic reaction, but also owing to their universal availability on the world market. The global success of wheat as a food crop must be attributed to its tolerance to

diverse climate and soil conditions. The popularity of soybean remains high and may even increase, primarily in Asia. In contrast, protein ingredients from other plants such as pea and lupin, which are major crops in the Mediterranean Basin and Central Europe, have emerged on a commercial scale only recently. To date, the widespread application of such proteins in the food industry has foundered due to lack of adequate knowledge about the necessary processing parameters. On account of the increasing demand of consumers for convenient and healthy foodstuffs of vegetable origin, and due to the opportunities to modify and improve functional properties and nutritive value of plant proteins by cross-linking with MTG, this enzyme will find an adequate place as a processing aid for the manufacture of innovative plant-based foodstuffs, particularly in the production of vegetarian meat-like texturised products and sweet products such as confectionery.

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