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Microbial Transglutaminase in Bakery Products: Mechanisms, Applications, and Safety

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ABSTRACT

Microbial transglutaminase (MTGase; EC 2.3.2.13), produced primarily by *Streptomyces mobaraensis*, is a calcium-independent acyltransferase that catalyzes the formation of ε-(γ-glutamyl)-lysine covalent crosslinks between glutamine and lysine residues in food proteins. This enzymatic crosslinking substantially modifies the structural and functional properties of both gluten and non-gluten proteins, establishing MTGase as a strategically important processing aid in the contemporary baking industry. The present review systematically examines evidence pertaining to MTGase applications across four principal bakery categories: conventional wheat breads, rye breads, sourdough fermented systems, and gluten-free formulations incorporating alternative grain and protein matrices. A structured literature search was conducted across Scopus, Web of Science, and Google Scholar databases, covering publications from 2006 to 2025. Findings consistently indicate that MTGase improves loaf-specific volume, crumb structure, and dough handling properties in wheat-based systems, while its efficacy in gluten-free formulations remains contingent upon the availability and reactivity of suitable protein substrates. Synergistic combinations with sourdough fermentation, hydrocolloids, and advanced processing technologies including high-pressure processing and ultrasound — demonstrate enhanced rheological and shelf-life outcomes. However, the enzyme's potential interaction with celiac-immunogenic gluten peptides and the possible generation of crosslinked neoantigens remain incompletely characterized, warranting structured clinical evaluation. Future research priorities include high-yield substrate-specific MTGase variants, standardized dosage protocols for gluten-free applications, and comprehensive safety assessments for immunocompromised populations.

KEYWORDS: Bakery applications; dough rheology; gluten-free formulation; microbial transglutaminase; protein crosslinking

1. INTRODUCTION

Consumer preferences in the global bakery sector have undergone substantial transformation over the past two decades, with mounting demand for high-protein, reduced-allergen, and gluten-free products (Fatima & Khare, 2018; Monteiro et al., 2021). Despite significant progress in ingredient reformulation, persistent challenges remain in replicating the structural, textural, and sensory characteristics of conventional wheat-based baked goods. The gluten network formed by the interaction of glutenins and gliadins upon hydration and mechanical work is primarily responsible for dough viscoelasticity, gas retention during proofing, and the characteristic open crumb structure of leavened bread (Bauer et al., 2003; Ogilvie et al., 2021). Its absence in gluten-free systems, or its modification in high-protein blends, creates significant technological barriers that conventional baking aids address only incompletely (Renzetti et al., 2008).

Among enzyme-based processing aids, microbial transglutaminase (MTGase; EC 2.3.2.13) has attracted sustained scientific and industrial interest since its initial characterization from *Streptomyces* species in the early 1990s (Motoki & Seguro, 1998). Unlike mammalian transglutaminases, which require calcium for activation, MTGase operates independently of divalent cations across a broad range of food processing conditions typically pH 5–8 and temperatures of 40–70°C (Miwa, 2020; Kolotylo et al., 2023). By catalyzing acyl-transfer reactions between glutamine (Gln) and lysine (Lys) residues, the enzyme generates covalent ε-(γ-glutamyl)-lysine bonds that reinforce protein networks and substantially

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alter functional properties such as gelation, emulsification, water-holding capacity, and foam stability (Mostafa, 2020; Muhammad et al., 2021).

In wheat bread systems, the consequences of MTGase action on the gluten network have been well characterized: enhanced dough elasticity and gas-holding capacity, improved loaf volume, and reduced crumb hardness (Ogilvie et al., 2021; Meybodi et al., 2020). In gluten-free formulations encompassing rice, millet, buckwheat, quinoa, and oat flour matrices MTGase crosslinks non-gluten proteins such as sesame, pea, and whey to partially reconstruct gas-retaining structures, producing measurable improvements in specific loaf volume, crumb softness, and shelf-life (Moore et al., 2006; Renzetti et al., 2008; Diowksz & Sadowska, 2021). The magnitude of these improvements, however, depends critically on protein composition, substrate concentration, and processing conditions.

Despite documented technological benefits, MTGase applications are not without complications. The enzyme's interaction with structural gluten proteins raises questions about the digestibility of immunogenic peptides relevant to celiac disease (Ogilvie et al., 2021), and crosslinked products have been proposed as potential neoantigens in sensitized individuals (Duarte et al., 2020; Lerner et al., 2024). Furthermore, regulatory status varies across markets, and the processing-aid classification in many jurisdictions may understate consumer exposure as enzyme concentrations in commercial formulations increase.

This review aims to: (i) describe the biochemistry and reaction mechanisms of MTGase; (ii) survey its technological applications across wheat, rye, sourdough, and gluten-free bakery categories; (iii) evaluate its interactions with hydrocolloids, alternative proteins, and novel processing technologies; (iv) critically assess evidence pertaining to technological benefits and safety risks; and (v) identify priority areas for future research.

2. LITERATURE REVIEW

2.1 Biochemical Properties and Enzymatic Mechanism of MTGase

Transglutaminases (protein-glutamine γ-glutamyltransferases; EC 2.3.2.13) constitute a family of enzymes that catalyze acyl-transfer reactions using the γ-carboxamide group of peptide-bound glutamine as the acyl donor (Motoki & Seguro, 1998). The microbial variant produced by *Streptomyces mobaraensis* now in widespread commercial use is a monomeric globular protein of approximately 38 kDa, operating optimally at pH 6–7 and temperatures between 50–55°C (Góes-Favoni & Bueno, 2014). Its calcium-independence distinguishes it mechanistically from mammalian transglutaminases, which require Ca^{2+} for conformational activation, and substantially broadens its practical utility across diverse food processing contexts.

The MTGase catalytic mechanism proceeds through a cysteine-protease-like ping-pong mechanism. In the first half-reaction, the active-site cysteine (Cys^{64}) attacks the γ-carboxamide of a Gln residue, displacing ammonia (NH_3) to form a covalent thioester (acyl-enzyme) intermediate. In the second half-reaction, when a primary amine — most importantly the ε-amino group of a Lys residue is available, aminolysis of the thioester generates the ε-(γ-glutamyl)-lysine isopeptide bond (Kolotylo et al., 2023; Muhammad et al., 2021). This crosslink is resistant to proteolysis and to reducing conditions that disrupt disulfide bonds, imparting exceptional stability to crosslinked protein networks. In the absence of a suitable amine acceptor, the thioester is hydrolyzed by water, resulting in net deamidation of Gln to Glu. Figure 1 presents a schematic summary of the enzymatic mechanism and its functional consequences.

2.2 Microbial Production and Commercial Sources

Commercial MTGase production relies principally on *Streptomyces mobaraensis* through submerged fermentation, yielding the enzyme initially as an inactive zymogen (pro-MTGase) that requires extracellular processing by a co-secreted subtilisin-like protease for activation (Akbari et al., 2021). Fermentation optimization encompassing carbon and nitrogen source selection, pH control, dissolved oxygen management, and medium composition has been an active research focus to maximize yield and reduce production costs (Fatima & Khare, 2018). Alternative production platforms, including recombinant expression in *Bacillus subtilis*, *Lactococcus lactis*, and *Escherichia coli*, have been explored to improve yield and simplify downstream processing (Ma et al., 2022). Genetic engineering approaches targeting pro-sequence modification and codon optimization have yielded variants with improved thermostability and substrate specificity (Akbari et al., 2021; Miwa, 2020).

2.3 Protein Substrate Specificity in Bakery Systems

The effectiveness of MTGase crosslinking is highly dependent upon protein conformation, the accessibility of reactive Gln and Lys residues, and the physical conditions of the reaction environment (Kolotylo et al., 2023). In wheat systems, glutenins particularly high-molecular-weight glutenin subunits (HMW-GS) appear to be the primary reactive substrates, while gliadins, which lack sufficient accessible Lys residues and exhibit a more compact tertiary structure, show comparatively limited reactivity (Bauer et al., 2003; Ogilvie et al., 2021). Dairy proteins (sodium caseinate, whey proteins), soy proteins, sesame proteins, and legume proteins are generally considered excellent MTGase substrates owing to their substantial content of accessible Gln and Lys residues and greater conformational flexibility (Rahim et al., 2023;

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Mohammad et al., 2018). Protein denaturation — induced by heat, ultrasound, or high pressure — generally improves substrate accessibility and MTGase crosslinking efficiency (Mohammad et al., 2018; Tomić et al., 2020).

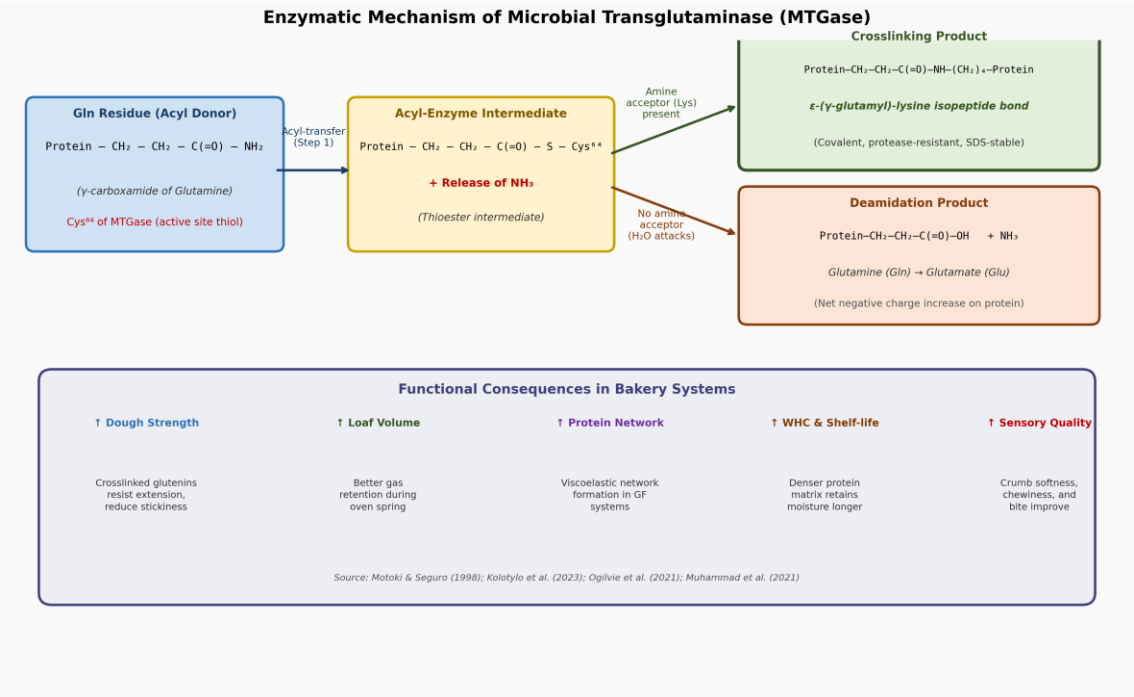


Figure 1. Enzymatic mechanism of microbial transglutaminase (MTGase). The acyl-enzyme intermediate formed at Cys[64] undergoes aminolysis (when Lys is present) to generate the ε-(γ-glutamyl)-lysine isopeptide bond, or hydrolysis (in the absence of a primary amine acceptor) resulting in deamidation. Both pathways alter protein functional properties in food systems. Sources: Motoki & Seguro (1998); Kolotylo et al. (2023); Muhammad et al. (2021).

3. METHODOLOGY

3.1 Literature Search Strategy

This narrative review was conducted in accordance with established systematic review principles to ensure comprehensiveness and transparency of evidence synthesis. Electronic databases searched included Scopus, Web of Science (Core Collection), PubMed/MEDLINE, and Google Scholar. The following keyword combinations were used in structured Boolean searches: ("microbial transglutaminase" OR "MTGase" OR "mTG") AND ("bakery" OR "bread" OR "dough" OR "gluten-free" OR "cake" OR "biscuit") AND ("mechanism" OR "crosslinking" OR "protein" OR "rheology" OR "texture" OR "safety" OR "allergenicity" OR "celiac"). Reference lists of all retrieved articles were manually screened to identify additional relevant studies not captured by automated database searches.

3.2 Inclusion and Exclusion Criteria

Publications considered for inclusion were: (i) peer-reviewed original research articles, systematic reviews, or meta-analyses published in indexed journals; (ii) published between January 2006 and September 2025, with seminal foundational works from earlier dates retained where methodologically significant; (iii) written in the English language; and (iv) addressing MTGase in the context of food processing with specific relevance to bakery applications. Exclusion criteria comprised studies focusing exclusively on non-food industrial applications (leather, textile processing), non-peer-reviewed conference abstracts or industry reports, and articles unavailable in full-text through institutional access. A total of 28 primary sources were identified as satisfying all inclusion criteria and are cited throughout this review, with emphasis placed on publications from 2018 onward to reflect the current state of knowledge.

3.3 Data Extraction and Evidence Synthesis

Data were extracted from each included study on: enzyme type and commercial source, protein substrate composition, processing conditions (enzyme dosage, pH, temperature, time), measured physicochemical outcomes (specific loaf volume, crumb hardness, water absorption, rheological parameters, sensory scores), and safety-relevant findings. Evidence was synthesized thematically across four application domains: wheat and rye systems; sourdough systems; gluten-free systems; and ingredient interaction and processing technology studies. The strength of evidence for each major claim was assessed descriptively based on study design, sample size, reproducibility of reported outcomes across independent groups, and presence or absence of mechanistic data.

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4. RESULTS AND DISCUSSION

4.1 Effect of MTGase on Wheat and Rye Bread Systems

MTGase's most extensively documented application in bakery technology concerns its modification of the gluten network in wheat dough. At doses typically ranging from 50–500 U/kg flour, MTGase-catalyzed crosslinking of glutenin subunits increases resistance to extension, reduces dough stickiness, and reinforces the continuous protein matrix in a manner analogous to chemical oxidizing improvers but through a fundamentally different mechanism (Bauer et al., 2003; Meybodi et al., 2020). The enzyme consistently improves loaf volume, crumb cohesion, and textural uniformity in pan bread at optimal dosages (Table 1). However, dose-response relationships are demonstrably non-linear: excessive crosslinking at very high doses (>1000 U/kg in some systems) generates an overly rigid network that resists oven spring, ultimately reducing loaf volume (Motoki & Seguro, 1998; Lerner et al., 2024). This critical feature of MTGase kinetics necessitates formulation-specific dose optimization and represents one of the principal practical constraints on its commercial deployment.

Rheological studies employing farinograph, extensograph, and dynamic oscillatory measurements have demonstrated dose-dependent increases in dough consistency and resistance to deformation in MTGase-treated wheat doughs, with corresponding reductions in extensibility at higher enzyme concentrations (Meybodi et al., 2020). These outcomes have been attributed primarily to polymerization of glutenin macro-polymers (GMP) and, to a lesser extent, crosslinking between glutenins and other dough proteins including albumins and globulins (Ogilvie et al., 2021). Scanning electron microscopy of MTGase-treated crumb structures reveals a denser, more interconnected protein matrix with better-defined, more uniformly sized gas cells compared to controls, providing a structural basis for the observed improvements in specific volume and crumb firmness (Bauer et al., 2003).

In rye dough systems inherently more challenging owing to the high pentosan content and comparatively limited gluten-forming capacity MTGase similarly improves protein network continuity and batter consistency, yielding measurable improvements in loaf volume and crumb structure at optimal dosages (Beck et al., 2011). The high content of soluble arabinoxylans in rye may create steric barriers to direct protein-protein crosslinks, and the interaction between MTGase and rye arabinoxylan-protein complexes has been proposed as a contributing mechanism for some of the observed rheological changes, though this requires further mechanistic characterization (Beck et al., 2011).

4.2 MTGase in Sourdough Wheat Bread

The combination of MTGase and sourdough fermentation represents a particularly well-studied and commercially relevant application that illustrates the context-dependency of enzyme functionality. In sourdough systems, lactic acid bacteria (LAB) and yeasts produce acidic conditions and secrete proteolytic enzymes that partially degrade the gluten network — simultaneously enhancing flavor development but potentially impairing crumb structure, particularly at advanced acidification stages (Scarnato et al., 2017). MTGase counterbalances this proteolytic weakening by forming additional covalent crosslinks within the gluten fraction, maintaining structural integrity and gas-retention capacity throughout fermentation and baking.

Table 1. Functional Effects of Microbial Transglutaminase (MTGase) in Major Bakery Product Categories

Product Type	Main Effect of MTGase on Quality	Key References
Wheat bread / pan bread	Strengthened gluten network; ↑ crumb cohesion, ↑ water absorption, ↑ loaf volume; ↓ stickiness; modified glutenin polymerization; dose-dependent non-linear response	*Lerner et al. (2024); Meybodi et al. (2020); Ogilvie et al. (2021)*
Sourdough wheat bread	Synergistic action with LAB: counterbalances proteolysis; ↑ protein aggregate size; improved crumb structure, aroma, and shelf-life vs. either treatment alone	*Scarnato et al. (2017); Ogilvie et al. (2021); Muhammad et al. (2021)*
GF buckwheat bread	Crosslinking of buckwheat globulins; with sourdough: ↑ volume, ↓ crumb density, ↓ bitterness, ↑ cohesiveness and springiness	*Diowksz & Sadowska (2021); Ebling et al. (2022)*
GF millet / oat bread	With non-gluten proteins (BSA, WPC): ↑ G' and G" (rheology), ↑ specific volume, ↓ crumb hardness; limited improvement in protein-deficient oat without supplementation	*Tomić et al. (2020); Li et al. (2021); Redd et al. (2024)*
GF quinoa bread	RSM-optimised 0.41% MTGase + 1.28% HPMC: enhanced batter rheology, ↑ moisture, ↑ overall acceptability; replicated functional gluten properties	*Ghodosipoor et al. (2025); Redd et al. (2024)*
GF rice-based cakes	With sesame protein isolate: ↑ batter viscosity, ↑ volume, ↓ density, ↓ staling rate, ↑ sensory acceptance; MTGase-crosslinked sesame protein provides structural scaffold	*Rahim et al. (2023); Ebling et al. (2022)*

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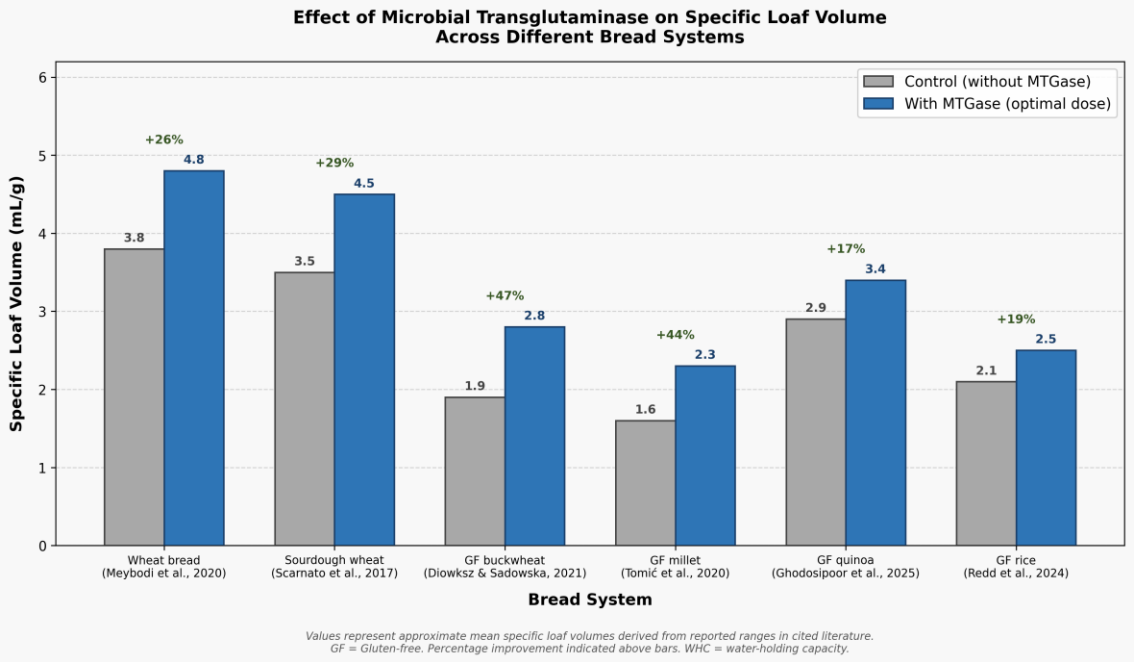


Figure 2. Effect of microbial transglutaminase (MTGase) on specific loaf volume across wheat and gluten-free bread systems. Values represent approximate mean specific volumes derived from literature-reported ranges for each system. Percentage improvement (MTGase vs. control) indicated above bar pairs. GF = gluten-free. Sources: Meybodi et al. (2020); Scarnato et al. (2017); Diowksz & Sadowska (2021); Tomić et al. (2020); Ghodosipoor et al. (2025); Redd et al. (2024).

Scarnato et al. (2017) demonstrated that combined treatment with a defined sourdough starter (*Lactobacillus sanfranciscensis* and *Candida milleri*) and MTGase produced larger protein aggregates, a stronger crosslinked network dominated by ε-(γ-glutamyl)-lysine bonds primarily within the gluten fraction, and a bread with superior shelf-life compared to either treatment alone. The combined system yielded rheological properties comparable to conventionally fermented wheat bread, suggesting that MTGase effectively compensates for the structural weakening induced by LAB proteolysis. Ogilvie et al. (2021) subsequently reported that while MTGase alone increased crumb hardness and chewiness at higher doses a potentially undesirable textural outcome sourdough fermentation partially counteracted this excessive firmness through controlled network degradation, ultimately producing a more balanced and consumer-acceptable texture profile.

These findings underscore a fundamental principle governing MTGase efficacy: outcomes are not determined by the enzyme's action in isolation but arise from complex interactions with the dough system's pH, temperature history, substrate availability, and the competing enzymatic activities of co-applied microbial agents (Muhammad et al., 2021). This systemic context-dependence complicates straightforward dosage recommendations and argues strongly for empirical optimization within each specific bakery formulation.

4.3 MTGase in Gluten-Free Bakery Systems

4.3.1 Rice- and Corn-Based Systems

Rice flour-based systems, among the most commercially prevalent GF formulations, contain relatively low protein concentrations and hence offer limited reactive substrate for MTGase crosslinking. Renzetti et al. (2008) reported that in GF batters from six different flour types (rice, buckwheat, amaranth, sorghum, teff, and oat), MTGase treatment altered batter rheology and improved bread microstructure across all substrates, but the magnitude of improvement varied considerably with protein content and composition. Rice-based systems showed relatively modest volume improvements, while buckwheat and oat systems, being comparatively protein-richer, responded more substantially. Ebling et al. (2022) demonstrated that in a mixed GF system (cornstarch, rice flour, buckwheat), MTGase at 0.25 g/100 kg flour particularly when combined synergistically with amyloglucosidase and glucose oxidase reduced baking loss, decreased crumb chewiness by 73%, and produced finer, more uniform crumb porosity.

4.3.2 Buckwheat GF Systems

Buckwheat flour presents a more favorable substrate profile for MTGase than most GF alternatives, owing to its substantial protein content (approximately 12–15%) and the presence of globular storage proteins (13S legumin type) with accessible Gln and Lys residues (Diowksz & Sadowska, 2021). Multiple studies have reported that MTGase, particularly when combined with sourdough fermentation using Lactiplantibacillus plantarum strains, markedly improves buckwheat bread specific volume, reduces crumb density, decreases characteristic buckwheat bitterness, and enhances springiness and cohesiveness (Diowksz & Sadowska, 2021; Ebling et al., 2022). The sourdough-induced proteolysis partially unfolds

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buckwheat globulins, increasing their accessibility to MTGase and thereby enhancing crosslinking efficiency again illustrating productive synergy between enzymatic and fermentative interventions.

4.3.3 Millet, Quinoa, and Oat GF Systems

In millet (Panicum miliaceum) flour systems, Tomić et al. (2020) demonstrated that the combination of supplementary non-gluten proteins (bovine serum albumin, whey protein concentrate) with MTGase significantly improved dough rheological parameters increasing storage modulus (G') and loss modulus (G") and produced breads with higher specific volume and reduced crumb hardness relative to millet flour controls. This study reinforced a critical principle applicable to all GF MTGase applications: the enzyme requires adequate reactive protein substrate to generate a functional network, and supplementation with reactive exogenous proteins is typically necessary in protein-deficient GF flours.

Ghodosipoor et al. (2025) employed response surface methodology (RSM) to optimize MTGase concentration (0–1.5%) and hydroxypropyl methylcellulose (HPMC) concentration (0–2%) in quinoa-based GF bread, identifying optimal conditions of approximately 0.41% MTGase and 1.28% HPMC. This combined treatment enhanced batter rheology, improved moisture retention, and maximized overall acceptability scores, demonstrating that the complementary action of protein crosslinking (MTGase) and hydrocolloid network formation (HPMC) can successfully replicate functional gluten properties in a quinoa matrix. Redd et al. (2024) confirmed this substrate-dependency across five GF sourdough systems (white rice, brown rice, quinoa, chickpea, oat): quinoa-based bread achieved a 6% improvement in specific volume, rice-based systems showed significant stiffness reductions, while oat systems without additional protein supplementation showed no measurable benefit. Li et al. (2021) further demonstrated that oat GF bread fortified with skim milk powder and treated with MTGase (12 mg/100 g) achieved viscoelastic dough characteristics with improved texture, specific volume, and prebiotic functionality.

4.4 Interactions with Other Ingredients and Processing Technologies

4.4.1 Hydrocolloids and Starches

In GF formulations, hydrocolloids such as HPMC, xanthan gum, and carrageenan contribute to batter viscosity, gas cell stability, and crumb structure through mechanisms that are mechanistically distinct from protein crosslinking. Evidence from Ghodosipoor et al. (2025) and the systematic review of Monteiro et al. (2021) indicates that MTGase and HPMC interact in a complementary, rather than redundant, manner: MTGase reinforces the protein scaffold while HPMC stabilizes the water-starch matrix and foam structure. Optimal MTGase-HPMC combinations consistently outperformed either additive used alone in quinoa and rice systems. This complementarity reflects the spatial segregation of protein and starch phases in GF batters, wherein MTGase-crosslinked proteins create load-bearing scaffolds within the HPMC-stabilized starch matrix.

4.4.2 Alternative and Supplementary Proteins

The substrate specificity of MTGase renders the selection of supplementary protein critically important in GF system design. Sesame protein isolate, employed by Rahim et al. (2023) in rice-based GF cakes, substantially increased batter viscosity, improved volume, reduced density and staling rate, and enhanced sensory acceptance. The MTGase-crosslinked sesame protein network provided structural support broadly analogous to though not equivalent to a gluten matrix, suggesting that sesame protein deserves wider investigation as an MTGase-compatible GF structural protein. Pea protein, whey protein concentrate, and sodium caseinate have demonstrated comparable substrate suitability in several independent systems, while highly glycosylated or conformationally constrained proteins such as native rice glutelins without prior denaturation show limited crosslinking response (Mohammad et al., 2018; Renzetti et al., 2008).

Table 2. Recent Gluten-Free Bread Systems Improved by Microbial Transglutaminase (MTGase)

GF Flour System	Key Findings with MTGase	Reference
Quinoa + HPMC	RSM optimization: ~0.41% mTG + 1.28% HPMC maximized rheology, moisture retention, and overall acceptability; successfully replicated gluten functionality in quinoa matrix	*Ghodosipoor et al. (2025)*
GF sourdough (rice, quinoa, chickpea, oat)	MTGase increased specific volume by 6% in quinoa; stiffness reduced by 28% and 13% (24 h) in white and brown rice; oat without added protein showed no benefit	*Redd et al. (2024)*
Oat + skim milk powder (GF)	12 mg TG/100 g + 8 g MP/100 g: viscoelastic dough with improved texture, specific volume, and prebiotic functionality on gut microbiota	*Li et al. (2021)*
Mixed GF (cornstarch, rice, buckwheat)	0.25 g mTG/100 kg flour combined with other enzymes: ↓ baking loss, ↓ crumb chewiness by 73%, finer crumb porosity	*Ebling et al. (2022)*
Buckwheat GF + Lactiplantibacillus sourdough	mTG-sourdough combination improved flavor, ↓ buckwheat bitterness, ↓ crumb density, ↑ cohesiveness and springiness	*Diowksz & Sadowska (2021)*

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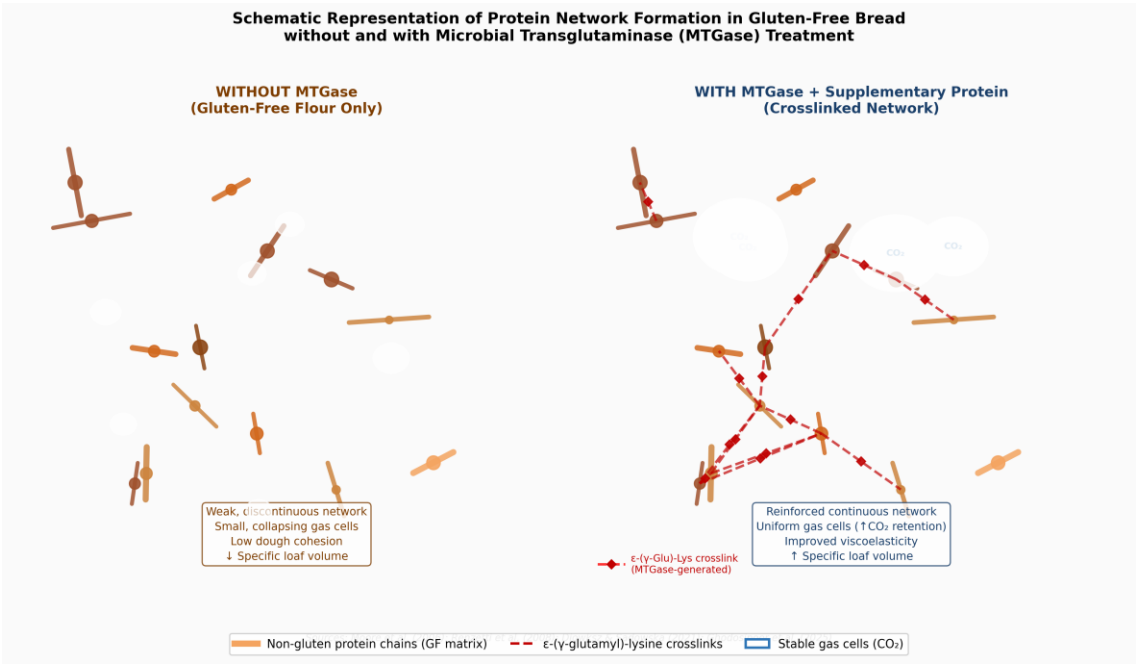


Figure 3. Schematic representation of protein network formation in gluten-free bread without (left) and with (right) microbial transglutaminase (MTGase) treatment and supplementary protein. Without MTGase, protein chains remain dispersed with a weak, discontinuous network and small, unstable gas cells. With MTGase and supplementary proteins, covalent ε-(γ-glutamyl)-lysine crosslinks (shown as red dashed lines) create a reinforced, interconnected network with larger, stable gas cells, resulting in improved loaf volume and crumb structure. Sources: Moore et al. (2006); Renzetti et al. (2008); Diowksz & Sadowska (2021); Ghodosipoor et al. (2025).

4.4.3 Advanced Processing Technologies

High-pressure processing (HPP), ultrasound, and microwave treatment have each been demonstrated to enhance MTGase crosslinking efficiency in food protein systems by inducing partial protein unfolding and increasing the accessibility of reactive residues (Mohammad et al., 2018; Tomić et al., 2020). Mohammad et al. (2018) comprehensively reviewed evidence for these synergistic technology-enzyme combinations and documented improvements in gel strength, water-holding capacity, and viscoelasticity in MTGase-treated protein matrices subjected to prior or concurrent physical processing. The translation of these process intensification approaches to commercial bakery operations remains limited by equipment cost and scale-up constraints, but the scientific evidence firmly supports the principle of using physical pre-treatments to enhance MTGase effectiveness in recalcitrant protein substrates.

4.5 Technological Benefits and Processing Limitations

The accumulated evidence supports MTGase as an effective functional ingredient across a range of bakery applications, offering improvements in loaf volume, crumb structure, water-holding capacity, and shelf-life without substantially compromising nutritional value (Mostafa, 2020; Muhammad et al., 2021). In lysine-fortified wheat or wheat-soy composite breads, MTGase has been reported to improve protein quality indices and increase available lysine, suggesting a nutritional benefit that extends beyond texture modification alone (Fatima & Khare, 2018). Commercially, MTGase is cost-competitive with chemical oxidizing improvers and is processed under mild, food-compatible conditions.

Several limitations, however, merit explicit acknowledgment. The non-linear dose-response relationship necessitates formulation-specific optimization, increasing development time and cost. At high doses or in systems with already robust gluten networks, excessive crosslinking impairs dough extensibility and reduces loaf volume (Motoki & Seguro, 1998; Lerner et al., 2024). In protein-deficient GF systems, meaningful improvements require supplementary reactive proteins, raising formulation costs. The growing "clean-label" consumer movement also creates a potential marketability conflict, as increasing numbers of consumers seek ingredient lists free of processing aids and enzymes regardless of regulatory classification (Lerner et al., 2024). These constraints collectively suggest that MTGase's commercial utility is substantial but bounded, and that transparent labeling practices and clear communication of the evidence base are important components of responsible deployment.

4.6 Safety Considerations: Allergenicity, Celiac Risk, and Regulatory Status

The safety of MTGase for individuals with celiac disease and wheat allergy represents an area of active scientific debate that warrants careful evidence-based discussion (Table 3). Tissue transglutaminase (tTG2) is the principal autoantigen in celiac disease pathophysiology: it deamidates specific Gln residues in gluten peptides (particularly in α-gliadin 33-mer

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epitopes), generating negatively charged neoepitopes that bind HLA-DQ2/DQ8 molecules and activate T-cell-mediated immune responses in genetically predisposed individuals (Duarte et al., 2020). The critical question is whether food-grade MTGase, with its structurally distinct active site and different substrate specificity, could modify gluten peptides in ways relevant to celiac immunopathology.

Ogilvie et al. (2021) directly investigated this question in a controlled wheat bread study, applying MTGase at concentrations up to 2000 U/kg and analyzing the deamidation state and proteolytic digestibility of key immunogenic gluten peptides in the resulting bread. Their findings indicated that MTGase did not significantly increase the deamidation of principal celiac-toxic peptides and did not alter their susceptibility to intestinal proteolysis, leading the authors to conclude that MTGase treatment at typical bakery doses does not generate "activated" celiac-toxic epitopes under those experimental conditions. The authors explicitly noted, however, that this conclusion relates specifically to deamidation and does not exclude potential effects from crosslinking of immunogenic peptides into novel crosslinked structures.

This distinction is critical to the broader safety debate. Duarte et al. (2020) and Lerner et al. (2024) present evidence that MTGase-mediated crosslinking may generate ε-(γ-glutamyl)-lysine crosslinked products involving celiac-relevant gluten peptides, and that these crosslinked structures as well as the MTGase enzyme protein itself may possess immunogenic properties in sensitized individuals. Scarnato et al. (2019) demonstrated that gluten-sensitized sera showed immunoreactivity toward wheat, rice, corn, and amaranth proteins treated with MTGase, suggesting that crosslinking may generate or expose new antigenic epitopes. Lerner et al. (2024) have further argued that the GRAS classification of MTGase was established under conditions that inadequately considered these crosslinked neoantigen scenarios, and that current risk assessment frameworks are insufficiently sensitive to the needs of celiac disease and non-celiac gluten sensitivity populations.

The evidence on allergenicity presents a further paradox: in certain experimental models, MTGase treatment has been shown to reduce in vitro allergenicity by crosslinking or blocking known allergenic epitopes (Duarte et al., 2020). This apparent duality MTGase potentially both generating novel immunogenic structures and masking existing ones underscores the genuine complexity of the safety evidence and the inadequacy of categorical safety or risk assertions. The weight of current evidence suggests cautious continued use: MTGase is unlikely to pose generalized risk to the non-celiac population but may warrant more rigorous structured evaluation in celiac disease and non-celiac gluten sensitivity cohorts than has thus far been conducted.

Table 3. Summary of Evidence on Safety and Technological Functionality of MTGase in Bakery Applications

Claim / Finding	Evidence Strength	Key References
MTGase strengthens dough/bread structure and improves loaf volume	Strong – consistent across wheat and GF systems in numerous experimental and review studies	*Lerner et al. (2024); Meybodi et al. (2020); Tomić et al. (2020)*
MTGase does not increase deamidation of key celiac-immunogenic gluten peptides	Moderate – supported by one controlled wheat bread study at ≤2000 U/kg; limited to specific peptides tested	*Ogilvie et al. (2021)*
MTGase and its crosslinked products are immunogenic/celiac-relevant	Emerging – supported by mechanistic and immunological evidence; clinical trials in celiac cohorts lacking	*Duarte et al. (2020); Lerner et al. (2024); Scarnato et al. (2019)*
MTGase can reduce allergenicity via transamidation of allergenic epitopes	Weak/Inconsistent – context-dependent; conflicting results across model systems	*Duarte et al. (2020)*
MTGase does not compromise protein digestibility or nutritional value	Moderate – supported in several studies; stronger crosslinking may reduce digestibility until combined with sourdough	*Ogilvie et al. (2021); Muhammad et al. (2021)*

4.7 Future Perspectives

Several priority research and development directions emerge from this synthesis. First, regarding sustainable and plant-based baking: as the food industry transitions toward reduced animal-protein formulations, MTGase's demonstrated ability to crosslink diverse plant proteins — soy, sesame, pea, lentil, hemp — positions it as a key enabling technology for high-protein plant-based bakery development. Research into MTGase substrate specificity with emerging novel protein sources, including microalgae proteins, insect protein flours, and fermented plant proteins, represents a compelling frontier (Mohammad et al., 2018; Fatima & Khare, 2018).

Second, novel MTGase variants and production optimization: genetic engineering has produced MTGase variants with improved thermostability, broader pH optima, and altered substrate preferences that may address current formulation limitations. Further development of recombinant expression systems in food-grade hosts (*Lactococcus lactis*, *Bacillus subtilis*) could reduce production costs and simplify regulatory approval pathways in multiple jurisdictions (Ma et al., 2022; Akbari et al., 2021; Miwa, 2020).

Third, precision dosage and process integration: the non-linear dose-response behavior of MTGase demands more precise, flour-type-specific dosage recommendations tailored to protein composition and processing conditions. Integration with continuous baking processes, 3D food printing platforms, and precision fermentation systems represents a promising direction for process innovation that has received limited attention to date. Fourth, and arguably most urgently, mechanistic

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safety research: given the unresolved questions regarding MTGase's interactions with celiac-immunogenic peptides and the potential immunogenicity of crosslinked products, there is a compelling need for structured clinical studies in well-characterized celiac disease cohorts. Development of validated in vitro digestion models designed specifically for MTGase-crosslinked proteins with immunotoxicological rather than purely nutritional endpoints would substantially advance the evidence base and inform more appropriately calibrated regulatory guidance.

5. CONCLUSIONS

Microbial transglutaminase has established a well-evidenced role in improving the structural, textural, and sensory quality of a broad range of bakery products, from conventional wheat and rye breads to gluten-free formulations based on buckwheat, millet, quinoa, and rice. Its capacity to catalyze ε-(γ-glutamyl)-lysine crosslink formation under mild, food-compatible conditions makes it a technically versatile and commercially viable ingredient that can substitute for or complement chemical oxidizing improvers across diverse bakery applications. In gluten-free systems, MTGase's ability to partially reconstruct gluten-like protein networks from non-wheat substrates addresses a fundamental barrier to product quality, particularly when combined synergistically with sourdough fermentation, hydrocolloids, and supplementary reactive proteins.

These technological benefits, however, must be evaluated alongside persistent uncertainties regarding MTGase's safety for celiac disease patients and individuals with non-celiac gluten sensitivity. While the evidence to date does not support a simple conclusion that MTGase is unsafe for celiac populations, neither does it fully establish safety particularly with respect to crosslinked neoantigen formation and the immunoreactivity of MTGase-modified proteins in sensitized individuals. The regulatory classification of MTGase as a processing aid with GRAS status, while commercially enabling, may not adequately reflect current mechanistic knowledge of its biochemical interactions with immunogenic food proteins.

Future progress in this field will require coordinated advances in enzyme engineering, formulation science, clinical safety research, and regulatory science. The trajectory toward more sustainable, plant-based, and allergen-aware baking systems creates both an opportunity and a responsibility to deploy MTGase with greater precision, more transparent labeling, and a more robust evidence base than the current state of knowledge provides.

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Conflict of Interest

The authors declare no conflict of interest.

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