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BIOTECHNOLOGICAL METHODS FOR IMPROVING QUALITY OF GLUTEN-FREE BREAD: A REVIEW

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Abstract

The manufacture of gluten-free bread remains a major technological and nutritional challenge due to the absence of the viscoelastic gluten network, which often results in products with reduced volume, weak crumb structure, rapid staling and limited sensory acceptance. In this review, recent biotechnological approaches applied to the improvement of gluten-free bread quality are critically analyzed, with emphasis on technological performance, nutritional enhancement and shelf-life extension. A systematic literature review was conducted in accordance with PRISMA guidelines in order to identify relevant scientific studies published within the last five years. The search focused on peer-reviewed articles addressing microbial fermentation strategies , enzyme-based technologies, and the incorporation of alternative protein sources and hydrocolloid systems. Additionally, the strategic combination of plant proteins and hydrocolloids was reported to compensate for the lack of gluten, especially when integrated with fermentation-based approaches, leading to synergistic improvements in technological and sensory quality. Overall, the findings support the potential of integrated biotechnological strategies to guide the development of gluten-free breads with improved quality and commercial viability.

Key words*: biotechnological methods, bread quality improvement, food biotechnology, gluten-free bread, gluten-free sourdough, selected microbial cultures, shelf-life extension, starter cultures, sourdough fermentation, texture enhancement.*

INTRODUCTION

The greatest challenge faced by those engaging in gluten-free (GF) baking is related to the absence of the bipartite gluten network, as this is what provides extensibility as well as elastic strength to regular baking products. Monomeric gliadins act as plasticizers that give viscosity and extensibility to wheat dough, thus, the dough matrix is able to expand continuously under the pressure of CO_2 generated continuously during the process of yeast fermentation (Šmídová & Rysová, 2022). At the same time, polymeric glutenins are able to supply the essential elasticity which is required to retain gas cells without any structural damage. This is only possible due to the robust, crosslinked macromolecular network they form via inter-chain disulphide bonds. This is the primary rheological hurdle faced in GF matrix design, extensibility without collapse (Calle et al., 2020). Furthermore, the shelf life of many GF products is compromised due to the heavy reliance on refined starches such as potato, rice, or corn. This leads to a plethora of effects such as moisture migration, rapid staling, and increased crumb firmness (Capriles et al., 2020). These starches are also known to undergo full gelatinization during the thermal baking process, however, there is a rapid re-association and crystallization process (retrogradation) due to the disruption of amylose and amylopectin chains, which happens during cooling. Gluten would provide a protein continuum which physically interrupts this recrystallization and permanently binds free water, but, due to its absence in GF products, this leads to a rapid transition of the crumb into a rigid, brittle structure within 24 to 48 hours of said GF products (Šmídová & Rysová, 2022). Xanthan and guar gums were initially used to mitigate these structural deficits, and while these polysaccharides successfully increase the viscosity of the batter and trap free water in order to simulate raw dough consistency, they are unable to form a hear-coagulable, continuous, three-dimensional network during baking. As a result, while it is possible for simple hydrocolloids to temporarily mimic the hydration properties of

gluten, they are unable to provide some of its benefits, such as, structural chewiness, mechanical resistance, or prolonged anti-staling properties characteristic of a true protein-based matrix, which leads to a necessity of using advanced biotechnological interventions (Manik & Nur, 2021). The use of certain enzyme treatments to cross-link proteins, the application of physical stressors like ultrasound to induce gelation, and the exploitation of microbial fermentation are among the most notable of said interventions (Azizi & Azizi, 2023). Sourdough technology is also such a tool, which has long since evolved from its rudimentary form of only being used as a traditional methods, as now selected lactic acid bacteria strains are used to degrade anti-nutritional factors and generate exopolysaccharides that function as natural texturizers (Ramos et al., 2021).

The purpose of this review is to critically analyze the latest biotechnological advancements that have been made in GF bread production. To be more specific, it tackles the efficacy of novel protein isolates, such as pea and zein, combined with the process of ultrasonic modification, the synergistic role of hydrocolloid-protein systems and the integration of sustainable, upcycled ingredients like apple pomace. As it is ultimately the acceptance of the consumers which determines the success of such a product, this paper aims to identify some of the most promising technological methods for producing high-quality, nutritionally balanced gluten-free bread by synthesizing data from the most recent peer-reviewed studies.

MATERIALS AND METHODS

A systematic literature review was conducted in accordance with PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to identify relevant scientific studies. The search was deliberately restricted to articles published within the previous five years in order to capture the quick, recent advancements in precision food biotechnology, such as focused enzymatic cross-linking, ultrasonic cavitation, and specific lactic acid bacteria (LAB) fermentation. This specific time frame was chosen because it represents a significant technological paradigm change from more outdated, traditional hydrocolloid-substitution methods to active molecular modification of gluten-free matrices. The initial search yielded 850 records, which were systematically filtered down to 32 highly relevant, peer-reviewed studies focused on biotechnological improvements to the gluten-free matrix (Figure 1).

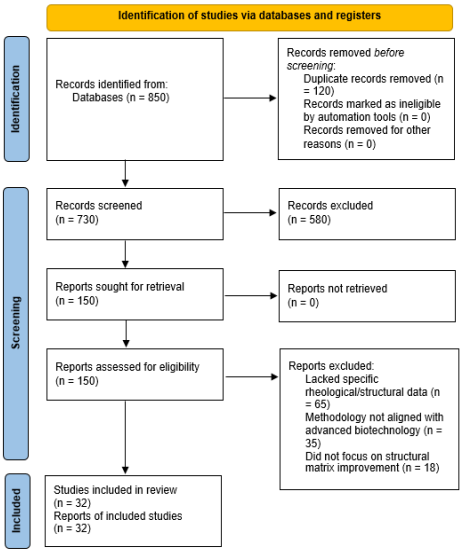

Figure 1. PRISMA flow diagram detailing the literature search, screening, and selection process for the systematic review

Major international databases such as Science Direct, Springer, PubMed and Web of Science were used in our search for relevant scientific articles. To ensure comprehensive coverage of the topic, the literature search was conducted using specific combinations of keywords and Boolean operators. The primary search strings utilized were: (“Gluten-free bread” or “GF bread” or “Gluten-free dough”) and (“Biotechnological methods” or “Sourdough fermentation” or “Enzymatic treatment” or “Alternative protein isolates” or “Hydrocolloid synergy” or "Ultrasound"). A secondary filter was applied in order to spare the peer-reviewed studies, and to further focus the current manuscript on methods such as microbial fermentation strategies, enzymatic treatments, or the integration of plant proteins in order to compensate for the lack of gluten.

References from the selected articles served as additional bibliographic sources for this paper as they ensured a thorough overview of the current methods being used.

RESULTS AND DISCUSSIONS

Diversity of additives and mechanisms of action

There are a number of practical issues in regards to the formulation of optimal gluten-free bread as it is considered that the technological requirements depend on the selected matrices (Calle et al., 2020). Generally speaking, the structure of a successful formulation is as follows: a major ingredient, an additive, and other ingredients (Djeukeu et al., 2024). The major ingredient commonly used is a source of carbohydrates, which helps in the formation of the basic structure of the bread. It is possible to simplify the formulation and potentially reduce production costs by using a whole matrix as the only major ingredient. The most frequently used matrix is rice flour, which has a usage percentage of 41. Following it are various combinations of corn starch and potato starch (15%), rice flour and cassava starch (6%), and corn starch and rice flour (4%) (Djeukeu et al., 2024).

It is necessary to use specific additives in order to satisfy the structural and sensory requirements of GF bread as optimal volume continues to be one of the biggest challenges in the field (Monteiro et al., 2021). It has been determined that consumers deem a bakery product to be satisfactory when it well-risen and regular in shape, as proper volume is a sign of quality to them. Proteins and protein sources (28.4%), enzymes (21.6%), fibers and fiber sources (12.7%), and hydrocolloids (11.8%) are some of the most commonly used additive groups to achieve this effect (Table 1; Djeukeu et al., 2024).

The role of enzymes in the development of high-volume gluten-free bread has been highlighted by a considerable number of researchers. Macromolecules are broken down into simpler compounds by amylases and proteases, this action provides yeast with carbon source to increase their activity and gas production, which, in turn, contributes to bread rising. Additionally, there is a possible improvement of the texture and volume of the bread due to the fact that transglutaminase can create bonds between proteins (Monteiro et al., 2021).

Furthermore, there is a correlation between the incorporation of fibers and bread volume due to mechanisms such as water absorption, gas retention, structural support, and starch gelatinization (Djordjević et al., 2021). Soluble fibers, such as inulin, lead to increased dough hydration and improved gas retention during fermentation because of their water absorption properties. An enhanced leavening and subsequent expansion of the dough is the result of this incorporation. On the opposite end of the spectrum, insoluble fibers, such as *Plantago psyllium* whole seed and oat fiber, may be used to prevent excessive collapse during baking as they provide structural support to the dough matrix, which results in them maintaining the shape, texture, and volume of the bread (Djeukeu et al., 2024). Researchers often try to find certain combinations of multiple mechanisms of action, such as a Protein and Enzyme Mix or a Protein and Fiber Mix in order to achieve a better synergistic volume effect (Parsamajd et al., 2025).

Table 1. Usage frequency of main matrices and additive groups in gluten-free formulations (Adapted from Djeukeu et al., 2024)

Category	Ingredient / Additive Type	Usage Frequency
Major Matrix	Rice flour	41%
	Corn starch and potato starch	15%
	Rice flour and cassava starch	6%
	Corn starch and rice flour	4%
Additive Group	Proteins and protein sources	28.4%
	Enzymes	21.6%
	Fibers and fiber sources	12.7%
	Hydrocolloids	11.8%

Functional Protein Isolates and Ultrasonic Modification

Plant-based protein isolates have recently been used in research in order to mimic the viscoelastic properties of gluten. Fan et al. (2026) studied these effects and has found out, via a comparison of the effects of pea protein, chickpea protein, and zein on rice dough, that zein exhibits a strong hydrophobicity which enhances the structural integrity of the dough through network formation, while due to the

highly hydrophilic nature of pea and chickpea proteins, their best use is in regards to water absorption. Furthermore, while there are universal improvements in the specific volume of gluten-free rice bread when plant-based proteins are used, their microstructural mechanisms of action diverge significantly depending on the profiles of their amino acids (Skendi et al., 2021). Pea and chickpea proteins, similarly to most legume proteins, are rich in polar amino acids and display strong hydrophilicity (Jiang et al., 2026). During the process of dough mixing, rice starch competes with them for available water, which ultimately results in a restriction of the swelling and gelatinization of starch granules. This, in turn, allows proteins to form a soft, hydrophilic gel network that significantly improves dough viscoelasticity. Whereas zein, which is rich in non-polar amino acids, forms a highly hydrophobic, film-like three-dimensional network during the process of heating as it undergoes self-assembly. Said structure is able to form a barrier which encapsulates the starch granules while at the same time inhibiting starch retrogradation far more effectively than legume proteins (Fan et al., 2026).

When taking the extension of shelf-life into account, another hurdle in GF bread-making, the addition of these proteins, which fundamentally alters water mobility within the dough matrix, must be paid close attention to (Capriles et al., 2020). Magnetic Resonance analysis has revealed that optimal amounts of pea protein, chickpea protein, and zein additions can shift free water into bound water. This enhanced hydration capacity prevents another problem that has been mentioned in this paper, namely moisture migration during storage, and there is a direct improvement of yeast fermentation rheology due to this stable viscoelastic structure (Haixi et al., 2025). When put in direct comparison to unfortified gluten-free dough, it has been determined that the addition of zein and chickpea protein at a 12% substitution level increases CO_2, resulting in a highly porous and aerated crumb (Fan et al., 2026).

Despite all of this, over-fortification has been identified as a potential issue in these studies, particularly the threshold of protein over-fortification (Tandazo et al., 2021). In an ideal scenario, exact additions of these proteins improve the matrix, however, excessive protein incorporation instead lead to reduced gas retention and collapsed loaf volumes. This results in a steady degradation of the structural integrity of the dough when protein additions exceed 6% for pea protein, 12% for chickpea protein, and 12% for zein. Ultimately, this causes the bread to become excessively hard and chewy (Fan et al., 2026).

On this note, the type of protein used has several effects on the final visual quality of the bread. Due to the fact that pea and chickpea proteins possess distinct natural pigments, an undesirable darkening may be noticed in the bread crumb when higher level of additions are implemented (Jiang et al., 2026). Zein would be a more suitable substitute in this regard, as it possesses high thermal stability and a pale-yellow hue that blends better with baking crusts than pea and chickpea. Thus, it results in the lowest total colour difference and is the best in terms of preserving the traditional appearance of the bread (Ozturk et al., 2025).

Furthermore, ultrasound treatment, among other physical modification techniques such, is used alongside protein fortification to enhance functionality (Haixi et al., 2025). Zhang et al. (2026) demonstrated how rice flour blended with Soy Protein Isolate (SPI) resulted in the oxidation of thiol groups to form disulfide bonds, constructing a gluten-like 3D network due to having undergone ultrasonic treatment. This synergistic treatment (optimized at 9% SPI with ultrasound) had favourable results compared to untreated samples, as the treatment helped in significantly reducing bread hardness by 6% and increasing specific volume by 10%.

Mechanistic and Microstructural Insights

The microstructure and rheology of the dough must be drastically altered for this synergistic method to be most effective. According to a plethora rheological studies, the majority of gluten-free doughs have significant elastic and loss moduli, but, when ultrasonic treatment is combined with SPI, these moduli are significantly reduced, making the dough behave much more like regular wheat flour (Aguiar et al., 2021; Ronda et al., 2023).

Amylose is liberated and attaches to the exposed amino and carboxyl groups of the SPI when the surface of starch granules is microscopically scratched by ultrasonic cavitation. Starch granules in untreated GF dough are mostly exposed, according to scanning electron microscopy (SEM), however, after ultrasonic treatment, these granules firmly wrap within the newly created protein network, greatly enhancing the structural stability of the dough (Zhang et al., 2026).

The Critical Role of Baking Parameters

Biotechnological changes can improve the raw dough matrix, however these benefits could be totally negated if heat processing is not precisely regulated (Table 2).

Table 2. Impact of thermal processing parameters on ultrasonic-SPI modified gluten-free dough (Adapted from Zhang et al., 2026)

Baking Temperature	Baking Time	Structural & Rheological Outcome
180°C	25 minutes	Increased specific volume and decreased baking loss to 9.19% due to enhanced water-holding capacity.
190°C	25 minutes	Excessive moisture evaporation, excessive starch gelatinization, and irreversible protein coagulation into a rigid network. Bread hardened and lost elasticity.
180°C	30 minutes	Excessive moisture evaporation, excessive starch gelatinization, and irreversible protein coagulation into a rigid network. Bread hardened and lost elasticity.

Zhang et al. (2026) claim that the structural advantages of the SPI-ultrasound synergy are significantly influenced by baking temperature and duration. Baking at 180°C for 25 minutes increased the specific volume of the loaf and decreased baking loss to 9.19% thanks to the modified protein network's enhanced capacity to hold water.

In reverse, due to excessive moisture evaporation, excessive starch gelatinization, as well as the irreversible protein coagulation into a rigid network brought on by raising the temperature to 190°C or extending the baking time by as little as 5 minutes, to 30 minutes, the bread eventually hardened and lost its elasticity.

These findings suggest that the disadvantages of inert starch matrices can be overcome by changing protein structure through cavitation effects if the subsequent heat processing is properly regulated (Nie et al., 2019).

Synergistic Hydrocolloid-Protein Systems

Even though individual functional additions provide partial improvements to gluten-free matrices, recent formulation techniques emphasize the necessity of multi-component systems to accurately replicate the complex viscoelasticity of gluten (Haixi et al., 2025). A recent study by Mensah et al. (2026) found that when a hydrocolloid, such as xanthan gum, is used with two distinct protein sources, like egg powder and gelatin, there are certain notable synergistic effects on dough rheology and final bread construction. Pure rice flour doughs are rather weak, incredibly sticky, and easily compressible due to the lack of structural support (Šmídová & Rysová, 2022). However, the employment of this ternary additive system significantly changes the mechanical profile of the dough, maximizing dough cohesiveness and firmness while reducing undesired adhesiveness, this effect has also been evidenced in other products, such as pasta (Culetu et al., 2021).

This particular combination works because of the complementary mechanisms of each individual ingredient during the stages of mixing, proofing, and baking. Xanthan gum primarily functions in the aqueous phase during proofing, forming an entangled network of polysaccharides that enhances dough viscosity, delays starch gelatinization, and inhibits the coalescence of gas bubbles (Mensah et al., 2026). Simultaneously, adding the thermoreversible protein gelatin creates a hydrated gel that expands to bind water and, when heated, changes into a denatured elastic network that allows the dough to stretch without rupturing. Finally, but perhaps just as importantly, egg powder provides vital structural proteins like albumin, which coagulate during baking to form a continuous, heat-set film around the expanding gas cells, increasing the volume of the loaf and stabilizing the crumb structure (Mensah et al., 2026).

Beyond the aspect of maintaining structural integrity, this hydrocolloid-protein synergy fixes two of the main sensory flaws in GF

bread, namely light crust hue and quick staling (Osei Tutu et al., 2024). The ideal bread formulation's final moisture content was raised to about 37% by the combined water-binding ability of xanthan, egg proteins, and gelatin (Mensah et al., 2026). This reduced water mobility and significantly enhanced both the shelf life and the crumb softness. Standard rice-based breads also have pale crusts because they often don't contain enough reducing sugars and reactive amino acids. During the process of thermal processing, the high amounts of glycine, proline, and other amino acids supplied by the egg powder and gelatin readily take part in Maillard browning reactions (Khan et al., 2025). Higher consumer acceptability scores are produced by this particular biochemical interaction, which successfully produces a rich, golden-brown crust and complex olfactory profile that more closely resembles conventional wheat bread (Capriles et al., 2020).

Advanced Fermentation and Sustainable Ingredients

The use of focused enzymatic biotechnology provided the largest increase in gluten-free bread volume, up to 122.79% when compared to control samples, according to a comprehensive review which detailed several formulation approaches (Djeukeu et al., 2024). This perfect formulation specifically used rice flour that was coated with a mold source, which cooperated with ordinary yeast and sugars. During the pretreatment and enzymatic action of mold preparations, including those derived from koji, very active proteases and amylases are released, cleaving native proteins into free amino acids and highly functional, and tastier peptides (Moradi et al., 2025). Additionally, the addition of these biological enzymes encourages the creation of complex molecular bonds between proteins, particularly when combined with cross-linking agents such as transglutaminase. In order to maximize carbon dioxide gas retention during proofing and produce improved structural aeration, this enzyme-driven structural alteration efficiently substitutes the missing gluten network (Šmídová & Rysová, 2022).

Furthermore, it has been shown that the addition of organic acids, such as lactic or acetic acid, can further optimize this process by controlling the dough's pH, which lowers the molecular weight of proteins and starches to enhance gas retention capacity. Sourdough fermentation remains one of the greatest microbiological techniques for improving the quality of gluten-free bread. Unlike ordinary baker's yeast (*Saccharomyces cerevisiae*), sourdough relies on a complex symbiotic culture of yeasts and lactic acid bacteria (LAB). The main mechanism that can lead to improvement is the dough's acidification; as LAB produces lactic and acetic acids, the pH drops, triggering natural cereal proteases and modifying the batter's viscosity in a positive manner (Seyedahmadi et al., 2025).

Recent studies indicate that this acidity solubilizes pentosans (arabinoxylans), increasing the water-holding capacity of rye and other similar GF flours (Zhang et al., 2026). Additionally, through their metabolic processes, some LAB strains, such as *L. plantarum* and *L. brevis*, can create exopolysaccharides (EPS), which serve as naturally occurring hydrocolloids (Parsamajd et al., 2025). By simulating the structural role of gluten, these microbial gums stabilize the gas bubbles during proving, producing a loaf with a higher specific volume and lower staling rates. Interestingly, a recent study indicates that through the act of concealing the "grassy" off-flavors that are frequently associated with novel plant proteins, like leaf protein concentrates, sourdough fermentation may potentially enhance the sensory profile of breads enriched with these proteins, potentially serving as a new synergistic system (Zhang et al., 2026).

The utilization of repurposed ingredients is also being driven by sustainability. Apple pomace and flaxseed were assessed by Cantero-Ruiz de Eguino et al. (2025) as potentially sustainable sources of fiber. Customers strongly favored compositions with up to 6% apple pomace and 12% flaxseed, according to the sensory analysis carried out by them, which assessed features like "crispy" crust and "artisan-like" appearance. However, an overabundance of 10% apple pomace in the mix resulted in unfavorable textural remarks like "rubbery" underscoring the necessity of optimizing fiber-rich residues in this field.

A sequential schematic of these integrated biotechnological solutions is visualized in Figure 2, while Table 3 provides a comprehensive overview of the novel protein sources driving these advancements.

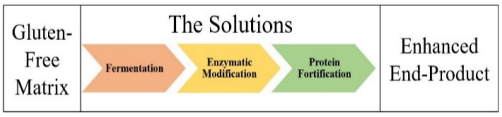

Figure 2. Schematic representation of several biotechnological strategies applied to improve the structural and nutritional quality of gluten-free bread

Table 3. Overview of novel biotechnological sources for gluten-free matrices

Additive/ Source	Processing Method/Intervention	Primary Rheological & Structural Functional Property	Reference
Zein (Corn Protein)	Physical blending (6–12% substitution)	Hydrophobic film-forming capability; significantly improves gas retention and specific volume by mimicking the gluten network.	Fan et al. (2026)
Soy Protein Isolate (SPI)	Ultrasound treatment (360 W, 40 kHz, 20 min)	Promotes thiol oxidation to form disulfide bonds; creates a "pseudo-gluten" 3D network that reduces crumb hardness.	Zhang et al. (2026)
Chickpea Protein	Sourdough fermentation (*L. plantarum* LB-1)	Formation of "weak gel" structures (G' > G"); significantly enhances batter viscosity and delays retrogradation (staling).	Jiang et al. (2026)
Egg Powder, Xanthan, & Gelatin	Synergistic composite blending	Distributes structural roles: increases viscosity, provides heat-coagulable proteins, and maximizes moisture retention and loaf volume.	Mensah et al. (2026)
Apple Pomace & Flaxseed	Dehydration and particle size reduction	High functional fiber content improves water-holding capacity; yields an "artisan-like" crispy crust at optimized inclusion levels (≤6%).	Cantero-Ruiz de Eguino et al. (2025)

Synergistic Fermentation of Legume-Based Matrices

Proteins derived from legumes, such as those found in peas, lentils, lupine, and chickpeas, are a novel method of giving GF doughs structural support (Imam et al., 2024). However, the fermentation step is where these legume matrices realize their full technological potential in regards to the aforementioned issues. The inclusion of these hydrophilic bean proteins significantly changes the dough's capacity to hold CO_2, in accordance with rheological fermentation tracking (Zeng et al., 2024). Because pure rice doughs lack a cohesive network, gas escapes rather quickly during proofing. On the other hand, robust molecular cross-linking is promoted by the targeted addition of pea protein, at 6% substitution, and chickpea protein, at 12% substitution, which changes the matrix into a viscoelastic structure with a high expansion potential. In addition to increasing the maximum dough height and overall gas retention coefficient by up to 1.15 times, this legume-protein network significantly prolongs the time at which carbon dioxide gas starts to escape the dough increasing it from 25 minutes in unfortified doughs to up to 50 minutes in chickpea-fortified formulations (Fan et al., 2026).

Modern biotechnological methods combine specialized sourdough fermentation with legume fortification to further optimize these structural advantages to their full potential. The addition of sourdough starters promotes the binding of starch granules by collaborating with the legume proteins in these matrices. As such, certain lactic acid bacteria, including *Lactobacillus plantarum*, alter the protein structure to create a "weak gel" network during this biological acidification process(Lin et al., 2022). In addition to maximizing the retention of CO_2 gas produced by yeast, this synergistic fermentation greatly delays staling and enhances the final gluten-free product's overall texture and volume, addressing some of GF bread's greatest hurdles (Fan et al., 2026).

Recent studies show that this synergistic application of legume flours and targeted sourdough fermentation significantly optimizes GF dough structure, whereas the sole application of alternative proteins and microbial fermentation can deliver quantifiable benefits. Recent research examined this interaction by carefully assessing the effects of *Lactobacillus plantarum* LB-1-fermented chickpea flour on a rice-based GF matrix (Jiang et al., 2026).

In terms of rheology, this chickpea sourdough greatly decreased the peak viscosity and setback values of the batter. The fermentation process improved the short-range order of starch molecules and actively helped in the stabilization of the conformations of protein molecules (Ramos et al., 2021). As a result, according to Jiang et al. (2026), the fermented batter presented remarkable viscoelastic behavior, maintaining a well-balanced loss tangent tan δ = 0.38-0.58 and reaching a

maximum storage modulus of G' = 11,347 Pa. These microscopic and rheological changes were strongly associated with improved macroscopic baking characteristics. The chickpea sourdough gluten-free (CSGF) bread had its baking moisture loss reduced to just 5.03% while achieving an amazing specific volume of 2.50 mL/g when compared to standard unfermented rice-based standards (Jiang et al., 2026). The crumb hardness was only 3.93 N due to the structural matrix that the fermented chickpea proteins and dietary fibers provided to the structure as a whole.

Additionally, a continuous, dense crumb porosity (AF = 29.94%) in macroscopic image analysis shown by SEM confirmed the creation of a very homogenous and compact structural matrix in the tested bread (Jiang et al., 2026).

Consumer Acceptance of Novel Ingredients

The *L. plantarum* fermentation greatly improved the physical rheology and antioxidant activity of the tested bread. These results demonstrate that using legume-based sourdoughs offers a dual-purpose technological approach that improves the nutritional and sensory qualities of GF bread, which improves its chances on the market, while simultaneously addressing its mechanical shortcomings (Seyedahmadi et al., 2025). The success of biotechnologically modified gluten-free products ultimately depends on consumer acceptance, even though technological functionality is crucial. Recent studies highlight the importance of sensory evaluation for products enriched with novel protein sources, as it is ultimately the acceptance of the consumers that will determine the success of any such experiment (Aguiar et al., 2023).

For example, Duta et al. (2025) stressed that in order to gain market acceptance, enhancing bread matrices, such as grissini, with protein by-products necessitates careful balance of nutritional benefits with sensory qualities.

Similarly, Scheibenzuber et al. (2023) showed that the presentation of "sustainability" and "nutritional" claims has a considerable impact on consumers' readiness to test foods containing industrial by-products, as markets change and these aspects are becoming more and more important.

CONCLUSIONS

The development of gluten-free bread quality has progressed far beyond the levels of simple starch substitution thanks to the application of sophisticated, cooperative biotechnological techniques. According to recent research, a multimodal strategy involving functional proteins, intricate physical alterations, and focused biological therapies is needed to replicate the viscoelastic characteristics of the absent gluten network.

Plant-based protein isolates, such as soy, pea, chickpea, and zein, have been shown to be highly successful in inducing molecular cross-linking and generating a functional, pseudo-gluten three-dimensional network when paired with techniques like ultrasonic cavitation. Additionally, by optimizing CO_2 gas retention during proofing, customized enzymatic treatments and especially those that combine organic acids with proteases and amylases derived from mold, have been able to produce record-breaking loaf volume increases of up to 122.79% in perfect environments. Further bridging the technological gap, there are formulation synergies, such as triple-additive systems made up of microbial hydrocolloids, heat-coagulable proteins, and thermoreversible gels. In addition to increasing specific volume and lowering crumb density, these intricate systems effectively restore the amino acids required for Maillard browning, so this is the best present method of overcoming the ongoing problem of pale crusts in gluten-free baking. In a similar vein, advanced sourdough fermentation combined with legume-based matrices demonstrates how biochemical acidification can modify protein structures to postpone staling and significantly increase gas retention, another longstanding issue.

Nevertheless, successful industrial application is still quite sensitive to processing conditions in spite of these important structural advancements. Over-fortification beyond essential ingredient limits (usually between 6% and 12% for new proteins) or even inadequate heat processing during the baking phase can completely offset the structural benefits of biotechnology. As such, the room for error is rather small and one must be careful when balancing these factors.

In the end, customer approval plays a major role in the commercial success of these innovative formulas, as they have the final word. Formulators must use focused sensory evaluations to remove important negative drivers, such as rubbery or too dense textures, before positive traits, such as crispiness and crumb lightness, can positively influence purchase intent in order to compete in the market. This is a tall task considering the competitors do not have to work around such inconveniences. A gluten-free bread that closely resembles the quality, appearance, and flavor profile of conventional wheat bread can be produced by striking a balance between these complementary biotechnological methods, exact thermal control, and stringent consumer validation.

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