

Technological Aspects of the Bakery Industry Affecting Gluten Structure, Digestibility, and Immunogenicity

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ABSTRACT: Wheat is traditionally consumed after technological modifications, and both the traditional processes used in baking—milling, mixing, heat treatments, extrusion, and fermentation—and food additives can induce structural changes in gluten. After these modifications, the gluten protein network may become more or less prone to the action of digestive enzymes, as well as having more or less epitopes capable of inducing immunogenic responses in susceptible individuals. Although the technological aspects regarding the gluten network are well established, the effects on digestibility and the resulting immunogenic peptide profile require further elucidation. Thus, this review provides an overview of the effects of the main technological modifications on the physicochemical structure of gluten and their impacts on digestibility and immunogenicity, aiming to assist in the adequate selection of ingredients and processing parameters in order to reduce the immunogenic potential of gluten-containing foods.

KEYWORDS: *Gluten network, Protein structure, Bakery industry, Technological modification, In vitro digestibility, Immunogenicity*

1. INTRODUCTION

Wheat is a staple food and one of the main food crops cultivated, commercialized, and consumed worldwide, as it is an important source of energy and nutrients. Its relevance is mainly due to gluten viscoelasticity, which determines the quality of bakery and pasta products, and other wheat-based foods.^{1,2} Furthermore, gluten is important for providing specific texture and sensory characteristics to an important variety of foods such as meat products, ice creams, candies, French fries, processed meals, sauces, soups, dressings, etc.³ In baking, gluten proteins form a continuous network, providing cohesion as well as supplying emulsifying and dough-binding properties. The enhanced dough strength contributes to a better capacity to retain fermentation gases and water, resulting in bakery products with greater volume and softness.⁴

From a public health perspective, exposure to gluten can induce adverse reactions in susceptible individuals, such as celiac disease (CD), wheat allergy (WA), and non-celiac gluten sensitivity (NCGS).⁵ Such diseases are mediated by the immune system, in which the reactions are triggered by epitopes from specific fractions of gluten peptides (known as immunogenic peptides) that are resistant to gastrointestinal digestion.⁶ CD is an autoimmune disease, in which specific serological autoantibodies are involved, such as anti-tissue transglutaminase and anti-endomysial antibodies.⁷ It is related to damage to the small intestinal mucosa and villous atrophy and is associated with poor digestion and nutrient absorption that can lead to several systemic consequences.^{8,9} In WA, IgE antibodies play a fundamental role in triggering pathogenesis, releasing histamine as a chemical mediator from basophils and mast cells. Until now, NCGS is poorly understood and the diagnosis is made by exclusion, when individuals experience symptoms when ingesting gluten-containing foods, although

they are not diagnosed with CD or WA.^{5,7} Currently, a gluten-free diet is the only safe solution for individuals with gluten-associated diseases and is based on the total exclusion of products derived from wheat, barley, and rye.¹⁰

Wheat and cereals are rarely consumed without processing. However, physicochemical modifications resulting from the processes can alter the digestibility and immunogenicity of gluten proteins.¹¹ An increase, a reduction, or even no impact on gluten digestibility and immunogenicity can be observed using different processing techniques. Therefore, processing effects are far from predictable.^{12,13} Among the main structural modifications induced by processing are the unfolding, aggregation, cross-linking of proteins, hydrolysis of peptide bonds, oxidation, and Maillard reactions.¹² Structural changes in gluten epitopes can be conformational, due to alterations in the secondary and tertiary structures of proteins or related to changes in the primary structures of the peptide chains. Conformational changes in gluten proteins can expose or hide epitopes, besides epitopes with a modified primary structure being recognized differently by the immune system.¹⁴ The extent of modifications to the gluten structure depends on the type and intensity of processing.^{15,16} However, to date, there are no clear indications for the complete elimination of immunogenic gluten peptides; this may be partial, depending on an appropriate selection of process parameters.¹⁴

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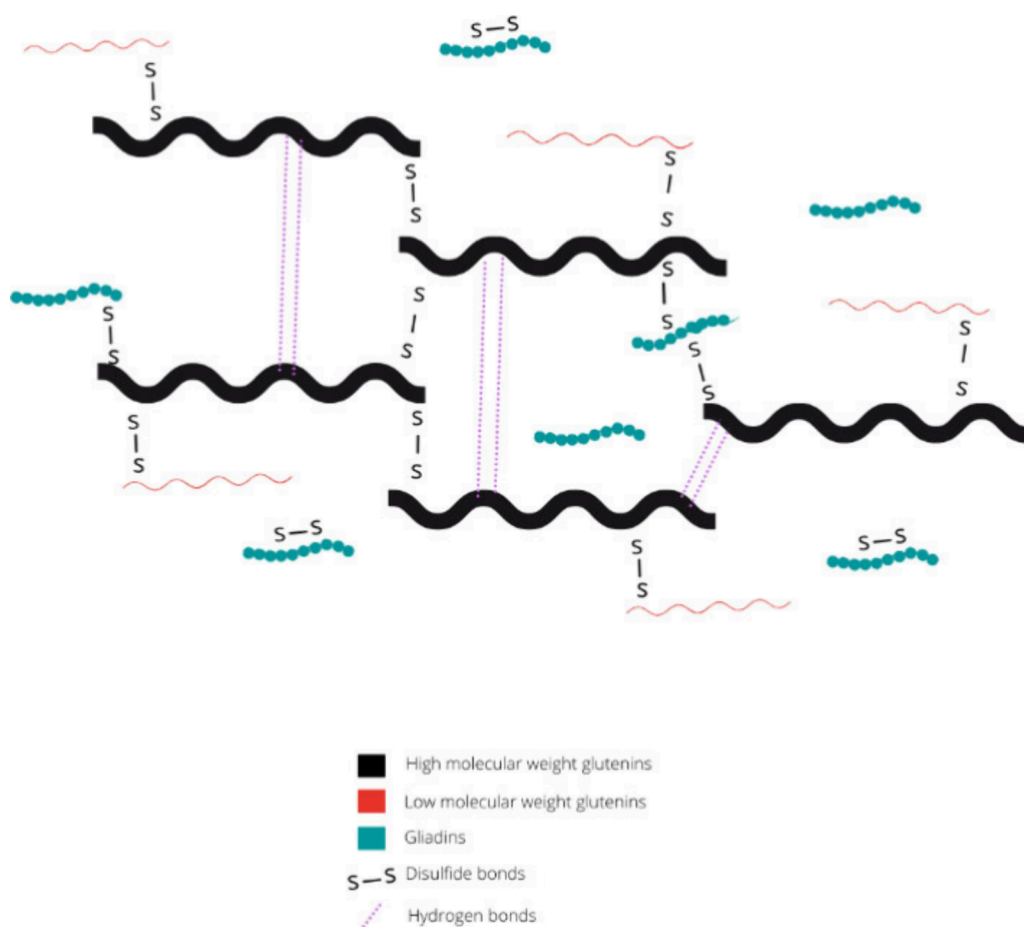


Figure 1. Scheme depicting the accepted structure of the gluten. Overall, the structure is stabilized by disulfide bonds.^{25–27}

Some reviews have described the effects of processing specifically focused on reducing gluten immunoreactivity, such as the use of emerging treatments to reduce the immunoreactivity of gliadin¹⁷ and other gluten-targeted approaches with medicinal focuses.^{18,19} Ye et al.²⁰ discuss gluten modifications in final products such as bread, noodles, and beer. However, no review study has extensively addressed the effects of traditional processing conditions on the physicochemical structure of the protein and the impact on the digestibility and immunogenicity of gluten. This knowledge would contribute to risk assessments and investigations of potential alternative strategies focused on reducing the immunogenicity of gluten-containing products. This article aims to review the effects on the physicochemical structure of the gluten network and the consequent impact on digestibility and generation of epitopes from the main conventional processes used in the wheat-based-product industry, such as grain milling, dough development, thermal processing (baking, drying, and cooking), extrusion, and biochemical processing, in addition to the use of additives and processing aids, commonly applied to improve the technological quality of flours and doughs. In order to have a complete understanding of structural changes in proteins and their impact on triggering gluten adverse reactions, a topic related to the basic structure of gluten will be addressed.

2. SEARCH SELECTION

The literature review was based on the search for scientific publications indexed in the Web of Science Core Collection (WoS) database in the advanced search section through the

use of keywords. To obtain articles covering structural modifications of gluten proteins by processing, keywords such as (“gluten” OR “wheat”) AND “protein structure” AND (“milling” OR “mixing” OR “mechanical process*” OR “thermal process*” OR “extrusion” OR “sourdough fermentation” OR “additives” OR “processing aids”) were used, including synonyms. To search for manuscripts investigating the effects of processes on digestibility or immunogenicity, the keywords “digest*” OR “immuno*” were also included. Other more specific and conceptual research was also carried out throughout the writing. The (*) was used to include suffixes, and manuscripts were considered up to the date of 2025. The Food Science and Technology category filter was used to include manuscripts within the theme. From the resulting manuscripts, the selection for full reading was made based on titles, abstracts, and keywords, with an approach to the structural characteristics of proteins, their alteration by processing, and impacts on the digestibility or immunogenicity of gluten.

3. GLUTEN STRUCTURE

Although gluten specifically refers to gliadins and glutenins from wheat, other similar proteins, such as hordeins (from barley) and secalins (from rye), often have the same nomenclature. Gluten is composed of gliadins, responsible for the viscosity and extensibility of the gluten network, and glutenins, which promote the cohesiveness and elasticity of the dough.²¹ Glutenins are stabilized mainly by intermolecular disulfide (SS) bonds and are classified as (1) high molecular

Table 1. Milling Process Affecting Macropolymer Structure, Gluten Digestibility, and Immunogenicity

Milling	Process conditions	Properties modification	Gluten digestibility or immunogenicity	References
Milling	Planetary ball milling; 170 rpm for 5 min, 15 min, or 30 min	↓ Particle size with increasing process intensity	Not assessed	39
		↓ Dough viscosity		
	Jet milling; 4–8 bar; 0.67–5.18 kg/h	↓ Protein solubility	Not assessed	40
		Displacement of starch from the gluten network		
		Protein denaturation		
		↓ Particle size with increasing process intensity		
		↑ Water absorption		
		Displacement of starch from the gluten network		
		Weaker dough structure		
		↓ Dough mixing stability		
Ball-milling; mechanical starch modification 4.78 g 100 g ⁻¹ flour–8.15 g 100 g ⁻¹ flour	↓ Gas-holding capacity	Not assessed	41	
	↓ Bread specific volume			
	↑ Crumb hardness			
	Poor network connectivity			
	Heterogenous gluten structure with protein agglomerates			
	Displacement of starch from the gluten network			
	↓ Dough extensibility and resistance to extension			
	↓ Dough height during fermentation			
	↓ Bread specific volume			
	↑ Water holding capacity			
Roller milling; 33 different streams	↓ Protein solubility	Not assessed	42	
	Weaker dough structure			
	↓ Dough elasticity, extensibility, and resistance to extension			
	↓ Dough mixing stability			
	↑ Water absorption			
	↓ Gas-holding capacity			
	↓ Bread specific volume			
	↓ Particle size			
	↓ Disruption of aleurone cells			
	↑ Protein extractability			
Factory-scale airflow impact mill	Not assessed	↑ Digestibility of proteins in superfine bran ↑ Degree of protein hydrolysis	46	
	Not assessed			
	↑ Proportion of α -20 epitope per unit of protein in flours generated by breaking streams			
	↓ Proportion of α -20 epitope per unit of protein in flours generated by reducing streams			
	↑ Protein digestibility in flours by phytic acid content			
	Not assessed			
	Not assessed			
	↑ Dissociation of glutenin particles			
	↑ SDS soluble glutenin particles			
	Better reassembly during resting for optimally mixed doughs			
Roller milling; 14 separate mill streams	↑ Viscoelasticity for optimally mixed dough	↑ Proportion of α -20 epitope per unit of protein in flours generated by breaking streams ↓ Degree of protein hydrolysis	45	
	↓ Viscoelasticity for undermixed doughs			
	Continuous protein matrix in optimally mixed and overmixed doughs			
	Not assessed			
	Not assessed			
	Not assessed			
	Not assessed			
	Not assessed			
	Not assessed			
	Not assessed			
Mixing and dough development	Not specified	Not assessed	44	
	Farinograph; 63 rpm			Not assessed
	Not assessed			
Not specified	Not assessed	48		

Table 1. continued

Process conditions	Properties modification	Gluten digestibility or immunogenicity	References
1.0–18.0 Wh/kg	Noncontinuous protein matrix in undermixed doughs ↑ SDS soluble glutenin particles in overmixed doughs ↓ Molecular weight of the glutenin macropolymer	Not assessed	49
Couette cell; 10–50 rpm; 25 min	↑ Free SH groups and rupture of SS bonds during mixing mixed doughs ↑ Storage modulus and extensional stress for optimally mixed doughs ↑ SDS soluble glutenin particles ↓ Dough strength and strain hardening with the increase in shear rate Continuous protein matrix and well-developed dough at intermediate shear levels Noncontinuous protein matrix at low shear Less aggregates Continuous protein matrix ↑ Protein surface area	Not assessed	47
Modern high speed mechanical dough development; 10%–180% of optimum work input; 63 rpm, 120 rpm, or 200 rpm	↑ Sulfur-rich gliadin extractability ↑ Bread volume with higher mixing degree and speed Shear did not affect hydrophobicity, electrophoretic pattern, and FTIR spectrum ↑ At pH 7, high shear rate + temperature (100 °C): hydrophobicity, and α -helix, random coil, and β -sheet structures	Mechanical process did not alter the immunogenic peptide release profile or concentration nor ELISA immunogenicity profile under any mixing conditions.	52
Shear rates 500, 1000, or 1500 s ⁻¹ ; temperatures 80, 90, or 100 °C; pH 3, 5, or 7	↑ Intermolecular SS bonds between the S-rich gliadins and glutenin macropolymer at higher temperatures and longer time ↓ α / β - and γ -gliadins extractability at higher temperatures ↓ Protein solubility ↑ Protein aggregates	Mechanical process alone did not affect gluten antigenicity but had a synergistic influence at pH 7 and 100 °C, resulting in increased antigenicity.	53
150 °C or 230 °C for 25, 35, or 45 min	↑ Protein aggregates in bread crust	After baking, the content and release profile of the celiac immunogenic peptides were changed during <i>in vitro</i> digestion. However, process time and temperature had no influence.	58
Not specified	↑ Protein denaturation ↑ Protein aggregates ↓ Protein solubility ↓ Extractability in dry and cooked pasta ↑ Protein polymerization	Baking reduced the digestibility of wheat gluten proteins, including sequences containing active epitopes in CD.	59
220 °C for 30 min	↑ Protein aggregates in bread crust	Baking affected the extent of proteolysis and the immunogenic characteristics of the digestion products regarding the degree of heat treatment.	54
Drying at 20, 60, 85, 110, and 180 °C	↑ Protein denaturation ↑ Protein aggregates ↓ Protein solubility ↓ Extractability in dry and cooked pasta ↑ Protein polymerization	Sample treated at 180 °C was much less digestible, due to protein aggregates resulting from Maillard reactions.	37
Cooking in deionized water at optimal cooking time	↑ Viscosity in high temperature drying ↑ β -sheet structures and α -helix and random structures ↑ Protein aggregates ↓ Protein solubility	Aggregates were recognized by IgE during the entire digestion process. Not assessed	62
Cooking in boiling water at optimal cooking time	↑ β -sheet structures and α -helix and random structures ↑ Protein aggregates ↓ Protein solubility	Not assessed	63
100, 120, 140, 160, and 180 °C; 11.4.94–145.62 Wh·kg ⁻¹ ; extrusion moisture 25%; screw speed 120 rpm	↓ Gluten extractability as increasing temperature ↑ Protein aggregation and particle size ↓ Free SH groups and formation of SS bonds ↑ β -sheet structures and α -helix	Not assessed	70
40–170 °C; 1.5–3.5 MPa and specific mechanical energy from 32 to 206 kJ·kg ⁻¹	↓ Gluten extractability as increasing temperature ↑ Viscosity and molecular weight ↑ Polymerization reactions	Not assessed	67

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Table 1. continued

Process conditions	Properties modification	Gluten digestibility or immunogenicity	References
160, 170, and 185 °C	<p>Only temperature influenced polymerization behavior</p> <p>↓ Protein solubility</p> <p>↑ Protein aggregation and fragmentation</p> <p>↑ SS bonds and hydrophobic interactions</p> <p>↑ Molecular weight</p>	Not assessed	71
65, 90, 130, 145, and 170 °C; screw speed 250 rpm; extrusion moisture 26.5%, 29.3%, and 32.0%	<p>↓ Extractability of textured wheat protein</p> <p>↑ Protein denaturation, aggregation, and texturization</p> <p>↑ Degree of texturization as extrusion moisture increases</p> <p>↓ Cohesiveness large gaps as extrusion moisture increases</p>	Digestion of textured wheat protein was greater than untreated sample and it increased with the degree of texturization and moisture content.	72
60, 130, 150, and 140 °C; screw speed 280 rpm; and specific mechanical energy 1714 kJ kg ⁻¹	<p>↑ Protein aggregation</p> <p>↑ SS bonds, noncovalent interactions, and Maillard reaction products</p> <p>↓ Free SH groups</p> <p>↓ Nitrogen solubility index</p> <p>↑ Water holding capacity</p> <p>↑ Antiparallel β-sheets and α-helices</p>	Increase in the protein digestibility of extruded wheat gluten products.	66
150 °C; screw speed 135 rpm; extrusion moisture 20%	<p>↑ Denaturation</p> <p>↑ β-turns and α-helical structures</p> <p>Looser structure of extruded wheat gluten</p>	Pancreatic hydrolysis efficiency of extruded wheat gluten was increased significantly.	73

weight glutenins (HMW) and (2) low molecular weight glutenins (LMW).² On the other hand, gliadins are classified according to the amount of sulfur: (3) sulfur-rich gliadins, such as α/β - and γ -gliadins, and (4) sulfur-poor gliadins, ω -gliadins, containing no cysteine. Gliadins have intramolecular disulfide (SS) bonds (except ω -gliadin), in addition to hydrophobic interactions and hydrogen bonds, which give rise to their compact structures.²²

Both gliadins and glutenins are responsible for the unique structure of gluten. All gluten proteins contain repetitive amino acid sequences with a high content of proline and glutamine and; for this reason, they are included in the “prolamin” family.^{5,11} While gliadins are ethanol-soluble monomeric prolamins, glutenins are ethanol-insoluble polymeric prolamins. After hydration and mixing, gluten proteins rearrange into a large continuous protein network known as the glutenin macropolymer, which is the largest polymer known in nature.²³ The quaternary structure of the glutenin macropolymer cannot be defined due to its size, polymeric nature, and complexity.²⁴ Figure 1 suggests a schematic of the structural model of the glutenin macropolymer. This structure highlights HMW glutenin dimers with LMW glutenin branches. Furthermore, glutenins form a backbone due to their propensity to form intermolecular SS bonds. Regarding gliadin proteins, α/β - and γ -gliadins cross-link to the glutenin macropolymer via intermolecular SS bonds, as extenders or terminators of the gluten macropolymer chains.²³ On the other hand, ω -gliadins, which contain no cysteine residues, are retained as monomers within the structure and are not incorporated into the macropolymer via SS bonds. Therefore, gluten structure is stabilized by different forces: physical entanglement of proteins, SS bonds, and noncovalent forces such as hydrogen bonds. SS bonds are those with the highest energy and, consequently, those most responsible for stabilizing and strengthening the protein conformation, in addition to providing resistance to thermal and proteolytic denaturation, among others.²²

The structure of the gluten network brings unique technological attributes and dictates the sensory qualities of wheat-based products. In breadmaking, the gluten network resists the pressure exerted by the release of carbon dioxide (CO₂) during the fermentation process, slowly increasing the dough volume. A stable network structure is essential for gas retention during fermentation and baking, contributing to the structure, volume, and texture of final products.^{28,29} The gluten network can also retain water, preventing moisture loss during storage.^{1,30} As gluten structure can be altered by the glutenin:gliadin ratio, different flours form structurally different polymers and products. Notably, increasing glutenin content produces a stronger network.³¹

4. STRUCTURAL CHANGES IN GLUTEN PROTEINS AFFECTING THEIR TECHNOLOGICAL PROPERTIES, DIGESTIBILITY, AND IMMUNOGENICITY

Protein digestibility is, in short, the susceptibility to proteolysis (by digestive enzymes) of ingested proteins. The releasing rate of peptides and amino acids in digestion depends on the structure of the protein.³² Digestion products may present bioactive components with beneficial effects on human health or induce adverse responses, as occurs with peptides with immunogenic characteristics.³³ Immunogenicity is characterized by the ability of a peptide to induce an immune response,³⁴ which is the focus of this review.

During digestion, gluten proteins are cleaved into free amino acids and peptides in a way similar to that for all proteins. However, many proteases fail to cleave certain peptide sequences that are highly resistant to gastrointestinal digestion due to their high content of proline and glutamine. These specific immunogenic sequences, known as epitopes, create compact structures that can mediate immune disorders in susceptible individuals.^{6,35} In WA, IgE-binding epitopes play a central role in the disease pathogenesis.⁷ For CD, there is a revised list of 38 celiac immunogenic peptides recognized by CD4+ T cells.³⁶

Thus, understanding the impact of the main technological strategies on protein structure and, consequently, on digestibility is important to measure their impact on the generation of epitopes with immunogenic potential. Such strategies include: (1) physical processes, such as milling, mixing, heat treatments, and extrusion; (2) fermentation; and (3) the use of food additives and processing aids. Next, the technological purposes and effects of these techniques on the gluten network structure will be addressed, and consequently, it will be described how they can affect digestibility and immunogenicity of the product.

4.1. Physical Processes. The processes of milling, mixing, and heat treatments of wheat are essential steps in the processing of bakery products to obtain safe food suitable for human consumption. In general, these processes or processing steps tend to cause changes in the structure of gluten protein, which can hinder or facilitate the action of digestive enzymes.^{14,37} Therefore, the impacts on the release of peptides and their immunogenic response are still unclear, and some aspects elucidated are described below. Table 1 summarizes some impacts caused by the physical process.

4.1.1. Milling. The most common wheat milling methods used by the food industry include stone milling and roller milling,³⁸ although techniques such as ball milling³⁹ and jet milling⁴⁰ have been studied to obtain superfine powders. The increase in the milling intensity leads to a reduction in particle size, impacting both flour and bread properties.

Mechanical changes alter the microstructure of protein networks as well as protein–starch interactions, impacting both the rheological behavior of the dough and the final quality of bakery products. Due to the restructuring of S–S bonds, doughs produced with high mechanical changes had a reduction in the protein branching rate and an increase in lacunarity. If the milling time increases, the structure of the gluten network becomes more heterogeneous and the protein threads become shorter and thicker, resulting in a structure with gaps and agglomerated proteins.⁴¹ In addition, starch granules are removed from the gluten network and become available as free particles.³⁹ These structural changes in the protein network cause a reduction in extensibility and resistance to extension, due to poor network connectivity.⁴¹ Furthermore, the greater the intensity of the milling process, the lower the consistency values and the greater the loss of mechanical stability of the doughs.⁴⁰ During baking, weaker networks do not allow the dough to expand optimally, reducing the gas retention capacity and volume of the bread.⁴² Thus, in general, with a smaller flour particle size, the bread structure becomes more compact, with reduced specific volume and increased crumb hardness.⁴⁰

Overall, different studies have evaluated starch digestibility in flours obtained from different milling conditions,^{39,40,43} while few studies have evaluated the effect of milling on

protein digestibility and immunogenicity.^{44,45} The improvement in protein digestibility by the milling process has been attributed to the removal of anti-nutritional factors present in bran and hull layers. Phytic acid, for example, due to its chelating property, reduces mineral bioavailability and protein absorption.⁴⁴ Superfine milling also allowed the disruption of aleuronic cells, causing the release of proteins from wheat bran, increasing their extractability and digestibility, including those of prolamins. The reduction in particle size was responsible for the increase in the degree of hydrolysis by 69.6% of superfine bran intestinal digesta, which had greater accessibility to pancreatic enzymes.⁴⁶ The impact of milling steps, such as grain breaking and particle size reduction, on the content of α -gliadin epitopes in flours was also evaluated. Flours that only underwent the breaking step showed more α -gliadin epitopes per unit of protein than flours subjected to reduction streams, which contributed to significantly lower proportions of these epitopes.⁴⁵ The authors suggested that the combination of selected flour streams may contribute to the production of flours with a reduction in α -gliadin epitopes, which are known to elicit immune responses in genetically predisposed individuals.⁴⁵ No studies were found that analyzed combined digestibility and immunogenicity nor their relationship with gluten network structure and different processing conditions.

4.1.2. Mixing and Dough Development. During mixing and dough development, several changes occur, depending on time and shear conditions, as shown in Table 1. Depending on the process conditions, the dough can be under, over, or optimally mixed (Figure 2).⁴⁷ At the optimum mixing conditions, particles are well dissociated, resulting in a continuous network after rest with a preserved internal structure. In undermixed doughs, glutenin structures are still present in “patches” and are unevenly distributed throughout the dough volume, not being able to create a continuous network as in optimally mixed doughs. In overmixed doughs, both kinetics and extent of reassembly as well as the internal structure of the particles are affected.⁴⁸ Excessive mechanical stress is responsible for the breakdown of structural elements and disruption of SS bonds, causing loss of macropolymer structure, due to the dissociation of glutenin into soluble particle sizes.⁴⁹ A softer, less consistent, and more sticky dough is formed compared to optimally mixed doughs.⁵⁰ Mixing time also impacts dough rheology. Developed doughs typically have a higher storage modulus (G') and extensional stress than undermixed doughs.⁴⁷

Measurement of the extractability/solubility of gluten proteins in different solvents is normally used to assess the level of polymerization, since cross-linking prevents the extraction of proteins from the food matrix. Therefore, the more proteins extracted from the macropolymer, the lower the degree of polymerization of the gluten network.⁵¹ The effect of different mixing speeds and stages of dough development on gluten structure, digestibility, and immunogenicity has been evaluated.⁵² At 200 rpm, the extractability of α/β - and γ -gliadins increased by about 40% when comparing different stages of dough development. However, at 120 rpm, the increase was only about 25%. Such results indicate that the work input causes the breakdown of part of the sulfur-rich gliadins from the macropolymer, increasing the extractability. Although mixing was able to alter the protein macropolymer structure, the immunogenic peptide profile as well as the ELISA antigenicity were equivalent for the different process conditions. Results suggested that structural modifications did

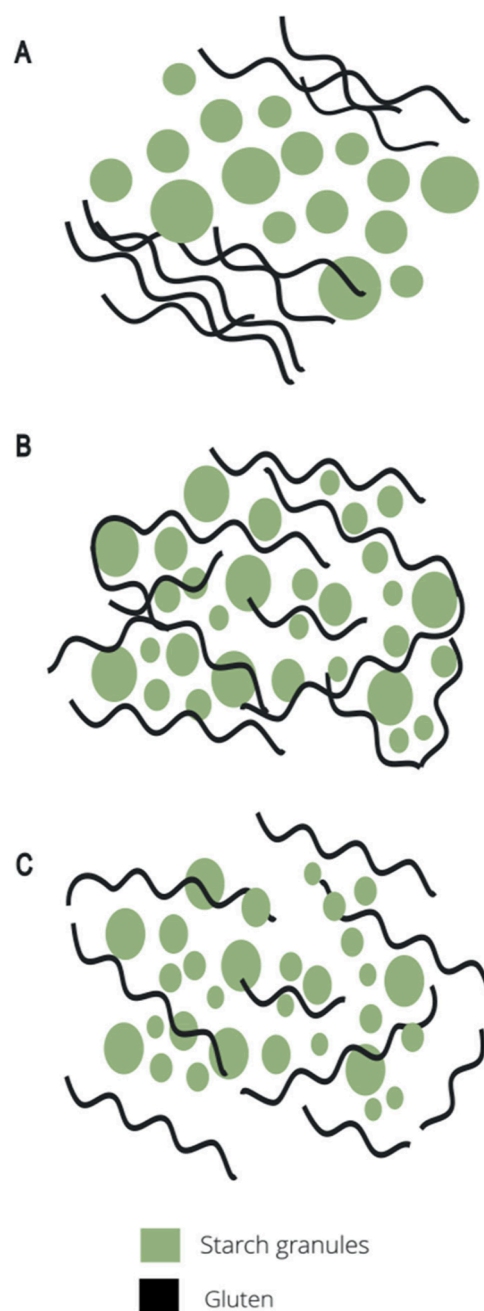


Figure 2. Structural modifications caused by mixing the dough under conditions of a) undermixing, b) ideal mixing, and c) overmixing.

not influence the digestibility or immunogenicity of celiac gluten peptides. The authors suggested that the epitopes were likely buried within the protein structure and were not very susceptible to changes in the protein macropolymer.⁵² Similarly, Rahaman et al.⁵³ identified that the shear rate at 500, 1000, and 1500 s^{-1} did not affect gluten antigenicity. However, the synergy between high shear rate and temperature resulted in alteration of the gluten secondary structure, which may have led to greater exposure of epitopes, resulting in increased immunogenicity.⁵³ Although shearing alone apparently did not result in changes in gluten immunogenicity, further studies are needed on the combined effect with other common process variables in the bakery industry.

4.1.3. Heating. As wheat is often consumed after thermal processing, such as baking, drying, cooking, etc., it is important

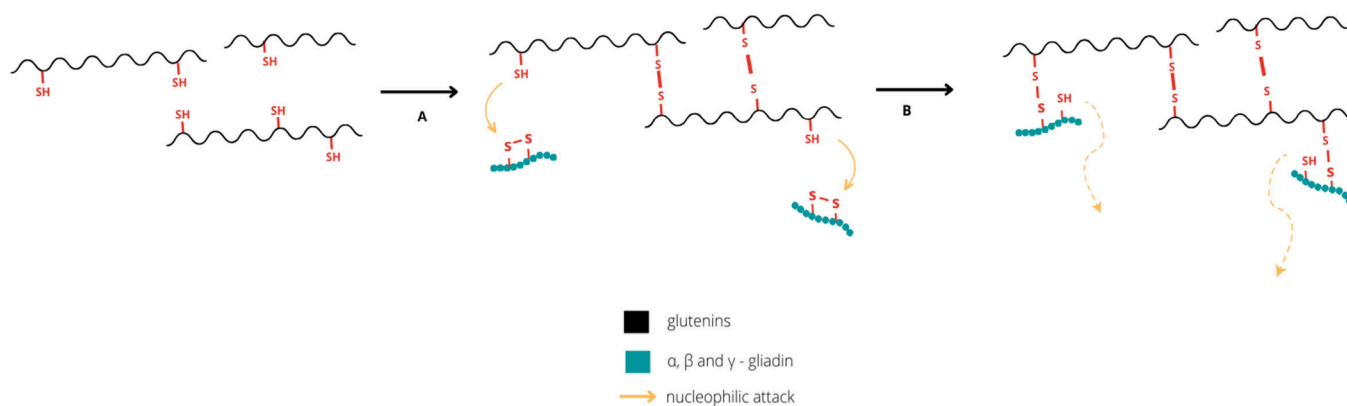


Figure 3. Polymerization of the gluten network promoted by heating. A- Polymerization of glutenins below 100 °C; B- Nucleophilic attack of glutenin free-SH groups and incorporation of gliadins into the macropolymer, starting at 90 °C.⁵⁶

to understand how gluten structure and digestion products are affected by these treatments.⁵⁴ When gluten is heat treated, gliadin and glutenin form large protein aggregates in two different ways. First, glutenin sulfhydryl groups (SH) are oxidized into disulfide bonds (SS) at temperatures below 100 °C. Subsequently, gliadins are incorporated into the macropolymer at higher temperatures, through sulfhydryl–disulfide exchange reactions.⁵⁵ Close to 100 °C, some of the glutenin SH groups remain free, inducing the gliadin–glutenin covalent bond. Sulfur-rich gliadins have intramolecular SS bonds, which undergo nucleophilic attack by the remaining glutenin free-SH groups and are then introduced into the gluten network via SH–SS exchange reactions (Figure 3). ω -Gliadins do not react since they do not have cysteine residues, demonstrating that polymerization occurs by SS bonding.⁵⁶

Polymerization during heating is followed by a loss of extractability of both proteins (glutenins and gliadins), which can be attributed to steric hindrance due to the formation of a three-dimensional network.⁵⁷ Similar results were found by Ogilvie et al.,⁵⁸ who found that increasing temperature during baking in a bread matrix affected the extractability of α/β - and γ -gliadins. At 150 °C, 20-fold more gliadins were extracted compared to 230 °C. Changing the structure of the gluten macropolymer also affects its digestibility. During chewing, a reduction in the absorption of digestive fluids into the bolus is related to the formation of an aggregated protein network.⁵⁹

During baking, the temperature inside the bread, due to the presence of residual water, is lower than that on the crust surface, which reaches the oven temperature. Interestingly, the protein digestibility of the crust is lower compared with the dough and bread crumb. The resistance of crust proteins to digestive enzymes could be explained by the reduced accessibility of proteases in strongly aggregated structures.⁵⁴ Smith et al.⁵⁹ observed that baking modified the physicochemical and immunological properties of gluten proteins, which were less digested after baking, including peptide sequences that are active for CD. Structural rearrangements caused by baking alter both gluten digestibility and immunogenicity, since the concentration and release profile of celiac immunogenic peptides are different for dough and bread. However, once the breads were baked, process temperature and time did not affect the profile of immunogenic peptides released during *in vitro* digestion.⁵⁸

Pasta processing involves temperatures different from those used in breadmaking, in addition to including steps such as shaping, drying, and cooking.⁶⁰ Furthermore, over the years,

technological changes in pasta processing have included increasing drying temperature from 75 to 100 °C or more, aiming at reducing processing time, which was originally 48 h, to only 2 to 3 h. However, high temperatures can lead to protein modifications, which may affect the digestibility and allergenic properties of wheat proteins. Pasta drying at different temperatures was studied by De Zorzi et al.³⁷ As expected, increasing the drying temperature results in greater protein denaturation and aggregation, affecting food digestion. Samples dried up to 100 °C showed similar degradation; however, those treated at 180 °C were poorly digested due to the presence of aggregates derived from protein cross-linking and Maillard reactions with carbohydrates present in the food matrix. High molecular weight protein aggregates were recognized by IgE antibodies during the *in vitro* digestion process. Therefore, it is suggested that aggregates induced by thermal processing have allergenic potential and are resistant to the action of digestive enzymes.³⁷

In pasta, protein polymerization occurs during both industrial processing and the cooking step at home. After high drying temperatures were applied to the paste, a large drop in solubility occurred (from 75.5% to 22.6%). The drop in solubility was less significant after cooking, reaching 18.2% and 14.5% for the overcooked pastes.⁶¹ In agreement, about 80–85% of the native semolina proteins were extracted, while during drying, proteins became more polymerized and, consequently, the extractability was reduced to 31–56%. After cooking, this value dropped to 12.4–20.8%.⁶² Evaluation of the gluten secondary structure during pasta processing indicates that β -sheet structures are the most affected by the processing conditions. After cooking, there is a significant change in the secondary structure of proteins, with an increase in the β -sheet structure at the expense of the α -helix and random coil structures, due to heat-induced denaturation and protein aggregation.⁶³

Depending on the complexity of the food matrix, the gluten disintegration and protein hydrolysis profiles are different. In this sense, the *in vitro* digestion profile of wheat proteins in bread, pasta, and cereal matrixes and their relationship with digestibility were evaluated. In general, although protein release in *in vitro* digestion was slower for pasta, it obtained a higher percentage of hydrolysis at the end of the intestinal phase. In bread and cereals, protection against proteolysis was probably due to a combination of structural changes caused by the processes or the preservation of structures resistant to digestion of the native protein.⁶⁴

Table 2. Sourdough Fermentation Affects Macropolymer Structure, Gluten Digestibility, and Immunogenicity

Process conditions	Structure modification	Gluten digestibility or immunogenicity	References
Sourdough fermentation; 10^9 CFU ^a of lactic acid bacteria strains mL ⁻¹ ; 28 °C for 24 h and 37 °C for 4 h; sourdough levels (20 and 40%)	<ul style="list-style-type: none"> ↑ Protein hydrolysis ↑ Dough softness ↑ Development times and ↓ dough stability ↓ Water absorption ↓ Resistance to extension as the sourdough level increases ↑ Specific bread volume and satisfactory sensory properties for moderate sourdough fermentation ↓ Bread volume, harder texture, unsatisfactory crumb and flavor, sticky, and weak doughs for intense sourdough fermentation 	Not assessed	84
Sourdough fermentation; 10^9 CFU/g of lactobacilli and 200 ppm of fungal proteases; 37 °C for 48 h	<ul style="list-style-type: none"> ↑ Protein hydrolysis ↓ Gluten content to less than 10 ppm ↑ Water/salt soluble protein fractions ↑ Low molecular weight peptides ↑ Free amino acids release 	In a clinical challenge, patients with CD showed normal values of hematology, serology, and intestinal permeability during 60 days of sourdough bread consumption.	78
Partial sourdough fermentation; selected lactobacilli at 10^9 CFU/g of each strain; fungal proteases (0, 50, 100, and 200 ppm); 30 °C for 15, 24, and 48 h	<ul style="list-style-type: none"> ↑ Protein hydrolysis ↑ Water/salt soluble protein fractions ↑ Free amino acids release ↓ Bread specific volume ↑ Bread hardness Similar bread moisture values ↑ Gluten depolymerization ↑ Gliadin extractability after the baking of sourdough bread compared to control 	The protein digestibility of bread with intermediate gluten content, by partial sourdough fermentation, increased 5% compared to the control, and immune reactive gluten decreased by 28%.	77
Partial sourdough fermentation; sourdough starter raised on white flour; 22 °C for 12–16 h	<ul style="list-style-type: none"> ↑ Protein solubility as pH is deviated from pI (6.4) ↓ Hydrophobicity ↑ Protein net charge ↓ Free SH groups exposed ↑ β-turn structures and ↓ α-helix at lower pH ↑ Protein deamidation in acidic pH ↑ Protein hydrolysis in acidic pH 	Partial sourdough fermentation changed the peptide release profile during <i>in vitro</i> digestion. However, the contents of immunogenic gluten peptides did not change compared to conventional bread.	82
Dough acidification; pHs 3, 5, 7; temperature 80, 90, 100 °C and shear rate 500, 1000, 1500 s ⁻¹		At pH 3, up to 90 °C, samples had reduced antigenicity by one-third compared to control, due to acidic deamidation with partial hydrolysis of peptide bonds.	53

^aCFU: colony-forming unit.

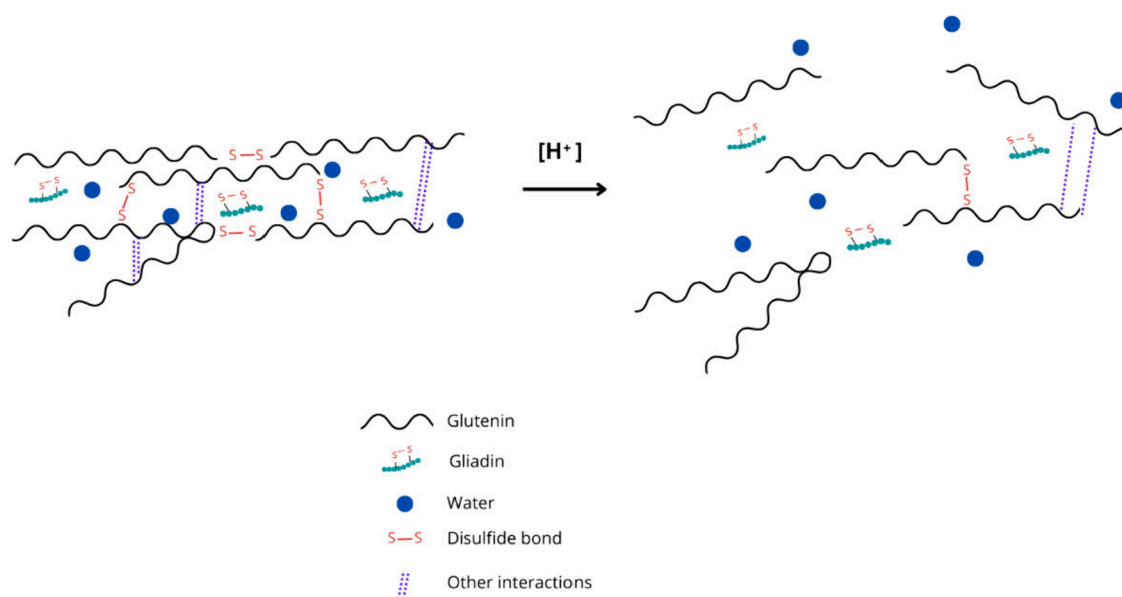


Figure 4. Effect of increasing acid concentration on protein interactions in the gluten network.

4.1.4. Extrusion. Wheat thermoplastic extrusion has two different objectives, depending on the composition of the materials. This process is used for the production of expanded food, breakfast cereals, and biscuits in starch-based raw materials, while extrusion has been used for texturing to obtain meat-like characteristics in protein-based materials. Starch-based products, such as wheat flour, melt during extrusion, trapping air bubbles that expand and subsequently form a rigid cell structure after rapid water evaporation.⁶⁵ Parameters such as high temperature, pressure, and shear forces contribute to the manufacture of expanded products.⁶⁶ The effect of extrusion conditions, pressure (between 1.5 and 3.5 MPa), and mechanical energy (from 32 to 206 kJ·kg⁻¹) on the structural properties of gluten proteins has been studied.⁶⁷ Within the range investigated, pressure and mechanical energy had no significant influence on polymerization reactions and only temperature influenced structural changes.⁶⁷ However, other studies have shown that polymerization behavior can also be affected by mechanical parameters.^{68,69} Some extrusion effects related to the gluten structure are summarized in Table 1.

The effect of the temperature on extrusion is clear and similar to that observed in other processes that involve heating. For instance, increasing extrusion temperature from 100 to 180 °C resulted in a reduction in the amount of free SH, accompanied by an increase in the level of SS bonds, also contributing to the reduction of gluten extractability.⁷⁰ Furthermore, the increase in β -sheet secondary structure and a decrease in α -helix demonstrate the occurrence of protein aggregation.⁷⁰ It is assumed that, first, there is a protein unfolding and an exposure of hydrophobic groups and reactive sites and, subsequently, there is aggregation and increase in the molecular weight of the protein.⁷¹ Despite the formation of protein aggregates, *in vitro* digestion studies demonstrate that extrusion significantly increases the digestibility of gluten proteins.^{66,72,73} After extrusion, denatured gluten has a looser and more flexible structure, which facilitates the action of digestive enzymes.⁷³ In addition, the unfolding of hydrophobic groups contributes to more sites being exposed to proteases.⁷²

In the extrusion of protein-based materials, such as vital gluten, extrusion moisture input is considered an important parameter, influencing the formation of fibrous structures and, consequently, the rate of protein disintegration during digestion. The presence of gaps between textured wheat protein fibers decreased with reduced extrusion moisture as adjacent fibers became more compact. The more compact the fiber structure and the greater the cohesiveness, the more restricted the access of digestive enzymes, which hinders the hydrolysis of gluten proteins and reduces the digestion rate. Higher extrusion moisture translates into greater breakdown of textured proteins during digestion, which could be verified by the smaller protein particle size, higher amount of soluble nitrogen, and increased release of free amino acids. Therefore, the results suggest that the digestibility of textured proteins under higher extrusion moisture conditions is superior to those with reduced moisture, and is even higher than nonextruded proteins.⁷²

4.2. Fermentation. Fermentation techniques are used in bakery products to obtain specific physicochemical and sensory characteristics such as volume, flavor, and texture. Currently, two leavening agents are commonly used on an industrial scale, chemicals (e.g., sodium bicarbonate and other salts in cakes and cookies) and yeast (e.g., *Saccharomyces cerevisiae* in bread).⁷⁴ However, natural fermentation has been gaining ground in the bakery market as it is considered a traditional, artisanal technique and is associated with products with better sensory and nutritional quality.^{75,76} Although baker's yeast has a certain impact on physicochemical structural modifications, sourdough breads have longer fermentation times, with greater production of organic acids and pH reduction, which can significantly modify the structural conformation of gluten and, consequently, its digestibility and immune response (Table 2).^{53,74,77,78}

4.2.1. Sourdough Fermentation. Sourdough fermentation is a traditional process carried out to improve the quality of wheat bread, due to physicochemical and sensory benefits.⁷⁹ These advantages are directly related to the compounds generated during the process such as organic acids, alcohols, aldehydes, and esters. In general, sourdough fermentation

Table 3. Additives and Processing Aids Affecting Macropolymer Structure, Gluten Digestibility, and Immunogenicity

Oxidizing agents	Process conditions	Structure modification	Gluten digestibility or immunogenicity	References
	5–100 ppm of AH ₂ ; ^a 5–45 ppm of ADA ^b	<ul style="list-style-type: none"> ↓ Free SH groups as increasing O₂ level ↑ SS bonds and polymerization ↑ Gas retention ↑ Bread specific volume at optimal concentrations ↑ Dough tenacity at optimal concentrations ↑ Baking strength ↑ Bread specific volume ↓ Free SH groups by ADA addition ↑ Farinograph stability ↑ Extensograph and mixograph areas ↑ Apparent biaxial extensional viscosity ↑ Compressive stress ↑ Hardness and cohesiveness ↑ Resistance to extension and ↓ extensibility ↑ Storage moduli (<i>G'</i>) ↑ SS bonds Cohesive network and homogeneous protein structure 	Not assessed	88
	120 ppm of AH ₂ ; 30 ppm of ADA		Not assessed	92
	20 and 40 ppm of potassium bromate; 100 and 200 ppm of AH ₂ and 20 and 40 ppm of potassium iodate		Not assessed	91
	100 and 200 ppm of AH ₂	<ul style="list-style-type: none"> ↑ Resistance to extension and ↓ extensibility ↑ Storage moduli (<i>G'</i>) ↑ SS bonds Cohesive network and homogeneous protein structure ↑ High molecular weight aggregates 	No major changes were observed in the electrophoretic profile and immunogenic gliadin content of wheat proteins at the end of the <i>in vitro</i> digestion.	93
Emulsifiers	0.25, 0.5, and 1.0% (w/w, flour basis) of SSL, ^c DATEM, ^d or SSL + DATEM (1:1)		Not assessed	96
	0.25%, 0.50%, and 0.75% of SSL (w/w, flour basis)	<ul style="list-style-type: none"> ↑ Peak viscosity ↑ Dough development and stability times ↑ Gluten strength ↑ Connectivity of the gluten network ↑ Noodle cohesiveness 	Not assessed	95
	1.0%, 1.3%, and 1.6% (w/w) of SSL, polysorbate 80, and DATEM	<ul style="list-style-type: none"> ↑ Dough development and stability times ↑ Loaf volume of breads ↓ Lysine content 	<i>In vitro</i> digestibility reduced as the level of emulsifiers increased.	97
Enzymes	mTg ^e and tTg ^f stock solutions (0.1–0.5 U·mL ⁻¹) and gliadin were added to form a complex	Not assessed	In a clinical challenge, mTG was immunogenic in children with CD ^g and it was enhanced when mTG was complexed to gliadin.	102
	300 ppm of mTG in wheat bread and gluten-free bread	Not assessed	The addition of mTG in wheat-based and gluten-free breads increased the IgA reactivity in CD patients ^h as compared to untreated breads.	101
	mTG (0–2000 U·kg ⁻¹) in a bread system	<ul style="list-style-type: none"> ↑ Resistance and ↓ extensibility ↓ Bread volume in high mTG concentrations ↑ Protein aggregation and ↓ free SH groups with increased levels of mTG 	mTG did not deaminate the celiac immunogenic peptides analyzed and did not alter their release profile in a bread system.	99

^aAH₂: Ascorbic acid. ^bADA: Azodicarbonamide. ^cSSL: Sodium stearoyl-lactylate. ^dDATEM: Diacetyl tartaric acid esters of monoglycerides. ^emTG: Microbial transglutaminase. ^ftTG: Tissue transglutaminase. ^gCD: Celiac disease.

consists of initial preparation of a combination of wheat flour, water, and a pool of microorganisms, such as lactic acid bacteria and yeast, which can be selected to obtain specific properties. The dough produced is fermented slowly and gradually with controlled biological activity until the desired characteristics are obtained.^{76,80}

During sourdough fermentation, significant depolymerization of the gluten macropolymer and partial hydrolysis of glutenin subunits may occur. The depolymerization process occurs in two different ways: proteolysis from the secretion of proteases by lactic bacteria and added yeasts and, more expressively, from the activation of endogenous flour enzymes due to the pH reduction by organic acids generated during fermentation. Endogenous wheat proteases have optimal activity at $\text{pH} \leq 4$.^{81,82} Higher concentration of hydrogen cations therefore causes several physicochemical changes in the structure of the protein with the deviation from its isoelectric point, pI (pH 6.4), reducing noncovalent interactions, promoting destabilization of SS bonds and partial hydrolysis of glutenin. All of these factors contribute to the depolymerization and disruption of the gluten network (Figure 4).⁸³ When the pH was far from the pI , the hydrophobicity of the proteins tended to decrease, caused by the increase in the net electric charge and the burial of some hydrophobic sites. Free SH values also had the same behavior, as far from the pI , SH groups participate in SS/SH reactions.⁵³ Sourdoughs showed an overall decrease in viscosity caused by intense proteolytic degradation of proteins.⁸¹ In this sense, the gluten network is altered, resulting in weaker and stickier doughs, with reduced stability and less resistance to extension as the level of sourdough increases.⁸⁴ As a result of the destabilization and reduction of protein interactions, a more open and less stable structure emerges that can alter protein digestion, increasing interactions between digestive enzymes and substrates as well as degrading immunogenic peptides. The content of immunogenic peptides can be reduced if the protein structure is hydrolyzed in a region containing an epitope sequence.⁷⁵

When a pool of microorganisms is selected for sourdough fermentation, it is important to consider the expected results for the product. The *Lactococcus lactis* LLGKC18 strain was able to hydrolyze gluten, reducing food allergenicity for application in hypoallergenic baked products.⁸⁵ A pool of selected lactobacilli and fungal proteases, for example, can even degrade gluten to less than 10 ppm during sourdough fermentation.⁷⁸ Synergistic interactions of lactobacilli and endogenous enzymes present in the grain further contribute to depolymerization of the gluten network. Proline-rich polypeptides released by endogenous enzymes are exposed to lactobacilli and proline-specific peptidases.⁸³ A clinical challenge with eight patients with CD showed that the use of selected lactobacilli and fungal proteases in bread sourdough fermentation led to normal values of hematology, serology, and intestinal permeability during 60 days of continuous consumption.⁷⁸

Although sourdough fermentation has managed to reduce the immunogenicity of gluten to safe levels, this is achieved only with a selection of microorganisms optimized for this objective, often compromising the technological characteristics. Therefore, commercially used cultures of microorganisms differ from those with a better degradation capacity. In commercially sourdough breads, cultures are used, aiming at good sensory structural quality and baking performance. Their capacity to degrade gluten proteins is reduced compared to

that of optimized cultures, which results in some intact proteins after fermentation. They are therefore known to induce a partial sourdough fermentation. Partial sourdough fermentation has been able to change protein structure, digestibility, and peptide release profile during *in vitro* digestion. Differences in peptide release kinetics translated into a change in the way the breads are digested, although partial sourdough fermentation was unable to disrupt epitopes. Therefore, traditional sourdough breads consumed by the public probably have contents of immunogenic gluten proteins similar to those of conventional breads. However, it is necessary to investigate other sourdough cultures to confirm such a hypothesis.⁸²

4.3. Food Additives and Processing Aids. The quantity and quality of proteins that make up the gluten network are directly related to the quality of wheat flour.⁸⁶ Briefly, more elastic and cohesive doughs with higher glutenin content are related to stronger and higher quality flours. On the other hand, more extensible and plastic doughs are obtained from weaker flours, which have higher proportions of gliadin, and are considered of inferior quality for breadmaking.³¹ To overcome the quality deficiencies of weaker flours, food additives and processing aids, such as oxidizing agents, emulsifiers, and enzymes, can be used. These ingredients aim to strengthen the gluten network by inducing more intra- and intermolecular interactions between the amino acid residues present in gliadins and glutenins. This contributes to increasing dough cohesion and results in desirable characteristics, such as increased volume, greater crumb uniformity, softness, and color of the bread.⁸⁷

However, these chemical and rheological changes resulting from the application of food additives and processing aids to flours are being associated with changes in food digestibility and gluten immunogenicity.⁸³ In this sense, this section brings a survey of studies that address the influence on the digestibility and immunogenicity of proteins by the use of food additives and processing aids that modify the gluten structure. To clarify these effects, Table 3 brings some results.

4.3.1. Oxidizing Agents. Oxidizing agents act by oxidizing SH groups of the gluten network, providing new SS bonds and other protein interactions and increasing the molecular weight of the glutenin macropolymer. These interactions contribute to the strengthening and stability of the gluten network (Figure S1 - Supporting Information).^{31,88} Different oxidizing agents are used in the bakery industry, the most common being ascorbic acid (AH_2) and azodicarbonamide (ADA).^{87,89}

ADA has an immediate oxidizing action on the free SH groups of wheat flour proteins in contact with water. It is generally used to fortify lower-quality flours and promote greater volume and softness in breads without changing the dough mixing time. However, excessive use can cause adverse effects due to excessive dough strengthening. Another problem related to the use of ADA is its partial transformation into a semicarbazide (SEM). During baking, ADA is converted into biurea (Figure S1 - Supporting Information) which, in turn, is partially transformed into SEM during baking. SEM is considered harmful to health, resulting in this additive being banned in some countries.^{87–89}

On the other hand, AH_2 has slower action and depends on the incorporation of oxygen (O_2) in dough development to act as an oxidizing agent. O_2 oxidizes AH_2 to dehydroascorbic acid (DHA) (Figure S1 - Supporting Information) in a reaction catalyzed by an endogenous wheat flour enzyme (ascorbic acid

oxidase). Dehydroascorbic acid oxidizes the SH groups of the protein into SS bonds, thus helping to stabilize the gluten network.^{88–90} Despite being less effective in increasing the dough strength and promoting greater volume and softness in bread, AH₂ tolerates an excessive addition. It is considered GRAS (Generally Recognized As Safe), and because of this, it is widely used in countries where other oxidizing agents are prohibited.⁸⁷

The influence of oxygen on the functionality of ADA and AH₂ in breadmaking based on the quantification of free SH content and bread volume has been evaluated.⁸⁸ Increasing the ADA level in the dough reduced the free SH content due to oxidation forming SS bonds. However, the addition of more than 20 ppm of ADA in the dough leads to a reduction in bread volume, even with a continuous reduction in the free SH content. In contrast, AH₂ provided a gradual increase in the bread volume even in larger quantities (40 ppm). Furthermore, no significant reduction of free SH was observed at short mixing times when using AH₂. However, opposite results were found in longer mixing times, when there was greater incorporation of O₂, emphasizing the need to incorporate air in dough development for better AH₂ action. At optimal concentrations, oxidizing agents are also known to improve dough rheological properties, such as toughness, baking strength, farinograph stability, extensograph and mixograph areas, compression stress, extensional viscosity, and cohesiveness.^{91,92}

In general, the addition of oxidizing agents to wheat flour leads to the formation of a compact and dense polymeric network due to molecule approximation by new SS interactions. This more stable and dense structure may interfere with the digestibility and immunogenicity of gluten, since polymer hydrolysis will be more difficult, protecting epitopes from digestive enzymes.^{22,87} Protein stability to gastrointestinal digestion is an important feature that keeps epitopes intact, which may promote immune reactions.^{14,34} Although AH₂ is capable of altering the rheological properties of the dough, SDS-PAGE and ELISA immunoassays did not indicate differences between the electrophoretic profile and gliadin content in the digesta of samples with addition of 200 ppm of AH₂ and control without addition of AH₂.⁹³ These results show that the relationship between structural changes in proteins and their digestibility is not always clear and direct, which highlights the need for a more in-depth assessment and the use of other techniques to expand knowledge of the issue of gluten immunogenicity.

4.3.2. Emulsifiers. The main function of emulsifiers is to reduce tension at oil–water interfaces, although they are also widely used in the baking industry to assist in the complexation of starch and proteins.⁹⁴ Furthermore, emulsifiers promote better dough handling properties, such as greater tolerance to mixing, resting, and fermentation time, as well as better gas retention capacity and uniform cell structure, consequently contributing to breads with greater loaf volume and better crumb structure.⁹⁰ Among the main emulsifiers used in this segment are diacetyl tartaric acid esters of monoglycerides (DATEM) and lactylates, which act as dough strengtheners.⁸⁹

SSL are lactylate esters of fatty acids and lactic acid. Their emulsifying action is due to the presence of polar and nonpolar regions, represented by the ionic chains of lactic acid and fatty acids, respectively. They interact with gluten proteins through hydrophobic bonds between nonpolar regions of the proteins and the stearic acid of SSL and ionic interactions between

charged amino acids of the proteins and the carboxylic group of SSL. The neutralization of charges promotes an increase in protein aggregation, and thus, SSL promotes a strengthening effect. DATEM are esters derived from fatty acids and mono- and diacetyl tartaric acid. Because they are ionic, they form hydrogen bonds with the amidic groups of proteins.⁸⁹

Niu et al.⁹⁵ observed that sodium stearoyl-lactylate (SSL) promoted longer development time and stability of noodle dough due to the increased strength of the gluten network. This emulsifier, due to its anionic character, could cause aggregation of gluten proteins in addition to interacting with hydrophobic regions and establishing hydrogen bonds with the amino groups of the proteins. Moreover, the interaction with amylose and amylopectin is also related to their property of reducing bread staling.⁹⁵ At low concentrations of SSL (0.25% and 0.50% w/w, flour basis), the aggregation mechanism occurs through the incorporation of a negative charge that allows interaction with the positively charged amino acids of gluten proteins. On the other hand, excess emulsifier (1% SSL w/w, flour basis) promotes different phenomena. Due to electrostatic repulsion caused by excess negative charges, protein unfolding and aggregation occur due to hydrophobic interactions.⁹⁶ On the other hand, DATEM, even at low concentrations (0.25% and 0.50% w/w, flour basis), favored the formation of hydrophobic interactions. Ionic interactions are less effective in the case of DATEM, since the hydrophilic portion of the emulsifier acts as a weak acid, being partially ionized. Hydrophobic interactions help in the formation of a structured gluten network but with a low level of aggregation, due to the lower proportion of ionic interaction. Higher concentrations of DATEM (1% w/w, flour basis) favor more intense hydrophobic interactions.⁹⁶ To date, Ajibade et al.⁹⁷ were the only ones to evaluate the effects of SSL, polysorbate 80, and DATEM treatments (in proportions of 1.0%, 1.3%, and 1.6% w/w) on the *in vitro* protein digestibility of breads. Emulsifiers contributed to the formation of complexes and networks with gluten proteins, and for this reason, a reduction in the levels of the amino acid lysine was observed. With increasing proportions of emulsifiers, protein digestibility was reduced.⁹⁷ The interactions promoted by emulsifiers apparently reduce enzymatic access and, consequently, protein digestibility. However, investigation of the effects of emulsifiers on gastrointestinal behavior, as well as their effects evaluated individually, is still scarce.

4.3.3. Enzymes. With the growing trend of “clean label” products, alternatives to the use of chemical additives have been studied and are widely used in the bakery industry. Enzymes are processing aids used to improve rheological properties, mainly in weak wheat flours with low protein content.^{86,98} To strengthen the gluten network, the use of glucose oxidase and transglutaminase (TG) can be highlighted.

Glucose oxidase acts by breaking down the glucose molecule into gluconic acid, releasing hydrogen peroxide (H₂O₂) which, in turn, will act as an oxidizing agent by oxidizing the SH groups of cysteine and promoting greater protein interactions.⁸⁷ Transglutaminase isolated from *Streptomyces mobaransensis* (microbial transglutaminase - mTG) catalyzes the acyl transfer reaction between the γ -carboxamide groups of I-glutamine and the ϵ -amino group of I-lysine, deamidating glutamine in a side reaction (Figure S2 - Supporting Information), which results in an ϵ -(γ -glutamyl)-lysine isopeptide bond resistant to the action of digestive enzymes.^{86,89,99} As a result of these reactions, there is a

reinforcement of protein interactions and the formation of more stable, dense, and resistant polymeric networks, which can interfere with the performance of digestive enzymes.¹⁰⁰

Some studies also argue that the deamidated gluten peptides generated by the action of mTG may be the same as those produced by tissue transglutaminase (tTG), an endogenous enzyme involved in CD pathogenesis.^{101,102} In this sense, Matthias et al.¹⁰² investigated the immunogenicity of mTG in individuals with and without CD. For this, the serological activity of mTG was analyzed and compared with tTG and an mTG–gliadin complex. They found that mTG showed immunogenicity in children with CD that was potentialized when mTG was complexed with gliadin. On the other hand, mTG immunoreactivity can be modified when used in a food matrix. Lerner et al.¹⁰³ also defend that the complexes formed by the cross-link between mTG and gliadin are immunogenic and are a potential public health concern. In this regard, the literature is contradictory, since some studies have shown that the use of mTG for the transamidation of wheat flour by mTG was effective in reducing the immunotoxicity of gliadin in *in vitro* assays, blocking a harmful immune response for individuals with CD.^{104,105}

Gluten deamination and digestibility were evaluated in breads made with flours added from 0 to 2000 U·kg⁻¹ of mTG, showing that the weight, volume, and specific volume of the breads tended to increase with increasing mTG concentration from 0 to 20 U·kg⁻¹. However, addition of 20 to 2000 U·kg⁻¹ had the opposite effect, indicating changes in the structure of gluten proteins within the food matrix.⁹⁹ Furthermore, gliadin extractability was reduced up to the addition of 100 U·kg⁻¹ of mTG, correlating with a reduction of bread volume. The authors associated these results with glutenin aggregation through lysine–glutamine cross-links. Although there were changes in the gluten structure, the authors did not find changes in the profile of immunogenic peptides released during *in vitro* digestion. Three hypotheses were cited to justify the observed results: (i) mTG does not deaminate the immunogenic peptides studied within a bread system; (ii) no protein–protein cross-links were formed in α -gliadin regions that contained the immunogenic peptides studied; and (iii) the observed changes in protein structure did not alter digestibility of the α -gliadins containing the analyzed peptides.⁹⁹

Although mTG, related to glutamine deamination in baking technology, is often associated with tTG, involved in CD pathogenesis, there are still few studies that address the peptide release profile and immunogenicity of mTG in food matrices.⁹⁹ Therefore, further studies are needed to evaluate the immune responses of different enzymes used in bakery products and other food matrices, since the immune response conferred by mTG may exist independently of the matrix.

Wheat-based food products are widely appreciated in many countries, but gluten consumption is known to induce adverse immune reactions in susceptible individuals. Wheat is rarely consumed without technological modifications, but the induction of such structural changes can affect gluten digestion and trigger immunological reactions. This review discussed in depth the impacts on gluten protein structure, digestibility, and immunogenicity of major traditional baking technologies. Processing generates conformational changes in the gluten network, altering the protein aggregation state, distribution throughout the macropolymer, particle size, or modulating SS–SH exchange reactions. Depending on the intensity and

conditions of the process, the structure becomes more or less accessible to digestive enzymes.

Maillard reactions, favored by high temperatures, contribute to the generation of new compounds and epitopes, which may increase the affinity of IgE antibodies related to allergic reactions. On the other hand, sourdough fermentation alters the primary structure of gluten through hydrolysis. This is one of the few treatments studied to date that are capable of reducing celiac immunogenic peptides to safe levels. However, complete degradation of epitopes is still a challenge in wheat products, since such treatments cause technological losses, reducing the baking capacity of the dough. Partial hydrolysis of wheat proteins may be an alternative for the development of hypoallergenic products for individuals at risk, maintaining functional properties, although it does not eliminate all immunogenic peptides. Finally, food additives and processing aids used to strengthen the gluten network contribute to the formation of more stable, dense, and resistant polymers that may hinder proteolysis by digestive enzymes. However, in some cases, studies are still contradictory. Some research indicates that the use of mTG in wheat-based products can generate complexes with immunogenic characteristics, similar to the triggering of the immune response in CD by the endogenous tTG enzyme. However, other studies indicate that transamidation by mTG blocks the immune response, indicating that more clinical studies should be performed to confirm the hypotheses.

Although the technological aspects are well established, the effects on digestibility and the resulting immunogenic peptide profile require further elucidation. Literature lacks studies that analyze all aspects, which are generally addressed separately. While processing may not be able to completely abolish epitopes, it is important to understand the role of different technologies, regardless of whether they increase or reduce risks. The appropriate selection of process parameters or the combined use of techniques can contribute to the development of gluten-containing products with lower immunogenicity. A better understanding of the topic is extremely important for an adequate risk assessment and exploration of possible alternatives by the food industry.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsfoodscitech.4c01007>.

Images relating to the effect of oxidizing agents and processing aids on protein interactions in the gluten network (PDF)

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