

1 **Title:** Wheat gluten: a functional protein still challenging to replace in gluten-free cereal-based foods

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7 **Abstract:**

8 Background and Objectives: Wheat gluten in cereal-based products has unique functionality derived
9 from its viscoelastic properties. Nevertheless, many food applications required its replacement to
10 obtain gluten-free foods but keeping similar quality characteristics. This review analyzes the
11 distinctive characteristics of wheat gluten, and the technological strategies implemented to mimic its
12 behavior within the gluten-free systems.

13 Findings: The viscoelastic behavior of wheat gluten is due to the interplay of glutenins and gliadins
14 after being hydrated and subjected to mechanical stress. Disulfide bonds and non-covalent
15 interactions are key in holding its structure and explaining its solubility and hydrophobicity. Gluten-
16 free flours and starches have represented the first adopted strategies for gluten replacement, but results
17 have not been completely satisfactory. To tackle this issue, non-wheat protein addition, physical
18 treatments, hydrocolloids, enzymes, and emulsifiers have allowed to recreate a pseudo gluten network
19 of the cereal-based foods.

20 Conclusions: Despite technological sensorial achievements, a gap still exists when gluten-free
21 products are compared with their wheat-based counterparts. A better comprehension about the
22 coactions of different processing aids and technologies could offer future answers.

23 Significance and Novelty: The review points out the main characteristics of the wheat gluten
24 uniqueness, shedding light on its replacement strategies to guide future research.

25 **Keywords:** wheat, starch, protein, gluten-free, hydrocolloids, enzymes

26 **1. Introduction**

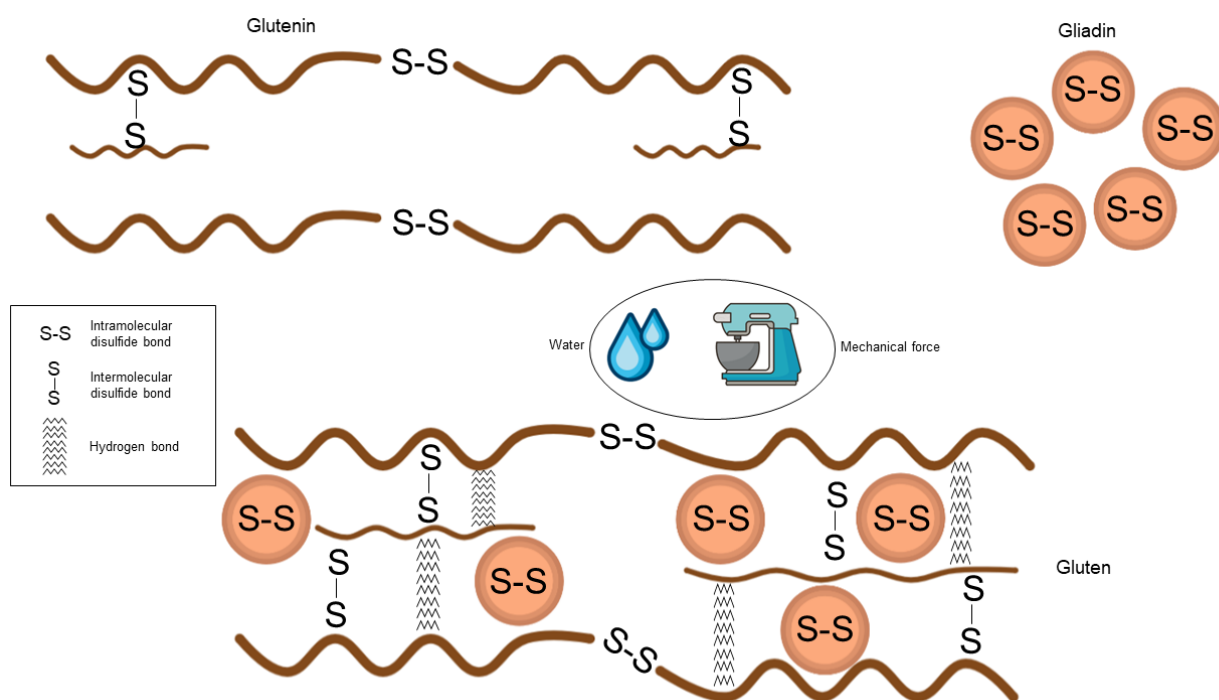
27 Going from spelt, mainly consumed during the Bronze Age, to the modern baked and extruded goods
28 of nowadays, wheat has always been a staple of mankind's diets over the millennia (Bell, 1987). But
29 it was not until the mid-eighteenth century that by kneading a wheat dough under water, Jacopo
30 Beccari observed the presence of a viscoelastic matter after starch had flowed away. In doing so, the
31 Italian chemist discovered the wheat gluten (WG) (Beccari, 1745). Since then, much research has
32 progressed with the aim of explaining and clearing up the contribution of gluten to the final quality
33 of the wheat-based products. In parallel, a quick look at the search interest relative to the word "gluten
34 free" in Google (June 2022) during the last fourteen years, confirms its great popularity (Google
35 Trends, 2022). That reveals the increasing interest in finding gluten substitutes that would imitate its
36 role within the food matrices and optimizing process strategies to create WG-like structures. During
37 the last two decades, new unconventional GF flours, physical processes and processing aids have
38 been explored with the goal of getting as close as possible to the WG-containing products, especially
39 in terms of texture and sensory features. Owing to that, much scientific knowledge has been generated
40 regarding the WG and its potential substitutes, while the grocery stores shelves have been filled with
41 new GF products. This review analyzes the distinctive characteristics of WG, and the technological
42 strategies implemented to mimic its behavior within the GF systems.

43 **2. Wheat gluten performance in the main food technologies**

44 **2.1 Wheat gluten chemistry**

45 Gluten has been the focus of many researchers trying to understand its unique physical properties.
46 Focusing on the protein content of wheat (*Triticum aestivum*), around 70-80% represent the storage
47 proteins (Wieser et al., 2022), that when subjected to hydration and mechanical energy give rise to
48 the WG. They contain high number of non-polar amino acids and glutamine residues with a high
49 hydrogen-bonding potential, and low content of charged side groups, which explain their poor
50 solubility in water or dilute salt solution (Veraverbeke & Delcour, 2002). WG has been separated by

sodium dodecyl sulfate-polyacrylamide gel electrophoresis into two main fractions, monomeric gliadins and glutenins, which have higher molecular weight (MacRitchie et al., 1990; Kasarda, 1989). Fractions from the first group are soluble in alcohol-water solutions (60% ethanol) while, the second ones have proven more hydrophobicity (Schmid et al., 2016). In a dough-system, gliadins provide viscosity and extensibility, whereas strength and elasticity are dependent on glutenins. WG could be seen as “two-components glue” in which gliadins act as plasticizer for glutenins (Wieser, 2007). The functionality of this protein depends on the balance of these two components. Cysteine residues are pivotal for the creation of a three-dimensional structure (Grosch & Wieser, 1999; Shewry & Tatham, 1997). In fact, they can be found as free sulfhydryl or can participate in the formation of disulfide bonds within the same polypeptide (intra-chain disulfide bonds) or between different polypeptides (inter-chain disulfide bonds) (Fig.1) (Shewry et al., 1986). Additional covalent bonds (tyrosine-tyrosine crosslinks) between WG protein and tyrosine-dehydroferulic acid of arabinoxylans contribute to the WG network formation (Tilley et al., 2001; Piber & Koehler, 2005). Non-covalent bonds, such as hydrogen bonds, ionic bonds, hydrophobic bonds also contribute to the structure (Wieser, 2007). Large consensus has been obtained by the “loop and train” model proposed by Belton (1999) (Fig.1). According to the author, the glutenin elasticity is mainly caused by hydrogen bonds occurring between and within its chains. Specifically, protein-protein interactions (interchain hydrogen bonding) are considered trains, while loops are referred to some zones generated by the hydrogen bonds between water and glutamine. With the hydration increase, more loops regions are developed. The application of low extension produces a double effect, firstly it stretches the loops out and after unpacks the trains zone. When additional extension is applied, protein-protein interactions increase, chains become stiffer, and loops disappear (Belton, 1999). After removing the extension, polymer relaxes and loops can be formed again (Jekle & Becker, 2015).



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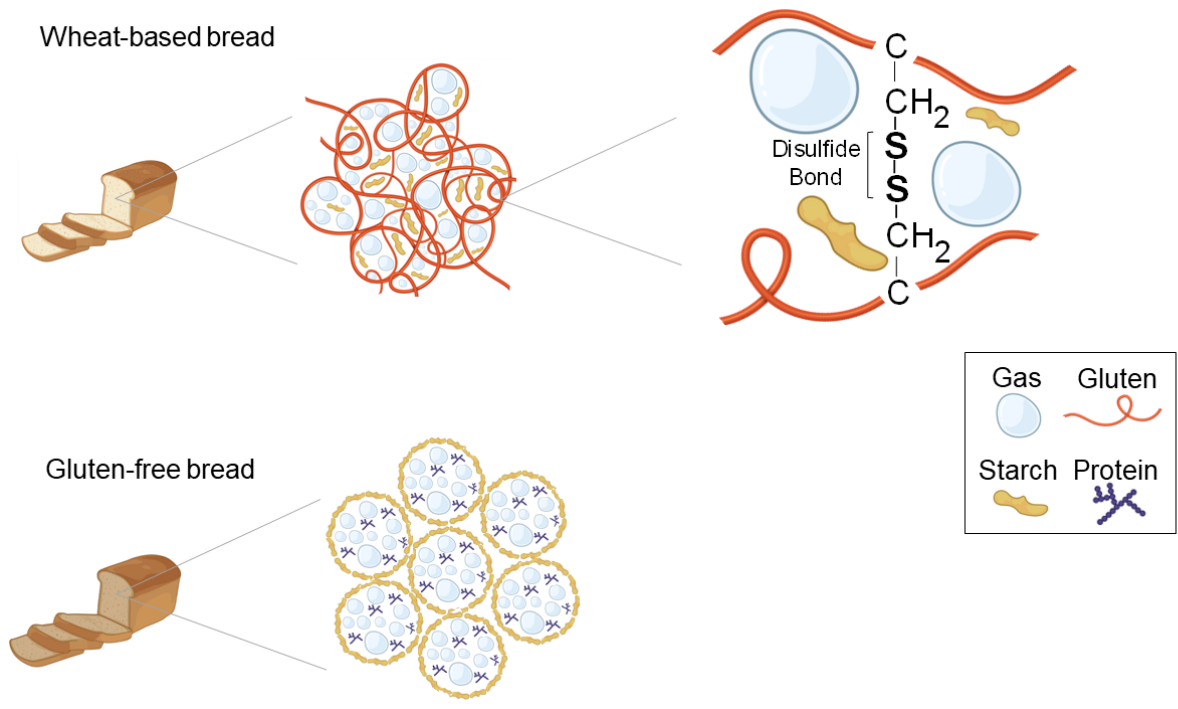
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76 Fig. 1. The development of gluten network according to Shewry et al. (1986), Belton (1999) and
 77 Veraverbeke & Delcour (2002).

78 2.2 Wheat gluten in breadmaking

79 Bread loaves with high volume and well-defined aerated crumb always embody the gold standard in
 80 terms of product acceptability. Apart from starch, which leads a key role in crumb formation, WG
 81 network is required, throughout the process, to ensure the creation of the desired product. To increase
 82 the interactions between proteins, WG polymers must be in their rubber-like state (above their glass
 83 transition temperature). Usually, glass transition temperature is reduced with the moisture content
 84 increase (Toufeili & Kokini, 2004). At room conditions, dry WG (moisture content < 20%) is on its
 85 glassy state below its glass transition temperature; as hydration increases, WG gets fully hydrated
 86 (moisture content > 35%), assuming the typical aspect of a rubbery viscoelastic mass (Toufeili et al.,
 87 2002). In operating circumstances, precisely during mixing and kneading, water acts as plasticizer,
 88 increasing the interaction rate among WG chains, while shear contributes to give strength to the dough

89 system. In fact, some studies have highlighted the importance of different parameters, such as mixing
 90 time, speed and quantity of applied mechanical energy for the ideal development degree of the WG
 91 network (Baudouin et al., 2020). Because of that, during dough formation, over-mixing conditions
 92 would cause a disulfide bond breaking with losses in elasticity, whereas poorly developed WG
 93 network with sticky dough would occur in under-mixing conditions. During proofing step, tiny air
 94 pockets, already formed during the mixing stage, become filled with carbon dioxide produced by the
 95 yeasts. The continuous bubble size growth reduces the thickness of their starch-WG walls, generating
 96 coalescence phenomena (Hayman et al., 1998). Uncontrolled bubble volume increase would lead to
 97 a loss of gas retention with consequent reduced volume and coarse crumb after baking (Grenier et al.,
 98 2021). Above 60°C, water loss, WG denaturation and especially the heat-induced crosslinking
 99 contribute to make stiffer the network until its rupture, occurring at normal baking temperatures
 100 (Rosell et al., 2013). Unquestionably, WG is fundamental for baking performances of wheat flours
 101 and any action on WG has direct impact on doughs. A good balance between elasticity and viscosity
 102 is of primary importance to allow higher gas retentions and breads with more attractive features (Fig.
 103 2).



105 Fig. 2. Structural differences between wheat-based and gluten-free breads

106 **2.3 Wheat gluten in pasta and noodles making**

107 A high visco-elasticity network is essential for the optimal development of pasta and noodles with
108 superior quality. Usually, products with high firmness, lack of stickiness and minimal cooking solid
109 losses are guaranteed by the presence of a strong WG network. Pasta manufacturing involves the use
110 of durum wheat semolina and water that are subjected to various steps such as, hydration, mixing,
111 forming, and drying before the home cooking (Pagani et al., 1986). Extrusion cooking is the main
112 technology utilized by the food industry to produce the major types of dried pasta. Following the
113 water addition, kneading and extrusion, starch becomes increasingly hydrated while WG starts
114 unfolding. In these stages, hydrated WG mass is stabilized by weak hydrogen, ionic, as well as
115 hydrophobic bonds, forming a continuous network that enclose the starch granules (Guerrero et al.,
116 2014). Usually, during these steps, temperature is kept below 50°C as a way of protecting WG and
117 starch from any heating-related damage. Notably, disulfide/sulphydryl exchange reactions occur at
118 temperatures beyond 55°C for glutenins and 70°C for gliadins. These phenomena take place mostly
119 during the drying steps, in which WG network is reticulated (protein solubilization) resulting pivotal
120 in limiting starch swelling throughout the next cooking stage (Lamacchia et al., 2007). Martin et al.
121 (2019) highlighted the role of high temperatures during drying, promoting higher number of covalent
122 bonds that improve the cooking performance of the pasta. During this last stage, managed by the final
123 consumer, a strong water competition between starch and protein occurs (Cubadda et al., 2007) (Fig.
124 3). Boiling water allows starch gelatinization and WG coagulation, and higher pasta firmness is
125 obtained when protein interactions prevail over the starch gelatinization. By doing so, WG network
126 traps the starch granules, preventing their spill and the associated final adhesiveness.

127 The importance of a fully developed and visco-elastic WG network in pasta goods is also reflected in
128 noodles making. In fact, Yao et al. (2020) reported that the hardness of wheat starch-based sheeted
129 noodles improved after wheat WG addition (14%). Definitively, by controlling starch swelling and

gelatinization along with the formation of a strong network, WG is of a great significance in pasta and noodles manufacturing, helping to prevent solids release during cooking and sticky texture in the end-products (Fig. 3).

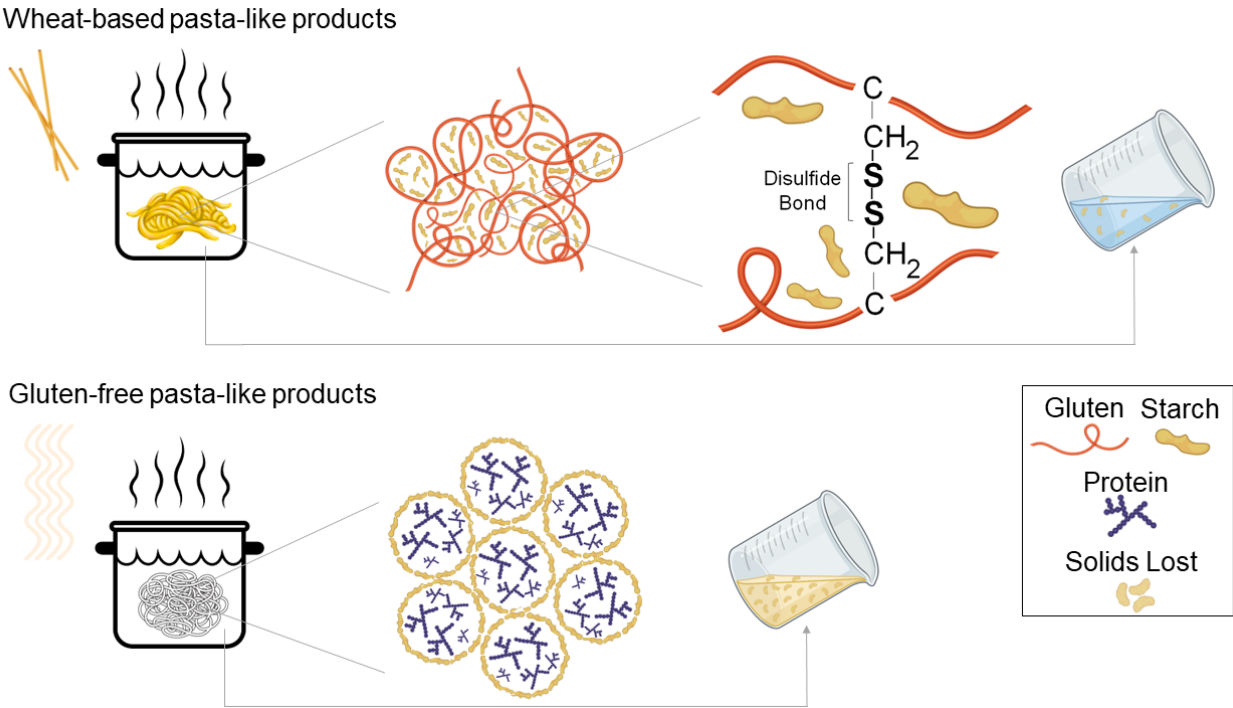


Fig. 3. Structural differences between wheat-based and gluten-free pasta-like products.

3. Strategies for wheat gluten replacement

In the previous sections, the importance of WG in guaranteeing certain technological characteristics has been pointed out. The necessity of producing GF foods has prompted much effort both in academia and food industry. Different strategies have been reported during the last thirty years with the main purpose of substituting WG to create GF food products with similar characteristics of those wheat-based ones. In fact, using “gluten replacement” or “gluten substitution” as retrieval keywords in the Food Science Technology category of Web of Science™ scientific database, five hundred ninety-five documents came up from 1991 to 2021. Keywords co-occurrence network displayed by Fig. 4 was obtained through VOSviewer version 1.6.18 after picking the first one hundred keywords with an occurrence frequency greater than eight. The analysis of the keywords found in the retrieved

160 Fig. 4. Keywords co-occurrence map of “gluten replacement” or “gluten substitution” in Web of
161 Science™.

162 3.1 Gluten-free flours and starches

163 To tackle the rising demand for GF product, naturally GF flours was one of the early approaches and
164 extensive literature has been published proposing different GF recipes. Only fundamental concepts
165 have been selected in relation with the functional replacement of gluten. Rice, maize, soy, pulses and
166 pseudocereals have constituted the main raw materials for bakery and pasta-like GF products. Particle
167 size of the flours is crucial for GF breadmaking. According to De la Hera et al. (2013), GF breads
168 made with coarser maize flours (up to 106 μm) had higher volumes, whereas finer flours ($<106 \mu\text{m}$)
169 showed lower dough development capacity; authors also reported that G''/G' was positively correlated
170 with starch content and negatively with protein. Despite the existence of that correlation, no
171 fundamental studies have been pursued to explore the chemistry and functionality of those flours’
172 proteins and how to improve it. The potential role of the hydrophobicity and hydrophilicity of those
173 proteins have been pointed out (Renzetti, & Arendt, 2009; Renzetti & Rosell, 2016), but other
174 properties like glass transition temperature and the extent of energy contribution in creating a network
175 remain unexplored. Considering the existing knowledge about gluten proteins interactions, focus on
176 other plant-based proteins, and to promote protein-protein interactions besides their performance
177 when physical constraints (energy strain, temperature, pH) have been neglected. Oftentimes, blending
178 GF flours with starches ensures higher volume and better texture to GF breads (Roman et al., 2019),
179 as well as improved firmness to GF pasta-like products (Gao et al., 2018). In flours, their larger and
180 more compact particles kept the integrity during kneading, resulting more resistant to the mechanical
181 stress and producing lower expansion. From the other hand, the lower consistency but higher gas
182 holding capacity and volume expansion observed in starch-based GF breads are related with their
183 different morphology, water absorption capacity and pasting temperature that determine the
184 interaction of the starch granules within the matrix (Martínez & Gómez, 2017). Similarly, GF pasta-

185 like products have extensively adopted starches, utilizing the knowledge based on some oriental
186 practices for starch noodles making.

187 In GF breadmaking, the amylose content of the flour has been an issue for debate. Baking
188 performances of GF breads made from long-grain rice flours were influenced mostly by water binding
189 capacity, swelling power, swelling volume and thermal properties like gelatinization temperature
190 (Cornejo & Rosell, 2015). From the other hand, Yano et al. (2020) remarked that the expansion ratio
191 of rice flour-based GF breads was amylose content-dependent; the greater amylose content the better
192 expansion, as well as batter viscosity and the maintenance of its foamy structure during proofing. In
193 GF pasta-making, high-amylose rice varieties are sought, because of their better structuring properties
194 that guarantee superior performances during cooking (Marti & Pagani, 2013). In fact, amylose chains
195 limit the swelling of granules during heating, promoting molecular reassociation upon cooling. Some
196 authors stressed the role of amylose in gel formation; in fact, significant positive correlations were
197 found between gel (formed at 95-140°C), hardness and amylose content in starches from maize,
198 potato and pea (Liu et al., 2019). Indeed, the capacity of high amylose starches in reassociating with
199 each other and forming harder gels, seems to be key for a development of stronger network that reduce
200 cooking loss and help to achieve GF pasta-like products with higher firmness.

201 Also the role of naturally present lipids in pseudocereal's flours have been pointed out for their role
202 in the gas cell stabilization before the starch gelatinization or even the protein aggregation upon
203 heating that could create stronger protein network (Alvarez-Jubete et al., 2009). Likewise, amino
204 acids composition of pulse flours has been related to the high foam expansion and stability, and in
205 consequence high bread volume and crumb softness obtained with chickpea flour (10%) blended with
206 rice flours and starches (maize and cassava) (Collar et al., 2015). Presumably, amino acids contained
207 in chickpea might form an interfacial layer that keep the air bubble in suspension with a consequent
208 formation of a well-defined structure (Miñarro et al., 2012).

209 By summing up, GF matrix with lower consistency have been produced to hold a significant amount
210 of carbon dioxide during proofing and to achieve higher volumes loaves, as opposed to certain GF
211 pasta-like products, in which stronger structure with high capacity to retrograde is needed to assure
212 better performances during cooking and final firmer textures. Research has been focused on flours
213 properties and their starch functionality, assigning to starch the structuring role in the GF
214 dough/batter. Structure, amylopectin/amylose ratio and granules morphology of starch are pivotal in
215 determining water absorption and pasting behavior, which underlie the rheological properties.
216 However, additional knowledge about the nature of the proteins in gluten free flours would allow
217 defining the process conditions that could promote proteins interactions and viscoelastic properties.
218 Currently the same GF breadmaking conditions are applied independently on the flours origin and
219 their composition, but likely the distinctive characteristics of their constituents could show different
220 performance under adapted process settings. More knowledge about the interactions among proteins
221 and starch chains, and other macromolecules in GF system, as well as the influence of temperature,
222 pressure, pH and ionic concentration is needed to clarify the relationship between process conditions,
223 ingredients and final product quality.

224 **3.2 Non-wheat proteins**

225 Initially, the inclusion of proteins in GF foods was studied solely for nutritional enrichment purposes,
226 and only later on its functionality as network forming agent was considered (Horstmann et al., 2017).
227 Non-gluten proteins functionality has been tested in rice flour-based cake batters observing lower
228 density and higher viscosity after the addition of pea protein concentrate, egg white powder and whey
229 protein isolate (15, 30 and 45%). Both animal proteins incorporation dramatically increased hardness
230 and final volume, while the vegetables ones reduced the cohesiveness (Sahagún et al., 2018). Egg
231 white and casein proteins (13%) led to rice based batters with a stronger network. Likely, due to their
232 capacity in reducing surface tension, better stabilization of the foam at gas-liquid interface is attained,
233 retaining more air within the structure of rice flour GF muffins (Matos et al., 2014).

234 In the GF pasta field, liquid egg albumen (15%), replacing rice flour, originated a stronger network,
235 stabilized by hydrophobic interactions and disulfide bonds that was accountable for the better
236 appearance and texture of the final GF macaroni (Marti et al., 2014). Encouraging results were
237 reported after the incorporation of rice protein concentrate (up to 10%) for rice flour spaghetti
238 manufacture, in particular cooking time, cooking loss and firmness were reduced while stickiness
239 increased. However, the cooking performances and the textural features, as well as the overall
240 acceptability of the GF samples were quite far from that of wheat control (Detchewa et al., 2022).

241 Soybean protein has been widely utilized in many food applications because of their well-known
242 emulsification, foaming, gelation, and water-fat absorption properties. These qualities are favored by
243 its two major protein fractions, glycinin (11S globulin) and β -conglycinin (7S globulin). The first one
244 enable to develop harder gels owing to its higher content of thiols groups. The second one, β -
245 conglycinin, has more foldable structure and higher hydrophobicity and emulsifying ability
246 (Fukushima, 2011), which makes it potentially useful for GF breadmaking. In fact, no significant
247 differences were reported about the textural properties of rice flour-based GF breads containing 10%
248 of vital WG and those made with β -conglycinin (10%) (Espinosa-Ramirez et al., 2018). Scanning
249 Electron Microscope micrographs remarked the similarities of both protein enriched matrices; but β -
250 conglycinin samples were characterized by a higher number of larger pores and thinner lamellae,
251 confirming its suitability in strengthening the protein-starch network for a better carbon dioxide
252 holding during proofing and baking (Espinosa-Ramirez et al., 2018).

253 Overall, the addition of different protein fractions can be a valid approach for the technological
254 challenge of recreating the WG network, particularly after their isolation and characterization as it
255 has been done with soybean proteins. Stabilization of the protein network (mostly via hydrophobic
256 interactions and disulfide bonds) allows improving the cooking performance of GF pasta-like
257 products and increase the gas hold retention in GF bread making. This is acceptable when the
258 comparison is made with samples solely prepared by GF flours, but when it moves towards wheat-

259 based models, the quality gap is still in there. The WG polymerization mechanism has been widely
260 discussed but regarding non-wheat proteins, very little has been made so far. The understanding of
261 their amino acid composition, identifying those sulfur-rich that have proven to be pivotal in the
262 creation of disulfide bonds, could give more valuable information for the understanding of the
263 network creation. By simulating the main process conditions, the study of the protein structure
264 changes and the different interactions among the different protein chains with starch and lipids could
265 give more insights about the key points and limiting factors involved in the creation of a pseudo-WG
266 network.

267 **3.3 Physical treatments**

268 The important role of starch in GF food matrices is driven primarily by its functional changes
269 occurring during food products processing. In fact, in its native state, starch is insoluble in water,
270 while in excess of water, and under heating conditions it swells and gelatinizes giving viscosity and
271 then retrogrades during the cooling stage. Physical treatments of GF flours and starches have been
272 implemented for technological purposes to bring GF products closer to those WG containing.
273 Heating, pre-gelatinization, annealing and high-moisture treatment have been the most explored ones
274 (Iuga & Mironeasa, 2020). Starch is the principal target of these types of treatments that, by changing
275 its native structure, promote interactions between the chains, generating end-products with changed
276 features in terms of crystallinity, water absorption capacity and pasting properties (Shi et al., 2018).
277 Heating starch suspension above the onset temperature represents the key step in the pre-
278 gelatinization treatment. For instance, the incorporation of 30% of pre-gelatinized maize flour
279 followed by sheeting provides stronger and more cohesive dough but with low extensibility
280 (Khuzwayo et al., 2020). GF breads with lower crumb hardness and chewiness were obtained from
281 doughs with low elasticity and improved resistance to deformation after adding pre-gelatinized
282 cassava starch (10%) to jasmine rice flour (Pongjaruvat et al., 2014). Nevertheless, by blending pre-
283 gelatinized *Colocasia* spp. Cormels' flour (50%) with its raw counterpart caused higher losses during

284 baking and the final GF breads showed lower expansion with higher, hardness, cohesiveness,
285 chewiness, resilience, and springiness (Calle et al., 2020). Therefore, despite knowing starch
286 gelatinization process, differences among starch sources make necessary adapted treatments and
287 processes, which have not sufficiently explored.

288 Concerning GF pasta making, pre-gelatinizing GF flours is the most common process currently
289 adopted by the manufacturers because of it does not require major changes in the production process
290 and the same press before extrusion could be used. Yalcin & Basman (2008) reported the effect of
291 the pre-gelatinization in rice noodles also highlighting the importance of the gelatinization level; in
292 fact, lower cooking losses and better sensory properties were found in samples with a 25% of
293 gelatinization level as opposed to those with 15, 20 and 30%, respectively. The application of heating
294 above the glass transition temperature but below the gelatinization one usually applies to heat
295 moisture treatment in low-moisture condition ($\leq 35\%$ w/w) and annealing in intermediate/excess of
296 water content (from 40 up to 65%) (Jacobs & Delcour, 1998). Treated glutinous rice flour at 90°C for
297 30 min at powder to water ratio of 3:7 (w:w) was employed for GF noodles production; dough resulted
298 softer and more extensible, while the final samples showed very fragile structure compared to those
299 made from wheat flour (Cai et al., 2016). For the technological improvement of GF breads, rice and
300 corn slurries, previously gelatinized, have been successfully used as improver, obtaining GF breads
301 with higher volume, less chewiness and softer crumb (Bourekoua et al., 2016).

302 As part of physical treatments, low-pressure homogenization has recently been applied with
303 interesting results in GF breadmaking. Boulemkahel et al. (2022) reported that long/medium grain
304 rice flours showed reduced particle size and an increased content of damaged starch after being
305 homogenized at low pressure (30 MPa, 3 passes). The higher level of damaged starch might have
306 contributed to increase the gas production during proofing with consequent higher specific volume
307 of the samples after baking; moreover, lower hardness, as well as higher cohesiveness and resilience
308 were found in the GF breads after the incorporation of the homogenized flours.

309 The aforementioned physical treatments, as well as others like cold plasma, microwave, electric
310 pulses, extrusion and so on, can contribute to the improvement of GF breads and pasta-like products,
311 but more efficient process must be developed to avoid that energy requirements for these technologies
312 limit their applicability. As a future step, physical treatments could be valuable allies to favor green
313 labelling in GF food products, making the ingredient lists shorter. However, more insights are needed
314 to characterize the microstructure changes of the food biopolymers, so as to identify the most effective
315 physical treatment to apply. **3.4 Hydrocolloids and gums**

316 This category probably encompasses the most utilized processing aids in GF formulations.
317 Hydrocolloids are a macro group of different water-soluble polysaccharides with several chemical
318 structure that determine their functional properties (Zoghi et al., 2021). The functionality of these
319 macromolecules can vary depending on the temperature of the system in which they are acting (Mir
320 et al., 2016). For instance, carboxymethylcellulose, guar and xanthan gums are soluble in cold water,
321 whereas carrageenan, locust bean gum and various alginates exert their action in hot water (Mir et
322 al., 2016). The ability to hold water of those hydrocolloids/gums competing with the starches during
323 gelatinization and their interactions with them might be responsible of the results obtained with the
324 extensive number of scientific papers describing specific recipes containing hydrocolloids (McCarthy
325 et al., 2005; Rosell et al., 2001; Padalino et al., 2013). Through their hydrophilic long-chains
326 molecules, hydrocolloids can control the rheology of aqueous systems; specifically, they firstly
327 interact with water molecules via hydrogen bonds, incorporating them in the inter-molecular or intra-
328 molecular voids of their three-dimensional structure (Salehi, 2019). GF formulations require higher
329 amount of water to develop an essential viscosity, as opposed to their wheat-based counterparts that
330 need less water, however, their effect is greatly dependent on the pair of starch/flour and the specific
331 hydrocolloid (Rosell et al., 2011). In this regard, Morreale et al. (2018) pointed out the key role of
332 hydration level (90, 100 and 110%) in defining the viscoelastic behavior of GF batter based on rice
333 flour and hydroxypropylmethylcellulose (1, 2 and 3%) with different viscosities. According to
334 findings, GF breads with better texture features were obtained by adding 2% of high viscosity HPMC

335 (15,000 mPa.s) and applying a hydration of 110%. In GF noodles based on tiger nut powder,
336 hydrocolloids type (xanthan gum, guar gum and carboxymethylcellulose) and hydration level
337 (constant and adjusted on the mix requirement) significantly affected dough thermomechanical
338 properties, cooking performances and final texture. Samples with adapted hydration (24%) and
339 xanthan gum (0.5% w/w) showed the lowest value of cooking loss and higher firmness (Gasparre &
340 Rosell, 2019). Nevertheless, in fermented rice noodles, xanthan gum incorporation (up to 0.1%) did
341 not show any significant effect in terms of final firmness, as compared with guar gum (up to 0.1%)
342 samples that obtained the highest values (Srikaeo et al., 2018).

343 The development of a three-dimensional structure, which ensures GF end-products with higher
344 quality is attributable to the structure of the hydrocolloids. In this framework, the high presence of
345 hydroxyl groups promises more interaction via hydrogen bonds with water molecules, giving to the
346 GF system an increased capability of binding water. In practice the result is an enhancement of the
347 structure viscosity, which is also involved in reducing phase separation and keeping the foamy
348 formation entire. Still, the complete comprehension of their structures, their higher water
349 requirements, the interactions between them and different raw materials, as well as the role of certain
350 processing variables are worthy of further elucidations.

351 **3.5 Enzymes**

352 Different enzymes, promoting linkages within proteins, have been considered for the creation of a
353 three-dimensional network as close as possible to that WG-based. Remarkable results have been
354 reported about the effect of some cross-linking enzymes, such as glucose oxidase (indirect cross-
355 linking) and transglutaminases (direct cross-linking). After the conversion of glucose to gluconic acid
356 operated by the glucose oxidase, the generated hydrogen peroxide interacts with the thiol groups of
357 the WG, with consequent creation of more disulfide bonds and gelation of water-soluble pentosans
358 that modify the rheology of the dough system (Gujral & Rosell, 2004a; Ebling et al., 2022). This
359 tendency was also described when rice flour was used for GF breadmaking, in fact, the addition of

360 glucose oxidase (up to 0.03%) caused a decrease of the free sulfhydryl groups (Gujral & Rosell,
361 2004a). This reduction reflected the development of new disulfide bonds that improved the dough
362 consistency, the elastic and viscous moduli, creating samples with greater volumes (Gujral & Rosell,
363 2004a). By catalyzing the cross-linking between glutamine and lysine protein residues,
364 transglutaminase is accountable for the formation of valuable protein network within the GF matrices
365 (Moore et al., 2006; Marco et al., 2008). Increasing quantities of transglutaminase (0.5, 1.0 or 1.5%
366 w/w) improve viscous (G'') and elastic (G') moduli of GF rice flour batter; and 1% of transglutaminase
367 provides the highest bread volume and softest crumb (Gujral & Rosell, 2004b). Same increase of G''
368 and G' was reported by Kim et al. (2014) after the addition of 1% (w/w) of transglutaminase to rice
369 flour alone or blended to 10% (w/w) of rice protein isolate for the GF sheeted noodles. However, GF
370 fava bean pasta treated with transglutaminase (20 nkat/g flour dm) showed higher cooking loss and
371 reduced water absorption compared to durum wheat semolina pasta, but in terms of cohesiveness,
372 resilience, adhesiveness, no significant differences were found (Rosa-Sibakov et al., 2016). Also the
373 implementation of proteases has allowed the achievement of attracting outcomes. Renzetti & Arendt
374 (2009) stated that brown rice breads with increased specific volume, as well as lower crumb hardness
375 and chewiness were obtained by utilizing protease (up to 0.01%). According to the authors, the
376 improved breadmaking performances depended on the reduction of complex modulus and initial
377 consistency, as well as a diminished peak viscosity actuated by the proteases, but impact with
378 proteases is highly dependent on the type or protease and the flour nature (Hamada et al., 2013).

379 To sum up, enzymatic technology has resulted compelling in the context of mimicking the WG
380 behavior. As a matter of fact, the higher elasticity and resistance to deformation, as well as the
381 continuity of the protein complex promoted by the cross-linking enzymes are key in a GF system, but
382 more research is needed to elucidate the role of the enzymatic dosage and the function of the protein
383 source. In addition, more attention should be paid to the polymerization degree after the enzymatic
384 treatment; higher levels of protein aggregation may lead to a stiff system most prone to breakage and
385 incapable to retain gas during proofing and baking. Worthy of attention in GF products manufacturing

386 is the similarity of the active site of microbial transglutaminase to that of the tissue transglutaminase,
387 because it could provoke immunoreactivity in celiac patients (Matthias et al., 2016). On the other
388 side, the proteolytic activity, carried out by the proteases, releases low molecular weight proteins that
389 would interlink with starch, forming a fine network which is responsible for the changes of the
390 rheological behavior and pasting properties. These modifications seem to be related to greater batter
391 deformability that would stabilize the gas cell walls, preventing their early rupture during proofing
392 and oven spring with consequent higher expansion after baking (Renzetti & Rosell, 2016). The great
393 variability of the raw materials and the different classes of enzymes utilized in the GF technology
394 avoid to make general statements and make the scaling up a tough challenge. Future directions should
395 integrate a holistic approach to optimize the enzymatic concentrations and the process conditions,
396 with special emphasis on the synergy and antagonism among enzymes in food matrices.

397 **3.6 Emulsifiers**

398 Different types of emulsifiers have found application in GF products development, appearing as a
399 feasible path for the WG replacement (Nunes et al., 2009; Gasparre et al., 2019). In GF bread making
400 they act as gas bubbles stabilizers during proofing (Matos & Rosell, 2015), while in extrusion they
401 behave as lubricants and by regulating starch swelling and amylose leaching during cooking, they
402 increase firmness and reduce adhesiveness in GF pasta-like products. Nevertheless, given the diverse
403 chemical structure of the emulsifier compounds, functionality must be checked in the specific GF
404 system. The addition of emulsifiers might favor new interactions increasing dynamic moduli (G' and
405 G'') (Sciarini et al., 2010). Nevertheless, emulsifiers contribution is not sufficient to resembling wheat
406 pasta in terms of elasticity and sensory properties (Schoenlechner et al., 2010). Their interactions with
407 starch, especially with amylose chains is of critical importance not only for the technological features,
408 but also regarding the shelf-life of baked GF products. In the case of pasta-like goods, water
409 distribution is affected by the amphiphilic nature of these compounds and consequently more
410 knowledge is required to clarify their effect during the drying steps.

411 **4. Conclusion remarks and future trends**

412 Gluten development is essential for imparting desirable qualities to the cereal-based products like
413 bread or pasta. The creation of non-covalent interactions and disulfide bonds, between gliadins and
414 glutenins are pivotal in building an elastic network. The three-dimensional structure of the wheat
415 gluten network assures the essential gas retention required for expanded and spongy loaves, while in
416 pasta-like products it helps to prevent solid loss during cooking, guaranteeing final higher firmness
417 and less stickiness. If wheat gluten is lacking, the dough system losses extensibility and elasticity
418 with consequent deterioration of the food product sensory properties. This is the case of the gluten-
419 free food products that have been at the center of numerous technological advances in the twenty
420 years. In fact, their basic formulations, mainly flours and/or starches from different sources are not
421 able to replicate the wheat-based food products features. Protein addition, physical treatments,
422 enzymatic technology, hydrocolloids and emulsifiers have been successfully applied to generate a
423 pseudo wheat gluten network. This progression has improved the gluten-free products, but the
424 comparison with their wheat-based counterparts still highlights deep differences in terms of
425 appearance, texture and sensory properties. One potential path for the future, therefore, might be
426 deepening the understanding of the synergies between the adoption of different physical technologies
427 and various processing aids. In addition to that, a greater deepening about the role of non-gluten
428 protein polymerization, in defining the quality of GF end products is needed for a better understanding
429 of the weight of all the existing variables in a complex GF system. The optimization gluten-free
430 formulations could also come from the search for raw materials already containing those functional
431 ingredients, which have proven to be fundamental in building a three-dimensional network. In this
432 regard, minor cereals, such as wild rice, fonio, teosinte and canary seeds have been little explored.
433 Powders from acorn and carob have shown a good potential in GF food technology but their presence
434 in the market is still limited. By utilizing oilseeds by-products (rapeseed and sunflower), which
435 contain sulfur-rich amino acids not only could be a valuable strategy under a technological and

436 nutritional standpoint, but also can help the shift towards a more sustainable production in accordance
437 with the circular economy principles.

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