

Advances in quantification and analysis of the celiac-related immunogenic potential of gluten

Miguel Ribeiro^{1,2,3}  | Telma de Sousa^{1,2,3}  | Carolina Sabença^{1,2,3}  |
 Patrícia Poeta^{3,4}  | Ana Sofia Bagulho⁵  | Gilberto Igrejas^{1,2,3} 

¹ Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

² Functional Genomics and Proteomics Unity, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

³ LAQV-REQUIMTE, Faculty of Science and Technology, University Nova of Lisbon, Caparica, Lisbon, Portugal

⁴ Microbiology and Antibiotic Resistance Team (MicroART), Department of Veterinary Sciences, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal

⁵ National Institute for Agrarian and Veterinarian Research, Elvas, Portugal

Correspondence

Gilberto Igrejas, Department of Genetics and Biotechnology, University of Trás-os-Montes and Alto Douro, 5000-801, Vila Real, Portugal.

Email: gigrejas@utad.pt

Abstract

Gluten-free products have emerged in response to the increasing prevalence of gluten-related disorders, namely celiac disease. Therefore, the quantification of gluten in products intended for consumption by individuals who may suffer from these pathologies must be accurate and reproducible, in a way that allows their proper labeling and protects the health of consumers. Immunochemical methods have been the methods of choice for quantifying gluten, and several kits are commercially available. Nevertheless, they still face problems such as the initial extraction of gluten in complex matrices or the use of a standardized reference material to validate the results. Lately, other methodologies relying mostly on mass spectrometry-based techniques have been explored, and that may allow, in addition to quantitative analysis, the characterization of gluten proteins. On the other hand, although the level of 20 mg/kg of gluten detected by these methods is sufficient for a product to be considered gluten-free, its immunogenic potential for celiac patients has not been clinically validated. In this sense, in vitro and in vivo models, such as the organoid technology applied in gut-on-chip devices and the transgenic humanized mouse models, respectively, are being developed for investigating both the gluten-induced pathogenesis and the treatment of celiac disease. Due to the ubiquitous nature of gluten in the food industry, as well as the increased prevalence of gluten-related disorders, here we intend to summarize the available methods for gluten quantification in food matrices and for the evaluation of its immunogenic potential concerning the development of novel therapies for celiac disease to highlight active research and discuss knowledge gaps and current challenges in this field.

KEYWORDS

celiac disease, gluten, immunochemical methods, in vitro models, in vivo models, proteomics

1 | INTRODUCTION

Wheat is one of the oldest and most cultivated cereals in the world and occupies an essential space in human's diet. One of the decisive factors for the success of this cereal is the fact that it contains a group of storage proteins with

viscoelastic properties, the gluten, which makes it possible to transform wheat into various foods, including common bakery and pastry products (Ribeiro, Nunes-Miranda, et al., 2013). In turn, gluten is not a single protein but a complex mixture of two types of proteins, gliadins and glutenins. As the main characteristics, gliadins (28–55 kDa)

are mostly monomeric, establishing intramolecular disulfide bonds (Wieser, 2007). They are soluble in alcohol solutions and can be divided into α -, β -, γ -, and ω -gliadins based on their mobility in acid-polyacrylamide gel electrophoresis (A-PAGE) and lately in ω 5-, ω 1,2-, α/β -, and γ -gliadins by reversed-phase high-performance liquid chromatography (RP-HPLC) (Wieser et al., 1994). In rheological terms, they mainly provide viscosity to the wheat dough, functioning as a lubricant for the large polymeric network established by glutenins (Uthayakumaran & Wrigley, 2017). The latter, with a higher molecular weight, are mainly divided by this factor into high molecular weight glutenins (HMW-GS; 70–90 kDa) and low molecular weight glutenins (LMW-GS; 30–45 kDa). They establish intra- and intermolecular disulfide bonds that are decisive for the formation of the glutenin macropolymer, which is correlated with the strength of the dough (Ribeiro, Bancel, et al., 2013; Uthayakumaran & Wrigley, 2017). Glutenins are soluble in dilute acid or alkali solutions, or with the help of reducing agents to break the disulfide bonds (Wieser, 2007).

The amino acid composition of gluten proteins is dominated by glutamine (>30%) and proline (>10%) residues. In particular, proline residues confer some resistance to gluten proteins to proteolysis by digestive enzymes in the gastrointestinal tract (Shan et al., 2002). Unlike other proteins commonly found in our diets, they can be absorbed in an almost structurally intact form, which contributes to the disease-inducing properties of gluten (Mittag et al., 2004). The best known and studied example is given by 33-mer peptide. A peptide derived from α -gliadins that is resistant to gastric/pancreatic digestion and is believed to have a crucial role in the pathogenesis of the celiac disease (Shan et al., 2005). In celiac disease, the partially hydrolyzed peptides can enter the lamina propria through mechanisms that include paracellular and transcellular routes, which remain to be better elucidated (Schumann et al., 2008). Glutamine residues are deamidated by tissue transglutaminase, which decisively increases the immunogenic potential of these peptides through a stronger binding to DQ2 or DQ8 heterodimeric surface receptors present in antigen-presenting cells, which in turn will activate a response from intestinal CD4⁺ T cells resulting in the damage of the intestinal mucosa (Caio et al., 2019). In addition to celiac disease, gluten is also the main trigger of other autoimmune disorders, such as dermatitis herpetiform and gluten ataxia, and allergic conditions such as gluten allergy (Lerner et al., 2019). Nonceliac wheat/gluten sensitivity is a condition in which people who test negative for both celiac disease and gluten allergy experience some symptoms similar to celiac disease, but which resolve after excluding wheat/gluten from their diets. Nevertheless, there is still an extensive debate about the main trigger of this condition, with oligosaccharides such as fruc-

tans recently been pointed out, rather than gluten (Skodje et al., 2018).

Gluten-free products have emerged with the growing concern associated with the wide spectrum of gluten-related disorders. These can be characterized as food products that try to imitate the corresponding traditional foods, replacing gluten by other ingredients, such as polysaccharide hydrocolloids and proteins of various origins, in order to provide an organoleptic experience as close as possible to gluten-containing counterparts (Ribeiro et al., 2019). With the development of this type of products and to ensure the safety of the diet of people suffering from health problems related to gluten, specific regulation was created (Administration, 2013; Commission, 2008; Regulation, 2009) defining gluten-free products; the statement “gluten-free” may only be made when the food contains no more than 20 mg/kg of gluten. In this context, the quantification of gluten takes on particular importance, and the methods intended for this purpose must be reliable, reproducible, and with adequate limits of detection and quantification (Martínez-Esteso et al., 2017).

The first method endorsed by Codex Alimentarius for the quantification of gluten was the R5 monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) (Commission, 2008). This antibody was raised against rye ω -secalins and recognizes potential celiac-toxic pentapeptide epitopes (Kahlenberg et al., 2006; Osman et al., 2001). Later, another ELISA, based on an antibody raised against the 33-mer peptide from wheat α -gliadins, the G12 monoclonal antibody, has been accepted, in addition to the R5-based ELISA, by the Association of Official Analytical Chemists (AOAC) International and the American Association for Clinical Chemistry (AACC) as a method for the detection of gluten (Halbmayer-Jech et al., 2019). Although some authors have obtained comparable results for the amount of gluten in routine samples labeled as gluten-free by ELISA methodologies based on these antibodies (Hocheegger et al., 2015), there are some concerns about the heterogeneous response of these and other commercially available antibodies particularly concerning wheat, barley, and rye glutelins (Lexhaller et al., 2016; Martínez-Esteso et al., 2017). The major criticism comes essentially from the lack of uniformity of the different commercial kits available, which due to factors such as the nonexistence of a globally accepted reference material for the calibration of the assay, different procedures for the extraction of gluten proteins or the different specificity of the antibodies may show inconsistent results (Díaz-Amigo & Popping, 2013).

New methods of analysis of gluten for quantitative purposes have been explored and are based on genomic and proteomic approaches. Polymerase chain reaction (PCR) is the technique of choice in genomic approaches, following

the principle of identifying contaminating DNA sequences from wheat, rye, or barley in gluten-free products (Dahinden et al., 2001; Mujico et al., 2011). On the other hand, the proteomic approaches supported by sophisticated mass spectrometry technologies have demonstrated the ability to be used for the quantification of gluten, adding the possibility to analyze the nature of the proteins/peptides and thus providing important insights into their immunogenic potential, for example, for celiac disease (Schalk et al., 2017). Nevertheless, it is important to note that there are also significant limitations, such as the analysis of complex hydrolyzed samples and without forgetting the cost of this technology (Alves et al., 2019).

On the other hand, fundamental research on celiac disease and the search for new alternatives to the gluten-free diet or a possible treatment for this disease require more sophisticated approaches than just the quantification of gluten. In fact, in recent times some new generation therapies have been developed, mostly relying on down-regulation of gliadin expression, proteolysis of immunodominant peptides, transamidation of glutamine residues, and sequestering of gliadin proteins, which essentially aim to improve the quality of life of the people who have celiac disease (Ribeiro et al., 2018). Thus, we have witnessed the development of increasingly complex *in vitro* models that are able to mimic in a closer way the pathophysiological processes involved in the disease and with crucial importance for the preclinical validation of most of these technologies. Besides, these studies are also supported by some *in vivo* models, spontaneous or induced, and even genetically engineered (Stoven et al., 2013).

The present work aims to critically review the main techniques and methodologies related to the quantification of gluten, its legal framework, and perspectives on the evolution of immunochemical methods up to advanced approaches based on omics tools. It is also the objective of this work to review the *in vitro* and *in vivo* models currently available for both fundamental research and the search for a nondietary treatment for celiac disease.

2 | IMMUNOCHEMICAL DETECTION METHODS

2.1 | Brief legal context

In 2009, the European Commission approved the Commission Regulation No. 41/2009 (Regulation, 2009), which was updated under the new Commission Regulation No. 828/2014 in 2014 (Regulation, 2014), both stating that products can only be labeled “gluten-free” if the final product sold to the consumer contains no more than 20 mg/kg of

gluten. The “very low gluten” label is used if foods containing ingredients made from wheat, barley, rye, oat, or their crossbred varieties, which are specially processed to reduce gluten content, which does not exceed 100 mg/kg of gluten in the final product sold to the consumer. Furthermore, it is also stated that food products containing oat must have been specially processed to prevent contamination by wheat, barley, rye, or their crossbred varieties, and their gluten content must not exceed 20 mg/kg (Regulation, 2014). Other countries such as China, Switzerland, South Africa, Canada, Australia, and the United States also have their legislation regarding gluten-free products labeling (Haraszi et al., 2011). For example, the U.S. Food and Drug Administration (FDA) approved, in 2013, the final rule stating that food may only be labeled gluten-free if the final product meets the following requirements: (a) must contain less than 20 parts per million (ppm) of gluten; (b) cannot contain wheat, rye, barley, or their crossbred varieties; (c) cannot contain ingredients derived from wheat, barley, and rye that have not been processed to remove gluten; and (d) contains ingredients derived from wheat, rye, barley, and triticale that have been processed to remove gluten (e.g., wheat starch) (Administration, 2013). In 2020, FDA has issued a final rule concerning “gluten-free” labeling for foods that are fermented or hydrolyzed or that contain fermented or hydrolyzed ingredients. The rule requires manufacturers of these food products to make and keep records providing adequate assurance that the food meets the definition of “gluten-free” before fermentation or hydrolysis; the manufacturer has adequately evaluated the potential for cross-contact with gluten during the manufacturing process; and if necessary, measures are in place to prevent the introduction of gluten into the food during the manufacturing process (Administration, 2020). Thus, the gluten content of foodstuff must be properly measured to ensure that gluten-free or very low gluten products are accordingly labeled.

2.2 | Antibodies used for detection and quantification of gluten

Immunochemical tests are the most common methods used to detect gluten proteins since they are sensitive, easy to use, and provide rapid results (Slot et al., 2016). These tests are based on the specific reaction between antibodies (immunoglobulins) and the substance to be determined, the antigens (celiac disease toxic proteins and peptides) (Wieser, 2008). The ELISA and the lateral-flow devices (LFD), also known as dipstick tests, are the two immunoassays most used to analyze the gluten content of food products. The differences between them are that ELISA is a quantitative test used to screen foodstuff, and LFD is a

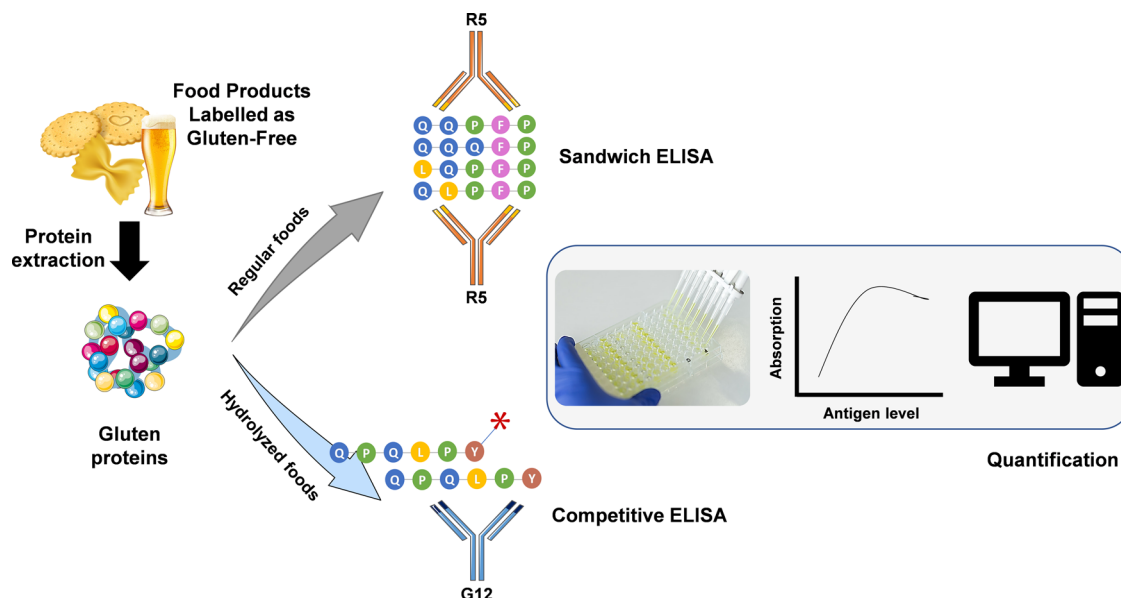


FIGURE 1 General procedure of an ELISA analysis using the two most common antibodies for the detection of gluten, R5 and G12

qualitative or semiquantitative test used to evaluate cleaning/decontamination techniques (Slot et al., 2016). Thus, the ELISA systems are used for the labeling of food products taking into account the rules established in terms of food security for the different countries (Figure 1).

Table 1 summarizes the main antibodies used in the detection of gluten. In 1991, a monoclonal antibody, named 401/21, was used to develop a gluten detection method whose target was ω -gliadins (Skerritt & Hill, 1990) and has also been shown to recognize HMW glutenins (Table 1) (Allred & Ritter, 2010). The fact that the ω -gliadin fraction does not denature during the heating process is an advantage of using this antibody. In other terms, this test can be employed to estimate the gluten content of foods containing both native (i.e., protein in their native state) and heated proteins (Haraszi et al., 2011). However, the glutelin recognition, together with the low amount and large variation of ω -gliadins present among cereal species (Wieser et al., 1994), leads to a fallible detection of the total prolamins content. An underestimation of the total gluten content in foods containing barley happens because the 401/21 antibody responds weakly to hordeins (Thompson & Méndez, 2008).

The R5 ELISA method, developed by Valdes et al. (2003) and validated by Mendez et al. (2005), is the current Type I Codex method for gluten assessment, which is used by official control systems throughout Europe and calibrated against the Prolamin Working Group (PWG) gliadin standard (Hochegger et al., 2015). The R5 antibody (Table 1) was produced against rye secalins and strongly binds to the QQPFP, LQPFP, QQQFP, and QLFPF epitopes which are present in α -/ β -, γ -, and ω -gliadins (Mendez et al.,

2005) as well as in hordeins (from barley) and secalins but not in avenins (from oat) (Valdes et al., 2003). Nevertheless, the number of epitopes varies with the type of protein and cereal. Attention should be given when selecting a standard since the R5 method overestimates hordein in barley-contaminated foods unless a hordein standard is used (Kanerva et al., 2006).

The G12 antibody (Table 1) was produced against the synthetic 33-mer peptide (LQLQPF-PQPQLPYPQPQLPYPQPQLPYPQPQPF) of $\alpha 2$ -gliadin by Moron, Cebolla, et al. (2008), recognizing the QPQLPY epitope of the peptide, which is present in prolamins from wheat, barley, rye, and some oat varieties. However, its sensitivity for oat is over a 100-fold lower than for barley and wheat and almost 50-fold lower than for rye (Moron, Bethune, et al., 2008). Therefore, the sensitivity for oat is overly low in the legally relevant concentration range. Additionally, this cross-reactivity inhibits the detection of wheat, barley, and rye contaminations in oat (Slot et al., 2016).

Recently, a new anti gliadin antibody, named X6, has been proposed (Table 1). This antibody strongly binds to the QXQFPFPXP epitope, and the PFP part is critical for antibody recognition. The structurally recognizable epitope is similar to R5 but with a larger size, which can result in higher specificity. The authors claimed that this new antibody has a high potential for using in test systems designed to determine the gluten content in foods based on the ELISA format (Shatalova et al., 2020).

At this standpoint, a recent study identified sequences of gliadins and glutenins from wheat, which are appropriate for the generation of new antibodies. Novel celiac

TABLE 1 Enzyme-linked immunosorbent assays (ELISA) and antibodies for prolamins and gluten detection

Antibody	Type	Target	Format	Standard	Gliadin		Reference
					LOD (ppm)	LOQ (ppm)	
401/21	Monoclonal	PQPQPFPE; PQQPFPEE	Sandwich	“Timgalen” variety	5 ^a	–	(Skerritt & Hill, 1990)
PN3	Monoclonal	QQQFPF	Sandwich	“Kolibri” variety	0.08 ^a	–	(Ellis et al., 1998)
			Competitive	PWG gliadin	0.128	–	(Bermudo Redondo et al., 2005)
R5	Monoclonal	QQFPF; QQQFP; LQFPF; QLFPF	Sandwich	Gliadin from Sigma	1.56	3	(Valdes et al., 2003)
			Competitive	PWG gliadin	0.36	1.22	(Mena et al., 2012)
Glia α –2/9	Monoclonal	LQPFQPPQ	Competitive	PWG gliadin	2.4	–	(Spaenij-Dekking et al., 2004)
Glia γ –1	Monoclonal	QQRPFPI	Competitive	PWG gliadin	2.4	–	(Spaenij-Dekking et al., 2004)
Glia- α 20	Monoclonal	RPQQYPY	Competitive	Synthetic peptide containing the α 20-gliadin epitope ^b	–	3.6 ^b	(Mitea et al., 2008)
G12	Monoclonal	QPQLPY	Sandwich	Gliadin from Sigma	0.6	1.5	(Moron, Cebolla, et al., 2008)
			Competitive		0.44	3.95	
A1	Monoclonal	QLPYQP	Competitive	Gliadin from Sigma	1.63	6.98	(Moron, Bethune, et al., 2008)
INRA-DG1	Monoclonal	QPEEPFPE	Competitive	Gluten sample with a 60% deamidation level	25 ^c	–	(Tranquet et al., 2015)
2D4	Monoclonal	Deamidated gliadin	Sandwich	Prolamins from wheat, barley, rye, and oats; deamidated wheat protein	1	–	(Benoit, Meshgi, et al., 2016)
Total gluten (four monoclonal antibodies including the R5)	Monoclonal	Prolamins from wheat, rye, and barley; HMW glutelins from wheat and rye; LMW glutelins from wheat	Sandwich	Total gluten extract of 4 wheat varieties	$\leq 5^d$	$\leq 5^d$	(Lacorn et al., 2019)
X6	Monoclonal	QQQPFPP	Sandwich	Gliadin from Sigma	2.5	5	(Shatalova et al., 2020)

Abbreviations: HMW, high molecular weight; LMW, low molecular weight; LOD, limit of detection; LOQ, limit of quantification; PWG, prolamins working group.

^aSensitivity value.

^bGluten-Tec kit manufactured by Europroxima.

^cng/ml.

^dppm of gluten.

disease-active target sequences include the peptide motifs PQQSFP for γ -gliadins, QQQYPS for α/β -gliadins, QPGQGQQG for LMW-GS, and QPGQGQQG for HMW-GS. These peptides are unique to wheat gluten, and, as a result, cross-reactivity with maize or rice proteins is very unlikely (Rockendorf et al., 2017).

In processed foods, prolamins can be partially or totally hydrolyzed, for example, in malted barley. Short pep-

tides that lack two antibody binding sites cannot be detected by sandwich ELISA because they require two epitopes to bind. Nevertheless, the presence of only one immunopathogenic element is enough to the onset of celiac disease, thus making it possible to miss the toxic gluten peptides in hydrolyzed foods by sandwich ELISA (Panda, Fiedler, et al., 2015; Panda, Zuerb, et al., 2015). The competitive ELISA format (see Figure 1), since it

recognizes a single epitope, was specifically developed to overcome this limitation and may be more effective in detecting immunopathogenic peptides derived from gluten in hydrolyzed foods (Panda & Garber, 2019). Competitive ELISA based on R5 and G12 antibodies are commercially available to detect and quantify gluten in hydrolyzed foods. Combining a competitive R5 ELISA and peptic-tryptic digestion of prolamins was proven to be appropriate for the determination of partially hydrolyzed gluten in wheat, barley, and rye (Gessendorfer et al., 2009; Mahroug et al., 2019). Nevertheless, the validation of this method was based on the detection of the reference material spiked into various foods, thus depending on the fermentation conditions and resulting proteolysis, the validation performed may not be scientifically valid (Mattarozzi & Careri, 2019; Panda & Garber, 2019).

A few studies have shown that the reactivity of the G12 to peptides relates to potential celiac immunotoxicity in fermented beverages such as beer. This antibody showed to recognize T cell epitopes in beer (Comino et al., 2013; Real et al., 2014). Moreover, when compared to the R5 antibody, the G12 showed to be more reactive at immunocapturing a hydrolyzed gliadin present in wheat and the T cell active peptides present in barley beer (Moreno et al., 2016). This evidence suggests that for the analysis of fermented-hydrolyzed gluten, the G12 ELISA could be more suitable, compared to the R5-based ELISA. Meanwhile, it was produced a novel multiplex-competitive ELISA, which included gluten-specific antibodies from nine different commercial ELISA test kits. Thus, the use of different antibodies that target different gluten epitopes allowed the authors to differentiate the protein characteristics of different fermentation processes; it was possible to distinguish between the sourdough breads, the soy-based sauces, the wheat beers, and the barley beers (Panda et al., 2017).

Deamidation can also take place during food processing, and monoclonal antibodies have been produced to detect deamidated gluten for food safety. It should be noted that the deamidation of glutamine residues has a vital role in the pathogenesis of the celiac disease as this reaction increases the binding affinity of gluten peptides to the human leukocyte antigen (HLA) receptors in antigen-presenting cells (Di Sabatino et al., 2012). Tranquet et al. (2015) produced the antibody INRA-DG1, which can be used to detect deamidated gluten sequences (Table 1). Likewise, 2D4 monoclonal antibody was developed to recognize deamidated gliadin (Benoit, Cox, et al., 2016; Benoit, Meshgi, et al., 2016), and 2B9 monoclonal antibody-based LFD was recently proposed as an immunodiagnostic assay for rapid detection of deamidated gluten (Masiri et al., 2016).

As previously mentioned, LFD is used to detect the presence of gluten in food products in a qualitative or semi-

quantitative way. They are commercially available since they perform a rapid analysis, are user friendly, and are useful for on-site analysis (Panda & Garber, 2019). Most of these tests use the R5 or G12 antibodies and the sandwich methodology. Recently, an LFD, named Nima™, was created to help the consumers to detect the presence of gluten in food products. This device incorporates food processing, gluten detection, result interpretation, and data transmission in a portable device for consumers. Compared with the reference R5, Nima™ antibodies 14G11 and 13F6 showed a 6.6- and 35-fold higher affinity for gliadin proteins, respectively (J. Zhang et al., 2019). Also, some biosensors for detecting gliadin contamination in gluten-free foods have been developed. Weng et al. (2016) have developed a microfluidic biosensor that significantly reduces the use of costly reagents and samples (up to 20-fold). It also reduces the detection time from 4 h to 20 min while maintaining superior sensitivity (Weng et al., 2016).

2.3 | General limitations

In ELISA or LFD systems, gluten proteins are generally divided into two groups according to their solubility in alcohol-water solutions: the soluble prolamins and the insoluble glutelins. Both techniques detect epitopes within the prolamins group and use a correlation factor of 2 to obtain the total gluten content. The calculation of gluten content is normally performed based on the assumption of a 50:50 ratio between prolamins and glutelins. Nonetheless, a few studies have reported some differences in the ratio between prolamins and glutelins, indicating a factor around 65:35 mainly for rye and barley and depending on the species of the cereal and variety, meaning that when calculating the gluten content, it would be more accurate to use a correlation factor of 1.6 (Mena & Sousa, 2015; Thompson & Méndez, 2008). Besides the ELISA R5 method being the current Type I Codex method for gluten assessment, it only measures the prolamins fraction using a correlation factor of 2 to obtain the total gluten content. The recently developed Total Gluten ELISA detects both gluten fractions and, thus, measures the gluten content (Lacorn et al., 2019). However, the PWG believes that it cannot exist two type I methods because the definition of a type I method as “the only method” should exclude approval of a second type I method. On the contrary, if there is a method on the market that meets all the criteria of the Codex and has been approved by suitable collaborative studies, it should not be excluded due to the fact that another method has already been approved by the Codex Alimentarius and should decide soon how to proceed (Scherf et al., 2020).

Furthermore, other relevant limitations of the immunochemical methods are related to (1) the extraction of gluten proteins, (2) the choice of which epitopes should be targeted by the detection methods, and (3) the production of a standardized reference material (Slot et al., 2016). The extraction of gluten proteins is the first step for gluten analysis; however, gluten proteins are insoluble in water, but prolamins are soluble in ethanol and glutelins in dilute acid and alkali solutions. Due to the difference in solubility characteristics of these different gluten protein groups, full extraction remains problematic. As mentioned before, ELISA-based test systems aim to extract and detect the prolamins and apply a correlation factor to estimate the total gluten content; nevertheless, incomplete extraction of prolamins can occur in some extraction protocols. Consequently, gluten detection methods only provide a relative indication of the true gluten content in food (Wieser, 2008). Despite this, new efforts have been made regarding the development of new solutions for the solubilization of gluten proteins. For example, the Universal Gluten Extraction Solution (UGES) has the advantage of using more environmentally friendly components and is compatible with the competitive format of the ELISA technique when compared with the cocktail solution recommended by Codex Alimentarius (Segura et al., 2021). The second major problem with gluten detection is that all the methods use antibodies raised against a specific prolamins epitope. Hence, the estimation of the total gluten content can be miscalculated because it is probable that they miss other epitopes, leading, therefore, to false-negative results (Mena et al., 2012). A final problem is the lack of a standardized reference material representative of gluten proteins. The reference should be used to reduce the variations between the assays and to enable the comparison of results from different laboratories and/or obtained with different techniques. In recent years, it is prevalent the use of various standard reference materials by the different commercial ELISA kits available to quantify the gluten content. Still, the standard of the Working Group on Prolamins Analysis and Toxicity (WGPAT) for gluten analysis has been the most widely used. Nevertheless, cereals contain a more significant number of proteins than those present in the WGPAT standard (Haraszi et al., 2011; Mena & Sousa, 2015; Slot et al., 2016; Wieser, 2008). Overall, these problems limit the reproducibility of this widely used technique.

3 | PROTEOMICS-BASED METHODS

In the past years, mass spectrometry techniques have created high expectations for the quantification of gluten, essentially due to the resolution of some problems associ-

ated with the use of immunochemical methods (Manfredi et al., 2015), allowing accurate quantification and identification of the source of cereals, the type of protein, and the identification of celiac-related immunogenic sequences (Colgrave et al., 2016; Schalk et al., 2018a). Figure 2 shows an overview of a possible proteomic approach for gluten analysis, and Table 2 shows the different proteomics-based approaches for gluten analysis developed in recent years.

In general, during the proteomics-based approaches, some aspects should be considered. For example, the appropriate extraction considering the solubility of gluten proteins, resorting to the use of reducing and denaturing agents (Alves et al., 2019) and the enzymatic digestion because the size of the peptides, the ability to be ionized and fragmented may be taken into consideration since they interfere in the sensibility of the methods (Fiedler et al., 2014). Furthermore, the ionization source and the type of mass spectrometry analyzer can interfere with the analysis and, consequently, the identification and quantification of proteins (Alves et al., 2019). For example, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) is a robust and sensitive technique that has been used in the characterization of wheat storage proteins; however, it cannot be used in conjunction with liquid chromatography (LC) methods (Ferreira et al., 2014). On the other hand, electrospray ionization (ESI) has been used as an adjunct to LC in the detection of immunogenic gluten marker peptides in a variety of foods since it has a good potential for the analysis of complex proteins (Jira & Munch, 2019; Manfredi et al., 2015).

LC-MS or high-performance liquid chromatography (HPLC)-MS is an analytical technique and an important tool for the quantification and identification of immunoreactive cereal proteins (Alves et al., 2019). It consists of a separation process based on the differential interaction of the sample components with a stationary phase or column, allowing its separation before being introduced into the ion source (ionization) for the mass spectrometer. The ions are separated and detected based on their mass/charge ratio (m/z). Thus, this method allows us to quantify several peptides and proteins in the same experiment and to identify and differentiate the different species of cereals and protein subunits (Ferreira et al., 2014). In order to increase the ability to study and analyze complex samples, tandem mass spectrometry (MS/MS) is often used. In general, two or more mass analyzers are coupled together. Precursor ions are introduced into a collision cell to undergo specific fragmentation by collision-induced dissociation (CID). This collision occurs by an inert gas, usually nitrogen or argon, resulting in the formation of product ions, that is, the identified peptides are selected and subjected to fragmentation to elucidate the sequence of amino acids, allowing the confirmation and identification of different sequences (Graves

TABLE 2 Recent proteomics-based approaches for wheat gluten analysis and related proteins from other cereals

Study	Food matrix	Technique	Database	Reference
Method for the quantitative detection of trace levels of physiologically relevant wheat gluten peptides	Quinoa, corn, soy, vital wheat gluten, wheat, rye, barley, rice, and oat flours; different conventional food products and beverages, including gluten-free items	HPLC-ESI-TQS-MS; MRM-MS/MS; LC-ESI-Ion Trap MS ⁿ	Peptides were identified by comparison with synthetically prepared peptides as well as NCBI protein database searches (https://www.ncbi.nlm.nih.gov/)	(Sealey-Voyksner et al., 2010)
Monitoring target peptides associated with clinically relevant wheat allergens and carrying immunologically relevant epitopes	“Viginta” wheat variety	NanoUPLC-MS ^E -QTOF-MS/MS	Wheat allergen database (http://www.allergenonline.org/); nonredundant Triticeae plant protein UniProt database (https://www.uniprot.org/)	(Uvackova et al., 2013b)
Method based on MS ^E for quantifying gliadin and glutenin proteins in wheat grain	“Viginta” wheat variety	NanoUPLC-MS ^E -QTOF-MS/MS	Nonredundant Triticeae plant protein UniProt database (https://www.uniprot.org/)	(Uvackova et al., 2013a)
Detection of gluten by mass spectrometry	Flour samples of wheat, barley, rye, and oat	nanoUPLC-pSRM-MS/MS	BEP clade (Bambusoideae, Ehrhartoideae, Poioideae) and PACMAD clade (Panicoideae, Arundinoideae, Chloridoideae, Micrairoideae, Aristidoideae, Danthonioideae) of the Poaceae family (https://www.uniprot.org/)	(Fiedler et al., 2014)
Method for detection and quantification of celiac-related epitopes	two hexaploid wheat varieties (“Minaret” and “Toronto”) and one tetraploid wheat variety (“Dibillik sinde”)	On-line 2D-nanoLC-ESI-MS/MS; UPLC-MRM-MS	Searches were performed using the UniProt database (https://www.uniprot.org/)	(van den Broeck et al., 2015)
Detection of wheat, oat, barley, and rye prolamins	Unprocessed raw materials as flours and seeds; processed foods, including pasta, biscuits, cookies, crackers, beverages, breads, breakfast cereals, and snacks	UPLC-LTQ-ESI-pSRM-MS/MS	Reviewed and unreviewed entries for the Poaceae family listed in the UniProt database (https://www.uniprot.org/)	(Manfredi et al., 2015)
Defining analytical targets for wheat gluten	Cargill GluVital vital wheat gluten; flours of rye, barley, oat, wheat, rice, corn, and soy	nanoUPLC-nanoESI-SRM-MS/MS	Custom database for <i>Triticum</i> spp. downloaded from NCBItr (https://www.ncbi.nlm.nih.gov/protein/); <i>Coeliac protein database</i> (http://www.allergenonline.org/)	(Martinez-Esteso et al., 2016)

(Continues)

TABLE 2 (Continued)

Study	Food matrix	Technique	Database	Reference
Detection of barley contamination	Grains of barley, wheat, rye, oats, millet, maize, rice, green wheat, amaranth, chia, quinoa, sorghum, and tef; flours of rye, sorghum, buckwheat, soy, oats, and millet; breakfast cereals	nanoUPLC-MRM-MS	In silico tryptic digests of Poaceae proteins in a specific database (https://www.uniprot.org/)	(Colgrave et al., 2016)
Quantification of the 33-mer peptide	Twenty-three modern and 15 old common wheat varieties; one rye, two spelt, two durum wheat, two emmer, and two einkorn varieties	RP-HPLC; untargeted LC-CID-MS/MS; LC-MRM-MS/MS	A stable-isotope-labeled *33-mer standard was used; pepsin and trypsin/chymotrypsin hydrolysate of the 33-mer was analyzed based on the NCBI database (https://www.ncbi.nlm.nih.gov/)	(Schalk et al., 2017)
Identification and quantification of barley, rye, and oat marker peptides to determine gluten concentrations	Flour mixtures containing individually four barley, four oat, and four rye varieties	RP-HPLC; LC-MRM-MS/MS	Peptides were identified based on the NCBI database (https://www.ncbi.nlm.nih.gov/)	(Schalk et al., 2018a)
Identification and quantification of wheat marker peptides representative of all types of gluten proteins	Flour mixture containing four common wheat varieties	RP-HPLC; untargeted LC-CID-ESI-MS/MS; LC-MRM-MS	Peptides were identified based on the NCBI database (https://www.ncbi.nlm.nih.gov/)	(Schalk et al., 2018b)
Detection and quantification of celiac-related immunogenic epitopes	Thirty wheat varieties with different release years (1910–2013)	RP-HPLC; LC-SRM-MS	Nr_triticeae database and a database of common contaminants 1648030_20150930_cRAP123 (https://www.ncbi.nlm.nih.gov/protein/)	(Malalagoda et al., 2018)
Monitoring allergenic and immunogenic profile	Wheat flours from nine Brazilian common wheat genotypes varying in technological quality	nanoUPLC-MS ^E -nanoESI; nanoUPLC-UDMS ^E	Triticum aestivum database (https://www.uniprot.org/); prolamin-specific database (https://propepper.net)	(Alves et al., 2018)

Abbreviations: CID, collision-induced dissociation; ESI, electrospray ionization; LC, liquid chromatography; LTQ, linear trap quadrupole; MS, mass spectrometry; MS^E, data independent quantitative mass spectrometry; MS/MS, tandem mass spectrometry; MRM, multiple reaction monitoring; MALDI-TOF MS, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry; nanoUPLC, nano ultra-high performance liquid chromatography; nanoESI, nano electrospray ionization; On-line 2D-nanoLC, on-line two-dimensional nano liquid chromatography; pSRM, pseudo-selected reaction monitoring; Q-TOF, the quadrupole time-of-flight; RP-HPLC, reversed-phase high performance liquid chromatography; SRM, selected reaction monitoring; TQS, triple quadrupole; UDMSE, ultra-definition data independent quantitative mass spectrometry.

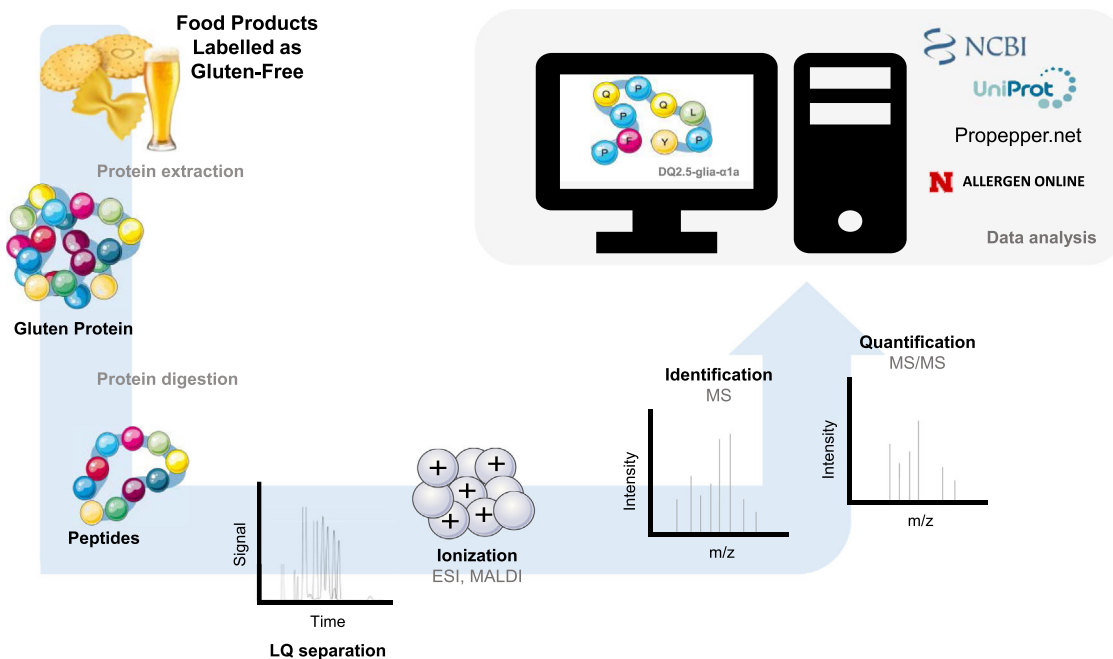


FIGURE 2 Proteomics-based workflow for gluten analysis

& Haystead, 2002). When associated with LC, the analysis of biomolecules in complex samples is more accurate due to their high levels of sensitivity and specificity (Alves et al., 2017). Still, its wider use is hampered by the high monetary value of the equipment, and not all laboratories are specialized for such analyses (Graves & Haystead, 2002).

In celiac disease, the prolamin storage proteins, namely gliadins, are responsible for triggering serious health problems (Caio et al., 2019). Nevertheless, the gliadins and glutenins share a high degree of similarity, mainly due to a large number of repetitive sequences, which makes it difficult to identify and analyze immunoreactive peptides (Alves et al., 2019; Juhasz et al., 2015). At this standpoint, LC-MS/MS for gluten detection has been used for identifying celiac-related immunogenic peptides, such as α - and γ -gliadin derived peptides, and often the proteolytically resistant 33-mer peptide (Alves et al., 2018; Malalgoda et al., 2018), which is believed to have a pivotal role in the pathogenesis of the celiac disease (Shan et al., 2002, 2005). In the same way, this technique has been used in the search for gluten markers. The combination of LC strategies can be used for a primary study of gluten proteins using RP-HPLC or size exclusion (SE)-HPLC followed by multi-enzymatic digestion of the resulting protein fractions and high-resolution measurements by MS or MS/MS (Jira & Munch, 2019; Schalk et al., 2018a). The gluten marker peptides obtained can be identified by comparison via *in silico* (using various databases) and with experimental results (Juhasz et al., 2015). For the selection of gluten markers, selected reaction monitoring (SRM) and multiple reaction

monitoring (MRM) are methods that allow targeted analyses, especially for quantification that aids in the selection of gluten markers (Alves et al., 2017; Martínez-Esteso et al., 2017). In these techniques, there are projections of transitions for the target analyte, involving the selection of precursor ions and their corresponding product ions based on predefined criteria (Kitteringham et al., 2009). The high sensitivity of this monitoring allows the precise quantification of proteins, as well as different isoforms. In the case of allergens, this has a great advantage since they have several different isoforms and cannot be easily detected using antibody-based methods (Koeberl et al., 2014).

Various types of mass analyzers are used for the selection of gluten markers, such as TOF, where the mass/charge ratio is determined via a time-of-flight measurement (Mejías et al., 2014), or in triple quadrupole (QqQ), which allow precise quantification of target proteins in complex samples with a wide dynamic range (Martínez-Esteso et al., 2017). In short, for triple quadrupole, the precursor ions will be selected and focused on the first quadrupole (Q1). In the second quadrupole (Q2), a collision gas (usually argon) is injected, leading to ion fragmentation. This cell is a collision cell. In the last quadrupole (Q3), the collision cell fragments are analyzed according to their m/z (Kitteringham et al., 2009).

On the other hand, recent developments in the field of quantitative proteomics such as MS^E -based multiplex label-free proteomics may prove to be important for the validation of new methodologies aimed at the exact quantification of gluten in different food matrices (Alves et al., 2019). Also known as data-independent mass

spectrometry, MS^E approaches are based on alternation between energy levels scans on a Q-TOF instrument. More specifically, the original technique uses high energy scans that are MS/MS scans with no precursor isolation, and the low energy scans are MS scans where all generated ions in the ionization source are transmitted to the collision cell, which alternates between high and low energy. After this process, both precursors and fragments of the peptides are sent to the TOF analyzer (Egertson et al., 2015). For instance, this technology has already enabled the identification and quantification of critical allergenic proteins and peptides carrying known epitopes involved in gluten-related disorders such as baker's asthma, allergy, wheat dependent exercise-induced anaphylaxis (WDEIA), atopic dermatitis, and celiac disease (Uvackova et al., 2013b). Another study used an ultra-definition mass spectrometry (UDMS^E) label-free quantitative approach, to analyze the expression of metabolic proteins (albumins and globulins) of wheat flours varying in technological quality. These authors found a total of 414 proteins being differentially expressed from nine cultivars of different qualities (Victorio et al., 2018).

4 | DNA-BASED METHODS

PCR is a highly specific and sensitive method based on the determination of specific DNA and its quantification (Dahinden et al., 2001), and when compared with protein analysis, these DNA-based tests are more sensitive. For these reasons, DNA tests have proven to be very useful for authenticating the varieties used in food production (Hernandez et al., 2005). In general, the presence of proteins in food indicates the presence of DNA. Therefore, a DNA-based method for detecting DNA fragments encoding toxic epitopes in food can be applied. In addition, the PCR-based method has a high yield, and it is timesaving (Dahinden et al., 2001; X. Zhang et al., 2011).

The quantitative PCR method (Q-PCR) has been used for the simultaneous detection of contamination of wheat, barley, and rye in gluten-free foods (Mujico et al., 2011). Some Q-PCR systems are based on TaqMan probes. These probes produce a fluorescence signal only if they hybridize specifically with the target DNA sequence; if the probe is not specific for the DNA sequence, there is no signal production (Hernandez et al., 2005; Mustorp et al., 2011). Overall, Q-PCR systems are efficient at detecting prolamins. Nevertheless, its use is limited when it comes to hydrolyzed and highly processed food samples because the DNA is practically undetectable due to its massive degradation and, therefore, impossible to be amplified by Q-PCR (Mujico et al., 2011). Thus, Q-PCR is a nonimmunological method that allows the detection and quantification of

DNA present in various foods that contain wheat and can be used for the detection of allergens or other sequences associated with gluten-related disorders based on DNA. Its use is more oriented toward the detection of gluten contamination or authentication of food than to quantify gluten, which in fact does not preclude its use as a complement to traditional methods based on protein (Mustorp et al., 2011; X. Zhang et al., 2011).

5 | *IN VITRO* AND *IN VIVO* MODELS WITHIN THE CONTEXT OF CELIAC DISEASE

It is of great importance to quantify gluten for the correct labeling of food products so that they are not potential sources of health problems for people suffering from gluten-related diseases. Although less than 20 ppm of gluten is at a level where its intake by celiac patients is potentially safe (Elli et al., 2020; Syage et al., 2018), there are certain assumptions that require caution: (1) The quantification of gluten by the methods approved by the Codex Alimentarius is based on immunochemical methods, which despite using antibodies that recognize potential immunogenic sequences, there will be others that will be under quantified (Lester, 2008); (2) some celiac-related epitopes present immunodominance, such as 33-mer, however, there is heterogeneity in relation to the immune response to different epitopes, which means that some may trigger more severe responses in some patients and not in others (Camarca et al., 2009); and (3) there are currently several research lines to develop products based on gluten devoid of toxicity for celiac patients. These are essentially based on chemical and physical treatments, which, by altering the structure of gluten, may have consequences on its solubility and thus affect its quantification by traditional methods (Mahroug et al., 2019; Ribeiro et al., 2018). In this context, it should be noted that the methods reviewed above are predominantly quantitative in nature and, with the exception of techniques based on mass spectrometry, which also allow a qualitative analysis, their results have not been clinically validated for celiac disease. Obviously, a reduction in the amount of gluten measured by traditional methods will have positive effects on the celiac disease. Still, it becomes insufficient for the study of new targets or therapeutic agents, or for the development of nondietary therapies that could lead to clinical trials. They are not able to mimic more complex intestinal functions that are required for drug development and disease research. Thus, different *in vitro* and *in vivo* models have been developed, which attempt to mimic both the innate and adaptive immune responses and their relationship in the pathogenesis and manifestation of celiac disease.

5.1 | In vitro models

In general, in vitro models include cell lines, patient-derived cells, and intestinal biopsy cultures. More recently, organoid and gut-on-chip technologies have provided new opportunities for the development of in vitro models that mimic more closely the pathophysiological processes characteristic of celiac disease.

5.1.1 | Cell lines

Different cells have been used to study particular events associated with the pathogenesis of the celiac disease. For example, cell lines such as Caco-2 or IEC-6 have been widely used to study the function of the epithelial barrier and, in turn, intestinal permeability. Their exposure to gluten can result in a wide range of responses, which include cytoskeletal rearrangement and tight junction dysfunction, as well as changes in the expression of inflammatory cytokines (Clemente et al., 2003). Moreover, Caco-2 cells have allowed some insights into the relationship between the intestinal microbiome and the development of the celiac disease. In particular, *Bacteroides fragilis* strains showed a strong adverse effect on the integrity of the Caco-2 cell monolayer. It was hypothesized that the gliadin-hydrolyzing activity of these bacteria is decisive in the generation of immunogenic peptides, increasing the production of inflammatory cytokines (Sanchez et al., 2012).

5.1.2 | T-cell lines and clones

T-cell lines or clones derived from small intestinal biopsy specimens of celiac patients have been used to further study adaptive immune responses to gluten proteins (Lundin et al., 1993). Its use allowed to know the function of specific cells and the elucidation of some processes in the pathogenesis of the celiac disease, as the role of CD11c⁺ dendritic cells on the activation of gluten-reactive T cells (Raki et al., 2006), the development of DQ2 tetramers for characterization and diagnosis of celiac disease (Raki et al., 2006) or to demonstrate the role of tissue transglutaminase in enhancing the immunogenic potential of gluten proteins through the deamidation process (Molberg et al., 1998). More recently, several gluten T-cell epitopes restricted by celiac disease-associated HLA-DQ molecules have been identified (Sollid et al., 2012; Tye-Din et al., 2010). This knowledge is fundamental to our understanding of the immunogenic potential of gluten and similar proteins derived from cereals other than wheat. On the one hand, it allows the development of antibodies that do the eventual screening of these epitopes or the devel-

opment of dedicated databases for approaches based on mass spectrometry, making the quantification of gluten more comprehensive in relation to the celiac disease for example. On the other hand, the use of these T cell-based assays has been widely used to validate the results of different technologies focused on gluten detoxification, such as the use of the down-regulation of gliadin expression (Gil-Humanes et al., 2010), proteolysis of immunodominant peptides (Wolf et al., 2015), or the previous transamidation of glutamine residues (Ribeiro et al., 2020).

5.1.3 | Monocytes and dendritic cells

Monocytes and dendritic cells are in vitro models focused on innate immune response, allowing the study of other cells that contribute to gluten induced intestinal damage (Marietta et al., 2009). Some authors reported that contrary to other food antigens, peptic fragments of gliadin induced the production of IL-8 and TNF- α by human monocytic line THP-1 cells (Jelinkova et al., 2004). Likewise, the peptic digests of gliadin also led to enhanced expression of maturation markers and increased secretion of chemokines and cytokines by human monocyte-derived dendritic cells (Palova-Jelinkova et al., 2005). In addition, a recent study aimed to investigate the role of intestinal bacteria and gliadin on the modulation of phenotypic and functional maturation of dendritic cells suggested that the gut microbiota may influence phenotypic and functional maturation of dendritic cells and their interactions with epithelial cells (De Palma et al., 2012). As previously mentioned for Caco-2 cells, dendritic cells also allowed important insights into the role of the microbiome in celiac disease progression. In general, cellular assays have been fundamental to the knowledge we have about the pathogenesis of celiac disease and have been used as an important tool for the study of the immunogenicity of gluten. Despite all its potential, its poor genomic integrity, and the fact that the immortalized lines do not faithfully represent the genetic component associated with the disease, can be pointed out as the main disadvantages of this model (Moerkens et al., 2019).

5.1.4 | Intestinal biopsy cultures

Intestinal biopsy cultures derived from celiac patients are the oldest model used to study celiac disease and comprehend the genetic background and the cell-cell interactions present in the small intestine, recapitulating more closely the complexity of celiac disease (Stoven et al., 2013). This model allowed the initial studies on the toxicity of specific peptides and gliadin fractions in celiac disease

(Ciclitira et al., 1984; de Ritis et al., 1988). Other relevant studies include the increased release of zonulin by celiac epithelial cells when exposed to a peptic tryptic digest of gliadin (Drago et al., 2006), how interactions between secretory immunoglobulin A (SIgA) and transferrin receptor (CD71) promote transepithelial transport of gliadin peptides (Lebreton et al., 2012) or the contribution of IL-15 to maintain and expand the local inflammatory response in celiac disease (Zanzi et al., 2011). On the other hand, as previously mentioned for the T cell-based assays, this model was also used in the validation of novel methodologies aiming the gluten detoxification for celiac patients, particularly concerning the previous transamidation of glutamine residues (Elli et al., 2012; Lombardi et al., 2013). Nevertheless, the availability of this model is low and limited, and this is essentially due to its invasive nature. Besides, tissue inflammation and limited life span comprise other limitations, which together, hinder a more widespread use of this model (Moerkens et al., 2019).

5.1.5 | Organoid and gut-on-chip technologies

In the past years, thanks to the development of new techniques, particularly related to the stem cell technology, it is possible to generate organoids from the human intestine (Kretzschmar & Clevers, 2016; Spence et al., 2011). A recent study showed important differences between intestinal organoids generated from duodenal biopsies of nonceliac and celiac patients, namely altered expression of genes associated with the gut barrier, innate immune response, and stem cell functions. In addition, by administering a peptic-tryptic digest of gliadins to the monolayers derived from the organoids generated from celiac patients, the authors found that this treatment increased the paracellular permeability and the secretion of proinflammatory cytokines when compared to nonceliac controls (Freire et al., 2019), which makes this model particularly useful for modeling and studying the pathogenesis of celiac disease and for developing potential treatments. The main limitations are often related to inconsistency in size and shape of the organoids, the fact they are cultured in a static system that does not mimic the intestine's physical environment, for example, the peristaltic movement, and the necessary use of an extracellular matrix to maintain the organoids hinders the physiological interactions with other components of the intestine (Moerkens et al., 2019; Rossi et al., 2018). In this context, new opportunities are coming with the development of gut-on-chip systems. These are microfluidic devices in which intestinal cells are seeded into channels under static culture conditions, but then media is pumped through the channels using, for example, peristaltic pumps to mimic the in vivo

dynamic microenvironment. A common chip uses one channel to simulate the lumen, and it is lined with epithelial cells, whereas the other channel simulates a blood vessel lined with vascular endothelial cells (Ashammakhi et al., 2020). Interestingly, it was found that the physiological peristalsis-like motions and liquid flow of a gut-on-chip system were decisive to induce Caco-2 cells to undergo three-dimensional (3D) villus differentiation, containing different types of differentiated epithelial cells (absorptive, mucus-secretory, enteroendocrine, and Paneth) (Kim & Ingber, 2013). On the other hand, these devices offer a more amenable platform to use organoid technology (Workman et al., 2018) and enhancing it as transcriptomic analyzes have shown that their combined use resulted in a system that more closely mimics whole human duodenum in vivo (Kasendra et al., 2018).

5.2 | *In vivo* models

In vivo models comprise a scientific tool that uses the whole organism, that is, with the presence of hormonal, neurological, and metabolic signals from other organs or cellular systems. Although the cellular models are fundamental for our understanding of processes related to the pathogenesis of celiac disease, modeling the systemic development of the disease is logically limited to the *in vivo* systems (Moerkens et al., 2019; Stoven et al., 2013).

5.2.1 | Spontaneous animal models

As spontaneous models in which sensitization is not necessary for the disease to develop in these organisms dog and monkey models are the most widely used, and more recently, a horse model has also been reported (Stoven et al., 2013). In the latter case, a study analyzed the concentration of antibodies important for the diagnosis of celiac disease in humans in blood from horses with inflammatory small bowel disease with a gluten-rich diet and two control groups with and without a gluten-rich diet. All groups with a gluten-rich diet had higher values for tissue transglutaminase, and particularly, a horse with inflammatory small bowel disease with markedly increased antibody levels presented a clinical recovery associated with improved duodenal histopathology and reduction of antibody levels after the introduction of a gluten-free diet (van der Kolk et al., 2012). The potential role of wheat in the pathogenesis of a naturally occurring enteropathy in Irish setter dogs was also studied. A cereal-containing diet induced partial villus atrophy, increased intraepithelial lymphocytes, and distinct biochemical abnormalities in the brush border of these animals; the clinical recovery was achieved on a wheat-free diet, and subsequent wheat

challenge resulted in relapse (Batt et al., 1987; Hall & Batt, 1992). Similarly, rhesus macaques with chronic diarrhea of noninfectious origin, showed symptoms compatible with celiac disease, namely intestinal lesions, and antigliadin antibodies when fed with a gluten-containing diet. Celiac-related histological and serological features were reversed with a gluten-free diet, whereas gluten reintroduction conducted to a rapid relapse (Bethune et al., 2008). Besides, the gluten-sensitive rhesus macaque model was recently used to study the effect of gluten on the level of intestinal microbiota, showing for example that some families of bacteria (Streptococcaceae and Lactobacillaceae) increased in gluten-sensitive macaques while others such as Coriobacteriaceae increased in healthy animals after consumption of gluten (Mohan et al., 2016).

5.2.2 | Induced animal models

There are also in vivo models where enteropathy is induced. The administration of gliadin from birth by intragastric probe in inbred germfree rats resulted in the shortening of jejunal villi, crypt hyperplasia, and increased number of intraepithelial lymphocytes (Stepankova et al., 1996). Later, using a similar gliadin-induced rat model, it was found that the administration of *Bifidobacterium longum* can attenuate the production of inflammatory cytokines and the T-cell mediated immune response (Laparra et al., 2012), as well as the capacity to modulate jejunal proteome (Olivares et al., 2012). Another study evaluated the role of intestinal microbiota on the permeability of the intestinal mucosa. The authors showed that the co-administration of *Shigella* CBD8 or *Escherichia coli* CBL2 with peptic fragments of gliadin could result in an increased impairment of tight junctions and translocation of gliadin peptides into the lamina propria (Cinova et al., 2011). Another induced animal model was developed by transferring gliadin-presensitized CD4⁺CD25⁻ T cell fractions from C57BL/6 donor mice to Rag1^{-/-} recipient mice, which showed deterioration of mucosal histological features typical of celiac disease when challenged with oral gluten (Freitag et al., 2009). Gluten sensitivity in mice was also established by feeding BALB/c mice with gluten-containing commercial food pellets for 30 days. The mice on this diet developed villous atrophy, crypt hyperplasia, and infiltration of T cells and macrophages in the small intestine (Papista et al., 2012).

5.2.3 | Genetically-engineered animal models

Humanized mouse models, including transgenic and knock out mice have been developed mainly to study specific pathways involved in the pathogenesis of the celiac

disease. Of these, mice that express the HLA genes DQ2 or DQ8, which are fundamental genetic factors for celiac disease development, have been the most widely used (Dieli-Crimi et al., 2015; Stoven et al., 2013). This model has been instrumental in our understanding of the importance of HLA receptors in the activation of T cells and the development of immune response in celiac disease and other HLA-associated immunological questions (Black et al., 2002; Chen et al., 2003; D'Arienzo et al., 2009; Hovhannisyan et al., 2008; Verdu et al., 2008). Also, transgenic gliadin-sensitive HCD4-DQ8 mice were used for the prevalidation of a technology that aims to create an adjuvant therapy within the context of celiac disease, for example, in a putative involuntary event of gluten exposure. It uses a synthetic polymer that binds to gluten proteins, thus decreasing its digestibility and the release of immunogenic sequences during digestion (Pinier et al., 2009).

Other studies include the identification of major immunodominant epitopes of α -gliadins (Senger et al., 2005) or the evaluation of conjugated linoleic acid in the activation of nuclear factor erythroid 2-related factor2 (Nrf2) within the context of gliadin-mediated toxicity (Bergamo et al., 2011). On the other hand, some studies with transgenic mice model have pointed out the importance of the innate immune response in the development of gluten dependent enteropathy (Stoven et al., 2013). In this context, a recent study described a mouse model that combines an adaptive response to gluten with a chronic perturbation of the innate immune system. It reproduces the overexpression of IL-15 in the gut epithelium, and lamina propria expresses the predisposing genetic factor HLA-DQ8 and develops villous atrophy when challenged with gluten (Abadie et al., 2020).

6 | CONCLUSIONS

ELISA systems are mostly used to monitor the gluten content in products labeled as gluten-free. There are multiple ELISA test kits available using different reference materials for calibration, extraction protocols, different formats (sandwich and competitive), and even different antibodies. All these differences make it almost impossible to compare and reproduce the results reported by other researchers or laboratories. The use of a standard reference material that is representative of the analyte would help to uniform all the results that researchers and laboratories report and assuring that the analytical methods are accurate. Another drawback is that commercial kits available only measure the prolamin fraction and use a correlation factor of 2 to obtain the total gluten content. This starts from a wrong assumption as it is well known that the ratio of gliadins to glutenins is quite heterogeneous for different varieties

or foods based on wheat. In this sense, some efforts have been made in the development of immunochemical tests that do the total quantification of gluten instead of quantifying only the prolamins group.

Other technologies, such as those based on DNA or proteomic approaches, seem to have other purposes than that of being a method of widespread use for the labeling of food products based on gluten content. On the one hand, DNA-based methods are highly sensitive and will have more use in authentication and detection of contamination in raw materials. On the other hand, the advanced proteomic approaches for the analysis of gluten allow its quantification and the monitoring of sequences with clinical importance. In any case, mainly due to the cost and the fact that it is a highly specialized analysis, they will hardly reach the generalized test status like the current ELISA, but they will be of great importance for the study of the celiac disease, as well as in investigations focused on its treatment. From this point of view, it is expected that the development of highly advanced in vitro and in vivo preclinical models such as the combination of organoid technology and the gut on a chip, and transgenic humanized mouse models, respectively, will precede the clinical validation of new therapies for celiac disease in the years to come. The greatest expectation is that this can contribute decisively to overcome the difficulties experienced by people who suffer from this common autoimmune disease.

ACKNOWLEDGMENTS

This work was supported by the R&D Project GLUTEN2TARGET—Optimizing natural low toxicity of wheat for celiac patients through a nano-/microparticles detoxifying approach, with reference POCI-01-0145-FEDER-029068 and PTDC/BAA-AGR/29068/2017, financed by the European Regional Development Fund (ERDF) through COMPETE 2020—Operational Program for Competitiveness and Internationalization (POCI) and by Foundation for Science and Technology (FCT). The authors also acknowledge the support by the Associate Laboratory for Green Chemistry-LAQV, which is financed by FCT under the Partnership Agreement UIDB/50006/2020.

AUTHOR CONTRIBUTIONS

Conceptualization: Miguel Ribeiro. *Investigation:* Miguel Ribeiro, Telma de Sousa, and Carolina Sabença. *Data curation:* Miguel Ribeiro, Telma de Sousa, and Carolina Sabença. *Writing—original draft preparation:* Miguel Ribeiro, Telma de Sousa, and Carolina Sabença. *Writing—review and editing:* Miguel Ribeiro and Gilberto Igrejas. *Project administration:* Gilberto Igrejas. *Funding acquisition:* Patrícia Poeta, Ana Sofia Bagulho, and Gilberto Igre-

jas. All authors have read and agreed to the published version of the manuscript.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

ORCID

Miguel Ribeiro  <https://orcid.org/0000-0002-6497-8337>

Telma de Sousa  <https://orcid.org/0000-0003-2326-6226>

Carolina Sabença  <https://orcid.org/0000-0002-7317-9123>

Patrícia Poeta  <https://orcid.org/0000-0003-0925-689X>

Ana Sofia Bagulho  <https://orcid.org/0000-0003-2430-6410>

Gilberto Igrejas  <https://orcid.org/0000-0002-6365-0735>

REFERENCES

- Abadie, V., Kim, S. M., Lejeune, T., Palanski, B. A., Ernest, J. D., Tastet, O., Voisine, J., Discepolo, V., Marietta, E. V., Hawash, M. B. F., Ciszewski, C., Bouziat, R., Panigrahi, K., Horwath, I., Zurenski, M. A., Lawrence, I., Dumaine, A., Yotova, V., Grenier, J. C., ... Jabri, B. (2020). IL-15, gluten and HLA-DQ8 drive tissue destruction in coeliac disease. *Nature*, 578(7796), 600–604. <https://doi.org/10.1038/s41586-020-2003-8>
- Administration, U. F. a. D. (2013). Food Labeling; Gluten-Free Labeling of Foods 78 FR 47154.
- Administration, U. F. a. D. (2020). Food Labeling; Gluten-Free Labeling of Fermented or Hydrolyzed Foods 21 CFR Part 101
- Allred, L. K., & Ritter, B. W. (2010). Recognition of gliadin and glutenin fractions in four commercial gluten assays. *Journal of AOAC International*, 93(1), 190–196. <https://www.ncbi.nlm.nih.gov/pubmed/20334180>
- Alves, T. O., D'Almeida, C. T. S., & Ferreira, M. S. L. (2017). Determination of gluten peptides associated with celiac disease by mass spectrometry, celiac disease and non-celiac gluten sensitivity. In L. Rodrigo (Ed.), *Celiac disease and non-celiac gluten sensitivity*. (pp. 43–58)IntechOpen.
- Alves, T. O., D'Almeida, C. T. S., Scherf, K. A., & Ferreira, M. S. L. (2019). Modern approaches in the identification and quantification of immunogenic peptides in cereals by LC-MS/MS. *Frontiers in Plant Science*, 10, 1470. <https://doi.org/10.3389/fpls.2019.01470>
- Alves, T. O., D'Almeida, C. T. S., Victorio, V. C. M., Souza, G. H. M. F., Cameron, L. C., & Ferreira, M. S. L. (2018). Immunogenic and allergenic profile of wheat flours from different technological qualities revealed by ion mobility mass spectrometry. *Journal of Food Composition and Analysis*, 73, 67–75. <https://doi.org/10.1016/j.jfca.2018.07.012>
- Ashammakhi, N., Nasiri, R., Barros, N. R. d., Tebon, P., Thakor, J., Goudie, M., Shamloo, A., Martin, M. G., & Khademhosseini, A. (2020). Gut-on-a-chip: Current progress and future opportunities. *Biomaterials*, 255, 120196. <https://doi.org/10.1016/j.biomaterials.2020.120196>
- Batt, R. M., McLean, L., & Carter, M. W. (1987). Sequential morphologic and biochemical studies of naturally occurring wheat-sensitive enteropathy in Irish setter dogs. *Digestive Diseases and Sciences*, 32(2), 184–194. <https://doi.org/10.1007/BF01297107>

- Benoit, L., Cox, D., Katepalli, M., Masiri, J., Nadala, C., Gendel, S., & Samadpour, M. (2016). *Development and characterization of a novel monoclonal antibody directed against gluten*. International Association for Food Protection. <https://iafp.confex.com/iafp/2016/webprogram/Paper11379.html>
- Benoit, L., Meshgi, M., Cox, D., Katepalli, M., Masiri, J., Sung, S., Gendel, S., & Samadpour, M. (2016). *Development and validation of a gluten-specific sandwich ELISA based on a novel monoclonal antibody, 2D4*. International Association for Food Protection. <https://iafp.confex.com/iafp/2016/webprogram/Paper11380.html>
- Bergamo, P., Gogliettino, M., Palmieri, G., Cocca, E., Maurano, F., Stefanile, R., Balestrieri, M., Mazzarella, G., David, C., & Rossi, M. (2011). Conjugated linoleic acid protects against gliadin-induced depletion of intestinal defenses. *Molecular Nutrition & Food Research*, 55(S2), S248–S256. <https://doi.org/10.1002/mnfr.201100295>
- Bermudo Redondo, M. C., Griffin, P. B., Garzon Ransanz, M., Ellis, H. J., Ciclitira, P. J., & O'Sullivan, C. K. (2005). Monoclonal antibody-based competitive assay for the sensitive detection of coeliac disease toxic prolamins. *Analytica Chimica Acta*, 551(1), 105–114. <https://doi.org/10.1016/j.aca.2005.07.023>
- Bethune, M. T., Borda, J. T., Ribka, E., Liu, M. X., Phillippi-Falkenstein, K., Jandacek, R. J., Doxiadis, G. G. M., Gray, G. M., Khosla, C., & Sestak, K. (2008). A non-human primate model for gluten sensitivity. *Plos One*, 3(2), e1614. <https://doi.org/10.1371/journal.pone.0001614>
- Black, K. E., Murray, J. A., & David, C. S. (2002). HLA-DQ determines the response to exogenous wheat proteins: A model of gluten sensitivity in transgenic knockout mice. *Journal of Immunology*, 169(10), 5595–5600. <https://doi.org/10.4049/jimmunol.169.10.5595>
- Caio, G., Volta, U., Sapone, A., Leffler, D. A., De Giorgio, R., Catassi, C., & Fasano, A. (2019). Celiac disease: A comprehensive current review. *BMC Medicine*, 17(1), 142. <https://doi.org/10.1186/s12916-019-1380-z>
- Camarca, A., Anderson, R. P., Mamone, G., Fierro, O., Facchiano, A., Costantini, S., Zanzi, D., Sidney, J., Auricchio, S., Sette, A., Troncone, R., & Gianfrani, C. (2009). Intestinal T cell responses to gluten peptides are largely heterogeneous: implications for a peptide-based therapy in celiac disease. *Journal of Immunology*, 182(7), 4158–4166. <https://doi.org/10.4049/jimmunol.0803181>
- Chen, D., Ueda, R., Harding, F., Patil, N., Mao, Y., Kurahara, C., Platenburg, G., & Huang, M. (2003). Characterization of HLA DR3/DQ2 transgenic mice: A potential humanized animal model for autoimmune disease studies. *European Journal of Immunology*, 33(1), 172–182. <https://doi.org/10.1002/immu.200390020>
- Ciclitira, P. J., Evans, D. J., Fagg, N. L. K., Lennox, E. S., & Dowling, R. H. (1984). Clinical testing of gliadin fractions in coeliac patients. *Clinical Science*, 66(3), 357–364. <https://doi.org/10.1042/cs0660357>
- Cinova, J., De Palma, G., Stepankova, R., Kofronova, O., Kverka, M., Sanz, Y., & Tuckova, L. (2011). Role of intestinal bacteria in gliadin-induced changes in intestinal mucosa: Study in germ-free rats. *Plos One*, 6(1), e16169. <https://doi.org/10.1371/journal.pone.0016169>
- Clemente, M. G., De Virgiliis, S., Kang, J. S., Macatagney, R., Musu, M. P., Di Pierro, M. R., Drago, S., Congia, M., & Fasano, A. (2003). Early effects of gliadin on enterocyte intracellular signaling involved in intestinal barrier function. *Gut*, 52(2), 218–223. <https://doi.org/10.1136/gut.52.2.218>
- Colgrave, M. L., Byrne, K., Blundell, M., & Howitt, C. A. (2016). Identification of barley-specific peptide markers that persist in processed foods and are capable of detecting barley contamination by LC-MS/MS. *Journal of Proteomics*, 147, 169–176. <https://doi.org/10.1016/j.jprot.2016.03.045>
- Comino, I., Real, A., Moreno, M. D., Montes, R., Cebolla, A., & Sousa, C. (2013). Immunological determination of gliadin 33-mer equivalent peptides in beers as a specific and practical analytical method to assess safety for celiac patients. *Journal of the Science of Food and Agriculture*, 93(4), 933–943. <https://doi.org/10.1002/jsfa.5830>
- Commission, C. A. (2008). Standard for foods for special dietary use for persons intolerant to gluten.
- D'Arienzo, R., Stefanile, R., Maurano, F., Luongo, D., Bergamo, P., Mazzarella, G., Troncone, R., Auricchio, S., David, C., & Rossi, M. (2009). A deregulated immune response to gliadin causes a decreased villus height in DQ8 transgenic mice. *European Journal of Immunology*, 39(12), 3552–3561. <https://doi.org/10.1002/eji.200839161>
- Dahinden, I., von Buren, M., & Luthy, J. (2001). A quantitative competitive PCR system to detect contamination of wheat, barley or rye in gluten-free food for coeliac patients. *European Food Research and Technology*, 212(2), 228–233. <https://doi.org/10.1007/s002170000252>
- De Palma, G., Kamanova, J., Cinova, J., Olivares, M., Drasarova, H., Tuckova, L., & Sanz, Y. (2012). Modulation of phenotypic and functional maturation of dendritic cells by intestinal bacteria and gliadin: Relevance for celiac disease. *Journal of Leukocyte Biology*, 92(5), 1043–1054. <https://doi.org/10.1189/jlb.1111581>
- de Ritis, G., Auricchio, S., Jones, H. W., Lew, E. J., Bernardin, J. E., & Kasarda, D. D. (1988). *In vitro* (organ culture) studies of the toxicity of specific A-gliadin peptides in celiac disease. *Gastroenterology*, 94(1), 41–49. <http://www.ncbi.nlm.nih.gov/pubmed/3335296>
- Di Sabatino, A., Vanoli, A., Giuffrida, P., Luinetti, O., Solcia, E., & Corazza, G. R. (2012). The function of tissue transglutaminase in celiac disease. *Autoimmunity Reviews*, 11(10), 746–753. <https://doi.org/10.1016/j.autrev.2012.01.007>
- Diaz-Amigo, C., & Popping, B. (2013). Accuracy of ELISA detection methods for gluten and reference materials: A realistic assessment. *Journal of Agricultural and Food Chemistry*, 61(24), 5681–5688. <https://doi.org/10.1021/jf3046736>
- Dieli-Crimi, R., Cenit, M. C., & Nunez, C. (2015). The genetics of celiac disease: A comprehensive review of clinical implications. *Journal of Autoimmunity*, 64, 26–41. <https://doi.org/10.1016/j.jaut.2015.07.003>
- Drago, S., El Asmar, R., Di Pierro, M., Grazia Clemente, M., Tripathi, A., Sapone, A., Thakar, M., Iacono, G., Carroccio, A., D'Agate, C., Not, T., Zampini, L., Catassi, C., & Fasano, A. (2006). Gliadin, zonulin and gut permeability: Effects on celiac and non-celiac intestinal mucosa and intestinal cell lines. *Scandinavian Journal of Gastroenterology*, 41(4), 408–419. <https://doi.org/10.1080/00365520500235334>
- Egertson, J. D., MacLean, B., Johnson, R., Xuan, Y., & MacCoss, M. J. (2015). Multiplexed peptide analysis using data-independent acquisition and Skyline. *Nature Protocols*, 10(6), 887–903. <https://doi.org/10.1038/nprot.2015.055>
- Elli, L., Bascunan, K., di Lernia, L., Bardella, M. T., Doneda, L., Soldati, L., Orlando, S., Ferretti, F., Lombardo, V., Barigelletti, G., Scricciolo, A., Fabiano, S., Vecchi, M., & Roncoroni, L. (2020). Safety of occasional ingestion of gluten in patients with celiac disease: A real-life study. *BMC Medicine*, 18(1), 42. <https://doi.org/10.1186/s12916-020-1511-6>

- Elli, L., Roncoroni, L., Hils, M., Pasternack, R., Barisani, D., Terrani, C., Vaira, V., Ferrero, S., & Bardella, M. T. (2012). Immunological effects of transglutaminase-treated gluten in coeliac disease. *Human Immunology*, 73(10), 992–997. <https://doi.org/10.1016/j.humimm.2012.07.318>
- Ellis, H. J., Rosen-Bronson, S., O'Reilly, N., & Ciclitira, P. J. (1998). Measurement of gluten using a monoclonal antibody to a coeliac toxic peptide of A gliadin. *Gut*, 43(2), 190–195. <https://doi.org/10.1136/gut.43.2.190>
- Ferreira, M. S. L., Mangavel, C., Rogniaux, H., Bonicel, J., Samson, M.-F., & Morel, M.-H. (2014). A MALDI-TOF based study of the in-vivo assembly of glutenin polymers of durum wheat. *Food Research International*, 63, 89–99. <https://doi.org/10.1016/j.foodres.2013.12.025>
- Fiedler, K. L., McGrath, S. C., Callahan, J. H., & Ross, M. M. (2014). Characterization of grain-specific peptide markers for the detection of gluten by mass spectrometry. *Journal of Agricultural and Food Chemistry*, 62(25), 5835–5844. <https://doi.org/10.1021/jf500997j>
- Freire, R., Ingano, L., Serena, G., Cetinbas, M., Anselmo, A., Sapone, A., Sadreyev, R. I., Fasano, A., & Senger, S. (2019). Human gut derived-organoids provide model to study gluten response and effects of microbiota-derived molecules in celiac disease. *Scientific Reports*, 9(1), 7029. <https://doi.org/10.1038/s41598-019-43426-w>
- Freitag, T. L., Rietdijk, S., Junker, Y., Popov, Y., Bhan, A. K., Kelly, C. P., Terhorst, C., & Schuppan, D. (2009). Gliadin-primed CD4+CD45RBlowCD252-T cells drive gluten-dependent small intestinal damage after adoptive transfer into lymphopenic mice. *Gut*, 58(12), 1597–1605. <https://doi.org/10.1136/gut.2009.186361>
- Gessendorfer, B., Koehler, P., & Wieser, H. (2009). Preparation and characterization of enzymatically hydrolyzed prolamins from wheat, rye, and barley as references for the immunochemical quantitation of partially hydrolyzed gluten. *Analytical and Bioanalytical Chemistry*, 395(6), 1721–1728. <https://doi.org/10.1007/s00216-009-3080-6>
- Gil-Humanes, J., Piston, F., Tollefsen, S., Sollid, L. M., & Barro, F. (2010). Effective shutdown in the expression of celiac disease-related wheat gliadin T-cell epitopes by RNA interference. *Proceedings of the National Academy of Sciences of the United States of America*, 107(39), 17023–17028. <https://doi.org/10.1073/pnas.1007773107>
- Graves, P. R., & Haystead, T. A. (2002). Molecular biologist's guide to proteomics. *Microbiology and Molecular Biology Reviews*, 66(1), 39–63. <https://doi.org/10.1128/mmbr.66.1.39-63.2002>
- Halbmayer-Jech, E., Rogers, A., Don, C., & Prinster, M. (2019). Gluten in rice flour and baked rice products by G12 sandwich ELISA: First action 2014.03. *Journal of AOAC International*, 98(1), 103–111. <https://doi.org/10.5740/jaoacint.14-197>
- Hall, E. J., & Batt, R. M. (1992). Dietary modulation of gluten sensitivity in a naturally occurring enteropathy of Irish setter dogs. *Gut*, 33(2), 198–205. <https://doi.org/10.1136/gut.33.2.198>
- Haraszi, R., Chassaigne, H., Maquet, A., & Ulberth, F. (2011). Analytical methods for detection of gluten in food-method developments in support of food labeling legislation. *Journal of AOAC International*, 94(4), 1006–1025. <https://www.ncbi.nlm.nih.gov/pubmed/21919334>
- Hernandez, M., Esteve, T., & Pla, M. (2005). Real-time polymerase chain reaction based assays for quantitative detection of barley, rice, sunflower, and wheat. *Journal of Agricultural and Food Chemistry*, 53(18), 7003–7009. <https://doi.org/10.1021/jf050797j>
- Hochegger, R., Mayer, W., & Prochaska, M. (2015). Comparison of R5 and G12 antibody-based ELISA used for the determination of the gluten content in official food samples. *Foods*, 4(4), 654–664. <https://doi.org/10.3390/foods4040654>
- Hovhannisyan, Z., Weiss, A., Martin, A., Wiesner, M., Tollefsen, S., Yoshida, K., Ciszewski, C., Curran, S. A., Murray, J. A., David, C. S., Sollid, L. M., Koning, F., Teyton, L., & Jabri, B. (2008). The role of HLA-DQ8 beta57 polymorphism in the anti-gluten T-cell response in coeliac disease. *Nature*, 456(7221), 534–538. <https://doi.org/10.1038/nature07524>
- Jelinkova, L., Tuckova, L., Cinova, J., Flegelova, Z., & Tlaskalova-Hogenova, H. (2004). Gliadin stimulates human monocytes to production of IL-8 and TNF-alpha through a mechanism involving NF-kappaB. *FEBS Letters*, 571(1–3), 81–85. <https://doi.org/10.1016/j.febslet.2004.06.057>
- Jira, W., & Munch, S. (2019). A sensitive HPLC-MS/MS screening method for the simultaneous detection of barley, maize, oats, rice, rye and wheat proteins in meat products. *Food Chemistry*, 275, 214–223. <https://doi.org/10.1016/j.foodchem.2018.09.041>
- Juhász, A., Haraszi, R., & Maulis, C. (2015). ProPepper: A curated database for identification and analysis of peptide and immune-responsive epitope composition of cereal grain protein families. *Database*, 2015. <https://doi.org/10.1093/database/bav100>
- Kahlenberg, F., Sanchez, D., Lachmann, I., Tuckova, L., Tlaskalova, H., Mendez, E., & Mothes, T. (2006). Monoclonal antibody R5 for detection of putatively coeliac-toxic gliadin peptides. *European Food Research and Technology*, 222(1–2), 78–82. <https://doi.org/10.1007/s00217-005-0100-4>
- Kanerva, P. M., Sontag-Strohm, T. S., Ryyppy, P. H., Alho-Lehto, P., & Salovaara, H. O. (2006). Analysis of barley contamination in oats using R5 and ω -gliadin antibodies. *Journal of Cereal Science*, 44(3), 347–352. <https://doi.org/10.1016/j.jcs.2006.08.005>
- Kasendra, M., Tovaglieri, A., Sontheimer-Phelps, A., Jalili-Firoozinezhad, S., Bein, A., Chalkiadaki, A., Scholl, W., Zhang, C., Rickner, H., Richmond, C. A., Li, H., Breault, D. T., & Ingber, D. E. (2018). Development of a primary human small intestine-on-a-chip using biopsy-derived organoids. *Scientific Reports*, 8(1), 2871. <https://doi.org/10.1038/s41598-018-21201-7>
- Kim, H. J., & Ingber, D. E. (2013). Gut-on-a-chip microenvironment induces human intestinal cells to undergo villus differentiation. *Integrative Biology*, 5(9), 1130–1140. <https://doi.org/10.1039/c3ib40126j>
- Kitteringham, N. R., Jenkins, R. E., Lane, C. S., Elliott, V. L., & Park, B. K. (2009). Multiple reaction monitoring for quantitative biomarker analysis in proteomics and metabolomics. *Journal of Chromatography. B, Analytical Technologies in the Biomedical and Life Sciences*, 877(13), 1229–1239. <https://doi.org/10.1016/j.jchromb.2008.11.013>
- Koeberl, M., Clarke, D., & Lopata, A. L. (2014). Next generation of food allergen quantification using mass spectrometric systems. *Journal of Proteome Research*, 13(8), 3499–3509. <https://doi.org/10.1021/pr500247r>
- Kretzschmar, K., & Clevers, H. (2016). Organoids: Modeling development and the stem cell niche in a dish. *Developmental Cell*, 38(6), 590–600. <https://doi.org/10.1016/j.devcel.2016.08.014>
- Lacorn, M., Weiss, T., Wehling, P., Arlinghaus, M., & Scherf, K. (2019). Quantification of wheat, rye, and barley gluten in oat and oat

- products by ELISA RIDASCREEN® total gluten: Collaborative study, first action 2018.15. *Journal of AOAC International*, 102(5), 1535–1543.
- Laparra, J. M., Olivares, M., Gallina, O., & Sanz, Y. (2012). Bifidobacterium longum CECT 7347 modulates immune responses in a gliadin-induced enteropathy animal model. *Plos One*, 7(2), e30744. <https://doi.org/10.1371/journal.pone.0030744>
- Lebreton, C., Ménard, S., Abed, J., Moura, I. C., Coppo, R., Dugave, C., Monteiro, R. C., Fricot, A., Traore, M. G., Griffin, M., Cellier, C., Malamut, G., Cerf-Bensussan, N., & Heyman, M. (2012). Interactions among secretory immunoglobulin A, CD71, and transglutaminase-2 affect permeability of intestinal epithelial cells to gliadin peptides. *Gastroenterology*, 143(3), 698–707. <https://doi.org/10.1053/j.gastro.2012.05.051>
- Lerner, A., O'Bryan, T., & Matthias, T. (2019). Navigating the gluten-free boom: The dark side of gluten free diet. *Frontiers in Pediatrics*, 7, 414. <https://doi.org/10.3389/fped.2019.00414>
- Lester, D. R. (2008). Gluten measurement and its relationship to food toxicity for celiac disease patients [Editorial]. *Plant Methods*, 4, 26. <https://doi.org/10.1186/1746-4811-4-26>
- Lexhaller, B., Tompos, C., & Scherf, K. A. (2016). Comparative analysis of prolamin and glutelin fractions from wheat, rye, and barley with five sandwich ELISA test kits. *Analytical and Bioanalytical Chemistry*, 408(22), 6093–6104. <https://doi.org/10.1007/s00216-016-9721-7>
- Lombardi, E., Bergamo, P., Maurano, F., Bozzella, G., Luongo, D., Mazzarella, G., Rotondi Aufiero, V., Iaquinto, G., & Rossi, M. (2013). Selective inhibition of the gliadin-specific, cell-mediated immune response by transamidation with microbial transglutaminase. *Journal of Leukocyte Biology*, 93(4), 479–488. <https://doi.org/10.1189/jlb.0412182>
- Lundin, K. E., Scott, H., Hansen, T., Paulsen, G., Halstensen, T. S., Fausa, O., Thorsby, E., & Sollid, L. M. (1993). Gliadin-specific, HLA-DQ(alpha 1*0501,beta 1*0201) restricted T cells isolated from the small intestinal mucosa of celiac disease patients. *Journal of Experimental Medicine*, 178(1), 187–196. <http://www.ncbi.nlm.nih.gov/pubmed/8315377>
- Mahroug, H., Ribeiro, M., Rhazi, L., Bentallah, L., Zidoune, M. N., Nunes, F. M., & Igrejas, G. (2019). How microwave treatment of gluten affects its toxicity for celiac patients? A study on the effect of microwaves on the structure, conformation, functionality and immunogenicity of gluten. *Food Chemistry*, 297, 124986. <https://doi.org/10.1016/j.foodchem.2019.124986>
- Malalgoda, M., Meinhardt, S. W., & Simsek, S. (2018). Detection and quantitation of immunogenic epitopes related to celiac disease in historical and modern hard red spring wheat cultivars. *Food Chemistry*, 264, 101–107. <https://doi.org/10.1016/j.foodchem.2018.04.131>
- Manfredi, A., Mattarozzi, M., Giannetto, M., & Careri, M. (2015). Multiplex liquid chromatography-tandem mass spectrometry for the detection of wheat, oat, barley and rye prolamins towards the assessment of gluten-free product safety. *Analytica Chimica Acta*, 895, 62–70. <https://doi.org/10.1016/j.aca.2015.09.008>
- Marietta, E. V., Schuppan, D., & Murray, J. A. (2009). In vitro and in vivo models of celiac disease. *Expert Opinion on Drug Discovery*, 4(11), 1113–1123. <https://doi.org/10.1517/17460440903307417>
- Martínez-Esteso, M. J., Brohée, M., Nørgaard, J., & O'Connor, G. (2017). Label-free proteomic analysis of wheat gluten proteins and their immunoreactivity to ELISA antibodies. *Cereal Chemistry*, 94(5), 820–826. <https://doi.org/10.1094/CCHEM-11-16-0266-R>
- Martínez-Esteso, M. J., Nørgaard, J., Brohée, M., Haraszi, R., Maquet, A., & O'Connor, G. (2016). Defining the wheat gluten peptide fingerprint via a discovery and targeted proteomics approach. *Journal of Proteomics*, 147, 156–168. <https://doi.org/10.1016/j.jpro.2016.03.015>
- Masiri, J., Benoit, L., Katepalli, M., Meshgi, M., Cox, D., Nadala, C., Sung, S. L., & Samadpour, M. (2016). Novel monoclonal antibody-based immunodiagnostic assay for rapid detection of deamidated gluten residues. *Journal of Agricultural and Food Chemistry*, 64(18), 3678–3687. <https://doi.org/10.1021/acs.jafc.5b06085>
- Mattarozzi, M., & Careri, M. (2019). The role of incurred materials in method development and validation to account for food processing effects in food allergen analysis. *Analytical and Bioanalytical Chemistry*, 411(19), 4465–4480. <https://doi.org/10.1007/s00216-019-01642-3>
- Mejías, J. H., Lu, X., Osorio, C., Ullman, J. L., von Wettstein, D., & Rustgi, S. (2014). Analysis of wheat prolamins, the causative agents of celiac sprue, using reversed phase high performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). *Nutrients*, 6(4), 1578–1597. <https://doi.org/10.3390/nu6041578>
- Mena, M. C., Lombardia, M., Hernando, A., Mendez, E., & Albar, J. P. (2012). Comprehensive analysis of gluten in processed foods using a new extraction method and a competitive ELISA based on the R5 antibody. *Talanta*, 91, 33–40. <https://doi.org/10.1016/j.talanta.2011.12.073>
- Mena, M. C., & Sousa, C. (2015). Analytical tools for gluten detection: Policies and regulation. In E. A. Sanz, F. Fernández-Bañares, A. S. Peña, L. Rodrigo, & C. M. Rosell (Eds.), *Advances in the understanding of gluten related pathology and the evolution of gluten-free foods* (pp. 527–564). OmniaScience. <https://doi.org/10.3926/oms.248>
- Mendez, E., Vela, C., Immer, U., & Janssen, F. W. (2005). Report of a collaborative trial to investigate the performance of the R5 enzyme linked immunoassay to determine gliadin in gluten-free food. *European Journal of Gastroenterology & Hepatology*, 17(10), 1053–1063. <https://doi.org/10.1097/00042737-200510000-00008>
- Mitea, C., Kooy-Winkelaar, Y., van Veelen, P., de Ru, A., Drijfhout, J. W., Koning, F., & Dekking, L. (2008). Fine specificity of monoclonal antibodies against celiac disease-inducing peptides in the gluteome. *American Journal of Clinical Nutrition*, 88(4), 1057–1066.
- Mittag, D., Niggemann, B., Sander, I., Reese, I., Fiedler, E. M., Worm, M., Vieths, S., & Reese, G. (2004). Immunoglobulin E-reactivity of wheat-allergic subjects (baker's asthma, food allergy, wheat-dependent, exercise-induced anaphylaxis) to wheat protein fractions with different solubility and digestibility. *Molecular Nutrition & Food Research*, 48(5), 380–389. <https://doi.org/10.1002/mnfr.200400016>
- Moerkens, R., Mooiweer, J., Withoff, S., & Wijmenga, C. (2019). Celiac disease-on-chip: Modeling a multifactorial disease in vitro. *United European Gastroenterology Journal*, 7(4), 467–476. <https://doi.org/10.1177/2050640619836057>
- Mohan, M., Chow, C.-E. T., Ryan, C. N., Chan, L. S., Dufour, J., Aye, P. P., Blanchard, J., Moehs, C. P., & Sestak, K. (2016). dietary gluten-induced gut dysbiosis is accompanied by selective upregulation of microRNAs with intestinal tight junction and bacteria-binding

- motifs in rhesus macaque model of celiac disease. *Nutrients*, 8(11), 684. <https://www.mdpi.com/2072-6643/8/11/684>
- Molberg, Ø., McAdam, S. N., Körner, R., Quarsten, H., Kristiansen, C., Madsen, L., Fugger, L., Scott, H., Norén, O., Roepstorff, P., Lundin, K. E. A., Sjöström, H., & Sollid, L. M. (1998). Tissue transglutaminase selectively modifies gliadin peptides that are recognized by gut-derived T cells in celiac disease. *Nature Medicine*, 4(6), 713–717. <https://doi.org/10.1038/nm0698-713>
- Moreno, M. D., Munoz-Suano, A., Lopez-Casado, M. A., Torres, M. I., Sousa, C., & Cebolla, A. (2016). Selective capture of most celiac immunogenic peptides from hydrolyzed gluten proteins. *Food Chemistry*, 205, 36–42. <https://doi.org/10.1016/j.foodchem.2016.02.066>
- Moron, B., Bethune, M. T., Comino, I., Manyani, H., Ferragud, M., Lopez, M. C., Cebolla, A., Khosla, C., & Sousa, C. (2008). Toward the assessment of food toxicity for celiac patients: Characterization of monoclonal antibodies to a main immunogenic gluten peptide. *Plos One*, 3(5), e2294. <https://doi.org/10.1371/journal.pone.0002294>
- Moron, B., Cebolla, A., Manyani, H., Alvarez-Maqueda, M., Megias, M., Thomas, M. D. C., Lopez, M. C., & Sousa, C. (2008). Sensitive detection of cereal fractions that are toxic to celiac disease patients by using monoclonal antibodies to a main immunogenic wheat peptide. *American Journal of Clinical Nutrition*, 87(2), 405–414.
- Mujico, J. R., Lombardia, M., Mena, M. C., Mendez, E., & Albar, J. P. (2011). A highly sensitive real-time PCR system for quantification of wheat contamination in gluten-free food for celiac patients. *Food Chemistry*, 128(3), 795–801. <https://doi.org/10.1016/j.foodchem.2011.03.061>
- Mustorp, S. L., Dromtorp, S. M., & Holck, A. L. (2011). Multiplex, quantitative, ligation-dependent probe amplification for determination of allergens in food. *Journal of Agricultural and Food Chemistry*, 59(10), 5231–5239. <https://doi.org/10.1021/jf200545j>
- Olivares, M., Laparra, M., & Sanz, Y. (2012). Oral administration of *Bifidobacterium longum* CECT 7347 modulates jejunal proteome in an *in vivo* gliadin-induced enteropathy animal model. *Journal of Proteomics*, 77, 310–320. <https://doi.org/10.1016/j.jprot.2012.09.005>
- Osman, A. A., Uhlig, H. H., Valdes, I., Amin, M., Mendez, E., & Mothes, T. (2001). A monoclonal antibody that recognizes a potential toxic repetitive pentapeptide epitope in gliadins. *European Journal of Gastroenterology & Hepatology*, 13(10), 1189–1193.
- Palova-Jelinkova, L., Rozkova, D., Pecharova, B., Bartova, J., Sediva, A., Tlaskalova-Hogenova, H., Spisek, R., & Tuckova, L. (2005). Gliadin fragments induce phenotypic and functional maturation of human dendritic cells. *Journal of Immunology*, 175(10), 7038–7045. <https://doi.org/10.4049/jimmunol.175.10.7038>
- Panda, R., Boyer, M., & Garber, E. A. E. (2017). A multiplex competitive ELISA for the detection and characterization of gluten in fermented-hydrolyzed foods. *Analytical and Bioanalytical Chemistry*, 409(30), 6959–6973. <https://doi.org/10.1007/s00216-017-0677-z>
- Panda, R., Fiedler, K. L., Cho, C. Y., Cheng, R., Stutts, W. L., Jackson, L. S., & Garber, E. A. (2015). Effects of a proline endopeptidase on the detection and quantitation of gluten by antibody-based methods during the fermentation of a model sorghum beer. *Journal of Agricultural and Food Chemistry*, 63(48), 10525–10535. <https://doi.org/10.1021/acs.jafc.5b04205>
- Panda, R., & Garber, E. A. E. (2019). Detection and quantitation of gluten in fermented-hydrolyzed foods by antibody-based methods: Challenges, progress, and a potential path forward. *Frontiers in Nutrition*, 6, 97. <https://doi.org/10.3389/fnut.2019.00097>
- Panda, R., Zoerb, H. F., Cho, C. Y., Jackson, L. S., & Garber, E. A. E. (2015). Detection and quantification of gluten during the brewing and fermentation of beer using antibody-based technologies. *Journal of Food Protection*, 78(6), 1167–1177. <https://doi.org/10.4315/0362-028x.Jfp-14-546>
- Papista, C., Gerakopoulos, V., Kourelis, A., Sounidaki, M., Kontana, A., Berthelot, L., Moura, I. C., Monteiro, R. C., & Yiangou, M. (2012). Gluten induces coeliac-like disease in sensitised mice involving IgA, CD71 and transglutaminase 2 interactions that are prevented by probiotics. *Laboratory Investigation*, 92(4), 625–635. <https://doi.org/10.1038/labinvest.2012.13>
- Pinier, M., Verdu, E. F., Nasser-Eddine, M., David, C. S., Vezina, A., Rivard, N., & Leroux, J. C. (2009). Polymeric binders suppress gliadin-induced toxicity in the intestinal epithelium. *Gastroenterology*, 136(1), 288–298. <https://doi.org/10.1053/j.gastro.2008.09.016>
- Raki, M., Tollefsen, S., Molberg, O., Lundin, K. E., Sollid, L. M., & Jahnsen, F. L. (2006). A unique dendritic cell subset accumulates in the celiac lesion and efficiently activates gluten-reactive T cells. *Gastroenterology*, 131(2), 428–438. <https://doi.org/10.1053/j.gastro.2006.06.002>
- Real, A., Comino, I., Moreno Mde, L., Lopez-Casado, M. A., Lorite, P., Torres, M. I., Cebolla, A., & Sousa, C. (2014). Identification and *in vitro* reactivity of celiac immunoactive peptides in an apparent gluten-free beer. *Plos One*, 9(6), e100917. <https://doi.org/10.1371/journal.pone.0100917>
- Regulation, E. C. (2009). Commission Regulation (EC) No 41/2009 of 20 January 2009 concerning the composition and labelling of foodstuffs suitable for people intolerant to gluten.
- Regulation, E. C. (2014). Commission Implementing Regulation (EU) No 828/2014 of 30 July 2014 on the requirements for the provision of information to consumers on the absence or reduced presence of gluten in food.
- Ribeiro, M., Bancel, E., Faye, A., Dardevet, M., Ravel, C., Branlard, G., & Igrejas, G. (2013). Proteogenomic characterization of novel x-type high molecular weight glutenin subunit 1Ax1.1. *International Journal of Molecular Sciences*, 14(3), 5650–5667. <http://www.mdpi.com/1422-0067/14/3/5650>
- Ribeiro, M., Lopes, S., Picascia, S., Gianfrani, C., & Nunes, F. M. (2020). Reinventing the nutraceutical value of gluten: The case of l-theanine-gluten as a potential alternative to the gluten exclusion diet in celiac disease. *Food Chemistry*, 324, 126840. <https://doi.org/10.1016/j.foodchem.2020.126840>
- Ribeiro, M., Nunes-Miranda, J. D., Branlard, G., Carrillo, J. M., Rodriguez-Quijano, M., & Igrejas, G. (2013). One hundred years of grain omics: Identifying the glutes that feed the world. *Journal of Proteome Research*, 12(11), 4702–4716. <https://doi.org/10.1021/pr400663t>
- Ribeiro, M., Nunes, F. M., Rodriguez-Quijano, M., Carrillo, J. M., Branlard, G., & Igrejas, G. (2018). Next-generation therapies for celiac disease: The gluten-targeted approaches. *Trends in Food Science and Technology*, 75, 56–71.
- Ribeiro, M., Picascia, S., Rhazi, L., Gianfrani, C., Carrillo, J. M., Rodriguez-Quijano, M., Branlard, G., & Nunes, F. M. (2019). Effect of *in situ* gluten-chitosan interlocked self-assembled supramolecular architecture on rheological properties and functionality of reduced celiac-toxicity wheat flour. *Food Hydro-*

- colloids, 90, 266–275. <https://doi.org/10.1016/j.foodhyd.2018.12.026>
- Rockendorf, N., Meckelein, B., Scherf, K. A., Schalk, K., Koehler, P., & Frey, A. (2017). Identification of novel antibody-reactive detection sites for comprehensive gluten monitoring. *Plos One*, 12(7), e0181566. <https://doi.org/10.1371/journal.pone.0181566>
- Rossi, G., Manfrin, A., & Lutolf, M. P. (2018). Progress and potential in organoid research. *Nature Reviews Genetics*, 19(11), 671–687. <https://doi.org/10.1038/s41576-018-0051-9>
- Sanchez, E., Laparra, J. M., & Sanz, Y. (2012). Discerning the role of *Bacteroides fragilis* in celiac disease pathogenesis. *Applied and Environmental Microbiology*, 78(18), 6507–6515. <https://doi.org/10.1128/AEM.00563-12>
- Schalk, K., Koehler, P., & Scherf, K. A. (2018a). Quantitation of specific barley, rye, and oat marker peptides by targeted liquid chromatography-mass spectrometry to determine gluten concentrations. *Journal of Agricultural and Food Chemistry*, 66(13), 3581–3592. <https://doi.org/10.1021/acs.jafc.7b05286>
- Schalk, K., Koehler, P., & Scherf, K. A. (2018b). Targeted liquid chromatography tandem mass spectrometry to quantitate wheat gluten using well-defined reference proteins. *Plos One*, 13(2), e0192804. <https://doi.org/10.1371/journal.pone.0192804>
- Schalk, K., Lang, C., Wieser, H., Koehler, P., & Scherf, K. A. (2017). Quantitation of the immunodominant 33-mer peptide from alpha-gliadin in wheat flours by liquid chromatography tandem mass spectrometry. *Scientific Reports*, 7, 45092. <https://doi.org/10.1038/srep45092>
- Scherf, K. A., Catassi, C., Chirido, F., Ciclitira, P. J., Feighery, C., Gianfrani, C., Koning, F., Lundin, K. E. A., Schuppan, D., Smulders, M. J. M., Tranquet, O., Troncone, R., & Koehler, P. (2020). Recent progress and recommendations on celiac disease from the working group on prolamins analysis and toxicity. *Frontiers in Nutrition*, 7, 29. <https://doi.org/10.3389/fnut.2020.00029>
- Schumann, M., Richter, J. F., Wedell, I., Moos, V., Zimmermann-Kordmann, M., Schneider, T., Daum, S., Zeitz, M., Fromm, M., & Schulzke, J. D. (2008). Mechanisms of epithelial translocation of the alpha(2)-gliadin-33mer in coeliac sprue. *Gut*, 57(6), 747–754. <https://doi.org/10.1136/gut.2007.136366>
- Sealey-Voyksner, J. A., Khosla, C., Voyksner, R. D., & Jorgenson, J. W. (2010). Novel aspects of quantitation of immunogenic wheat gluten peptides by liquid chromatography-mass spectrometry/mass spectrometry. *Journal of Chromatography A*, 1217(25), 4167–4183. <https://doi.org/10.1016/j.chroma.2010.01.067>
- Segura, V., Díaz, J., Ruiz-Carnicer, Á., Muñoz-Suano, A., Carrillo-Carrión, C., Sousa, C., Cebolla, Á., & Comino, I. (2021). Rapid, effective, and versatile extraction of gluten in food with application on different immunological methods. *Foods*, 10(3), 652. <https://doi.org/10.3390/foods10030652>
- Senger, S., Maurano, F., Mazzeo, M. F., Gaita, M., Fierro, O., David, C. S., Troncone, R., Auricchio, S., Siciliano, R. A., & Rossi, M. (2005). Identification of immunodominant epitopes of alpha-gliadin in HLA-DQ8 transgenic mice following oral immunization. *Journal of Immunology*, 175(12), 8087–8095. <https://doi.org/10.4049/jimmunol.175.12.8087>
- Shan, L., Molberg, O., Parrot, I., Hausch, F., Filiz, F., Gray, G. M., Sollid, L. M., & Khosla, C. (2002). Structural basis for gluten intolerance in celiac sprue. *Science*, 297(5590), 2275–2279. <https://doi.org/10.1126/science.1074129>
- Shan, L., Qiao, S. W., Arentz-Hansen, H., Molberg, O., Gray, G. M., Sollid, L. M., & Khosla, C. (2005). Identification and analysis of multivalent proteolytically resistant peptides from gluten: Implications for celiac sprue. *Journal of Proteome Research*, 4(5), 1732–1741. <https://doi.org/10.1021/pr050173t>
- Shatalova, A., Shatalov, I., & Lebedin, Y. (2020). X6: A novel antibody for potential use in gluten quantification. *Molecules*, 25(14), 3107.
- Skerritt, J. H., & Hill, A. S. (1990). Monoclonal-antibody sandwich enzyme immunoassays for determination of gluten in foods. *Journal of Agricultural and Food Chemistry*, 38(8), 1771–1778. <https://doi.org/10.1021/jf00098a029>
- Skodje, G. I., Sarna, V. K., Minelle, I. H., Rolfsen, K. L., Muir, J. G., Gibson, P. R., Veierød, M. B., Henriksen, C., & Lundin, K. E. A. (2018). Fructan, rather than gluten, induces symptoms in patients with self-reported non-celiac gluten sensitivity. *Gastroenterology*, 154(3), 529–539. <https://doi.org/10.1053/j.gastro.2017.10.040>
- Slot, I. D. B., Van der Fels-Klerx, H. J., Bremer, M. G. E. G., & Hamer, R. J. (2016). Immunochemical detection methods for gluten in food products: Where do we go from here? *Critical Reviews in Food Science and Nutrition*, 56(15), 2455–2466. <https://doi.org/10.1080/10408398.2013.847817>
- Sollid, L. M., Qiao, S. W., Anderson, R. P., Gianfrani, C., & Koning, F. (2012). Nomenclature and listing of celiac disease relevant gluten T-cell epitopes restricted by HLA-DQ molecules. *Immunogenetics*, 64(6), 455–460. <https://doi.org/10.1007/s00251-012-0599-z>
- Spaenij-Dekking, E. H. A., Kooy-Winkelaar, E. M. C., Nieuwenhuizen, W. F., Drijfhout, J. W., & Koning, F. (2004). A novel and sensitive method for the detection of T cell stimulatory epitopes of α/β - and γ -gliadin. *Gut*, 53(9), 1267–1273. <https://doi.org/10.1136/gut.2003.037952>
- Spence, J. R., Mayhew, C. N., Rankin, S. A., Kuhar, M. F., Vallance, J. E., Tolle, K., Hoskins, E. E., Kalinichenko, V. V., Wells, S. I., Zorn, A. M., Shroyer, N. F., & Wells, J. M. (2011). Directed differentiation of human pluripotent stem cells into intestinal tissue *in vitro*. *Nature*, 470(7332), 105–120. <https://doi.org/10.1038/nature09691>
- Stepankova, R., Tlaskalova-Hogenova, H., Sinkora, J., Jodl, J., & Fric, P. (1996). Changes in jejunal mucosa after long-term feeding of germfree rats with gluten. *Scandinavian Journal of Gastroenterology*, 31(6), 551–557. <https://doi.org/10.3109/00365529609009127>
- Stoven, S., Murray, J. A., & Marietta, E. V. (2013). Latest *in vitro* and *in vivo* models of celiac disease. *Expert Opinion on Drug Discovery*, 8(4), 445–457. <https://doi.org/10.1517/17460441.2013.761203>
- Syage, J. A., Kelly, C. P., Dickason, M. A., Ramirez, A. C., Leon, F., Dominguez, R., & Sealey-Voyksner, J. A. (2018). Determination of gluten consumption in celiac disease patients on a gluten-free diet. *American Journal of Clinical Nutrition*, 107(2), 201–207. <https://doi.org/10.1093/ajcn/nqx049>
- Thompson, T., & Méndez, E. (2008). Commercial assays to assess gluten content of gluten-free foods: Why they are not created equal. *Journal of the American Dietetic Association*, 108(10), 1682–1687. <https://doi.org/10.1016/j.jada.2008.07.012>
- Tranquet, O., Lupi, R., Echasserieau-Laporte, V., Pietri, M., Larre, C., & Denery-Papini, S. (2015). Characterization of antibodies and development of an indirect competitive immunoassay for detection of deamidated gluten. *Journal of Agricultural and Food Chemistry*, 63(22), 5403–5409. <https://doi.org/10.1021/acs.jafc.5b00923>
- Tye-Din, J. A., Stewart, J. A., Dromey, J. A., Beissbarth, T., van Heel, D. A., Tatham, A., Henderson, K., Mannering, S. I., Gianfrani, C., Jewell, D. P., Hill, A. V., McCluskey, J., Rossjohn, J., & Anderson, R.

- P. (2010). Comprehensive, quantitative mapping of T cell epitopes in gluten in celiac disease. *Science Translational Medicine*, 2(41), 41ra51. <https://doi.org/10.1126/scitranslmed.3001012>
- Uthayakumaran, S., & Wrigley, C. (2017). Wheat: Grain-quality characteristics and management of quality requirements. In C. Wrigley, I. Batey, & D. Miskelly (Eds.), *Cereal grains* (pp. 91–134, 2nd ed.). Woodhead Publishing. <https://doi.org/10.1016/B978-0-08-100719-8.00005-X>
- Uvackova, L., Skultety, L., Bekesova, S., McClain, S., & Hajduch, M. (2013a). The MSE-proteomic analysis of gliadins and glutenins in wheat grain identifies and quantifies proteins associated with celiac disease and baker's asthma. *Journal of Proteomics*, 93, 65–73. <https://doi.org/10.1016/j.jprot.2012.12.011>
- Uvackova, L., Skultety, L., Bekesova, S., McClain, S., & Hajduch, M. (2013b). MSE based multiplex protein analysis quantified important allergenic proteins and detected relevant peptides carrying known epitopes in wheat grain extracts. *Journal of Proteome Research*, 12(11), 4862–4869. <https://doi.org/10.1021/pr400336f>
- Valdes, I., Garcia, E., Llorente, M., & Mendez, E. (2003). Innovative approach to low-level gluten determination in foods using a novel sandwich enzyme-linked immunosorbent assay protocol. *European Journal of Gastroenterology & Hepatology*, 15(5), 465–474. <https://doi.org/10.1097/01.meg.0000059119.41030.df>
- van den Broeck, H. C., Cordewener, J. H. G., Nessen, M. A., America, A. H. P., & van der Meer, I. M. (2015). Label free targeted detection and quantification of celiac disease immunogenic epitopes by mass spectrometry. *Journal of Chromatography A*, 1391, 60–71. <https://doi.org/10.1016/j.chroma.2015.02.070>
- van der Kolk, J. H., van Putten, L. A., Mulder, C. J., Grinwis, G. C., Reijm, M., Butler, C. M., & von Blomberg, B. M. (2012). Gluten-dependent antibodies in horses with inflammatory small bowel disease (ISBD). *Veterinary Quarterly*, 32(1), 3–11. <https://doi.org/10.1080/01652176.2012.675636>
- Verdu, E. F., Huang, X., Natividad, J., Lu, J., Blennerhassett, P. A., David, C. S., McKay, D. M., & Murray, J. A. (2008). Gliadin-dependent neuromuscular and epithelial secretory responses in gluten-sensitive HLA-DQ8 transgenic mice. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, 294(1), G217–G225. <https://doi.org/10.1152/ajpgi.00225.2007>
- Victorio, V. C. M., Souza, G., Santos, M. C. B., Vega, A. R., Cameron, L. C., & Ferreira, M. S. L. (2018). Differential expression of albumins and globulins of wheat flours of different technological qualities revealed by nanoUPLC-UDMS(E). *Food Chemistry*, 239, 1027–1036. <https://doi.org/10.1016/j.foodchem.2017.07.049>
- Weng, X., Gaur, G., & Neethirajan, S. (2016). Rapid detection of food allergens by microfluidics ELISA-based optical sensor. *Biosensors*, 6(2), 24. <https://doi.org/10.3390/bios6020024>
- Wieser, H. (2007). Chemistry of gluten proteins. *Food Microbiology*, 24(2), 115–119. <https://doi.org/10.1016/j.fm.2006.07.004>
- Wieser, H. (2008). Detection of gluten. In E. K. Arendt & F. Dal Bello (Eds.), *Gluten-free cereal products and beverages* (pp. 47–80). Academic Press. <https://doi.org/10.1016/B978-012373739-7.50005-8>
- Wieser, H., Seilmeier, W., & Belitz, H. D. (1994). Quantitative determination of gliadin subgroups from different wheat cultivars. *Journal of Cereal Science*, 19(2), 149–155. <https://doi.org/10.1006/jcrs.1994.1020>
- Wolf, C., Siegel, J. B., Tinberg, C., Camarca, A., Gianfrani, C., Paski, S., Guan, R., Montelione, G., Baker, D., & Pultz, I. S. (2015). Engineering of Kuma030: A gliadin peptidase that rapidly degrades immunogenic gliadin peptides in gastric conditions. *Journal of the American Chemical Society*, 137(40), 13106–13113. <https://doi.org/10.1021/jacs.5b08325>
- Workman, M. J., Gleeson, J. P., Troisi, E. J., Estrada, H. Q., Kerns, S. J., Hinojosa, C. D., Hamilton, G. A., Targan, S. R., Svendsen, C. N., & Barrett, R. J. (2018). Enhanced utilization of induced pluripotent stem cell-derived human intestinal organoids using micro-engineered chips. *Cellular and Molecular Gastroenterology and Hepatology*, 5(4), 669–677. <https://doi.org/10.1016/j.jcmgh.2017.12.008>
- Zanzi, D., Stefanile, R., Santagata, S., Iaffaldano, L., Iaquinto, G., Giardullo, N., Lania, G., Vigliano, I., Vera, A. R., Ferrara, K., Auricchio, S., Troncone, R., & Mazzarella, G. (2011). IL-15 interferes with suppressive activity of intestinal regulatory T cells expanded in Celiac disease. *American Journal of Gastroenterology*, 106(7), 1308–1317. <https://doi.org/10.1038/ajg.2011.80>
- Zhang, J., Portela, S. B., Horrell, J. B., Leung, A., Weitmann, D. R., Artiuch, J. B., Wilson, S. M., Cipriani, M., Slakey, L. K., Burt, A. M., Dias Lourenco, F. J., Spinali, M. S., Ward, J. R., Seit-Nebi, A., Sundvor, S. E., & Yates, S. N. (2019). An integrated, accurate, rapid, and economical handheld consumer gluten detector. *Food Chemistry*, 275, 446–456. <https://doi.org/10.1016/j.foodchem.2018.08.117>
- Zhang, X., Liu, D., Jiang, W., Guo, X., Yang, W., Sun, J., Ling, H., & Zhang, A. (2011). PCR-based isolation and identification of full-length low-molecular-weight glutenin subunit genes in bread wheat (*Triticum aestivum* L.). *Theoretical and Applied Genetics*, 123(8), 1293–1305. <https://doi.org/10.1007/s00122-011-1667-8>

How to cite this article: Ribeiro, M., de Sousa, T., Sabença, C., Poeta, P., Bagulho, A. S., & Igrejas, G. (2021). Advances in quantification and analysis of the celiac-related immunogenic potential of gluten. *Compr Rev Food Sci Food Saf*, 1–21. <https://doi.org/10.1111/1541-4337.12828>