Proceedings of the 37th Meeting

Working Group on Prolamin Analysis and Toxicity

26 – 28 September, 2024
Darmstadt, Germany

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WORKING GROUP on PROLAMIN ANALYSIS and TOXICITY

Edited by Carmen Gianfrani and Knut Lundin

Impressum

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26-28 September 2024

Darmstadt, Germany

Scientific Organization

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Preface

Dear Colleagues,

the 37th meeting of the Working Group on Prolamin Analysis and Toxicity (WGPAT) was held in Darmstadt, Germany, from 26 to 28 September 2024.

On behalf of Prolamin Working Group, we are very grateful to R-Biopharm AG for being the local host of the meeting. The warm hospitality has been deeply appreaciated by all participants. A special thanks to Sigrid Haas-Lauterbach, Stefan Schmidt and Johanna Meder for taking care of every aspect of the meeting, thus making the stay in Darmstadt very pleasant.

A special thanks also to Alisha Fimmler from Deutsche Zöliakie-Gesellschaft (DZG) for taking over the registration process.

We had a symposium on “Gluten contamination: how real is the risk in commercially and freshly prepared gluten-free products”, a very timely topic.

So far, the R5/G12-ELISA has been globally the gold standard procedure for a detection of gluten protein content, with a large application in gluten free food industry. However, ELISA has some limitations, above all the sensitivity to detect gluten concentration lower than few ppm or in malt beverages containing partially hydrolyzed gluten proteins. At symposium, more advanced analytical techniques, such as mass spectrometry (MS)-based proteomics, were extensively explored.

We would like to thanks all speakers of symposium, WGPAT members’ and industry sessions who gave high-level oral communications that stimulated a deep discussion among all participants.

Naples, April 30, 2025 Carmen Gianfrani

Oslo, April 30, 2025 Knut Lundin

Table of Contents

1 Executive summary

We had overall sixteen presentations by chemists, gastroenterologists, pediatricians, nutritionists, epidemiologists and representative of celiac patients' associations, all recognized experts in the field.

Four presentations were given at the symposium, nine presentations at analytical and clinical WGPAT members' sessions and, finally, three presentations were given from industry and patients associations delegates.

A special overview of « Historical origin of the Prolamin working group » was given by Conleth Fighery, Emeritus Professor of Gastroenterology, Trinity College of Dublin.

2 List of Participants

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3 Programme

Thursday, 26 September, 2024

20:00 Arrival of Prolamin Working Group and all participants

Informal get-together with dinner (included in registration)

Location: Restaurant Gaumenfreund, Eschollbrücker Str. 16, 64295 Darmstadt, located at the H+ Hotel.

Friday, 27 September, 2024

08:15 Bus transfer to R-Biopharm AG

09:00 Opening of the meeting (*Carmen Gianfrani, Naples, Italy)*

09:10 R-Biopharm introductive lecture: New assay developments for gluten quantitation. (*Thomas Weiss, R-Biopharm AG, Darmstadt)*

09:35 Analytical research reports

- *Kim Lorenz, Eleonora Tissen, Paul Ciclitira*

10:50 *Coffee break*

11:20 **Analytical research reports (continued)**

- *Renè Smulders, Detlef Schuppan, Fernando Chirdo*

13:00 Lunch

14:00 Clinical research reports

- *Nancy Odden, Chiara Monachesi*

14:50 Coffee break

15:20 Production tour of R-Biopharm AF for all participants

16:00 The Prolamin Working Group Executive Meeting (members only)

19:30 Dinner at Restaurant Sitte, Karlstr. 15, 64283 Darmstadt

Saturday, 28 September, 2024

09:00-10:40 Symposium

"Gluten contamination: how real is the risk in commercially and freshly prepared gluten-free products"

- *Chair: Peter Koehler, Esslingen, Germany*

09:10 Analytical approaches to determine prolamin contents in foods: role of mass spectrometry and proteomics in the characterization of celiac epitopes

- *Gianfranco Mamone, Avellino, Italy*

09:40 Risk of gluten contamination when dining out: is it always a safe gluten-free experience? The International Celiac Disease MUlticenter Pizza Project (CD-MUPP).

- *Chiara Monachesi, Ancona, Italy*

10:10 Role of GIP examinations in clinical practice and research

- *Knut Lundin, Oslo, Norway*

10:40 Coffee break

11:10 How challenging is the compliance to gluten free diet? The nutritionists' perspective

- *Nick Trott, Sheffield, UK*

11:40 General discussion

12:00 Update on regulatory issues.

- *Hertha Deutsch, AOECS Codex Delegate, Vienna, Austria*

12:20 Update from industry: Legal aspects and certification of gluten-free sourdough.

- *Karoline Terberger, BÖCKER GmbH & Co., Minden, Germany*

12:40 Outline: Action plan 2025 of the Prolamin Working Group.

Carmen Gianfrani, Naples, Italy

13:00 Lunch box and farewell

Afternoon activity:

15:00 Walking tour of Darmstadt

19:00 Joint Dinner at City Braustüb'l, Wilhelminenstr. 31, 64283 Darmstadt

4 Introductive lecture

New assay developments for gluten quantitation

Thomas Weiss[1]

[1] *R-Biopharm AG, Darmstadt, Germany*

Abstract

To meet different requirements for gluten testing along the food production chain, R-Biopharm AG has developed two new products: the ELISA RIDASCREEN®EASY Gluten RAE7071 and the **quantitative** lateral flow device (LFD) RIDA®QUICK Gluten quant. RAL7073. Both products contain the monoclonal R5 antibody and are calibrated to the MoniQA wheat flour (1). Sample extraction is performed with an easy-to-use extraction tablet in combination with 60% ethanol. The measurement range is 3-48 mg/kg gluten for the ELISA and 2-40 mg/kg gluten for the LFD. The latter is read using the SMART®APP on an android cell phone.

Both assays were validated according to the new AOAC Gluten Validation Guidance Document (2). One of the main features of the new validation guideline is the major usage of incurred matrices, which means that the contamination with gluten takes place **prior** to the main processing step (usually heat treatment). The different gluten sources wheat, rye and barley are rotated across the different matrices.

Matrix	Processing	Mean recoveries (%) in ELISA RAE7071	Mean recoveries (%) in LFD RAL7073
Cookies	25 min 150°C/302°F	109 (w); 176 (r); 156 (b)	118 (w); 144 (r); 103 (b)
Cake	55 min 170°C/338°F	94 (w); 124 (r)	78 (w); 100 (r); 82 (b)
Sauce	5 min 100°C/212°F, 10 min 80°C/176°F	85 (w)	97 (w)
Dessert	10 min 100°C/212°F	97 (w); 166 (b)	92 (r)
Spices	Mixed / blended	102 (w)	88 (w)

Table 1: Summary of recoveries of the different incurred matrices tested. Gluten sources used for contamination: (w) wheat; (r) rye; (b) barley.

108 substances were tested for potential cross reactivities in both assays. Except for soy drink, no cross reactivities were observed.

Conclusion: the ELISA RIDASCREEN®EASY Gluten RAE7071 and the **quantitative** LFD RIDA®QUICK Gluten quant. show very good recoveries in highly processed materials within the AOAC requirement of 50 - 200% for wheat, rye and barley.

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1. Schall *et al.* Characterisation and comparison … *Food Chemistry 2020* **Vol. 313**, 126049
2. AOAC International Guidance for Validation of Quantitative Gluten Methods, Draft for Review, October 2023

5 Analytical research reports

5.1 Exploring Gluten Digestibility: LC-MS/MS Analysis of Immunoreactive Peptides in Flour and Bread Following In Vitro Digestion

Kim Karolin Lorenz[1], *Katharina Anne Scherf*[1,2]

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Abstract

Wheat-related disorders, including celiac disease, non-celiac gluten sensitivity, and wheat allergy, affect an increasing number of individuals, causing a variety of symptoms. These disorders are triggered by eating gluten-containing grains due to some gluten peptides being resistant to proteolysis in the gastrointestinal tract. Yet, as long as the exact molecular structures of these gluten immunoreactive peptides (GIP) are unknown, it is impossible to track their fate in the human body. Our project aims to unravel these peptide sequences after gastrointestinal digestion and investigate the influence of different factors, like food processing or ingredient ratios, on gluten digestibility and the GIP profile.

The GIPs from the digesta were identified using an untargeted nano-liquid chromatography-tandem mass spectrometry (nLC-MS/MS) experiment conducted in data-dependent acquisition mode. A proteomics workflow was developed and optimized to ensure reliable peptide identification. This workflow involves several steps: centrifugation, peptide clean-up, drying, dilution, nLC-MS/MS measurement, and data evaluation. The peptide clean-up and the data evaluation were optimized to increase the number of identifiable GIPs from the digesta.

Multiple peptide clean-up methods were evaluated, including filter-aided sample preparation, solid phase extraction (SPE), a precipitation method, and single-pot solid-phase-enhanced sample preparation. Among these, the SPE method proved to be most effective for peptide identification. Consequently, various columns with different sorbent materials were tested for SPE, including reversed-phase (RP) SPE, cation-exchange SPE, and mixed-mode SPE. Most GIPs were identified using the mixed-mode SPE, which was chosen as the preferred peptide clean-up method.

Significant improvements in peptide identification were achieved by refining the settings in the proteomics software, adjusting digestive enzymes, missed cleavages, peptide modifications, and peptide size. Up to 60% of identified peptides were modified, mainly by oxidations, deamidations, and amidations. Peptide lengths ranged from 6 to 41 amino acids, with an average length of 17 and up to 15 missed cleavages per peptide.

The optimization of data evaluation proved to have the most substantial effect on the number of identifiable peptides. Only 15 GIPs were identified without any optimizations, whereas an optimized data evaluation increased this number to 90. Furthermore, by combining all workflow optimizations, the total number of identified GIPs rose to 136.

5.2 Quantitation of Celiac Disease-Active Peptides in Beer by LC-MS/MS

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Abstract

Celiac disease (CeD) is an inflammatory disease of the small intestine that causes serious health issues in patients due to foods containing gluten. Currently, the only therapeutic option is a gluten-free (GF) diet. Therefore, affected individuals rely on the correct declaration of GF foods. GF beers made from barley could potentially cause symptoms in CeD patients due to CeD-active peptides occurring from partially hydrolyzed gluten. Routine methods like the competitive enzyme-linked immunosorbent assay (ELISA) may not detect all small yet harmful peptide fragments, leading to a potential risk for individuals with CeD. The current study aimed to develop a liquid chromatography-tandem mass spectrometry (LC-MS/MS) method to identify CeD-active peptides in GF beers.

Single-pot solid-phase-enhanced sample preparation (SP3) was used for the untargeted LC-MS/MS analysis of 21 commercially available GF barley beers and four common or carbohydrate-reduced barley beers. Based on the guidelines for risk assessment of allergenicity by the European Food Safety Authority (EFSA), the identified peptides with a complete CeD-active epitope sequence were considered CeD-hazardous [1]. We identified complete CeD-active epitopes in different gluten-free barley beers, which can, therefore, be classified as hazardous, as the peptides can be recognized by CD4+ T cells of CeD patients [2]. In total, 44 CeD-active peptides were identified in seven GF beers and four non-GF beers with LC-MS/MS. Seventeen of the identified CeD-active peptides did not contain a recognition epitope of the R5 or G12 ELISA and would thus not be detected by the competitive ELISAs.

The results of this work indicate that GF foods such as beers containing partially hydrolyzed gluten may pose a risk to CeD patients due to the presence of CeD-active peptides. By analyzing CeD-active peptides by LC-MS/MS, modification and hydrolysis of peptides can be identified and tracked in processed products. This represents a major advantage over currently used methods to detect gluten. However, the method needs further optimization and must be applied and validated on other foods containing partially hydrolyzed gluten. This highlights the ongoing need for research and development in the field of food safety and celiac disease.

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5.3 Worldwide gene editing policy: implication for development of wheat cultivars with reduced gluten immunogenicity

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Abstract

In wheat, coeliac disease (CD) epitopes occur mostly in gliadins, while the baking quality is determined predominantly by glutenins. As bread wheat varieties contain around 100 gliadin and glutenin genes, most of which contain one or more CD epitopes, genetically linked on chromosomes 1 and 6, traditional breeding cannot efficiently generate bread wheat that is safe for coeliac disease patients while retaining baking quality. However, employing targeted mutagenesis by gene editing with CRISPR/Cas has made it feasible to edit and/or delete gliadin genes[1,2,3]. Once hypoallergenic loci of multiple genes have been obtained, they may be combined through regular crossing and selecting. An intermediate product will be low-gluten wheat varieties, not yet safe for CD patients, but of interest to people who want to lower their gluten intake, e.g., people with (self-diagnosed) non-coeliac gluten wheat sensitivity.

CRISPR/Cas gene editing does not introduce foreign genes in the end-product. For that reason, many countries have relaxed the legislation on release of genetically modified plants in the environment for gene-edited plants. In the EU, the European Commission published a proposal for a revised regulation in July 2023. As part of the process of developing a new regulation, an inception impact assessment was made of the socio-economic impacts, including the potential contribution of low-gluten, CD-safe wheat for food security, nutrition and public health[4].

In the presentation we provided an outline of the proposal of the Commission and a description of the legislative process that follows a Commission proposal to the European Parliament and to the Council of Member States, all of which must agree with it, and then they have to combine the amended proposals into one text that is agreed by the three bodies.

The ongoing discussions do not center on safety of the changes, which are more precise than generated by random mutagenesis, but include freedom of choice (labelling)[5] and power issues (patents on methodology and traits that may limit the use of the varieties for further breeding)[6].

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5.4 Clinical Studies in CeD Therapeutics

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Abstract

Celiac disease is the most common chronic inflammatory disease of the small intestine. Classic symptoms are abdominal pain, diarrhea, malabsorption with anemia or osteoporosis, weight loss, and in children failure to thrive. Non-specific symptoms such as poor performance, headaches and joint pain are also common. Up to 30% of adult coeliac patients suffer from associated autoimmune diseases, including thyroid and rheumatoid diseases or type 1 diabetes. The pathogenesis of coeliac disease is well studied. Incompletely digested gluten peptides reach the immune system of the intestinal mucosa and activate glute-reactive T cells, which lead to inflammation and atrophy of the absorptive villi. The prerequisite for the development of celiac disease is the carrier status for HLA-DQ2 or DQ8, as well as the enzyme and coeliac disease autoantigen transglutaminase-2 (TG2) expressed in the intestine, which modifies the gluten peptides by deamidation and thus increases their binding to HLA-DQ2/DQ8 and subsequent T-cell activation. Despite the gluten-free diet, 30-40% of diagnosed patients continue to have moderate symptoms with signs of inflammation, often due to unavoidable minimal gluten contamination in everyday life, classified as non-responsive celiac disease (NRCD). Therefore, supportive pharmacological therapy is needed (1,2). Several promising therapeutic approaches with a clear mechanism of action and signals of efficacy are currently in clinical phase 2 development, including an oral inhibitor of intestinal TG2 (3; Falk Pharma/Takeda), blocking antibodies against interleukin (IL)-15, IL-2/IL-15 (AMG/ProventionBio, Calypso/Novartis, AnaptysBio/Teva), Ox40 ligand (4; Sanofi), the HLA-DQ2/gliadin peptide complex (5;Chugai), an oral sirtuin-6 agonist to stabilize the intestinal barrier (6; Immunic), a highly active oral gluten degrading enzyme (7), as well as nanoparticular therapies that can induce tolerance to gluten by activating tolerogenic immune cells in the spleen or liver (8,9; Takeda, Anokion). Current results indicate protection from gluten challenge induced intestinal damage and symptoms. How far patents with NRCD will profit from these drugs is evaluated in some current studies.

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5.5 Update on ATIs: Mechanisms and Clinical Studies

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Abstract

Wheat amylase trypsin inhibitors (ATIs), a family of up to 17 non- gluten wheat proteins, are allergens in Baker's Asthma and triggers of inflammatory non-celiac wheat sensitivity (NCWS). Thus, dietary ATIs ingested via wheat products stimulate Toll-Like Receptor 4 (TLR4) on intestinal myeloid cells, which promotes intestinal and extra-intestinal inflammatory diseases, such as autoimmune diseases, metabolism associated steatotic liver disease and type 2 diabetes, both in mouse experimental (1-5) and in human studies (6-8). Due to their strong disulfide bonds that endow them with resistance to intestinal proteases and heat during food processing, ATIs possess strong immunogenic potential even after heating and cooking (1,7). Several attempts proved to be ineffective in degradation of bioactive ATIs in wheat flours. While the highly expressed recombinant ATIs CM3 and 0.19 display high TLR4 stimulating activity in cellular reporter assays (1,2), our unpublished data indicate that other ATIs, such as 0.53, CMX, CM2 and CM16 also display modest TLR4 stimulating activity (9-10). Importantly, optimal stimulation depends not only on ATI isoforms but also on post-translational processing that provide a better fit for the TLR4 binding pocket. ATIs can also interact non-covalently with other wheat proteins. This explains, why mere ATI quantification by mass spectrometry does not predict ATI bioactivity and that different wheat species and cultivars (and resultant wheat flours) can differ widely in their content of bioactive ATIs despite comparable ATI protein content. We also refined prior studies on the impact of germination and dough fermentation methods on ATI bioactivities, revealing an up to 50% bioactivity reduction, while even prolonged yeast fermentation was ineffective (11). Here, quantitative Western blotting for some ATI subspecies showed effective degradation, which could not be demonstrated by mass spectrometry. While TLR4 activating potential remains stable in defined wheats and flours, these studies reveal a highly complex interplay of different factors that determine final ATI bioactivity and disease promoting potential.

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5.6 Role of MHC class II expression in intestinal epithelial cells.

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Abstract

Intestinal epithelial cell (IEC) damage is a hallmark of celiac disease (CeD). However, the role of IECs in gluten dependent T-cell activation is unknown. Using a mouse model, we investigated IEC-gluten-T cell interactions in organoid monolayers from intestinal epithelium. This epithelial organoids express human MHC class II (HLA-DQ2.5), molecule required for gluten recognition by $CD4^+$ T cells.

To this end, intestinal organoid monolayers from gluten-sensitized DR3-DQ2.5 mice, non-sensitized and naïve mice, were treated with or without IFNγ, and MHC class II was determined by flow cytometry. Organoid monolayer incubated with gluten and murine $CD4^+$ T cell expressing human CD4 from gluten-immunized mouse, were co-cultured. T cell function was assessed by cell proliferation and expression of activation markers.

Monolayers derived from gluten-sensitized mice expressed MHC class II (HLA-DQ), which was upregulated by IFN-γ. In monolayer-T cell co-cultures, gluten increased the proliferation of $CD4^+$ T cells, which was paralleled by increased expression of T cell activation markers. These changes were not observed when zeins was used as antigen.

In conclusion, MHC class II-expressing IECs activate gluten-specific $hCD4^+$ T cells, We described IECs as non-conventional antigen presenting cell which may play a relevant role in the amplification of the gluten-specific immune response in CeD.

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6 Clinical research reports

6.1 Clinical short-term challenges of intact and hydrolysed barley gluten peptides of CeD patients in remission

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Abstract

Background: Patients with celiac disease (CeD) are restricted to a gluten free diet and are advised to limit the gluten intake to no more than 10 mg per day to prevent symptom recurrence and long-term complications[1]. The threshold for a safe gluten intake remains debated, with current guidelines primarily based on research focused on wheat rather than rye and barley. It is largely unknown whether hydrolyzed gluten proteins from barley, which are frequently used in food industry, retain immunogenetic properties. Due to the lack of reliable quantitative results, it remains challenging to assess whether these trace amounts pose immunologically risk for CeD-patients. Recently, the release of IL-2 into blood from activated gluten specific CD4+ T-cells, has protruded as a sensitive and objective biomarker of a rapid immune activation 4 hours after one-dose gluten challenge in CeD-patients[2,3].

Objective: To examine serum IL-2 production following oral barley challenge as a marker of T-cell activation and to determine if this correlates with symptom onset.

Study design: This study used a randomized single-blinded crossover design with five periods and 20 sequences. Participants received five one-dose challenges (wheat 1 g, barley 1g, barley 0,05 g, hydrolyzed barley 0,05 g and placebo), each followed by a 4-week wash out period. The challenge vehicle was gluten flour mixed into flavoured lactose-reduced chocolate milk.

Endpoints: Primary endpoint: Immune response to low-dose barley vs. low-dose hydrolyzed barley, assessed by serum IL-2 levels. Secondary endpoints: 1) immune response to high-dose wheat vs. high dose barley 2) immune response to high- vs. low-dose barley 3) gastrointestinal symptom pre- and post-challenge measured by VAS-score 4) gluten immunogenic peptides in urine and feces pre- and

post-challenge, assessed by G12 antibody 5) baseline cytokine response to *in vitro* gluten peptide stimulation (wheat and barley) in whole blood.

Preliminary results: A total of 20 women and 8 men CeD were enrolled in the study. The median age was 54 years for women and 52.5 years for men. All participants had been diagnosed with CeD and had been following a gluten-free diet for at least two years (median years living on a gluten free diet = 13). To date, 12 participants have completed five challenges, and we aim to complete the study by March 2025. There were two dropouts after the first challenge.

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6.2 Monitoring gluten in food: results from the real world and future perspectives for celiac safety

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Abstract

Celiac disease (CD) management requires a multidisciplinary approach, combining strict adherence to a gluten-free diet (GFD), professional dietary counseling, and addressing social challenges.

The meaning of the “gluten-free” label varies throughout the world. A gluten threshold of 20 ppm is used in the USA, Canada, the UK, and the EU. Argentina allows up to 10 ppm in “gluten-free” products. In contrast, Australia, New Zealand, and Chile have the strictest standard, requiring no detectable gluten in a product to be labeled “gluten-free”. Despite these standards, there remains a longstanding and unresolved scientific debate about the maximum daily gluten intake that can be safely tolerated in CD patients to prevent clinical symptoms and histological damage.

Our research has investigated gluten contamination in commercially available foods. Among 200 tested products, 18 exceeded the 20 ppm threshold, with a median gluten content of 32 ppm. None of the contaminated items carried the "crossed grain ear" certification, confirming the reliability of this certification. Additionally, higher-priced gluten-free products were less likely to be contaminated, suggesting a correlation between cost and quality control.

We also extended our analysis to oral hygiene and cosmetic products, such as toothpastes and lip balms. Among the 66 items tested, only 4 exceeded 20 ppm, indicating that these products pose minimal risk for CD patients.

In another study, we evaluated gluten exposure in 69 children adhering to a strict GFD. Of 448 food samples analyzed, only 2.7% showed detectable gluten contamination, with just one exceeding the 20 ppm threshold. Preliminary results from a similar study currently ongoing in the United States yielded comparable findings.

Lastly, in an ongoing study involving European and non-European countries, we are exploring gluten contamination risks in dining out, focusing on pizza, a popular yet challenging choice for CD patients. Further findings from this research will be shared as the study progresses.

While a total daily gluten intake below 10–50 mg appears to be tolerable for most CD patients, individual sensitivities vary. Some patients have shown inflammatory changes, including elevated interleukin-2 levels, after consuming as little as 3 mg of gluten. These findings underscore the need for further randomized controlled trials to re-evaluate the tolerable daily gluten threshold. Strengthening the scientific foundation in this area is essential to enhance the reliability of CD management and optimize dietary recommendations.

6.3 Assessment of Novel Dietary Treatment for Coeliac Disease

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Abstract

Coeliac disease (CD) involves aberrant adaptive and innate-immune response to wheat gluten proteins in wheat and related cereals. The condition affects 10.4 Million people in North America, 8 Million in Europe and 670,00 in the U.K, including children. The only accepted treatment is a gluten-free diet. Alternatives involving in-vitro digestion or immunisation with gluten-proteins that have to date been shown not to work. Glutens are mixtures of hundreds of proteins termed gliadin, low (LMWG) and high molecular weight (HMWG) glutenins that provide wheat with specific baking and sensorial characteristics. Some of them are CD-toxic. Because there is no process to separate CD-toxic from CD non-toxic glutens, gluten-free products are frequently wheat flour based without protein, that cannot be used to bake bread that is sensorially acceptable.

Therefore, gluten-free products are not easily accepted by CD affected patients. However, we noted that wheat strains differ in their gluten composition widely, with natural gluten variants presumed to be non-toxic. This presumption was tested with synthetic small gluten fragments involving culture with CD gluten-sensitive. T-cells and CD duodenal-biopsy organ-culture. This revealed that point substitution of certain amino acids (AA), glutamine or proline, reduces CD toxicity and concomitant replacement of both AA obviates CD toxicity. As a unique testing platform, in collaboration, we expressed single gluten genes in maize, that lacks gluten, but is not suitable for bread making. This approach has enabled us to introduce CD non-toxic wheat proteins into maize that we wish to assess to confirm lack of CD-toxicity, in collaboration, we have subsequently used mutagenesis with deletion of CD-toxic proteins in wheat to generate non-GMO wheat that is CD-safe. Confirmation of a “good” gluten will then provide the breakthrough for the gluten-free market, which could either be realised by breeding a new non GMO CD-safe wheat, that variety or modified corn with bread-baking quality. We are primarily seeking to generate in collaboration non-GMO wheat that is CD-safe. Thus having developed wheat grain radiation mutants and CRISPR Cas9 technology this has allowed us to generate CD non-toxic 1Dy10 and 1Dx5 high molecular weight glutenins as a prelude to generation of non-GMO wheat that is hypothesised to be CD-safe. We propose to assess for absence of CD in-vitro toxicity as a prelude to confirmation with an in-vivo feeding study.

The in vitro assessment will involve assessment of the flour with our panel of monoclonal antibodies to coeliac toxic motifs, employing dot and potentially Western blotting. Following this we would seek to confirm the flour is non-toxic to small intestinal biopsies obtained from treated coeliac affected subjects, cultured with extracts of the flour, followed by histological assessment of the biopsies. Assuming we found no evidence of in vitro coeliac toxicity, we would then proceed to undertaking in vivo feeding studies as we have previously described. This would involve undertaking assessment of treated coeliac affected subjects, involving with written informed consent undertake

clinical assessment of the subjects, appropriate blood tests and an upper gastrointestinal endoscopy with small intestinal biopsies obtained.

The subjects would then be asked to ingest the flour as either pancakes or porridge. They would be clinically assessed 2 weekly and after 6 weeks undergo a further endoscopy with small intestines biopsies taken.

We suggest this is important as the outcome would permit not only improved treatment of coeliac disease, but also improved quality of life for the 10.4 Million affected people in North America, 8 Million in Europe and 670,000 in the U.K, including children.

7 Symposium - Gluten contamination: how real is the risk in commercially and freshly prepared gluten-free products

7.1 Analytical approaches to determine prolamin contents in foods: role of mass spectrometry and proteomics in the characterization of celiac epitopes

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Abstract

Prolamins are storage proteins found in cereals and are responsible for triggering adverse immune responses in individuals with celiac disease. The immune system of affected individuals recognizes specific immunogenic peptides within prolamins, particularly gliadins and glutenins in wheat, leading to an inflammatory response that damages the intestinal lining. Since celiac disease is a lifelong autoimmune disorder requiring strict dietary gluten exclusion, accurate prolamin analysis is essential for elucidating the disease's pathophysiology and ensuring the safety of gluten-free foods.

Historically, enzyme-linked immunosorbent assay (ELISA) has been the gold standard for gluten protein analysis due to its accessibility, cost-effectiveness, and ability to detect gluten at low concentrations. However, ELISA has notable limitations, including variable sensitivity depending on the gluten source and cross-reactivity. These shortcomings have led to the exploration of more advanced analytical techniques.

Mass spectrometry (MS)-based proteomics has revolutionized gluten analysis by providing highly specific and sensitive detection of prolamins and their immunogenic peptides. This technology enables the precise characterization of gluten-derived peptides that trigger immune responses, including those resistant to gastrointestinal digestion, which persist in the small intestine and are recognized by T cells in individuals with celiac disease. High-resolution MS allows for comprehensive mapping of celiac-related epitopes, improving our understanding of gluten toxicity and aiding in the search for wheat varieties with reduced immunogenicity.

MS-based proteomics also plays a critical role in evaluating enzymatic or microbial treatments aimed at degrading gluten proteins in food products—a potential strategy for reducing gluten immunogenicity. Additionally, MS enables accurate quantification of gluten in complex food matrices, including thermally processed or fermented products. Emerging techniques allow for the highly specific quantification of known immunogenic peptides, enhancing food safety and quality control.

Furthermore, the integration of high-resolution MS, targeted proteomics, and bioinformatics tools has significantly improved the detection of celiac-related epitopes, contributing to the development of safer food products. As research advances, the synergy between proteomics, food science, and clinical studies will be essential in deepening our understanding of celiac disease and ensuring the safety and quality of gluten-free products.

7.2 Risk of gluten contamination when dining out: is it always a safe gluten-free experience? The International Celiac Disease MUlticenter Pizza Project (CD-MUPP)

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Abstract

Background: Exposure to gluten while dining out is a major barrier to quality of life for celiac disease (CD) patients. Gluten-free pizza is a typical example of a food item that can easily be contaminated by gluten, especially in kitchens where both gluten-free and traditional wheat-based pizzas are prepared.

Aim: To quantify rates of gluten contamination in supposed gluten-free pizza restaurants and identify risk factors associated with the presence of gluten across a European context.

Methods: In an ongoing multi-center, cross-sectional study, a total of 140 pediatric celiac disease (CD) patients (2-18 years old) following a gluten-free diet (GFD) from 11 different European and non-European countries are proposed to be enrolled. CD patients will be requested to collect a representative portion of pizza and store it frozen until delivery to the participating center personnel. Pizza samples will be processed for gluten quantification by Ridascreen gliadin sandwich R5 ELISA. To avoid bias, pizzeria personnel will not be aware of the study. Collected data will be used only for statistical purposes and restaurants' identification will not be disclosed.

Results: So far, 98/140 pizza samples have been collected in Italy, Sweden and Norway. Only two pizza samples from Italy, and one from Sweden have been quantified with more than safe threshold of gluten (i.e. 50, 58, and 29 mg/kg, respectively). The contaminated pizza samples from Italy were collected from a pizzeria declaring a gluten-free claim but not included in the list of restaurants recommended by the national celiac association (AIC) and were not cooked in a dedicated oven.

The contaminated pizza samples from Sweden was collected from a restaurant using ready-made gluten-free pizza crust, prepared in designated area separate from regular pizzas, but with the same ladle to put regular and gluten-free pizzas in/out of oven. Reassuringly, the presence of gluten traces did not lead to exceed the tolerable daily intake of 50 mg gluten.

Conclusion: The very preliminary results suggest that in countries characterized by high awareness of CD and GFD, chances of gluten cross-contamination in restaurants and pizzerias are quite low. In the coming months, study results with a greater number of gluten-free pizzas from all the participating centers will provide much needed data for CD patients and the healthcare providers who treat and advise them, to develop interventions on reducing gluten contamination in restaurants.

7.3 Gluten immunogenic peptides in urine and stool – all problems solved?

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Abstract

The only approved treatment for coeliac disease (CeD) is the strict gluten-free diet based on food with less than 20 ppm of gluten. The acceptable total amount of gluten is by and large not known but presumably in the range 50-100 or less on a daily basis, but compliance among treated CeD patients is far less than perfect [1]. To investigate actual gluten exposure is difficult. Structured interview by clinical nutritionalist is a good option [2]. Recently, demonstration of gluten immunogenic peptides (GIP) in urine and/or stool has been introduces as an objective biochemical marker [3].

A hallmark paper from 2017 by Moreno and colleagues using urine assessment showed that dietary transgressions correlated well with incomplete mucosal healing – the most important endpoint for CeD [4]. Secretion of peptides in urine has been studied and the antigenic peptides, although in their native, non-deamidated forms, are found [5]. Evidence suggests abundance of peptides and other peptides in urine from CeD patients than in healthy. Whereas urine testing is convenient the signal in urine is relative short-lived (hours) whereas the signal in stool lasts days [6]. Stool determinations has been claimed to be superior to urine testing, as judged from controlled gluten challenge [7]. The significance of GIP has recently been reviewed [8-10]. It serves as a low-cost, non-invasive tool for investigating gluten compliance in CeD.

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7.4 How Challenging is Compliance with a Gluten-Free Diet? A Dietitian's Perspective.

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Abstract

Adherence to a gluten-free diet (GFD) among patients with coeliac disease varies widely, influenced by a complex interplay of individual, societal, and systemic factors. This talk explores adherence rates, key barriers and facilitators, the role of dietitians, and the broader impact of national policies on supporting effective GFD compliance. Rates of adherence range from 40% to 92%. Barriers include the cost and availability of gluten-free products, social challenges, and emotional factors. Conversely, adherence is enhanced by regular dietetic follow-up, supportive communities, and associated improved knowledge of the GFD.

Dietitians are pivotal in addressing challenges at both individual and systemic levels. By providing education on label reading, meal planning, and cross-contamination prevention, alongside psychosocial adaptation, dietitians support improved adherence.

National policies also play a critical role in shaping adherence rates and quality of life for individuals with coeliac disease. Such policies that ensure clear labelling laws, promote accessibility of gluten-free foods, and provide tailored healthcare services have been shown to significantly improve adherence and patient outcomes. These findings underscore the importance of combining policy initiatives with patient education to optimise care.

Emerging evidence highlights the need for a more nuanced approach to dietary management of coeliac disease not only focusing on adherence to the GFD balancing this with dietary strategies to enhance metabolic health. In the era of personalised medicine, further research is essential to refine dietary strategies, reduce treatment burden, and enhance patient-centred care.

8 Statements on current developments concerning gluten analysis, clinical and legal aspects

8.1 Update on Codex issues

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Abstract

AOECS, the Association Of European Coeliac Societies, has Observer status in the Codex Alimentarius Commission and the Codex Committees since 1992 and worked successfully to improve Codex Standards for the benefit of coeliacs. In 1999, the Codex Alimentarius Commission adopted the list of foods and ingredients which are known to cause hypersensitivity and shall always be declared. The first on the list are “Cereals containing gluten; i.e. wheat, rye, barley, oats, spelt or their hybridized strains and products of these”.

Proposed Draft Revision of the General Standard for the Labelling of Prepackaged Foods - Provisions relevant to Allergen Labelling

In October 2021, the Codex Committee on Food Labelling (CCFL) began the work and finalised it in 2024. AOECS participated in the sessions, in pre-meetings and elaborated several comments which were distributed to all CCFL participants.

The main important results: We worked successfully to delete coeliac disease from the definition of food allergy, coeliac disease is separately described in the chapter definitions. The substances which are causing adverse reactions to foods are defined as “allergen” and include also “other specific immune-mediated reactions”, which is coeliac disease, and not only IgE-mediated reactions. Cereals containing gluten are specified: wheat and other Triticum species, rye and other Secale species, barley and other Hordeum Species and products thereof. A footnote provides further information and complies with the Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CXS 118-1979). To conclude: Cereals containing gluten and products thereof must always be declared, this had not changed, however “Specified names” (wheat, rye, barley) have been inserted. Oats is deleted from the list of the most important allergens but is added in the list of foods which are up to national authorities to consider.

Proposed Draft Guidelines on the use of Precautionary Allergen Labelling (PAL)

AOECS did not agree to the draft published by CCFL because coeliac disease was not considered, only consumers with food allergy. The proposed RfD of 5 mg wheat does not match with the threshold of gluten-free <20 mg/kg gluten as defined in the Codex Standard for Foods for Special Dietary Use for Persons Intolerant to Gluten (CXS 118-1979). CCFL requested the Codex Committee on Methods of Analysis and Sampling (CCMAS) to recommend suitable analytical methods. AOECS succeeded in the CCMAS sessions that gluten containing cereals are written in the report of CCMAS and not only wheat. Finally CCFL accepted the addition of coeliac disease in the purpose of the PAL. An EWG of CCMAS is continuing the work to recommend validated analytical methods, the results are expected in spring 2025.

The reports of the CCFL and CCMAS are published on the Codex website www.fao.org.

8.2 From Fermentation to Certification: Legal Aspects of Böcker's Gluten-Free Sourdough Products

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Abstract

Ernst Böcker has been producing sourdoughs for the bakery industry in Minden, Germany, since 1910. To produce sourdough, flour and water are fermented under defined conditions, during which parts of the flour are metabolized by lactobacilli and yeasts and organic acids, CO2, aromatic substances and other components are formed, which have many advantages for the finished bread. The use of sourdough results in many benefits such as improved texture and taste, increased bioavailability of nutrients, better digestibility, extended shelf life and a lower glycemic index [1]. This also applies to gluten-free baked goods.

Böcker offers various forms of sourdough, including gluten-free options since 2004. In the gluten-free range we have sourdough starters, which are used to make your own sourdough of consistent quality, as well as dried sourdough which is used directly in bread. During sourdough fermentation, the gluten network is broken down and hydrolyzed; during drying, the gluten is additionally heat-processed.

To ensure the absence of gluten in raw materials, we utilize lateral flow assays and ELISA (R5) tests. Given the presence of hydrolyzed gluten in sourdough, the competitive R5 ELISA is specifically employed. The demand for gluten-free sourdoughs continues to grow and new raw materials are constantly being requested and tested. Certain raw materials like margarine, oil, pumpkin seeds, sultanas, and linseed may not be validated by ELISA tests. For this reason, we must carry out a separate validation for each new raw material to ensure food safety.

The regulations for gluten detection in Europe are clearly regulated by EU standard (EU) No 828/2014. Products are considered gluten-free if they contain less than 20ppm gluten. The R5-competitive ELISA must be used for hydrolyzed samples.

In the USA, the regulation is similar, but the U.S. Food and Drug Administration (FDA) regulation; 21 CFR § 101.91 stipulates that special methods must be used for fermented samples and that a test of the final product is not sufficient.

In Japan, the Ministry of Health, Labor and Welfare (MHLW) does not issue any regulations regarding gluten content, only wheat is listed as an allergen. For gluten-free products, however, it is still recommended that a value of less than 20 ppm be maintained.

However, there are countries such as Australia that deviate from these rules and require less than 20ppm. For example, the Food Standards Australia New Zealand (FSANZ) requires that a product can only be labeled as "gluten-free" if it contains no detectable gluten. However, this is not possible with current methods, and we are learning more and more that it is almost impossible to avoid even minor contamination in the field and during transportation of cereals.

This overview highlights the rigorous processes and regulatory hurdles involved in ensuring gluten-free products meet international standards, thereby maintaining consumer trust and product integrity.

Reference

1. Arendt, E.K., Shwaiki, L.N., Zannini, E. (2023). Sourdough and Gluten-Free Products. In: Gobbetti, M., Gänzle, M. (eds) Handbook on Sourdough Biotechnology. Springer, Cham. https://doi.org/10.1007/978-3-031-23084-4_11

9 Perspectives and action plan of the WGPAT

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The Prolamin Working Group executive meeting and joint discussion held on 28 September 2024, led to the decisions and statements outlined below.

Action plan

I. Analytical

- The PWG gliadin reference material is available from Arbeitsgemeinschaft Getreideforschung – AGF (Association of Cereal Research), Schuetzenberg 10, 32756 Detmold, Germany. Please contact Dr Jörn Weiler, e-mail: info@agf-detmold.de.
- The price for one batch (100 mg) is 300 Euro.
- Material for at least 4 years is still on stock.
- Plans to prepare new PWG gliadin reference material are actively on progress.

II. Clinical

- The PWG is considering to build up a working group for a project to assess the minimal gluten content (threshold) for a safe accidentally exposure in celiac patients. A fundraising action is also on plan.

III. Members, Policy

- Prof Conleth Feighery, Emeritus fellow of Trinity College Dublin (gastroenterology) and Prof Peter Koehler, Biotask Esslinghen, left the PWG. All members are very grateful to Profs Feighery and Koehler for their great and valid contribution given to Prolamin working group activities and achievements over the years.
- Proceedings of this meeting will be available free of charge in electronic form from the PWG website (http://www.wgpat.com).

PWG 2025 Meeting

We are very pleased to announce that 38th PWG meeting will be held in Ancona, 23-25 October 2025.

Invitation and Programme

38th Meeting

Working Group on Prolamin Analysis and Toxicity

23 – 25 October, 2025
Ancona, Italy

Scientific Organisation

Prof Carlo Catassi
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Prof Maria Elena Lionetti
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Congress Venue

Polytechnic University of Marche, Ancona, Italy.
Studentato del Buon Pastore