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XV INTERNATIONAL GLUTEN WORKSHOP

GLUTEN & KERNEL COMPONENTS

Role in Processing, Health and Diseases

Palermo - Italy
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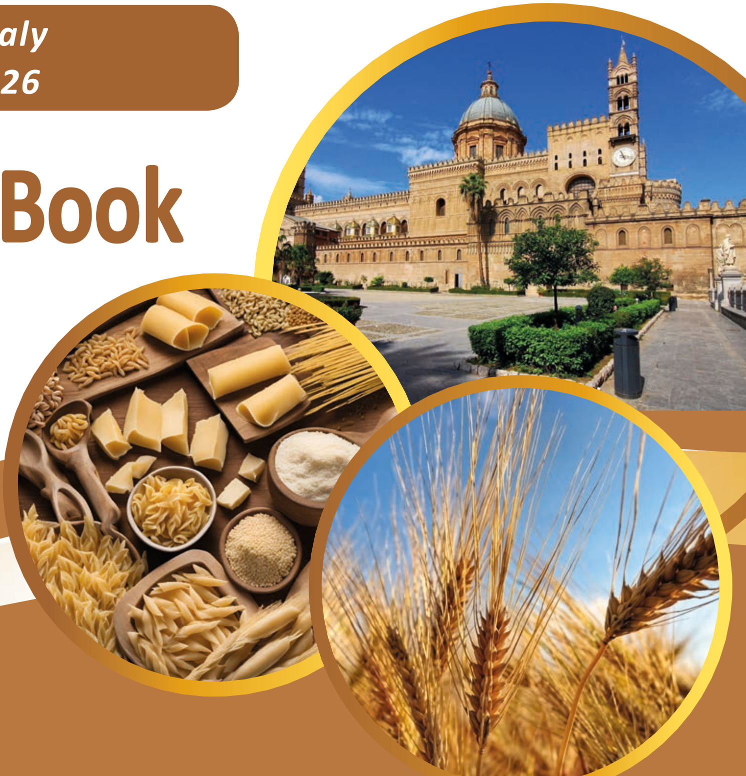
Abstract Book



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SPEAKERS

OPENING KEYNOTE LECTURE

01 - Wheat Gluten proteins: an historical perspective on Structure, Function and Impact

S. Masci, D. Lafiandra

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The study of wheat gluten proteins has evolved through a series of conceptual and technological advances that have progressively clarified their genetic control, structure-function relationships, and impact on quality. Early work by Osborne (1924) established the foundational classification of wheat proteins based on solubility, distinguishing gliadins and glutenins as the principal gluten fractions. Subsequent research further resolved these groups into distinct classes, notably high and low molecular weight glutenin subunits (HMW-GS and LMW-GS), and gliadin groups, all of which contribute to dough viscoelasticity.

The use of genetic lines, such as substitution and aneuploid lines, along with Near Isogenic Lines (NILs) or biotypes, enabled researchers to map gluten protein loci to specific chromosomes and to associate superior quality traits to particular protein components. This progress was further supported by the development of two-dimensional electrophoresis and chromatographic techniques.

Advances in protein chemistry, including N-terminal sequencing and, later, mass spectrometry, enabled a more precise characterization of gluten protein composition and its relationship with technological properties. These techniques have provided high-resolution insights into protein diversity and interactions within the gluten network, including the detailed mapping of disulfide bonds.

More recently, biotechnological approaches have expanded the capacity to manipulate gluten composition. Genetically modified (GM) wheat plants, and more specifically genome-editing technologies such as CRISPR/Cas systems, have enabled to modify the gluten protein composition and improved our understanding of the roles of these proteins in both quality traits and adverse human reactions to wheat.

The advent of whole-genome sequencing marked a turning point, offering comprehensive catalogs of gluten protein genes and revealing their complex organization and redundancy within the wheat genome.

Together, these innovations open new avenues for tailoring wheat quality, improving processing properties, and potentially reducing components implicated in human adverse reactions. This historical overview underscores the integration of classical genetic and modern molecular tools, along with biochemical approaches, in advancing our understanding of wheat gluten proteins.

SHORT PANEL

02 - From the Rippling Wheat Fields: An Anthropology of Wheat Consumption in the Mediterranean Region

M.F. Fontefrancesco

Catholic University of Sacred Heart, Italy

The talk investigates the enduring centrality of wheat in Mediterranean cultures, examining its dual significance as both a material staple and a symbolic cornerstone of social life. Through an anthropological lens, it interrogates the symbolic dimensions of wheat as a mediator of fertility, prosperity, and collective identity across rural and urban contexts. The analysis proceeds by exploring prevailing modalities of consumption-most notably bread and pasta-as cultural texts that materialize notions of community, continuity, and everyday conviviality. In the concluding section, the study situates these practices within a broader transformation of dietary regimes across the region. While contemporary European societies appear to be decentering wheat-based foods from their everyday meals and identity narratives, the Arab Mediterranean continues to reaffirm the centrality of cereals in both domestic and public food systems, a persistence underscored by the socio-economic disruptions of the 2022 food crises

CONCLUDING KEYNOTE LECTURE

03 - Challenges for the Production and Utilisation of Wheat

Peter Shewry (1)

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The global production of wheat continues to increase, from 6.84 million tonnes in 2009 to 8.0 million tonnes in 2024, and the increased production over the past 60 years has been achieved without an increase in the area under cultivation, by increasing yields through a combination of genetic enhancement and improved agronomy. Furthermore, the average global yield of wheat (about 3.6 tonnes/ha) is still fall far below the yields achieved in high input systems such as those used in in Western Europe (8-9 tonnes/ha). Hence, wheat production has the potential to keep up with population growth. However, the production and utilisation of wheat face significant challenges. Firstly, the yields in high input systems are driven by nitrogen fertilisation with additional nitrogen being required to achieve the protein contents required for breadmaking. Secondly, the consumption of wheat and bread is decreasing in some countries due to concerns about adverse effects of wheat, particularly gluten, and of breadmaking ingredients on health. The scientific basis for these concerns is discussed and strategies proposed to reduce nitrogen requirements and provide a scientific basis to provide advice to regulatory authorities and consumers.

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ORALS

01 CUTTING-EDGE RESEARCH & TECHNOLOGIES ON KERNEL COMPONENTS

- Integrated genome editing and breeding strategies to develop ultra-low immunogenic wheat for gluten-related disorders

***Miriam Marín-Sanz* (1) *- Francisco Barro* (1) *- Susana Sánchez-León* (1)**

(1) *IAS-CSIC, Plant Breeding, Córdoba, Spain*

Adverse reactions to wheat, including celiac disease (CD), are primarily triggered by immunogenic epitopes present in gliadin proteins. Due to the high redundancy and complexity of gliadin gene families in hexaploid wheat, reducing gluten immunogenicity while maintaining technological quality remains a major challenge.

Here, we present a multi-layered strategy combining CRISPR/Cas genome editing, RNA interference (RNAi), and doubled haploid (DH) technology to efficiently target gliadin gene families and accelerate the development of low-immunogenic wheat.

Multiplex CRISPR/Cas9 editing of γ- and ω-gliadins generated wheat lines with up to 97.7% reduction in gluten content. In parallel, precise editing of α-gliadins using paired sgRNAs enabled targeted excision and replacement of the highly immunogenic 33-mer peptide, achieving excision rates up to 74.2% and significantly reducing immunoreactivity. This approach allows modification of protein sequences while preserving structural features relevant for processing quality.

To overcome genetic segregation and fix multiple edits, CRISPR-edited lines were combined with RNAi-silenced genotypes using doubled-haploid technology. This enabled the rapid generation of genetically stable lines exhibiting>70% reduction in α-gliadin immunogenic epitopes, with some lines approaching near-depletion of gliadins. Notably, compensatory changes in storage protein composition were observed, suggesting potential mitigation of negative effects on dough functionality.

Overall, this work demonstrates that integrating gene editing, gene silencing, and accelerated breeding strategies provides an effective route to develop wheat varieties with substantially reduced immunogenic potential. These results contribute to ongoing efforts to produce wheat compatible with gluten-related disorders, aligning with current advances in new genomic techniques and their application in cereal breeding.

This work was funded by MCIN/AEI/10.13039/501100011033 (grant no. PID2022-142139OB-I00)

- Computational methods and AI - the future of bread-making quality breeding and processing

Eva Johansson ***[1] - Ramune Kuktaite [1] - Aakash Chawade [1]***

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Currently we see an AI and computational methods boom in the society, with these methods gaining momentum in all sectors everywhere. Agricultural studies have been the driver for the early development of statistical methods and these are still used to understand effects of selection of genotypes and environment on yield and quality aspects. With the predicted climate change scenarios, opportunities to understand requirements of future wheat genotypes in terms of baking quality will thereby be increasingly important. Polymerization of the gluten proteins in the grain and during mixing and baking is highly impacted by both temperature and drought stress during cultivation. Recent studies have also shown the importance of prediction models to determine bread-making quality, where a study under Swedish conditions indicated grain protein content as the most important determinant of bread-making quality, followed by protein polymerisation (strength) measured by HPLC or Alveograph.

The increase in computer power and technology have resulted in novel opportunities to use AI and computational methods to develop genomic selection models for all types of breeding characters, although bread-making quality is complicated due to the high impact of environmental and processing related inputs. Additionally, avenues has opened to understand protein behaviour through simulations of protein structures, although cross-linking of large protein subunits such as those in wheat is still complicated.

The most recent AI technology that will most likely have an impact on bread-making quality is the use of digital twin systems in the lines of bread production in bakeries. There, quality parameters will be measured continuously in the inlet on the raw material, most likely using NIR, and this information will be sent to the digital twin system that will continuously update models and give signals on how to modify procedures to fit the quality of the raw material. Thus, quality parameters possible to measure on NIR, additionally to protein content that is the current major measurement of NIR, will be of high importance.

- Broad characterisation of compositional and technological properties of spelt wheat grown in different environments

Sándor Tömösközi **(1) - *Edina Jaksics* (1) - *Marietta Szentmiklóssy* (1) - *Eszter Schall* (1) - *Péter Mikó* (2) - *Viola Tóth* (2) - *Marianna Rakszegi* (2)**

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There are many claims made about the production, nutritional and technological quality of spelt. Among others these include its more valuable composition, beneficial effects on health, and certain food safety issues. In contrast to modern wheat, which is widely characterized in terms of biochemical, genetic, compositional, nutritional and technological aspects, much less information available on spelt. Moreover, comparing the existing information is not easy due to the different number of samples and varieties tested, their genetic background, and the use of different flours and testing methods.

The main aim of the four years project is to compare compositional and processing properties of spelt and common wheats on a wide range of sample sets, some of them grown in both conventional and organic environment. In addition to the examination of the general compositional properties, the dietary fiber and protein composition, as well as the study of the variability of short-chain carbohydrates (FODMAP). Wide range of technological properties and baking quality using small-scale methods were also investigated.

Our results confirmed that, the spelt wheat samples had a slightly higher protein (and gliadin) content and a significantly lower fiber (arabinoxylan), than common wheats. Minor differences could be identified in the short-chain carbohydrate composition. The mixing properties of doughs made from spelt wheat flours were weaker and did not show great genetic variability. However, there was a greater diversity in the viscous behaviour, and there are also differences in the results of the baking tests. It is a proven fact that in the case of spelt samples, the cultivation method has a significant impact on the development of technological properties, the extent and direction of the change depend not only on the cultivation method, but also on the genotype and the year.

This work was supported by the OTKA 135211 and OTKA 135343 projects. The research is the part of the BME-TKP2021-EGA-02 and TKP2021-NKTA-06 projects supported by the Ministry for Innovation and Technology of Hungary from the National Research, Development and Innovation Fund. The research was carried out with the tools of the National Cereal Science Infrastructure Network (3-110-H).

- *Reducing the Immunogenic Potential of Wheat Flour: Advanced Strategies Targeting WDEIA and Celiac Disease*

Jong-Yeol Lee *(1)*

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Wheat (Triticum aestivum L.) gluten provides unique viscoelastic properties but triggers Wheat-Dependent Exercise-Induced Anaphylaxis (WDEIA) and Celiac Disease (CD). This study utilized the US wheat cultivar ‘Butte 86’ as a model cultivar, selected for its comprehensive omics resources, including high-quality gluten gene sequences, proteomic maps, and expression profiles. Based on this comprehensive dataset, we applied radiation mutagenesis to develop wheat lines with reduced immunogenicity. For WDEIA, we successfully developed double deletion mutants lacking both 1B and 1D ω-5 gliadins. For Celiac Disease, our epitope analysis of ‘Butte 86’ α-gliadins revealed that the Gli-D2 locus (encoding the 33-mer peptide) is the most toxic, followed by Gli-A2 as the next significant source of immunogenicity. Based on this hierarchy, we prioritized and successfully identified mutant lines lacking Gli-D2 and Gli-A2 loci. Finally, recognizing that ω -1,2 gliadins are major immunodominant triggers comparable to α-gliadins in Celiac Disease, we identified and characterized deletion lines lacking these specific proteins. These novel genetic stocks, harboring defined deletions of major celiac-toxic and WDEIA-triggering epitopes, represent invaluable resources for stacking traits to develop hypoallergenic wheat varieties.

02 GENETICS, GENOMICS & BREEDING OF GLUTEN CONTAINING CEREALS

- Integrated GWAS and metabolomic analyses provide novel details about the genetic basis of free asparagine accumulation in durum wheat grains

Andrea Tafuri (1) ***- Raul Pirona*** (1) ***- Agostino Fricano*** (2) ***- Marie Gasser*** (3) ***- Elisabetta Mazzucotelli*** (2) ***- Estelle Maret*** (4) ***- Laura Ruth Cagliani*** (5) ***- Stefano Ravaglia*** (6) ***- Roberto Consonni*** (5) ***- Aurelien Thomas*** (3) ***- Aldo Ceriotti*** (1) ***- Federica Gilardi*** (3) ***- Elena Baldoni*** (1)

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Free asparagine content is a key factor in acrylamide formation in wheat derivatives after high temperature processing. Therefore, the control of free asparagine levels is of interest in crop and food sciences. Our study aims to explore free asparagine natural variation in durum wheat grain to identify candidate genes controlling this trait. Two hundred and one durum wheat genotypes were selected from an international germplasm collection and sown in an experimental field for three years. Free asparagine content was measured on whole grain using an enzymatic assay. A genome-wide association study identified six associated quantitative trait nucleotides. Moreover, the whole grain metabolome of one-year samples was investigated to identify metabolic pathways associated to free asparagine accumulation. Specific enriched pathways involved in amino acids metabolism were detected and four candidate genes were identified. This study paved the way to characterize the genetic determinants of free asparagine accumulation in wheat grain.

- Slicing into the Genetic Regulation of Wheat Gliadin Proteins

Zachary Jones *(1)* ***- Gaganjeet Kalra*** *(1)* ***- Gautam Saripalli*** *(1)* ***- Eric Olson*** *(2)* ***- Sachin Rustgi*** *(1)*

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Common wheat constitutes a large fraction of the global daily diet. However, this is not the case for everyone, as an increasing number of individuals have been diagnosed with wheat-related disorders. In many of these cases, including celiac disease and wheat allergy, the primary causative agents are gluten proteins-particularly the gliadin fraction. Although gliadin loci are well characterized, with Gli2 encoding alpha/beta gliadins and Gli1 encoding gamma- and omega-gliadins, the regulatory elements controlling their expression remain incompletely understood. To gain deeper insight into the regulatory network governing gliadin expression, several genetic resources were utilized. First, nullisomic/tetrasomic lines, wild wheat relative, and a set of deletion mutants were used to assign specific genes (using a combination of targeted sequencing and gene-specific markers) and protein bands to sub-chromosomal locations. Subsequently, a 420-line D-subgenome nested association mapping (DNAM) panel, originating from eight Aegilops tauschii D-subgenome donors and a common hexaploid wheat parent, was employed. This panel, selected for its enriched genetic diversity, was extensively genotyped and screened for variability in gliadin protein composition. Genetic mapping through single-marker regression of both quantitative and qualitative gel analyses identified several genomic regions associated with gliadin variation. Significant marker associations were detected on chromosomes 1DS (cis to Gli-D1), 2DL, 4DL, 5DL, 6DS (cis to Gli-D2), 7DS, and 7DL, highlighting key genomic regions influencing gliadin accumulation. Notably, the observed regulatory effects extended beyond individual subgenomes. Furthermore, mutants of two previously identified gliadin regulators, Storage Protein Activator (SPA) and Prolamin Binding Factor (PBF), were profiled for their gliadin content and pyramided, producing a reduced gliadin phenotype. Altogether, this research advances our understanding of gluten regulation in hexaploid wheat. Ultimately, the findings aim to support the development of molecular markers for breeding programs targeting improved end-use quality and the production of lines with reduced levels of immunogenic gluten proteins.

- Pan-genomics of hordeins

Volodymyr Radchuk [(1)] ***- Jason Wilke*** [(2)] ***- Patrick Koenig*** [(3)]

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Hordeins, the gluten proteins of barley, are major targets for breeding due to their role in triggering adverse immune responses in individuals with celiac disease. These storage proteins comprise four subfamilies, B-, C-, D- and γ-hordeins, most of which are encoded by multigene families. Using long-read sequencing of 76 cultivated, landrace and wild barley accessions, we performed a comprehensive pan-genome analysis of hordein loci. All hordein genes were confirmed to cluster on chromosome 1H, with substantial variation in gene copy number, structure and chromosomal organization. γ-Hordeins ranged from three to seven copies per genome, shaped by gene loss or duplication, with some copies likely representing pseudogenes. Variation in chromosomal positioning of these genes among accessions suggests local structural rearrangements. Substantial intragenic diversity was also observed where 79 SNPs, of which 40 lead to amino acid substitutions, along with at least four larger indels was observed only in γ-hordein 1. B-hordeins are encoded by a large gene family comprising 9-24 members. Some genes producing identical protein sequences indicative for recent duplication events. A similar gene number was observed for C-hordeins. In contrast, D-hordein is encoded by a single gene but exhibits considerable intragenic variation in the number of direct repeats in the C-terminal region which vary between 10 and 16 copies. Interestingly, the number of repeats appears to be inversely correlated with the expression level of the corresponding gene. Together, these findings reveal extensive structural and sequence variation at hordein loci, providing a foundation for targeted breeding strategies aimed at reducing immunogenic gluten content in barley.

- Chromosomal engineering of the Glu-A1 locus with Glu-D1 alleles improves rheological properties in Italian bread wheat

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Two Italian bread wheat lines, N11 and N18, were used as donor genotypes to generate novel lines through a chromosomal engineering approach, with the aim of improving technological quality. This approach led to the development of two promising lines expressing six High Molecular Weight glutenin subunits, obtained through the replacement of the Glu-A1 locus with Glu-D1 with the introgression of the Glu-D1a or Glu-D1d alleles into N18 and N11, respectively. Additionally, two lines carrying a double dose of the parental allelic variants, 2+12 in N11 and 5+10 in N18, were developed.

Grains harvested over two experimental seasons were analysed at the biochemical and genetic levels using UPP, A-PAGE, SDS-PAGE, and gene expression assays, and at the technological level through alveograph and farinograph tests. The introduction of the 5+10 glutenin subunit pair did not result in improved dough quality. This outcome may be attributed to (i) excessive dough tenacity in lines already carrying the 5+10 subunits at the Glu-D1 locus, and (ii) the influence of the specific genetic backgrounds of the parental lines.

A different behaviour was observed for lines containing the Glu-D1a (2+12) allele. In these lines, gluten quality was significantly improved, particularly in N11-derived lines. The results showed that the improvements in rheological properties were more strongly associated with the substitution of the Glu-A1 locus with Glu-D1 alleles than with the genetic background of the lines, highlighting the key role of targeted chromosomal introgression in modulating wheat quality traits.

- The impact of stripe rust resistance genes Yr9, Yr10, and Yr15 on gluten protein fractions and SDS sedimentation volume in wheat

Maryke Labuschagne (1) - **Ridokunda Sivhada** (1) - **Willem Boshoff** (1) - **Angeline Jacoby** (1)

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Breeding programs have successfully increased wheat production by increasing yield potential and resistance to biotic and abiotic stress. Although often seen as secondary, good end-use quality is crucial in wheat breeding. Enhancing crop resistance to diseases remains the most cost-effective and environmentally friendly strategy to manage diseases and decrease yield loss. Therefore it is essential to explore its influence on end-use quality. This study used reverse phase high - performance liquid chromatography (RP-HPLC) and sodium dodecyl sulphate - sedimentation (SDSS) to investigate the potential influence of stripe rust disease and the presence of the resistance genes Yr9, Yr10 and Yr15 on end-use quality by quantifying gluten protein fractions and measuring SDS sedimentation volume. The presence of the resistance genes showed significant effects on ωb-, α-, and γ-gliadins, although diverse responses of these fractions to genotype, treatment, and environmental interaction effects were noted. Similar effects of the resistance genes were also noted for the glutenin fractions. Disease inoculation did not have a significant effect on the fractions. An increase in SDSS was observed in the presence of the disease as well as in the presence of Yr9 and Yr15 resistance genes. The observed effects of the stripe rust disease and resistance genes indicate their effects on protein quality, which may affect end-use quality.

IMPLICATIONS OF CLIMATE CHANGE ON QUALITY & NUTRITIONAL PROPERTIES

- Contrasted end-use quality response of two bread wheat varieties to combined sulfur supply and post-anthesis heat stress

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Bread wheat is mostly consumed after milling and breadmaking processes, which require a specific grain quality, mainly determined by protein concentration and composition. However, crops are increasingly subject to post-anthesis heat stress due to global warming, which may severely decrease yield and alter quality. Environmental changes can impact the quality of some varieties which are considered unstable compared to varieties whose quality remains stable in any growing conditions. Sulfur (S) nutrition has been identified as a potential lever to better tolerate abiotic stresses. S is part of antioxidant molecules like glutathione which help maintain cellular redox status. Thus, the aim of the study was to evaluate whether S supply could mitigate negative impacts of post-anthesis heat stress on yield and end-use quality. Two varieties differing in quality stability and high molecular weight glutenin Glu-D1 alleles were grown in a field experiment (i) with or without S supply at heading and (ii) with or without heat stress treatment during grain filling via portable heat tents which elevated daytime temperatures by more than 3°C on average. Heat stress treatment induced a decrease in yield and thousand kernel weight and an increase in grain protein concentration. S supply did not mitigate the negative effects of heat stress, but had a positive effect on yield without heat stress. Storage protein composition was modified by heat stress and S supply, with a significant increase in the percentage of unextractable polymeric proteins under heat stress. The quality of variety KWS Extase, in terms of baking strength, tenacity and elasticity, was more stable in response to heat stress and S supply than the quality of variety Rubisko, possibly due to differences in Glu-D1 alleles.

- The relationship between weather, genotype, and elastic properties of gluten in winter wheat - a study of historical data from Norway

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The viscoelastic properties of gluten are crucial for breadmaking quality in wheat and are influenced by both genotype and environment. However, it remains unclear how weather and genotype influence gluten quality and, in particular, at which stage during grain filling the effects of weather become critical. This study, therefore, investigated how genotype and weather during grain filling influence the viscoelastic properties of gluten.

Winter wheat cultivars with a high market share in each season were grown between 2005 and 2025. Field trials were conducted at up to eight locations per year, including farmers' fields and research farms at the Norwegian Institute of Bioeconomy Research, in collaboration with the Norwegian Agricultural Advisory Services. Agronomic practices, including sowing time, fertilization, weed control, and harvest time, were tailored to each location. Fungicides were applied at the heading stage to control fungal diseases. Weather data, including daily values for rainfall, air temperature (mean, maximum, and minimum), and relative humidity, were collected from the closest weather stations to the field locations (lmt.nibio.no). Gluten was prepared from wholemeal flour with 2% NaCl in the Glutomatic 2100, and the maximum resistance to extension (Rmax) and extensibility were measured by the SMS/Kieffer Dough and Gluten Extensibility Rig attached to the TA.XT plus Texture Analyzer.

The results showed that the elastic properties of gluten varied not only across seasons but also among various locations within the same season. Genotype and environment each account for approximately 30% of the variance in Rmax. Approximately 20% of the variation in Rmax was explained by the differing sensitivities to environmental conditions among the cultivars. Weather conditions around physiological maturity have been shown to be critical for gluten quality for spring wheat [1]. To investigate whether this applies to winter wheat, the physiological maturity date was estimated using temperature data from local meteorological stations when the dates were unavailable. We could not determine the critical period for Norwegian winter wheat as we did for spring wheat; instead, the data suggest that weather conditions after physiological maturity are equally important for gluten elasticity.

[1] Kollstrom et al., 2025, JCS 121, DOI: 10.1016/j.jcs.2024.104095

- Climatic Drivers of Quality trait Performance in Spanish Bread Wheat Landraces

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Quality and agronomic performance are central goals for wheat breeding. Contradictory findings regarding the influence of climatic variables on wheat quality point out the need of further research on the factors driving genotype-by-environment interaction (GEI) to facilitate selection of stable genotypes. This study investigated the main climatic drivers of GEI of wheat quality-related traits across four Mediterranean environments, and identified stable wellperforming landraces, which are increasingly valuable for lowinput agricultural systems.

Sixty spring wheat landraces were evaluated for flour protein content (PC), sedimentation volume (SDSS), and test weight (TW), key indicators of functional and market quality. Trials were conducted over two seasons in two Spanish locations (Centre and South). Genotype, environment and GEI effects were highly significant for all traits. Location exerted a stronger influence on GEI than seasonal variation. Between localities, GEI was mainly associated with temperature during the vegetative growth period, while seasonal differences were mostly driven by post-heading conditions. PC and SDSS shared broadly similar climatic drivers of GEI. In contrast, TW was influenced by additional factors -particularly water availability before heading- which did not affect functional quality traits. Among the highestyielding landraces, both stable genotypes and accessions with specific environmental adaptation were identified.

The study demonstrates the value of comprehensive climatic characterization in multi-environment trials to investigate the contribution of individual variables to GEI and for predicting genotype performance in untested environments. Selection of genotypes with stable functional quality appears more feasible in the South location, whereas multi-season evaluations encompassing wide ranges of post-heading thermal and hydric conditions are advisable to identify specifically adapted genotypes to the Centre. Achieving broad adaptation for processing quality across both regions seems more challenging and requires trials incorporating contrasting temperatures during the vegetative phase.

- How to assess the wheat quality in a changing climate: Can multi-target approach by LC-MS/MS, SE-HPLC, NIR, the sedimentation methods and Ai provide a desired outcome?

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Assessing the gluten quality is a demanding task requiring great efforts from breeders and wheat food producers. Climate change makes this task more difficult due to the imposed instability in the wheat gluten protein material. Climate-change events, such as heat and drought, induce changes in the gluten protein amount and composition(1) leading to complex differentiation of genotypes' quality in breeding process. Furthermore, the bread baking industry is desiring robust methods to assess the gluten quality and quickly predict the flour, dough and bread baking performance. Here, a multi-target approach assessing the gluten protein quality is highly desired. For bread producers, reliable methods to assess flour, dough and baking performance are of the highest importance. With this study, we evalute the gluten protein quality in wheat from diverse climates using LC-MS/MS, SE-HPLC, NIR and the sedimentation methods with the aim to measure the drought stress impact. The polymeric proteins evaluated by LC-MS/MS have shown specific the HMW and LMW-glutenins subunits in the varying in climate (2). The polymer proteins (%UPP) indicated a significant correlation (0.52, $P<0.01$) with the Extensograph parameters and the Glutomatic gluten index (0.71, $P<0.001$) as studied by SE-HPLC. From the sedimentation tests, the swelling index of glutenin (SIG) showed the greatest correlation (0.62, $P<0.001$) with the Extensograph values and with the Glutomatic wet gluten (0.67, $P<0.01$). NIR spectra of wheat flours showed no significant correlation with the gluten parameters (TOTE) and gluten strength (%UPP) as evaluated by SE-HPLC. Further steps including Ai-based prediction of gluten protein quality using a multi-targeted approach that includes data from several techniques might be a way forward to map gluten quality in fluctuating future climates.

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04 NUTRITIONAL IMPACT & HEALTH EFFECTS

- Amylase trypsin inhibitors activation of toll-like receptor 4 revisited: The dominance of lipopolysaccharides contamination

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Amylase-trypsin inhibitors (ATIs) found in cereals may contribute to symptoms in people with irritable bowel syndrome (IBS) and nonceliac wheat sensitivity (NCWS). These proteins are thought to interact with the TLR4MD2CD14 receptor complex in the gut and trigger inflammation, but more detailed cellbased studies are needed to fully understand this mechanism.

In this study, we prepared and characterized an ATIenriched fraction (AEF) and tested whether it could activate TLR4MD2CD14 using a human TLR4 reporter cell line (HEKBlueTM). To separate the effects of ATIs from those of lipopolysaccharides (LPS), we used Polymyxin B (PMB) to block LPS binding to TLR4. We also treated the AEF with Proteinase K to break down the proteins and remove any proteindriven effects.

Our experiments showed that PMB reduced the AEFinduced signal by 92%, indicating that much of the activation came from LPS rather than from the proteins themselves. Unexpectedly, when the AEF proteins were fully hydrolyzed, the TLR4 activation doubled, suggesting that complexes between protein and LPS may actually limit LPSdriven activation.

These findings highlight the importance of accounting for nonprotein components-especially LPS contamination-in cellbased ATI studies. Overall, the results show that LPS is a strong activator of the TLR4MD2CD14 pathway and raise questions about how much ATIs alone contribute to intestinal inflammation in IBS and NCWS. In future studies the complex of ATI/LPS should be taken into account.

- Breeding Health-Promoting Wheat: Enhanced Phenotyping, Germplasm Mining, and Marker-Assisted Selection

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Epidemiological evidence indicates that staple foods play a critical role in human health. In wheat, arabinoxylans (AXs), ferulic acid, and alkylresorcinols are major health-promoting components, alongside yield potential and processing quality. This presentation summarizes our recent progress in developing and utilizing health-promoting wheat varieties to improve human health.

(1) We developed enhanced, eco-friendly phenotyping methods for phenolic acids and alkylresorcinols, and successfully adapted the CIMMYT protocol for AX assessment. These methods increased analytical throughput by 3-5× while reducing costs by ~50%.

(2) We evaluated health-promoting traits in diverse germplasm across multiple environments, including 400 Chinese varieties, 200 Pakistani varieties, and 267 advanced breeding lines from CIMMYT's 53rd IBWSN. This work broadened our understanding of parental resources for improving bioactive profiles. Notably, Taishan 1 and Shan 715 showed higher WE-AX content than Yumai 34 (a benchmark for AX). In spring wheat, several "Jinqiang" lines with pedigree traceable to the Canadian cultivar "Wildcat" exhibited AX profiles comparable to Yumai 34. We also identified 16 CIMMYT lines combining high ferulic acid content with high thousand-kernel weight.

(3) Using linkage mapping in a Zhongmai 578/Jimai 22 RIL population, we identified stable QTLs for AX (1), ferulic acid (3), and alkylresorcinols (4). We developed and validated KASP markers for these loci, and marker-assisted selection enabled the development of CA17169 (registered as Zhongmai 39) with improved health-promoting and bread-making qualities. In parallel, we optimized variety-specific processing for Zhongmai 578: pearling increased loaf volume by ~30% compared with wholemeal flour while maximizing retention of AX, ferulic acid, and alkylresorcinols. We observed a significant positive correlation between wholemeal and refined flour bioactive levels, indicating refined flour can still provide meaningful amounts of ferulic acid and AX. Interestingly, newly developed varieties showed bioactive profiles comparable to, or exceeding, those of landraces.

Overall, our results highlight the value of integrating wheat genetics, food science, and human nutrition to enhance dietary intake of AX and phenolics for better health outcomes. International collaboration in germplasm exchange, high-throughput phenotyping, and molecular breeding will be essential to advance global nutrition and health security.

- Sprouting of Wheat Reduces Immunogenic Gluten Peptide Release but Increases Acrylamide Formation in Baked Products

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Celiac disease, an autoimmune enteropathy, is triggered by the formation of immunogenic gluten peptides due to the presence of proline and glutamine amino acids in gluten. Sprouting process of grains initiates a series of metabolic reactions, including the hydrolysis of macronutrients into peptides and amino acids increasing bioaccessibility of proteins. Consequently, sprouting process might provide the hydrolysis of gluten peptides. On the other hand, the hydrolysis of proteins also accelerates the Maillard reaction in baked goods, potentially increasing acrylamide formation. Therefore, investigating the sprouting process from a food safety perspective is crucial, as acrylamide is a probable carcinogen and formed in bakery products. This study aimed to assess the impact of sprouting wheat on immunogenic gluten peptide release and acrylamide formation. Wheat grains were sprouted for up to 72 h at 20°C and 95% relative humidity under dark conditions. Sprouted wheat flours were subjected to in vitro digestion process to monitor the immunogenic gluten peptides. Among the various immunogenic gluten peptides, 33-mer, the well-known and most immunogenic gluten peptide, was detected by high resolution mass spectrometry analysis. In the concentrations of 33-mer peptide a 68% decrease was observed by the end of 24 h of sprouting. Prolonged sprouting time to 36 h could provide a 79% decrease, while reaching an 85% reduction at the end of 60 h of sprouting. Furthermore, free amino acids and reducing sugars increased during sprouting, accelerating the Maillard reaction and resulting in acrylamide formation. Sprouted wheat wholemeal was incorporated into biscuits and crackers to evaluate their food safety attributes. Acrylamide formation increased by 36% in biscuits prepared with 48-h sprouted wholemeal. In addition to this, a significant increase in acrylamide was observed in crackers formulated with the incorporation of sprouted wholemeal for a 72-h period. These indicate that sprouting reduces the immunogenicity of wheat flour but increase its acrylamide forming potential. While sprouted wheat flours could be useful in developing low-gluten products for individuals with celiac disease or gluten sensitivity, their use should be combined with certain mitigation strategies to minimize acrylamide risk.

- What does untargeted metabolomics from in vitro digestates of wheat bread tell us?

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Metabolomics offers a powerful means to investigate metabolic profiles. It has been extensively used to study biological matrices like biological fluids and is now used as a phenotyping tool for identifying diagnostic or prognostic biomarkers. However, untargeted metabolomic analyses of in vitro digestate matrices are still scarce.
In this work, we explored the metabolic response associated with an in vitro digestion of different breads, each made with a given process (yeast or sourdough) and a given cultivar (four bread wheat (Triticum aestivum) cultivars contrasted for their protein digestibility). Each bread was masticated by the AM2 artificial masticator. Bolus were then digested thanks to a dynamic digester (DIDGI©). AM2 and DIDGI© mimicked the physiological conditions of the adult oral and gastrointestinal digestion. In vitro digestates were collected at four sampling times (5, 30, 60 and 120 min). Their analysis, using an untargeted high-resolution mass-spectrometry (HRMS) based approach allowed the identification of 1,146 and 197 robust metabolic features, in positive and negative ionization modes respectively. The Principal Component Analysis (PCA) based on the positive ions (1/3 being annotated) showed a clear discrimination of metabolites based on digestion time, independent of the wheat varieties and breadmaking processes, highlighting the dynamic nature of the digestion process and the evolution of the metabolomic profile over time. Orthogonal Projection to Latent Structure - Discriminant Analysis (OPLS-DA) revealed that these 1,146 metabolites also discriminated the process and at a less extend the wheat variety. Interestingly, it also clearly discriminated between cultivars exhibiting high versus low protein digestibility. Four unannotated metabolites were significantly more abundant in high digestible varieties.
Taken together, these results indicate that metabolite profiles can be used to distinguish in vitro gastric digestates derived from different breads, as well as from cultivars with high or low protein digestibility. In particular, four metabolites emerge as candidate markers of wheat bread resistance to gastric digestion.

- Genetic Dissection and Targeted Modification of Wheat Gluten Proteins for Reduced Immunogenicity

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Gluten proteins determine the nutritional value, functional properties, and immunoreactivity of wheat. These proteins are encoded by 12 loci on group 1 and 6 chromosomes. The Gli1, Gli2, and Glu3 loci are structurally complex and, in the Chinese Spring reference genome, contain 27 α-gliadin, 12 γ-gliadin, 6 ω-gliadin, and 15 low-molecular-weight glutenin genes. Despite detailed genomic characterization, the correspondence between specific gluten genes and protein bands resolved on acid- and SDS-polyacrylamide gels remains poorly understood. Previous studies identified key regulators of gluten protein expression. Transcription factors such as PBF and SPA bind conserved motifs, including the prolamin box and GCN4-like motif, to regulate prolamin genes, while epigenetic regulators such as DEMETER and DRE2 selectively demethylate gliadin gene promoters in the endosperm, modulating gluten protein accumulation. This study expands current knowledge by identifying cis- and trans-regulatory loci controlling gluten protein expression. Wheat aneuploid stocks, lines with terminal deficiencies and interstitial deletions, ABA-insensitive and hypersensitive mutants, and a D-subgenome-confined nested association mapping population were used to establish gene-protein correspondence and map novel regulators. These analyses clarified relationships between genes and their protein products, identified cis- and trans-regulatory protein quantity loci (PQLs) associated with variation in gliadin and glutenin content, and revealed new regulatory loci on chromosome 2A in addition to a strong PQL on chromosome 7D. These findings informed strategies to reduce immunogenic gluten proteins. Crossbreeding approaches combined mutants lacking ω-gliadins (gli1 triple null) with lines carrying reduced α-gliadins (gli-A2 and gli-D2 null), along with additional gli1 double nulls and pbf triple-null and spa single-null mutants, to generate reduced-gluten wheat lines. In parallel, CRISPR-Cas12a and a Cas12a-RRV variant targeted 12 sites across four gluten gene families, with constructs delivered to wheat embryogenic calli via biolistics and edited plants screened for mutations. Additional approaches include RNAi-mediated suppression of the epigenetic regulator DEMETER and gluten detoxification through endosperm expression of prolyl endopeptidase and glutamine-specific endoprotease. These strategies are being evaluated through genetic, biochemical, immunological, agronomic, and end-use assessments. Public perceptions of conventionally bred and gene-edited wheat lines were also examined. These findings and the lessons learned will be discussed.

- Svevo high-amylose enriched diet improves the quality of life of patients affected by Chron’s disease and metabolic syndrome

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Wheat genotypes with high amylose content have been proposed as healthy ingredients for the development of functional foods lowering postprandial plasma glucose response and improving postprandial metabolism.
The objective of the present study was to investigate the effects of the durum wheat Svevo improved for high amylose content in a pilot clinical trial involving patients affected by low to moderate symptoms of concomitant inflammatory bowel disease (Chron’s disease) and metabolic syndrome. The first group of patients received a low calorie diet including Svevo high amylose wheat-based products (i.e: pasta: 100 g/die, crackers: 20 g/die and biscuits: 25 g/die), whereas the control group received the same diet in which the same type and quantity of durum wheat-based products was given, but made using traditional Svevo semolina. Several inflammatory markers and metabolic parameters were monitored every six weeks on patients, including Bio-Impedance Analysis, Skeletal muscle mass (SMI) index, nutrition risk screening (MUST), Crohn’s disease activity index (CDAI), means of anthropometry, bioelectrical impedance vector analysis (BIA). Moreover, feces were collected at the beginning of the study and after twelve weeks to be analyzed for variation in the microbiota composition as depending on the diet. The results showed that the high amylose diet was associated with a higher tendency to reduce circulating markers associated with metabolic syndrome including cholesterol, glycemia and triglycerides, together with fat mass. The group receiving the Svevo high amylose wheat products showed a significant improvement of intestinal-related functions, improvement of muscle mass/SMI index and reduction of malnutrition, improvement of stool consistency (Bristol index), reduction of daily evacuation number, thus resulting in a significant improvement in the quality of life of patients.

- The Role of Gluten/Wheat-Free Diet in IBS Patients: Assessing Symptom Severity and Well-being

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Background: Irritable Bowel Syndrome (IBS) is a complex functional gastrointestinal disorder characterized by chronic abdominal pain and altered bowel habits. Wheat consumption can often exacerbate symptoms due to intolerance to Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols or to innate immune activation by α-Amylase/Trypsin Inhibitors. These components are more prevalent or bioactive in modern, highly processed wheat varieties than in ancestral strains.

Objective: To assess the impact of Gluten/Wheat-Free Diet (G/WFD) on gastrointestinal (GI) symptoms, extraintestinal manifestations, and Quality of Life (QoL) in patients diagnosed with IBS.

Methods: This study includes 28 patients with IBS (aged 18-75 years). Participants adhered to a high-content wheat diet, consuming at least 100 grams of wheat daily for the first month (T0). Then, they followed a G/WFD for 3 months (T1). Symptom severity and clinical outcomes were evaluated at the end of each period using validated questionnaires: the Modified Gastrointestinal Symptom Rating Scale (GSRS-m) for pain and bloating, the Visual Analogue Scale (VAS) for extraintestinal symptoms, the IBS Symptom Severity Scale (IBS-SSS), the IBS-QoL, and the FANTASTIC Lifestyle Questionnaire (FLQ).

Results: Preliminary data indicate a strong correlation between wheat exclusion and symptomatic relief. To date, 10 of the 28 enrolled patients have completed the T1 phase, while 18 are still following the G/WFD. Among those who completed T1, 8 patients (80%) demonstrated significant clinical improvement and could be suspected as wheat-sensitive IBS according to the Salerno Criteria (improvement ≥ 30%), if they will have a worsening at the following wheat challenge. Conversely, 2 patients showed no benefit. The GSRS-m analysis revealed an improvement exceeding 30% across several parameters, i.e., abdominal pain (54%), heartburn (37.5%), bloating/swelling (44.4%), nausea (40%), borborygmi (38.6%), and abdominal distension (39%). Improvements were also noted in stool consistency and passage, including urgency (47.4%) and incomplete evacuation (33.3%). Regarding extraintestinal symptoms (assessed by VAS), significant reductions were observed in fatigue (57.7%), dermatitis (45.45%), "foggy mind" (41.2%), and joint pain (37.14%). Overall QoL improved, particularly body weight perception (35.5%) and psychological status (33.3%).

Conclusions: These preliminary data showed that G/WFD significantly alleviated both GI and extraintestinal symptoms while enhancing the QoL in IBS patients.

- Interdependences between Gluten Friendly technology, technological performances of wheat flour, and beneficial effect on gut microbiota of celiac subjects

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Gluten Friendly (GF) technology is a microwave-driven process applied to wheat kernel capable of tackling the celiac disease (CD) through physicochemical changes generated on gluten structure. The modifications significantly affect the rheological properties of the flours while maintaining a high quality of both dough and bread. GF technology also has been demonstrated to increase starch digestibility, especially for the slowly digestible starch (SDS) rather than the rapid (RDS), while the resistant starch (RS) has been reduced significantly; these modifications are related with the thermal properties exhibiting a reduction in starch gelatinization. Furthermore, the changes in protein structure reduce immunogenicity in treated flours by R5 Elisa test and cross-reactivity toward antibodies recognizing the antigenic epitope of gluten protein in treated wheat kernels. In vitro and in vivo studies carried out on celiac patients, showed a significant reduction of GF bread inflammatory potential along with enhanced gluten digestibility. Results also indicate the establishment of a positive environment for the proliferation of beneficial gut bacteria, as well as the modulation of the celiac gut microbiota towards homeostasis by boosting butyrate-producing species and promoting cluster-specific reorganization of microbial community, as also showed by networking analysis. Further analyses, such as Z-potential and FTIR spectroscopy, are currently being conducted to characterize Gluten-Friendly (GF) protein modifications. In parallel, epigenetic analyses of duodenal biopsies from patients with celiac disease are underway to further elucidate the anti-inflammatory effects of GF proteins, alongside immunohistochemical analyses to study in situ the protein expressed by the FOXP3 gene, a key transcriptional regulator involved in the development and inhibitory function of regulatory T cells (Tregs). This presentation describes in details the effect of Gluten Friendly Technology on compositional and technological properties of wheat flour proteins and starch and the bioactive properties of the GF proteins and their potential as possible future therapy for CD.

05 QUALITY OF GLUTEN-CONTAINING CEREALS

- End-use quality of Spanish landraces under organic cropping

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Wheat landraces adapted to a wide range of climatic conditions represent highly valuable genetic resources for breeding varieties suited to organic farming. Previous studies have shown that Spanish bread wheat landraces harbor extensive genetic diversity and are structured into four distinct genetic subpopulations [1]. Phenotypic screening of these materials identified a set of sixteen cultivars with competitive values for flour protein content (PC), gluten strength (measured by SDSS-test), and thousand-kernel weight (TKW), in addition to a high degree of variability for adaptability-related traits [2]

The selected landraces were genotyped for HMW and LMW-GS and grown under organic management during the season 2024-2025 at two locations (Experimental fields of ETSIAAB-UPM, Madrid; 40 25’ N, 3 42’ W and experimental station ITACyL, Valladolid; 41º. 42’ 8” N, 4º 42’ 31” O/W)). Wholemeal flour samples from the trials were evaluated using small-scale tests related to end-use quality, such as flour protein content (FPCSDSS-test and Glutopeak analysis). Additional grain milling was performed, and the proportions of the different obtained fractions: bran, semolina, and white flour were quantified. White flour performances were evaluated by alveograph and falling number analysis. Subsequently, bread-making was carried out following the UNE 34902 (AENOR) protocol with minor modifications.

The analysis confirmed the substantial phenotypic diversity present in this set, with dough strength (W) ranging from 312 to 53, tenacity (P) from 125 to 24, extensibility from 146 to 52, and the tenacity/extensibility ratio (P/L) from 2.5 to 0.27. Statistical analysis revealed the effect of the genotype, environment and genotype-by-environment (GxE) interaction on each of the parameters analyzed, as well as the correlations between quality traits of wholemeal and white flour with those determined for the final obtained bread.

Finally, the effect of the different HMW and LMW-GS alleles on quality parameters was explored providing the first assessment of the new alleles identified within this germplasm set.

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- Determining the influence of the wbm gene on gluten protein fractions using near-isogenic lines grown under greenhouse and speed breeding conditions

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This study focused on the relationship between the wheat bread-making (wbm) gene and gluten protein fractions in South African wheat cultivars and compared gluten protein fractions from near-isogenic lines (NILs) grown under conventional greenhouse and speed-breeding conditions to confirm that these environments don't affect protein composition and quality. The NILs were developed using cultivars Krokodil and PAN3471 as donor parents that carried the gene, and Koedoes and PAN3497 as recurrent parents that lacked the gene. Protein content was analyzed using NIR spectroscopy, and protein fractions were analyzed by reverse-phase HPLC. Results demonstrated variation in protein content and fractions among the NILs and their recurrent parents, in both the ARC-SG and Pannar backgrounds. The contribution of the gene was relatively small compared with that of major quality loci such as Glu-D1. Differences observed indicated that individual cultivar genetic backgrounds strongly affect the quantity and expression of protein subunits, even when glutenin composition was similar. The lines differed from their recurrent parent in several protein fractions, indicating that the gene influenced specific protein characteristics. The NILs group together in PCA biplots showed higher values for some gliadin and glutenin fractions than the recurrent parent, thus suggesting the gene contributed to changes in protein composition. In the Pannar population, NILs grown under different environmental conditions grouped more closely in the biplot with their respective recurrent parents. This indicated that environmental conditions, rather than the presence of the gene, had a greater influence on protein fraction variation for this population. The gene significantly affected protein fractions in the ARC-SG population. Changes in these fractions are meaningful, as gliadins and LMW-GS are known to influence loaf volume and bread-making performance. Overall, the study concluded that the gene had an inconsistent effect on protein fractions and quality across different genetic backgrounds. Therefore, the gene may not be a reliable target for improving bread-making quality in South African wheat breeding programs. Furthermore, speed breeding did not consistently increase or decrease protein fractions, but it remains a valuable tool for accelerating breeding cycles and developing improved wheat varieties.

06 PROCESSING & FOOD PRODUCTION

- Overcoming soft kernel texture and weak gluten in einkorn for couscous and wholegrain pasta via parboiling and micronization processes

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Einkorn (Triticum monococcum) offers acknowledged nutritional value but presents two major technological limitations: an extremely soft kernel texture and a weak gluten network. Kernel texture strongly influences flour yield, particle size, starch damage, water absorption and rheological performance. Although total gluten content in einkorn is comparable to modern wheat varieties, its gluten quality is peculiarly weak mainly due to an unbalanced gliadin/glutenin ratio, with gliadins largely prevailing. The objective of this study was to evaluate two targeted processes capable of enhancing gluten functionality in two einkorn varieties: (i) pre milling parboiling (hydrothermal treatment under pressure) to improve kernel texture and enable the production of coarse wholegrain grits (particle sizes >500 µm) suitable for couscous agglomeration; (ii) ultrafine grinding (micronization) to obtain wholegrain flours for the production of 100% einkorn pasta, spaghetti shape, with improved techno-functional and sensory properties. Products analyses included compositional and rheological tests with a specific focus on gluten functionality, cooking quality, texture and sensory evaluation by trained panelists. Nutritional traits such as protein, fiber and resistant starch content and total antioxidant capacity were also assessed. Parboiling significantly increased kernel hardness, consistent with the displacement of puroindoline proteins from starch granule surfaces, leading to a transition toward a hard texture endosperm (hardness index >70). This modification enabled the production of coarse einkorn grits and successful couscous agglomeration. Wholegrain pasta by micronized flour showed high protein (>18% d.m.) and total dietary fiber (>6%) content. Despite elevated protein levels, gluten strength remained scarce, with gluten index values ranging from negligible (0-5%) to moderate (40-50%). The weak gluten matrix of whole grain spaghetti contributed to shorter optimal cooking time, reduced water absorption and higher cooking losses compared to semolina pasta, though all samples remained within acceptable technological standards. Antioxidant capacity was markedly higher in both einkorn pasta and couscous (+43% vs semolina) and resistant starch levels were enhanced. Parboiling and micronization effectively address the inherent softness and weak gluten functionality of einkorn, enabling the development of high quality couscous and wholegrain pasta with valuable nutritional properties.

- Market and labeling of oat-based products with and without gluten in Brazil: a study of characterization and compliance

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Oats are one of the main winter cereals produced in Brazil, and in recent years, there has been an increase in the availability of oat-based products labeled as gluten-free. However, to date, no studies have characterized this market in Brazil. Gluten labeling in Brazil is regulated by Law No. 10.674/2003, which requires the declaration “contains gluten” when gluten is intentionally present or occurs due to cross-contamination, or “does not contain gluten” when its presence is undetectable. In addition, oats are considered an allergenic food according to RDC No. 727/2022, requiring mandatory declaration on food labels. This study aimed to characterize the Brazilian market for oat-based products with and without gluten, as well as to assess labeling compliance and price variation. Data collection was conducted between March and April 2026 through data collection in physical retail stores and digital platforms. Oat-based products were included, and information was recorded on brand, net content, the presence of mandatory gluten and allergen statements, the presence of certification seals, mentions of cross-contamination prevention protocols, and retail price (collected in triplicate). Imported products, bulk products, and composite formulations such as granolas and flavored beverages were excluded. A total of 202 products from 84 brands were identified, with 70% labeled as “contains gluten” and 30% as “does not contain gluten.” Products were classified into nine categories (with/without gluten): oat grains (4/0), bran (18/6), flakes (87/28), flour (17/9), instant oats (2/1), beverages (11/12), cream (1/1), concentrate (1/3), and condensed (0/1). Gluten-free products showed an average price 49.6% higher than products containing gluten. All products containing gluten displayed the mandatory “contains gluten” statement. However, six products labeled as gluten-free indicated the presence of traces of wheat, rye, and/or barley, suggesting inconsistencies in risk communication. No specific certification seals related to “gluten-free” claims were observed. Given that labeling is a primary tool for consumer risk communication, the identified inconsistencies-particularly the presence of allergen traces in products labeled as gluten-free-highlight gaps in labeling reliability within the Brazilian market. Additionally, the substantial price premium observed for gluten-free products underscores a potential economic burden for individuals requiring gluten-free diets.

- Studying Factors Affecting Wet Milling of Wheat Flour into Starch and Gluten and Developing Wheat for Gluten Production

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Utilization of wheat may be divided into four categories: food, feed, seed and industrial uses. Food is the major use of wheat, providing more than 20% of the calories for the world population. A major industrial use of wheat is wet-processing of wheat flour into vital wheat gluten and wheat starch. In general, hard red winter wheat or hard white winter wheat is acceptable for wet-processing into gluten and starch but not all hard wheat varieties result in desirable high yield and high quality of wheat gluten. Protein level and quality in wheat can also vary during wheat harvest. Th e objectives of this research are to (1) determine the factors affecting wet-processing of wheat flour into gluten and starch; (2) identify and select Kansas wheat varieties that are best suitable for production of wheat gluten. Hard red winter wheat using genomics-assisted selection were assembled to understand the genetic factors that influence both protein content, extractability of gluten and the effects of wheat starch. This panel is the result of the intermating of twenty-six parent lines that were selected for agronomic performance, high protein content and gluten functionality. The population was intermated three times and 330 lines were generated through double haploid production. Factors affecting wet-processing of wheat flour into gluten and starch were studied. A dough washing method was used to separate wheat flour into four main fractions: starch, gluten, tailings and water-solubles. The protein content, total starch content, and ash content of each fraction were determined. It is found that the starch yield for the selected samples ranged from 67.1-80.1% and starch recovery ranged from 69.3-84.2%, while the protein yield is between 8.1-13.6% and protein recovery is between 80.4-96.6%. Multiple analytical methods were applied to determine flour functionality, including the farinograph, GlutoPeak, Glutomatic system, falling number, and solvent retention capacity tests, and used to correlate wheat gluten yield and recovery. An economic analysis is performed to guide the best path forward for utilization of these wheats for value capture by the starch and gluten industry and Kansas wheat producers.

- Urutrigo: a practical system for rapid baking strength classification in Uruguayan wheat

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For wheat-exporting countries, meeting industrial quality standards is essential to remain competitive in international markets. Uruguay initiated wheat exports approximately two decades ago, triggering heightened quality requirements-particularly regarding baking strength. However, national varietal registration policy mandates that new varieties demonstrate agronomic performance data within the country, without allowing poor industrial quality to restrict their release. This regulatory framework created the need for a practical, cost-effective classification system enabling rapid quality assessment upon grain delivery to storage facilities. While physical quality (test weight), absence of pre-harvest sprouting (Falling Number), low mycotoxin levels (via low Fusarium damage), and protein content (via near-infrared spectrophotometry) can be swiftly evaluated, baking strength assessment presented a significant bottleneck. To address this challenge, Urutrigo was developed: a branded designation for premium Uruguayan bread wheat, combining “Uru-” (Uruguay) and “trigo” (wheat). The program operates through self-financing by seed companies and has been scientifically validated. Using a standardized set of trials, the relationship between gluten baking strength (defined as Alveograph W) and protein content is determined for each genotype. When the linear Pearson correlation is significant ($P<0.01$) and the predicted Alveograph W value exceeds 250 Joules/10,000 at 11.5% protein (dry basis), the cultivar qualifies for “Urutrigo” designation. Despite this being a demanding requirement that most adapted genotypes cannot meet, seed companies are actively promoting its adoption. Although not yet universally applied, some companies now offer premium prices for Urutrigo-certified wheat grain. Commercial grain from Urutrigo varieties has demonstrated superior baking strength, consistently achieving quality standards of W >250 J/10,000.

3

POSTERS

01 CUTTING-EDGE RESEARCH & TECHNOLOGIES ON KERNEL COMPONENTS

01 - *Targeting Major Celiac Disease Epitopes: Sequential Deletion of Gli-D2 and Gli-A2 Loci in Wheat*

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α-gliadins harbor the primary immunogenic epitopes triggering Celiac Disease (CD), most notably the highly toxic 33-mer peptide. To address this, we established a precision mutagenesis strategy using the model wheat cultivar ‹Butte 86›, selected for its comprehensive genomic and proteomic resources. Our epitope profiling of ‹Butte 86› revealed a clear toxicity hierarchy: the Gli-D2 locus on chromosome 6D contributes the highest immunogenic load (including the 33-mer), followed by the Gli-A2 locus on chromosome 6A. Guided by these insights, we employed proton beam and gamma-ray irradiation to sequentially target these high-risk loci. Initially, we successfully isolated deletion lines lacking the Gli-D2 locus, confirming the complete removal of the 33-mer peptide. Building on this, we subsequently screened for Gli-A2 null mutants using 6A-specific markers. Validation via Copy Number Variation (CNV) analysis and 2D-immunoblotting confirmed the elimination of both Gli-A2 genes and their corresponding proteins. These genetic stocks facilitate the pyramiding of deletion traits, enabling the development of hypoallergenic wheat that retains essential end-use quality.

02 - *Targeting Immunodominant Omega-1,2 Gliadins in Wheat: Antibody Validation and Identification of Deletion Mutants*

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While α-gliadins are widely recognized triggers of Celiac Disease (CD), ω-1,2 gliadins also harbor major immunodominant epitopes that induce comparable immune responses in genetically predisposed individuals. Consequently, the elimination of ω-1,2 gliadins is considered a prerequisite for the development of wheat varieties with significantly reduced toxicity for celiac disease patients. In this study, we employed a two-step strategy involving precise antibody validation followed by mutant screening.

First, to establish a reliable screening system, we characterized polyclonal antibodies raised against the N-terminal region of ω-1,2 gliadins. The specificity of these antibodies was rigorously validated using the reference wheat cultivar ‘Chinese Spring’ and its group 1 aneuploid lines. 2D-immunoblotting analysis confirmed that the antibody specifically react with ω-1,2 gliadins encoded by the Gli-A1 and Gli-D1 loci.

Leveraging this validated antibody, we screened a mutant population of the model cultivar ‘Butte 86’ generated by radiation mutagenesis. We successfully identified and characterized deletion lines lacking three specific ω-1,2 gliadin proteins encoded by the Gli-A1 and Gli-D1 loci. These novel genetic resources will be useful for further breeding programs aimed at reducing the immunogenic potential of wheat.

02 GENETICS, GENOMICS & BREEDING OF GLUTEN CONTAINING CEREALS

03 - *A genetic toolkit to reduce wheat immunogenicity and the incidence of Celiac Disease*

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The amino acid sequences within gluten proteins that elicit immune responses in CeD patients are designated as immunogenic epitopes. Multiple studies have shown that the amount of consumed immunogenic wheat epitopes correlates with the incidence of CeD. These results indicate that the quantity of ingested immunogenic epitopes may affect the onset of the disease and that, by extension, a reduction of wheat immunogenic epitopes may reduce the incidence of the disease at the population level. Complete removal of all gluten proteins from wheat is not possible without affecting the viscoelastic properties of bread and pasta dough. Gluten is composed of glutenins and gliadins, with glutenins playing the most substantial role in the formation of the gluten polymer that is critical for dough strength and gliadins having the majority of the immunodominant epitopes. Gliadin and glutenin genes are distributed in 9 main loci on the hexaploid wheat genome, and we have developed lines combining radiation-induced deletions, chemical-induced mutations, and CRISPR-edited mutations that eliminate CeD epitopes. We follow two different strategies to address research and breeding objectives. For our research objective, we are combining the loci without any epitopes to generate “clean” genetic stocks that will be tested in CeD patients to confirm the complete elimination of immunogenic epitopes. For our breeding objective, we are combining the deletions that eliminate the immunogenic gliadins but not the glutenins to generate breeding lines with adequate breadmaking quality and reduced immunogenicity. Finally, we are initiating transgenic experiments to introduce edited glutenins without known immunogenic epitopes in lines lacking the endogenous glutenin proteins to generate CeD-safe lines with restored breadmaking quality. Our epitope-free genetic stocks can then be used to test the immunogenicity of synthetic glutenins and their ability to restore breadmaking quality.

04 - *AMIGRANO - Obtaining new resistant-starch durum wheat varieties for the development of a high nutritional value supply chain*

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The AMIGRANO project aims to develop new durum wheat varieties with high amylose content, enabling the establishment of a specialized, high-nutritional-value supply chain-the first of this kind in Europe. AMIGRANO is a three-year project led by CGS Sementi and funded under the SRG08 Intervention of the Emilia-Romagna Co-PSR. High-amylose foods exhibit increased resistance to enzymatic starch digestion and are a major source of resistant starch. A diet rich in resistant starch, a fermentable dietary fibre, provides several health benefits: it helps regulate blood glucose levels and positively influences the intestinal microbiota. In pasta, a high resistant starch content also ensures improved firmness and cooking stability.

The project methodology follows an integrated approach, starting from molecular breeding and progressing to field trials, technological validation and business model analysis. Based on crosses between the elite durum wheat varieties from CGS Sementi and donor lines carryng the resistant starch (RS) trait, the research and experimental activities include: (i) development of molecular markers for KASP assays and identification of target mutations; (ii) screening of F2 progenies; (iii) backcrossing with parental lines and fixation of the “high amylose” trait; (iv) advancement to the BC1F2 generation and large-scale screening; (v) analysis of starch content and composition of the selected lines; vi) comparative field trials and small multiplication strips for sufficiently stable RS lines and vii) quality evaluation of both seed material and durum wheat milling products.

AMIGRANO activities will enable the development of RS varieties suited to the Mediterranean area supporting the production of milling products with a high level of resistant starch and dietary fibre. To ensure market alignment and the competitiveness of the supply chain arising from the project outcomes, AMIGRANO will develop a comprehesive analysis of the value proposition, focusing on four targets: the processing industry, retail, catering, and end consumer, including a business model analysis.

05 - Investigating potential bZIP-mediated amylose regulation in durum wheat through CRISPR-Cas9 knockout of ZmbZIP22 orthologues

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With the aim of developing durum wheat lines with enriched health-promoting properties in downstream products, we targeted ZmbZIP22 orthologues in Triticum turgidum ssp. durum cv. Svevo for knockout. Indeed, in maize, ZmbZIP22 has been reported as a negative regulator of amylose biosynthesis. Based on this evidence, we aimed to generate loss-of-function mutants to potentially increase amylose content.

Orthologues genes were knocked out via precise genome editing using the CRISPR-Cas9 system mediated by Agrobacterium tumefaciens transformation.

To do so, two sgRNAs were designed to target exon 1 of both orthologous gene copies (AABB) to achieve a complete knockout with a single transformation event and to obtain NGT1-type lines that should not be considered genetically modified materials.

Sanger sequencing of the resulting lines confirmed the loss of genes function through i) minor editing, ii) inversion, and iii) large deletion between the guides' targeted regions, which introduced a premature stop codon due to a detrimental frameshift in the amino acid sequences.

Preliminary analysis of starch content and composition revealed no differences between edited lines and controls, suggesting either functional redundancy among these transcription factors or possible neofunctionalization that remains to be explored via RNASeq analyses. Studies on gluten composition and technological properties are in progress.

06 - Common winter wheat cultivars released in Europe during the second part of the XXth century present clear space-based structure as studied using the polymorphism at the GLI-1A locus.

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Grain storage proteins, particularly gliadins, have historically been among the most widely used genetic markers for the characterization and differentiation of wheat genotypes. Owing to their extensive intraspecific polymorphism and the multiple allelic variants of their encoding genes [1], gliadins provide a powerful tool for genotype identification and for broader genetic studies in wheat.

In this study, gliadin genotypes in more than 600 winter wheat cultivars released throughout the second part of the 20th century in 14 European countries were analyzed. Allelic variation at six main gliadin-encoding loci locus was assessed using acid polyacrylamide gel electrophoresis (A-PAGE) and the pedigrees of the cultivars studied. No one pair of non-related cultivars had identical or even similar gliadin genotypes when the six main gliadin loci were considered. At the Gli-A1 locus, about 30 alleles were identified, and all of them were described, being shown in different genetic backgrounds, in more than 100 electrophoregrams. Moreover, at the Gli-A1 locus, strong differences in allelic frequencies were observed among cultivars bred in different countries. The relationship between genetic and geographic distances were examined, and potential factors underlying the spatial genetic structure observed in European wheat germplasm were discussed.

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Reference

[1] Metakovsky, E.; Melnik, V.A.; Pascual, L.; Wrigley, C.W. (2024). How important are genetic diversity and cultivar uniformity in wheat? The case of gliadins. Genes, 15, 927. https://doi.org/10.3390/genes15070927

07 - Structural organization, expression and functional analysis of prolamin genes in durum wheat cultivar Svevo.

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Durum wheat prolamins are known to play an important role in determining the characteristics of durum wheat semolina. Variants in the prolamin electrophoretic profile have been associated with dough functional properties, while the presence of specific epitopes is known to determine the immunogenic potential of foods produced using durum wheat flours. In general, efforts to improve the features of durum wheat flours have to deal with the complexity of the prolamin family, which is encoded by a large set of genes clustered in few chromosomal loci. Recent advances in genome sequencing and assembly allowed to obtain a complete picture of the family of prolamin genes expressed in the endosperm of durum wheat cultivar Svevo, which was found to comprise 112 members located on chromosomes 1A, 1B, 4A, 6A and 6B, and 7A. About half (51%) of these genes was classified as pseudo genes, characterized by low or null mRNA levels. Full size genes mRNAs were evident starting at 5 or 11 days post anthesis (DPA), and mRNA levels remained high till advanced stages of seed maturation (30 DAP). Here we show that considerable variation in mRNA levels is evident within each prolamin subfamily, suggesting that a restricted set of highly transcribed genes is likely to give a major contribution to the final protein composition of durum wheat flours. In addition, we identified epitopes associated with coeliac disease, and we examined their distribution across all prolamin gene families in relation to expression patterns.
Altogether, this information may be useful for the genetic improvement of durum wheat quality using genomic techniques.

04 NUTRITIONAL IMPACT & HEALTH EFFECTS

08 - Modifying alpha-amylase/trypsin inhibitors (ATIs) composition in wheat: from existing variation to genome editing

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Alpha-amylase/trypsin inhibitors (ATIs) are a cereal multigenic protein family with defence role for the plant. Wheat ATIs are the cause of IgE-mediated allergy and are putatively involved in Non-Coeliac Wheat Sensitivity (NCWS). Although the exact mechanism of the latter is still unclear, it has been hypothesized that ATIs could be the triggering factor through their binding with Toll-like Receptor 4 (TLR4). In this context, the reduction of ATIs in wheat is useful for understanding their role and improve wheat safety. Since some studies reported a potential increased tolerability of “ancient grains” in NCWS patients, we investigated the ATI composition of five “ancient” Sicilian durum wheats (Bidì, Perciasacchi, Russello, Timilia reste bianche and Timilia reste nere) in comparison with three modern cultivars (Iride, Orizzonte and Simeto) in a multi environment trial. Our results showed no significant variability in total ATI content and a significant effect of the environment on their accumulation. Significant differences were found for some less abundant ATIs: 0.28, CMX 1/2/3 and WTI.
Using a TILLING approach, we found two durum wheat lines of the cv. Kronos containing a stop codon mutation for the two ATI genes CM3 and 0.19. Through crossing, we obtained double mutant lines and analyses of their inflammatory potential are ongoing.
In parallel, genome editing was used for silencing the two ATI genes CM3 and CM16 in the durum wheat Italian cultivar Svevo. Previous results showed a reduction in inflammatory potential in comparison with the wild type control. On these bases, a multiplex genome editing approach, based on polycistronic gRNA arrays, is under development for the silencing of additional ATI genes. Overall, the genetic material we are developing will allow us to elucidate the potential ATIs biological activity on human health.

09 - Phytochemicals, nutritional and genetic profiling of advanced wheat mutants for enhanced flour quality

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Wheat plays a vital role in ensuring global food security, with its nutritional and phytochemical attributes significantly impacting flour quality and health benefits. This study assessed 30 advanced wheat mutant lines based on their nutritional composition, bioactive profiles and variations in the PIN gene linked to grain hardness. Results revealed that mutant SG1/12-41 showing the highest total phenolic content and catalase activity, while SE5/12-19 exhibited maximum peroxidase levels. Ascorbate peroxidase was highest in SE5/12-7, and SE4/12-1 showed high carotenoid content. The seed protein fraction showed the highest glutenin in SE5/12-19 and SG1/12-41, with higher globulin and albumin levels in SG1/12-41 and SE4/12-1. Micronutrient profiling indicated more zinc in KCT7/12-44, iron in SE5/12-12 and potassium in SE4/12-1. Mutants SE5/12-12 and SG4/12-35 showed the highest phosphorus and nitrogen, respectively. Moreover, milling trait assessment revealed variability in grain hardness index (56-89) and gluten content (37-45%). Mutant SG2/12-27 was exhibited the highest Zeleny sedimentation value (87.3) and SE4/12-5 recording the highest falling number (729 seconds). In addition, Allelic variation of the PIN gene associated with kernel texture, showed clear allelic patterns across the mutant panel. Mutants SE4/12-5 and SG4/12-35 exhibited higher HI values (89). Overall, these mutants show promising potential to enhance wheat flour quality for health benefits, functional food applications.

06 QUALITY OF GLUTEN-CONTAINING CEREALS

10 - *Structural analysis of wheat glutenins by diagonal electrophoresis for disulfide bond detection*

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Glutenins form large complexes through intermolecular disulfide bonds. The large size of glutenins prevents their analyses by proteomic techniques (e.g., sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), high performance liquid chromatography, or mass spectrometry) in intact or nearly intact states. Therefore, it has been difficult to easily grasp the overall picture of the glutenin structure or determine the differences between various wheat varieties. Herein, we found that Glutenins could be fragmented to the appropriate size by cleaving them with cyanogen bromide (CNBr). CNBr works very efficiently under acidic conditions, suppressing artificial disulfide bond scrambling and cleaving methionine residues that are rarely present in glutenin, resulting in peptides with molecular weights close to those of intact high-molecular-weight glutenin subunits (HMW-GS). And the diagonal electrophoresis of the obtained fragments, which involves separation under non-reducing condition in the first dimension and reducing condition in the second dimension, enabled the visualization of the disulfide bond patterns of glutenins. This structure was present in four varieties (including bread wheat, noodle wheat and cake wheat), with its composition depending on the cultivar-specific glutenin genotype. The constituent proteins were identified as HMW-GSs, low-molecular weight glutenin subunits (LMW-GSs), α-gliadin, and serpin using liquid chromatography-tandem mass spectrometry. Until now, the HMW-GSs in glutenins were thought to be linearly disulfide-bonded via head-to-tail bonds, while the linear branches comprise LMW-GSs linked to y-type HMW-GSs. However results in this study demonstrated that HMW-GSs formed a complex with disulfide bonds, even though the cysteines used for head-to-tail bonding were cleaved by CNBr. These suggest that the cysteines in the central part are also disulfide-bonded, forming a branched structure. Therefore, we added the newly predicted disulfide bonds to previously established glutenin structure models. In the future, by visualizing and comparing the gluten network structures of various flours and doughs using the method established herein, one can identify the dough property-affecting differences in the glutenin structure.

11 - Gluten quality and pasta-making performance in two Caucasian wheat species

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This study provides an in-depth characterization of gluten quality and functionality in two Caucasian hulled wheat species, the tetraploid Triticum timopheevi and the hexaploid Triticum zhukovskyi, grown in Italy. The research was carried out within the framework of the RGV-FAO project, which supports the preservation and valorization of plant genetic resources. Kernel physical traits relevant to commercial processing, including 1000-kernel weight, test weight, hardness index, ash content and color, were assessed to evaluate milling behavior and flour yield. Despite having a kernel weight approximately half that of durum wheat, both species showed acceptable test weight and good milling performance. Notably, T. timopheevi exhibited a markedly higher yellow index (+32%) compared with durum wheat.

Wholemeal and refined flours were subsequently analyzed with a focus on gluten properties determining processing aptitude. Storage protein allele combinations at the HMW-GS and LMW-GS loci strongly influenced gluten quality. SDS sedimentation test values differed significantly between the two species: T. zhukovskyi showed “poor gluten quality” with SDS ≤20 mL, whereas T. timopheevi displayed intermediate values (30-40 mL). These results aligned with the very low gluten index values observed in both species (average <35%). The weak gluten strength was further confirmed by alveograph and farinograph parameters, with W values $<50\times10^{-4}$ J and farinographic quality index IQ <50.

Despite their unconventional gluten characteristics, both species were successfully processed into refined and wholemeal spaghetti using a low-temperature drying cycle (Tmax = 58°C). Cooked pasta was evaluated for technological properties (optimal cooking time, cooking loss, firmness), sensory attributes (bulkiness, stickiness and firmness), and selected nutritional traits (protein, total starch, total dietary fiber, antioxidant capacity). Weak gluten strength did not compromise pasta technological performance in both species, providing products with acceptable sensory quality. Wholemeal pasta showed enhanced nutritional value due to higher fiber content (9.0% on average) and greater antioxidant capacity.

Overall, these findings highlight the distinctive gluten functionality of Caucasian wheat species and their potential value as genetic resources for the development of niche, nutritionally enriched cereal-based foods and for their use in breeding programs.

12 - *Investigating the role of dietary fiber components in gluten-containing and gluten-free model food matrices*

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In our work, the functionality of the two main cereal dietary fibre components, β-glucans (BG) and arabinoxylans (AX) was investigated in different food model systems. The basic idea of our research was to determine how generalizable or unique the effect of fibers on rheological properties is in food matrices containing macromolecules (proteins and starch) with different compositions.

Wheat based gluten containing, and buckwheat- and millet-based gluten free suspension and dough model matrices were created. Fiber concentrates from different sources (AX: maize, barley; BG: oat, barley) were added to the flours in equal amounts. The viscous (RVA) and mixing properties (micro-doughLab) were determined. The composition and size distribution of fiber components were investigated with HPLC methods while the changes in protein profiles were followed with SDS-PAGE. The modifications in the structure of the dough were examined using a scanning electron microscope.

During viscosity measurements, both AX and oat BG resulted in a decrease, while barley BG resulted in an increase, in all three matrices. In the case of mixing properties, the effect of AX is uniform, causing an increase in consistency in the gluten-containing matrix, while a decrease in consistency in the gluten-free systems. As a result of the addition of BG, we observed an increase in consistency in the wheat and millet dough, while no significant change was observed in the buckwheat matrix. It can be concluded that it is not possible to speak in general terms about dietary fibers and their effects; their technological (and let's add, nutritional) role strongly depends on the fiber source, their composition, the ingredients and other properties of the food matrices. The current work might contribute to a better understanding of the functionality of dietary fibre and other macromolecules in cereal and pseudocereal food systems.

The research is related to the implementation of the objectives of the following programs: ÚNKP-22-4-II-BME-129, EKÖP-24-2-BME-301, BME-EGA-02-TKP2021 project supported by the Ministry for Innovation and Technology of Hungary from the National Research, Startup Program of the Varga József Foundation BME VBK. The research was carried out with the tools of the National Cereal Science Infrastructure Network (3-110-H).

13 - Breadmaking quality of Norwegian milling wheat cultivars and the effect of environment

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Norway aims to increase the self-sufficiency of milling wheat. Increased knowledge about factors influencing the breadmaking quality of wheat will help the industry utilize Norwegian wheat more effectively. This study aims to identify factors that affect the quality of Norwegian milling wheat. Breadmaking quality can be defined in several ways, but for the baking industry, factors such as bread volume, gluten strength, and water absorption (WA) of the flour are important. Moreover, it is crucial for the baking industry that flour quality is stable throughout the season. Both genotype and environment can contribute to differences in these quality aspects. In this study, we evaluate how genotype and environment affect water absorption, resistance to extension (Rmax), extensibility, and breadmaking characteristics using refined flour. Three spring wheat cultivars (Betong, Helmi, and Festus) and two winter wheat cultivars (Lizzie and Informer) grown in multiple locations in Norway for two years were included. The combination of location and year was defined as environment.

Differences in WA were observed both between cultivars and environments, suggesting both an environmental and genotype effect. This could be due to differences in protein, fiber, and damaged starch content. The Rmax and extensibility also varied across genotypes, showing that the cultivars differed in gluten strength. The strongest cultivars were Betong and Informer, while Festus, Helmi, and Lizzie had the highest extensibility. Overall, these results show that there is variation in factors that affect the breadmaking quality of wheat because of genotype, but also that the environment affects some of these. The flour and protein compositions, which influence breadmaking characteristics, will also be investigated.

14 - Pearl millet flour incorporation has increased wheat-based bread rheological properties, nutritional quality, mineral bioavailability while decreasing gluten content

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A set of composite flour prepared from wheat flour and different levels of pearl millet flour ranging from 10 to 30%, were subjected to analysis of gluten composition, flour functionality, dough rheology, bread quality, and micronutrient availability. The nutritional value of composite flour was significantly improved ($p < 0.05$) compared to the control (100% wheat flour). Pearl millet flour substitution of 30% has increased the protein content by 21.2% compared to the control. Iron (Fe) and Zinc (Zn) have almost doubled with significant increases from 8.71 to 19.52 ppm for Fe and 7.34 to 13.07 ppm for Zn, recorded respectively for Control and 30% compsite flour. Functional and rheological properties of the composite flour were significantly affected by substitution level ($p < 0.05$). Water absorption decreased from 61.75% to 52.45%, while dough development time has increased from 99.5 s to 503 s. Gluten-related parameters, including wet gluten content and SDS-sedimentation values, showed significant reductions beyond 15% substitution ($p < 0.05$), indicating weakening of gluten structure. Bread quality attributes were also significantly affected ($p < 0.05$), with loaf volume decreasing from 1490.5 mL (Control) to 1015.0 mL at 30% substitution and specific volume from 3.45 to 2.96 mL/g. Sensory evaluation revealed that bread containing 10-15% millet flour did not show significant differences compared to the control in overall acceptability. The level of 20% substitution represented a transitional level with acceptable sensory quality but significantly reduced technological performance ($p < 0.05$). Higher substitution levels (≥25%) have significantly affected quality attributes. Phytic acid content increased significantly ($p < 0.05$) from 0.03 to 0.13 g/100 g, resulting in higher PA:Fe and PA:Zn molar ratios. These ratios remain below the critical limits for impaired mineral bioavailability. These findings demonstrate that partial substitution of wheat flour with pearl millet flour at 10-15% provides a statistically optimal balance between nutritional enhancement and technological performance, while 20% represents a viable compromise for further nutritional gains with moderate quality loss.

PROCESSING & FOOD PRODUCTION

15 - Processing Strategies to Improve Breadmaking Performance of Whole-Grain Triticale-Wheat Blends Across Contrasting Production Environments

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Background / Objective: Whole-grain breads offer nutritional benefits but often have reduced loaf volume and weaker structure due to altered gluten functionality. Triticale (× Triticosecale) provides sustainability and nutritional advantages over wheat, and multi-environment analyses suggest wheat-triticale co-production and flour blending can improve water and nitrogen productivity. However, triticale exhibits inherently weaker gluten and greater sensitivity to rapid gas production during fermentation, and protein concentration alone does not reliably predict breadmaking performance. This study evaluated whether fermentation strategy and formulation can compensate for weaker gluten functionality in whole-grain triticale systems across contrasting agronomic environments.
Methods: Two wheat and three triticale varieties were grown at two California locations under standard management and resource-limited conditions (soil nitrogen limitation in Yolo County; terminal drought in Fresno County). Whole-grain flours were milled and evaluated in pup loaves using a sponge-and-dough fermentation method. Formulations included a 60% triticale: 40% wheat blend compared with 100% whole-wheat controls. Sponge-and-dough was selected based on preliminary trials indicating improved dough handling and gluten development. Dough strength and mixing tolerance were assessed by Mixograph, and loaf volume served as the primary quality indicator.
Results: Across environments, triticale-containing doughs showed lower mixing tolerance and loaf volume than wheat controls, confirming weaker gluten functionality. Higher protein was generally associated with higher loaf volume; however, loaf volume plateaued despite substantial protein differences in wheat (~9% vs ~12%), indicating diminishing returns of protein concentration and highlighting the importance of gluten quality and fermentation dynamics. The 60% triticale: 40% wheat formulation produced commercially acceptable loaf volume and crumb structure across environments, with improvements largely scaling with wheat inclusion. Relative to 100% triticale, blending with wheat under sponge-and-dough fermentation consistently increased mixing tolerance and loaf volume. Environmental management altered protein levels but did not change crop-specific gluten performance patterns.
Conclusions: Sponge-and-dough fermentation partially compensates for weaker triticale gluten in whole-grain triticale-wheat blends, enabling acceptable bread quality across contrasting environments. Protein concentration alone is insufficient to predict performance. Integrating fermentation strategy with formulation and agronomic context is a practical pathway to translate triticale's sustainability potential into consumer-acceptable whole-grain breads.

16 - Beyond Recovery: Advanced Processing of Barley Spent Grain and B. clausii Spores for Innovative Functional Bread

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Advancing the processing for gluten-containing cereals is a key strategy for developing value-added foods. This study integrates the recovery and utilization of brewer's spent grain (BSG), microwave-drying precision, and postbiotic compounds for functional bread reformulation strategies. An advanced microwave drying protocol has been designed, tested and validated to stabilize BSG while preventing biological and chemical degradation thereby obtaining fiber-rich ingredients to be incorporated into the dough formulations. Furthermore, the addition of Bacillus clausii spores during baking enabled to significantly increase the functional properties of the product with new health benefits. In conclusion, the study demonstrates how advanced processing of cereal by-products can transform industrial waste into a functional ingredient capable of improving the nutritional and health profile of the finished product.

17 - Gluten-Free and Gluten-Containing Bakery Products in the Portuguese Market: A Comparative Assessment of Nutritional Quality and Gluten Content

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Background

Gluten-free diets are essential for individuals with gluten-related disorders, but their popularity has grown among the general population, often due to perceived health benefits. However, growing evidence suggests that gluten-free products may differ significantly from their gluten-containing counterparts in terms of nutritional quality, composition, and cost.

Aim

This is study evaluated the nutritional quality of gluten-free bakery products relative to their gluten-containing equivalents using the Nutri-Score system, and assessed the accuracy of gluten-free labelling claims through laboratory analysis of gluten content.

Methodology

Nutritional information from 223 bakery products in Portuguese supermarkets was collected and analysed. Products were categorized as gluten-free (n=107) or gluten-containing (n=116) and further grouped into breads; cakes, cookies, and biscuits; and toasts, crackers, and snacks. Nutritional quality was assessed using the Nutri-Score algorithm. A subset of 23 gluten-free products was randomly selected for laboratory analysis, and gluten content was quantified by the ELISA-R5 sandwich method (RIDASCREEN® Gliadin, R-Biopharm) following Codex Alimentarius guidelines.

Results

Gluten-free products generally have lower nutritional quality, higher prices, and lower protein content than their gluten-containing counterparts. A statistically significant association was found between the Nutri-Score nutritional profile and the presence of gluten in the Breads category ($p=0.048$). In this category, 50% (n=20) of gluten-containing breads were classified as Nutri-Score categories A and B, indicating high nutritional quality, whereas 79% (n=30) of gluten-free breads were classified as Nutri-Score categories C and D, reflecting medium-to-low nutritional quality, often associated with higher fat content. Across all categories, 82% (n=95) of gluten-containing bakery products were classified in Nutri-Score categories C, D, and E, while 92% (n=98) of gluten-free products fell into the same categories, which are recommended for moderate or occasional consumption. Laboratory analysis showed that all analysed gluten-free samples contained gluten at levels below the method's limit of quantification and met the legal threshold.

Conclusion

Gluten-free bakery products in the Portuguese market are safe for consumers with gluten intolerance but generally have lower nutritional quality than gluten-containing alternatives. While essential for celiac patients, they are not necessarily healthier for the general population, highlighting the need for improved formulations and increased consumer awareness.

18 - Fabrication of functionally enhanced gluten-free products using artichoke derived ingredients

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The growing demands of gluten-free foods due to the therapeutic requirements and the changing preferences of consumers has prompted substantial research to enhance the nutritional and functional value of gluten-free cereal foods. Nevertheless, these products tend to be lower in nutritional content and optimality of textural properties in comparison to their gluten containing alternatives (1). Thus, the production of gluten-free formulations with special nutritional value and functionality is one of the major challenges in food science and technology. The study suggests a development of the agro-industrial byproducts of globe artichoke (Cynara scolymus) into gluten free cereal systems with a background to the previous research of the underutilized cereal sorghum and a valorization of food processing residues. The artichoke-derived ingredients, especially inulin and polyphenol-rich fractions may be attributed with the possibility to increase technological and nutritional properties. Dietary fiber such as inulin is a popular ingredient that can help boost retention of water and texture of gluten-free items, as well as mouth feel along with prebiotic functionality (2). Alternatively, polyphenols can increase the antioxidant level and the overall functionality of the final product. Based on literature and previous experimental insights, the combination of these ingredients into gluten-free matrices should enhance the quality of the products. At the same time, it will also help to overcome nutritional deficiencies that are widely known in gluten-free foods. This approach also promotes sustainable food production via valorization of agro-industrial byproducts. The suggested idea accentuates the possibility of utilizing gluten-free cereals and incorporating the functional ingredients of artichoke waste to formulate value-added edibles. Subsequent experimental confirmation is expected to streamline the formulation parameters and assess the performance of the processing, physicochemical characteristics, and nutritional effects.

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