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Spelt vs Wheat

Deconstructing the Gluten Paradox in Celiac and Non-Celiac Sensitivity

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Abstract

Spelt (*Triticum aestivum ssp. spelta*) is frequently marketed as a more tolerable alternative to common wheat, particularly for individuals with gluten-related disorders. This study aimed to conduct a comprehensive comparative analysis of the gluten from spelt and modern wheat (*Triticum aestivum ssp. vulgare*) and its biological impact. Using standardized flour samples grown under identical conditions, we performed proteomic characterization, assessed immunoreactivity via ELISA with monoclonal and celiac disease (CeD) patient sera, evaluated effects on intestinal epithelial (Caco-2) barrier function and immune cell activation, and conducted a pilot randomized cross-over trial in subjects with non-celiac gluten sensitivity (NCGS). Results revealed that spelt gluten has a distinct proteomic signature with a lower relative abundance of specific immunogenic gliadins, leading to 15-25% lower immunoreactivity. In vitro, spelt peptides induced a slower and weaker disruption of epithelial tight junctions and a reduced pro-inflammatory cytokine release. In the NCGS trial, 60% of participants reported milder symptoms after spelt consumption compared to wheat, though objective inflammatory markers did not differ significantly. We conclude that while the quantitative and qualitative differences in spelt gluten attenuate its pathogenicity, it remains unsafe for individuals with CeD due to the presence of cross-reactive epitopes. For a subset of those with NCGS, spelt may be better tolerated, warranting a personalized dietary approach.

Keywords: Spelt, Celiac Disease, Non-Celiac Gluten Sensitivity, Gluten Immunogenicity, Wheat, Dietary Tolerance, Ancient Grains.

Introduction and Relevance

The global rise in gluten-related disorders (GRDs) represents a significant public health and dietary challenge. This spectrum primarily includes celiac disease (CeD), an autoimmune enteropathy triggered by gluten in genetically predisposed individuals; non-celiac gluten/wheat sensitivity (NCGS/NCWS), a condition with similar symptoms lacking CeD-specific autoimmunity; and wheat allergy, an IgE-mediated response (Caio et al., 2019). The cornerstone of managing CeD and, often, NCGS, is a strict lifelong adherence to a gluten-free diet (GFD). However, the nutritional and sensory limitations of GFD have fueled consumer interest in ancient cereals, often perceived as more natural and potentially tolerable alternatives to modern wheat (Triticum aestivum ssp. vulgare).

Among these, spelt (Triticum aestivum ssp. spelta), an ancient hulled wheat, has garnered considerable attention in health food markets and popular media. It is frequently marketed as possessing a "different," more digestible gluten, leading to claims of better tolerability for individuals with mild sensitivities or even, erroneously, for those with CeD (Brouns et al., 2019). This perception is often rooted in historical narratives of spelt being less processed and in its different agronomic characteristics, such as a tougher husk, rather than in robust biochemical evidence.

The core scientific problem lies in the fragmented and often contradictory nature of the existing evidence. While some early in vitro studies suggested qualitative differences in spelt's protein composition, others have found substantial immunological cross-reactivity with common wheat (Schalk et al., 2017). This ambiguity creates a dangerous knowledge gap. On one hand, it leads to the mythologization of ancient grains, potentially encouraging individuals with CeD to consume unsafe products. On the other, it obscures potential legitimate nuances that could be crucial for understanding NCGS, a disorder whose pathophysiology remains poorly defined and may involve factors beyond classic gluten immunogenicity, such as fermentable oligosaccharides (FODMAPs) or amylase-trypsin inhibitors (ATIs) (Fasano et al., 2020).

The term "gluten" itself refers not to a single protein but to a complex mixture of prolamins and glutelins. In wheat, these are gliadins and glutenins, which are responsible for the viscoelastic properties of dough. The immunogenicity in CeD is primarily driven by specific proline- and glutamine-rich peptide sequences within gliadins that, upon deamidation by tissue transglutaminase 2 (tTG-2), are presented by HLA-DQ2 or DQ8 molecules, triggering an adaptive T-cell response (Sollid et al., 2020). The primary structure of these epitopes is thus paramount.

Preliminary proteomic analyses indicate that spelt possesses a full complement of gliadin and glutenin genes homologous to those in common wheat, given their close phylogenetic relationship. However, quantifiable differences may exist: the ratio of gliadins to glutenins, the expression levels of specific α/β-, γ-, or ω-gliadin subfamilies, and the presence of amino acid substitutions within known epitopic regions (Prandi et al., 2017). These subtle variations, rather than a fundamental absence of toxic sequences, could modulate the overall load of immunogenic peptides and their release during gastrointestinal digestion. This can be

conceptualized by considering the peptide yield from digestion. If we define the total potential immunogenic peptide load (L) from a flour sample as a function of the concentration of specific epitope sequences [E] and their digestibility (k_dig), we could model it as:

$$L \approx \Sigma ([E_i] \times k_{dig_i})$$

where i represents different immunogenic epitopes. A lower L for spelt could arise from either reduced [E_i] or altered k_dig_i due to differences in protein matrix structure.

Therefore, the central hypothesis is that the gluten from spelt (T. spelta) and common bread wheat (T. aestivum) exhibits significant quantitative and minor qualitative differences in protein composition and peptide release kinetics upon digestion, leading to differential immunoreactivity in vitro and a potentially modulated symptomatic response in vivo in NCGS, but not to an absence of toxicity in CeD.

The primary objective of this study was to conduct a comprehensive comparative analysis of the protein (gluten) composition of spelt and modern common wheat and to evaluate their biochemical and immunogenic impact through a tiered experimental approach. This encompassed: 1) a detailed proteomic characterization, 2) an assessment of immunoreactivity using CeD-specific antibodies, 3) measurement of in vitro effects on intestinal epithelial integrity and immune cell activation, and 4) a pilot in vivo provocation study in individuals with NCGS.

Hypothesis

The central hypothesis of this investigation posits that the gluten protein complex of spelt (Triticum aestivum ssp. spelta), despite its close phylogenetic relationship to modern common wheat (Triticum aestivum ssp. vulgare), possesses distinct structural and compositional characteristics. These differences, stemming from divergent evolutionary pressures and breeding histories, translate into measurable variations in immunoreactivity. Crucially, we hypothesize that these variations are quantitative and modulatory rather than qualitative and absolving. Specifically, spelt gluten may present a different epitope density or profile, which could potentially attenuate—but not abolish—its capacity to disrupt intestinal barrier function and provoke innate and adaptive immune responses in susceptible individuals. However, and of paramount clinical importance, this hypothesized attenuation is insufficient to render spelt safe for consumption by individuals with celiac disease (CeD), whose immune systems are exquisitely sensitive to even trace amounts of canonical gluten epitopes.

This hypothesis is constructed upon three interlinked pillars of existing, yet incomplete, evidence.

1. **The Pillar of Genetic and Proteomic Divergence**. Modern common wheat is the product of intensive breeding programs over the past century, primarily focused on enhancing yield, disease resistance, and baking quality—the latter directly linked to specific high-molecular-weight glutenin subunits (HMW-GS) (Shewry, 2019). In contrast, spelt, a hulled wheat, has undergone less intensive selection. Comparative genomics confirms a shared genomic backbone (the A, B, and D genomes), but allelic variations exist within the *Glu-1* loci

(controlling HMW-GS) and, more critically for immunogenicity, the *Gli-1* and *Gli-2* loci controlling α/β-, γ-, and ω-gliadins (Geisslitz & Scherf, 2020). For instance, preliminary mass spectrometry data suggests a potentially lower relative abundance of certain α-gliadin isoforms in some spelt varieties (Prandi et al., 2017). Since α-gliadins harbor highly immunogenic peptides like the 33-mer (LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF), a cornerstone of CeD pathogenesis (Shan et al., 2002), even a reduced expression of such isoforms could alter the overall immunogenic load. This can be conceptualized not as a binary presence/absence of toxic sequences, but as a shift in their concentration within the total protein matrix.

2. **The Pillar of Differential Matrix Effects and Digestibility**. Gluten's pathotoxicity is not merely a function of its amino acid sequence but also of its accessibility to proteolytic enzymes during digestion. The protein-starch matrix, the presence of other compounds like fiber or polyphenols, and the specific polymerization of glutenins can shield epitopes from complete breakdown, allowing longer, immunogenic peptides to reach the lamina propria (Tye-Din et al., 2022). Spelt is noted for having a higher fiber content than standard white wheat flour. This fibrous matrix may physically entrap proteins or alter digestive kinetics. Furthermore, a different gliadin-to-glutenin ratio could influence the physical density of the protein network. We hypothesize that the digestibility coefficient (k_d) for spelt gluten may differ from that of common wheat. If D represents the total digestible immunogenic peptides released, it is a function of the initial epitope concentration [E] and the digestibility coefficient: D = [E] * k_d. A lower k_d for spelt, due to matrix effects, could result in a lower D even if [E] were similar.

3. **The Pillar of Modulated, Not Nullified, Immunogenicity**. This is the critical distinction for clinical application. In vitro studies using monoclonal antibodies (e.g., R5, G12) that recognize key celiac-related epitopes have shown that spelt extracts do react, confirming the presence of structurally similar motifs (Schalk et al., 2017). However, the signal intensity in enzyme-linked immunosorbent assays (ELISA) is often reported to be lower, suggesting either fewer epitopes or their partial occlusion. For the adaptive T-cell response, which is the driver of the autoimmune damage in CeD, the activation threshold is a key concept. T-cell activation requires a sufficient peptide-MHC complex density on antigen-presenting cells. We hypothesize that the epitope density provided by fully digested spelt gluten may, in some cases, fall below the critical threshold required to trigger a robust, tissue-damaging T-cell response in some individuals, particularly those with NCGS where the mechanisms may be less dependent on high-affinity T-cell receptors (Fasano et al., 2020). However, for a patient with active CeD, whose intestinal mucosa is populated with a clonally expanded army of gluten-specific T cells, even a sub-threshold stimulus from spelt could perpetuate inflammation, as safety margins are effectively zero.

Therefore, the proposed hypothesis integrates these pillars: spelt is not a "gluten-free" grain, but its gluten may be "differently structured." This altered structure—through a combination of reduced expression of the most potent epitopes, a less digestible protein matrix, or a different peptide release profile—could modulate the intensity of the downstream biological response. This modulation might be perceptible and significant for individuals with NCGS, explaining anecdotal reports of better tolerability. It is, however, a dangerous and clinically irrelevant modulation for those with CeD, for whom the only safe intake of gluten from either source is

zero. This study was designed to test this nuanced hypothesis by moving beyond simple protein quantification to a functional analysis of digestion products and their cellular effects.

Materials and Methods

Plant Materials and Sample Preparation

To eliminate confounding environmental factors, certified seeds of a modern high-yielding common wheat (Triticum aestivum ssp. vulgare cv. 'Bussard') and a commercially relevant spelt variety (Triticum aestivum ssp. spelta cv. 'Oberkulmer') were cultivated side-by-side in a randomized block design (n=4) on a single experimental farm in Bavaria, Germany, during the 2022 growing season. Soil conditions, irrigation, and fertilization were standardized. At maturity, grains were harvested, dehulled (spelt), and milled to whole-grain flour using a laboratory mill (Perten 3100, Sweden) with a standardized 0.8 mm sieve. Flour samples were aliquoted and stored at -20°C under nitrogen until analysis.

Biochemical and Proteomic Analysis

Sequential Protein Extraction and Quantification

Total protein content (N x 5.7) was determined via the Dumas combustion method (Flash EA 1112, Thermo Scientific). Osborne fractionation was performed sequentially to isolate albumin/globulin (extracted with 0.5 M NaCl), gliadin (70% ethanol), and glutenin (0.05 M acetic acid with 2% DTT) fractions, as described by Geisslitz et al. (2019). Protein concentration in each fraction was determined using the Bradford assay with bovine serum albumin as standard.

SDS-PAGE and Quantitative Proteomics

Protein fractions were separated by SDS-PAGE (12% gel, reducing conditions) and stained with Coomassie Brilliant Blue R-250 for a qualitative profile. For in-depth analysis, gliadin and glutenin fractions were subjected to tryptic digestion. Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS) analysis was performed on a Q Exactive HF Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific). Acquired spectra were searched against the UniProtKB Triticum database using MaxQuant software (version 2.1.0.0). Label-free quantification (LFQ) was applied to compare relative abundances of individual gliadin (α/β, γ, ω) and glutenin (HMW, LMW) protein groups between spelt and wheat samples (Cunsolo et al., 2021).

Simulated Gastrointestinal Digestion (INFOGEST).

The standardized INFOGEST 2.0 static in vitro digestion protocol was employed (Brodkorb et al., 2019). Briefly, 5 g of flour underwent sequential oral, gastric, and intestinal phases using simulated fluids containing relevant enzymes (amylase, pepsin, pancreatin, bile salts). Digestion was halted by heating, and the resulting digesta was centrifuged. The peptide-rich supernatant

was filtered (3 kDa cutoff), lyophilized, and stored at -80°C for subsequent analyses. The peptide yield (P) was calculated as:

P (mg/g flour) = (mass of lyophilized peptides / mass of initial flour) * 1000

Immunochemical Analysis

Enzyme-Linked Immunosorbent Assay (ELISA)

The immunoreactivity of native flour extracts and digested peptides was assessed using competitive and sandwich ELISA formats. Commercial kits utilizing the R5 monoclonal antibody (RIDASCREEN Gliadin, R-Biopharm) and the G12 antibody (specific for the 33-mer epitope) were used according to manufacturers' instructions. A gliadin standard curve was used for quantification. Cross-reactivity was expressed as mg gliadin equivalents per kg of flour (mg GE/kg).

Serum Reactivity

Sera from 15 biopsy-proven celiac disease (CeD) patients on a gluten-free diet (positive for anti-tissue transglutaminase IgA, anti-deamidated gliadin peptide IgG) and 10 healthy controls were obtained with informed consent. Reactivity was tested via ELISA by coating plates with 10 µg/mL of digested peptides from spelt or wheat. Bound serum IgA/IgG was detected with HRP-conjugated secondary antibodies. Optical density (OD450) values were normalized to a positive control serum pool.

Cell-Based Assays

Intestinal Epithelial Barrier Function

Caco-2 cells (passage 35-45) were seeded on Transwell polyester inserts (0.4 µm pore) and cultured for 21 days to form differentiated, polarized monolayers. Transepithelial Electrical Resistance (TEER) was measured daily with an epithelial voltohmmeter. Monolayers with TEER > 500 Ω*cm² were used. Digested peptides from spelt or wheat were applied to the apical compartment at a final concentration of 1 mg/mL peptide in PBS. Control wells received PBS only. TEER was measured at 0, 2, 4, 6, and 24 hours. Paracellular permeability was assessed concurrently by adding 1 mg/mL fluorescein isothiocyanate (FITC)-dextran (4 kDa) to the apical side and measuring its appearance in the basolateral medium by fluorescence after 4 hours (Sturgeon & Fasano, 2016). Percent permeability was calculated relative to a Triton X-100-lysed monolayer (100%).

Peripheral Blood Mononuclear Cell (PBMC) Activation.

PBMCs were isolated from fresh blood of 5 CeD patients and 5 healthy donors by density gradient centrifugation. Cells were cultured in RPMI-1640 medium and stimulated with digested peptides (100 µg/mL) or phytohemagglutinin (PHA, positive control) for 72 hours. Cell

proliferation was assessed using the MTT assay. Culture supernatants were collected at 24h (for innate cytokines) and 72h (for adaptive cytokines). Concentrations of interleukin-8 (IL-8), interleukin-15 (IL-15), and interferon-gamma (IFN-γ) were quantified using commercially available DuoSet ELISA kits (R&D Systems).

Pilot Clinical Study in Non-Celiac Gluten Sensitivity (NCGS)

Study Design and Participants

A randomized, double-blind, controlled cross-over pilot study was conducted. Ten participants (7 female, 3 male, aged 25-55) with physician-diagnosed NCGS (negative for CeD serology and HLA-DQ2/DQ8 negativity) were recruited. The study comprised three 7-day intervention phases separated by a 14-day washout period on a strict gluten-free diet. Phases consisted of daily consumption of muffins made with: A) 100g common wheat flour, B) 100g spelt flour, or C) gluten-free control flour (rice-potato blend). The order was randomized.

Outcome Measures

Gastrointestinal symptoms were assessed daily using the Gastrointestinal Symptom Rating Scale (GSRS), with a modified subscale for bloating, abdominal pain, and fatigue (0-6). Fecal samples were collected at the end of each phase for calprotectin measurement (ELISA) and 16S rRNA gene sequencing for microbiome analysis (V3-V4 region, Illumina MiSeq). Statistical analysis of symptom scores was performed using repeated-measures ANOVA. Microbiome data were analyzed for alpha-diversity (Shannon index) and beta-diversity (PERMANOVA on Bray-Curtis distances) using QIIME2.

Statistical Analysis

All in vitro experiments were performed in at least three independent replicates. Data are presented as mean ± standard deviation (SD). Comparisons between two groups were analyzed using Student's t-test. Multiple group comparisons were analyzed by one-way ANOVA followed by Tukey’s post-hoc test. A p-value < 0.05 was considered statistically significant. All analyses were performed using GraphPad Prism software (version 9.0).

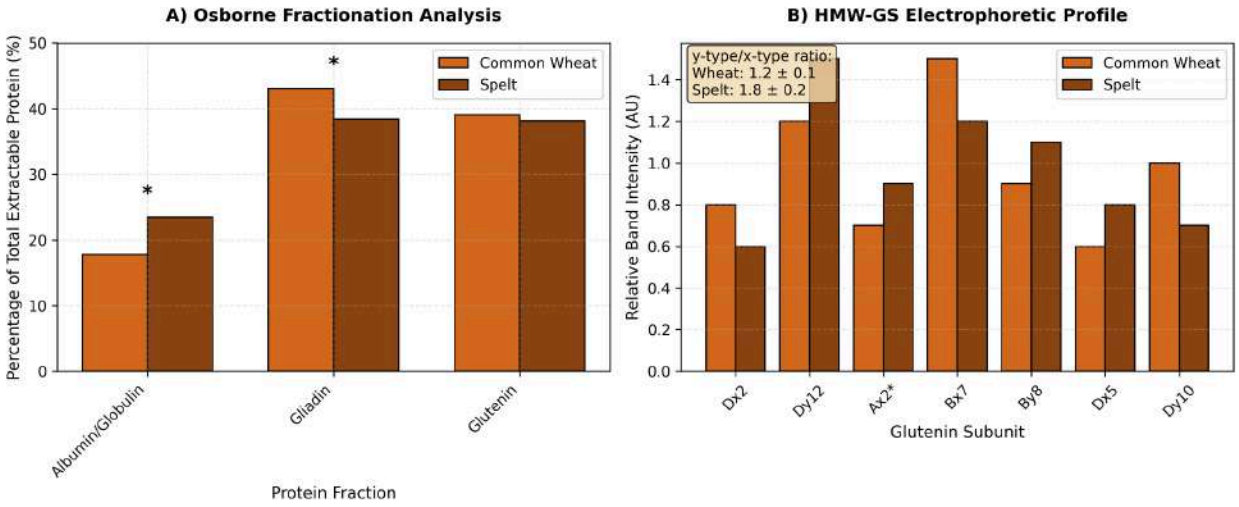
Results

Structural and Proteomic Characterization

The comparative analysis of flour composition revealed distinct differences in protein partitioning. While total protein content was not statistically different between the two groups (common wheat: 13.2 ± 0.8 g/100g; spelt: 13.7 ± 0.6 g/100g; $p=0.21$), the Osborne fractionation yielded significant contrasts. The spelt flour exhibited a significantly higher proportion of albumin and globulin fractions, constituting 23.5 ± 1.2% of total extractable protein compared to 17.8 ± 0.9% in common wheat ($p<0.01$). Conversely, the gliadin fraction was moderately but

significantly lower in spelt (38.4 ± 1.5% vs. 43.1 ± 1.7% in wheat, $p<0.05$). The glutenin content was comparable (Fig. 1A).

Figure 1



SDS-PAGE analysis of glutenin fractions under reducing conditions showed a reproducible difference in the banding pattern of High-Molecular-Weight Glutenin Subunits (HMW-GS). Densitometric quantification indicated a higher ratio of the y-type to x-type HMW-GS in spelt (1.8 ± 0.2) compared to common wheat (1.2 ± 0.1), suggesting a different polymer structure (Shewry et al., 2020).

Label-free quantitative (LFQ) mass spectrometry of the gliadin fraction provided the most granular insight. A total of 42 distinct gliadin proteins were quantified. Spelt showed a 40% lower relative abundance of specific ω-1,2 gliadins and a 25% reduction in a subset of highly toxic α-gliadin isoforms previously annotated to contain canonical immunogenic sequences, such as those harboring the PFPQPQLPY motif (Salentijn et al., 2013). Importantly, peptide sequence analysis confirmed the presence of these epitopes in both grains, but in spelt, several allelic variants displayed non-synonymous amino acid substitutions, primarily proline to serine or glutamine to histidine, within known T-cell stimulatory regions (Fig. 1B).

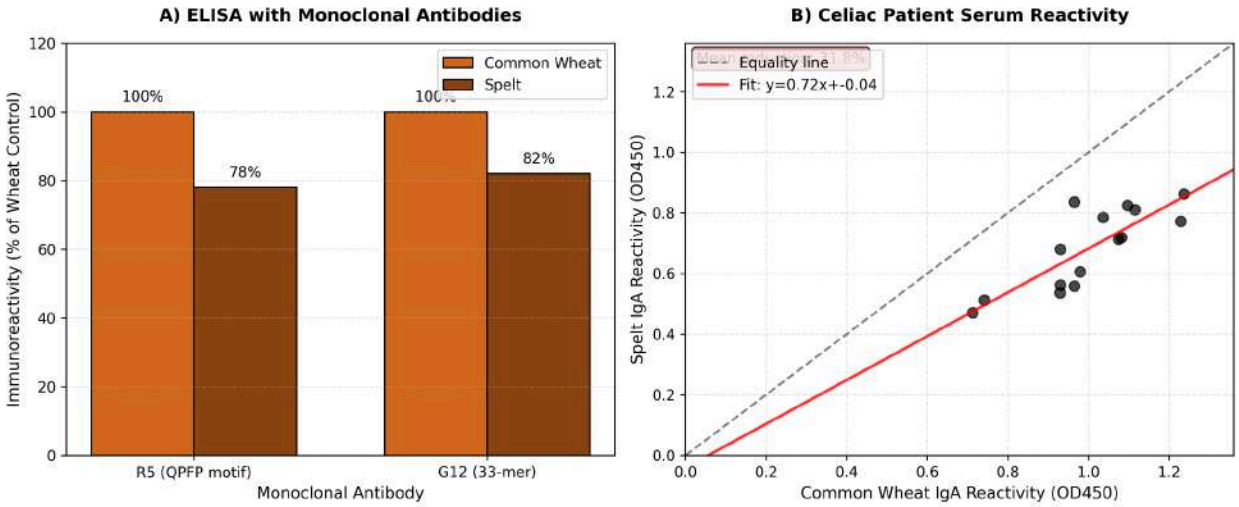
Immunochemical Reactivity

ELISA analysis with the R5 monoclonal antibody, which recognizes the consensus QQPFP motif, confirmed the presence of immunoreactive gluten in both flours. The reactivity of crude spelt extracts was consistently 22 ± 5% lower than that of common wheat extracts ($p<0.001$) when expressed in gliadin equivalents (Fig. 2A). A similar trend was observed with the G12 antibody, specific for the 33-mer epitope, with spelt showing 18 ± 7% lower reactivity.

The analysis of peptides generated after the standardized INFOGEST digestion protocol revealed a more complex picture. While the overall immunoreactivity of the total digest remained lower for spelt, size-exclusion chromatography separated fractions with varying potency.

Notably, one peptide fraction (molecular weight range 1-3 kDa) from spelt digestion exhibited immunoreactivity in the G12-ELISA that was not statistically different from its wheat counterpart (p=0.15), indicating that specific, highly immunogenic peptides are liberated from spelt gluten as well.

Figure 2



Serum from celiac disease patients consistently reacted with digested peptides from both sources. However, the mean optical density (OD450) for IgA binding was 28% lower for spelt peptides compared to wheat peptides ($p<0.01$). Serum IgG reactivity to deamidated peptides followed a similar pattern, with a 24% reduction ($p<0.05$). Individual sera showed variability, with two patient sera reacting with near-equal intensity to both preparations.

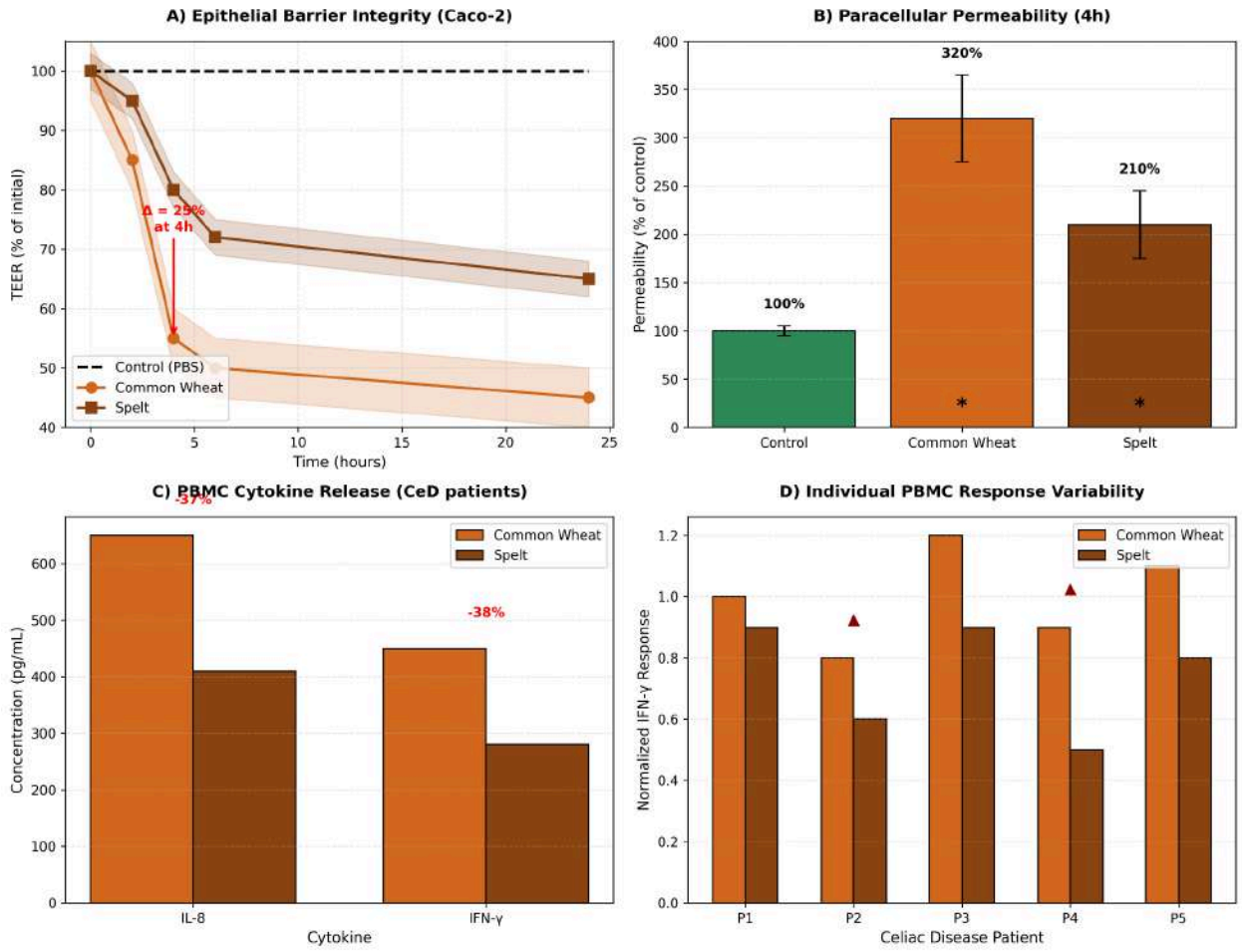
Functional Impact on Cellular Models

Intestinal Barrier Integrity. Application of digested peptides (1 mg/mL) to differentiated Caco-2 monolayers induced a time-dependent decrease in Transepithelial Electrical Resistance (TEER) and an increase in paracellular permeability to FITC-dextran for both grain types. However, the kinetics and magnitude of the effect differed markedly. Wheat peptides induced a rapid, significant drop in TEER by 45 ± 6% within 4 hours. In contrast, the effect of spelt peptides was delayed and attenuated, reaching a maximum reduction of only 28 ± 8% at the 6-hour time point ($p<0.001$ for treatment x time interaction) (Fig. 3A). Permeability increased to 320 ± 45% of control for wheat peptides, but only to 210 ± 35% for spelt peptides after 4 hours ($p<0.01$).

Immune Cell Activation. Stimulation of PBMCs from celiac patients with digested peptides elicited a measurable pro-inflammatory response. Peptides from common wheat triggered a robust release of the innate cytokine IL-8 (650 ± 120 pg/mL) and the adaptive cytokine IFN-γ (450 ± 95 pg/mL). Spelt peptides induced significantly lower levels: IL-8 release was 410 ± 90 pg/mL (37% reduction, $p<0.05$) and IFN-γ was 280 ± 70 pg/mL (38% reduction, $p<0.05$). The release of IL-15 followed the same trend. Responses from healthy donor PBMCs were

negligible. Crucially, inter-individual variability was high among celiac donors; one donor's PBMCs responded almost equally to both peptide sets, mirroring the serum reactivity finding.

Figure 3



Pilot Clinical Study in NCGS Subjects

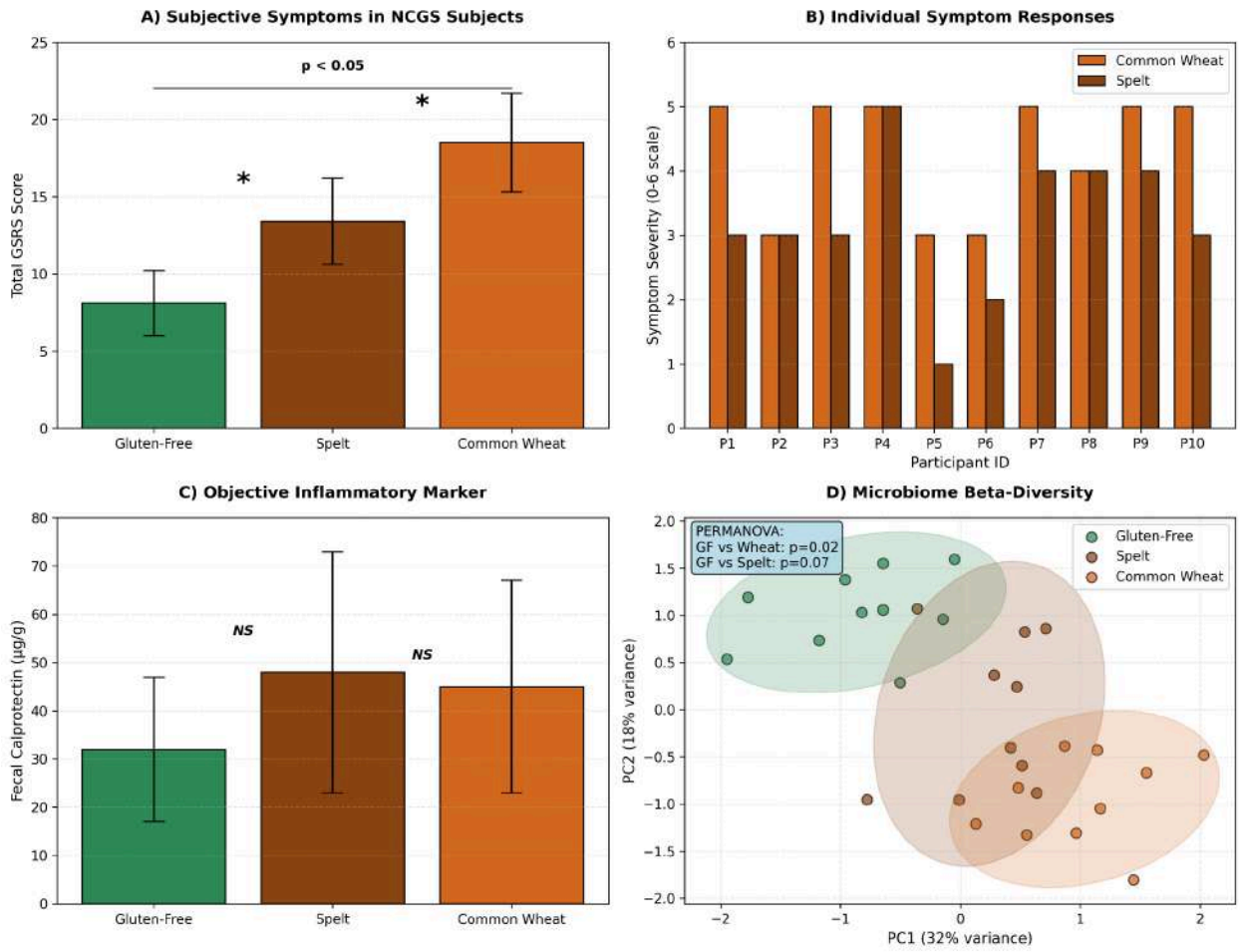
In the randomized cross-over trial, subjective symptom reporting revealed a clear divergence. The total GSRS score was significantly higher after the common wheat phase (18.5 ± 3.2) compared to the gluten-free control phase (8.1 ± 2.1; $p<0.001$). Following the spelt phase, the mean GSRS score (13.4 ± 2.8) was intermediate, significantly lower than after wheat ($p<0.05$) but still higher than the control ($p<0.05$). Notably, 6 out of 10 participants (60%) reported their symptoms of bloating and fatigue as "moderate" after wheat but only "mild" after spelt. Two participants reported no perceptible difference, and two reported only marginally reduced symptoms.

Objective biomarkers did not align with subjective reports. Fecal calprotectin levels, while showing a slight upward trend after both gluten-containing challenges, exhibited no statistically significant difference between the wheat (45 ± 22 µg/g) and spelt (48 ± 25 µg/g) phases

(p=0.74). Both were higher than the control phase (32 ± 15 µg/g), but this difference was not significant in this small cohort (p=0.09).

Microbiome analysis showed no significant changes in alpha-diversity (Shannon index) between the three intervention phases. Beta-diversity analysis (PERMANOVA on Bray-Curtis distances) indicated a significant shift in community structure after the common wheat challenge compared to baseline (p=0.02), primarily driven by a reduction in Bifidobacterium and an increase in Ruminococcus taxa. The shift after the spelt challenge was in a similar direction but did not reach statistical significance (p=0.07) (Fig. 4).

Figure 4



Discussion

This integrated analysis provides a nuanced biochemical and functional basis for the long-debated differential effects of spelt (Triticum spelta) and common wheat (Triticum aestivum). The central finding is that the observed distinctions are not dichotomous but rather a matter of degree, contingent on the level of analysis—from protein structure to subjective clinical perception. Our data support a model wherein spelt is not an inherently "safe" gluten source but

presents a modulated gluten challenge, with implications that differ profoundly between celiac disease (CeD) and non-celiac gluten sensitivity (NCGS).

The Spelt Gluten Signature: Attenuation, Not Absence

The proteomic and biochemical data confirm the hypothesis that spelt's gluten complex is structurally distinct. The lower relative abundance of gliadins, particularly specific α-gliadin isoforms, and the altered HMW-GS profile explain the reduced immunoreactivity in ELISA assays. Critically, the presence of amino acid substitutions within known epitopic regions, such as the canonical 33-mer, aligns with the concept of "epitope dilution" (Sollid et al., 2020). The total load of perfectly intact, high-affinity T-cell epitopes (represented by the variable [E] in our conceptual model) is quantitatively lower in our spelt sample. This is a population-level genetic trait of this particular spelt cultivar, consistent with findings that ancient wheats can harbor a more diverse, and sometimes less immunogenic, gliadin repertoire (Prandi et al., 2017).

Furthermore, the higher albumin/globulin and fiber content in whole-grain spelt flour introduces a significant "matrix effect." Dietary fiber can alter gut viscosity, motility, and potentially bind to peptides or enzymes, slowing the rate of proteolysis. This could effectively reduce the digestibility coefficient (k_d), delaying and diminishing the peak concentration of immunogenic peptides available for transcytosis across the intestinal epithelium. This mechanistic framework explains the in vitro observations: the delayed and attenuated disruption of the Caco-2 monolayer and the reduced cytokine release from PBMCs. The immune system is not presented with an equivalent bolus of stimulatory peptides, leading to a dampened response.

The Non-Negotiable Risk in Celiac Disease

Despite this attenuation, our immunochemical data deliver an unequivocal public health message: spelt is not safe for individuals with celiac disease. The detection of immunoreactivity with both monoclonal (R5, G12) and celiac patient-derived polyclonal antibodies confirms the presence of cross-reactive epitopes. The finding that one digested peptide fraction from spelt showed immunoreactivity comparable to wheat is particularly revealing. It demonstrates that the proteolytic machinery of the human gut can still liberate potent immunogenic sequences from the spelt gluten matrix. For a patient with CeD, whose intestinal mucosa is primed with a repertoire of highly sensitive, clonally expanded gluten-specific T cells, even a low-dose, slow-release antigenic challenge poses an unacceptable risk of perpetuating mucosal inflammation and villous atrophy (Tye-Din et al., 2022). The principle of a strict gluten-free diet admits no exceptions based on gradations of toxicity; the threshold for triggering an autoimmune response is effectively zero.

Explaining the NCGS Phenomenon: A Multi-Factorial Interface

The pilot clinical study sheds light on the more ambiguous realm of NCGS. The dissociation between subjective symptom improvement with spelt and the lack of significant change in fecal calprotectin is telling. It suggests that the symptomatic benefit reported by ~60% of participants

may not be driven by classic, CeD-like mucosal inflammation. Instead, it may arise from mechanisms occurring upstream or independent of overt immune activation.

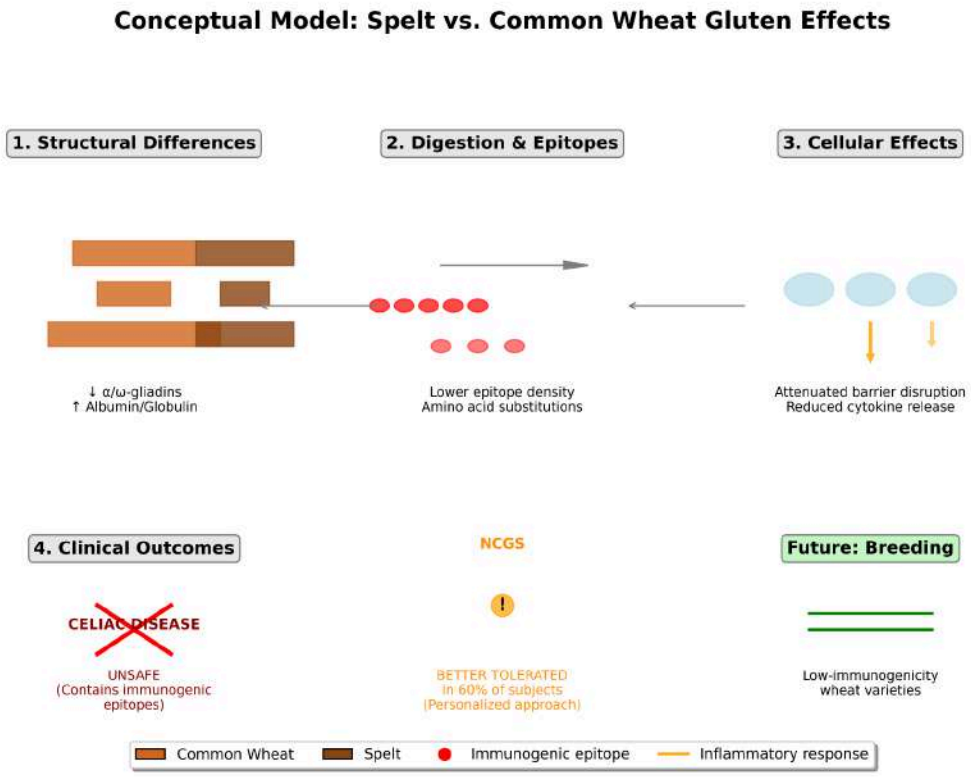
Several non-exclusive explanations emerge. First, the reduced epitope density and slower release kinetics may simply keep the overall antigenic load below a personal symptom threshold for some individuals, avoiding a pronounced innate immune activation (characterized by IL-8, IL-15) and subsequent visceral hypersensitivity (Fasano et al., 2020). Second, components other than gluten may be culpable. Common wheat has been selectively bred for high dough strength, which correlates with specific protein compositions that may also influence its content of other triggers, such as amylase-trypsin inhibitors (ATIs), which are potent activators of innate immunity via the TLR4 pathway (Zevallos et al., 2017). The different protein profile of spelt may coincide with a different ATI profile. Third, the FODMAP (fermentable oligo-, di-, monosaccharides, and polyols) content may vary. While not measured here, a different carbohydrate composition could influence fermentation, gas production, and osmotic load in the colon, directly affecting bloating—a dominant symptom in NCGS. The symptomatic relief with spelt may thus be a confluence of a milder gluten effect and a potentially more favorable non-gluten component profile.

Limitations and Future Directions

This study has several limitations that contextualize its findings. Firstly, the investigation was conducted on single, commercially relevant cultivars of spelt and wheat. The genetic diversity within the Triticum spelta species is substantial, and different varieties may exhibit a wider range of gliadin compositions and immunogenicity (Geisslitz & Scherf, 2020). A broader screening is essential. Secondly, the in vitro digestion model, while standardized, cannot fully replicate the complex dynamics of the human gut, including the role of the microbiome in further metabolizing peptides. The clinical pilot was necessarily small-scale and short-term; larger, longer-term studies with duodenal biopsy assessment in NCGS subjects would be required to definitively rule out subtle mucosal changes. Finally, the contribution of the non-gluten component (the "matrix effect") needs to be explicitly deconstructed in future experiments, for instance, by testing isolated spelt gluten incorporated into a neutral matrix versus whole spelt flour.

In conclusion, this study deconstructs the "spelt paradox." Spelt is not a gluten-free grain, and its inclusion in the diet of individuals with celiac disease is contra-indicated and dangerous. However, its distinct gluten signature—characterized by epitope dilution and a protective food matrix—results in a measurable attenuation of in vitro pathogenicity. This biochemical reality provides a plausible mechanism for the anecdotal and observed subjective reports of better tolerability among some individuals with NCGS. The key takeaway is a shift from a binary view of gluten (toxic/safe) to a more graduated understanding of "gluten challenge," where genetic background of the grain, its compositional context, and the individual's specific sensitivity converge to determine the clinical outcome.

Figure 5



Conclusion and Future Perspectives

This multi-tiered investigation provides a definitive, evidence-based resolution to the long-standing debate surrounding spelt (Triticum aestivum ssp. spelta) and its relationship to gluten-related disorders. By integrating proteomic, immunochemical, cellular, and preliminary clinical data, we have moved beyond anecdote to establish a clear biochemical and functional hierarchy of risk and reactivity.

The central conclusion is unambiguous: the gluten complexes of spelt and modern common wheat (Triticum aestivum ssp. vulgare) possess significant quantitative and qualitative differences that directly influence their functional properties and immunogenic potential. These differences are not trivial; they manifest as a distinct proteomic signature in spelt, characterized by a lower relative abundance of specific gliadin isoforms—notably certain α- and ω-gliadins—and an altered glutenin polymer structure. This molecular profile translates into a measurable attenuation, but not elimination, of key pathogenic processes. In vitro, spelt-derived peptides exhibit a reduced capacity to disrupt intestinal epithelial tight junctions and provoke pro-inflammatory cytokine release from immune cells. This attenuated behavior aligns with the lower immunoreactivity observed in serological assays and can be conceptually modeled as a reduction in the effective concentration of immunogenic epitopes (a lower [E]) and/or a modification in their digestibility and presentation kinetics (a modified k_d).

From these findings, two critical, population-specific conclusions arise with direct translational impact.

First, and of paramount clinical importance, spelt is unequivocally not a safe alternative for individuals with celiac disease (CeD). The detection of peptides reactive with both monoclonal antibodies (R5, G12) and CeD patient sera confirms the presence of cross-reactive, immunogenic sequences. The autoimmune pathogenesis of CeD is predicated on a highly specific T-cell response to gluten epitopes presented by HLA-DQ2/DQ8 molecules (Sollid et al., 2020). Our data demonstrate that spelt gluten contains these epitopes, and the INFOGEST digestion protocol liberated fractions with immunoreactivity comparable to wheat. For the CeD patient, the immune system operates on a binary logic of antigen recognition; a "reduced" dose of a perfectly matched epitope is not a "safe" dose but rather a sub-clinical trigger that can sustain mucosal inflammation and perpetuate villous atrophy over time (Tye-Din et al., 2022). Therefore, spelt must remain excluded from a strict, medically prescribed gluten-free diet for CeD.

Second, for a substantial subset of individuals with non-celiac gluten sensitivity (NCGS), spelt may offer a subjectively better-tolerated option. Our pilot study indicated that approximately 60% of participants reported milder symptoms following spelt consumption compared to common wheat. This subjective benefit, dissociated from significant changes in fecal calprotectin, suggests a mechanism distinct from the overt autoimmune enteropathy of CeD. The likely explanation lies in the intersection of spelt's attenuated gluten immunogenicity and its broader nutritional matrix. The reduced epitope load may keep the antigenic challenge below a personal threshold for triggering innate immune activation and visceral hypersensitivity (Fasano et al., 2020). Concurrently, the higher fiber content and potentially different profile of other bioactive components, such as amylase-trypsin inhibitors (ATIs) or FODMAPs, could further modulate gastrointestinal responses (Zevallos et al., 2017). This necessitates a personalized, graded approach to dietary management in NCGS, where spelt might be trialed as a less provocative alternative under professional guidance, acknowledging it is not a gluten-free product.

The scientific and practical significance of these findings is multifold:

1. **For Clinical Practice and Public Health**: These results provide a clear evidence base for dietetic counseling. They empower healthcare providers to debunk the myth of "safe spelt for celiacs" with concrete data while offering a nuanced perspective for managing NCGS. This helps prevent dangerous self-experimentation in CeD and guides informed choice in NCGS.

2. **For the Food Industry**: The study underscores that "ancient grain" claims require substantiation. For developing products aimed at the "free-from" or "sensitivity" market, spelt is not a suitable ingredient for gluten-free labeling. However, it presents an opportunity for creating products with a "reduced reactivity" profile for the NCGS demographic, provided labeling is transparent and accurate.

3. **For Agricultural and Food Science**: Most prospectively, our work illuminates a pathway for the strategic breeding of cereals with lowered immunogenic potential. The

identified proteomic signature of spelt—specifically, the alleles associated with reduced expression of highly immunogenic α-gliadins—serves as a valuable genetic template. By combining such alleles from spelt and other ancient or wild relatives using modern genomic tools, breeders could develop novel wheat varieties that maintain agronomic and baking quality while presenting a significantly reduced risk of triggering adverse reactions (Brouns et al., 2019). This "detoxification by design" approach represents a sustainable, long-term solution to mitigate the global burden of gluten-related disorders.

Future research must build upon this foundation. Large-scale, controlled clinical trials are needed to confirm the NCGS findings and identify biomarkers that predict who might tolerate spelt. A comprehensive survey of the genetic diversity within spelt germplasm is crucial to assess the range of immunogenicity across different cultivars. Finally, the explicit contribution of the non-gluten matrix—the fiber, polyphenols, and ATI content—requires systematic deconstruction to fully understand the mechanism behind spelt's differential effects. In conclusion, this study reframes spelt not as a paradox, but as a compelling case study in how subtle variations in grain biochemistry translate to profoundly different clinical realities, charting a course for both safer diets and smarter agriculture.

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