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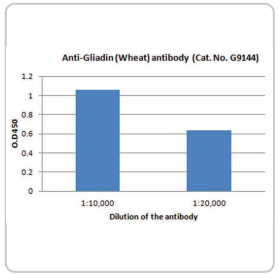

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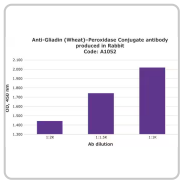

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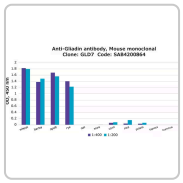

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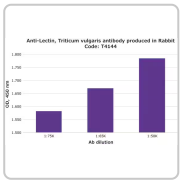

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T4144

Anti-Lectin, *Triticum vulgaris* antibody...

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PROPERTIES

biological source	rabbit
Quality Level	**200**
conjugate	unconjugated
antibody form	fractionated antiserum
antibody product type	primary antibodies
clone	polyclonal
form	buffered aqueous solution
species reactivity	wheat

technique(s)	dot blot: 1:1,500 indirect ELISA: 1:5,000
shipped in	dry ice
storage temp.	−20°C
target post-translational modification	unmodified
Gene Information	wheat ... **LOC543191(543191)** , **LOC543191(543191)** , **Loc543191(543191)**

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DESCRIPTION

General description

Gliadin is a protein found in wheat and other cereals. The T cells recognize the gliadin epitopes and activate the innate immune response similar to the response to pathogens. This response is more intense in coeliac disease wherein the gut-derived T cells produced in response to gliadin induce damage to the small intestine. Therefore, patients affected by coeliac disease or gluten-sensitivity should avoid food that produce gliadin

Gliadin is an important component of wheat gluten. It is composed of single-chain polypeptides and has a molecular weight of 25−100 kDa. It is associated by intramolecular disulfide bonds.

Specificity

Rabbit Anti-Gliadin shows specificity for native wheat gliadin.

Immunogen

native and heat-treated wheat gliadin

Application

Anti-Gliadin (Wheat) antibody produced in rabbit has been used in immunoblot and enzyme-linked immunosorbent assay (ELISA).

Biochem/physiol Actions

Gliadin is a class of glutamine containing, alcohol soluble proteins separable from wheat and rye glutens known as the prolamins. These proteins are associated with the harmful effects of celiac disease and gluten sensitive enteropathy in humans, causing characteristic changes in the small intestinal mucosa. In such cases, strict compliance to a gluten-free diet is usually recommended.

Physical form

Solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide

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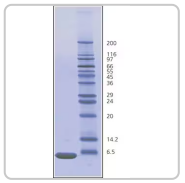

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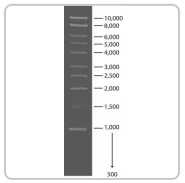

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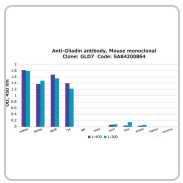

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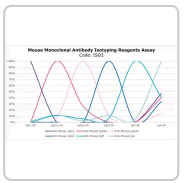

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Gliadin Monoclonal Antibody (14D5)

Catalog Number **HYB 314-01-02**

Product data sheet

Details	
Size	200 µL
Host/Isotope	Mouse / IgG2a, kappa
Class	Monoclonal
Type	Antibody
Clone	14D5
Immunogen	Synthetic peptide corresponding to residues K(58) L Q P F P Q P E L P Y P Q P Q(73) of gliadin peptide.
Conjugate	Unconjugated
Form	Liquid
Concentration	1.05 mg/mL
Storage Conditions	4° C, store in dark

Tested Applications	Dilution *
ELISA (ELISA)	1:64,000
Western Blot (WB)	1:1,000

* Suggested working dilutions are given as a guide only. It is recommended that the user titrate the product for use in their own experiment using appropriate negative and positive controls.

Product specific information

HYB 314-01-02 detects the Gliadin peptide in wheat samples. The immunogen corresponds to a deamidated form of a region that includes the T-cell epitopes, including the immunodominant PQPQLPY region and two PXPQP motifs associated with binding to IgA from patients with celiac disease. It reacts specifically with the deamidated peptide when coated directly in an immunoplate. NOTE: Concentration is lot-dependent and can vary from 0.85-1.15 mg/mL

Background/Target Information

Celiac disease is associated with a CD4+ T-cell response to epitopes of gliadin presented by HLA-DQ2 or -DQ8 class II MHC molecules. These epitopes are present in a 33-mer peptide of wheat alpha-gliadin, residues 56-88, which is resistant to digestion and forms a substrate for tissue transglutaminase (TG2), generating the glutamic acid residues essential for binding to HLA-DQ2. Complete homology exists between residues 63-73, 70-80 and 77-87 of wheat alpha gliadin.

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Dr Saini

by anu b

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File name: Final-Gliadin__pAb__mAb_ms.docx (49.51K)
Word count: 4261
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Association between gliadin content and celiac disease antigenicity using polyclonal and monoclonal antibodies in Indian wheat cultivars

ABSTRACT

Objectives: Wheat, the second most consumed staple food after rice, is vital for global food security, providing sustenance to 30% of the human population. However, despite its remarkable viscoelastic properties in the form of gluten composed of glutenins and gliadins, wheat has been linked to celiac disease in genetically susceptible individuals.

Methods: In this study, we analyzed 62 Indian wheat varieties released over six decades (1961-2020) for gliadin content using BSA and gliadin as calibration standards, and assessed their antigenicity using celiac disease-specific polyclonal antibody (pAb).

Results: In addition, a set of 25 wheat varieties were scanned using monoclonal antibody (mAb) raised against gliadin. Large variations in gliadin content (2.75 to 6.98 g 100^{-1} g whole wheat flour) were observed with the lowest in PBW 34 and the highest in HS 420. Comparative analysis revealed that gliadin content measured using the standard gliadin calibrant was 1.5 times higher than using BSA as calibrant. A positive correlation was observed between total antigenic gliadin and total gliadin content using pAb (r^2 = 0.5841; r = 0.763**) and mAb (r^2 = 0.4923; r = 0.728**) indicating that total gliadin content is the major factor for eliciting celiac antigenicity.

Conclusion: The study also exhibited that celiac causing potential of wheat is not different among the varieties developed across different periods. The findings contribute to our understanding of the relationship between gliadin content and antigenicity, and thus have potential implications in management of celiac disease.

Key words: Gliadin correlation, Celiac disease, Antigenicity, Poly- and Monoclonal antibody

1. Introduction

Wheat, a widely consumed crop in nearly 100 countries, is an annual herb belonging to the Gramineae or Poaceae family. It plays a crucial role in ensuring global food security, providing food for 2.5 billion human population across the world (Ramadas et al., 2019). It is the second most consumed staple after rice and contributes approximately 20% of the calorie needs and 25% of the dietary protein (Shewry, 2009). Alongside carbohydrates, wheat contains 10-15% protein, as well as significant amount of dietary fiber and micronutrients (Shewry and Tatham, 2016; Zhao et al., 2009). The protein in wheat grains is comprised of various fractions including albumins, globulins, gliadins (prolamins) and glutenins identified based on their solubility. When wheat flour is mixed with water a viscoelastic complex called gluten composed of glutenins and gliadins is developed which determines the end-product quality.

Wheat gluten, comprising 75-80% of total proteins, is a complex mixture consisting of α/β, γ-, and ω-gliadins, as well as high- and low molecular weight (HMW; LMW) glutenins. These proteins are encoded by medium to large multigene families (Shewry, 2009; Shewry, 2019). Gluten's viscoelastic and extensible properties provide binding and shape-forming characteristics that are highly desirable in the processed food industry and essential for dough making. It imparts a chewy and palatable texture to baked and processed foods. However, for genetically predisposed individuals, gluten can be associated with certain disorders and allergies. Some people experience harmful immune responses against wheat gluten proteins after consumption, leading to an inability to tolerate wheat and its products (Cabanillas, 2020; Shewry and Tatham, 2016). One notable disorder linked to gluten is celiac disease (CD), an autoimmune condition directly associated with gluten proteins (Stamnaes and Sollid, 2015). Celiac disease affects approximately 1% of the global population (pre-dominant in females), with variations based on sex, age, and geographical location (Caio et al., 2019; Narwal et al., 2020). The disease afflicts individuals having HLA-DQ2 and DQ8 genetic backgrounds, and show intolerance towards gluten proteins of wheat along with prolamins of barley (hordiens), rye (secalins), and oats (avenins) (McAllister et al., 2019; Narwal et al., 2020) Among all the protein fractions, α- and γ-gliadins play significant roles in the progression of CD (Balakireva and Zamyatnin, 2016; Garcia-Calvo et al., 2021).

The toxicity of CD is primarily attributed to the presence of large repeat domains containing homologous and repetitive sequences of six-to-eight amino acids, rich in proline (P) and glutamine (Q) (Shewry, 2019). Within the α-gliadin protein, the central domain contains two key sequences: the P and Q-rich heptapeptide PQPQPFP and the pentapeptide PQQPY. Extensive *in vitro* and *in vivo* studies conducted in rats and humans have revealed that this domain encompasses a 33-mer peptide of gliadin (LQLQPFPQPQLPYPQPQLPYPQPQLPYPQPQPF), consisting of six overlapping epitopes that play a significant role in the pathogenesis of CD. Remarkably, this 33-mer peptide resists digestion by gastric, pancreatic, and intestinal brush-border membrane endo-proteases (Shan et al., 2002; Ozuna et al., 2015). The former along with similar peptides, serve as a primary stimulator of the inflammatory response in genetically predisposed individuals (Narwal et al., 2020; Shan et al., 2002). Therefore, it is important to identify varietal differences if any with respect to antigenicity using celiac disease-specific polyclonal antibody (pAb) and monoclonal antibody (mAb). In addition, it is also important to quantify gliadins and assess its relationship with CD antigenicity. In this investigation, 62 Indian wheat varieties released over the last six decades were used for assessing gliadin content and antigenic epitopes (using polyclonal antibodies) to find out the relationship between them. In addition, a subset of 25 varieties was used for monoclonal antibody (mAb) analysis.

2. Materials and methods

2.1 Chemicals

The standard gliadin, bovine serum albumin (BSA), polyclonal antibody (Sigma-Aldrich; G9144), and 3,3′,5,5′-Tetramethylbenzidine (TMB) Horseradish Peroxidase Substrate (soluble) (TMB/E) employed in this experiment were acquired from Sigma-Aldrich, Steinheim, Germany. All chemicals utilized in the study were of the highest purity and obtained from reputable sources such as Invitrogen (HRP-labeled secondary antibody, monoclonal antibody) and other well-established firms.

2.2. Experimental material

Sixty-two wheat varieties (59 *Triticum aestivum* and 3 *T. durum*) released in India during 6 decades (1961-2020) for commercial cultivation across different wheat growing regions of the country were employed for pAb antigenic study, while a subset of 25 varieties (*T. aestivum*) were used for mAb antigenic study. All the varieties were grown at the ICAR-IIWBR experimental farm at Karnal location, following standard agricultural practices. Further details of wheat varieties utilized in this study have been provided in Table 1.

2.3. Extraction of gliadin

Gliadins were extracted from whole wheat flours (WWF) obtained using a Cyclotec mill (FOSS) with a 0.5 mm screen. 100 mg of WWF was mixed with 1.0 mL of 60% alcohol and homogenized in a water bath for 30 min at 35±2 $^{\circ}$C followed by centrifugation at 3000 g for 15 min (Narwal et al., 2020). The supernatant obtained was used for measurement of gliadin content as well as antigenicity studies. To ensure robustness, two biological replicates with two technical replicates were performed.

2.4. Determination of gliadin content

Gliadins were quantified by using the Bradford assay method (1976) as modified by Rekowski et al. (2021) and standardized using a Gen5 BioTek Absorbance Microplate Reader (Biotek, USA) on a 96-well microplate. Standard gliadin obtained from Sigma-Aldrich was dissolved in a solution consisting of 60% alcohol and 10% acetic acid (@1 μg/μL), with mild heating and constant shaking. The assay was conducted using a total reaction mixture volume of 300 μL containing 5 μL of diluted standard or sample, 95 μL of double-distilled water, and 200 μL of Bradford reagent. The reaction mixture was incubated at room temperature for 11 min, and then the absorbance was recorded at 595 nm. Standard curves were made using both bovine serum albumin (BSA) and gliadin as calibrant and the concentration was expressed as g gliadin 100 g^{-1} WWF.

2.5. Determination of total protein

The protein content of the samples was measured using near-infrared (NIR) instrument (Foss Infratech® 1241 Grain Analyzer, Denmark) and expressed at 12% moisture basis.

2.6. Estimation of antigenicity using polyclonal (pAb) and monoclonal antibody (mAb)

For estimation of antigenicity, indirect ELISA coupled with microplate assay was employed following a standardized protocol (Gregorini et al., 2009). The pAb [Sigma-Aldrich, G9144; Anti-Gliadin, polyclonal (Wheat) antibody, IgG] raised against native and heat-treated wheat gliadin in rabbits while mAb (HYB-314-01-02) raised against gliadin antigen having immunogen synthetic peptide corresponding to residues K(58)LQPFPQPELPYPQPQ(73) of gliadin peptide produced in mouse were employed in this study. The experiments were performed in duplicate and repeated twice for accurate estimation. Separate calibration curves were prepared using standard gliadin for pAb as well as mAb to quantitate antigenicity. The dilutions of the antigen, primary antibodies, and secondary antibody (In vitrogen; Pierce Goat Anti-Mouse IgG (H+L), HRP; 31430; peroxidase conjugated, 1:10000) were optimized using the standard checkerboard method. Primary pAb was 1000 times diluted while for mAb the dilution was 1500 times. The horse radish peroxidase (HRP)-labelled secondary antibody was used at 7500 times dilution for both the experiments. The data were calculated as the total antigenic gliadins in g 100 g^{-1} WWF (%).

3. Results

3.1. Comparative analysis of gliadin quantification using bovine serum albumin (BSA) and gliadin as calibrants

The gliadin content and antigenicity of 62 wheat varieties released during 1961-2020 were assessed. Gliadin is a component of wheat gluten known to be implicated in CD. The variation in gliadin content was determined using BSA and gliadin as calibrants (Fig. 1). Table 1 presents the detailed data on the average total gliadin content, total protein content, and the ratio of gliadin to total protein content. The total gliadin content (measured by using gliadin as standard) varied from 2.75 (PBW 34) to 6.98 (HS 420) g 100 g^{-1} WWF. Protein content ranged from 9.10 (GW 89) to 15.40 (HPW 251) g 100 g^{-1} and gliadin to total protein ratio ranged from 24.8% (PBW 34) to 58.2% (HS 420) (Table 1). To compare the quantification of gliadin using different standards, the gliadin content was also determined using BSA as a standard which ranged from 2.22 to 4.29 g 100 g^{-1} WWF (Table 1). Though there was highly significant

positive correlation (r=0.99) between gliadin content measured by both BSA and gliadin as calibrant, the absolute values were different. Gliadin content measured using the standard gliadin calibrant was 1.5 times higher than using BSA as standard. Similar variations were observed in gliadin content of old and new cultivars indicating no correlation between the cultivar release year and the gliadin content.

3.2. Determination of total antigenicity employing polyclonal antibody (pAb)

To assess antigenicity of celiac toxic epitopes, gliadins extracted from all 62 wheat varieties were analysed for total antigenic gliadin content using polyclonal antibodies (Fig. 2). Among the varieties, AKAW 4627 (2011-20) exhibited the highest antigenicity (0.067 g 100 g^{-1} WWF), while Rohini (1981-90) recorded the lowest one (0.016 g 100 g^{-1} WWF) (Fig. 2). Furthermore, the antigenic/total gliadin ratio ranged from 0.0042 (Rohini) to 0.0119 (HD 4672) (Fig. 2). Overall averages for total antigenicity, total gliadin, and ratio of antigenic *vs*. total gliadin for the varieties taken under investigation were 0.044 (%), 4.93 (%) and 0.009, respectively. There was a significant positive correlation (r= 0.763**) between total antigenic gliadin (determined via ELISA-based assay) and total gliadin (measured using the Bradford assay) (Fig. 3). Strong positive correlation between total gliadin content and CD antigenicity indicated that CD toxicity depends on gliadin content and not on variety or the release year of the variety.

3.3. Determination of total antigenicity utilizing monoclonal antibody (mAb)

The celiac toxic epitopes were also screened and quantified using more specific mAbs in the gliadin extracts of a subset of 25 wheat varieties (Fig. 4). Among the varieties under investigation, MACS 6145 (2005) exhibited the highest % antigenicity (0.092), while PBW 34 (1985) recorded the lowest one (0.036). The antigenic/total gliadin ratio ranged from 0.0083 (WB 2) to 0.0143 (DBW 222). Overall averages for total antigenicity, total gliadin, and ratio of antigenic *vs*. total gliadin were 0.054 (%), 4.78 (%) and 0.0113, respectively. A significant positive relationship (r^2= 0.4923; r= 0.728**) was observed between total antigenic gliadin and total gliadin present (Fig. 5). In the same subset, the correlation between antigenic gliadin *vs*. pAb mediated total antigenicity was also highly significant (r=0.843**). In addition, the correlation between mAb and pAb mediated antigenicity was found statistically significant (r=0.686**).

4. Discussion

Wheat the second most important cereal crop, consumed widely in developed and developing countries. For developing world, it acts as protein and energy source (Govindan et al., 2023). It is a staple crop that contains approximately 10-15% grain protein content, along with significant amount of carbohydrates, dietary fiber, and micronutrients (Shewry and Tatham, 2016). Dough viscoelasticity and extensibility necessary for an array of processed products (bread, pasta, biscuit *etc*.) and their quality, are attributable to wheat seed storage proteins (gliadins and glutenins) (Shewry and Hey, 2015; Sissons, 2008) which are stockpiled is seed endosperm clubbed with starch (Sharma et al., 2020). In the present study, the gliadin content (measured using gliadin as calibrant) in wheat varieties ranged from 24.8% to 58.2%. Some of the previous reports showed gliadin concentrations in the range of 30-40% as a proportion of total proteins in wheat grains (Malik, 2009; Urade et al., 2018). Other mentioned albumin-cum-globulin, gliadin and glutenin contents in the range of 10.7–44.0%, 18.6–34.3%, and 27.4–43.9% respectively for different wheat varieties (Mohan Kumar et al., 2017; Siddiqi et al., 2016; Siddiqi et al., 2021; Žilić et al., 2011). This shows that the content of gliadin and other protein fractions can vary depending on the variety as well as environmental factors.

Two methods of gliadin measurement, using bovine serum albumin (BSA) and gliadin as calibrant, were compared. The data showed that gliadin content measured using the standard gliadin calibrant was 1.5 times higher than using BSA as standard. However, in one of the recent study, Rekowski et al. (2021) reported a conversion factor of 4.25 (gliadin concentration estimated using BSA calibrant was 4.25 times lower than as with gliadin calibrant) by comparing wheat gliadins using standard BSA and gliadins extracted (used as gliadin calibrant) from wheat flour (cultivar Akteur). In our study, there was very high significant positive correlation (r=0.99) between gliadin content measured by both the calibrants using 62 diverse Indian wheat varieties indicating its high reliability of using BSA as standard by employing a factor of 1.5.

Though gliadin being the component of gluten important for dough making properties, it has been found accountable for developing certain intolerances to some people including CD. Celiac disease (CD) is an autoimmune disorder that primarily affects genetically predisposed individuals upon consumption of a gluten-containing diet (Kumar et al., 2024). Celiac occurs in individuals with specific genetic backgrounds, particularly those carrying the HLA-DQ2 and DQ8 genes, who exhibit intolerance to the gluten proteins found in wheat, particularly highly immunogenic α-gliadins (Caio et al., 2019; Sharma et al., 2020). For CD, serological testing can be accomplished using anti-gliadin or anti-deaminated gliadin antibodies, anti-tissue transglutaminase-2 antibodies and anti-endomysium antibodies (Al-Toma et al., 2019; Sharma et al., 2020; Tye-Din et al., 2018). In the current study, antibodies raised against native gliadins and/or heat-treated gliadins have been employed for calculating antigenicity. There was strong positive correlation (r=0.76) between total antigenic gliadin and total gliadin content, using pAb. Poirier et al. (2021) also used pAbs to discriminate wheat, barley, and oat prolamins in an indirect ELISA assessment. Schopf and Scherf (2018) compared different ELISA kits and found that the pAb ELISA was less affected by gluten variability and was able to detect gluten from einkorn wheat too. In addition, monoclonal antibodies have been employed by various researchers to demonstrate the CD mediated antigenicity in wheat protein fractions (Gregorini et al., 2009; Narwal et al., 2020; Ribeiro et al., 2016; Spaenij-Dekking et al., 2005). In this study, mAb destined against the α-gliadin peptides p58-73 (KLQPFPQPELPYPQPQ) containing a core region reported to cause CD toxicity was employed for screening antigenicity. A significant positive correlation (r^2= 0.4923; r= 0.728**) was also found in between total antigenic gliadin and total gliadin using mAb showing a strong correlation between antigenicity and gliadin content. However, there are few exceptions having higher gliadin content and lower antigenicity. It can be explained by the fact that antigenic peptides are not uniformly distributed among all wheat genetic resources as stated by Schalk et al. (2017) and Garcia-Calvo et al. (2021).

Conflicting results have been reported for old and modern bread varieties regarding their antigenic behaviour (Shewry, 2018). Van den Broeck et al. (2010) reported that ancient wheat varieties have a smaller number of CD antigenicity (gilaα9) compared to modern ones. While others reported that

modern wheat varieties have fewer CD antigenicity as compared to ancient varieties (Colomba and Gregorini, 2012; Prandi et al., 2017). On the other hand, Narwal et al. (2020) observed varietal differences in antigenicity, but they did not find noteworthy changes in antibody reactivity for the wheat varieties released after 1960 in India. Malalgoda et al. (2018) reported that the cultivar release year and the number of immunogenic epitopes and α-gliadin have no relation with each other. According to Pronin et al. (2021), the immune-reactive potential of old and modern wheat cultivars is similar. In addition, Ribeiro et al. (2016) stated that breeding has no contribution towards the prevalence of CD antigenic epitopes. Our results are in conformity with Malalgoda et al. (2018), Narwal et al. (2020) and Pronin et al. (2021) in that year of release has no relation to antigenicity.

5. Conclusion

In conclusion, this investigation provides comprehensive insights into the gliadin content and antigenicity in a diverse set of wheat varieties. The study reveals significant variations in gliadin content among the wheat varieties tested, emphasizing the potential implications for CD. The comparison of gliadin quantification using BSA and gliadin as standard calibration materials highlights the importance of establishing an appropriate conversion factor. These findings contribute to our understanding of the gliadin content, antigenicity, and their potential implications in celiac disease.

CRediT authorship contribution statement

Sunil Kumar: Research, Conceptualization, Writing – original draft, Formal analysis, Methodology, Supervision, Project administration, Data curation. **Ankush**: Research, Methodology. **Sewa Ram**: Writing – original draft, Review and editing, Formal analysis, Supervision, Project administration, Funding acquisition. **Arun Gupta**: Providing pedigree and germplasm material. **Om P Gupta**: Review and editing. **Vanita Pandey**: Writing and review. **Anuj Kumar**: Editing. **Gyanendra Singh**: Supervision, Project administration.

Declarations of interest: None

Table and Figure legends

Table 1. List of wheat varieties, release year, total gliadin content, total protein content, and gliadin-to-total protein ratio (WWF= whole wheat flour)

Fig. 1. Standard curves using gliadin and BSA as calibrant

Fig. 2. Total antigenicity and its ratio to total gliadin using polyclonal antibody: For decades 1961-2000 (2a); for decades 2001-2020 (2b)

Fig. 3. Correlation between antigenicity to total gliadin content using polyclonal antibody (Varieties: 62; period: 1961 to 2020)

Fig. 4. Total antigenicity and its ratio to total gliadin using monoclonal antibody (Varieties: 25; period: 1981 to 2020)

Fig. 5. Correlation between antigenicity to total gliadin using monoclonal antibody (Varieties: 25; period: 1981 to 2020)

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