

MS^E Based Multiplex Protein Analysis Quantified Important Allergenic Proteins and Detected Relevant Peptides Carrying Known Epitopes in Wheat Grain Extracts

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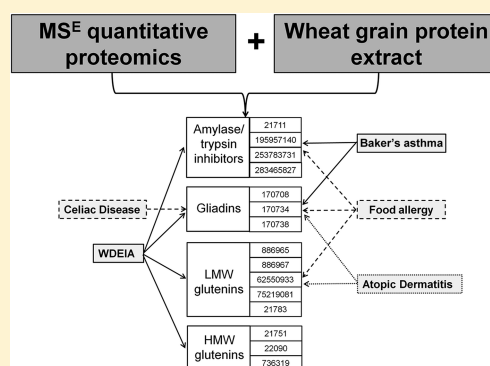
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Supporting Information

ABSTRACT: The amount of clinically relevant, allergy-related proteins in wheat grain is still largely unknown. The application of proteomics may create a platform not only for identification and characterization, but also for quantitation of these proteins. The aim of this study was to evaluate the data-independent quantitative mass spectrometry (MS^E) approach in combination with 76 wheat allergenic sequences downloaded from the AllergenOnline database (www.allergenonline.org) as a starting point. Alcohol soluble extracts of gliadin and glutenin proteins were analyzed. This approach has resulted in identification and quantification of 15 allergenic protein isoforms that belong to amylase/trypsin inhibitors, γ -gliadins, and high or low molecular weight glutenins. Additionally, several peptides carrying four previously discovered epitopes of γ -gliadin B precursor have been detected. These data were validated against the UniProt database, which contained 11764 *Triticaceae* protein sequences. The identified allergens are discussed in relation to Baker's asthma, food allergy, wheat dependent exercise induced anaphylaxis, atopic dermatitis, and celiac disease (i.e., gluten-sensitive enteropathy). In summary, the results showed that the MS^E approach is suitable for quantitative analysis and allergens profiling in wheat varieties and/or other food matrices.

KEYWORDS: wheat grain extract, epitope, allergens, proteomics, MS^E, quantification



1. INTRODUCTION

Wheat, soybean, egg, milk, fish, crustaceans, peanut, and nuts are responsible for a majority of food allergies.^{1–3} Thus, wheat belongs to a group of allergenic food ingredients that have to be labeled in the European Union for food products as potentially dangerous for the susceptible individuals.^{4,5} Wheat proteins are divided into two basic groups: water/salt-soluble (albumins, globulins) and the water/salt-insoluble gluten proteins. The gluten group represents about 80% of all wheat proteins, which is constituted by gliadins (Glia) and glutenins (Glu).⁶ Glutenins are the major determinants of dough elasticity and strength for bread making quality^{7,8} and are divided based on mobility in an electric field to high (HMW) and low molecular weight (LMW) subunits.⁹ Gliadins are classified into α , β , γ , and ω subgroups.¹⁰

Currently, food intolerance or allergy affects 5–8% of children and 1–3% of adults, and these numbers are increasing.^{11–13} Depending on the route of exposure, hypersensitivity reaction may develop into a variety of symptoms. The common food intolerance celiac disease, or gluten-sensitive enteropathy, is a permanent condition of the sensitive individuals.¹⁴ Celiac disease is triggered by ingestion of gluten proteins and is presented with a set of diverse clinical features, which typically includes weight

loss, diarrhea, fatigue, or anemia (for recent reviews see refs 11, 15, and 16). Gliadin proteins, alcohol-soluble components representing about half a gluten protein, are responsible for developing celiac disease.^{17–19} Food allergy to wheat is defined as hypersensitivity reactions to ingested wheat proteins (for review see ref 20). These reactions typically occur within an hour after wheat ingestion and may include gastrointestinal, cutaneous, or respiratory problems. These symptoms are clinically distinct from other allergenic reactions to wheat proteins, such as atopic eczema/dermatitis, urticaria, Baker's asthma, or wheat dependent exercise induced anaphylaxis (WDEIA). Atopic dermatitis is a chronic inflammatory skin disease that is associated with sensitization to food allergens from soy, fish, egg, or wheat (for reviews see refs 21 and 22). Major antigenic epitopes associated with atopic dermatitis²³ belong to gliadins²⁴ and to LMW glutenins.^{25,26} Baker's asthma^{27,28} is respiratory allergy to wheat proteins caused by inhalation of wheat flour and is one of the most common types of occupational asthma.^{29–32} WDEIA is a

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rare but severe anaphylactic reaction caused by combined wheat ingestion and physical exercise.^{33,34} Major antigenic epitopes associated with wheat allergies belong to gliadins²⁴ and LMW glutenins.^{25,26} The main WDEIA allergen is ω 5-gliadin.^{35,36} Furthermore, LMW glutenin can also trigger WDEIA and share same epitopes with ω 5-gliadin.³⁷

Determining accurate amounts of clinically relevant wheat proteins could be important for food source identification and clinical diagnostic studies. Among the available methods used in diagnostics and clinical practice are (i) the skin prick test, (ii) the double blind placebo controlled food challenge test, (iii) basophil activation tests, (iv) enzyme-linked immunosorbent assay (ELISA), (v) polymerase chain reaction (PCR), and (vi) immunoblotting assays (for review see refs 38 and 39). However, methods based on IgE depend on purity of wheat extracts that often do not include major allergens.⁴⁰ Furthermore, because of common IgE-reactive epitopes, cross-reactivity among wheat flour, other cereal (barley, rye, rice, and maize), and grass pollen allergens may cause additional problems.^{41,42} Therefore, a rapid and easy procedure for allergen identification is still needed.

In recent years, various methods of mass spectrometry (MS) were used for the detection and quantification of allergens. For instance, spectral counting and multiple reaction monitoring were used in order to quantify allergenic protein in soybean.⁴³ In wheat, MS has been used for the analysis of proteins associated with celiac disease.¹⁷ As the MS technology has advanced, we recently employed the quantitative LC/MS^E approach to monitor multiple target proteins and determine the amounts of GliA and Glu proteins isolated from wheat grain.⁴⁴ Quantifying proteins using MS^E technology^{45,46} is based on the observation that an exponentially modified protein abundance index (emPAI) is directly proportional to the protein content in a protein mixture.⁴⁷ The combined intensity of the charged ions for the three most abundant tryptic peptides of internal standard is compared with the observed response for identified peptides in a complex mixture. The average MS signal response from bovine hemoglobin, the internal standard in this study, was used to determine the universal signal response factor (USRF, counts/mol of protein) that was later used to determine the concentrations for each of the target proteins by dividing the MS by USRF. In the present study, we used MS^E proteomics to specifically monitor target peptides that in turn were used to determine the concentrations of clinically relevant wheat allergens⁴⁸ and to detect the peptides carrying known immunologically relevant epitopes.

2. EXPERIMENTAL PROCEDURES

2.1. Plant Material

Bread wheat cultivar "Viginta" was provided by SELEKT Ltd, Bučany. Sample preparation was performed as described earlier.⁴⁴ Briefly, one kilogram of wheat grain was divided into 4 equal parts and then each part was divided into five 50-g portions that were milled by electric grinder. Each 50-g portion of ground grain was considered a replicate sample.

2.2. Protein Extraction and "in-Solution" Digestion

Proteins were extracted from nine samples of milled wheat grains according to van den Broeck et al.⁴⁹ as we described recently.⁴⁴ Briefly, GliA were obtained after extraction with 1 mL of 50% (v/v) aqueous isopropanol followed by centrifugation at 2500g for 15 min at room temperature. The pellet was further extracted with 1 mL of 50% (v/v) aqueous isopropanol, 50 mM Tris-HCl (pH 7.5) containing 1% (w/v) DTT for 30 min at 60 °C, and Glu

were obtained. Because isopropanol interferes with determination of total protein concentration according to the Bradford method, protein extracts were divided into two equal aliquots. Total protein from the first aliquot was precipitated, resolved, and measured according to Bradford⁵⁰ to determine protein concentration. In the second aliquot, one hundred micrograms of protein were reduced with DTT, alkylated with IAA, and digested with Trypsin (Promega, Madison, WI, USA). The resulting tryptic digest was concentrated and desalted using Peptide Cleanup C18 spin columns (Agilent Technologies) according to the manufacturer's instructions and stored at -80 °C until the MS^E analysis.

2.3. Mass Spectrometry

Trypsinized proteins were spiked with predigested Bovine Hemoglobin (digested HBA P01966, HBB P02081) internal standard (Waters 186002327, U.K.) at a level of 1 pmol per 5 μ L (1.5 μ g of wheat proteins) and analyzed as we described recently.⁴⁴ Shortly, the peptide mixture was injected onto a reverse-phase ultraperformance liquid chromatography (UPLC) column (nanoAcquity, BEH 130 C18, 75 μ m $\times$ 150 mm, 1.7 μ m particle size) that was connected to the PicoTip emitters (New Objective, USA) mounted into the nanospray source (3.4 kV at 70 °C) of the quadrupole time-of-flight (Q-TOF Premier) mass spectrometer. Peptides were eluted with an acetonitrile gradient (10–45% B in 40 min; A = water with 0.1% formic acid, B = acetonitrile containing 0.1% formic acid) at a flow rate of 350 nL/min. The spectral acquisition scan rate was set to 1 s with a 0.05 s interscan delay. Glu-1-fibrinopeptide B (GFP, 500 fmol/mL) was infused via the Lock-mass source at a flow rate of 500 nL/min and sampled every 30 s as the external mass calibrant. Calibrant data were collected at a constant collision energy of 20 eV. The MS^E data were collected in alternating, low, and elevated energy modes.^{45,46} In the low energy MS mode, data were collected at a constant collision energy of 3 eV. In elevated energy MS^E mode, the collision energy was ramped from 20 to 38 eV during each integration.

2.4. Wheat Allergen Database, Data Processing, and Protein Quantification

The MS^E data were searched against the wheat allergen database containing 76 *Triticum* species entries (downloaded from <http://www.allergenonline.org> on February 7, 2011) using the IDENTITY^E search algorithm within the ProteinLynx Global Server v. 2.4 (PLGS 2.4; Waters, U.K.). Prior to searching, the sequence of the internal standard HBA_BOVIN Hemoglobin subunit α (NCBI: P01966) was added to the database. Time alignment was applied for initial correlation of a precursor (MS) and possible fragment ions (MS/MS). Search parameters included the "automatic" setting for mass accuracy (50 ppm for precursor ions and 0.1 Da for product ions), a minimum of one peptide match per protein, a minimum of three consecutive product ion matches per peptide, and a minimum of seven total product ion matches per protein. The maximum false positive rate (FPR) against the randomized forward database was set to 4%, and the protein quantification functionality was enabled using the internal standard. Only one missed tryptic cleavage site was allowed during the search. Modifications included the following: fixed, carbamidomethylation of Cys; variable, deamidation of Asn and Gln, oxidation of Met, and dehydration of Ser and Thr.

Following database searching, the quantification values between technical replicates were manually corrected by the normalizing peptide intensities of the protein of interest against

the intensities of peptides from the spiked internal standard. The minimal requirements for accepting of protein quantification data were as follow: (i) detection of at least three fragment ions per peptide, (ii) a minimum of two peptides matched to the protein sequence, (iii) PLGS score greater than 50, and (iv) quantification of the protein in at least three biological replicates. The similarity between the quantified allergen sequences was determined using the Local similarity program (SIM) available at <http://web.expasy.org/sim/>.⁵¹

3. RESULTS

3.1. MS^E Reliably Quantified Fifteen Wheat Allergen Protein Isoforms

The MS^E data, obtained from the analysis of Glia and Glu extraction fractions of milled wheat grains,⁴⁴ were bioinformatically compared against 76 wheat allergenic/ceciac sequences downloaded on February 7, 2011 (Figure 1). The feasibility of

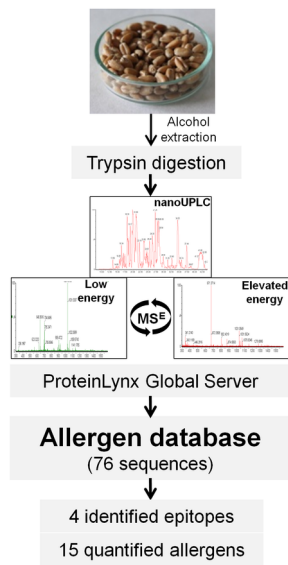


Figure 1. Workflow. A preparation of wheat grain protein was digested with trypsin. Peptides from the digestion were then separated from one another using ultraperformance liquid chromatography followed by quantitative data-independent mass spectrometry analysis of the targeted proteins (MS^E). Raw MS^E sequence data were searched against 76 known wheat allergenic sequences obtained from the AllergenOnline database (www.allergenonline.org) using the ProteinLynx Global Server (v. 2.4). This approach resulted in detection of peptides carrying four clinically relevant epitopes and quantification of 15 wheat allergenic protein isoforms.

quantifying a target protein has been demonstrated by determining the concentration of Bovine Hemoglobin B (P02081) spiked to the peptide mixture at a level of 10 ng/ μ g as a positive control. Only wheat protein isoforms that were quantified in a minimum of three independent extractions/MS^E analyses were accepted. In total, 15 wheat allergen/ceciac protein isoforms satisfied this criterion (Figure 1 and Supporting Information (SI) table). Quantified allergenic proteins were sorted into seed storage and proteolysis functional groups according to Bevan et al.⁵² (Figure 2) based on endogenous protein functionality. The most abundant proteins extracted to the Glia fraction were HMW glutenin and γ -gliadin (Table 1 and SI table). The most abundant proteins extracted to the Glu fraction were HMW and LMW glutenins (Table 1 and SI table).

HMW glutenins	21751	LMW glutenins	886965	Amylase/trypsin inhibitors	21711
	22090		886967		195957140
	736319		62550933		253783731
Gliadins	170708		75219081		283465827
	170734		21783		
	170738				

seed storage proteolysis

Figure 2. Functional classification of 15 quantified wheat clinically relevant proteins.

Despite the presence of HMW glutenin in the Glia fraction, which highlights problems in separating glutenins from gliadins, the extraction method was sufficient for this study because the focus was prefractionation of wheat grain proteins prior to MS^E analysis, which has the capacity to identify multiple proteins in this complex matrix. This is in accordance with the main aim of this study, that was an initial quantitative survey of clinically relevant proteins of wheat grain.

3.2. HMW Glutenins, LMW Glutenins, and Amylase/Trypsin Inhibitors Were Quantified in Wheat Grain Extract

Three HMW glutenins were quantified as shown in Table 1. The most abundant HMW glutenin in the Glia fraction was gi: 22090, with 56.6 ± 93.8 ng/ μ g (SI table) which was also highly abundant in the Glu fraction (76.5 ± 67.4 ng/ μ g). The HMW glutenin with NCBI accession gi: 21751, which shares 83% similarity to gi: 22090, was abundant in HMW glutenin in the Glu fraction (90.4 ± 24.9 ng/ μ g) that was found also in the Glia fraction (15.1 ± 8.3 ng/ μ g). The glutenin gi: 736319 was identified only in the Glia fraction at an abundance of 7.1 ± 0.8 ng/ μ g (SI table).

In total, five LMW glutenins were identified (Table 1). LMW glutenins were preferentially extracted to the Glu fraction in which gi: 886965 (63.3 ± 32.0 ng/ μ g) and gi: 886967 (80.6 ± 11.2 ng/ μ g) were the most abundant ones. These two LMW glutenins showed 90% overall similarity. LMW glutenin gi: 75219081 was observed only in the Glu fraction (6.7 ± 5.6 ng/ μ g). LMW glutenins, gi: 21783 (8.2 ± 6.3 ng/ μ g) and gi: 62550933 (1.6 ± 0.3 ng/ μ g) were observed only in the Glia fraction (SI table).

Four amylase/trypsin inhibitors were quantified in this study (Table 1). The gi: 21711 and gi: 253783731 were detected only in the Glu fraction in concentrations of 6.8 ± 5.6 ng/ μ g and 1.5 ± 1.7 ng/ μ g, respectively. The protein gi: 283465827 was detected only in the Glia fraction at a concentration of 3.4 ± 2.7 ng/ μ g. The amylase/trypsin inhibitor gi: 195957140 was detected at a concentration of 1.5 ± 1.3 ng/ μ g in the Glia fraction and 1.7 ± 1.8 ng/ μ g in the Glu fraction; these two concentrations were among the four lowest concentrations measured.

3.3. Peptides Carrying Four Known Epitopes of γ -Gliadin Were Identified

The α -, β -, γ -, and ω -gliadins were characterized as the major allergens for patients with wheat allergies; however, in concordance with another study,⁵³ we identified only γ -gliadin subunits with several peptides carrying four ("QPQQPFP",

Table 1. Table of Quantified Allergenic Proteins in Wheat Grain Extract

protein name	description	NCBI accession no.	MW (kDa)	pI	Gli ^a		Gli ^a		disease	ref
					avg conc (ng/ μ g of sample)	stDev	avg conc (ng/ μ g of sample)	stDev		
amylase/trypsin inhibitor	CM 17 protein	21711	15978	4.9			6.8	5.6	Baker's asthma WDEIA	65–67 36
	major allergen CM16	195957140	15772	4.7	1.5	1.3	1.7	1.8	food allergy Baker's asthma WDEIA	56, 57, 68 65–67 36
	α -amylase inhibitor CM1	253783731	13087	6.9			1.5	1.7	food allergy Baker's asthma WDEIA	56, 57, 68 65–67 36
	putative α -amylase inhibitor CM2	283465827	12996	5.8	3.4	2.7			food allergy Baker's asthma WDEIA	56, 57, 68 65–67 36
HMW glutenin	HMW glutenin subunit	21751	69586	7.6	15.1	8.3	90.4	24.9	food allergy	56, 57, 68
	HMW glutenin subunit	22090	75655	8.4	56.6	93.8	76.5	67.4	WDEIA	34–36
	glutenin	736319	89119	5.8	7.1	0.8			WDEIA	34
	γ -gliadin precursor	170708	32945	8.1	11.2	9	4.9	2.9	WDEIA	35, 36, 69, 70
gliadin	γ -gliadin	170734	27319	8.9	13.4	6.6	40.2	12.1	Baker's asthma celiac/food allergy WDEIA	71 58, 72–74 35, 36, 69, 70
	γ -gliadin	170738	37098	7.6	60.9	13.7	27.4	26.5	Baker's asthma celiac/food allergy WDEIA	71 58, 72–74 35, 36, 69, 70
	LMW glutenin	886965	30279	8.2	12.2	5.6	63.3	32	Baker's asthma celiac/food allergy WDEIA	71 58, 72–74 57, 74
	LMW glutenin	886967	32101	8.2	17.6	17.2	80.6	11.2	food allergy WDEIA	37 25, 26 57, 74
LMW glutenin	putative LMW glutenin subunit	62550933	37142	8	1.6	0.3			AD food allergy WDEIA	25, 26 57, 74 37
	LMW glutenin 1	75219081	32546	8.1			6.7	5.6	AD food allergy WDEIA	25, 26 57, 74 37
	triticum LMW glutenin	21783	40994	8.7	8.2	6.3			AD food allergy WDEIA	25, 26 57, 74 37
									AD	25, 26

^aProtein concentration from at least triplicate measurements in ng per μ g of protein extract.

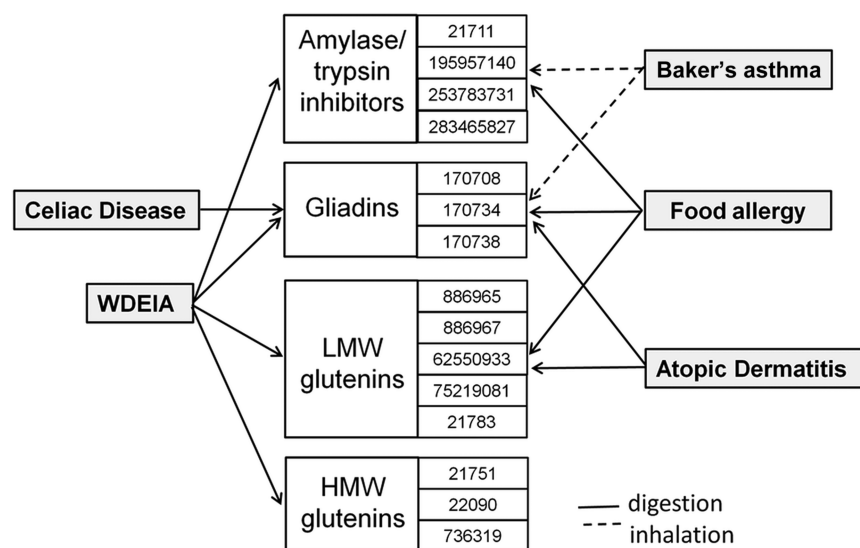


Figure 3. Associations of quantified wheat proteins with Baker's asthma, celiac disease, food allergy, atopic dermatitis, and WDEIA. Also shown is the relationship of the quantified proteins relative to the characterized diseases they cause.

"QTQQPQQFP", "LALQTLPMC", and "YIPPHCSTTI") previously characterized epitopes.⁵⁴ γ -Gliadin was found in three isoforms (gi: 170708, gi: 170738, and gi: 170734) presented in the "allergenonline.com" database (Table 1). The most abundant γ -gliadin in the Glia fraction was gi: 170738 (NCBI accession number) at 60.9 ± 13.7 ng/ μ g. This isoform was detected in the Glu fraction with a concentration of 27.4 ± 26.5 ng/ μ g (Table 1). The most abundant γ -gliadin isoform detected in the Glu fraction was gi: 170734 with 40.1 ± 12.1 ng/ μ g. This isoform was detected in a concentration of 13.4 ± 6.6 ng/ μ g in the Glia fraction. The less abundant isoform was the protein with gi: 170708, which was detected in the Glia (11.2 ± 9.0 ng/ μ g) and Glu (4.9 ± 2.9 ng/ μ g) fractions in relatively low concentration (Table 1 and SI table). Using a local similarity program (SIM), 46% similarity between gi: 170738 and gi: 170734 was observed.

3.4. MS^E Proteomics Quantified Allergens Associated with Five Diseases

Altogether, this study quantified 15 protein isoforms (Table 1 and SI table). Four of these belong to amylase/trypsin inhibitors, three to HMW glutenins, three to gliadins, and five to LMW glutenins (Table 1, Figure 2). These proteins are associated with five diseases, namely Baker's asthma, food allergy, WDEIA, celiac disease, and atopic dermatitis (Figure 3). Ingestion (food allergy and celiac disease), inhalation (respiratory allergy), and skin contact exposure (contact allergy) are three ways for exposure to occur and resultant sensitization to be caused by wheat grain proteins.⁵⁵ The mechanism of immunological reaction (intolerance, hypersensitivity reaction) depends on the type of allergen and how it is exposed to the immune system (Figure 3). For example, the inhalation of amylase/trypsin inhibitors might be associated with the clinically termed "Baker's asthma"^{27,28} and oral exposure would likely be associated with food allergy^{56,57} or WDEIA.³⁶ Similarly, gliadins might cause Baker's asthma when inhaled²⁸ or celiac disease,^{17–19} food allergy,⁵⁸ WDEIA,³⁶ or atopic dermatitis²⁴ when ingested orally (Figure 3). Other allergenic proteins cause an allergic reaction only upon ingestion by the oral route (Figure 3). HMW glutenins can cause WDEIA when ingested,³⁶ and LMW glutenins cause food-allergy-associated symptoms,⁵⁷ WDEIA,³⁷ or atopic dermatitis^{25,26} when ingested, typically as food.

4. DISCUSSION

The aim of this study was to perform an initial quantitative survey of clinically relevant proteins of wheat grain and their peptides carrying known epitopes in order to standardize the MS^E quantitative proteomic approach in wheat. For this purpose, we followed our recent work that quantified 34 gliadin and 22 glutenin in wheat grain extract.⁴⁴ Following database searching against 76 confirmed allergenic proteins from *Triticum* species, the quantification values between technical replicates were manually corrected by normalizing peptide intensities and validated against the spiked internal standard. In agreement and/or good correlation with previously published reports,^{40,44,59,60} we identified amylase/trypsin inhibitors, γ -gliadins HMW, LMW glutenins, and their sequential homologues as major allergens present in the water/salt-insoluble extracts of the gluten proteins.

Because a small database may generate misleading assignments (i.e., target proteins may not be accurate) and create estimates with an inaccurate false discovery rate,⁵³ our data were compared with previously published results,⁴⁴ in which the UniProt database containing 11764 *Triticeae* protein sequences was used to search MS^E data. The coefficients of variation (CV) of proteins quantified in this study ranged from 0.12 to 1.66 (SI table), which was almost identical with the one calculated from previous data (CV from 0.12 to 1.39)⁴⁴ and supports the use of the MS^E technique as a reproducible platform for multiplex protein analyses from complex matrices. Consensus has also been attained in the estimated amount of HMW glutenin (gi: 217751, UniProt: A9YSK3), CM17 (217711, Q41540), and CM16 (195957140, B9VRI3), which were found at similar levels (Figure 4) in both studies. Thus, these data not only confirm good reproducibility from a technical standpoint but also indicate that a small database can also be used to identify proteins for which there is interest in measuring the concentration of allergy-related proteins from matrices such as wheat grain.

The standard deviations of quantified proteins in this study ranged from 11.3% (736319) to 165.7% (22090) of the average amount (Table 1). A majority of quantified proteins displayed standard deviations between 20 and 100% of the average amount (Table 1). Three measurements showed standard deviations

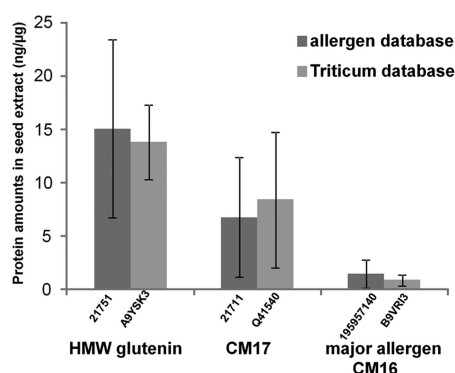


Figure 4. Quantifications of HMW glutenin (gi: 217751, UniProt: A9YSK3), CM17 (217711, Q41540), and CM16 (19S9S7140, B9VRI3) proteins using a database of 76 wheat allergen proteins (this study) and the UniProt database, which contained 11764 *Triticeae* protein sequences.⁴⁴ Error bars indicate standard deviations of biological triplicate analyses.

smaller than 20%, and only two proteins showed higher than 100% of the average amount (Table 1). These values are similar to those obtained from a recent study⁴⁴ and are still relatively high, especially when compared to ELISA, which showed a standard deviation less than 6% during the determination of walnut proteins.⁵⁴ However, techniques based on mass spectrometry are likely to provide more robust characterization of clinically relevant proteins.¹⁷ For instance, MS methods were found superior to immunochemical methods in the detection of gluten proteins in wines.⁶¹

Our results are in agreement with other proteomic data found in the literature for which concentration values are generated. For example, the determined concentration of the homologues (CM1, CM2, CM16, and CM17) amylase inhibitor ranged between 1.5 and 6.8 ng/μg in this study (Table 1), and similar levels ≤2.2 ng/μg were found in the published result of Sotkovsky et al.⁴⁰ In summary, the LC/MS^E based approach is a powerful tool for obtaining targeted, multiplexed, and proteome-scale quantification that can be applied to profile allergens in wheat cultivars.

In recent years, techniques of mass spectrometry are used for analysis of gliadin epitopes related to celiac disease.¹⁷ Within this effort, for instance, using peptide digests of gliadin and synthetic peptides, it was shown that gliadin peptide “VSFQQPQQYPSSQ” can induce specific immune responses.⁶² Similarly, two other peptides related to celiac disease were characterized by MS.⁶³ More recently, using IgE from sera of several groups of patients and synthetic peptides, epitopes relevant to atopic eczema/dermatitis syndrome (AEDS), anaphylactic shock (AS), and WDEIA were identified.⁵⁸ In our study, we detected four known epitopes: two related to AEDS (LALQTLPMC and YIPPHCSTTI), one related to AS (QPQQPFP), and one related to WDEIA (QTQQPQQPFP).⁵⁸ These epitopes were identified in the γ-gliadin protein gi:170708 that was observed to be more abundant in the GliA fraction (Table 1). These data support the conclusion that not only does the multiple reaction monitoring procedure that was successfully applied in quantitation of eight soybean allergens⁴³ work well, but also MS^E based proteomics is capable of detecting multiple peptides carrying clinically relevant epitopes and quantifying the parent proteins in a contiguous analysis.

5. CONCLUSIONS

This study used quantitative MS^E in combination with a small database of 76 confirmed allergy-related proteins. This approach detected peptides carrying four previously characterized gliadin epitopes and provided the basis for a quantitative measure of 15 allergenic wheat proteins. The impact of this work is highlighted by the foundation it provides for important future clinical research that could begin to associate a quantitative measure of protein exposure in the patient with the presence and severity of wheat-associated intolerance or allergy disease. Future studies will further examine the MS^E approach and compare the amount of allergenic proteins between wheat varieties, similar to recent targeted proteomic soybean studies using synthetic, isotope-labeled peptides.⁶⁴ The goal is to assess allergen expression variability as it may fluctuate with genetic and environmental factors.

■ ASSOCIATED CONTENT

Supporting Information

Table of quantified allergens from the gliadin (GliA) fraction. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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