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Characterization of wheat gluten subunits by liquid chromatography – Mass spectrometry and their relationship to technological quality of wheat

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ABSTRACT

The quality of common wheat is largely influenced by the composition of its storage proteins. The currently presented research explores factors influencing observed differences in quality and quantity between wheat cultivars, in particular in relation to gluten composition and its relationship to technological characteristics. Eight wheat cultivars (H. Wieser, Seilmeier, W., Belitz, H.D., 1994 Parsi, Sirvan, Sivand, Pishgam, Pishtaz)were selected for evaluation. Analysis results demonstrated that Morvarid and Sirvan cultivars yielded the highest quality of wheat, while the Chamran cultivar was indicated as the most favorable for baking Taftoon bread. Conversely, the Sepahan cultivar was deemed to have the worse quality in both categories. A Q Exactive LC-MS/MS system was employed to evaluate the most effective sub-fractions of gliadin and glutenin on wheat quality. Matching peptides resulting from trypsin digestion on gliadin and glutenin fractions, led to the identification of subunits α/β-gliadin, γ-gliadin, HMW-Dx5, HMW-Bx17, HMW-Dy3, HMW-Dy10, HMW-By15, LMW-m, LMW-s, and LMW-i. The obtained results indicated that the most influential subunits of glutenin on wheat quality were Dy10, Dy3 and Dx5, while the most effective gliadin subfraction was noted to be α/β-gliadin However, the most important subunit influencing the quality of flat breads in particular was identified as the x-HMW-GS, in particular the Bx17 subunit, and LMW-GS.

1. Introduction

The unique properties of hexaploid common wheat are primarily related to its gluten-forming storage proteins (Butow et al., 2003). Since the gluten network is mainly responsible for dough extensibility and elasticity, understanding the role of storage protein fractions on bread texture is crucial. Studies into the relationship between wheat flour quality and bread characteristics have mostly focused on applications related to pan breads, with loaf volume, in such cases, considered as the most important factor linked to bread quality. However, few investigations to date have explored this relationship as it applies to flat breads (Quail et al., 1990). In view of the unique attributes of flat bread that

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differentiate this commodity from pan bread, particularly in relation to taste and quality, it is important to consider whether the impact of gluten proteins on the end product characteristics of flat breads differs from how said proteins impact pan bread quality. For example, previous research has shown that highly elastic dough derived from high quality gluten are not compatible with the rapid expansion of gases at the high temperature and short time conditions mainly employed in the baking of flat breads (Faridi, 1982).

Indeed, various studies have shown that the global content of proteins in flour does not play the key determinant role in flour performance; rather, flour quality is determined by certain protein sub-fractions deduced from gluten (Anjum et al., 2007). These wheat gluten proteins can be classified into two main sub-groups: gliadins and glutenins. Gliadins can be further classified into different fractions, such as α/β-, γ- and ω-gliadins, in order of decreasing electrophoretic mobility. Moreover, it is quite established that the strength and elastic properties of dough are primarily imparted by glutenin proteins, whereas gliadin fractions have been indicated to play a role in determining dough extensibility. Gliadins account for 40–50% of wheat seed storage proteins, largely influencing both the technological and nutritional quality of dough and bread (Wieser and Kieffer, 2001).

Polymeric glutenin proteins, with molecular masses ranging from approximately 300 kDa to one million kDa, can be further classified into two subunit groups: low molecular weight glutenin subunits (LMW-GS) and high molecular weight glutenin subunits (HMW-GS) (Wieser, 2007). Low molecular weight glutenin subunits (LMW-GS) are similar in size and structure to γ-gliadin (30–40 kDa). LMW-GS subunits compose approximately 20% of total gluten proteins in wheat, with LMW-GS subunits being biochemically classified into B, C, and D types on the basis of SDS-PAGE mobility (Muccilli et al., 2010). B type subunits include mostly typical LMW-GS sequences, named according to their first amino acid residue (i.e. m = Methionine, s = Serine and i = Isoleucine), such as LMW-m, LMW-s, and LMW-i types, On the basis of LMW-GS's ability to form different numbers of intermolecular disulphide bonds, LMW-GS can be classified as chain extenders or chain terminators. Chain extenders are characterized by m-, s- and i-type subunits linked by interchain disulphide bonds, which may subsequently extend to produce glutenin polymers. Chain terminators are characterized by gliadin-like LMW-GS (i.e. α-, γ-, ω-gliadin), and block subunits from becoming extended polymer chains due to the lack of additional free cysteine molecules necessary for interchain linkages.

High molecular weight glutenin subunits (HMW-GS) range in molecular mass from ~65 to 90 kDa. Each wheat variety contains three to five HMW-GS that be further grouped into two different types: x- and y-type. x-type HMW-GS subunits are characterized by molecular weights that range from 83000 to 88000, while the molecular weights of y-type HMW-GS subunits range from 67000 to 74000 Da. All hexaploid wheat contains 1Bx, 1Dx, and 1Dy subunit, some cultivars also containing a 1By and 1Ax subunit as well. The composition of HMW-GS alone may account for up to 60% of variation observed in the quality of bread flour (Wieser and Kieffer, 2001).

There are ambiguous aspects of relationship between wheat protein quality and quantity, as was noted by Katyal et al. (2016), protein content of flour showed a strong positive relation with gluten index and sedimentation value, whereas, Kaur et al. (2013) represented that gluten index has not shown relationship with protein, and was even negatively correlated with gluten content, similarly, Bonfil and Posner (2012) reported there was no real correlation between wheat gluten index and protein content or SDS-sedimentation value.

In the currently presented research, the liquid chromatography-mass spectrometry (LC-MS) technique is applied towards the identification of the most effective subunits of gluten on wheat quality and quantity, so, help shed further light into the outstanding debate stemming from contradicting past research on the relationship between the characteristics of wheat and its impact on bread and wheat quality.

2. Materials and methods

2.1. Materials

All solvents/chemicals used were of analytical grade and obtained from Merck® (Germany).

Cultivars of wheat were collected from Iran, Morvarid, Chamran, Sepahan, Sirvan, Sivand, Parsi, Pishtaz and Pishgam.

2.2. Analysis

2.2.1. Chemical and physicochemical analysis

Ash, protein, wet gluten and gluten index percentage, falling number, zeleny sedimentation value, water absorption, dough development time, stability, degree of softening, and farinograph quality number were all determined according to methods established by the American Association of Cereal Chemists (AACC, 2000). The sensory characteristics of the studied breads were determined in collaboration with 16 trained panelists through the use of a hedonic scale. The textural profiles of the breads under study were also evaluated using a texture analyser by puncture test (Rochdale 350, England) in accordance to AACC methods (AACC, 2000).

2.2.2. Milling and baking

Each wheat cultivar was milled with Senior Quadromat milling (Brabender) to obtain suitable flour for Taftoon bread (i.e. soft white flour with 82–87% extraction rate). The resulting flour was then baked at 315 °C for 2–3 min.

2.2.3. Protein extraction and sample analysis

Gliadin and glutenins were extracted from whole meal according to the sequential procedure described by Singh et al. (1991). Wheat cultivars were subjected to three consecutive extraction steps so as to separate the two principal protein sub-fractions, namely gliadin and glutenin (Zhang et al., 2007). The procedure involved the extraction of 1 g of sample with 6 ml of solvent for 30 min at 60 °C with vortexing applied every 10 min, followed by centrifugation (25.000 × g, 10 min). Ground wheat was sequentially extracted with the following solvents: 50% 1-propanol (for gliadin fraction), 50% 1-propanol containing 4% Dithiothreitol, (DTT) (for glutenin fraction), 50% 1-propanol containing 4%-DTT, and 1% acetic acid (for residual HMW and LMW glutenin). All fractionation steps were carried out in duplicate.

Extracted gliadin and glutenin from wheat cultivars were dissolved in digestion buffer (1% sodium dodecyl sulphate in 50 mM ammonium bicarbonate (pH = 8.5). Protein samples were reduced using dithiothreitol (DTT) at 56 °C. Next, the free sulfhydryl groups in the sample were alkylated with iodoacetamide in the dark and room temperature for 30 min. The extracted proteins were then submitted to overnight digestion with trypsin at 37 °C. Digestion was terminated by adding formic acid 1% (v/v). C18 Zip Tip columns (Millipore) were used to desalt peptides prior to LC-MS/MS analysis.

LC-MS/MS analysis was performed by loading peptide mixtures onto a 50 cm × 75 μm ID C18 column (Millipore) placed in-line with an Easy nanoLC-electrospray 1000 coupled to a Q-Exactive hybrid quadrupole-orbitrap mass spectrometer (Thermo Fisher, San Jose,

CA). Solvent A was composed of HPLC gradient water with 0.1% formic acid, and solvent B was acetonitrile with 0.1% formic acid. Peptides were separated on the system by applying a 1-h gradient elution starting from 0% solvent B to 100% solvent B.

Positive precursor ions (100–2600 m/z) were subjected to data-dependent collision induced dissociation as the instrument cycled through one full scan at 60,000 full-width at half maximum, followed by successive MS/MS scans targeting the most intense precursors, with 225 s dynamic exclusion enabled. Ions with unassigned charge states (decided by high resolution precursor ion measurements) were rejected. The top 10 scans were chosen for MS/MS analysis. A fixed first mass of 100 Da and a dynamic exclusion time of 20 s were used for the MS/MS scans. Raw data files were acquired with XCalibur 2.2 software and processed with PEAKS version 7.5 (Bioinformatics Solutions Inc., Waterloo, Canada).

2.2.4. Proteome data analysis

Raw MS files were analyzed by PEAKS (version 7.5). MS/MS spectra were searched against a protein database (NCBI and swissprot) containing forward and reversed (decoy) sequences, allowing for variable modifications of methionine, glutamine and cycteine by oxidation, diamidation and carbamidomethylation, respectively. Parent mass and fragment ions were matched using a maximal initial mass deviation of 10 ppm and 0.5 h, and a retention time shift tolerance of 5 min. The protein false discovery rate was set to 1%.

2.2.5. Statistical analysis

The statistical significances of the differences among wheat cultivars were determined by one-way analysis of variance (ANOVA) with SPSS software. Pearson's correlation coefficients for quality and quantity parameters were obtained using SPSS software, version 24. Differences were judged to be significant at $p < 0.05$.

3. Results and discussion

3.1. Flour and bread analysis

The results of the chemical and physicochemical analyses shows Morvarid and Sirvan cultivars contained the highest wheat quality, while the Sepahan cultivar was deemed to have the lowest quality.

The quality of the assessed Taftoon breads was determined by employing 16 trained panelists, and through the use of puncture tests conducted with a Texture Analyser (Rochdale M350). The parameters used for the puncture tests on the Texture Analyser were: load cell: 500 N, probe speed: 1 mm/s, probe diameter: 4.5 mm. Evaluation of the flat breads by the trained panelists and through the puncture tests revealed the Chamran cultivar had the best texture, taste, odor, and shape. Conversely, Sepahan cultivar was identified to be the least favorable. Morvarid, consisted of a strong and very elastic texture, was deemed inappropriate for baking flat bread.

3.2. Selection of wheat varieties

Chemical and farinograph analyses of the wheat flours indicated Morvarid and Sirvan cultivars yielded the highest quality, while Sepahan cultivars were deemed the most unfavorable. In regards to bread-making quality, Chamran cultivar was shown to have the best flat bread quality while Sepahan, again, was appointed as the cultivar with the lowest quality. So, Morvarid, Sirvan, Chamran and Sepahan were selected to evaluate their gluten structure.

3.3. Protein identification

Taking into account the high complexity of the wheat grain protein matrix, and considering that two types of extractions are required to support detection of gliadin and glutenin proteins, MS proteomics can provide reliable quantification of proteins obtained from wheat grain. There are several difficulties associated with proteomic analysis of wheat storage proteins, including the limited available database, the limited number of basic residues, the complexity resulting from the presence of sets of homologous proteins, and the presence of repeating motifs. In spite of this, wheat proteins have been widely studied with the use of HPLC or mass spectrometry techniques, which are now widely applied in combination with each other in proteomic studies of wheat. In our study, each fraction was subjected to tryptic digestion and then analyzed by Q Exactive LC-MS/MS. The resulting peptide maps were then searched against a protein database (NCBI and SwissProt) for matching proteins. As each fraction contains multiple proteins, a mass error tolerance of 10.0 ppm, retention time shift tolerance of 5 min, and false discovery rate threshold of 1% was necessary for protein peak identification in order to reduce false positives.

Table 2 demonstrates substituted or deleted amino acid residues of peptide chain under the specified accession number in Uniprot. Basically both gliadins and glutenins are unique in terms of their amino acid compositions, i.e. high content of glutamine and proline and low content of amino acids with charged side groups such as lysine, histidine and arginine (Wieser, 2007), a matter that is evidenced in amino acid profile of the all gluten fractions. It is well-known that glutamine and somehow tyrosine may have a key role in the formation of hydrogen bonds and therefore gluten aggregation. On the other hand, lack of amino acids with charged side groups provide a lateral repulsion between them, help gluten to be more aggregated (Wrigley and Bietz, 1988). In general, difference between α/β-gliadin and γ-gliadin is related to their tyrosine content where the first fraction contains more tyrosine.

LMW-i and LMW-s have relatively similar peptide sequences; only serine, in LMW-s is replaced by isoleucine in LMW-i, makes it slightly more hydrophobic. All y-type HMW-GS (Dy3, Dy10 and By15) have a domain of AQQPATQLPTVCR, except in By15, alanine, threonine and valine are replaced with hydrophobic valine, isoleucine and methionine (M) amino acids, so, there is a slight difference between D and B genomes in peptide sequence. Common peptide in x-HMW-GS (Dx5 and Bx17) is YYPSVTCPQQVSYYPGQASPQRPGQGQQPGQGQQ-GYYPTSPQQPGQ, whereas, cycteine, proline and arginine in Dx5 are replaced with serine, serine and glutamine in Bx17, therefore, Dx5 has additional cycteine. The MW distribution of glutenins has been recognized as a major factor of dough quality which is governed by disulphide structure that depends on genetic factors i, e. presence of Dx5 (Wieser and Zimmermann, 2000).

So, the subunits α/β-gliadin, γ-gliadin, HMW-Dx5, HMW-Bx17, HMW-Dy10, HMW-Dy3, HMW-By15, LMW-s, LMW-i, and LMW-m were identified after matching the isolated peptide sequences with SwissProt or NCBI databases.

The quantities found for the identified gliadin and glutenin subfractions in four selective cultivars (Morvarid, Chamran, Sirvan and Sepahan) are summarized in Table 3.

The obtained results, summarized in Table 3, show that Morvarid yielded the greatest glutenin:gliadin ratio, while Sepahan cultivars had the lowest ratio. According to previous reports, glutenin plays a more important role than gliadin in determining dough properties. Indeed, Zhang et al. (2007) showed that wheat cultivars with higher dough stability had higher glutenin content, while research by Khatkar et al. (2002) indicated that increasing the levels of total gliadin and gliadin subgroups in flour decreased

Table 1
Chemical, physicochemical, sensory, and texture analysis of wheat and bread.

Characteristics	Chamran	Sivand	Sirvan	Parsi	Pishgam	Pishtaz	Sepahan	Morvarid
Water absorption (%)	71.65^{b} ± 0.15	67.95^{g} ± 0.05	71.3^{c}±0	68.3^{f}±0.2	69.85^{e}±0.05	70.05^{d} ± 0.05	72.65^{a}±0.05	56.55^{h} ± 0.05
development time (min)	2.75^{b} ± 0.05	2.25^{ef}±0.05	3.75^{a}±0.07	2.10^{f}±0.10	2.5^{c}±0.02	2.35^{de} ± 0.05	2.4^{cd} ± 0	1.75^{g} ± 0.05
Stability (min)	1.25^{e}±0.05	1.35^{de} ± 0.05	2.2^{b} ± 0.07	1.00^{f}±0.20	1.45^{cd} ± 0.05	1.55^{c}±0.05	1^{f}±0.05	5.45^{a}±0.05
Degree of softening (Butow et al., 2003)	171.53^{d} ± 4.5	146.00^{e}±0	113.51^{f}±0.7	197.57^{b} ± 2.5	184.5^{c}±2.50	142.5^{e}±6.50	248^{a}±20	56.5^{g} ± 1.50
Farinograph quality number	37.00^{c}±0	31.00^{e}±0	48.00^{b} ± 1.41	29^{f}±0.56	32.50^{e}±0.53	34.50^{d} ± 1.50	28.50^{f}±00	66.00^{a}±1.0
Protein (%)	12.66^{c}±0.16	13.63^{a}±0.06	12.65^{c}±0.04	10.88^{f}±0.22	11.87^{d} ± 0.5	11.88^{d} ± 0.03	11.59^{e}±0.42	13.21^{b} ± 0.01
Wet gluten (%)	29.05^{bc}±0.25	28.65^{cd} ± 0.15	31.35^{a}±0.50	26.95^{f}±0.25	28.05^{de} ± 0.25	29.25^{b} ± 0.25	27.66^{e}±0.3	25.0^{g} ± 0.50
Gluten index (%)	2.925^{de} ± 0.20	6.73^{c}±0.73	48.67^{b} ± 3.70	3.18^{de} ± 0.12	4.63^{ed} ± 0.32	6.52^{c}±0.08	1.82^{f}±0.03	80.58^{a}±2.28
Sedimentation value (Quail et al., 1990)	19.0^{c}±0	20.0^{bc}±00	24.55^{a}±0.70	20.0^{b} ± 0	20.5^{b} ± 0.5	12.5^{d} ± 0.5	12.0^{d} ± 0	21^{b} ± 0.5
Forced required to puncture (N)	3.18^{g} ± 0.01	4.58^{f} ±0.01	4.91^{b} ± 0.007	4.92^{b} ± 0.01	4.64^{d} ± 0.007	4.87^{c} ±0.01	4.98^{a} ±0.01	4.62^{c} ±0.01
Panelist score (on the basis of 20)	15.49^{a} ±0.02	14.11^{bc}±0.007	13.26^{a}±0.007	10.27^{d} ± 0.02	14.20^{bc}±0.14	13.80^{c}±0.03	7.65^{e}±0.007	15.10^{ab} ± 0.07

Values followed by a different letter in the same row are significantly different ($P < 0.05$).

Table 2
Identification of trypsin-digested gliadin and glutenin peptides, obtained with nanoLC-MS/MS analysis.

Identified peptide sequences	RT (min)	Mass (Da)	Accession number	Protein
DVVLQQPNIAHASSK	25.98	536.2906	383310741	α/β-gliadin
VSQQSYQLLQQLCCQQLWQTPEQSR	47.71	1046.1702		
SDCQNMQQQCCQQLAQIPR	31.88	793.3547	260401170	γ-gliadin
SQMLQQSICHVMQQQCCQQLPQIPQQSRYEAIR	36.87	1030.4786	537846572	LMW-i
VFLQQQCIPVAMQR	38.14	859.4478		
ILPTMCSVNNPLYR	43.10	554.9622		
TTTSVPFGVGTGVGAY	46.33	757.3801		
SQTLWQSSCHVMQQQCCR	27.42	775.3315	164470672	LMW-m
QLPQIPEQSRYDAIR	31.05	605.3241		
VFLQQQCSPVAMPQSLAR	34.75	692.6843		
SQMLQQSSCHVMQQQCCQQLPQIPQQSRYEAIR	32.51	1019.7261	164470668	LMW-s
VFLQQQCSPVAMPQSLAR	34.75	692.6843		
TTTSVPFDVGTGVGAY	47.32	786.3836		
ACQQVMDQQLR	21.62	688.8245	662357281	HMW-Dy3
ELKACQQVMDQQLR	25.25	582.9590		
AQQPATQLPTVCR	26.18	735.3805		
GGSFYPGETTPPQQLQQR	30.87	995.9858		
DISPECHPVVVSPVAGQYEQQIVVPPKGGSFYPGETTPPQQLQQR	42.53	1236.8748		
IFWGIPALLKP	46.78	438.6082		
CCQQLR	9.48	432.69	164457873	HMW-Dy10
SVAVSQVAR	18.12	458.7643		
ELQESSLEACR	22.31	661.3078		
QLQCERELQESSLEACR	25.34	712.6665		
AQQPATQLPTVCR	26.18	735.3805		
QQPVQGQQPEQGQQPGQWQQGYYPTSPQQLGQGQQPR	34.22	1048.2515		
LPWSTGLQMR	38.32	594.8136		
QVVDQQLAGRLPWSTGLQMR	41.81	761.7395		
ELQELQER	20.55	522.7695	288860106	HMW-Dx5
ACQQVMDQQLR	21.62	688.8245		
GGSFYPGETTPPQQLQQR	30.87	995.9858		
AQQLAAQLPAMCR	32.51	486.5837		
YYPSVTCPQQVSYYPGQASPQRPGQGQQPGQGQQGYYPTSPQQPGQWQQPEQGQPR	30.60	1261.7882		
DISPECHPVVVSPVAGQYEQQIVVPPK	41.12	991.5128		
IFWGIPALLKR	46.78	438.6082		
QYEQQPVVPSK	20.59	651.8374	109452233	HMW-Bx17
QQSGQGQQPGQGQQSGQGQQPGQGQQAYYPTSSQQSR	21.02	1406.6378		
DVSPGCRPITVSPGTR	24.12	849.9332		
RYYPSVTSSQQGSYYPGQASPQQSGQGQQPGQEQQPGQGQQDQQPGQR	27.66	1048.2810		
QQGYYPTSPQQPGQGQQLGQGQPGYYPTSQQPGQK	31.79	952.4514		
AQQLAAQLPAMCRLEGSDALSTRLEGSDALSTR	38.02	829.7531		
CCQQLR	9.49	432.6946	160425383	HMW-By15
QVVDQQLAGR	20.03	557.3046		
ELQESSLEQCR	22.06	661.3076		
QLQCERELQESSLEACR	25.18	712.6662		
VQQPATQLPIMCR	33.46	771.4003		
LPWSTGLQMR	38.03	594.8143		
ELQESSLEACRQVVDQQLAGRLPWSTGLQMR	44.19	897.2035		

overall dough strength. In this research, in addition to having the highest glutenin:gliadin ratio, Morvarid cultivar also demonstrated the lowest percentage of wet gluten. Based on the presented data, the overall content of gliadin would appear to be influenced by the percent of wet gluten present in the cultivar. Therefore, the quantity and quality of gluten can be determined to be affected by gliadin and glutenin fractions, respectively.

In all of cultivars, the percentage of LMW-GS was found to be higher than that of HMW-GS, with the exception of the Sirvan variety. As can be seen in Table 1, Sirvan and Morvarid cultivars, which

Table 3
Gluten subunits and their respective quantities in four wheat cultivars.

Gluten subunits			Morvarid	Sirvan	Chamran	Sepahan
glutenin:gliadin			1.21	1.07	0.79	0.67
HMW:LMW-GS			0.84	1.06	0.72	0.25
x:y-HMW-GS			0.92	0.80	1.30	0.44
glutenin	y-HMW	Dy10 (%)	6.44	5.30	4.53	0
		Dy3 (%)	2.03	2.11	1.75	0.08
		By15 (%)	0	3.34	0	0.17
	x-HMW	Bx17 (%)	4.57	3.87	3.94	0.11
		Dx5 (%)	3.22	3.34	2.77	0
	LMW	s (%)	6.67	5.47	7.33	3.70
		m (%)	7.08	5.80	7.12	0.11
		i (%)	3.44	2.65	4.68	0.34
gliadin	α/β (%)		16.78	13.50	8.92	3.00
	γ (%)		16.33	10.60	20.07	21.60

HMW-GS: high molecular weight glutenin.
LMW-GS: low molecular weight glutenin.

were noted to have the highest chemical and farinography qualities, had the highest HMW:LMW-GS ratios, 1.06 and 0.84, respectively. Considering that the Sirvan cultivar had the greatest zeleny sedimentation value and the highest HMW:LMW ratio, it can be surmised that HMW-GS have a significant impact on zeleny sedimentation values. Our results are in agreement with previous reports in the literature, where HMW:LMW ratios have been correlated with farinograph development time, stability, and mixing time (Cunsolo et al., 2003). As such, the findings of this study support that HMW-GS content is an important parameter for flour quality evaluation. Conversely, the Chamran cultivar was determined as the cultivar yielding the highest baking (sensory and texture) quality, despite having a lower HMW:LMW ratio than Sirvan and Morvarid. Since LMW-GS and gliadin composition affect dough extensibility (Maucher et al., 2009), but HMW-GS correlated strongly with dough strength (Sliwinski et al., 2004). On the other hand, in flat breads, highly elastic doughs, derived from high quality gluten, are not suitable for the conditions typically employed in the baking of flat breads (Marchetti et al., 2012). The obtained results are in agreement with He et al.'s (2003) report which asserts that Chinese Steamed Bread flour quality requirements are dependent on the processing conditions being employed; while doughs with medium protein content and medium to strong gluten strength with good extensibility are desirable for mechanized methods, doughs with weak to medium gluten strength are preferred for manual methods.

While a comparison of x:y-HMW-GS ratios in different cultivars indicated that wheat quality was positively correlated with the contents of x-HMW-GS and y-HMW-GS, in flat bread, the presence of x-HMW-GS was deemed as the most important determinant of flat bread quality.

Identification of subfractions of gliadin in the selected wheat cultivars revealed that all cultivars contained α/β-gliadin and γ-gliadin, with molecular weights ≈34000 and 33000 Da, respectively. Y-gliadin was found to be present in high quantities in Chamran and Sepahan cultivars, causing a decrease on wheat quality. Conversely, high quality wheat cultivars (Morvarid and Sirvan) were noted to contain high quantities of α/β-gliadin. These findings are consistent with those of Altenbach et al. (2010), who reported when γ-gliadins were silenced, resulting in the reduction of this fraction, which had no direct effect on the mixing and bread-making properties of the dough. However, the synthesis of other prolamins was noted to result in a stronger dough, with improved over-mixing resistance.

With regard to subfractions of glutenin, identified, y-HMW-GS included Dy3, Dy10, and By15, with average masses of 85285, 6970, and 77334 Da, respectively, with Dy10 (0–6.44%) identified as the most abundant HMW-GS subunit in all cultivars. It is according to finding of Leon et al. (2009), which, the expression of subunit Dy10 was associated with a greater effect on the proportion of high molecular mass glutenin polymers than subunit Dx5.

In this research, the percentage of Dy10 was relatively high for high quality cultivars; in contrast, the Sepahan cultivar, noted for its low quality, was shown to not contain this subunit. Subunits of Dy10 have at least one free cysteine on the N-terminal domain, which can participate in intermolecular disulfide bond formation. Moravarid and Sirvan cultivars had similar Dy3 content (≈2%), and a higher percentage than that of other cultivars.

Moreover, in Morvarid, Sirvan, Chamran and Sepahan cultivars, identified x-type HMW-GS included Dx5 and Bx17, with average masses of 90293 and 80069 Da, respectively. The most abundant x-type subunit was Bx17 (0.11–4.57%). The Sepahan cultivar had the lowest content of Bx17, while other cultivars contained relatively high amounts of this subunit. In Morvarid and Sirvan cultivars, noted for their high quality, Dx5 was present in relatively high quantities (≈3.20%); conversely, no Dx5 subunits were identified in Sepahan.

In view of these findings, it can be concluded that Dy3, Dy10, and Dx5 are the most effective subunits of HMW-GS in terms of wheat quality. The subunit Dx5 has an additional cysteine for an interchain crosslink when compared to all other x-type subunits (Cazalis et al., 2003). On the other hand, the molecular weights of Dx5 and Dy10 are greater than that of other HMW-GS subunits, as previously reported by Naeem et al. (2012). Wheat lines with HMW-GS, which are associated with dough strength (e.g. 5 + 10), begin to polymerize earlier and reach higher molecular weights than lines with HMW-GS, which are associated with dough weakness (e.g. 2 + 12) (Naeem et al., 2012). In addition, the Dx5/Dy10 pair, associated with elastic and strong doughs, may be mitigated by a concomitant increase in Dy10 (Popineau et al., 2001), owing to the over-strengthening effect of Dx5. Subunits 5 + 10 at GluD1 are desirable subunits for pan bread quality, while Dx5, Dy10 and Ax1 modify the proportions of high molecular mass glutenin polymers (Blechl et al., 2007). Also obtained results are consistent with findings reported by Leon et al. (2009) where the contribution ranks of HMW-GS encoded by the Glu-B1 locus to bread-making quality were reported as Bx17 + By18 > Bx14 + By15 > Bx7 +By8 > Bx7+By9.

The identified LMW-GS subunits, together with their respective percentages in different cultivars, are listed in Table 3. LMW-s, LMW-m, and LMW-I, with average masses of 27794, 39773, and 40051 Da, respectively, were identified in 4 selective wheat cultivars (Morvarid, Chamran, Sirvan, and Sepahan). As can be seen, the content of the LMW-i subunit was lower than s- and m-type LMW

Table 4
Correlation coefficients for relationships between fractions of gluten and wheat and bread quality.

	W.A.P	stability	F.Q.N	gluten	Protein	index	zeleny	sensory	texture
X:Y-HMW	-0.151	0.116	0.270	0.057	0.669*	0.043	0.475	0.820*	-0.894*
glutenin:gliadin	-0.760*	0.870*	0.963*	-0.270	0.848*	0.971*	0.664*	0.681*	0.241
HMW:LMW-GS	-0.309	0.455	0.686*	0.266	0.806*	0.655*	0.969*	0.903*	-0.092
Dx5	-0.444	0.541	0.748*	0.083	0.923*	0.651*	0.885*	0.981*	-0.304
Bx17	-0.537	0.598*	0.774*	-0.065	0.964*	0.642*	0.783*	0.980*	-0.406
Bx23	0.515	-0.414	-0.144	0.834*	0.214	-0.207	0.754*	0.612*	-0.476
Dy10	-0.620*	0.697*	0.858*	-0.124	0.977*	0.756*	0.785*	0.945*	-0.257
Dy3	-0.443	0.542	0.749*	0.085	0.922*	0.653*	0.887*	0.980*	-0.299
By15	0.308	-0.108	0.099	0.727*	0.073	0.240	0.749*	0.288	0.410
LMW-m	-0.471	0.509	0.685*	-0.047	0.935*	0.523	0.734*	0.982*	-0.548
LMW-s	-0.415	0.389	0.520	-0.144	0.835*	0.304	0.507	0.882*	-0.765*
LMW-i	-0.294	0.282	0.443	-0.006	0.795*	0.230	0.570	0.902*	-0.796*
α/β-gliadin	-0.745*	0.839*	0.957*	-0.247	0.938*	0.914*	0.718*	0.814*	0.024
γ-gliadin	0.170	-0.364	-0.565	-0.382	-0.513	-0.655*	-0.887*	-0.591*	-0.356

* correlation is significant at the 0.05 level (2-tailed).
W.A.P: water adsorption percent.
F.Q.N: farinograph quality number.
Index: gluten index.
Sensory: sensory evaluation.
Texture: texture analysis.

subunits for all four cultivars. This is in line with previously reported researches, in which LMW-i was identified as a relatively minor component of wheat (Gao et al., 2016). The Chamran cultivar yielded the highest content of all types of LMW-GS, while the Sepahan cultivar presented the lowest percentages. As such, it can be reasonably concluded that the presence of LMW-GS has a larger impact on flat bread quality than on wheat quality.

3.4. Statistical analysis

The correlation coefficients between different fractions of gluten with wheat and flat bread properties are given in Table 4.

Using a 95% confidence level, statistical analysis of the obtained data indicated that percent of protein, dough stability, farinograph quality number, gluten index, zeleny sedimentation of wheat, and sensory evaluation of Taftoon bread were all positively associated with glutenin:gliadin, while water absorption percent had a negative correlation with glutenin:gliadin. Percent of protein, zeleny sedimentation, and sensory evaluation were positively associated with HMW:LMW-GS. x:y-HMW-GS were also positively correlated with percent of protein and sensory evaluation, while being negatively correlated with firmness of bread texture. According to Veraverbeke and Delcour (2002), the strength of dough is related to the amount and type of HMW-GS subunits, with variations in both quantity and quality of glutenin being strong determinants of variations in bread-making performance.

In particular, water absorption percentage was shown to be negatively correlated to glutenin:gliadin, Dy10 and α/β-gliadin content, and positively correlated with γ-gliadin. Stability, in turn, showed a positive correlation with Dy10, Bx17, and α/β-gliadin. The farinograph quality number was positively correlated to Dx5, Bx17, Dy10, Dy3, LMW-m, and α/β-gliadin, while wet gluten percent yielded a positive correlation with By15. Further, the overall content of protein was positively correlated with that of Dx5, Bx17, Dy10, Dy3, LMW-m, LMW-s, LMW-I, and α/β-gliadin. In addition, zeleny sedimentation values were positively correlated with Dx5, Bx17, Dy10, Dy3, By15, LMW-m, and α/β-gliadin, while the gluten index demonstrated a positive correlation with Dx5, Bx17, Dy10, Dx3, and α/β-gliadin, but a negative correlation with γ-gliadin.

4. Conclusion

In this research, liquid chromatography coupled to high resolution Q Exactive MS/MS analysis was demonstrated as a suitable method to assist in the identification gliadin and glutenin structures present in wheat cultivars. By extension, such a technique can be used in the identification of differences between technological quality of wheat, as well as in the quantification of particular wheat compositions. A comparison of the obtained molecular weights of gliadins and LMW-GS demonstrated that these proteins have approximately equal molecular weights (≈30000Da), whereas HMW-GS are characterized by larger molecular weights (75000–97000). As the weight of molecules increase, the number of active sites in the molecule that are capable of interchain bonds increase accordingly, making it easier for polymers to form. Therefore, it can be concluded that high molecular weight subunits contribute to the viscosity of the wheat, while on the other hand, greater linkages aid in strengthening the gluten network, and thus, overall dough elasticity. As such, high molecular weight subunits are associated with the viscoelastic properties of the final end product.

Conflict of interest

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