Gliadin protein antigenicity and health benefitting potential of Indian bread wheat (*Triticum aestivum* L.) varieties

Asima Hamid¹ · S. A. Mallick¹ · Gupta Moni¹ · M. Iqbal Jeelani² · Gupta Sachin³

Revised: 28 August 2018 / Accepted: 5 September 2018 / Published online: 17 September 2018
© Association of Food Scientists & Technologists (India) 2018

Abstract Health benefitting potential of twenty leading wheat varieties was assessed for grain nutritional quality status in relation to antigenic reactivity level of gluten protein fractions. Among the nutritional parameters, macronutrients viz. starch, total sugar, total protein and gluten content were observed maximum in the varieties RAJ4120, RAJ4083, RAJ3077, and WH1021 respectively. Micronutrients- zinc and iron and phytochemicals- total phenolics and flavonoids were observed to be maximum in RAJ4083. Among the four protein fraction, albumin and globulin contents were the highest in RAJ3077, whereas gliadin and gluten content was maximum in GW322 and minimum in RAJ4120. The varieties were also characterized by SDS-PAGE and the results revealed significant polymorphism in all of the four protein fractions. The antigenic properties of flour gliadin proteins as evaluated by ELISA revealed that all the varieties possessed antigenicity with highest level in GW322 (0.217 OD). However, all the varieties possessed good baking qualities as studied by rheological measurements.

Keywords Wheat · Nutrition · Gluten proteins · Antigenicity

Introduction

A balance food with proper proportions of necessary parameters such as energy, growth and defense is indispensable for health and growth. Humans do prefer variety of plant and animal based food to meet the same purpose. Among the cereals, wheat (*Triticum aestivum* L.) is widely consumed globally as common food grain to cater the needs of energy and growth. India has been the second largest producer of wheat in the world with a production of 88.9 million tons in the year 2015–2016 (www.world-grain.com). Although, three species of wheat viz., *Triticum aestivum*, *T. durum* and *T. dicoccum* are cultivated in India, but the production of bread wheat *T. aestivum* is maximum. Being a widely consumed food, people were concerned about the nutritional quality parameters of wheat grains. Besides the presence of health benefitting food factors, some anti-nutritional and disease causing factors have also been reported in wheat (Inomata 2009). Wheat grains possess comparatively high protein contents and excellent baking ability which is essential for bakery products like bread and biscuit and chapatti besides its high energy content (Kasarda et al. 2013). Out of four major categories of grain protein fractions viz. albumin, globulin, glutenin and gliadin, first two fractions are water soluble having highest nutritive value and last two protein fractions together form gluten that confers a unique combination of elasticity and viscosity enabling the dough to be processed into the range of products (Shewry 2009). However, unfortunately these gluten proteins besides the baking character are reported to create serious health problems viz. gluten intolerance causing autoimmunity, celiac disease, insomnia, irritable bowel syndrome and wheat allergy (Foschia et al. 2016). Therefore, it is a great challenge to overcome these problems without affecting wheat's

✉ Asima Hamid
asmamir653@gmail.com

¹ Division of Biochemistry, Faculty of Basic Sciences, Sher-e-Kashmir University of Science and Technology-Jammu, Main Campus, Chatha, Jammu & Kashmir, India

² Division of Statistics and Computer Science, Faculty of Basic Sciences, Sher-e-Kashmir University of Science and Technology-Jammu, Main Campus, Chatha, Jammu & Kashmir, India

³ Division of Plant Pathology, Faculty of Agriculture, Sher-e-Kashmir University of Science and Technology-Jammu, Main Campus, Chatha, Jammu & Kashmir, India

bakery/chapati making quality. The present study was under taken to evaluate twenty leading bread wheat varieties in terms of gliadin allergy indices along with baking and health promoting characters so as to find out the best variety(-ies) with high baking quality but lowest/very low antigenic property and high nutritional character.

Materials and methods

Materials

Twenty leading wheat varieties viz. WH1021, PBW175, DBW17, HD2888, HD2932, HD2864, GW322, PBW596, HD2781, C306, RAJ4083, DBW14, RAJ4120, PBW396, HD2987, K0307, HI1531, RAJ3765, RAJ3077 and RSP561 procured from Directorate of Wheat Research (DWR) Karnal, Haryana and Division of Plant Breeding and Genetics, SKUAST- Jammu were used for the present study. The seeds of varieties were multiplied during Rabi season in the year 2015–2016 in Research Farm, SKUAST-J, India following recommended package of practice (Anonymous 2000). After harvest, the wheat grain samples of respective varieties were sun dried, grinded to fine powder and stored in airtight and moisture free containers for further biochemical analysis.

Methods

Biochemical analysis

Total sugar was extracted out from powdered grain sample by using 80% hot ethanol and was determined by phenol sulphuric acid method (Dubois 1956). The sugar free sample was then treated with cold 52% perchloric acid (4 °C) to hydrolyze starch of the sample and was determined by the method of Dubois (1956) using glucose standard. Starch content was obtained by multiplying the glucose content by 0.9. Fat content was determined following the method of extraction with petroleum ether in a soxhlet apparatus (AOAC 1990). Total soluble protein was extracted from wheat grain powder samples using phosphate buffer and estimated following the method of Lowry et al. (1951). Different protein fractions were extracted following the method of Dvoracek et al. (2001). Albumin and globulin were extracted in distilled water and 0.5 N NaCl solutions respectively and glutenin and gliadin were then extracted step-wise from the residue by homogenizing with Na-borate buffer (pH 10.0) and 70% ethanol respectively. Total polyphenol was extracted by refluxing powdered sample with 80% hot methanol for 1 h and was estimated colorimetrically by measuring blue colour developed due to reduction of phosphor-tungsto-molybdic

acid by the reaction of polyphenol (Schanderi 1970). Total flavonoids were extracted from grain flour samples and estimated following the method of Zhishen et al. (1999). Elements were extracted from wheat grain powder by wet digestion using nitric acid and perchloric acid in a ratio of 4:1 v/v. Zinc and iron compositions of the extract were measured by using Atomic Absorption Spectrophotometer (HITACHI, Z-2300, Japan) and comparing these absorbance values with corresponding standard values (Lindsay and Norvell 1978).

Characterization of wheat protein fractions by SDS-PAGE

Protein fractions were selectively extracted from wheat flour according to the method of Osborne (1907), further modified by Weiss et al. (1993). The concentration of proteins was determined by the Bradford method (Bradford 1976). Sodium Dodecyl Sulfate-Poly Acrylamide Gel Electrophoresis (SDS-PAGE) was carried out using 10% separating gels and 5% stacking gels following the method of Laemmli et al. (1970). Prior to the electrophoresis, the proteins fractions were diluted in the ratio 1:2 (v/v) with the sample buffer, heated at 90 °C for 5 min and cooled to room temperature. Aliquots of 40 µg of each albumin, globulin, gliadin and glutenin fractions were loaded per well. Gels were run at 50 mA for 5 h, fixed and stained with 1% coomassie brilliant blue (R-250), methanol, glacial acetic acid, distilled water in the ratio of 4.5:1:4.5. Destaining was performed with methanol, acetic acid and distilled water in the ratio of 1:0.5:3.5. Albumin, globulin, gliadin and glutenin fractions were characterized separately and molecular weights of the polypeptides were estimated by comparing with standard molecular weight marker (BLUeye Prestined protein Ladder, Sigma).

Immunological evaluation of gliadins by ELISA

Diluted gliadin extracts (1:100) were mixed in 1:1 ratio with 0.1 M carbonate-bicarbonate buffer (pH 9.6). 100 µl solution aliquots of gliadin antigens were used to coat micro-titer plates (Sigma Aldrich) overnight at 4 °C. After 24 h, the plates were washed four times with wash buffer. Thereafter, 100 µl of blocking solution was added to each well to block the free binding sites with 2% BSA in wash buffer and incubated for 2 h at room temperature. This was followed by removal of coating buffer, rinsing the plates four times with wash buffer and addition of 100 µl of unconjugated primary antibody (anti-gliadin antibody produced in rabbit obtained from Sigma Aldrich) to each well and incubated the plates for 2 h at room temperature. The plates were washed with wash buffer and 100 µl of conjugated secondary antibody (anti-rabbit IgG whole molecule—peroxidase) was added to the wells of micro-

titre plates. After incubation for 2 h, the plates were rinsed three times with wash buffer. Thereafter, 100 µl of the substrate solution (TMB for peroxidase) was added and plates were incubated for 30 min. The reaction was stopped by adding 50 µl of stop solution for TMB (Sigma Aldrich) and the output absorbance was measured using Multiscan RC reader at 450 nm.

Rheology of the wheat dough

Wheat dough samples were prepared with deionised water for measurement of rheological properties. The rheological characteristics (storage modulus, G' and loss modulus, G'') of wheat dough samples were obtained by using Modular Compact Rheometer (MCR-301, Anton Paar, Ostfildern, Germany), equipped with parallel plate system (40 mm diameter) at a gap of 1 mm with a coaxial geometry. The dough sample was placed between the plates and the edges were carefully trimmed with a spatula. Amplitude sweeps were conducted to establish the linear visco-elastic regions of the samples. Frequency sweeps from 1 to 50 10^{-5} angular frequency (ω) were performed with a target strain of 10^{-3} (0.1%) at 30 °C. Finally elastic (G') and loss (G'') modulus values were obtained. The storage modulus represents the non-dissipative component of mechanical properties. The elastic or “rubber-like” behaviour is suggested if the G' spectrum is independent of frequency and greater than the loss modulus over a certain range of frequency. The loss modulus represents the dissipative component of the mechanical properties and is characteristic of viscous flow. All the rheological experiments were performed at least twice and their averages were taken. The storage modulus (G') and loss modulus (G'') were calculated using the Origin software (version OriginPro 8.5). The power law model (Eqs. 1 and 2) was used to analyze the flow behavior of wheat doughs.

$$G' = K' (\omega)^{n'} \quad (1)$$

$$G'' = K'' (\omega)^{n''} \quad (2)$$

where K' and K'' indicate consistency coefficient while n' and n'' represent flow behavior index of G' and G'' respectively.

Statistical analysis

Analysis of the data and the fitting of power law model were done in R software version 3.4.1 and level was set to 0.05. Values of all parameters are expressed as mean $\pm$ SE of three independent measurements.

Results and discussion

Biochemical composition of wheat grains

The varieties varied inter-genotypically amongst each other in respect to the nutritional parameters assessed. The results recorded in Table 1 revealed that starch content was found maximum in RAJ4120 (75.47%) followed by K030 (75.33%) and minimum in GW322 (64.97%). Total sugar content was found highest in RAJ4083 (2.41%) followed by RAJ3077 (2.33%) and lowest in HI1531 (1.26%). So, RAJ4120 and K030 varieties were found to be highly potential in context to energy content. The total protein content which varied significantly among varieties was observed to be maximum in RAJ3077 (12.49%) and at par with HD2781 (12.44%). However, fat content was observed to vary in narrow range (1.4–1.8%) among the varieties. Both RAJ4120 and RAJ3077 possessed highest values of fat content. Mallick et al. (2013) reported variation in starch, fat and sugar contents in different wheat varieties from 75 to 66%, 1.3 to 2.0% and 3 to 4% respectively.

Among nutritional parameters, total protein, starch, and essential elements are important factors for growth and energy, whereas gluten, a complex protein made of protein gliadin and glutenin, capable of water and gas retention is essential factor for preparation of bread, biscuit and other bakery products. Water soluble albumin and globulin improves the biological value of protein and are considered as nutritionally superior (Stehno et al. 2008). In the present study, both albumin and globulin contents were also found to vary inter-genotypically with maximum content in RAJ3077 i.e. 2.11 and 2.07% respectively which was at par with HD2764. However, minimum albumin content was observed in GW322 (1.25%) and minimum globulin content in K030 (1.22%) (Table 1).

The levels of albumin and globulin in the present study are in conformity to the earlier reports. Merlino et al. (2009) reported that albumin and globulin content ranged from 2.0 to 2.5% of total proteins, whereas Chiang et al. (2006) reported albumin and globulin fractions to vary between 1.1 and 2.1% in the wheat flour. The combined content of albumin and globulin was found to vary from 1.80 to 2.6% in bread wheat grains (Gafurova et al. 2002) and about 3.8% in durum wheat of total protein (Zilic et al. 2011). Albumins and globulins are nutritionally better owing to their high lysine and methionine contents as compared to the rest of proteins in the wheat grain (Lasztity 1985). Therefore among the wheat varieties evaluated, RAJ3077 having highest values in total protein content as well in albumin and globulin (total 4.18%) showed maximum nutritional potential with highest protein biological

Table 1 Nutritional components (starch, total sugar, fat, total protein) and protein fraction content (%) in grains of twenty Indian wheat varieties

Variety	% Starch content	% Sugar content	% Total protein	% Fat content	% Albumin	% Globulin	% Gliadin	% Glutenin	% Gluten protein
HD2764	66.93 ± 14.966	2.04 ± 0.456	12.40 ± 2.772	1.6 ± 0.357	2.07 ± 0.0733	1.93 ± 0.0437	3.91 ± 0.0314	4.50 ± 0.0274	8.40 ± 0.0505
RAJ3765	65.05 ± 14.545	1.59 ± 0.355	11.81 ± 2.640	1.8 ± 0.402	2.05 ± 0.0726	1.80 ± 0.0407	3.67 ± 0.0294	4.29 ± 0.0261	7.96 ± 0.0478
GW322	64.97 ± 14.527	2.24 ± 0.500	11.35 ± 2.537	1.4 ± 0.313	1.25 ± 0.0443	1.69 ± 0.0383	3.97 ± 0.0319	4.56 ± 0.0278	8.44 ± 0.0507
RAJ3077	70.22 ± 15.701	2.33 ± 0.521	12.49 ± 2.792	1.8 ± 0.402	2.11 ± 0.0747	2.07 ± 0.0469	3.87 ± 0.0311	4.43 ± 0.0270	8.31 ± 0.0499
HD2781	71.93 ± 16.084	1.50 ± 0.335	12.44 ± 2.781	1.8 ± 0.402	2.01 ± 0.0712	2.01 ± 0.0455	3.88 ± 0.0311	4.53 ± 0.0276	8.42 ± 0.0506
WH1021	68.76 ± 15.375	1.58 ± 0.353	11.61 ± 2.596	1.8 ± 0.402	1.39 ± 0.0492	1.80 ± 0.0407	3.87 ± 0.0311	4.44 ± 0.0270	8.41 ± 0.0505
PBW175	69.15 ± 15.462	2.19 ± 0.489	11.86 ± 2.651	1.4 ± 0.313	1.75 ± 0.0620	1.86 ± 0.0421	3.74 ± 0.0300	4.51 ± 0.0275	8.25 ± 0.0496
RSP561	73.48 ± 16.430	2.26 ± 0.505	11.51 ± 2.573	1.6 ± 0.357	1.79 ± 0.0634	1.73 ± 0.0392	3.77 ± 0.0303	4.22 ± 0.0257	7.99 ± 0.0480
HD2888	74.55 ± 16.669	1.70 ± 0.380	11.55 ± 2.582	1.8 ± 0.402	1.72 ± 0.0609	1.76 ± 0.0398	3.82 ± 0.0307	4.23 ± 0.0257	8.07 ± 0.0485
K0307	75.33 ± 16.844	2.19 ± 0.489	10.76 ± 2.406	1.8 ± 0.402	1.62 ± 0.0574	1.22 ± 0.0276	3.61 ± 0.0290	4.27 ± 0.0260	7.88 ± 0.0473
RAJ4120	75.47 ± 16.875	2.18 ± 0.487	11.65 ± 2.605	1.8 ± 0.402	1.95 ± 0.0691	1.88 ± 0.0426	3.52 ± 0.0282	4.30 ± 0.0262	7.82 ± 0.0470
RAJ4083	68.56 ± 15.330	2.41 ± 0.538	11.54 ± 2.580	1.6 ± 0.357	1.73 ± 0.0613	1.81 ± 0.0410	3.63 ± 0.0291	4.37 ± 0.0266	8.00 ± 0.0481
HI1531	68.70 ± 15.361	1.26 ± 0.281	11.67 ± 2.609	1.6 ± 0.357	1.77 ± 0.0627	1.61 ± 0.0364	3.71 ± 0.0298	4.59 ± 0.0279	8.29 ± 0.0498
HD2932	70.87 ± 15.847	1.77 ± 0.395	11.90 ± 2.660	1.8 ± 0.402	1.85 ± 0.0655	1.67 ± 0.0378	3.84 ± 0.0308	4.54 ± 0.0276	8.38 ± 0.0503
HD2987	67.72 ± 15.142	2.26 ± 0.505	11.94 ± 2.669	1.8 ± 0.402	1.89 ± 0.0669	1.95 ± 0.0441	3.70 ± 0.0297	4.40 ± 0.0268	8.10 ± 0.0487
DBW17	68.90 ± 15.406	1.33 ± 0.297	11.44 ± 2.558	1.6 ± 0.357	1.36 ± 0.0482	1.93 ± 0.0437	3.77 ± 0.0303	4.51 ± 0.0275	8.28 ± 0.0497
PBW396	71.12 ± 15.902	1.60 ± 0.357	11.17 ± 2.497	1.6 ± 0.357	1.45 ± 0.0513	1.85 ± 0.0419	3.54 ± 0.0284	4.33 ± 0.0264	7.88 ± 0.0473
DBW14	71.79 ± 16.052	1.75 ± 0.391	11.29 ± 2.524	1.6 ± 0.357	1.57 ± 0.0556	1.72 ± 0.0389	3.63 ± 0.0291	4.37 ± 0.0266	8.00 ± 0.0481
PBW596	66.82 ± 14.941	1.28 ± 0.286	10.98 ± 2.455	1.8 ± 0.402	1.45 ± 0.0513	1.73 ± 0.0392	3.56 ± 0.0286	4.24 ± 0.0258	7.80 ± 0.0469
C306	68.37 ± 15.288	1.80 ± 0.402	11.03 ± 2.466	1.8 ± 0.402	1.30 ± 0.0460	1.66 ± 0.0376	3.60 ± 0.0289	4.48 ± 0.0273	8.07 ± 0.0485
CD ($p \leq 0.05$)	2.03	0.11	0.15	0.01	0.11	0.13	0.09	0.1	0.07

Data represented as mean value ± SE

Table 2 Total phenolic, total flavonoid, zinc and iron content in grains of twenty wheat varieties

Variety	Total phenolic content (mg/g dry wt)	Total flavonoid content (mg/100 g dry wt)	Zinc content (ppm)	Iron content (ppm)
HD2764	1.11 ± 0.0502	0.57 ± 0.0115	20.28 ± 1.3791	23.54 ± 1.8102
RAJ3765	1.52 ± 0.0384	0.56 ± 0.0113	23.34 ± 1.5872	44.80 ± 3.4450
GW322	1.50 ± 0.0723	0.54 ± 0.0109	15.82 ± 1.0758	62.25 ± 4.7869
RAJ3077	1.71 ± 0.0821	0.64 ± 0.0129	27.54 ± 1.8729	57.02 ± 4.3847
HD2781	1.39 ± 0.0345	0.63 ± 0.0127	21.67 ± 1.4737	39.55 ± 3.0413
WH1021	1.34 ± 0.0652	0.62 ± 0.0125	24.62 ± 1.6743	43.19 ± 3.3212
PBW175	1.50 ± 0.0723	0.54 ± 0.0109	26.90 ± 1.8293	32.85 ± 2.5261
RSP561	1.83 ± 0.0827	0.67 ± 0.0136	24.76 ± 1.6838	46.77 ± 3.5965
HD2888	1.61 ± 0.0775	0.58 ± 0.0117	23.65 ± 1.6083	38.32 ± 2.9467
K0307	1.62 ± 0.0410	0.64 ± 0.0129	23.11 ± 1.5716	23.61 ± 1.8156
RAJ4120	1.15 ± 0.0560	0.64 ± 0.0129	23.65 ± 1.6083	22.01 ± 1.6925
RAJ4083	1.97 ± 0.0945	0.78 ± 0.0158	50.31 ± 3.4213	75.76 ± 5.8250
HI1531	1.80 ± 0.0867	0.68 ± 0.0138	21.67 ± 1.4737	44.81 ± 3.4458
HD2932	1.16 ± 0.0567	0.64 ± 0.0129	25.78 ± 1.7532	39.12 ± 3.0083
HD2987	1.48 ± 0.0710	0.60 ± 0.0121	32.43 ± 2.2054	24.83 ± 1.9094
DBW17	1.46 ± 0.0508	0.70 ± 0.0142	21.87 ± 1.4873	50.24 ± 3.8634
PBW396	1.90 ± 0.0893	0.64 ± 0.0129	25.80 ± 1.7545	30.98 ± 2.3823
DBW14	1.21 ± 0.0339	0.62 ± 0.0125	17.42 ± 1.1846	29.11 ± 2.2385
PBW596	1.39 ± 0.0671	0.64 ± 0.0129	20.89 ± 1.4206	34.55 ± 2.6568
C306	1.70 ± 0.0814	0.60 ± 0.0121	40.04 ± 2.7229	44.33 ± 3.4089
CD ($p \leq 0.05$)	0.007	0.04	0.051	0.241

value. In gluten fraction, gliadin protein content was observed to be maximum in GW322 (3.97%) which was at par with HD2764 (3.91%) and lowest in RAJ4120 (3.52%), whereas glutenin content was observed to be highest in HI1531 (4.59%) and at par with GW322 (4.56%) and lowest in RSP561 (4.22%). Equivalent range of gliadin and glutenin protein contents of other wheat varieties has been reported by Mallick et al. (2013). Gluten content was observed to be highest in GW322 which possessed highest gluten content (gliadin 3.97% + glutenin 4.56% = 8.53%) and therefore expected to have highest gluten related properties i.e. baking and chapatti making properties.

Around three billion people throughout the world face problem of malnutrition to micronutrient deficiency (Anonymous 2000). Besides the high calories and high nutritive protein contents, micronutrients like zinc and iron and phytochemicals like total phenols and flavonoids are also needed for proper growth, development and defense of human body. Iron and zinc are directly involved in blood formation and brain development respectively, whereas phenolics and flavonoids are reported to have ability to fight against different diseases (Adil et al. 2012). So, these micronutrient and phytochemical contents in the wheat varieties were also studied to evaluate their nutritional status. Total phenols as well as flavonoid contents in grains were recorded maximum in RAJ4083 (1.97 mg/g dry wt

and 0.78 mg/100 g respectively) and minimum was found in HD2764 (1.11 mg/g dry wt) and GW322 (0.54 mg/100 g dry wt) respectively. RAJ-4083 also recorded the highest zinc (50.32 ppm) and iron (75.5 ppm) contents. However, GW322 variety showed minimum flavonoids (0.54 mg/100 g dry wt) and zinc content (15.80 ppm) (Table 2). RAJ4083 which recorded maximum contents of micronutrients (zinc and iron) and phytochemicals (total phenol and flavonoid) can thus be considered to the best variety for health benefitting nutrients. Therefore, RAJ4083 can be considered to be the best in terms of major and micronutrient content respectively. Although, GW322 showed lowest nutritional parameters but owing to its highest gluten content was expected to have highest baking quality.

Characterization of wheat grain protein fractions by SDS-PAGE

Electrophoresis of proteins is a powerful tool for identification of genetic diversity and the SDS-PAGE is predominantly considered as a consistent technology because seed storage proteins are highly independent of environmental fluctuations (Javid et al. 2004; Iqbal et al. 2005). The results from SDS-PAGE analysis of wheat endosperm proteins indicate differential banding pattern for different

Fig. 1 SDS-PAGE of **a** albumin protein fraction of twenty wheat varieties, **b** globulin protein fraction of twenty wheat varieties, **c** gliadin protein fraction of twenty wheat varieties, **d** glutenin protein fraction of twenty wheat varieties (*Lane 1*: HD-2764, 2: RAJ-3765, 3: GW-322, 4: RAJ-3044, 5: HD-2781, 6: WH-1021, 7: PBW-175, 8: PBW-561, 9: HD-2888, 10: K-030, 11: RAJ-4120, 12: RAJ-4083, 13: HI-1531, 14: HD-2932, 15: HD-2987, 16: DBW-17, 17: PBW-396, 18: DBW-14, 19: PBW-596, 20: C-306)

wheat varieties but the overall degree of variation is relatively low. The wheat protein fractions viz. albumins, globulins, gliadins and glutenins extracted from wheat

grain in different solubilizing mediums were characterized by SDS-PAGE. According to the results of the SDS-PAGE, the overall pattern of seed storage-proteins showed low

degree of heterogeneity. Electrophoretic patterns of albumin and globulin proteins of twenty wheat varieties showed little polymorphism (Fig. 1a, b). However, banding pattern of gliadin proteins revealed significant polymorphism amongst all of its four protein sub-fractions. The α/β gliadins showed two to three bands having molecular weight ranging from 20 to 40 kDa. However, three to four bands of γ - gliadins in the range of 41.9–62 kDa were observed and the ω - gliadins showed protein bands in the range from 70 to 75 kDa (Fig. 1c). GW322 and HD2781 showed a different kind of banding pattern for ω gliadins with presence of additional 71 kDa polypeptide which was absent in other varieties. The SDS-PAGE analysis of Katyal et al. (2018) revealed differential accumulation of different polypeptide (PPs) of gliadins and glutenins and storage of these PPs was varietal dependent. PPs ranging from 88 to 48 kDa were classified as ω - gliadins whereas, PPs ranging between 43 and 28 kDa were known as α , β , and γ gliadins. Hence it was inferred that the polymorphism in ω - gliadins in many durum accessions is associated with varied pasting properties. Similarly another study performed by the same research group correlated hard wheat (HW), medium-hard wheat (MHW) and extraordinarily soft wheat (Ex-SW) varieties with grain hardness index (GHI) for the assessment of pasting, protein molecular weight (MW) distribution, dough rheology and baking properties. It was found that accumulation of additional polypeptides (PPs) varied significantly in different varieties and further Ex- SW variety (QBP12-10) depicted accumulation of 98, 90, 81 and 79 kDa PPs, this pattern was distinct and different from other varieties (Katyal et al. 2017). The results of the present study are therefore supported by these findings and suggest different banding pattern resulting from polymorphism of ω gliadins that may have a correlation with differential rheological behaviour of wheat flour. Polypeptides with molecular weight between 31.03 and 54.50 kDa belonging to α , β , and γ subunits of gliadin have been reported by Chattopadhyay and Kumar (2016) and Nadeem et al. (2015). Glutenin proteins showed a differential banding pattern for different wheat varieties but the overall degree of variation was good having eight to nine bands in different varieties with two types of subunits viz. Low Molecular Weight (LM_r)-Glutenin Subunit (30–65 kDa) and High Molecular Weight-Glutenin Subunit, (HMr)-Glutenin Subunit (70–140 kDa) (Fig. 1d). Our results are in conformity with Shuaib et al. (2010) who reported eight to nine numbers of bands in different varieties with two types of subunits viz. LM_r-GS (30–65 kDa) and HMr-GS (70–140 kDa). Seed storage protein profiles show different polymorphic patterns amongst the four protein fractions, with gliadins depicting highest polymorphic nature.

Evaluation of antigenic property of gliadin proteins by ELISA

Gluten, a heterogeneous complex of proteins (gliadins and glutenins) gives viscoelasticity to dough and is reported to be involved in the pathogenesis of many disorders and diseases. Reliable analytical methods are needed to assess the gluten content of the wheat. Currently ELISA (Enzyme-Linked Immunosorbent Assay) is the most commonly preferred method for determining the antigenic potential of wheat. The study on evaluation of antigenicity of Indian wheat varieties is very scanty. Therefore, the leading Indian bread wheat varieties were assessed for gluten/gliadin antigenic reactivity besides baking quality. Leszczynska et al. (2003) reported antigenic reactivity of wheat flour due to gliadin fraction of the gluten. Therefore, the gliadin protein fractions were isolated from grains of wheat varieties and tested for antigenic effect and the varieties recognized for good bread and chapatti making abilities were assessed for health benefitting status through measurement of gluten/gliadin antigenicity levels. A significant variation in the immunoreactive properties by absorbance measurements (OD) of gliadin proteins was revealed by ELISA using Multiscan RC reader at 450 nm (Table 3). Highest OD values were recorded in GW322 (0.217), followed by HI1531 (0.212). Values were much lower in DBW17 (0.177 OD) and RSP561 (0.179 OD) but

Table 3 Immunoreactivity levels of flour gliadin fractions

Variety	Absorbance at 450 nm
HD2764	0.198 ± 0.0030
RAJ3765	0.185 ± 0.0028
GW322	0.217 ± 0.0033
RAJ3077	0.205 ± 0.0031
HD2781	0.203 ± 0.0031
WH1021	0.211 ± 0.0032
PBW175	0.183 ± 0.0028
RSP561	0.177 ± 0.0027
HD2888	0.188 ± 0.0028
K0307	0.203 ± 0.0031
RAJ4120	0.176 ± 0.0027
RAJ4083	0.208 ± 0.0031
HI1531	0.212 ± 0.0032
HD2932	0.182 ± 0.0027
HD2987	0.192 ± 0.0029
DBW17	0.179 ± 0.0027
PBW396	0.182 ± 0.0027
DBW14	0.195 ± 0.0029
PBW596	0.186 ± 0.0028
C306	0.181 ± 0.0027
CD($p \leq 0.05$)	0.011

the lowest one was recorded in RAJ4120 (0.176). However, Waga and Zientarski (2007) and Sherif et al. (2006) reported antigenic values in terms of OD values of gliadins from 3.3 to 1.1 and 0.216 to 1.31 respectively. As compared to earlier studies, the varieties of this experiment carried low antigenic values ranging from 0.176 to 0.217 and hence could be considered to be better in general and RAJ4120 was best among the varieties and at par with DBW17 and RSP561.

Rheological measurements

The baking quality of wheat flour is due to presence of good amount of gluten protein fractions (Branlard et al. 2001). Gluten protein develops visco-elasticity in dough when mixed with water and hence helps in baking property. To analyze baking quality of flour, rheological assessment was done. Rheology measurements showed that the storage

modulus (G') magnitude was much greater than loss modulus (G'') magnitude in all the evaluated wheat varieties indicating that the dough was elastic than being viscous i.e. $G' > G''$. At a constant temperature of 30 °C and throughout the frequency range studied, elastic behavior ($G' > G''$) was more pronounced for all the dough samples (Fig. 2a–d). This reflected that the behavior of the twenty dough samples was comparable to a gel like material (Muzaffer et al. 2016). Table 4 shows the power law parameters of the dough samples of the twenty wheat varieties. The G' and G'' data of all the varieties at 30 °C were fitted to the power law model and consistency coefficient and flow behavior index were determined. The results showed that all the twenty wheat genotypes displayed a solid like behavior because the magnitudes of consistency coefficient k' (17790) were much greater than those of k'' (11083) as in HD2764. Similar results for k' and k'' were found in all the other varieties with minor

Fig. 2 a–d Mechanical spectra showing frequency dependence of storage modulus (G') and loss modulus (G'') of wheat dough samples

Table 4 Power law model for storage and loss modulus of wheat dough samples as a function of angular frequency

Variety	G'			G''		
	η'	K'	R ²	η''	K''	R ²
HD2764	0.275	17,790.78	0.99	0.181	11,083.93	0.98
RAJ3765	0.317	6838.553	0.98	0.229	4443.567	0.99
GW322	0.335	13,952.29	0.98	0.238	9250.051	0.95
RAJ3077	0.305	19,014.22	0.95	0.232	11,692.68	0.83
HD2781	0.332	10,856.69	0.97	0.245	7502.329	0.95
WH1021	0.421	3964.065	0.99	0.342	2815.987	0.97
PBW175	0.257	15,321.76	0.98	0.187	8965.643	0.96
RSP561	0.262	5867.194	0.98	0.209	3216.416	0.97
HD2888	0.306	5098.898	0.99	0.255	3131.99	0.98
K0307	0.297	5264.737	0.98	0.227	3418.137	0.98
RAJ4120	0.306	5098.898	0.98	0.255	3131.990	0.97
RAJ4083	0.301	7825.764	0.99	0.263	4734.926	0.97
HI1531	0.321	14,505.62	0.97	0.218	10,390.26	0.93
HD2932	0.291	8377.038	0.96	0.200	5762.731	0.91
HD2987	0.303	9879.615	0.97	0.209	6725.584	0.93
DBW17	0.317	6838.553	0.98	0.229	4443.567	0.99
PBW396	0.303	10,359.25	0.97	0.224	6975.682	0.97
DBW14	0.394	3855.443	0.99	0.335	2451.716	0.98
PBW596	0.326	17,612.21	0.99	0.251	11,501.34	0.96
C306	0.337	14,543.91	0.98	0.241	9599.008	0.98

G': storage modulus, G'': loss modulus, η' and η'' : flow behaviour index of G' and G'', K' and K'': consistency coefficient of G' and G''

variations among different varieties. Flow behavior indexes (η' and η'') ranging from 0.257 to 0.421 and less than unity showed that all the dough samples displayed shear thinning (pseudoplastic or Non-Newtonian) behavior (Table 4). Our results are in agreement with previous studies (Singh and Singh 2013) which depicted that G' of dough from all the varieties was greater than G'', indicating predominance of elastic character. Similar results have been reported by Mohammed et al. (2011) and Koussa-Koffi et al. (2015) who studied the dynamic rheological properties of chickpea and wheat flour doughs and observed that storage modulus was greater than the loss modulus in all the wheat doughs, indicating viscoelastic and solid like behavior of all the doughs.

Conclusion

Inter-genotypically variation in terms of nutritional quality parameters was observed in Indian wheat varieties. The varieties varied significantly for the immunogenic potential of gliadin proteins. SDS-PAGE revealed significant polymorphic nature of the wheat proteins. Among the twenty Indian wheat varieties, RAJ4120 exhibited good values of

macronutrients, micronutrients and lowest value in antigenic property with high baking quality. However, GW-322 showed highest antigenic potential.

References

- Adil G, Wani SM, Masoodi FA, Gousia H (2012) Whole grain cereal bioactive compounds and their health benefits. *J Food Process Technol* 3:146
- Anonymous (2000) Fourth Report on the World Nutrition Situation, ACC/SCN (Administrative committee on coordination, subcommittee on Nutrition) and International Food Policy Research
- AOAC (1990) Official methods of analysis, 15th edn. Association of Official Analytical Chemists, Washington
- Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72:248–254
- Branlard G, Dardevet M, Saccomano R (2001) Genetic diversity of wheat storage proteins and bread wheat quality. *Euphytica* 59:119
- Chattopadhyay T, Kumar S (2016) Diversity analysis of wheat cultivars on the basis of solubility- fractionated seed storage protein polymorphism. *Indian J Biotech* 15:190–194
- Chiang SH, Chen CS, Chang CY (2006) Effect of wheat flour protein compositions on the quality of deep-fried gluten balls. *Food Chem* 97:666–673
- Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. *Anal Chem* 28:350–356
- Dvoracek V, Moudary I, Curn V (2001) Studies of protein fractions in grain of split wheat (*Triticum split* L.) and common wheat (*Triticum aestivum* L.). *Sci Agric Biochemica* 32:287–305
- Foschia M, Horstmann S, Arendt EK, Zannini E (2016) Nutritional therapy—facing the gap between celiac disease and gluten-free food. *Int J Food Microbiol* 239:113–124
- Gafurova DA, Tursunkhodzhaev PM, Kasymova TD, Yuldashev PK (2002) Fractional and amino-acid composition of wheat grain cultivated in Uzbekistan. *Chem Nat Compd* 38:462–465
- Inomata N (2009) Wheat allergy. *Curr Opin Allergy Clin Immunol* 9:238–243
- Iqbal SH, Ghafoor A, Ayub N (2005) Relationship between SDS-PAGE markers and *Ascochyta blight* in chickpea. *Pak J Bot* 37:87–96
- Javid A, Ghafoor A, Anwar R (2004) Seed storage protein electrophoresis in groundnut for evaluating genetic diversity. *Pak J Bot* 36:25–29
- Kasarda DD, Adalsteins E, Lew EJ, Lazo GR, Altenbach SB (2013) Characterization of a novel wheat endosperm protein belonging to the prolamin superfamily. *J Agric Food Chem* 61:2407–2417
- Katyal M, Singh N, Viridi AS, Kaur A, Chopra N, Ahlawat AK, Singh AM (2017) Extraordinarily soft, medium-hard and hard Indian wheat varieties: composition, protein profile, dough and baking properties. *Food Res Int*. <https://doi.org/10.1016/j.foodres.2017.08.050>
- Katyal M, Viridi AS, Singh N, Kaur A, Rana JC, Kumari J (2018) Diversity in protein profiling, pasting, empirical and dynamic-dough rheological properties of meal from different durum wheat accessions. *J Food Sci Technol*. <https://doi.org/10.1007/s13197-018-3036-y>
- Koussa-Koffi JD, Muresan V, Gnanui SN, Sturza A, Mudura E, Kouame LP (2015) Wheat flour dough rheological properties measurement: effects of the target values on the attained results. *J Texture Stud* 46:475–486

- Laemmli VK (1970) Cleavage of structural proteins during assembly of the head of bacteriophage T4. *Nature* 227:680–685
- Lasztity R (1985) The chemistry of cereal proteins. *Die Nahrung* 29:710
- Leszczynska J, Lacka A, Szemraj J, Lukamowicz J, Zegota H (2003) The influence of gamma irradiation on the immunoreactivity of gliadin and wheat flour. *Eur Food Res Technol* 217:143–147
- Lindsay WL, Norvell WA (1978) Development of a DTPA soil test for zinc, iron, manganese, and copper. *Soil Sci Soc Am J* 42:421–428
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin Phenol reagent. *J Biol Chem* 193:265–275
- Mallick SA, Azaz K, Gupta M, Sharma V, Sinha BK (2013) Characterization of grain nutritional quality in wheat. *Indian J Plant Physiol* 18:183–186
- Merlino M, Leroy P, Chambon C, Branlard G (2009) Mapping and proteomic analysis of albumin and globulin proteins in hexaploid wheat kernels (*Triticum aestivum* L.). *Theor Appl Genet* 18:1321–1337
- Mohammed I, Abdelrahman R, Ahmed Senge B (2011) Dynamic rheological properties of chickpea and wheat flour dough. *J Appl Sci* 11:3405–3412
- Muzaffer K, Dar BN, Kumar P (2016) Assessment of nutritional, physicochemical, antioxidant, structural and rheological properties of spray dried tamarind pulp powder. *J Food Meas Charact* 11:746–757
- Nadeem M, Anjum FM, Khan MR, Shabbir MA, Masud T (2015) Immunological appraisal of modified gluten to trim down celiac toxicity. *Food Agric Immunol* 26:394–409
- Osborne TB (1907) The protein of the wheat kernel. Carnegie Institute, Washington, DC, Publication No. 84
- Schanderi SH (1970) *Methods in food analysis*. Academic Press, New York, p 709
- Sherif ISM, Issa AH, Lafta AH (2006) Extraction and purification of gliadin from local variety wheat as specific antigen and using it in the enzyme linked immunosorbent assay (ELISA) as serological test diagnosis of celiac disease. *Basrah J Sci* 24:70–85
- Shewry PR (2009) Wheat. *J Exp Bot* 60:1537–1553
- Shuaib M, Jamal M, Akbar H, Khan I, Khalid R (2010) Evaluation of wheat by polyacrylamide gel electrophoresis. *Afr J Biotech* 9:243–247
- Singh S, Singh N (2013) Relationship of polymeric proteins and empirical dough rheology with dynamic rheology of dough and gluten from different wheat varieties. *Food Hydrocoll.* <https://doi.org/10.1016/j.foodhyd.2013.04.007>
- Stehno Z, Dvoracek V, Dotlacil L (2008) Wheat protein fractions in relation to grain quality characters of the cultivars. In *Proceedings of 11th international wheat genetics symposium*, vol 2, p 556
- Waga J, Zientarski J (2007) Isolation and purification of individual gliadin proteins by preparative Acid polyacrylmide gel electrophoresis (A-PAGE) for allergenic research. *Pol J Food Nutr Sci* 57:91–96
- Weiss P, Raymond F (1993) Red clover silage for fattening young meat bulls. *Fourrages* 134:283–286
- Zhishen J, Mengcheng T, Jianming W (1999) The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem* 64:555–559
- Zilic S, Barac M, Pesic M, Dodig D, Micic DI (2011) Characterization of Proteins from Grain of Different Bread and Durum Wheat Genotypes. *Int J Mol Sci* 12:5878–5894