



Diversity in quality traits amongst Indian wheat varieties I: Flour and protein characteristics



Mehak Katyal^a, Amardeep Singh Viridi^a, Amritpal Kaur^a, Narpinder Singh^{a,*}, Seeratpreet Kaur^{a,b}, Arvind Kumar Ahlawat^b, Anju Mahendru Singh^b

^a Department of Food Science and Technology, Guru Nanak Dev University, Amritsar 143005, India

^b Division of Genetics, Indian Agricultural Research Institute, New Delhi 110012, India

ARTICLE INFO

Article history:

Received 8 May 2015

Received in revised form 24 July 2015

Accepted 25 July 2015

Available online 6 August 2015

Keywords:

Wheat

Solvent retention capacity

Sedimentation value

Gluten index

HPLC

SDS–PAGE

ABSTRACT

The relationships of polymeric as well as monomeric proteins (unextractable and extractable) with various flour properties amongst Indian wheat varieties were evaluated. Unextractable polymeric proteins and unextractable monomeric proteins in flours ranged from 23.83% to 51.97% and 48.03% to 76.17%, respectively. Varieties with higher grain hardness index resulted into flours with higher α^* , ash content and protein content. Unextractable polymeric and monomeric proteins were related to grain hardness index. Unextractable polymeric proteins showed a positive correlation with gluten index and LASRC. Majority of varieties with HMW-GS combinations of 91 kDa + 80 kDa + 78 kDa + 74 kDa PPs showed very high grain hardness index (97–100).

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

The functionality of wheat flour is mainly attributed to the gluten proteins (gliadins and glutenins) present in it. Addition of water into wheat flour results in the formation of dough, which has unique viscoelastic properties (Singh, Singh, & MacRitchie, 2011). These properties (such as strength, tenacity and extensibility of wet gluten) are mainly attributable to the composition of gliadins and glutenins (Branlard & Dardevet, 1985a, 1985b; Fido, Bekes, Gras, & Tatham, 1997; Payne, Harris, Law, Holt, & Blackman, 1980; Sozinov, Popereya, & Stacanov, 1974). Wheat genome (A, B and D) and genetic analysis of wheat storage proteins revealed that genes encoding these proteins were present on several loci spanned on wheat chromosomes. Gliadins are monomeric alcohol soluble proteins having molecular weight ranging from

30 kDa to 60 kDa and are broadly categorized into four categories (ω -, γ -, β - and α gliadins). Three homologous loci *Gli-A1*, *Gli-B1* and *Gli-D1*, located at the distal end of chromosomes 1AS, 1BS and 1DS, contain tightly associated genes encoding for ω -, and many γ gliadins, respectively. Moreover, short arm of the group 6 chromosomes, named *Gli-A2*, *Gli-B2* and *Gli-D2*, encode genes responsible for the synthesis of α -, β -, and some γ gliadins, respectively (Shewry, Tatham, Forde, Kreis, & Mifflin, 1986). Except ω -gliadins, all other (α -, β -, and most γ gliadins) contain intramolecular disulfide bond linkages. Some exceptional γ gliadins are supposed to be associated with glutenin subunits (Kohler, Belitz, & Wieser, 1993). Glutenins are polymeric proteins and are divided into high molecular weight-glutenin subunits (HMW-GS) and low molecular weight-glutenin subunits (LMW-GS). HMW-GS are mainly responsible for dough strength in wheat (Ram, 2003). The long arm of homologous group chromosomes of wheat have complex *Glu-1* loci containing genes that encode HMW-GSs. *Glu-1* locus possesses two tightly associated genes encoding two HMW-GS subunits and is classified as type x and type y, according to their decreasing molecular weight. Hexaploid wheat possesses six genes (*1Ax*, *1Ay*, *1Bx*, *1By*, *1Dx* and *1Dy*) for HMW-GSs which are differentially expressed. Genes *1Bx* and *1Dx* are always expressed but genes *1Ax* and *1By* are expressed infrequently, however, *1Ay* is mostly unexpressed in hexaploid wheat though some

Abbreviations: TGW, thousand grain weight; GHI, grain hardness index; SV, sedimentation value; Ex-PP, extractable polymeric protein; Ex-MP, extractable monomeric protein; UnEx-PP, unextractable polymeric protein; UnEx-MP, unextractable monomeric protein; SRC, solvent retention capacity; NaSRC, sodium carbonate solvent retention capacity; SuSRC, sucrose SRC; LASRC, lactic acid SRC; WSRC, water SRC; WG, wet gluten; DG, dry gluten; GI, gluten index; PCA, principal component analysis.

* Corresponding author.

E-mail address: narpinders@yahoo.com (N. Singh).

landracs have been shown to express it (Lawrence & Payne, 1983). Lawrence and Payne (1983) classified these alleles and their respective subunits as *Glu-A1a* (1), *Glu-A1b* (2*), *Glu-A1c* (Null), *Glu-B1a* (7), *Glu-B1b* (7 + 8), *Glu-B1c* (7 + 9), *Glu-B1d* (6 + 8), *Glu-B1e* (20), *Glu-B1f* (13 + 16), *Glu-B1g* (13 + 19), *Glu-B1h* (14 + 15), *Glu-B1i* (17 + 18), *Glu-B1j* (21), *Glu-B1k* (22), *Glu-D1a* (2 + 12), *Glu-D1b* (3 + 12), *Glu-B1c* (4 + 12), *Glu-B1d* (5 + 10), *Glu-D1e* (2 + 10) and *Glu-D1f* (2,2 + 12). Ram (2003) documented allelic variation for HMW-GS in Indian wheat varieties. Sreeramulu and Singh (1994) evaluated 103 Indian wheat varieties for HMW-GS allelic combination, out of which 50 wheat varieties showed allelic combination of 2*(20 + 12)(7 + 8) and rest of the varieties showed 2*(2 + 12)(17 + 18) allelic combination of HMW-GS. Since Indian wheat is widely consumed in the form of chapattis and the breeding programs in India are broadly focused on the development of good chapattis, therefore, it is likely that these combinations may be selected for their favorable effect on quality and sensory attributes (Sreeramulu & Singh, 1994). Matuz (1998) reported that gluten quality and bread making properties of wheat flour were positively correlated with sedimentation value (SV) and solvent retention capacity (SRC). Wheat varieties with HMW-GS allelic combination of 2*(17 + 18) and (5 + 10) have higher SV as compared to 2*(2 + 12) and (17 + 18) HMW-GS subunit. In addition to SV, SRC and GI are also considered as important parameters for the initial evaluation of quality of wheat germplasm. At present the demand for wheat suitable for different products has increased, but the relationship of UnEx-PP, Ex-PP, UnEx-MP and Ex-MP as well as HMW-GS with physico-chemical, SV, SRC and protein characteristics of India wheat varieties is scarce. The objective of present study was to evaluate (i) diversity in UnEx-PP, Ex-PP, UnEx-MP and Ex-MP as well as HMW-GS among Indian wheat varieties. (ii) to see the relationships of UnEx-PP, Ex-PP, UnEx-MP and Ex-MP with flour characteristics.

2. Materials and methods

2.1. Materials

Twenty-eight wheat varieties namely, HD2329, HP2851, HD2894, WH542, HD3043, DPW621-50, WH1080, HD2733, HD 2824, HUW468, NW1012, Raj4120, K307, HP1761, DBW16, PBW373, WH1021, Raj3765, UP2425, WR544, HW2045, HUW 234, NW1014, NW2036, HP1744, PBW396, HDR77 and HW2004 were used for the study. These varieties have been released for cultivation during different years for one or the other of the six agro-climatic zones and different sowing conditions in India. The genotypes were grown at ICAR-Indian Agricultural Research Institute, New Delhi during 2013–14 crop season following standard agronomic practices and three random grain lots of 1000 gm each were drawn from the harvest providing three replications of each genotype. The three replications were subjected to quality analysis.

2.2. Grain characteristics

Various grain parameters such as thousand grain weight (TGW), grain diameter and grain hardness index (GHI), were analyzed with Single Kernel Characterization system (SKCS) 4100 (Perten Instruments, Australia) using AACC method 55-31 (2000). The average of 300 grains is reported. The moisture content of grains was ranged from 10% to 11%.

2.3. Wheat milling

The grains were conditioned for 48 h at 14% moisture and milled in the Quadrumat Senior mill (Brabender, Germany).

2.4. Flour characteristics

Color parameters (L^* , a^* and b^* values) of flour were determined using Ultra Scan VIS Hunter Lab (Hunter Associates Laboratory Inc, Reston, VA, USA). Ash content and protein contents were determined using AOAC (1990).

2.5. Sedimentation value

SV of flour was determined using the method of Axford, McDermott, and Redman (1979).

2.6. Protein characteristics

Total grain protein was measured using NIR of Foss Tecator, Sweden and expressed on as is basis.

SDS-extractable and un-extractable polymeric proteins (Ex-PP and UnEx-PP, respectively) were analyzed as described earlier by Gupta, Khan, and Macritchie (1993). Flour (10 mg) was suspended in 1 mL of 1% SDS and 0.1 M sodium phosphate buffer (pH 6.9) and stirred for 5 min using a pulsing vortex mixer. The mixture was centrifuged for 20 min at 12,000×g. The extractable protein was dissolved in supernatant and filtered through a 0.2 µm membrane filter. The un-extractable protein was obtained from the residue. The residues were sonicated for 60 s with 1 mL of extraction buffer. Then the mixture was centrifuged for 20 min at 12,000×g and the supernatant was filtered and 20 µL of sample was injected on HPLC (Agilent Technologies 1260 Infinity model) for analysis. SDS - extractable and un-extractable protein fractions were separated by a narrow bore column PROSEC 300S (300 × 7.5 mm). Eluting solution was 50% acetonitrile in water with 0.1% trifluoroacetic acid at a flow rate of 0.5 mL/min. Solutes were detected at 214 nm using a photodiode array detector.

2.7. Solvent retention capacity (SRC)

NaSRC, LASRC, SuSRC and WSRC tests were carried out by using sodium carbonate (5%), sucrose (50%), lactic acid (5%) and distilled water, respectively, as described in approved method 56-11 (AACC International, 2000). Flour (5.0 g) was poured in 50 ml Oakridge centrifuge tubes and 25 ml of an appropriate solvent was added. Centrifuge tubes containing suspension were vortexed for 20 min at RT with 1 min resting after every 5 min intervals followed by centrifugation at 3000 rpm for 10 min. Supernatant was discarded and wet pellet obtained was allowed to decant for 10 min and weighed.

2.8. Gluten content

Wet gluten, dry gluten and gluten index was analyzed using Glutomatic 2200 (Perten Instruments) according to AACC method 38-12A (2000).

2.9. SDS-PAGE analysis of gliadins and glutenins

Gliadins and glutenins were sequentially extracted from wheat flour as described earlier (Hou, Yamamoto, & Ng, 1996; Singh, Shepherd, & Cornish, 1991) respectively. Total gliadins and glutenins were estimated by CBBR-250 G dye binding assay as described by Bradford (1976). SDS-PAGE analysis of gliadins and glutenins were performed according to Laemmli (1970). Numbering and classification of gliadins were done according to Lagrain, Rombouts, Wieser, Delcour, and Koehler (2012), whereas, glutenins were classified according to Ram (2003) which was based on the methods of Lawrence and Payne (1983).

2.10. Statistical analysis

The data reported are average of three replications unless otherwise stated. Pearson correlation (r) and principal component analysis (PCA) were also carried out for determining the relationship between different variables using Minitab Release 14 Statistical Software (Soft College, PA, USA). The PCA results were graphically represented by the projection of the first two principal components using the same software.

3. Result and discussion

3.1. Grain characteristics

TGW and grain diameter of different varieties ranged from 29.11 to 48.35 g and 2.42 to 2.96 mm, respectively (Table 1). HP1761 showed the lowest TGW and grain diameter while UP2425 showed the highest. The varieties with higher grain diameter showed higher TGW and a strong correlation was observed between them ($r = 0.876$, $p \leq 0.005$). Similar correlation of TGW with grain diameter was reported earlier (Kaur et al., 2013). GHI ranged between 68 and 95 and was related to the milling performance of wheat varieties. GHI indicates the resistance of the grains to fracture as well as ability to produce flour of high quality. UP2425 and HP1744 showed lower GHI whereas K307, HD2851, NW1012, NW1014 and HW2004 showed higher value (90–95). GHI showed negative correlation with TGW and grain diameter ($r = -0.552$ and -0.464 , $p \leq 0.05$). PCA indicated that the varieties with lower grain weight and diameter had higher GHI might be due to the compact structured endosperm (Fig. 1).

3.2. Flour color characteristics

L^* , a^* and b^* values of flours from different varieties ranged from 92.12 to 94.58, 0.07 to 0.4 and 7.23 to 11.55, respectively (Table 2).

Table 1
Physical properties of grains from different wheat varieties.

Varieties	GHI	TGW (g)	Grain diameter (mm)
HD2329	89 ^{fg}	38.89 ^h	2.82 ^h
HD2851	90 ^{fg}	35.38 ^d	2.71 ^{fg}
HD2894	79 ^c	37.48 ^f	2.79 ^h
WH542	88 ^f	32.34 ^c	2.62 ^d
HD3043	89 ^f	31.56 ^c	2.63 ^d
DPW621-50	89 ^f	34.38 ^d	2.73 ^g
WH1080	80 ^{cd}	31.94 ^c	2.62 ^d
HD2733	83 ^e	35.34 ^{de}	2.77 ^{gh}
HD2824	80 ^{cd}	38.77 ^{gh}	2.88 ⁱ
HUW468	84 ^e	31.75 ^c	2.54 ^b
NW1012	94 ^h	30.91 ^b	2.63 ^d
Raj4120	89 ^f	34.17 ^d	2.6 ^c
K307	95 ^h	30.61 ^b	2.61 ^{cd}
HP1761	83 ^e	29.11 ^a	2.42 ^a
DBW16	77 ^c	39.5 ^h	2.82 ^h
PBW373	83 ^e	38.32 ^g	2.8 ^h
WH1021	78 ^c	38.21 ^g	2.76 ^g
Raj3765	81 ^{de}	43.15 ⁱ	2.91 ⁱ
UP2425	68 ^a	48.35 ^j	2.96 ^j
WR544	83 ^e	37.34 ^f	2.82 ^h
HW2045	85 ^e	40.14 ^h	2.7 ^f
HUW234	82 ^{de}	33.37 ^c	2.54 ^b
NW1014	92 ^g	33.49 ^c	2.61 ^{cd}
NW2036	78 ^c	36.62 ^{ef}	2.76 ^g
HP1744	72 ^b	36.21 ^e	2.83 ^h
PBW396	81 ^d	33.76 ^c	2.67 ^e
HDR77	83 ^e	39.48 ^g	2.78 ^g
HW2004	91 ^{fg}	38.68 ^g	2.83 ^h

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$).

GHI = grain hardness index; TGW = thousand grain weight.

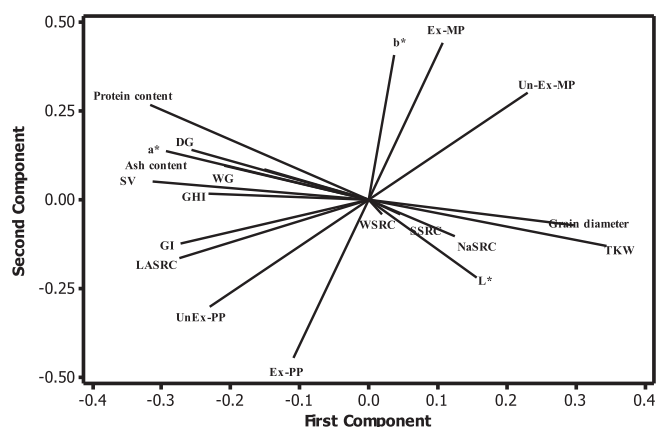


Fig. 1. Loading plot indicating the relationship of UnEx-PP and UnEx-MP with grain and flour characteristics of different wheat varieties.

DBW16, HD2851 and UP2425 showed higher L^* values (>94) and HUW468 and K307 showed the lower (92.12–92.21). L^* designated for lightness of flours. K307 showed the highest while NW2036 showed the lowest a^* value. a^* reflects redness to greenness of flours which means that flour having more a^* value showed greater redness. Kaur, Singh, Kaur, Ahlawat, and Singh (2014) reported L^* and a^* value ranged from 92.9 to 94.04 and 0.18 to 0.38, respectively for flours from Indian wheat varieties. The varieties with lower grain diameter and higher GHI showed higher a^* values. TGW and grain diameter showed negative correlation with a^* values ($r = -0.528$, $p \leq 0.005$ and -0.442 , $p \leq 0.05$, respectively). a^* was related to ash content of flours, as a significant correlation between them was observed ($r = 0.516$, $p \leq 0.005$). b^* reflects yellowness to blueness of flours. NW2036, PBW373, HD2824 and HW2004 showed higher b^* (>10.0) and HD2851 showed the lowest of 7.23. NW2036 flour showed more yellowness which might be due to presence of xanthophyll. PCA revealed that varieties with higher GHI resulted into flours with higher a^* and lower L^* value (Fig. 1). This reflected more contamination of flour with outer branny layers amongst varieties with higher GHI. The difference in color parameters of flours from different varieties might be due to the variation in xanthophyll, ash content and particle size distribution (Kaur et al., 2013).

3.3. Composition

Ash content of flours milled from different wheat varieties ranged from 0.31% to 0.50% (Table 2). UP2425 and HUW234 showed lower and DPW621-50 and WH542 showed higher ash content. Ash content of flour was reported to range between 0.29% and 0.57% for flour milled from hard winter wheat varieties by Xiao, Park, Chung, and Caley (2006). Singh et al. (2011) reported ash content of flours ranged from 0.49% to 0.61%. Ash content determines the contamination of flour with bran particles during milling. It also provides an estimate of the degree of separation of bran and germ from endosperm during milling, with the bran imparting a darker color to the products. In the present study also, the color of flours with higher ash content was darker as indicated by lower L^* values. a^* value of flour showed significant positive correlation with ash content ($r = 0.395$, $p \leq 0.05$). The brightness (L^*) was related inversely to ash and protein content. While redness (a^*) was significantly positively correlated with ash and protein content. This reflects that darker color of flour was due to higher bran contamination, since minerals are concentrated in branny layers. Yellowness (b^*) did not show significant correlation with ash and

Table 2
Physicochemical Composition and Solvent retention properties of flours from different wheat varieties.

Varieties	L^*	a^*	b^*	Ash content (%)	Protein content (%)	SV (ml)	NaSRC (%)	WSRC (%)	LASRC (%)	SuSRC (%)
HD2329	93.35 ^b	0.22 ^{de}	7.76 ^b	0.41 ^{bc}	10.38 ^d	32.50 ^{bc}	106.64 ^c	84.07 ^c	114.54 ^e	128.88 ^f
HD2851	94.21 ^{bc}	0.11 ^b	7.23 ^a	0.40 ^{bc}	10.38 ^d	35.50 ^c	98.13 ^c	83.15 ^c	125.00 ^f	122.11 ^{de}
HD2894	93.91 ^{bc}	0.15 ^c	8.63 ^d	0.37 ^b	11.08 ^e	40.00 ^e	92.32 ^{bc}	80.54 ^{bc}	112.95 ^e	127.53 ^{ef}
WH542	93.25 ^b	0.28 ^f	9.63 ^g	0.50 ^d	10.93 ^e	33.00 ^c	98.82 ^c	83.60 ^c	105.37 ^d	127.46 ^{ef}
HD3043	93.19 ^b	0.23 ^e	9.34 ^f	0.35 ^{ab}	12.35 ^g	36.50 ^c	114.09 ^c	88.51 ^d	114.65 ^e	134.12 ^g
DPW621-50	93.2 ^b	0.36 ^h	8.98 ^e	0.50 ^d	12.77 ^h	47.00 ^f	87.08 ^b	82.70 ^c	129.45 ^{fg}	119.86 ^d
WH1080	93.75 ^{bc}	0.33 ^g	8.01 ^c	0.48 ^d	12.55 ^h	48.50 ^f	85.48 ^b	72.93 ^{ab}	131.71 ^g	117.98 ^d
HD2733	93.5 ^b	0.30 ^f	9.73 ^g	0.48 ^d	12.48 ^{gh}	39.00 ^d	94.64 ^{bc}	81.95 ^c	103.74 ^d	111.93 ^{cc}
HD2824	92.88 ^{ab}	0.26 ^e	10.43 ^h	0.42 ^c	10.72 ^e	30.50 ^b	88.66 ^b	79.68 ^{bc}	97.73 ^{cd}	117.24 ^{dc}
HUW468	92.12 ^a	0.29 ^f	9.08 ^e	0.41 ^{bc}	11.46 ^f	31.00 ^b	95.31 ^c	75.37 ^{ab}	125.00 ^f	113.72 ^{cd}
NW1012	93.56 ^b	0.31 ^f	8.12 ^c	0.42 ^c	12.25 ^g	51.00 ^g	83.24 ^{ab}	82.13 ^c	127.00 ^f	104.13 ^b
Raj4120	93.54 ^b	0.25 ^e	7.99 ^{bc}	0.41 ^{bc}	10.86 ^e	33.00 ^c	92.08 ^{bc}	73.95 ^{ab}	101.99 ^d	107.65 ^{bc}
K307	92.21 ^a	0.40 ⁱ	10.01 ^g	0.43 ^c	11.36 ^f	34.25 ^c	90.05 ^{bc}	76.75 ^c	84.27 ^a	103.82 ^b
HP1761	93.44 ^b	0.25 ^e	9.42 ^f	0.35 ^{ab}	12.30 ^g	47.00 ^f	97.44 ^c	79.00 ^{ab}	109.40 ^e	124.82 ^e
DBW16	94.27 ^{bc}	0.16 ^c	7.84 ^b	0.39 ^b	8.89 ^a	28.25 ^a	92.92 ^{bc}	73.82 ^b	93.14 ^c	109.72 ^c
PBW373	93.57 ^b	0.16 ^c	10.36 ^h	0.44 ^c	11.15 ^e	33.50 ^c	94.94 ^{bc}	76.79 ^b	94.38 ^c	115.72 ^d
WH1021	93.88 ^{bc}	0.16 ^c	9.15 ^e	0.37 ^b	10.93 ^e	33.00 ^c	96.17 ^{bc}	76.77 ^b	94.87 ^c	118.40 ^d
Raj3765	93.81 ^{bc}	0.20 ^d	7.90 ^b	0.37 ^b	9.60 ^b	27.50 ^a	105.24 ^c	85.01 ^c	108.29 ^e	135.81 ^g
UP2425	94.58 ^c	0.14 ^c	8.09 ^c	0.31 ^a	10.57 ^d	31.50 ^b	88.89 ^b	77.00 ^b	95.20 ^c	106.46 ^{bc}
WR544	93.80 ^{bc}	0.23 ^e	7.91 ^b	0.37 ^b	11.81 ^f	34.00 ^c	98.15 ^c	80.00 ^{bc}	89.00 ^b	88.72 ^a
HW2045	93.96 ^{bc}	0.12 ^b	7.69 ^b	0.35 ^{ab}	10.12 ^c	36.00 ^c	105.20 ^c	90.03 ^d	107.38 ^e	119.19 ^d
HUW234	93.48 ^b	0.30 ^f	8.08 ^c	0.31 ^a	11.00 ^e	32.50 ^b	99.97 ^c	84.64 ^c	109.00 ^e	112.57 ^{cd}
NW1014	93.47 ^b	0.25 ^e	8.08 ^c	0.45 ^c	10.99 ^e	35.00 ^c	83.02 ^{ab}	73.60 ^{ab}	103.09 ^d	123.17 ^{de}
NW2036	93.05 ^{ab}	0.07 ^a	11.55 ⁱ	0.34 ^{ab}	11.09 ^e	32.75 ^b	78.07 ^a	71.77 ^a	101.34 ^d	119.48 ^d
HP1744	93.46 ^b	0.14 ^c	8.95 ^d	0.40 ^b	11.62 ^f	40.50 ^e	88.61 ^b	81.78 ^{bc}	97.71 ^{cd}	131.47 ^f
PBW396	93.68 ^b	0.22 ^{de}	7.77 ^b	0.34 ^{ab}	11.12 ^e	38.50 ^d	94.28 ^{bc}	73.86 ^{ab}	109.29 ^e	126.38 ^e
HDR77	92.64 ^{ab}	0.32 ^f	9.44 ^f	0.37 ^b	10.23 ^c	29.00 ^a	86.17 ^{ab}	76.06 ^{ab}	93.35 ^c	118.34 ^d
HW2004	93.15 ^{ab}	0.12 ^b	10.79 ^h	0.34 ^{ab}	10.84 ^d	35.50 ^c	102.15 ^c	86.47 ^{cd}	106.94 ^{de}	126.77 ^e

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$).

SV = sedimentation value; NaSRC = sodium carbonate solvent retention capacity; SuSRC = sucrose solvent retention capacity; LASRC = lactic acid solvent retention capacity; WSRC = water solvent retention capacity.

protein content. Ash content was correlated positively with protein content ($r = 0.356$, $p < 0.05$).

Protein content ranged between 8.89% and 12.77% (Table 2). DBW16 showed the lowest and DPW621-50 showed the highest value. Similar values of protein content were earlier reported for hard winter wheat (Xiao et al., 2006). Kaur et al. (2014) reported protein content ranging from 8.65% to 12.02%. Singh et al. (2011) reported that the protein content ranged from 8.26% to 12.85% in Indian varieties. While considering the wheat quality, the protein content is an important criterion. It is influenced by genetic as well as by non-genetic factors (Subda, 1991). Most of the varieties studied in present investigation had protein content $\geq 10\%$. Protein content of flour showed significant positive correlation with a^* value ($r = 0.438$, $p \leq 0.05$). Similar correlation was also reported earlier (Kaur et al., 2014). DPW621-50 with the highest protein content (12.77%) also had higher a^* value (0.36).

PCA reflected that GHI was related to flour protein and ash content, as all are located on same side of loading plot (Fig. 1). Varieties with higher GHI may have fragmented more during milling that led to more contamination of the aleurone layer causing increased protein and ash content of flour.

3.4. Sedimentation value

SV of flours from different wheat varieties ranged from 27.5 to 51 ml (Table 2). The highest value was observed for NW1012 and the lowest for RAJ3765. Kaur et al. (2013) also reported SV of flours from different Indian wheat varieties varied between 36 and 56 ml. SV indicated the quantity and quality of wheat flour protein (Carter, Morris, & Anderson, 1999). Protein content of flour showed a strong positive relation with SV ($r = 0.739$, $p \leq 0.005$). NW1012 showed the highest SV (51 ml) showing protein content of 12.25% whereas RAJ3765 with the lowest SV (27.5 ml) had lower protein content (9.60%). SV in SDS solution was related to swelling

of glutenins strand (Eckert, Amend, & Belitz, 1993) and it was found that flours with higher strength of gluten had more swelling. In earlier studies, SV were found to be significantly correlated with glutenin content and the glutenin to gliadin ratio (Gill-Humanes, Piston, Rosell, & Barro, 2012).

3.5. Protein characteristics

The proportions of various protein fractions from flours milled from different wheat varieties are reported in Table 3. The proportion of Ex-PP and Ex-MP ranged from 19.32% to 38.75% and 61.25% to 80.68%, respectively. NW2036 showed the lowest proportion of Ex-PP (19.32%) and the highest proportion of Ex-MP (80.68%) while HD2329 showed the least proportion of Ex-MP and the highest proportion of Ex-PP of 61.25% and 38.75%, respectively. The proportion of UnEx-PP and UnEx-MP for different varieties ranged from 23.83% to 51.97% and 48.03% to 76.17%, respectively. The proportion of Ex-PP, Ex-MP, UnEx-PP and UnEx-MP on average basis was reported to be 46%, 54%, 62% and 38%, respectively for bread wheat by Gupta et al. (1993). HUW468 showed the highest proportion of UnEx-PP ($\approx 52\%$) and the lowest proportion of UnEx-MP ($\approx 48\%$). WH542 showed the highest proportion of UnEx-MP ($\approx 76\%$) and the lowest proportion of UnEx-PP ($\approx 24\%$). Ex-PP showed highly significant negative correlation with Ex-MP ($r = -0.997$, $p \leq 0.005$). The proportion of Ex-PP and Ex-MP ranged from 28.14% to 40.44% and from 45.33% to 55.83%, respectively were reported earlier for Indian wheat varieties (Singh et al., 2011). Differences in extractability of PP in the dough from different varieties were attributed to the differences in degree of polymerization that resulted in the availability of varied amount of cysteine residues. Increase in availability of cysteine residues resulted into polymerization of proteins, increasing the size and decreasing the solubility (Don, Lookhart, Naeem, MacRitchie, & Hamer, 2005). The amount of Un-ExPP was reported to be

Table 3

Gluten index and protein characteristics of flour from different wheat varieties.

Varieties	GI	WG (%)	DG (%)	Ex-PP (%)	Ex-MP (%)	UnEx-PP (%)	Un-Ex-MP (%)
HD2329	87 ⁱ	30.46 ^d	10.20 ^d	38.75 ⁱ	61.25 ^a	46.90 ^h	53.10 ^b
HD2851	97 ^k	27.57 ^b	9.71 ^d	33.45 ^g	66.55 ^a	35.58 ^e	64.42 ^e
HD2894	72 ^f	32.94 ^e	11.09 ^e	35.97 ^h	64.03 ^{ab}	45.64 ^{gh}	54.36 ^b
WH542	64 ^e	26.70 ^b	9.02 ^c	22.55 ^b	75.45 ^d	23.83 ^a	76.17 ^h
HD3043	70 ^f	27.48 ^{bc}	11.91 ^f	23.63 ^b	76.37 ^d	31.72 ^c	68.28 ^{fg}
DPW621-50	98 ^k	33.43 ^e	11.81 ^f	24.23 ^{bc}	75.77 ^d	45.81 ^h	54.19 ^b
WH1080	99 ^k	30.04 ^d	11.82 ^f	35.71 ^h	64.29 ^a	43.63 ^g	56.37 ^c
HD2733	61 ^d	36.70 ^f	11.793 ^f	21.8 ^{ab}	78.20 ^{de}	30.43 ^c	69.57 ^g
HP2824	60 ^d	33.17 ^e	11.16 ^{ef}	24.4 ^{bc}	75.60 ^d	47.70 ^h	52.30 ^b
HUW468	97 ^k	31.06 ^d	11.24 ^{ef}	33.96 ^g	66.04 ^{ab}	51.97 ⁱ	48.03 ^a
NW1012	100 ^k	30.07 ^d	10.79 ^e	28.61 ^e	71.39 ^{bc}	50.53 ⁱ	49.47 ^a
Raj4120	77 ^g	30.32 ^d	10.14 ^d	29.20 ^e	70.80 ^{bc}	48.68 ^h	51.32 ^b
K307	52 ^b	33.89 ^e	11.23 ^{ef}	32.90 ^g	67.10 ^{ab}	33.20 ^d	66.80 ^f
HP1761	85 ⁱ	34.48 ^e	11.52 ^f	30.55 ^{ef}	69.45 ^b	30.98 ^c	69.02 ^g
DBW16	60 ^d	21.58 ^a	6.94 ^a	34.12 ^{gh}	65.88 ^{ab}	37.67 ^{ef}	62.33 ^{de}
PBW373	73 ^f	28.42 ^c	9.65 ^d	23.06 ^b	76.94 ^d	30.29 ^c	69.71 ^g
WH1021	49 ^a	33.08 ^e	10.94 ^e	25.25 ^c	74.75 ^{cd}	28.21 ^b	71.79 ^g
Raj3765	61 ^d	26.82 ^b	8.95 ^c	29.77 ^e	70.23 ^{bc}	32.87 ^d	67.13 ^f
UP2425	61 ^{de}	30.45 ^d	11.02 ^e	23.49 ^b	76.51 ^d	30.36 ^c	69.64 ^g
WR544	78 ^g	31.42 ^d	10.02 ^d	31.43 ^f	68.57 ^b	39.39 ^f	60.61 ^{cd}
HW2045	52 ^b	37.50 ^f	10.85 ^e	31.66 ^f	68.35 ^b	41.31 ^f	58.69 ^{cd}
HUW234	82 ^h	30.09 ^{cd}	10.62 ^e	27.89 ^d	72.11 ^c	41.83 ^f	58.17 ^{cd}
NW1014	91 ^j	28.90 ^c	10.31 ^{de}	28.77 ^d	71.23 ^{bc}	49.68 ^h	50.32 ^{ab}
NW2036	100 ^k	22.67 ^a	8.36 ^b	19.32 ^a	80.68 ^e	37.14 ^e	62.86 ^e
HD1744	64 ^e	33.71 ^e	11.22 ^e	35.47 ^h	64.53 ^a	39.78 ^f	60.22 ^d
PBW376	57 ^c	32.84 ^e	10.47 ^e	23.57 ^b	76.43 ^d	40.45 ^f	59.55 ^d
HDR77	62 ^{de}	30.59 ^d	10.17 ^{de}	28.04 ^d	71.96 ^{bc}	43.61 ^g	56.39 ^c
HW2004	52 ^b	30.49 ^d	10.42 ^e	27.48 ^d	72.52 ^c	39.92 ^f	60.08 ^d

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$).

WG = wet gluten; DG = dry gluten; GI = gluten index; Ex-PP = extractable polymeric protein; Ex-MP = extractable monomeric protein; UnEx-PP = unextractable polymeric protein; UnEx-MP = unextractable monomeric protein.

influenced by different quaternary structures that result from polymers involving disulphide bridges (Gobin, Ng, Buchanan, & Kobrehel, 1997; Rhazi, Cazalis, Lemelin, & Aussenac, 2003) and aggregates of different size (Aussenac, Carceller, & Kleiber, 2001). The loading plot indicates that varieties with higher GHI have higher UnEx-PP and lower UnEx-MP (Fig. 1).

3.6. Solvent retention capacity

NaSRC, SuSRC, LASRC and WSRC ranged from 78.07% to 114.09%, 88.72% to 135.82%, 84.27% to 131.71% and 71.77% to 90.03%, respectively (Table 2). NW2036 showed the lowest WSRC and NaSRC whereas HD3043 and HW2045 showed higher NaSRC and WSRC. WR544 showed the lowest SuSRC and Raj3765 showed the highest. K307 showed the lowest LASRC while NW1012 and WH1080 showed higher value (~132%). WSRC, NaSRC, SuSRC and LASRC value from 56% to 66%, 74% to 88%, 90% to 102% and 106% to 147%, respectively were reported earlier for European wheat varieties (Duyvejonck, Lagrain, Dornez, Delcour, & Courtin, 2012). NaSRC, LASRC and SuSRC between 62.23% and 91.35%, 74.35% and 139.73% and 82.67% and 122.29%, respectively for Argentina's soft wheat advance lines and cultivars were reported (Moiraghi et al., 2011). SuSRC ranged between 125% and 163% and LASRC ranged between 101.5% and 139% for fifty Pakistan's cultivars (Pasha, Anjum, & Butt, 2009). NaSRC, LASRC, SuSRC and WSRC were reported to be ranged from 111% to 168%, 84% to 143%, 111% to 168%, 81% to 124%, respectively for Indian wheat varieties (Kaur et al., 2014). NaSRC values were the indicator of the damage starch content of flours. A strong correlation of NaSRC with WSRC ($r = 0.796$, $p \leq 0.05$) and SuSRC ($r = 0.339$, $p \leq 0.05$) was observed. Similar correlation of NaSRC with WSRC and SuSRC was reported earlier (Pareyt & Delcour, 2008). LASRC showed significant correlation with the protein content ($r = 0.342$, $p \leq 0.05$), consistent with the earlier reported results

(Xiao et al., 2006). A positive correlation was observed between LASRC and SV of flour ($r = 0.542$, $p \leq 0.05$) and both were also correlated with protein content of flour. Guttieri, Bowen, Gannon, O'Brien, and Souza (2001) did not observe any relationship between the protein content and the glutenin composition, however, a relationship between the protein content and LASRC was observed. LASRC measurements have been related to the glutenin characteristics of flour (Gaines, 2000). The flours from varieties with higher LASRC may be attributed to greater swelling of glutenins that forms extensive gluten network. PCA indicated positive correlation of UnEx-PP and negative correlation of UnEx-MP with LASRC (Fig. 1). The r values of UnEx-PP and UnEx-MP with LASRC were 0.390 ($p \leq 0.05$) and -0.390 ($p \leq 0.05$), respectively.

3.7. Gluten characteristics

GI is an indicator of the quantity of polydisperse polymer present in developed gluten (Edwards et al., 2007). In the present study, GI ranged from 49 to 100 (Table 2). NW1012, NW2036, WH1080 and DPW621-50 showed higher while WH1021, HW2004, K307 and HW2045 showed the lower GI. GI showed strong positive correlation with LASRC ($r = 0.653$, $p \leq 0.05$). WG ranged from 21.58% to 37.5% (Table 2), DBW16 showed the lowest while HW2045 showed the highest value. DG ranged between 6.94% and 11.94% (Table 2), DBW16 showed the lowest while HD3043, DPW621-50 and WH1080 showed higher value. A positive correlation was found between WG and DG ($r = 0.779$, $p \leq 0.005$). Similar correlation between WG and DG was reported earlier by Zaidel, Chin, Yusof, and Abd Rahman (2009), Baslar and Ertugay (2011) and Desheva, Valchinova, Kyosev, and Stoyanova (2014). Protein content showed significant positive correlation with GI, WG and DG ($r = 0.403$, 0.442 and 0.753, respectively, $p \leq 0.005$). Kaur et al. (2013) reported similar results for WG and DG. GI, WG and DG showed positive correlation with SV

($r = 0.435$ and 0.377 respectively, $p \leq 0.05$; $r = 0.555$, $p \leq 0.005$). The loading plot (Fig. 1) shows a positive correlation of GI with Un-ExPP and negative correlation with Un-ExMP ($r = 0.472$ and -0.472 , respectively, $p \leq 0.05$). Earlier studies have also shown that variation in GI is related to unextractable glutenin content and gliadin to glutenin ratio (Sissons, Ames, Hare, & Clarke, 2005).

3.8. SDS-PAGE analysis of gliadins and glutenins

Protein profiling of gliadins by SDS-PAGE showed presence of 10 polypeptides (PPs) ranging from 25 kDa to 90 kDa. The accumulation of these PPs was differential and variety dependent (Fig. 2). NW1012 and RAJ4120 showed presence of a 90 kDa PP that was absent in other varieties. A 62 kDa PP was accumulated in HD2851, HUW468 and HP1761. Two other PPs of 81 kDa and 70 kDa showed varied banding pattern and storage of 81 PPs was higher in HD2894, HW1080, NW1012, Raj4120, HP1761, DBW16, Raj3765, HW2045, and HDR77. On the contrary, 70 kDa PPs was present in higher quantity in DPW-621-50, WH1080, HP1744, PBW396, HW2004 and HDR77. Accumulation of 50 kDa PP was significantly lower or absent in WH542, HD3043, DPW621-50, HD2733, HD2824, K307, and PBW396, whereas DBW16, PBW373, WH1021, HDR77 and HW2004 showed accumulation of 48 kDa and 50 kDa, instead of only 50 kDa PPs. Other PPs of 41 kDa, 36 kDa and 33 kDa were present in all varieties, however, intercultivar varied accumulation of these PP was also observed. SDS-PAGE analysis of glutenins resulted in visualization of 15 PPs ranging from 29 kDa to 91 kDa. These PP were classified into three major groups (i) High molecular weight glutenin subunits (HMW-GS) of 84 kDa, 80 kDa, 78 kDa and 74 kDa; (ii) low molecular weight glutenin subunits-basic (LMW-B) of 44 kDa, 41 kDa and 40 kDa, and (iii) low molecular weight glutenin subunits-C (LMW-C) of 39 kDa and 36 kDa, 35 kDa, 34 kDa, 32 kDa, 30 kDa and 29 kDa) as per molecular weight. All the varieties showed different

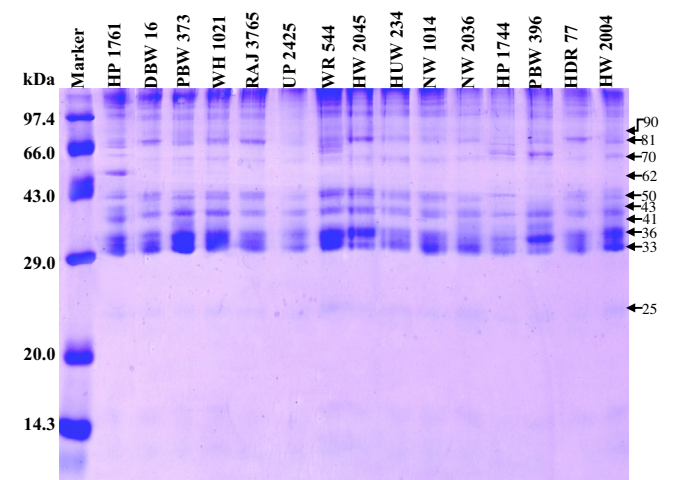


Fig. 2a. SDS-PAGE analysis of ethanol solubilized gliadins of Indian wheat varieties under non-reducing conditions. Flour (20 mg) was weighed and poured into sterile micro centrifuge tubes (MCT) and albumins were removed by three washings of flour with 1.0 ml of 400 mM NaCl each for 5 min at room temperature (RT). Gliadins were extracted twice from albumin free flour by using 60% ethanol at 40 °C. Equal volume of gliadins and 2× sample buffer (62.5 mM Tris-HCl, pH 8.0, 4% SDS, 40% glycerol and 0.04% bromophenol blue) were mixed together and loaded on the wells of SDS-PAGE gel. 10% resolving and 5% stacking gels were prepared according to Laemmli (1970). The electrophoresis was carried out at 120 volt for stacking and 25 mA for resolving gel followed by staining of the resolved gliadins with 5% ethanol +12% trichloro acetic acid and 0.2% coomassie brilliant blue R-250 staining solution. The gels were washed using deionized water before acquiring images on scanner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

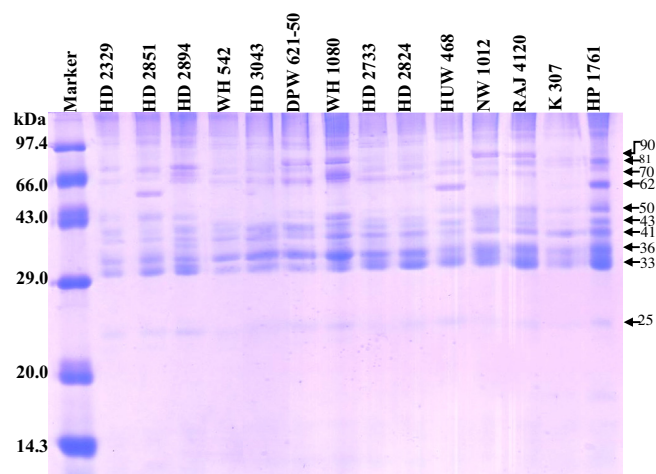


Fig. 2b. Non-reducing SDS-PAGE analysis of ethanol soluble gliadins of different Indian wheat varieties.

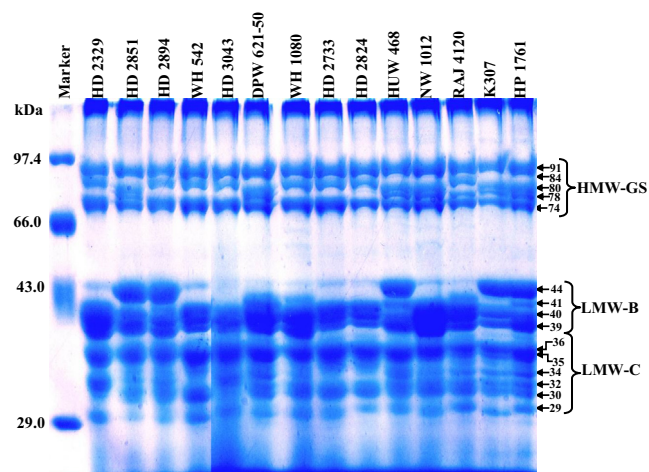


Fig. 2c. SDS-PAGE analysis of reduced and alkylated glutenins from different Indian wheat varieties. Glutenins were extracted from flour as described by Singh, Shepherd and Cornish (1991) and mixed with equal volume of 2× sample buffer (62.5 mM Tris-HCl, pH 8.0, 4.0% sodium dodecyl sulfate, 40% glycerol and 0.04% bromophenol blue) and loaded on the wells of stacking gels. 5% stacking and 10% resolving gels were prepared according to Laemmli (1970). The electrophoretic separation of proteins was accomplished as described in Fig. 2a. Resolved gels were stained with 50% methanol, 12% acetic acid and 0.2% Coomassie brilliant blue R-250 staining solution. The stained gels were de-stained using a solution comprising of 50% methanol and 12% glacial acetic acid and images of the stained gels were acquired on scanner. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

banding pattern for HMW-GS, LMW-B and LMW-C glutenin subunits. HD2329, HD2894, WH542, HD3043, WH1080, HD2733, HD2824, PBW373, UP2425, NW2036, HP1744, PBW396 and HW2004 showed presence of 91 kDa, 81 kDa, and 74 kDa PPs, whereas, HD2851, PBW621-50, HUW468, NW1012, K307, HP1761 and DL153-2 showed accumulation of 91 kDa, 80 kDa, 78 kDa and 74 kDa PPs. Contrary to these varieties, RAJ4120, DBW16, RAJ3765, HW2045, HUW234, NW1014 and HDR77 showed presence of 91 kDa, 84 kDa, 78 kDa and 74 kDa PPs. A significant inter-variety variation for LMW-B and LMW-C subunits at PPs levels was observed. HD2851, HD2894, HUW468, K307, HP1761, UP2425, HUW234, HP1744 and HW2004 showed higher accumulation of 44 kDa PP, whereas higher accumulation of 40 kDa, 38 kDa, and 37 kDa PPs was depicted in HD2329, WH542, DPW621-50, WH1080, NW1012, RAJ4120, NW1014,

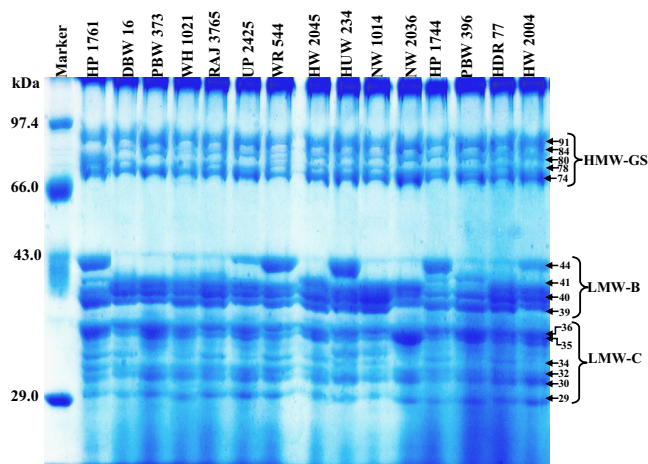


Fig. 2d. SDS–PAGE analysis of reduced and alkylated glutenins from different Indian wheat varieties.

HDR77 and HW2004. Accumulation 36 kDa, 35 kDa, 34 kDa, 32 kDa and 29 kDa PPs was varietal dependent and WH542, PBW550, HD3043, DPW621-50, WH1080, HD2733, HD2824, HUW468, PBW373, NW2036, PBW 396 and HW2004 showed lesser accumulation or absence of 36 kDa and 35 kDa PPs. Although 30 kDa and 29 kDa PPs were present in all varieties but storage of these PPs was also varietal dependent and these were classified as LMW-C glutenin subunits.

Based on studies carried out by Ram (2003) the HMW-GS architecture of all the wheat varieties were predicted in present study. HMW-GS combination of PPs 91 kDa + 80 kDa + 78 kDa + 74 kDa, 91 kDa + 84 kDa + 78 kDa + 74 kDa and 91 kDa + 84 kDa + 74 kDa belonged to $2^*(2+12)(13+16)$, $2^*(2+12)(7+8)$ and $2^*(2+12)(7+9)$, respectively. Whereas, PPs of 91 kDa + 84 kDa + 72 kDa

+ 74 kDa and 91 kDa + 80 kDa + 78 kDa + 74 kDa represented $1(5+10)(7+9)$ (Table 4). The relationship between different molecular weight HMW-GS and different locus is described in (Table 4). Wheat varieties with HMW-GS combination of 91 kDa + 80 kDa + 78 kDa + 74 kDa PPs showed very high GI (97–100) except HP1761 which showed GI value of 85. Other combination of HMW-GS combinations of 91 kDa + 84 kDa + 74 kDa PPs showed lower value of GI except WH1080 and NW2036 which had significantly higher values of GI (99–100).

4. Conclusion

Both UnEx-PP and UnEx-MP, HMW-GS as well as various wheat flour properties showed wide variation amongst Indian wheat varieties. These traits showed varied association with each other. Wheat varieties could be classified into distinct groups based on the types of UnEx-PP and UnEx-MP. The HMW-GS combinations of 91 kDa + 80 kDa + 78 kDa + 74 kDa PPs produced very high GI (97–100). Similarly, UnEx-PP showed positive correlation with GI and LASRC. Thus, GI may be useful in determining gluten strength.

Acknowledgements

NS acknowledges Ministry of Food Processing Industry (MOFPI), India for providing funds in the form of a research project. MK acknowledges UGC-BSR for providing financial assistance in the form of Fellowship. We are grateful to Dr. Howard G. Ohlhausen, Director, UNELKO CORPORATION, 14641 N 74th St, Scottsdale, AZ 85260, USA for providing us Rain Clear[®] Liquid and Repel[™] Windshield Wash Fluid Concentrate for the modification of surface of the glass plates for Acidic–PAGE and SDS–PAGE analysis of Gliadins.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.foodchem.2015.07.125>.

Table 4

Allele combination and HMW Glutenin subunits in flour from different wheat varieties.

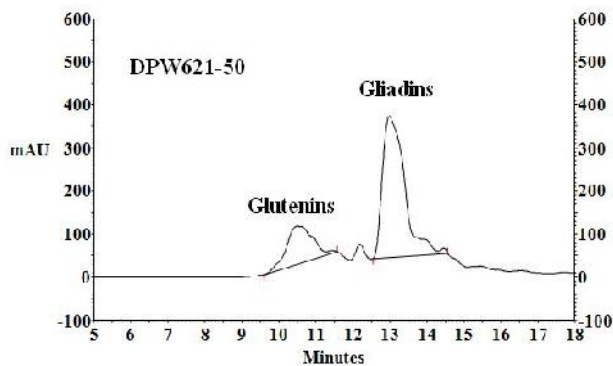
Varieties	Glu(A1)(D1)(B1)	Combination of HMW-GS PPs (MW)
HD2329	$2^*(2+12)(7+9)$	91 + 84 + 74
HD2851	$2^*(2+12)(13+16)$	91 + 80 + 78 + 74
HD2894	$2^*(2+12)(7+9)$	91 + 84 + 74
WH542	$2^*(2+12)(7+9)$	91 + 84 + 74
HD3043	$2^*(5+10)(7+9)$	91 + 84 + 74
DPW621-50	$2^*(2+12)(13+16)$	91 + 80 + 78 + 74
WH1080	$2^*(2+12)(7+9)$	91 + 84 + 74
HD2733	$2^*(2+12)(7+9)$	91 + 84 + 74
HD2824	$N(2+12)(20)$	91 + 84 + 74
HUW468	$2^*(2+12)(13+16)$	91 + 80 + 78 + 74
NW1012	$2^*(2+12)(13+16)$	91 + 80 + 78 + 74
Raj4120	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
K307	$2^*(2+12)(13+16)$	91 + 80 + 78 + 74
HP1761	$2^*(2+12)(13+16)$	91 + 80 + 78 + 74
DBW16	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
PBW373	$1(5+10)(7)$	91 + 84 + 74
WH1021	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
Raj3765	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
UP2425	$1(5+10)(17+18)$	91 + 84 + 74
WR544	NA	91 + 84 + 80 + 78 + 74
HW2045	$1(5+10)(7+9)$	91 + 84 + 72 + 74
HUW234	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
NW1014	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
NW2036	$2^*(2+12)(7+9)$	91 + 84 + 74
HD1744	$2^*(2+12)(7+9)$	91 + 84 + 74
PBW396	$1(5+10)(7+9)$	91 + 84 + 72 + 74
HDR77	$2^*(2+12)(7+8)$	91 + 84 + 78 + 74
HW2004	$N(2+12)(20)$	91 + 84 + 74

NA = available.

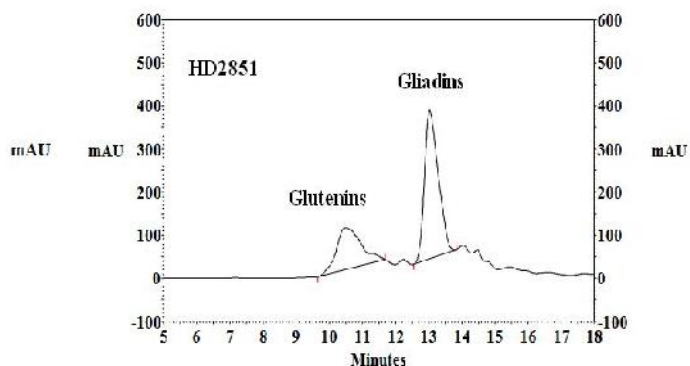
References

- AACC International (2000). *Approved methods of American association of cereal chemists* (10th ed.). St. Paul, MN: AACC International Press.
- AOAC (1990). *Official methods of analysis* (15th ed.). Washington, DC: Association of Official Analytical Chemists.
- Aussenac, T., Carceller, J. L., & Kleiber, D. (2001). Change in SDS-solubility of glutenin polymers during dough mixing and resting. *Cereal Chemistry*, 78, 39–45.
- Axford, D. W. E., McDermott, E. E., & Redman, D. G. (1979). Note on the sodium dodecyl sulfate test of breadmaking quality: Comparison with pelshenke and zeleny tests. *Cereal Chemistry*, 56, 582–584.
- Baslar, M., & Ertugay, M. F. (2011). Determination of protein and gluten quality-related parameters of wheat flour using near-infrared reflectance spectroscopy (NIRS). *Turkish Journal of Agriculture and Forestry*, 35, 139–144.
- Bradford, M. M. (1976). A rapid and sensitive for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry*, 72, 248–254.
- Branlard, G., & Dardevet, M. (1985a). Diversity of grain protein and bread quality. I. Correlation between gliadin bands and technological characteristics. *Journal of Cereal Science*, 3, 329–343.
- Branlard, G., & Dardevet, M. (1985b). Diversity of grain protein and bread quality. II. Correlation between high molecular weight subunits of glutenin and flour quality characteristics. *Journal of Cereal Science*, 3, 345–354.
- Carter, B. P., Morris, C. F., & Anderson, J. A. (1999). Optimizing the SDS sedimentation test for end use quality selection in a soft white and club wheat breeding program. *Cereal Chemistry*, 76, 907–911.
- Desheva, G., Valchinova, E., Kyosev, B., & Stoyanova, S. (2014). Grain physical characteristics and bread-making quality of alternative cereals towards common and durum wheat. *Emirates Journal of Food and Agriculture*, 26, 418–424.
- Don, C., Lookhart, G., Naem, H., MacRitchie, F., & Hamer, R. J. (2005). Heat stress and genotype affect the glutenin particles of the glutenin macropolymer-gel formation. *Journal of Cereal Science*, 42, 69–80.

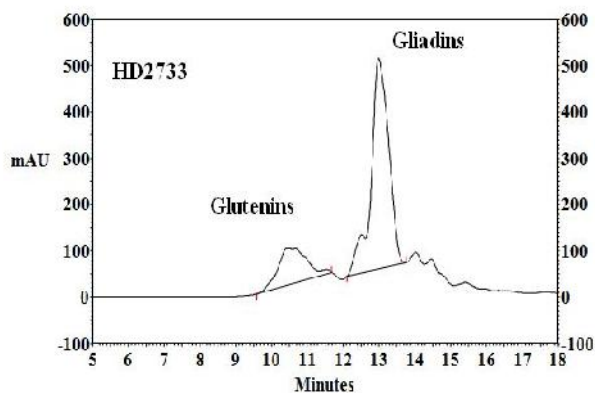
- Duyvejonck, A. E., Lagrain, B., Dornez, E., Delcour, J. A., & Courtin, C. M. (2012). Suitability of solvent retention capacity tests to assess the cookie and bread making quality of European wheat flours. *Journal of Food Science and Technology*, 47, 56–63.
- Eckert, B., Amend, T., & Belitz, H. D. (1993). The course of SDS and Zeleny sedimentation tests for gluten quality and related phenomena studied using the light microscope. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 196, 122–125.
- Edwards, N. M., Gianibelli, M. C., McCaig, T. N., Clarke, J. M., Ames, N. P., Larroque, O. R., & Dexter, J. E. (2007). Relationship between dough strength, polymeric protein quantity and composition for diverse durum wheat genotypes. *Journal of Cereal Science*, 45, 140–149.
- Fido, R. J., Bekes, F., Gras, P. W., & Tatham, A. S. (1997). Effects of α -, β -, γ - and ω -gliadins on the dough mixing properties of wheat flour. *Journal of Cereal Science*, 26, 271–277.
- Gaines, C. S. (2000). Collaborative study of methods for solvent retention capacity profiles (AACC method 56-11). *Cereal Foods World*, 45, 303–306.
- Gill-Humanes, J., Piston, F., Rosell, C. M., & Barro, F. (2012). Significant down-regulation of γ -gliadins has minor effect on gluten and starch properties of bread wheat. *Journal of Cereal Science*, 56, 161–170.
- Gobin, P., Ng, P. K. W., Buchanan, B. B., & Kobrehel, K. (1997). Sulphydryl-disulphide changes in proteins of developing wheat. *Plant Physiology and Biochemistry*, 35, 777–783.
- Gupta, R. B., Khan, K., & Macritchie, F. (1993). Biochemical basis of flour properties in Bread wheats. 1. Effects of variation in the quantity and size distribution of polymeric protein. *Journal of Cereal Science*, 18, 23–44.
- Guttieri, M. J., Bowen, D., Gannon, D., O'Brien, K., & Souza, E. (2001). Solvent retention capacities of irrigated soft white spring wheat flours. *Crop Science*, 41, 1054–1061.
- Hou, G., Yamamoto, H., & Ng, P. K. W. (1996). Relationships of quantity of gliadin subgroups of selected U.S. soft wheat flours to rheological and baking properties. *Cereal Chemistry*, 73, 352–357.
- Kaur, A., Singh, N., Ahlawat, A. K., Kaur, S., Singh, A. M., Chauhan, H., & Singh, G. P. (2013). Diversity in grain, flour, dough and gluten properties amongst Indian wheat cultivars varying in high molecular weight subunits (HMW-GS). *Food Research International*, 53, 63–72.
- Kaur, A., Singh, N., Kaur, S., Ahlawat, A. K., & Singh, A. M. (2014). Relationships of flour retention capacity, secondary structure and rheological properties with the cookie making characteristics of wheat cultivars. *Food Chemistry*, 158, 48–55.
- Kohler, P., Belitz, H. D., & Wieser, H. (1993). Disulphide bonds in wheat gluten: Further cystine peptides from high molecular weight (HMW) and low molecular (LMW) subunits of glutenin and from γ -gliadins. *Zeitschrift für Lebensmittel-Untersuchung und -Forschung*, 196, 239–247.
- Laemmli, U. K. (1970). Cleavage of structural protein during the assembly of the head of bacteriophage T4. *Nature*, 227, 680–685.
- Lagrain, B., Rombouts, I., Wieser, H., Delcour, J. A., & Koehler, P. (2012). A reassessment of the electrophoretic mobility of high molecular weight glutenin subunits of wheat. *Journal of Cereal Science*, 56, 726–732.
- Lawrence, G. J., & Payne, P. I. (1983). Detection by gel electrophoresis of oligomers formed by the association of high-molecular-weight glutenin protein subunits of wheat endosperm. *Journal of Experimental Botany*, 34, 254–267.
- Matuz, J. (1998). Inheritance of SDS sedimentation volume of flour in crosses of winter wheat (*Triticum aestivum* L.). *Cereal Research Communications*, 26, 203–210.
- Moiraghi, M., Vanzetti, L., Bainotti, C., Helguera, M., Leon, A., & Perez, G. (2011). Relationship between soft wheat flour physicochemical composition and cookie-making performance. *Cereal Chemistry*, 88, 130–136.
- Pareyt, B., & Delcour, J. A. (2008). The role of wheat flour constituents, sugar and fat in low moisture cereal based products: A review on sugar-snap cookies. *Critical Reviews in Food Science and Nutrition*, 48, 1–16.
- Pasha, I., Anjum, F. M., & Butt, M. S. (2009). Genotypic variation of spring wheats for solvent retention capacities in relation to end-use quality. *Journal of Food Science and Technology*, 42, 418–423.
- Payne, P. I., Harris, P. A., Law, C. N., Holt, L. M., & Blackman, J. A. (1980). The high-molecular-weight subunits of glutenin: Structure, genetics and relationships to bread-making quality. *Annales de Technologie Agricole*, 29, 309–320.
- Ram, S. (2003). High molecular weight glutenin subunit composition of Indian wheats and their relationships with dough strength. *Journal of Plant Biochemistry and Biotechnology*, 12, 151–155.
- Rhazi, L., Cazalis, R., Lemelin, E., & Aussenac, T. (2003). Changes in the glutathione thiol-disulfide status during wheat grain development. *Plant Physiology Biochemistry*, 41, 895–902.
- Shewry, P. R., Tatham, A. S., Forde, J., Kreis, M., & Mifflin, B. J. (1986). The classification and nomenclature of wheat gluten proteins: A reassessment. *Journal of Cereal Science*, 4, 97–106.
- Singh, N. K., Shepherd, K. W., & Cornish, G. B. (1991). A simplified SDS-PAGE procedure for separating LMW subunits of glutenin. *Journal of Cereal Science*, 14, 203–208.
- Singh, S., Singh, N., & MacRitchie, F. (2011). Relationship of polymeric proteins with pasting, gel dynamic- and dough empirical-rheology in different Indian wheat varieties. *Food Hydrocolloids*, 25, 19–24.
- Sissons, M. J., Ames, N. P., Hare, R. A., & Clarke, J. M. (2005). Relationship between glutenin subunit composition and gluten strength measurements in durum wheat. *Journal of the Science and Food Agriculture*, 85, 2445–2452.
- Sozinov, A. A., Poperelya, F. A., & Stacanova, A. I. (1974). Use of electrophoresis of gliadin for selection of wheat by quality (in Russian) *Vestnik Navki*, 7, 99–108.
- Sreeramulu, G., & Singh, N. K. (1994). High Mr glutenin subunits of Indian wheat cultivars: Association of subunits 5+10 with the 1BL/1RS wheat-rye translocation. *Journal of Cereal Science*, 20, 217–225.
- Subda, H. (1991). Biochemical and technological characteristics of spring and winter wheat I. Protein content and quality. *Hodowla-Roslin-Akli, atyczacja-i-Nasiennic*, 35, 69–82.
- Xiao, Z. S., Park, S. H., Chung, O. K., & Caley, M. S. (2006). Solvent retention capacity values in relation to hard winter wheat and flour properties and straight-dough bread making quality. *Cereal Chemistry*, 83, 465–471.
- Zaidel, A. D. N., Chin, N. L., Yusof, Y. A., & Abd Rahman, R. (2009). Analysis and correlation studies on gluten quantity and quality during production. *Journal of Applied Science*, 9, 1686–1694.



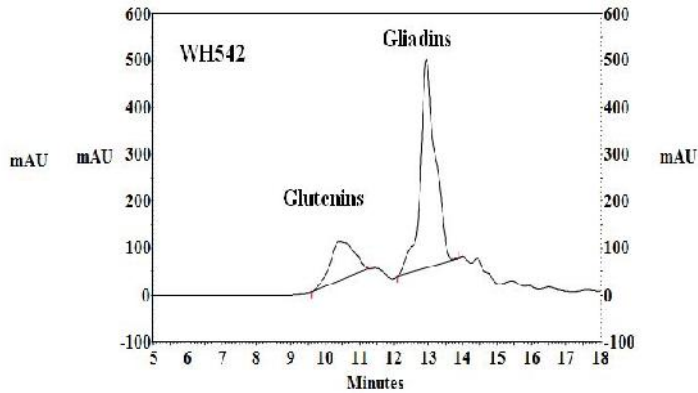
DPW621-50
2*(2+12)(13+16)



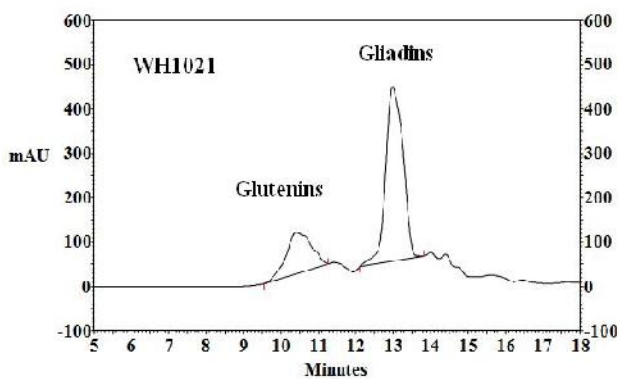
HD2851
2*(2+12)(13+16)



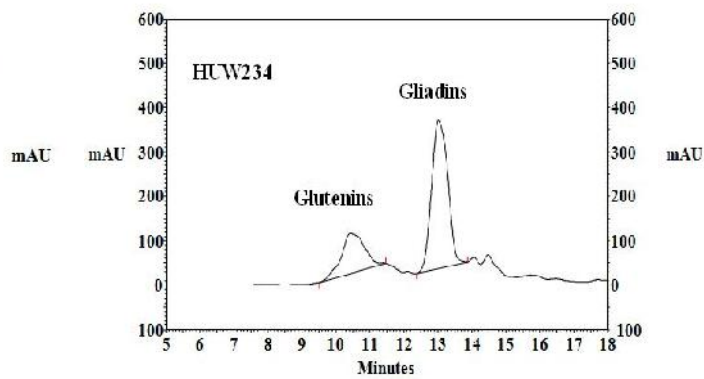
HD2733
2*(2+12)(7+9)



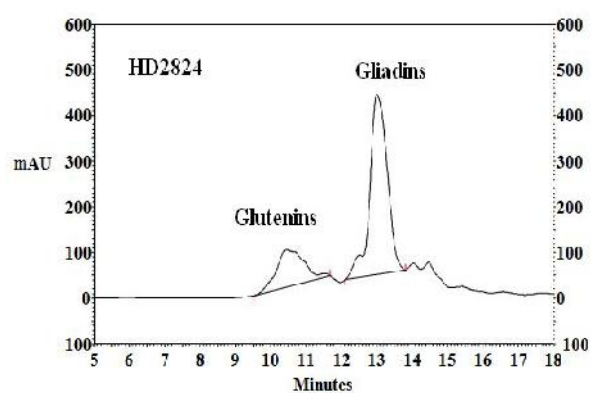
WH542
2*(2+12)(7+9)



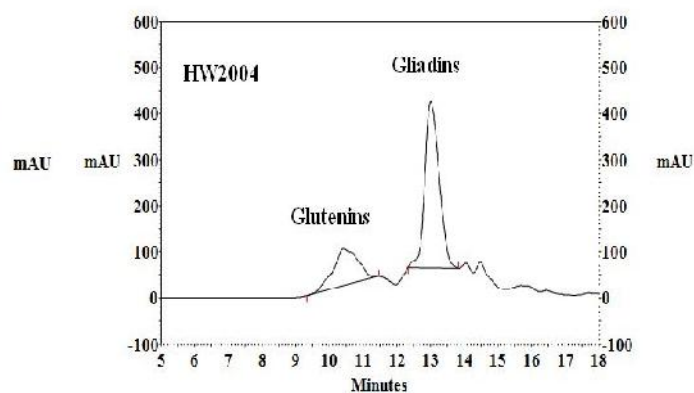
WH1021
2*(2+12)(7+8)



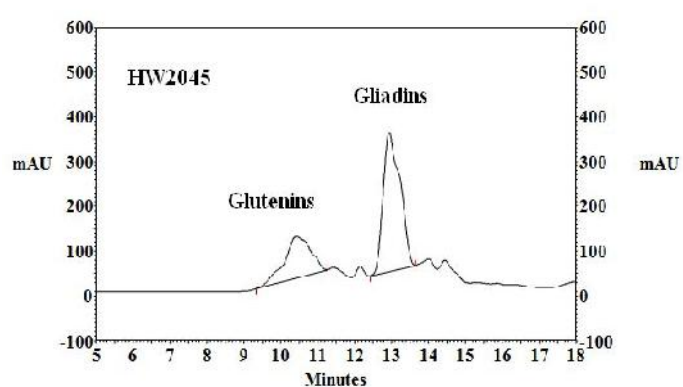
HUW234
2*(2+12)(7+8)



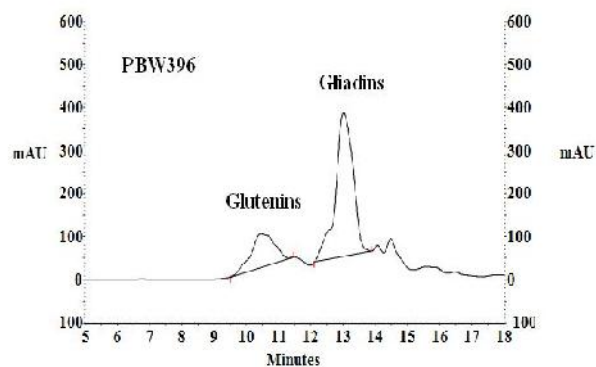
HD2824
N(2+12)(20)



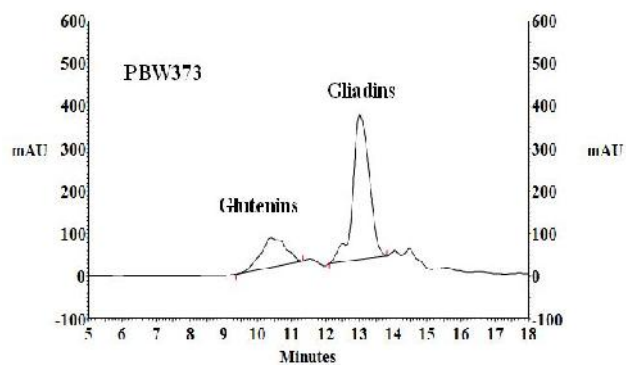
HW2004
N(2+12)(20)



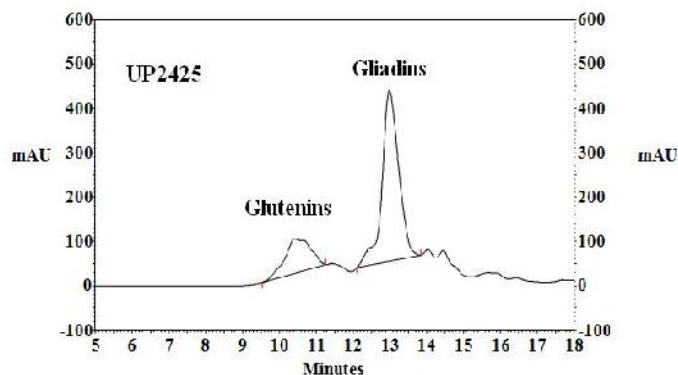
HW2045
1(5+10)(7+9)



PBW396
1(5+10)(7+9)



PBW373
1(5+10)(7)



UP2425
1(5+10)(17+18)

Supplementary Fig. 1s.HPLC chromatograms of flours from different wheat varieties