

Fractionation and grain hardness effect on protein profiling, pasting and rheological properties of flours from medium-hard and extraordinarily soft wheat varieties

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Abstract In the present study coarse fraction (CF), medium fine fraction (MFF) and fine fraction (FF) were separated from flours milled from medium-hard and extraordinarily soft wheat varieties and were evaluated for various quality characteristics. Grain hardness of medium-hard and extraordinarily soft wheat varieties varied from 77 to 80 and 17 to 18, respectively. Ash and protein content was the highest for FF and the lowest for CF. Varieties with greater hardness produced higher CF and lower of FF. FF showed higher unextractable polymeric protein (UnEx-PP) and dough stability as compared to MFF and CF. FF showed lower damage starch content as related by lower Sodium SRC (NaSRC) as compared to MFF and FF. CF showed higher paste viscosities than FF and difference were greater amongst fractions from varieties with lower grain hardness. FF with greater proportion of small size particles showed greater accumulation of 98 kDa and 85 kDa PPs than CF. This study demonstrated that fractionation of flours can be employed to produce fractions with varied gluten strength required for production of various products.

Keywords Wheat flour · Solvent retention capacity · SDS-PAGE · Pasting · HPLC · Farinograph

Abbreviations

CF	Coarse fraction
MFF	Medium fine fraction
FFF	Fine fraction
GHI	Grain hardness index
MHW	Medium-hard wheat
Ex-SW	Extraordinarily soft wheat
SRC	Solvent retention capacity
NaSRC	Sodium carbonate SRC
SuSRC	Sucrose SRC
LASRC	Lactic acid SRC
WSRC	Water SRC
WG	Wet gluten
DG	Dry gluten
GI	Gluten index
PT	Pasting temperature
PV	Peak viscosity
BDV	Breakdown viscosity
FV	Final viscosity
SBV	Setback viscosity
WA	Water absorption
DDT	Dough development time
DS	Dough stability
DOS	Degree of softening
LMW-GS	Low molecular weight glutenins subunits
HMW-GS	High molecular weight glutenins subunits

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Introduction

Wheat milling resulted into flour with varied particle size distribution and the proportion of fine and coarse particles depend upon grain hardness. A large number of flour streams varying in their composition; functionality and particle size distribution are produced during commercial milling of wheat. When flours are separated by sifting into two fractions (Coarse and Fine), the proportion and composition of fine and coarse fraction always vary. Fine fractions contain starchy endosperm fragments and small granules of starch, while the coarse fractions contain mainly large granules of starch and fragmented endosperm. As the break passes and reduction passes increased during wheat milling, the ash and protein content was increased (Dornez et al. 2006), these variations largely affect the rheological properties and quality of different products (Greffeuille et al. 2005). Flour yield was greatest for the coarse fraction of flour from softer wheat composed of mostly large unreduced endosperm pieces. The content of damaged starch was higher in the small particle size fractions. Flour from hard wheat does not have intracellular spaces around the starch granules and discontinuity formed in the starch–protein matrix while flour from soft wheat had intracellular spaces around the starch granules (Glenn and Saunders 1990). De La Hera et al. (2013) reported that particle size of flour affected the bread quality and observed that fine flour led to the poor gas retention during fermentation and produce lower specific volume bread. Ash content, gluten content, and sedimentation value were reported to be increased with increase in break system (Indrani et al. 2007). The variation in protein composition, falling number, rheology of dough and baking properties amongst flour streams obtained during wheat milling have also been reported (Menkovska et al. 2002; Every et al. 2002). The surface area to volume ratio increased in size reduction unit operation, sieving separates flours on the basis of particle size and gives range of particles size from coarse particles to fine particles differs in their composition and functional properties (Snow et al. 1999). The study of the particle size distribution has an important role in the functionality of final product (Ahmed 2014). Hayashi et al. (1976) found that cake is

prepared with coarse fractions while fine fractions are preferred for optimum bread volume of hard red spring wheat flour. Variation in various other composition such as fat, ash and damaged starch content greatly affect the rheological properties and end quality of food products (Bonnand-Ducasse et al. 2010; Greffeuille and Lullien-Pellerin 2007; Lazaridou and Duta 2007). By using sieves and air classification the distributions of particle size of hard and soft wheat flours were measured (Wu et al. 1990). Wang et al. (2013) investigated the rheological and physicochemical properties of wheat flours and observed that average gluten, damaged starch and protein content of standard flours were lower than that of filter flour. The wheat flour was passed through four different sizes of sieves to obtain flour of variable particle size and then prepared chapattis from these flours (Gujral and Pathak 2002). The fine flour fineness had more extensibility of chapatti and these chapattis were stored for 24 h. The objective of present study was to find out variation in Indian wheat varieties varied in hardness and their fractions (coarse and fine) of flour were studied for greater variation in particle size and their relationship with various physicochemical characteristics.

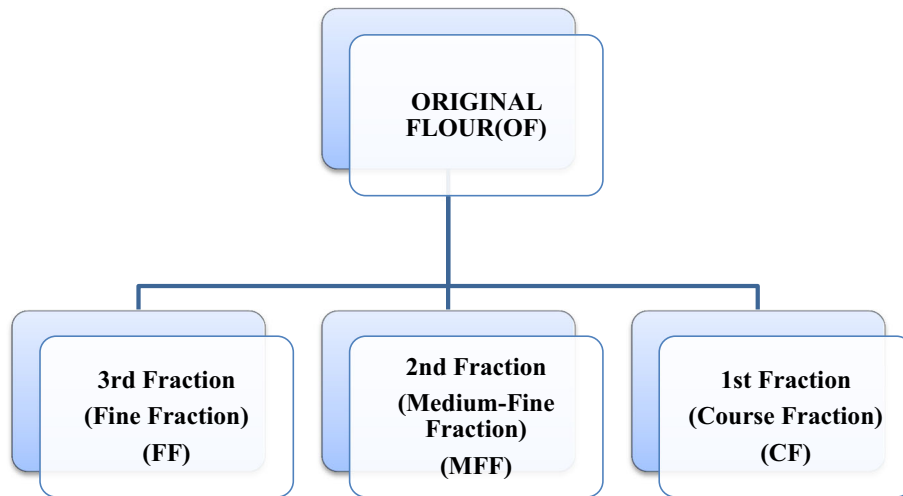
Material and methods

Fractionation of flours from MHW and Ex-SW varieties by using sieve shaker

Wheat varieties with GHI ranged from 77 to 80 and 17 to 18, respectively, were classified as MHW (HI977, HD2967) and Ex-SW (QBP12-11, QBP12-8) varieties.

Fractionation of flours

Flours from MHW and Ex-SW varieties were fractionated into coarse, medium fine and fine fractions using 3 sets of sieves with different mesh size (60, 100 and 120 μ). Flours obtained after sieving is termed as Coarse Fraction (CF), medium fine fraction (MFF) and fine fraction (FF).



Flour characteristics

Color characteristics and proximate composition

L^* , a^* and b^* values of different fractions of flours obtained from MHW and Ex-SW varieties were evaluated as described earlier by Katyal et al. (2016).

Particle size distribution

Particle size distribution of different fractions of flours obtained from MHW and Ex-SW varieties were evaluated as described earlier by Singh et al. (2016).

Protein characteristics

Protein characteristics (Ex-MP, UnEx-MP, Ex-PP and UnEx-PP, respectively) of different fractions of flours obtained from MHW and Ex-SW varieties were analyzed as described earlier by Katyal et al. (2016).

Solvent retention capacity (SRC)

WSRC, LASRC, SuSRC and NaSRC tests of different fractions of flours obtained from MHW and Ex-SW varieties were carried as described earlier by Kaur et al. (2016).

Pasting properties

Pasting properties of different fractions of flours obtained from MHW and Ex-SW varieties were evaluated as described Singh et al. (2016).

Farinographic characteristics

The dough mixing properties of different fractions of flours obtained from MHW and Ex-SW varieties were evaluated as described Singh et al. (2016).

SDS-PAGE analysis of proteins

Glutenins were extracted according to the method described by Ng and Bushuk (1987) with minor modifications. Briefly, glutenin extracts were prepared by solubilizing 40 mg wheat flour of different wheat varieties in 1 ml extraction buffer containing 0.063 M Tris-HCl pH 6.8, 10% (v/v) glycerol, 0.02% (w/v) bromophenol blue, 2% w/v SDS and 5% (v/v) β -mercaptoethanol. The electrophoresis of glutenins was done as described by Laemmli (1970).

Statistical analysis

The data obtained in triplicate were subjected to two-way ANOVA to find out significant difference in different properties amongst various cultivars. The relationship between different properties was established using Pearson correlation (r) coefficient. The data of CFF and FFF of different cultivars were subjected to paired t test to find out the significant difference. The statistical analysis was carried out using Minitab Release 14 Statistical Software (Soft College, PA, USA).

Table 1 Physicochemical properties and particle size distribution of various fractions obtained from flours of different MHW and Ex-SW varieties

Varieties	GHI	Fractions	L*	A*	B*	Ash content (%)	Protein content (%)	Small size particles (0 to < 55 μm)	Medium size particles (55–105 μm)	Large size particles (> 105 μm)
MHW	80	CF	87.46 ± 1.01 ^b	0.7 ± 0.01 ^d	8.89 ± 0.03 ^c	0.58 ± 0.02 ^d	11.65 ± 0.30 ^b	11.06 ± 0.02 ^b	47.06 ± 2.05 ^a	41.88 ± 0.10 ^c
		MFF	85.51 ± 1.02 ^a	0.56 ± 0.02 ^d	8.31 ± 0.02 ^b	0.62 ± 0.06 ^d	12.12 ± 0.50 ^b	20.92 ± 1.03 ^b	52.66 ± 2.06 ^d	26.42 ± 0.40 ^g
	77	FF	87.80 ± 1.01 ^b	0.42 ± 0.02 ^d	7.26 ± 0.02 ^c	0.65 ± 0.05 ^b	12.25 ± 0.20 ^b	32.29 ± 2.04 ^b	52.43 ± 1.05 ^b	15.28 ± 0.11 ^c
		CF	88.33 ± 0.11 ^a	0.65 ± 0.04 ^b	9.83 ± 0.62 ^d	0.45 ± 0.02 ^b	10.85 ± 0.10 ^a	6.70 ± 0.30 ^a	44.10 ± 2.04 ^b	49.20 ± 0.20 ^d
Ex-SW	18	MFF	87.46 ± 1.15 ^{ab}	0.51 ± 0.03 ^b	9.13 ± 0.04 ^d	0.40 ± 0.02 ^c	11.15 ± 0.40 ^b	11.77 ± 0.11 ^b	48.69 ± 2.10 ^b	39.54 ± 0.40 ^d
		FF	89.85 ± 0.19 ^b	0.42 ± 0.01 ^c	8.43 ± 0.03 ^d	0.35 ± 0.03 ^a	11.25 ± 0.45 ^b	26.16 ± 1.04 ^c	61.09 ± 1.05 ^d	12.75 ± 0.10 ^a
	8	CF	90.55 ± 2.09 ^a	0.43 ± 0.02 ^b	5.90 ± 0.02 ^a	0.42 ± 0.02 ^b	12.22 ± 0.55 ^a	29.81 ± 1.07 ^c	41.14 ± 0.09 ^{ab}	29.05 ± 0.01 ^c
		MFF	94.68 ± 1.01 ^b	0.36 ± 0.02 ^{ab}	5.60 ± 0.28 ^a	0.39 ± 0.01 ^b	12.69 ± 0.30 ^b	38.78 ± 0.10 ^b	41.90 ± 1.06 ^b	19.32 ± 0.30 ^b
QBPI2-11	17	FFF	90.47 ± 1.61 ^{ab}	0.33 ± 0.01 ^a	5.51 ± 0.09 ^{ab}	0.49 ± 0.01 ^c	12.46 ± 0.20 ^{ab}	39.15 ± 1.08 ^b	42.20 ± 0.20 ^b	18.65 ± 0.20 ^a
		CF	92.00 ± 1.19 ^b	0.36 ± 0.01 ^b	6.80 ± 0.05 ^b	0.30 ± 0.02 ^a	12.41 ± 0.40 ^a	32.37 ± 0.30 ^d	39.55 ± 0.10 ^c	28.08 ± 0.03 ^a
	Average values (different fractions of all varieties)	MFF	87.67 ± 2.49 ^b	0.33 ± 0.02 ^a	6.19 ± 0.14 ^b	0.36 ± 0.04 ^a	12.69 ± 0.10 ^{ab}	52.90 ± 1.09 ^d	33.33 ± 0.20 ^a	13.80 ± 0.20 ^b
		FFF	90.84 ± 2.88 ^a	0.37 ± 0.03 ^b	5.97 ± 0.20 ^a	0.46 ± 0.02 ^c	13.16 ± 0.11 ^b	48.36 ± 0.3 ^b	38.57 ± 0.4 ^b	13.07 ± 0.03 ^a
Average values	CF	MFF	89.59	0.54	7.86	0.44	11.78	19.99	42.96	37.05
		FF	89.67	0.44	7.31	0.44	12.16	31.09	44.15	24.77
	CF(MHW)	MFF	89.74	0.39	6.79	0.49	12.42	36.49	48.57	14.94
		FF	87.89	0.68	9.36	0.52	12.31	8.88	45.58	45.54
CF(EX-SW)	MFF	91.28	0.39	6.35	0.36	11.36	31.09	40.35	28.57	
	FF	86.49	0.54	8.72	0.51	12.69	16.34	50.67	32.98	
FF(MHW)	MFF	92.86	0.35	5.89	0.38	11.63	45.84	37.62	16.59	
	FF	88.82	0.42	7.84	0.50	13.09	29.22	56.76	14.01	
FF(EX-SW)	MFF	90.65	0.35	5.74	0.47	11.75	43.75	40.38	15.86	
	FF									

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$). For each parameter, means with similar subscript in a column do not differ significantly between CF, MFF and FF for each variety ($p \leq 0.05$). Results displayed are the means of three independent assays. Data represented as mean value ± SD

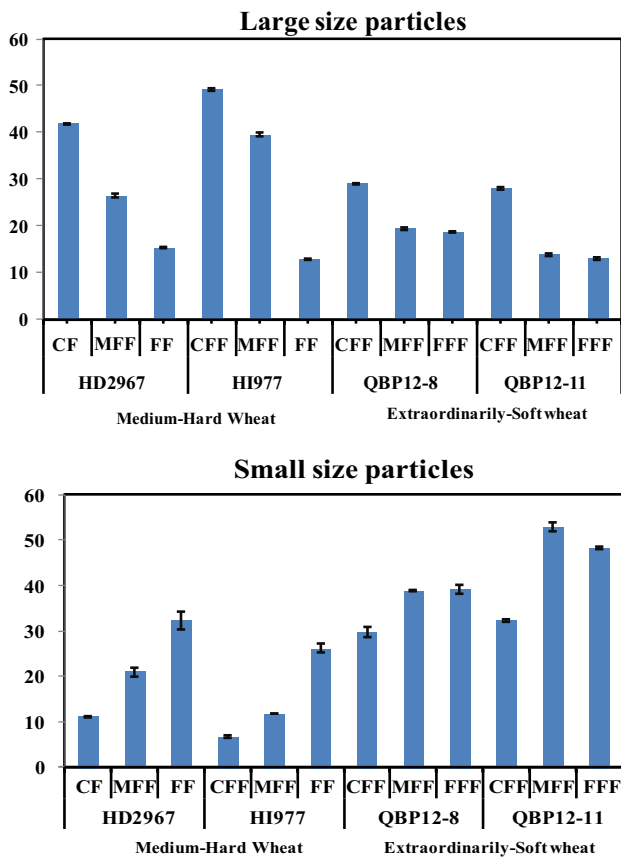


Fig. 1 Bar charts showing particle size distribution of different fractions of MHW and Ex-SW varieties flours obtained by sieve shaker

Results and discussion

Color characteristics

The statistical analysis revealed a significant difference in L^* , a^* and b^* values between varieties, grain hardness and fractions (Supplementary Table 2a). The effect of varieties and hardness was greater than fractions on L^* , a^* and b^* . Hunter color parameters of CF, MFF and FF from different wheat varieties are shown in Table 1. Average L^* , a^* and b^* values was 89.59, 0.54 and 7.86 for CF against 89.67, 0.44 and 7.31 for MFF and 89.74, 0.39 and 6.79 for FF. FF from different varieties showed lower b^* and a^* but higher L^* value than their corresponding MFF and CF. Average L^* value of CF was 87.89 and 91.28 for MHW and Ex-SW while L^* value of FF was 88.82 and 90.65, respectively for MHW and Ex-SW (Table 1). The results showed that L^* of CF, MFF and FF were higher for Ex-SW than MHW. While a^* and b^* values of CF, MFF and FF were lower for Ex-SW as compared to MHW. Average L^* value was 91.04 and 87.73, respectively for Ex-SW and MHW varieties (Supplementary Table 1). Average a^* value was 0.36 and

0.54, respectively for Ex-SW and MHW varieties. Average b^* value was 5.99 and 8.64, respectively for Ex-SW and MHW varieties. The results showed that L^* value increased while a^* and b^* value decreased with decrease in GHI. L^* value was negatively correlated with a^* and b^* value ($r = -0.575$ and -0.628 , respectively, $p \leq 0.05$). Singh et al. (2018) (accepted) also reported the similar correlation between L^* , a^* and b^* values. Darker colors were observed to be associated with the large size particles. The results reflected that color decreased with decrease in particle size as reported earlier by Dobaldo-Maldonado and Rose (2013). The proportion of small size particles were negatively correlated with a^* ($r = -0.881$, $p \leq 0.005$) whereas large size particles were positively correlated with a^* and b^* ($r = 0.818$ and 0.691 , respectively, $p \leq 0.005$) while small size particles were negatively correlated with a^* and b^* ($r = -0.881$ and -0.882 , respectively, $p \leq 0.005$). Sakhare et al. (2014) also reported that L^* value decreased with increase in the particle size while a^* and b^* value showed the opposite trend.

Particles size distribution

Particle size distribution of different fraction of flours milled from Ex-SW and MHW varieties obtained by sieving is shown in Fig. 1. ANOVA revealed a significant variation in the proportion of small, medium and large size particles due to fractions, grain hardness and varieties (Supplementary Table 2a). The effect of fractions and varieties was greater than fractions and hardness on small, medium and large size particles. The average proportion of small, medium and large size particles was 19.99, 42.96 and 37.05%, respectively for CF against 31.09, 44.15 and 24.77%, respectively for MFF and 36.49, 48.57 and 14.94%, respectively for FF (Table 1). CF contained higher proportion of large size particles than their corresponding MFF and FF. Average proportion of small size particles was 40.23 and 18.15%, respectively for Ex-SW and MHW varieties. Average large size particles of CF were 45.54 and 28.57 for MHW and Ex-SW while large size particles of FF were 14.01 and 15.86 for MHW and Ex-SW (Table 1). Average proportion of large size particles was 20.33 and 30.85%, respectively for Ex-SW and MHW varieties (Supplementary Table 1). The results showed that small size particles increased while medium and large size particles decreased with decrease in GHI. Results reflected that wheat varieties with high GHI resulted into flour with higher proportion of large size particles and lower proportion of small size particles. Singh et al. (2018) (accepted) also reported the similar correlation between proportion of large size particles and GHI. Small size particles were present in large proportion in Ex-SW varieties. Small size particles was positively correlated with

protein content ($r = 0.831$, $p \leq 0.005$) while negatively correlated with medium and large size particles ($r = -0.496$ and -0.631 , respectively, $p \leq 0.005$). Results showed that wheat varieties with lower GHI had less protein content and results into flour with higher proportion of small size particles. GHI was related to fine particle size proportion, recovery of wheat flour and grain weight (Kaur et al. 2013).

Composition

The statistical analysis revealed a significant effect on fractions, varieties and grain hardness on ash content (Supplementary Table 2a). The effect of fractions and varieties on ash content was greater than fractions and hardness. Ash content of CF, MFF and FF varied from 0.30 to 0.58, 0.36 to 0.62 and 0.35 to 0.65%, respectively (Table 1). An average ash content of 0.49% for FF, 0.44% for MFF and 0.43% for CF. FF had higher ash content as compared to MFF and CF and these differences might be because of more contamination of FF with bran particles during milling than MFF and CF. Average ash content was 0.40 and 0.51% for flour from Ex-SW and MHW varieties (Supplementary Table 1). Ash content of flour decreased with decrease in GHI. Average ash content of CF was 0.52 and 0.36 for MHW and Ex-SW (Table 1). Wang et al. (2013) earlier reported that fine flour had higher ash content. Indrani et al. (2007) also reported that ash content increased with increase in break system. Ash content was positively correlated with a^* ($r = 0.498$, $p \leq 0.005$) and negatively correlated with L^* ($r = -0.487$, $p \leq 0.005$). The greater darkening in color of flours might be attributed to contamination of flour with bran. ANOVA indicated significant differences in protein content between varieties and fractions (Supplementary Table 2a). Protein content of CF, MFF and FF ranged between 10.85 to 12.41, 11.15 to 12.69% and 11.25 to 13.16, respectively (Table 1) with average protein content of 11.78, 12.16 and 12.42%, respectively. Protein content of FF was higher than MFF and CF. Wang et al. (2013) and Schutyser and Goot (2011) also reported that fine flour had high protein content. Average protein content was 12.60 and 11.55%, respectively for flour from Ex-SW and MHW varieties (Supplementary Table 1). The results reflected that the protein content decreased with increase in GHI. Average protein content of CF was 12.31 and 11.36 for MHW and Ex-SW varieties (Table 1). Protein content showed negative correlation with a^* and b^* ($r = -0.605$ and -0.809 , respectively, $p \leq 0.005$).

Protein characteristics

The statistical analysis revealed a significant effect of fractions, grain hardness and varieties on UnEx-PP, UnEx-MP, Ex-PP and Ex-MP. There was a strong interaction between varieties and fractions on UnEx-MP and UnEx-PP (Supplementary Table 2b). The proportion of UnEx-PP of CF, MFF and FF ranged from 52.69 to 77.74%, 53.60 to 75.02 and 57.95 to 71.08%, respectively and proportion of UnEx-MP ranged from 22.26 to 47.31%, 24.98 to 46.40 and 28.92 to 42.05%, respectively (Table 2). QBP12-11 showed the highest Un-ExPP while HI977 showed the lowest Un-ExPP. Average UnEx-PP was 65.35, 63.17 and 63.53%, respectively for FF, MFF and CF (Table 2). Results reflected that FF had higher proportion of UnEx-PP than their corresponding MFF and CF. Average UnEx-MP and UnEx-PP was 34.19 and 65.81%, respectively for Ex-SW against 37.78 and 62.22%, respectively for MHW varieties. Average UnEx-PP of CF was 58.45 and 68.61 for MHW and Ex-SW varieties (Table 2). Average Ex-MP and Ex-PP was 64.56 and 35.44% for Ex-SW against 68.22 and 31.78%, respectively for MHW varieties (Supplementary Table 1). Results reflected that varieties with higher GHI had higher MP (both extractable and unextractable) while lower PP (both extractable and unextractable). The effect of fractionation on UnEx-PP, Ex-PP, UnEx-MP and Ex-MP of both Ex-SW and MHW varieties is shown in Supplementary Fig. 2. Average UnEx-PP, Ex-PP, UnEx-MP and Ex-MP, and were reported as 62, 46, 38 and 54%, respectively for bread wheat by Gupta et al. (1993). Ex-MP and Ex-PP was reported to be ranged from 45.33 to 55.83 and 28.14 to 40.44, respectively for Indian wheat varieties (Singh et al. 2011). The endosperm with high gliadin content showed an increase in continuous structure of the protein matrix around starch granules during grain desiccation that led to the formation of vitreous endosperm (Dexter and Edwards 2001; Johansson et al. 2013). It was most likely that grain vitreousness was associated with GHI amongst wheat varieties evaluated.

Solvent retention capacity (SRC)

The statistical analysis revealed a significant effect of varieties, fractions and grain hardness on NaSRC, SuSRC, LASRC and WSRC (Supplementary Table 2b). NaSRC, LASRC, SuSRC and WSRC of CF, MFF and FF obtained from Ex-SW and MHW varieties are shown in Supplementary Fig. 1. NaSRC of CF, MFF and FF ranged from 63.50 to 82.55, 62.24 to 81.12 and 60.15 to 74.83, respectively while SuSRC ranged from 98.05 to 118.59 to 91.66 to 111.68 and 84.37 to 109.68, respectively (Table 2). FF showed lower NaSRC and SuSRC than MFF and CF. NaSRC has been related to damage starch content

Table 2 Solvent retention capacity and protein characteristics of various fractions obtained from flours of different MHW and Ex-SW varieties

Varieties	GHI	Fractions	NaSRC (%)	LASRC (%)	SuSRC (%)	WSRC (%)	Ex-PP (%)	Ex-MP (%)	UnEx-PP (%)	UnEx-MP (%)
<i>MHW</i>										
HD2967	80	CF	82.06 ± 1.01 ^c	95.00 ± 1.03 ^a	103.33 ± 2.05 ^b	67.89 ± 1.03 ^a	26.20 ± 0.40 ^a	73.80 ± 1.06 ^d	64.21 ± 0.95 ^e	35.79 ± 0.99 ^b
		MFF	79.28 ± 1.20 ^b	96.00 ± 1.52 ^b	91.66 ± 1.21 ^b	69.16 ± 0.94 ^b	25.87 ± 0.55 ^b	74.13 ± 1.26 ^c	59.53 ± 0.88 ^b	40.47 ± 0.89 ^c
		FF	74.83 ± 1.19 ^c	97.00 ± 1.62 ^b	88.44 ± 1.02 ^b	69.25 ± 0.89 ^b	22.10 ± 0.80 ^a	77.90 ± 1.25 ^c	67.87 ± 1.15 ^b	32.13 ± 1.09 ^b
HI977	77	CF	82.54 ± 1.39 ^b	102.00 ± 1.72 ^c	118.59 ± 2.26 ^d	73.50 ± 1.42 ^d	39.25 ± 1.07 ^b	60.75 ± 1.50 ^a	52.69 ± 1.36 ^a	47.31 ± 1.10 ^d
		MFF	81.12 ± 1.22 ^{ab}	112.00 ± 1.77 ^d	111.68 ± 2.13 ^d	72.47 ± 1.36 ^d	38.93 ± 1.24 ^b	61.07 ± 1.70 ^b	64.52 ± 2.20 ^b	35.48 ± 1.49 ^b
		FF	72.38 ± 1.09 ^d	115.00 ± 1.82 ^c	109.68 ± 1.89 ^d	73.40 ± 1.22 ^d	38.33 ± 1.90 ^c	61.67 ± 1.99 ^b	64.51 ± 2.30 ^b	35.49 ± 1.52 ^c
<i>Ex-SW</i>										
QBPI2-8	18	CF	64.96 ± 0.60 ^b	86.00 ± 1.22 ^a	98.05 ± 1.24 ^e	57.03 ± 1.09 ^b	36.72 ± 1.50 ^{bc}	63.28 ± 2.05 ^b	59.48 ± 1.05 ^b	40.52 ± 1.66 ^c
		MFF	63.15 ± 0.88 ^a	95.00 ± 1.49 ^b	95.73 ± 1.20 ^b	57.20 ± 0.83 ^b	36.05 ± 1.69 ^{bc}	63.95 ± 2.04 ^{ab}	75.02 ± 2.60 ^d	24.98 ± 1.49 ^a
		FFF	62.78 ± 0.78 ^b	97.00 ± 1.56 ^c	90.90 ± 1.04 ^c	60.51 ± 0.78 ^b	34.75 ± 1.88 ^b	65.25 ± 2.38 ^b	71.08 ± 2.50 ^b	28.92 ± 1.79 ^b
QBPI2-11	17	CF	63.50 ± 0.81 ^a	104.00 ± 1.79 ^d	117.53 ± 2.22 ^c	55.12 ± 1.04 ^a	35.56 ± 1.60 ^b	64.44 ± 2.55 ^a	77.74 ± 2.75 ^d	22.26 ± 1.44 ^a
		MFF	62.24 ± 0.79 ^b	107.00 ± 1.81 ^b	100.72 ± 1.31 ^b	55.97 ± 0.74 ^b	34.55 ± 1.50 ^b	65.45 ± 2.22 ^b	53.60 ± 1.90 ^a	46.40 ± 0.90 ^d
		FFF	60.15 ± 0.89 ^a	112.00 ± 1.91 ^c	84.37 ± 1.13 ^a	55.93 ± 1.11 ^b	35.02 ± 1.60 ^{ab}	64.98 ± 1.87 ^{ab}	57.95 ± 1.77 ^b	42.05 ± 0.94 ^d
		Average values (different fractions of all varieties)	73.27	96.75	109.38	63.39	34.43	65.57	63.53	36.47
		Average values								
		MFF	71.45	102.50	99.95	63.70	33.85	66.15	63.17	36.83
		FF	70.00	105.25	93.35	64.77	32.55	67.45	65.35	34.65
		CF(MHW)	82.31	98.50	110.96	70.62	32.72	67.27	58.45	41.55
		CF(EX-SW)	64.23	95.00	107.79	56.07	36.14	63.86	68.61	31.39
		MF(MHW)	80.20	104.00	101.67	70.81	32.40	67.60	62.02	37.97
		MF(EX-SW)	62.69	101.00	98.22	56.59	35.30	64.70	64.31	35.69
		FF(MHW)	73.60	106.00	99.06	71.32	30.22	69.78	66.19	33.81
		FF(EX-SW)	61.46	104.50	87.64	58.22	34.88	65.11	64.52	35.48

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$). For each parameter, means with similar subscript in a column do not differ significantly between CF, MFF and FF for each variety ($p \leq 0.05$). Results displayed are the means of three independent assays. Data represented as mean value ± SD

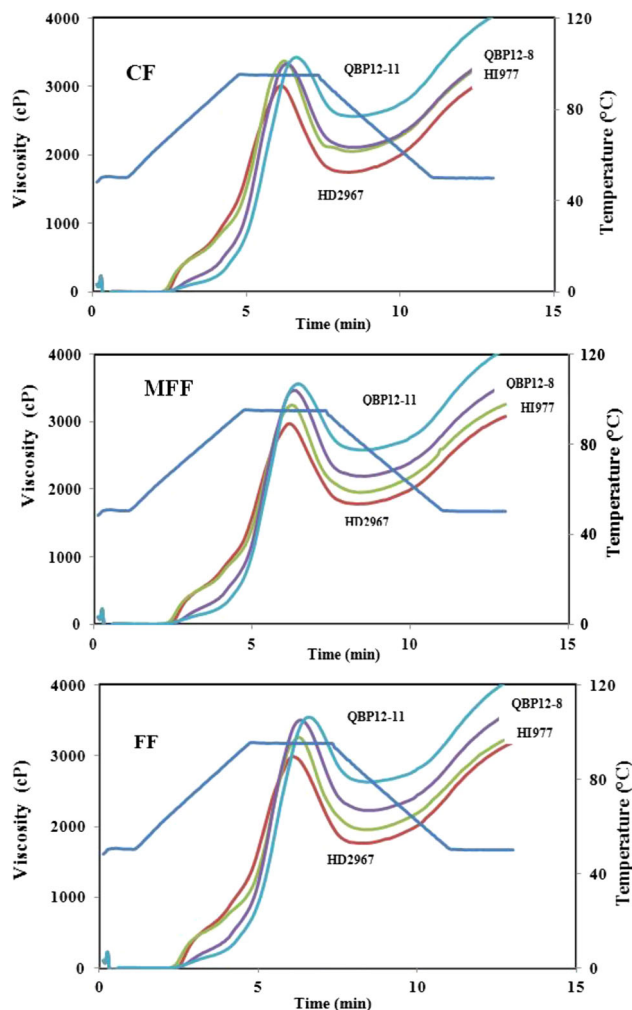


Fig. 2 Pasting profiles of different fractions of MHW and Ex-SW varieties flours obtained by sieve shaker

of flour (Guttieri et al. 2002). NaSRC was negatively correlated with protein content ($r = -0.783$, $p \leq 0.005$). The results reflected that decrease in GHI accompanied with the increase in protein content and decrease in NaSRC (damage starch content). Average NaSRC of CF was 82.31 and 64.23% for MHW and Ex-SW varieties (Table 2). Baasandorj et al. (2016) also reported that CF had lower protein content while higher damaged starch. SuSRC has been related to water soluble pentosan as well as gliadin content of flour (Guttieri et al. 2002). NaSRC was negatively correlated with small size particles ($r = -0.891$, $p \leq 0.005$) while positively correlated with medium and large size particles ($r = 0.714$ and 0.623 , respectively, $p \leq 0.005$). This indicated that varieties with more small size particles had high damage starch content. Average NaSRC for Ex-SW and MHW varieties was 62.79 and 79.70%, respectively against average SuSRC for Ex-SW and MHW varieties was 97.88 and 103.89%, respectively (Supplementary Table 1). The results also reflected that damage

starch content decreased while arabinoxylans content increased with decrease in GHI which means that Ex-SW had less damage starch and high arabinoxylans content. SuSRC was negatively correlated with protein content and Ex-MP ($r = -0.608$ and -0.514 , respectively, $p \leq 0.05$). SuSRC was negatively correlated with small size particles ($r = -0.555$, $p \leq 0.005$) and positively correlated with large size particles ($r = 0.611$, $p \leq 0.005$). LASRC indicated the gluten quality and swelling of glutenins subunit, an indicator of elasticity of dough (Guttieri et al. 2002). Average LASRC was 96.75, 102.50 and 105.25%, respectively for CF, MFF and FF against average WSRC of 64.77, 63.70 and 63.39%, respectively (Supplementary Table 1). FF showed lower WSRC and higher LASRC as compared to their corresponding MFF and CF. The results indicated that FF had better gluten quality as compared to their corresponding MFF and CF. Average LASRC for Ex-SW and MHW varieties was 100.17 and 102.83%, respectively against average WSRC for Ex-SW and MHW varieties was 56.96 and 70.94%, respectively. LASRC was negatively correlated with protein content and Ex-MP ($r = -0.375$, $p \leq 0.05$). WSRC was negatively correlated with small size particles ($r = -0.777$, $p \leq 0.005$) and positively correlated with medium size particles ($r = 0.809$, $p \leq 0.005$).

Pasting properties

ANOVA indicated significant differences in PV, FV, SBV, BDV and PT between varieties, fractions and grain hardness (Supplementary Table 2c). Pasting profiles of different fractions of MHW and Ex-SW varieties flour obtained by sieve shaker (Fig. 2). Average PV varied between 3328, 3309 and 3278cP, respectively for CF, MFF and FF. FF showed lower PV than their corresponding MFF and CF. Average PV for Ex-SW and MHW varieties was 3471 and 3139 cP, respectively (Supplementary Table 1). The results reflected that PV increased with decrease in GHI. PV was negatively correlated with medium size particles ($r = -0.671$, $p \leq 0.05$) and positively correlated with small size particles and protein content ($r = 0.530$ and 0.386 , respectively, $p \leq 0.05$). PV was negatively correlated with NaSRC and WSRC ($r = -0.677$ and -0.629 , respectively, $p \leq 0.05$). PV of CF and FF was positively correlated with Ex-PP ($r = 0.720$, $p \leq 0.05$) while negatively correlated with Ex-MP ($r = -0.751$, $p \leq 0.05$). BDV of CF, MFF and FF varied from 865 to 1319 cP, 987 to 1295 and 916 to 1303 cP, respectively. Average BDV for Ex-SW and MHW varieties was 1089 and 1265cP, respectively. The difference in these properties of FF, MFF and CF may be due to difference in lipids content. BDV was positively correlated with NaSRC and WSRC ($r = 0.533$ and 0.680 , respectively, $p \leq 0.005$). BDV was

Table 3 Pasting and farinographic properties of various fractions obtained from flours of different MHW and Ex-SW varieties

Varieties	GHI	Fractions	PV (cP)	BDV (cP)	FV (cP)	SBV (cP)	PT (. C)	WA (%)	DDT (min)	DS (min)	DOS (BU)
<i>MHW</i>											
HD2967	80	CF	3014 ± 101 ^a	1265 ± 15 ^b	3163 ± 84 ^{ab}	1414 ± 199 ^b	66.15 ± 0.10 ^a	60.60 ± 0.30 ^g	2.50 ± 0.20 ^b	7.10 ± 0.40 ^e	27 ± 1.02 ^a
		MFF	2965 ± 89 ^a	1190 ± 116 ^b	3087 ± 99 ^a	1312 ± 21 ^a	66.10 ± 0.01 ^b	60.30 ± 0.10 ^c	5.40 ± 0.40 ^b	4.90 ± 0.10 ^a	90 ± 2.50 ^d
		FF	2983 ± 95 ^{ab}	1217 ± 3 ^{ab}	3198 ± 100 ^b	1432 ± 193 ^b	66.85 ± 0.05 ^b	59.50 ± 0.40 ^c	1.50 ± 0.10 ^a	5.60 ± 0.20 ^g	72 ± 2.02 ^d
HI977	77	CF	3368 ± 90 ^b	1319 ± 181 ^c	3393 ± 102 ^b	1344 ± 11 ^a	65.25 ± 0.04 ^a	61.20 ± 0.20 ^d	1.90 ± 0.20 ^a	8.90 ± 0.20 ^c	27 ± 1.50 ^b
		MFF	3244 ± 100 ^a	1295 ± 193 ^c	3267 ± 90 ^b	1318 ± 3 ^a	65.25 ± 0.02 ^a	62.00 ± 0.30 ^d	7.50 ± 0.40 ^g	13.40 ± 0.40 ^d	10 ± 0.50 ^a
		FF	3258 ± 90 ^a	1303 ± 188 ^c	3289 ± 102 ^{ab}	1334 ± 199 ^a	65.30 ± 0.30 ^a	59.70 ± 0.30 ^e	11.20 ± 0.80 ^c	13.30 ± 0.20 ^g	10 ± 1.04 ^a
<i>Ex-SW</i>											
QBPI2-8	18	CF	3499 ± 91 ^g	1274 ± 23 ^b	3643 ± 84 ^g	1418 ± 16 ^c	70.25 ± 0.05 ^g	55.10 ± 0.10 ^g	4.70 ± 0.30 ^h	7.90 ± 0.10 ^b	56 ± 1.80 ^g
		MFF	3460 ± 106 ^g	1272 ± 51 ^g	3566 ± 96 ^{ab}	1378 ± 41 ^b	69.40 ± 0.40 ^g	54.80 ± 0.20 ^a	4.70 ± 0.31 ^h	8.60 ± 0.33 ^g	50 ± 1.03 ^g
		FF	3330 ± 115 ^{bc}	1218 ± 33 ^b	3446 ± 91 ^b	1334 ± 9 ^a	69.40 ± 0.20 ^g	55.50 ± 0.50 ^g	2.00 ± 0.10 ^a	7.70 ± 0.34 ^b	68 ± 2.70 ^g
QBPI2-11	17	CF	3429 ± 110 ^{bc}	865 ± 49 ^a	4045 ± 120 ^d	1481 ± 59 ^c	84.00 ± 0.20 ^g	57.70 ± 0.20 ^b	4.00 ± 0.20 ^g	7.30 ± 0.32 ^a	33 ± 1.40 ^b
		MFF	3566 ± 94 ^d	987 ± 50 ^c	4104 ± 125 ^d	1525 ± 75 ^c	84.00 ± 0.40 ^g	56.20 ± 0.20 ^b	5.00 ± 0.31 ^{ab}	8.10 ± 0.50 ^b	33 ± 1.02 ^b
		FF	3542 ± 112 ^{ab}	916 ± 640 ^a	4123 ± 119 ^d	1497 ± 77 ^{ab}	84.90 ± 0.45 ^g	56.40 ± 0.10 ^b	4.00 ± 0.12 ^a	7.80 ± 0.21 ^{ab}	48 ± 2.03 ^b
Average values (different fractions of all varieties)			3328	1181	3561	1414	71.41	58.65	3.28	7.80	35.75
Average values			3309	1186	3506	1383	71.19	58.33	5.65	8.75	45.75
			3278	1164	3514	1399	71.61	57.78	4.68	8.60	49.50
CF(HW)			3191	1292	3278	1379	65.70	60.90	2.2	8.00	27.00
CF(EX-SW)			3464	1069	2395	1449	77.12	56.40	4.35	7.60	44.50
MF(HW)			3105	1243	3177	1315	65.67	61.15	6.45	9.15	50.00
MF(EX-SW)			3513	1129	3835	1452	76.70	55.50	4.85	8.35	41.50
FF(HW)			3121	1260	3444	1383	66.08	59.60	6.35	9.45	41.00
FF(EX-SW)			3436	1067	3784	1416	77.15	55.95	3.00	7.75	58.00

Means with similar superscript in a column do not differ significantly between varieties ($p \leq 0.05$). For each parameter, means with similar subscript in a column do not differ significantly between CF, MFF and FF for each variety ($p \leq 0.05$). Results displayed are the means of three independent assays. Data represented as mean value ± SD

positively correlated with medium and large size particles ($r = 0.471$ and 0.335 , respectively, $p \leq 0.005$) and negatively correlated with small size particles ($r = -0.546$, $p \leq 0.005$). Average PV of CF was 3191 and 3464 cP for MHW and Ex-SW varieties (Table 3). FV of CF, MFF and FF ranged between 3163 to 4045 cP, 3087 to 4104 cP and 3198 to 4123 cP, respectively. CF showed higher FV than their corresponding FF. Average FV for Ex-SW and MHW varieties was 3821 and 3233 cP, respectively (Supplementary Table 1). FV was negatively correlated with WSRC and NaSRC ($r = -0.826$, and -0.799 , respectively, $p \leq 0.05$). FV was reported to be largely determined by soluble amylose retrogradation upon cooling (Olkku and Rha 1978). Average FV of CF was 3278 and 2395 cP MHW and Ex-SW varieties (Supplementary Table 1). Average SBV was 1414, 1383 and 1399cP, respectively for CF, MFF and FF. FF showed the lower SBV than their corresponding MFF and CF (Supplementary Table 1). Average SBV for Ex-SW and MHW varieties was 1438 and 1359 cP, respectively. SBV was negatively correlated with WSRC and NaSRC ($r = -0.439$ and -0.581 , respectively, $p \leq 0.005$). FV and SBV both was positively correlated with small size particles ($r = 0.725$ and 0.647 , respectively, $p \leq 0.05$) was negatively correlated with medium size particles ($r = -0.797$ and -0.660 , respectively, $p \leq 0.05$) and was negatively correlated large size particles ($r = -0.353$ and -0.430 , respectively, $p \leq 0.05$). Average PT for Ex-SW and MHW varieties was 76.99 and 65.82 °C, respectively. PT was positively correlated with small size particles ($r = 0.738$, $p \leq 0.005$) and negatively correlated with medium size particles ($r = -0.737$, respectively, $p \leq 0.005$). PT was negatively correlated with NaSRC and WSRC ($r = -0.740$ and -0.800 , respectively, $p \leq 0.005$). This clearly indicated that the presence of higher protein content delayed PT.

Farinographic properties

The statistical analysis revealed significant differences in WA, DDT, DS and DOS between varieties, fractions and grain hardness (Supplementary Table 2c). Farinographic characteristics of CF, MFF and FF obtained from MHW and Ex-SW varieties are shown in Supplementary Fig. 3. Farinograms of different fractions of MHW and Ex-SW varieties flour obtained by sieve shaker (Fig. 4). Average WA was 58.65, 58.33 and 57.78%, respectively for CF, MFF and FF. FF showed the lower WA than their corresponding MFF and CF (Table 3). Singh et al. (2018) (accepted) also reported that FF showed the lower WA than CF. Average WA of Ex-SW and MHW varieties were 55.95 and 60.55%, respectively. WA was positively correlated with NaSRC and WSRC ($r = 0.926$ and 0.884 ,

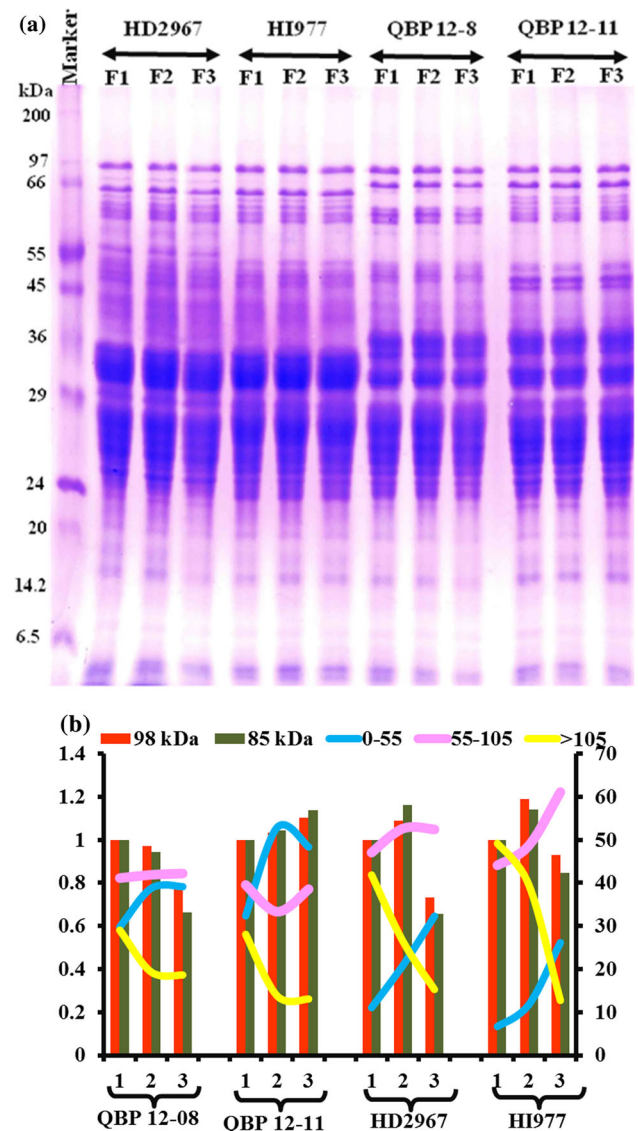


Fig. 3 a SDS-PAGE analysis of glutenins of MHW (HD2967 and HI977) and Ex-SW (QBP 12-8 and QBP 12-11) varieties. The glutenin were extracted from different wheat cultivars using 0.063 M Tris-HCl pH 6.8, 2% w/v SDS, 5% (v/v) β -mercaptoethanol, 10% (v/v) glycerol extraction buffer and resolved on SDS-PAGE under reducing conditions. The staining of the gel was done with a staining buffer contained 50% methanol and 12% glacial acetic acid and 0.2% coomassie brilliant blue R-250 dye followed destaining in solution of 50% methanol and 12% glacial acetic acid. The gels were documented with HP G4010 flatbed scanner and molecular weight analysis was done using Alpha Ease FC[®] gel analysis software. b Densitometry of 98 kDa and 85 kDa major glutenins in different fractions of wheat cultivars. The analysis was carried out by using AlphaEase FC[®] gel analysis software. The integrated densitometry value (IDV) of 98 kDa and 85 kDa PPs of CF of each cultivar was used to divide the IDV value of medium and fine fractions of each cultivar so as convert them into fold changes. The column filled red square and filled green square represents 98 kDa and 85 kDa proteins while filled blue rectangle, filled pink rectangle and filled yellow rectangle color line on secondary Y axis represent the 0–55, 55–105 and > 105 μ m particle sizes respectively

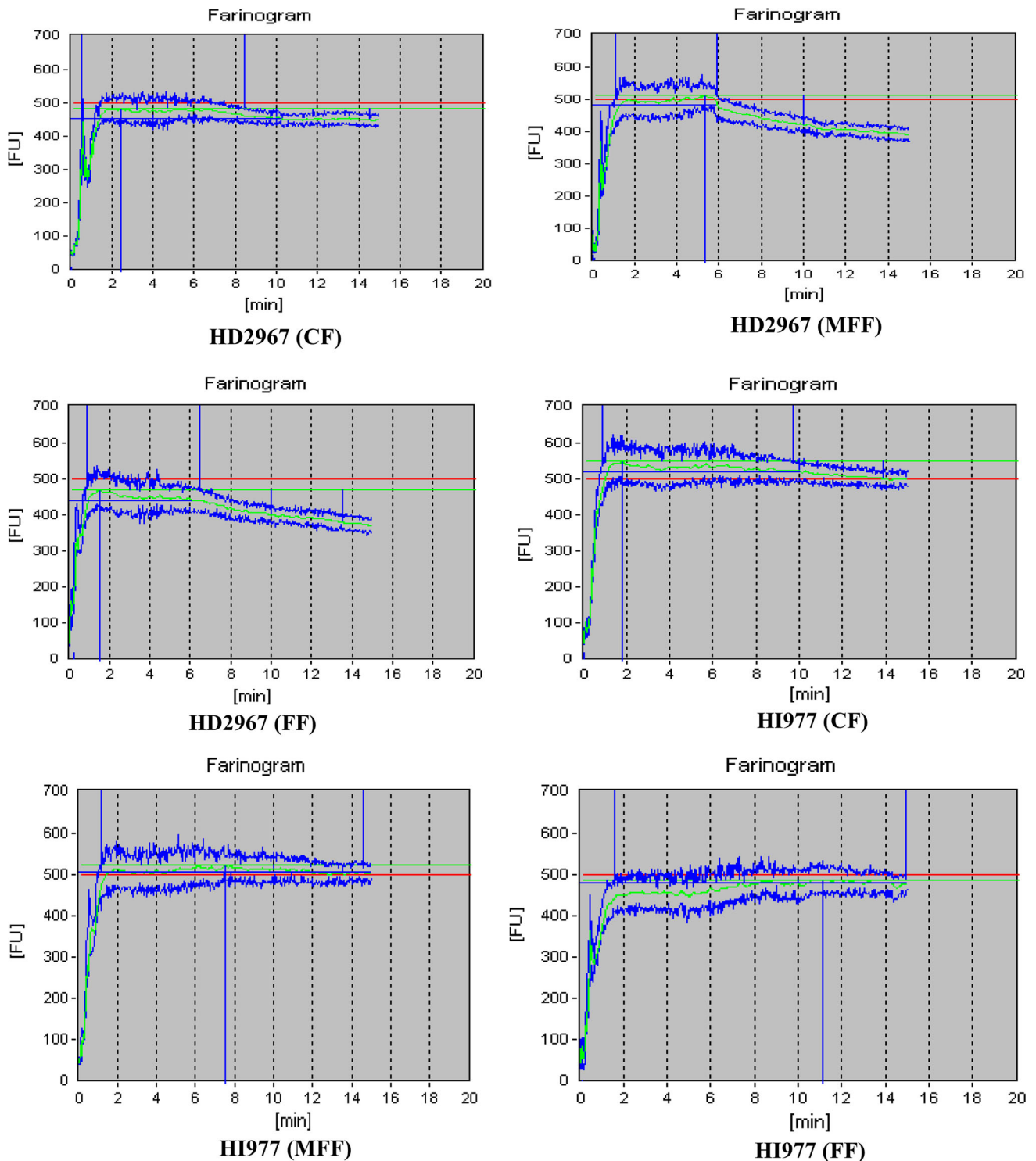


Fig. 4 Farinograms of different fractions of MHW and Ex-SW varieties flour obtained by sieve shaker

respectively, $p \leq 0.005$) while negatively correlated with protein content ($r = -0.767$, $p \leq 0.005$). WA was positively correlated with medium and large size particles ($r = 0.621$ and 0.586 , respectively, $p \leq 0.05$) while

negatively correlated with PV, FV, PT and small size particles ($r = -0.614$, -0.630 , -0.533 and -0.817 , respectively, $p \leq 0.05$). Average WA of CF was 60.90 and 56.40% for MHW and Ex-SW varieties (Table 3). DDT of

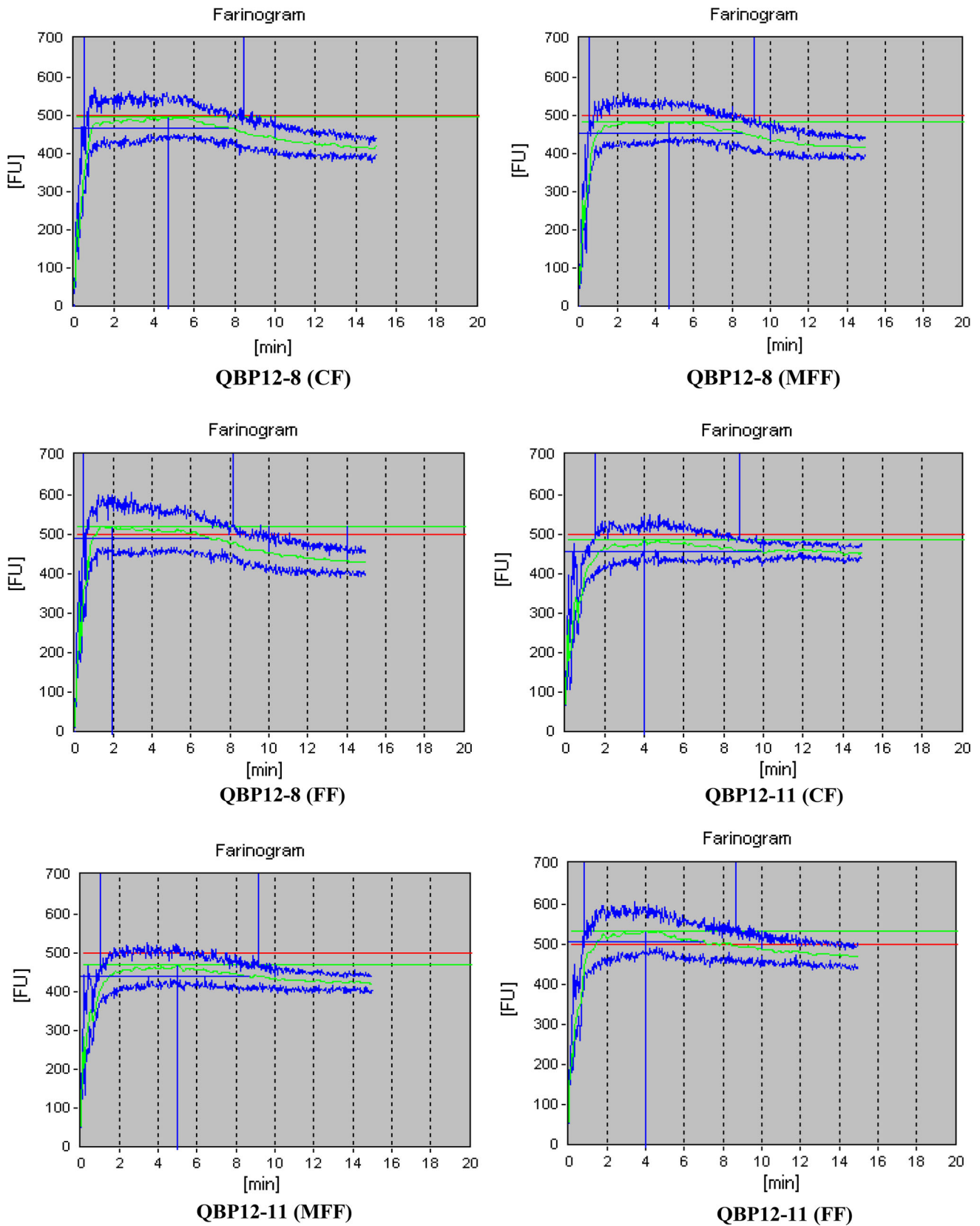


Fig. 4 continued

CF, MFF and FF ranged from 1.9 to 4.00 min, 4.7 to 7.5 and 2.00 to 11.2 (HI977) min, respectively. CF showed lower DDT and DS than MFF and FF (Table 3). Average DDT of Ex-SW and MHW varieties were 4.07 and 5.00 min, respectively (Supplementary Table 1). DS and DDT increased with decrease in grain hardness. DDT was positively correlated with LASRC and SuSRC ($r = 0.563$ and 0.641 , respectively, $p \leq 0.005$). DDT was positively correlated with Ex-PP ($r = 0.432$, $p \leq 0.005$) and negatively correlated with Ex-MP ($r = -0.400$, $p \leq 0.005$). DS of CF, MFF and FF ranged from 7.1 to 8.9 min, 4.9 to 13.4 (HI977) and 5.5 to 13.30 (HI977) min, respectively (Table 3). Average DDT of CF was 2.20 and 4.35 min for MHW and Ex-SW varieties (Supplementary Table 1). FF of HI977 showed exceptionally higher DS and DDT than their corresponding fractions and varieties. Average DS of Ex-SW and MHW varieties were 7.90 and 8.90 min, respectively (Supplementary Table 1). DS was positively correlated with SuSRC and LASRC ($r = 0.624$ and 0.641 , respectively, $p \leq 0.005$). The results indicated the correlation of arabinoxylans and glutenins content with DS. DS was positively correlated with Ex-PP ($r = 0.722$, $p \leq 0.005$) and negatively correlated with Ex-MP ($r = -0.722$, $p \leq 0.005$). Average DS of CF was 8.00 and 7.60 min for MHW and Ex-SW varieties (Table 3). DOS of CF, MFF and FF ranged from 27 to 56 BU, 10 to 90 BU and 10 to 72, respectively. Average DOS for Ex-SW and MHW varieties was 48 and 39.3 BU, respectively. DOS was negatively correlated with SuSRC, LASRC and Ex-PP ($r = -0.736$, -0.618 and -0.604 , respectively, $p \leq 0.05$) and positively correlated with Ex-MP ($r = 0.604$, $p \leq 0.05$). Average DOS of CF was 27.00 and 44.50 BU for MHW and Ex-SW varieties (Table 3).

SDS-PAGE analysis of proteins

SDS-PAGE analysis of protein extracts, isolated from the sieve classified fractions of different MH and Ex-SW wheat varieties showed major diversity in HMW-GS and LMW-GS polypeptides (PPs) ranging from 97–55 kDa and 55–24 kDa (Fig. 3a). Densitometry analysis revealed that concentration of HMW-GS of 98 kDa was higher in MFF of HD2967, HI977 and QBP 12-11 as compared to CF of same varieties and concentration of same proteins was declined in FF except QBP 12-11 (Fig. 3b). On contrary, the storage of 98 kDa PPs in QBP-12-8 was declined from CF to FF. The levels of HMW-GS PPs of 85 kDa was increased from CF to MFF followed by a decline in HD2967, HI977 and QBP12-11, however, rise in the levels of 85 kDa PPs was differential in MHW and Ex-SW varieties (Fig. 3b). HD2967, HI977 and QBP12-8 also showed lower levels of 98 kDa and 85 kDa PPs in FF, as compared to CF (Fig. 3b). Particle size analysis revealed

that the levels of 98 kDa and 85 kDa proteins were increased with an increase in small size particles in HD2967, HI977, QBP 12-8 and QBP 12-11 which was maximum up to MFF followed by a decline in the levels of 98 kDa and 85 kDa PPs in HD2967, HI977 and QBP-12-8. Whereas, distribution of small size particles was increased in HD2967, HI977 but the levels of levels of 98 kDa and 85 kDa PPs was declined. The distribution of small size particles in Ex-SW QBP12-8 and QBP 12-11 was declined in FF and it was associated with decline in the levels of 98 kDa and 85 kDa PPs in QBP 12-8 but not in QBP 12-11. On the contrary, the levels of large size particles were not appeared to be associated with the levels of 98 kDa and 85 kDa PPs since the levels of these particles in flours obtained from CF to FF was decreased. QBP 12-8 and QBP-12-11 showed decreased distribution of small size particles from CF to MFF while the levels of 98 kDa and 85 kDa PPs was increased. HD2967 and HI977 showed an increase in the levels of 98 kDa and 85 kDa PPs from CF to MFF, and the distribution of small size particles was also increased. The medium size particles distribution was increased in HI977 and QBP 12-11 but the levels of 98 kDa and 85 kDa PPs was decreased in HI977 while it was increased in QBP 12-11. It was, therefore, likely that the association of 98 kDa and 85 kDa PPs with small size particles was correlated directly, as the distribution of these particles along with 98 kDa and 85 kDa PPs was linear irrespective of HW and Ex-SW varieties. Intriguingly, the protein content in CF, MFF and FF, irrespective of varieties, was 11.78, 12.16, and 12.42%, respectively, whereas the particle size distribution of small, medium and large size particles in FF was 36.49, 48.57 and 14.94%, respectively. Although the FFs contained marginal difference in the distribution of small and medium size particles but protein content was varied up to a higher extent. These results thus revealed that the association of proteins with small particle size may be strong and thus affected the rheological behavior of dough to a greater extent.

Conclusion

CF, MFF and FF obtained from flours of different MHW and Ex-SW varieties varied in particle size were evaluated for various physicochemical characteristics. L^* increased while a^* and b^* value decreased with decrease in GHI. The small size particles increased while medium and large size particles decreased with decrease in GHI. Fractionation improved the protein content and paste properties of flour as FF had higher protein content, gluten content and UnEx-PP. Therefore FF obtained from flour might be more suitable for preparation of bread than original flour. The association of proteins with small size particles may be

strong and thus affected the rheological behavior of dough to a greater extent. The fractions obtained are very useful for the breeders, millers and baking industries.

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