Fractionation of unreduced gluten proteins on SEC and their relationship with cookie quality characteristics

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Abstract The size distribution assessment of unreduced wheat gluten proteins of commercial Indian wheat varieties was examined using Size-Exclusion Fast Protein Liquid Chromatography. Elution profiles were fractionated into five peaks, with the molecular weights of eluting peaks as 130–30, 55–20, 28–10 and <10 kDa (IV and V), respectively. Peak I (glutenins) and II (gliadins) exhibited contrary results with AWRC ($r = 0.928^{**}$ and $r = -0.831^{**}$), R/E ratio ($r = 0.745^{**}$ and $r = -0.869^{**}$), gluten index ($r = 0.959^{**}$ and $r = -0.994^{**}$), gliadin/glutenin ratio ($r = -0.952^{**}$ and $r = 0.948^{**}$), dough development time ($r = 0.830^{**}$ and $r = -0.930^{**}$), dough stability ($r = 0.901^{**}$ and $r = -0.979^{**}$) and dough weakening ($r = -0.969^{**}$ and $r = 0.986^{**}$). Significant statistical correlation was also observed for peak I and II with cookie hardness ($r = 0.948^{**}$ and $r = -0.924^{**}$) and cookie spread ($r = -0.837^{**}$ and $r = -0.743^{**}$) respectively. Peak III, IV and V occupied a minor fraction of whole and did not exhibit a statistically significant correlation with any of the quality parameters.

Keywords SEC · Dough rheology · Glutenin · Gliadin · Cookie quality

Introduction

Quality of a wheat variety for cookie making is largely adhered to the molecular weight distribution of proteins. Wheat containing lower protein content with poor gluten strength produces tender cookies with higher spread. Extensible dough with weak gluten network development is generally preferred for cookie making. During rising of the temperature in baking oven, cookie dough allows the formation of gluten network and flour undergoes an apparent glass transition which leads to expansion of the dough. The dough with strong gluten network restricts the movement of water, resulting in decreased dough viscosity during baking which prevents the expansion of the cookie dough and leads to cookie with smaller diameter. The glass transition temperature of the soft wheat flour which generally contains low protein content was significantly higher (Doescher et al. 1987). Barak et al. (2013a) reported that cookie hardness exhibited a negative association with gliadin/glutenin ratio indicating that higher gliadin content in the dough produced tender cookies.

In recent years, interest on the qualitative features in relation to proteins of wheat cultivars for specific baking quality, acquired special attention. Size-exclusion chromatography (SEC) or size-exclusion high pressure liquid chromatography (SE-HPLC) have become the most assessable and precise tool to evaluate the molecular weight distribution patterns of wheat proteins. Singh et al. (1990a) has studied variation in relative proportion of three main classes of wheat proteins by utilizing sonication treatment for enhanced protein extraction followed by SE-HPLC. The statistical significance of correlation of these wheat flour protein fractions were demonstrated with bread making quality (Singh et al. 1990b). Variation in the amounts of fraction with varying molecular size in wheat

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flour was conceded by Batey et al. (1991). Huebner et al. (1997) isolated the gliadin and glutenin fractions and analyzed them by SE-HPLC and gliadins by reversed phase high performance liquid chromatography (RP-HPLC) and correlation studies were applied on proportion of individual polypeptides, as determined by SE-HPLC/RP-HPLC with measurements of baking quality. Quantitative protein compositions of soft wheat varieties were analyzed by SE-HPLC and results were statistically correlated with numerous physical and chemical characteristics of wheat and quality characteristics of cookies baked from the flours (Huebner et al. 1999). Ohm et al. (2009) investigated the absorbance area and area percentage values of unreduced wheat protein fractions separated by SE-HPLC and reported the relationships with grain, flour, and end-use quality characteristics of soft white winter wheats that indicated associations of molecular weight distributions of proteins with cookie quality characteristics.

This investigation was performed to observe the fitness of SEC chromatograms of unreduced wheat gluten proteins on FPLC (fast protein liquid chromatography) system to evaluate the performance of wheat varieties in context to cookie making quality. The target objective was to report the impact of molecular weight distributions variation of gluten proteins on quality characteristics of wheat flour and cookie production.

Materials and methods

Wheat flour samples and their physicochemical analyses

Four Indian wheat varieties i.e. C306, DBW16, HI977 and HW2004 were selected for the study. Grain hardness was determined using Single Kernel Characterization System (Model 4100, Perten Instruments, Huddinge, Sweden) according to standard AACC procedures. Crude protein content was determined by standard AACC method (2000). The samples were tempered at 15.5 g/100 g moisture for 24 h and milled to 65% extraction rate using Chopin laboratory mill (Model CD1, Villeneuve la Garenne, France). Chopin SDmatic and Falling Number apparatus were used to evaluate damaged starch content and falling number value, respectively. SDS Sedimentation value of flours was determined according to Axford et al. (1979). Alkaline water retention capacity (AWRC) was carried out according to the AACC 56–10 method.

Dough rheological properties

Dough rheological characteristics were investigated using Mixolab Standard “Chopin S” protocol. The protocol is

based on constant mixing of dough at a speed of 80 rpm up to 30 min at 30 °C so as to produce a target torque of 1.1 ± 0.05 Nm. The information recorded from the curves revealed the water absorption (%), dough development time (min), dough stability (min) and dough weakening (FU).

Isolation and fractionation of gluten

Wheat flours were defatted by successive extraction with chloroform according to MacRitchie (1987). 100 g flour was defatted using 200 ml of chloroform repeatedly and filtered through filter paper at room temperature. The defatted flour was dried at room temperature. Gluten was isolated from defatted flour using Glutomatic instrument (Model GM 2200, Perten Instruments) as per standard ICC method. Gluten index was determined and measurements for gluten extensibility were performed with a TA-XT 2i Texture Analyzer (Stable Micro System) using Kieffer dough/gluten extensibility rig with the test speed of 3.3 mm/s and data acquisition rate of 200 pps under force in tension. Freeze dried gluten samples were ground in a pestle mortar and dissolved in 200 ml of 70% ethanol. The mixture was allowed to stir on a magnetic stirrer for 3 h at 25 °C followed by centrifugation for 30 min at $1000 \times g$ at 4 °C and the whole process was repeated thrice. The supernatants were pooled and ethanol was removed from the gliadin extracts using rotary evaporator at 30 °C. The gliadin and glutenin fractions, thus, obtained were freeze dried and powdered in pestle and mortar.

Size exclusion chromatography on FPLC system

Extraction of total proteins 20 mg gluten was mixed with 10 ml of 3 M urea (pH 4.6) buffer (Chaudhary et al. 2016a, b, c). The mixture was sonicated using Power Sonic 410 sonicator (Hwashin Technology) for 30 min. Afterwards, the mixture was centrifuged at $12,000 \times g$ for 30 min using Remi Cooling Centrifuge. The supernatant was filtered through 0.22 µm syringe filter (HV Millipore, Durapore). 2 ml of the sample extract was used for each run. **Run conditions** SEC was performed on Sephacryl S-200 column (HI Prep™ 16/60 Sephacryl S-200 HR, GE Healthcare). The protein fractions were eluted from the column using 3 M urea (pH 4.6) containing 0.15 M NaCl with a flow rate of 0.5 ml/min. Selected eluted fractions were then assayed by SDS-PAGE as described by Laemmli (1970).

Cookies making

Cookies were prepared according to AACC approved method 10–50D (2000) with slight modifications. The

ingredients used were flour (225 g), sugar (130 g), shortening (64 g), dextrose solution (33 ml), sodium bicarbonate (1.6 g), ammonium bicarbonate (0.9 g), sodium chloride (2.1 g) and distilled water (16 ml). The dough was prepared and sheeted to a thickness of 10 mm on a dough sheeter and round shaped cut was given using cutter of 60 mm diameter. Cookies were baked in a lightly greased tray in baking oven at 205 °C for 15 min followed by cooling and subsequently measured for diameter and thickness and average was calculated. The spread ratio was calculated by dividing diameter (mm) with thickness (mm). Cookies were prepared in triplicate.

Texture analysis

Textural analyzer (Stable Micro Systems TA-XT 2i, Godalming, U.K.) was used to measure the hardness of the cookie. The probe used was Knife Edge Insert (HDP/BS) in order to apply braking force required to fracture the cookies with 5 kg load cell Heavy Duty Platform (HDP/90) at pre-test speed, test speed and post-test speed of 1.5, 2.0 and 10.0 mm/s respectively with a data acquisition rate of 400 pps.

Statistical analyses

Data was analysed using SPSS software version 16.0 (SPSS Inc.). Correlation among various quality characteristics of flour, dough, gluten, area % and cookie were derived using Pearson's test ($p < 0.05$). The mean comparison was carried out using one way ANOVA with Duncan's multiple range test and the statistical significance was observed at $p < 0.05$. All the tests were performed in triplicates.

Results and discussion

Fractionation of wheat gluten proteins by SEC

SEC fractionated un-reduced gluten proteins into five peaks, namely, peak I, II, III, IV and V in order of decreasing molecular size of eluted proteins (Chaudhary et al. 2016b). Protein fraction eluted in peak I at 68.00–89.07 min was enriched in polymeric glutenin proteins i.e. HMW-GS and LMW-GS with molecular weight range of 130–30 kDa. Monomeric gliadins ranging from 55–20 kDa were eluted in peak II at 93.60–112.27 min. Peak III eluted at 120.27–128.53 min consists of very low molecular weight monomeric gliadins (28–10 kDa). Peak IV and V with elution time 136.80–157.60 and 212.00–224.27 min, respectively, corresponds to albumins and globulins collectively with molecular weight <10 kDa. The relative

proportion of each protein fraction in whole gluten protein eluted at a particular interval of time (peak I–V) is expressed in terms of area % which varied from 35.63 to 47.34, 38.93 to 55.47, 0.85 to 2.49, 5.27 to 8.97 and 0.48 to 2.40%, respectively, among the wheat cultivars. It is noteworthy that peak I and II constituted a major fraction of un-reduced gluten protein while peak III, IV and V represented a minor portion of whole.

Relationship of physicochemical, gluten characteristics and HMW-GS composition

Table 1 enlisted the physicochemical characteristics of wheat varieties. Among the four varieties, grain hardness was the lowest for DBW16, followed by HW2004, HI977 and the highest for C306. Damaged starch content followed the same trend as grain hardness in wheat varieties. HW2004 was observed to possess the highest amylase activity as indicated by its least falling number value (471 s). Protein content ranged from 8.68 to 12.56%, corresponding to HW2004 and C306 respectively. SDS sedimentation value showed large fluctuations among the varieties. It was recorded minimum for HW2004 (35 ml), followed by C306 (45 ml), DBW16 (46 ml) and maximum for HI977 (62 ml). The values for AWRC ranged between 84.60% for HW2004 and 90.21% for DBW16.

In addition to higher protein content, C306 also contained the highest proportion of dry gluten content (11.7 g/100 g). In contrast to this, HW2004 yielded the least content of dry gluten (7.8 g/100 g). R/E ratio varied considerably among the varieties with C306 and HI977 exhibiting the lowest and the highest ratio of 0.38 and 1.49, respectively. HI977 poses greater gluten strength as evident from its highest gluten index value (98.7) and lowest gliadin/glutenin ratio (0.76). It is a well-known fact that C306 and HW2004 had HMW-GS composition null, 20,

Table 1 Physicochemical and cookie quality characteristics of wheat varieties

	C306	DBW16	HI977	HW2004
GH (N)	87.27d	68.43a	76.30c	75.07b
DaS (%)	6.87d	5.92a	6.56c	6.13b
FN (s)	536c	501b	501b	471a
AWRC (%)	85.75b	90.21d	89.90c	84.60a
DG (g/100 g)	11.7d	9.6c	8.9b	7.8a
HD	2266.67b	2898.67c	3287.00d	1884.33a
SF	8.13c	5.54a	5.90b	10.28d

GH Grain hardness, DaS Damaged starch, FN Falling number, AWRC Alkaline water retention capacity, DG Dry gluten, HD Hardness and SF Spread factor

Values followed by different letters are significantly different at $p < 0.05$

2 + 12 at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively with a low *Glu-1* score of 4, while DBW16 and HI977 had alike HMW-GS composition at *Glu-A1* and *Glu-D1* i.e. 2* and 5 + 10 with only difference at *Glu-B1* loci expressing 7 + 8 and 17 + 18, respectively, with a high *Glu-1* score of 10 (Kaur et al. 2016, 2013). Though the *Glu-1* scores of HW2004 and C306 are similar, yet they differed widely with respect to the dry gluten content, R/E ratio, gluten index, gliadin and glutenin content. However, DBW16 and HI977 displayed the similar results. The cumulative results of these parameters, in addition to their HMW-GS composition strongly characterize variety HW2004, the weak and HI977 as the strong.

The statistical correlation analysis (as presented in Table 2) revealed that the association of overall protein content in a wheat variety with SEC peaks was poor, the correlation coefficients being statistically non-significant. Peak I and II displayed a strong positive and negative correlation coefficient with SDS sedimentation volume ($r = 0.818$ and $r = -0.893$). It is the ability of glutenin strands to swell on reaction with lactic acid and SDS (Carver and Rayburn 1995), which reflects a strong association of glutenins eluted in peak I with SDS sedimentation volume. However, Eckert et al. (1993) stated that gliadins dissolve completely in solution and thus could not contribute to swelling. An increase in glutenin (peak I) could accompany an increase in R/E ratio ($r = 0.745^{**}$) and gluten index ($r = 0.959^{**}$), but negatively related to gliadin/glutenin ratio ($r = -0.952^{**}$) by favouring development of extensive gluten network by means of intermolecular disulphide bridges. Gliadins, eluted in peak II, behaves like a plasticizer, interfering with glutenin–glutenin interaction and thereby displayed reciprocating results with R/E ratio ($r = -0.869^{**}$), gluten index ($r = -0.994^{**}$) and gliadin/glutenin ratio ($r = 0.948^{**}$). Similar results were observed by Gulia and Khatkar (2014), Khatkar and Schofield (1997). Statistical positive and negative correlation coefficient were observed among AWRC and peak I ($r = 0.928^{**}$) and peak II ($r = -0.831^{**}$). Moreover, low molecular weight gliadins eluted in peak III exerted a parallel outcome to peak I for various parameters discussed. Peak IV and V did not exert a significant outcome due to their very less amount as compared to other peaks, in addition to this; they are not known to have any technological importance.

Dough rheological properties and SEC

Water absorption ranged from 53.73 to 62.40%. The values for water absorption varied in accordance with damaged starch content. Dough development time was recorded to be the lowest for HW2004 (1.89 min) and the highest for HI977 (7.38 min). As expected, dough of HW2004 was

least stable and had the highest degree of softening, owing to its least protein and gluten content, presence of weaker HMW-GS and minimum gluten index, with a higher proportion of gliadins and greater gliadin/glutenin ratio suggesting a reduced tolerance of flour to mixing in comparison to flours of DBW16, C306 and HI977. An interesting fact emerging out is that varieties having similar HMW-GS composition, as in case of HW2004 and C306, differed widely in terms of their rheological properties. In a similar manner, dough weakening was much more pronounced in DBW16 than HI977. These results were in accordance with the studies of Barak et al. (2013b).

Table 2 cited correlation among mixograph parameters and SEC peaks. Peak I was positively correlated with dough development time ($r = 0.830^{**}$), dough stability ($r = 0.901^{**}$) and negatively with dough weakening ($r = -0.969^{**}$). Peak II conferred contrary results to peak I as for dough development time ($r = -0.930^{**}$), dough stability ($r = -0.979^{**}$) and dough weakening ($r = 0.985^{**}$). Hydration of gluten protein and formation of interactions between glutenins and gliadins is a prerequisite for dough development (Belton 1999). Song and Zheng (2008) concluded that at the time of mixing, glutenins tend to align themselves and form intermolecular disulphide bridges leading to the development of strong gluten network and resulted in increased dough strength. Khatkar et al. (2013) displayed a higher correlation of glutenin with dough development time and dough stability. However, gliadin promotes elongational resistance and dough extensibility and thereby brings about degree of softening to the dough. Analogous remarks were laid out by Barak et al. (2013c), Dhaka and Khatkar (2015), Khatkar et al. (2002a, b) who explained that the incorporation of gliadins cut down the dough development time and resulted in dough weakening. Water absorption was negatively influenced by SEC peak I ($r = -0.765^{**}$) and peak III ($r = -0.639^{*}$), whereas, positively influenced by peak II ($r = 0.624^{*}$). It could be attributed to the fact that hydrated gluten protein network also provides a framework for holding starch granules intact and diminish the water absorption accordingly. Peak IV and V did not demonstrate any marked relationship with rheological characteristics. These results suggested that SEC is well suited for predicting the rheological behaviour of wheat cultivars.

Relationships among cookie quality, wheat quality and SEC

Cookie quality parameters prepared from various wheat varieties differed significantly (Table 1). Hardness of cookies ranged from 1884.33 to 3287.00 g. Cookie spread was aligned from 5.54 to 10.28 in increasing order from DBW16, followed by HI977, C306 and HW2004. HW2004

Table 2 Correlation among flour, gluten, dough rheological characteristics, SEC area % and cookie quality attributes

	SDSV	DaS	AWRC	R/E	Glia/Glu	GI	WA	DDT	DS	DW	PI	PII	PIII	PV	HD	SF
SDSV	1															
DaS	ns	1														
AWRC	0.744**	−0.760**	1													
R/E	0.732**	ns	ns	1												
Glia/Glu	−0.935**	ns	−0.925**	−0.686*	1											
GI	0.928**	ns	0.810**	0.844**	−0.965**	1										
WA	ns	0.852**	−0.742**	ns	ns	ns	1									
DDT	0.977**	ns	0.681**	0.857**	−0.904**	0.950**	ns	1								
DS	0.936**	ns	0.730**	0.910**	−0.917**	0.981**	ns	0.980**	1							
DW	−0.931**	ns	−0.885**	−0.789**	0.985**	−0.992**	0.590*	−0.935**	−0.963**	1						
PI	0.818**	−0.593*	0.928**	0.745**	−0.952**	0.959**	−0.765**	0.830**	0.901**	−0.969**	1					
PII	−0.893**	ns	−0.831**	−0.860**	0.948**	−0.994**	0.624*	−0.930**	−0.979**	0.986**	−0.969**	1				
PIII	0.757**	−0.726**	0.986**	ns	−0.913**	0.796**	−0.639*	0.664*	0.685*	−0.852**	0.871**	−0.774**	1			
PV	0.615*	ns	ns	0.872**	ns	0.585*	ns	0.744**	0.722**	ns	ns	−0.589*	ns	1		
PV	0.737**	ns	ns	0.934**	ns	0.727**	ns	0.851**	0.839**	−0.664*	ns	−0.730**	ns	0.982**	1	
HD	0.914**	ns	0.951**	0.626*	−0.994**	0.944**	0.683*	0.869**	0.883**	−0.972**	0.948**	−0.924**	0.945**	ns	ns	1
SF	−0.764**	−0.689*	−0.970**	ns	0.902**	−0.774**	ns	−0.660*	−0.663**	0.832**	−0.837**	0.743**	−0.996**	ns	ns	−0.937**

* Correlation is significant at the 0.05 level

** Correlation is significant at the 0.01 level

SDSV Sedimentation volume, DaS Damaged starch, AWRC Alkaline water retention capacity, R/E Resistance to extension to extensibility ratio, Glia/Glu Gliadin to glutenin ratio, GI Gluten index, WA Water absorption, DDT Dough development time, DS Dough stability, DW Dough weakening, PIV Peak I, PII Peak II, PIII Peak III, PV Peak V, HD Hardness, SF Spread factor and ns non significant

resulted in cookies with higher spread and soft texture; however, cookies from HI977 were extremely hard with poor spread.

The correlation coefficients observed among cookie quality, wheat quality and SEC are summarized in Table 2. Damaged starch was negatively associated with cookie spread ($r = -0.689^*$) in agreement with the studies of Moiraghi et al. (2011). An increase in damaged starch content increases the water absorption of the flour as reflected by their positive correlation ($r = 0.852^{**}$). This leads to dough stiffness and resulted in cookie with lower spread. SDS sedimentation volume exhibited a positive correlation with cookie hardness ($r = 0.914^{**}$) and negative with cookie spread ($r = -0.764^{**}$). Barak et al. (2013a) concluded that SDS sedimentation volume symbolizes gluten quality; a higher value of which comprehends higher proportion of glutenin in flour and brings about more elastic dough and results in compact cookie. Dough development time and dough stability also affected cookie hardness ($r = 0.869^{**}$ and $r = 0.883^{**}$) and spread ($r = -0.660^*$ and $r = -0.663^*$) by virtue of developing stronger gluten network by glutenins. The results are in agreement with the studies of Kaur et al. (2014). The spread ratio of cookie followed an increasing trend with the increase in the gliadin/glutenin ratio as indicated by significant positive correlation ($r = 0.902^{**}$). This necessitates the requirement of wheat variety with poor gluten strength for superior quality cookies. It was previously reported by Kuragano et al. (1991) that incorporation of gliadins to the base wheat flour resulted in cookies with greater spread and softer texture. Furthermore, Uthayakumaran et al. (2001) concluded that γ -fraction of gliadins increases the extensibility of the wheat dough utmost and thus wheat flours with higher content of this gliadin sub-fraction improve the cookie making quality of the wheat cultivar. AWRC was observed to exert a positive and negative effect on cookie hardness ($r = 0.951^{**}$) and spread ($r = -0.970^{**}$), respectively. Flours with low AWRC values tend to produce superior quality cookies with large diameters.

Cookie hardness was strongly positively correlated with polymeric glutenins (peak I, $r = 0.948^{**}$) and low molecular weight monomeric gliadins (peak III, $r = 0.945^{**}$), however, negatively correlated with gliadins (peak II, $r = -0.924^{**}$). A likely explanation for this is the tendency of polymeric glutenins to build up a strong gluten network by covalent linkages which brings about an intensive competition for water among gluten hydration, starch swelling and gelatinization, thereby, raising the dough viscosity. This restricts the spreading of dough and a strong gluten network provides innate hardness to the cookie. Peak II (gliadins) diminish the detrimental effect caused by glutenins by acting as a plasticizer and resulted

in tender cookies. Cookie spread was favoured by peak II ($r = 0.743^{**}$), but negatively influenced by peak I ($r = -0.837^{**}$) and peak III ($r = -0.996^{**}$) in agreement with the studies of Ohm et al. (2009). The divergent relationship of these protein fractions with cookie quality parameters reflects that flour which forms strong dough leads to highly elastic gluten network formation and tends to cause shrinkage of dough making it inappropriate for cookie.

Conclusion

For years, HMW-GS composition is ruling the wheat province which evoked a major interest to develop a single reference tool for understanding the discrete behaviour of a specific wheat variety. This study was performed to investigate the association between technological and functional characteristics of a wheat variety with molecular weight distribution of gluten proteins. Presumably, wheat varieties used in this study contained varied levels of glutenins, gliadins and low molecular weight proteins as revealed from SEC results. The deviation in the chromatogram peak's area % exerted a pronounced alliance with damaged starch, SDS sedimentation volume, R/E ratio, gluten index, gliadin/glutenin ratio, dough development time, dough stability, dough weakening and cookie quality characteristics. Peak I and III induced a positive correlation coefficient with cookie hardness and negative with cookie spread while peak II recurred with a paradoxical trend. Wheat varieties with weak gluten network, owing to lower area % of peak I and higher area % of peak II have no intensive competition for water among gluten hydration, starch swelling and gelatinization, thereby, providing less resistance to flow and resulted in cookie with greater spread and tender texture. On this basis, variety HW2004 stood outstanding for cookie making. Subsequently, higher levels of peak I as in HI977 raised initial viscosity of the dough by extensive cross linkages and as a consequence, harder cookies with poor spread were produced.

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