

Physicochemical and Gluten Quality Characteristics of Commercial Wheat Varieties

Priya Dangi¹, B. S. Khatkar²

Research Scholar, Department of Food Technology, GJUS&T, Hisar, Haryana, India¹

Professor, Department of Food Technology, GJUS&T, Hisar, Haryana, India²

ABSTRACT: Five commercial wheat varieties, namely C 306, HI 977, HW 2004, PBW 550 and WH 542 of diverse baking performances were examined for their physicochemical parameters, gluten quality and HMW-GS composition. All the varieties presented a broad spectrum of different quality parameters. Based on the kernel dimensions, thousand kernel weight and hectolitre weight, WH 542 was observed to have smaller and less plumpy grains, while HW 2004 possessed sound, plumpy and bulky grains. The protein content was found maximum for PBW 550 with SDS sedimentation volume of 60 ml. The gluten quality parameters, particularly, gluten index (GI), R/E ratio and gli/glu ratio showed remarkable differences among the varieties. The exceeding values of GI, R/E ratio along with superior breadmaking HMW-GS composition in PBW 550 and HI 977 makes them strong variety. On the contrary, the lower values of GI, R/E ratio with higher gli/glu ratio and inferior HMW-GS makes C 306 and HW 2004 as weak varieties.

KEYWORDS: Wheat, Physicochemical characteristics, Gluten proteins, HMW-GS composition.

I. INTRODUCTION

Wheat (*Triticumaestivum*) is the prime cereal crop for majority of world's population. It is the dominant crop in temperate countries and is widely used for human consumption and livestock feed. India has witnessed as second largest producer of wheat in the world after China with a record production of 92.29 MMT, contributing a share of 11.78 % to global wheat production in year 2015-16. Despite the introduction of many species to cultivation, cereals (bread wheat, durum wheat, barley, rye, oat and triticale) remain the major nutritional source for the majority of global population. The wide adaptability and high yield of wheat are not the exclusive factors for its acceptance. Wheat is prominent by virtue of its ability to form viscoelastic dough that exhibits rheological properties required for the heterogeneity of foods such as chapatti, bread, biscuit, cakes, noodles and pasta (Khatkar *et al.*, 2016).

The end use utilization of wheat is related to its physicochemical characteristics, quantity and quality of starch and protein fractions. The type and quantity of the gluten proteins serves as an index for the prediction and evaluation of flour quality for end products (Chaudhary *et al.*, 2016a, 2016d). Wheat protein quality depends on the presence or absence of specific HMW-GS, LMW-GS (Gupta *et al.*, 1995), gliadins (Chaudhary *et al.*, 2016b), ratio of monomeric to polymeric proteins (Gupta *et al.*, 1992) and the molecular size distribution of glutenin (MacRitchie, 1999). The present study is aimed to analyse the physicochemical characteristics and gluten protein composition of wheat varieties.

II. MATERIALS AND METHODS

A. Procurement of wheat varieties

The selection of wheat varieties for the research was based on their quantitative and qualitative protein profiles. Five wheat varieties viz. C 306, HI 977, HW 2004, PBW 550 and WH 542 were used in this research. The varieties were procured from different research stations and agricultural universities as mentioned in Table I.

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Table I: Wheat varieties from various Research Institutions and Agricultural Universities

Wheat Varieties Procured	Research Station/ University	Location
C 306, PBW 550, WH 542	CCS HAU	Hisar, Haryana
HI 977, HW 2004	IARI Regional Centre	Indore, M. P.

B. Physical analysis of wheat grains

Grain characteristics like kernel dimensions, test weight and thousand kernel weight of the wheat varieties were estimated according to standard procedures. The length and width of the kernel of each variety were determined using digital Vernier calliper. Test weight is an index of the density and the soundness of kernels which symbolizes milling yield. For determination of test weight, grains were poured into a 100 ml stainless steel measuring cylinder, with excess wheat being leveled off using a round stroker. The weight of wheat grains in the cylinder was measured to determine the test weight. Kernel weight, a function of kernel size or kernel density, is usually expressed in grams per 1,000 kernels. Smaller and less dense kernels exhibited a lower endosperm to non-endosperm ratio in comparison to large and dense wheat counterparts that had a higher ratio of endosperm to non-endosperm components. Thousand kernel weight (TKW) is the observed weight of 100 seeds and multiplying it by a factor of 10. Grain hardness and moisture content were determined by using Single Kernel Characterization System as prescribed by AACC (2000).

C. Milling of wheat grains

The foremost step prior milling is cleaning of grains which was carried out manually so as to eliminate soil particles, broken kernels and foreign seeds. Properly cleaned wheat varieties were allowed to temper to 15.5 % moisture content for 48 h and subsequently underwent milling on a Chopin laboratory mill to produce flour. The flour samples of each variety were sealed in air-tight containers and placed at -18 °C in a deep freezer for storage. The flour samples were thawed at room temperature prior further analysis.

D. Chemical Analysis of wheat flour

a. Moisture

Moisture content of flour was determined by standard AACC (2000) procedure. 5 g of flour was weighed, placed in a pre-weighed moisture dish and was allowed to heat at 130 °C in hot air oven for 1 h or till a constant weight was obtained. The sample was then cooled to room temperature and the residue was weighed. As a result of moisture evaporation, there is a total loss in weight which was calculated and expressed in percentage. Moisture content of flour is an indicator of its safe storability. Flour with low moisture content is more stable during storage, however, higher moisture content (> 14.5 %) attracts mold, bacteria, and insects resulting in deterioration of wheat flour during storage.

Calculation:

$$\text{Moisture (\%)} = \frac{\text{Loss in weight (g)} \times 100}{\text{Weight of sample (g)}}$$

b. Ash

Ash was determined by standard AACC (2000) method. 5 g of flour sample was weighed and placed in a silica crucible. The sample was ignited till no charred particle remained in the crucible and further kept for heating at 550 °C in a muffle furnace for 5-6 h till the weight become stable. The residue was allowed to cool in a desiccator to room temperature and then weighed. The ash content or the mineral content present in a sample was expressed as a percentage of the initial sample weight. Ash includes non-combustible, inorganic minerals concentrated in the bran layer which imparts a darker color to finished products. During incineration, the high temperature purges out the moisture and burns away all the organic materials (starch, protein, and oil), leaving ash behind. The amount of ash present in a flour indicates its milling performance by indirectly revealing the amount of bran contamination in flour.

Calculation:

$$\text{Ash (\%)} = \frac{\text{Weight of ash (g)} \times 100}{\text{Weight of sample (g)}}$$

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c. Crude protein content

The nitrogen content of flour samples was determined by Kjeldahl's method (AACC, 2000). To 1 g flour sample, 1-2 g digestion mixture (K_2SO_4 : $CuSO_4 \cdot 6H_2O$: SeO_2 in ratio of 10:20:1) and 20 ml of concentrated sulphuric acid were added in Kjeldahl's flask and the contents were heated on hot plate till colorless or light yellow colored liquid is obtained. Once digestion is done and clear liquid was obtained, allow it to cool and dilute to 100 ml. 5 ml of liquid was pipetted out in distillation flask and 10 ml of 40 % sodium hydroxide was further added. Steam distillation was carried out, and about 25 ml of distillate containing ammonia was collected into 5 ml of 2 % boric acid along with three drops of mixed indicator. The titration of ammonium metaborate was carried out with standardized N/70 hydrochloric acid. The titer value involved in obtaining end point from blue to pink gives % of protein. Blank determination was carried out without sample as above and the amount of N/70 hydrochloric acid required subtracted from the sample reading. For wheat flour, the crude protein content was calculated by multiplying % N with protein factor of 5.7.

Calculation:

$$\text{Nitrogen (\%)} = \frac{(a-b) \times \text{normality} \times 1.4}{\text{weight of sample}}$$

where,

a – Volume of HCl used for sample,

b – Volume of HCl used for the blank

d. SDS Sedimentation volume

The sodium dodecyl sulphate (SDS) sedimentation volume of flour samples was estimated by Axford *et al.* (1979). 2 % SDS solution was prepared by dissolving 20 g SDS in distilled water and volume make-up was done to 1 L. For the preparation of lactic acid solution, 3 ml of 88 % lactic acid was taken and diluted (1:8 v/v) to 27 ml with distilled water. Now 20 ml of lactic acid solution was added to 1 L SDS solution to prepare SDS-lactic acid reagent. To a 100 ml stoppered graduated cylinder, 5 g of flour sample (14 % moisture basis) was added and mixed with 50 ml of distilled water. The cylinder was then shaken horizontally for 15 s at 0, 2 and 4 min intervals. Subsequently, 50 ml of SDS-lactic acid reagent was added to the cylinder and mixing was continued by inverting the cylinder four times at 0, 6, 8 and 10 min intervals. The contents of the cylinder were then allowed to settle for 40 min. The sedimentation values are expressed in millilitre. This test reveals information on the protein quantity and the quality of flour samples.

e. Falling number

Falling number is the measure of enzymatic activity (α -amylase) in wheat flour and determined using falling number apparatus (AACC, 2000). It is based upon the determination of viscosity by measuring the resistance of a flour and water paste to a falling stirrer. For measurement, 7 g of flour sample was mixed with 25 ml of distilled water in a falling number tube with a stirrer and shaken to form homogenous slurry. The slurry was allowed to heat in a boiling water bath at 100 °C and stirred constantly until the starch gelatinizes and forms a thick paste. The time in seconds needed by the stirrer to fall through the paste was recorded as the falling number value. A higher value of falling number (for example, above 300 s) indicates minimal enzymatic activity and sound quality of wheat or flour, however, low value of falling number (for example, below 250 s) indicates high enzymatic activity and thus sprout-damaged wheat or flour.

E. Isolation and fractionation of gluten

a. Isolation of gluten

Wheat flours were defatted using chloroform in accordance with MacRitchie (1987). Flour sample (100 g) was extracted with 200 ml of chloroform at room temperature. This mixture was allowed to shake for 1 min and filtered through filter paper. The whole process was repeated thrice and the resulted defatted flour was kept at room temperature until dry. Gluten was isolated from flour using Glutomatic instrument by adopting AACC (2000) procedure. Flour (10 g) was weighed and placed into the washing chamber of glutomatic system. The starch and other solubles were removed from the flour by washing with 2 % NaCl at 15 °C for 5 min and subsequent washing with distilled water. The wet gluten so obtained was collected and expressed on a 14 % moisture basis. Afterwards, the gluten was placed in the centrifuge holder, and was forced to pass through a sieve. The gluten percentage remaining on the sieve is defined as the Gluten Index (an indicator of gluten strength). Wet gluten was dried in gluten drier for 4 min

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to obtain dry gluten. The wet gluten was dried in a freeze dryer and converted into powder form using pestle and mortar and stored for further analysis. Wet gluten is an index of protein quality and is a common flour specification required by end-users in the food industry.

Calculation:

$$\text{Wet gluten (\%)} = \frac{\text{Weight of wet gluten} \times 100}{\text{Weight of sample taken}}$$

$$\text{Gluten index (\%)} = \frac{\text{Weight of gluten retained on sieve} \times 100}{\text{Weight of wet gluten}}$$

$$\text{Dry gluten (\%)} = \frac{\text{Weight of dry gluten} \times 100}{\text{Weight of sample taken}}$$

b. Gluten extensibility test

Uni-axial extensibility of gluten was determined by TA-XT plus Texture Analyzer using Kieffer dough and gluten extensibility rig (Ktenioudaki *et al.*, 2010). Gluten was rolled into a cylindrical shape and placed over three or four channels of the Teflon-coated block which divided the gluten into gluten strips of uniform shape and size. Gluten strips were given a resting period of 40 min at 25 °C, prior to the test. Subsequently, the gluten strips were drawn from Teflon block and positioned across the Kieffer rig dough holder. The force in tension was measured by adjusting the test speed to 3.3 mm/s and a trigger force of 1 g by using 5 kg load cell. The resistance to extension (g) to extensibility (mm) in tension mode was computed by documenting the peak force and the distance at the maximum and the extension limit.

c. Fractionation of gluten proteins

Gluten proteins were fractionated into gliadins and glutenins by following modified Osborne (1907) procedure. Gluten powder (50 g) was dissolved in 1 L of 70 % (v/v) ethanol and stirred on magnetic stirrer for 3 h at room temperature (~22 °C). Later, the mixture was centrifuged at 1000 x g for 30 min in cooling centrifuge at 4 °C. The extraction was repeated thrice. The precipitant was collected as glutenin and the supernatant was administered to rotary evaporator at 30 °C so as to remove ethanol and obtain the gliadins. Gliadins (%), glutenins (%) and gliadin/glutenin ratio were determined. The gliadin and glutenin fractions, thus, obtained were freeze dried and powdered in a pestle and mortar.

F. Statistical analysis

The data was analysed through SPSS software version 16.0 (SPSS Inc.). The mean comparison was accomplished by Duncan's multiple range test and the statistical significance was observed at $p < 0.05$.

III. RESULTS AND DISCUSSION

A. Intervarietal variation in grain quality

All the five varieties, namely C 306, HI 977, HW 2004, PBW 550 and WH 542 presented a broad spectrum of different quality parameters. The physical attributes of kernels of various wheat varieties have been presented in Table II. Considerable deviation was noticed in the grain quality characteristics of wheat varieties. The grain length was found maximum for variety HI 977 i.e. 7.4 mm, and the least for WH 542 i.e. 6.2 mm. The grain diameter or width ranged from 2.5 mm in variety WH 542 to 3.6 mm in case of HW 2004. Thousand kernel weight was recorded as the lowest in the cultivar WH 542 (26.6 g) while the highest for HW 2004 (48.2 g). Hectolitre weight which reflects the grain bulk density was minimum for cultivar WH 542 (77.2 kg/hl), while maximum for the cultivar HW 2004 (84.7 kg/hl). In other words, smaller and less plumpy grains were found for WH 542 as reflected by its least diameter, kernel weight and hectolitre weight. Contrary, HW 2004 possessed sound, plumpy and bulky grains. The moisture content of kernels varied from 7.2 to 11.2 % being minimum in case of HW 2004, while it was the highest in WH 542. It is postulated that kernel with greater width were bulkier as compared to the thinner grains. The cultivar WH 542 (91.9 N) had the hardest kernels, whereas cultivar HI 977 had softest grains among the studied varieties. On the basis of hardness data, varieties HI 977 and HW 2004, may be regarded as medium hard, while PBW 550, WH 542 and C 306 were hard wheat cultivars.

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Table II. Physical characteristics of grains of wheat varieties

Varieties	GL (mm)	GD (mm)	TKW (g)	HLW (kg/hl)	GM (%)	GH (N)
C 306	6.5 ^b	3.2 ^d	39.5 ^c	80.1 ^b	10.9 ^c	86.6 ^d
HI 977	7.4 ^d	3.1 ^c	40.3 ^d	80.8 ^c	8.2 ^b	75.2 ^a
HW 2004	6.9 ^c	3.6 ^e	48.2 ^e	84.7 ^e	7.2 ^a	76.5 ^b
PBW 550	6.9 ^c	2.7 ^b	36.8 ^b	81.9 ^d	11.1 ^d	83.4 ^c
WH 542	6.2 ^a	2.5 ^a	26.6 ^a	77.2 ^a	11.2 ^e	91.9 ^e

GL- Grain length; GD- Grain diameter; TKW- Thousand kernel weight; HLW- Hectolitre weight; GM- Grain moisture and GH- Grain hardness. *Mean values followed by different letters within a same column differ significantly ($p < 0.05$)

B. Flour quality characteristics

The quality characteristics of flour of wheat varieties have been summarized in Table III. The moisture, ash content, protein content, sedimentation volume and falling number varied significantly among the varieties. The moisture content ranged from 11.8 to 13.8%, for WH 542 and HW 2004, respectively. The ash content was found minimum and maximum in PBW 550 and C 306, respectively. The protein content of flour varied greatly among the varieties from 8.4 to 14.6 %. HW 2004 had lower protein contents ($< 10.5\%$), while C 306, PBW 550 and WH 542 had higher protein contents ($> 12\%$). The quality of gluten proteins was assessed by SDS sedimentation volume, which is considered as an index of protein quality. It indicates the swelling ability of glutenin fractions, assorted from 37 to 61 ml. The highest SDSV were noticed for HI 977 and PBW 550, while lower SDSV was demonstrated by the HW 2004, WH 542 and C 306. The measure of amylase activity is signified as falling number, with HW 2004 having a lower value (472 s), followed by WH 542 (502 s), HI 977 (510 s), C 306 (535 s) and lastly PBW 550 (615 s).

Table III: Chemical characteristics of wheat varieties

Varieties	MC (%)	Ash (%)	PC (%)	SDSV (ml)	FN (s)
C 306	12.8 ^b	0.75 ^e	12.8 ^d	46 ^c	535 ^d
HI 977	13.2 ^c	0.68 ^b	12.6 ^c	61 ^e	510 ^c
HW 2004	13.8 ^e	0.74 ^d	8.4 ^a	37 ^a	472 ^a
PBW 550	13.5 ^d	0.65 ^a	14.6 ^e	60 ^d	615 ^e
WH 542	11.8 ^a	0.70 ^c	12.1 ^b	44 ^b	502 ^b

MC- Moisture content; PC- Protein content; SDSV- Sedimentation volume and FN- Falling number. *Mean values followed by different letters within a same column differ significantly ($p < 0.05$)

C. Variation in gluten protein composition

Table IV displayed the gluten content, quality and composition of different wheat varieties. The quality of gluten proteins was assessed by gluten index, R/E ratio and proportion of glutenins and gliadins in the flour. The dry gluten content ranged from 7.7 to 11.7 %. The wheat varieties HI 977 and HW 2004, had a dry gluten content of less than 9%, while the varieties C 306, PBW 550 and WH 542 had higher dry gluten content ($> 10\%$). Gluten index reflects the strength and quality of gluten proteins. A higher value of gluten index indicates the presence of stronger and elastic gluten proteins in a cultivar that are challenging to pass through the glutomatic sieve. However, varieties with lower value of gluten index showed the presence of weaker proteins which exhibited lower gluten strength and can pass through easily. Gluten index holds a minimum value of 60.6 corresponding to HW 2004 and a maximum value of 98.0 for HI 977. R/E ratio is defined as the ratio of Resistance to extension (R) to Extensibility (E) of gluten protein, where R and E are governed by the elastic proteins (glutenins) and viscous proteins (gliadins). R/E ratio of glutes of different varieties varied markedly from 0.37 to 1.48, being reported as the highest for PBW 550 and the lowest for C 306. The content of gluten proteins, gliadins and glutenins, ranged from 4.2 to 6.0 % and 3.5 to 5.5 %, respectively. Glutenins, being the largest polymeric proteins, administered continuity and elasticity to the dough system. Nevertheless, gliadins contribute to the dough extensibility by the cause of their plasticizing effect. They may interfere with glutenin-glutenin interaction which resulted in decrease in the elasticity of gluten proteins. The ratio of gliadins to glutenins regulates the

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equilibrium between dough viscosity and elasticity which is known to affect the viscoelasticity of gluten proteins as well as dough. Gli/glu ratio varied from 0.76 to 1.71, displaying significant variability in the viscoelastic properties of glutes of various varieties. Furthermore, the exceeding values of SDSV, GI and R/E ratio alongwith the limiting values of Gli/glu ratio in varieties PBW 550 and HI 977, confirms the presence of strong, elastic and good quality proteins as prime determinants of strength in these cultivars. Another exceptional consideration was regarding variety HI 977, which despite of having lower gluten content, exhibited stronger dough properties which may be owed to the quality of its gluten proteins and HMW-GS composition.

Table IV: Gluten quality characteristics of wheat varieties

Varieties	DG (%)	GI	R/E	Gli (%)	Glu (%)	Gli/ Glu
C 306	11.7 ^e	64.1 ^b	0.37 ^a	5.7 ^c	3.8 ^b	1.5 ^c
HI 977	8.6 ^b	98.0 ^e	1.45 ^d	4.2 ^a	5.5 ^d	0.76 ^a
HW 2004	7.7 ^a	60.6 ^a	0.75 ^c	6.0 ^d	3.5 ^a	1.71 ^d
PBW 550	10.3 ^d	96.8 ^d	1.48 ^e	4.2 ^a	5.5 ^d	0.76 ^a
WH 542	10.2 ^c	79.6 ^c	0.61 ^b	5.3 ^b	4.3 ^c	1.23 ^b

DG- Dry gluten; GI- Gluten index; R/E- Resistance to extension/ extensibility; Gli- Gliadin; Glu- Glutenin and Gli/glu- Gliadin/ glutenin ratio. *Mean values followed by different letters within a same column differ significantly (p<0.05)

D. HMW-GS composition of wheat varieties

The HMW-GS composition of the varieties was altogether different, as shown in Table V. Varieties C 306 and HW 2004 expressed alike subunits Null, 20 and 2+12 at *Glu-A1*, *Glu-B1* and *Glu-D1* loci, respectively. PBW 550 and WH 542 also expressed similar subunits 2*, 7+9 and 5+10. HI 977 slightly differed at *Glu-B1* loci with the expression of 17+18 subunit. Overall, the *Glu-1* score for C 306 and HW 2004 was 4, PBW 550 and WH 542 was 9 and maximum of 10 for HI 977. Conclusively, varieties with 1 and 2* HMW-GS at *Glu-A1* exhibited stronger dough properties as compared to C 306 and HW 2004 which expressed Null and 20 HMW-GS at *Glu-A1* and *Glu-B1*, respectively. HMW-GS 5+10 subunit expressed at *Glu-D1* loci also conferred greater strength and elasticity to the dough. From the results, it is apparent that HMW-GS and *Glu-1* score in a cultivar could not be solely held responsible for the variation in the quality of isolated gluten (Chaudhary *et al.*, 2016c; Chaudhary *et al.*, 2017).

Table V: HMW-GS composition of wheat varieties

Varieties	<i>Glu-A1</i>	<i>Glu-B1</i>	<i>Glu-D1</i>	<i>Glu-1</i> score
C 306	Null	20	2+12	4
HI 977	2*	17+18	5+10	10
HW 2004	Null	20	2+12	4
PBW 550	2*	7+9	5+10	9
WH 542	2*	7+9	5+10	9

IV. CONCLUSIONS

The deviations in the quantitative and qualitative composition of gluten proteins is responsible for the varied end-use quality of the varieties. From the gluten quality attributes and HMW-GS composition, it is clearly indicated that C 306 and HW 2004 are comparatively weak varieties owing to the presence of lower proportions of glutenins and higher proportions of gliadins alongwith inferior HMW-GS. Variety WH 542 is directed to be an intermediate variety in terms of strength by the virtue of low level of difference in gliadin and glutenin contents. Nonetheless, varieties HI 977 and PBW 550 are concluded as strong varieties with the expression of superior HMW-GS and higher levels of glutenins with comparatively low levels of gliadins.

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