

EVALUATING DOUGH AND GLUTEN STRENGTH IN WHEAT VARIETIES: A COMBINED QUALITATIVE AND QUANTITATIVE APPROACH

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ABSTRACT

This study investigates the relationship between gluten characteristics and dough strength in selected wheat varieties, focusing on the performance of 'DBW88' and 'HI1500'. Through comprehensive quantitative and qualitative analyses, we assessed key rheological parameters, revealing that 'DBW88' demonstrated superior dough and gluten traits compared to the weaker characteristics of 'HI1500'. A positive correlation was found between dough stability (DST), dough development time (DDT), energy at peak (EP), and both wet and dry gluten content, along with the percentage of glutenin, highlighting the pivotal role of gluten quantity in determining dough strength. Further, multiple linear regression analysis validated the influence of protein content, glutenin content, DST, DDT, and EP on gluten and dough strength. Principal component analysis identified significant quality factors—including sodium dodecyl sulfate (SDS) sedimentation volume, glutenin percentage, resistance/extension (R/E) ratio, DDT, and DST—as critical determinants of gluten strength, with respective contributions quantified. Our findings indicate that both quantitative measures (e.g., glutenin content, DST, EP, DDT) and qualitative traits (e.g., R/E ratio, SDS sedimentation volume, gluten index) are essential for assessing wheat dough and gluten quality. This research provides valuable insights for wheat breeding and quality assessment, aiming to enhance dough performance in baking applications.

Keywords: Dough development time, Gluten index, Protein content, Resistance/Extension Ratio

Among all cereals, wheat is considered unique due to its inherent viscoelastic properties, which significantly influence the rheological performance and quality of various wheat-based products. This intrinsic characteristic is attributed to the gluten-forming storage proteins found in the wheat endosperm. The dough rheological attributes of wheat flour primarily depend on both the quantity and quality of gluten proteins (Dhaka *et al.*, 2013). Wheat gluten proteins consist of gliadins and glutenins, where gliadins contribute to viscosity, while glutenins impart elasticity to dough and gluten (Khatkar and Schofield, 2002). Various quantitative and qualitative parameters of wheat flour, such as gluten index (GI), gliadin-to-glutenin ratio, sodium dodecyl sulfate (SDS) sedimentation volume, and farinographic and extensographic characteristics, play crucial roles in determining gluten and dough strength. The SDS sedimentation test evaluates the swelling capacity of glutenin (Clarke *et al.*, 2010), and the content of insoluble glutenin is anticipated to serve as a quality forecasting measure (Sapirstein and Hussain, 2000), though it has yet to be assessed for early generation selection. The relationship between protein quantity and quality with GI has been well documented (Edwards *et al.*, 2007). Horvat (2002) demonstrated that GI can be recognized as a rapid and reliable parameter for assessing gluten

strength, given its correlation with extensographic characteristics. The GI value serves as a classification parameter for wheat varieties based on gluten strength, categorized as fragile (GI < 30%), standard (GI = 30–80%), or strong (GI > 80%) (Cubadda *et al.*, 1992). Oikonomou *et al.* (2015) established a statistical model highlighting a strong correlation between GI, fertilization levels, and protein content. However, this model does not adequately address the relationships between gluten index and other variables, such as specific wheat varieties and the presence of distinct gliadin and glutenin subunits. While literature has documented the impact of various parameters on dough and gluten strength, it is essential to explore the interactions among these parameters, as they may hold significant implications for wheat quality assessment and improvement.

The rheological behavior of dough provides insights into its water absorption capacity, performance during mixing, and suitability for end products. Ferrari *et al.* (2014) attempted to measure gluten properties and strength through a comparative analysis using a wheat gluten quality analyzer (WGQA), extensograph, and farinograph. Their study concluded that these instruments effectively predict the strength of strong or weak flours, but are less reliable for medium-strength flours. The importance of high molecular weight glutenin subunits (HMW-GS) in dough rheology and gluten

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strength is well established. Subsequent research highlighted the superior significance of HMW-GS over low molecular weight glutenin subunits (LMW-GS) in forming a robust network in durum wheat. HMW-GS plays a critical role in providing greater gluten strength by establishing a polymer-like connective structure (Edward *et al.*, 2007). Zhao *et al.* (2010) noted that the rheological properties of gluten do not solely align with *Glu-D1* glutenin subunits, indicating that wheat class alone is insufficient for predicting gluten strength. Isaak (2019) further supported the strong correlation between the molecular size distribution of polymeric proteins and gluten strength. Abu Hammad *et al.* (2012) conducted a comparative assessment of dough and gluten strength across durum wheat cultivars, aiming to correlate these attributes with pasta cooking quality. Their findings indicated that alveograph, gluten index, and glutograph were the primary tests for predicting gluten and dough strength, as well as for distinguishing between medium-strong and strong durum wheat cultivars. These studies highlight a robust relationship between the quality factors of wheat flour and the strength of dough and gluten. It is evident that no single parameter can serve as the sole criterion for predicting gluten and dough strength; rather, these parameters are interrelated and dependent on one another. Therefore, it is essential to determine the relative significance of various wheat flour quality factors in governing gluten and dough strength across different wheat varieties. However, there is limited research that considers the comprehensive physico-chemical, rheological, and gluten characteristics of diverse *Triticum aestivum* varieties to establish their relative significance concerning the factors affecting gluten and dough strength. Consequently, the present study was designed to assess the relative importance of both qualitative and quantitative parameters of wheat varieties in relation to dough and gluten strength.

MATERIALS AND METHODS

Procurement of wheat varieties

Eleven wheat varieties, namely HD3059, HD2967, DBW88, HD3086, CBW38, C306, WH711, HS562, WH1025, HI1500, and HI1531, were procured from various research stations and State Agricultural Universities across India. These winter varieties were harvested during the 2019-2020 growing season. The selection of these wheat varieties was based on their diverse quality characteristics, including grain, flour, protein, and rheological properties. Information regarding the high molecular weight glutenin subunit (HMW-GS) composition of the selected wheat varieties was obtained from the literature (Ram, 2003; Gulia and Khatkar, 2015; Barak *et al.*, 2013).

Physical analysis of wheat grains

Kernel characteristics, including dimensions, test weight (hectoliter weight), and thousand kernel weight (TKW), for the wheat varieties were assessed following standard procedures outlined by the AACC (2000). The length and breadth of the kernels for each variety were measured using a digital vernier caliper. Test weight serves as an index of kernel density and soundness, reflecting potential milling yield. To determine test weight, grains were filled into a 100 mL measuring cylinder, with excess grains leveled off, and then weighed. Kernel weight is typically expressed in grams per 1,000 kernels, calculated by weighing 100 kernels and multiplying the result by a factor of 10. Grain hardness and moisture content were measured using a Single Kernel Characterization System (Model 4100, Perten Instrument, Australia).

Milling of wheat grains

Cleaned grains were tempered to achieve a moisture content of 15.5% over a period of 48 hours at 30°C. Following this, the grains were ground using a Chopin laboratory mill (Model CD1, Villeneuve la Garenne, France) to produce flour at the Indian Agricultural Research Institute (IARI) in New Delhi. Flour samples from each variety were stored in hermetically sealed containers and maintained at -18°C in a deep freezer (Panghal *et al.*, 2018). For analysis, the flour samples were defrosted at room temperature prior to use.

Chemical Analysis of wheat flour

The straight grade flour samples were analyzed for ash content, moisture content, protein content (calculated as nitrogen $\times$ 5.7), and falling number, following standard methods outlined by the AACC (2000). The SDS sedimentation volume of the wheat flour samples, which indicates both the quantity and quality of the protein, was assessed according to the method described by Axford *et al.* (1979).

Quantity and quality evaluation of gluten proteins

Defatting of the wheat flour was done using chloroform, following the method described by MacRitchie (1987). A 10 g sample of flour was weighed and placed into the washing section of the Glutomatic system. Starch and other soluble components were removed by washing with a 2% NaCl solution at 15°C for 5 minutes, followed by a rinse with distilled water. The collected mass was weighed as wet gluten. The wet gluten was then passed through a sieve in the rotating chamber to measure the gluten index (GI), which serves as an indicator of gluten strength. The wet

gluten was dried in a gluten dryer for 4 minutes to obtain dry gluten. The separation of gluten from the flour, along with the calculations of wet gluten, dry gluten, and GI, was performed using the Glutomatic instrument in accordance with AACC (2000) protocols. Additionally, lyophilized gluten was fractionated into gliadins and glutenins using a modified Osborne (1907) procedure. The percentages of gliadins and glutenins, as well as the gliadin-to-glutenin ratio, were calculated based on the methodology outlined by Barak *et al.* (2013).

Gluten extensibility test

Uni-axial extensibility of gluten was assessed using a TA-XT plus Texture Analyzer with the Kieffer dough and gluten extensibility rig, as described by Ktenioudaki *et al.* (2010). The gluten was folded over four channels of a Teflon-coated block, which divided the gluten into strips of uniform shape and size. Silicon oil was applied to the Teflon block to facilitate the clean detachment of the gluten strips. The gluten strips were allowed to rest for 40 minutes at 25°C before testing. Following this resting period, a gluten strip was positioned across the Kieffer rig dough holder. The tensile force was measured at a test speed of 3.3 mm/s, with a trigger force set to 1 g using a 5 kg load cell. The resistance (in grams) to extension (in millimeters) during the test was represented by the peak force and the distance at which the gluten strip reached its maximum stretching limit before breaking.

Rheological analysis of wheat flour

Dough rheological characteristics, including water absorption capacity (WAC) (%), dough development time (DDT) (min), dough stability (DST) (min), dough softening (DS) (FU), and energy at peak (EP) (Wh/K), were assessed using a 4g microdough LAB (Perten Instruments, Huddinge, Sweden), following the methodology outlined by Barak *et al.* (2014). Based on the flour's water absorption capacity, the software of the microdough LAB calculated the required amount of flour (less than 4 g). The dough was kneaded in the microdough LAB mixer with the appropriate amount of water needed to achieve a dough consistency equivalent to 500 Brabender Units (BU) on a farinograph.

Statistical analysis

The data were analyzed using SPSS software version 16.0 (SPSS Inc.). Mean comparisons were done using Duncan's multiple range tests to assess statistical significance. The data were further subjected to correlation analysis, principal component analysis, and multiple linear regression analysis to explore relationships among the variables.

RESULTS AND DISCUSSION

Intervarietal variation in kernel quality

The grain quality parameters of the various wheat varieties are presented in Table 1. Kernel moisture content ranged from 8.03% to 12.63%, with the lowest moisture observed in variety HI1531 and the highest in variety HS562. The longest grain was recorded for variety HI1500 at 6.97 mm, while the shortest was found in variety HD2967 at 6.32 mm. Grain diameter varied between 3.02 mm for HI1500 and 3.53 mm for WH711. The thousand kernel weight (TKW) was lowest in variety CBW38 and highest in variety C306. Hectoliter weight, an indicator of bulk density, ranged from 72.8 kg/hl for CBW38 to 84.6 kg/hl for HI1531. This indicates that CBW38 had smaller and less plump grains, whereas HI1531 possessed sound and bulky grains. Smaller, less dense kernels exhibited a lower endosperm-to-non-endosperm ratio compared to larger, denser counterparts, which had a higher ratio of endosperm to non-endosperm components. Upon analyzing the grain quality parameters of the eleven diverse wheat varieties, the kernel moisture, grain length, grain diameter, TKW, and hectoliter weight of varieties C306, WH711, and WH1025 were largely consistent with findings by Gulia and Khatkar (2013). Additionally, the protein content and SDS sedimentation volume for C306 and CBW38 were in agreement with the results reported by Barak *et al.* (2014). Hectoliter weight, also referred to as test weight, is determined by weighing a measured volume of grains. A higher hectoliter weight indicates better grain quality and correlates with higher milling yield (Nuttall *et al.*, 2017). It is suggested that for industrial applications, hectoliter weight should exceed 76 kg/hl, as this reflects the soundness of the grains and leads to higher milling yields (Devi *et al.*, 2020). All selected varieties, except CBW38, had hectoliter weights above 76 kg/hl, indicating their suitability in terms of grain health and milling yield.

Milling yields for the wheat varieties ranged from 67.47% for HD2967 to 80.56% for C306. Notably, C306 exhibited the hardest kernels, along with higher TKW and hectoliter weight, resulting in the highest milling yield. Harder kernels were associated with greater milling yields, as corroborated by Khatkar *et al.* (1996), which indicated that hard-textured grains produce higher milling yields, while soft kernels yield less. The hardest kernels among the varieties studied were found in C306, with a hardness index of 94.8 N, whereas HD2967 had the softest kernels. Based on the hardness index, varieties HD3059, HD2967, and HD3086 were classified as medium hard, while DBW88, CBW38, C306, WH711, WH1025, HS562, HI1500, and HI1531 were categorized as hard wheat varieties.

Table 1. Grain, flour and dough rheological characteristics of different wheat varieties

Wheat variety	Grain characteristics					Flour characteristics				Dough rheological characteristics [#]					
	GM* (%)	GL (mm)	GD (mm)	TKW (g)	HLW (k g/hl)	KHI	Milling yield (%)**	MC (%)	AC (%)	FN (s)	WAC (%)	DDT (min)	DST (min)	DS (FU)	EP (Wh/kg)
C306	10.23 ^f	6.77 ^{b cde}	3.39 ^{def}	44.17 ^g	83.4 ^h	94.80 ^h	80.56	14.03 ^e	0.43 ^d	480 ^a	60.03 ^e	1.16 ^a	0.76 ^a	138.27 ^d	1.50 ^{ab}
HI1531	8.03 ^a	6.75 ^{bcd}	3.25 ^{cd}	38.96 ^{cde}	84.6 ⁱ	90.86 ^g	75.02	11.95 ^{ab}	0.27 ^b	632 ^b	60.26 ^e	1.63 ^{abc}	2.46 ^{ab}	85.00 ^{bc}	2.46 ^c
HI1500	8.90 ^b	6.97 ^e	3.02 ^a	37.40 ^{bcd}	81.0 ^g	90.76 ^g	73.21	11.73 ^{ab}	0.29 ^b	605 ^b	56.63 ^c	2.2 ^{cd}	2.36 ^{ab}	78.33 ^b	2.56 ^c
HS562	12.63 ^j	6.79 ^{b cde}	3.19 ^{bc}	35.23 ^{ab}	76.7 ^b	90.60 ^g	77.04	11.76 ^{ab}	0.18 ^a	490 ^a	53.23 ^{ab}	1.53 ^{ab}	1.93 ^a	60.00 ^{ab}	2.03 ^{abc}
DBW88	9.70 ^d	6.79 ^{b cde}	3.17 ^b	34.37 ^{ab}	77.2 ^{bc}	88.70 ^f	68.31	12.04 ^{abc}	0.44 ^d	618 ^b	58.16 ^{cd}	5.33 ^d	7.93 ^d	65.00 ^{ab}	7.32 ^d
CBW38	11.50 ^h	6.91 ^{de}	3.05 ^{ab}	33.76 ^a	72.8 ^a	87.30 ^{ef}	72.14	14.59 ^f	0.39 ^{cd}	604 ^b	52.70 ^a	1.56 ^{abc}	2.56 ^{ab}	53.33 ^{ab}	2.13 ^{abc}
WH711	11.70 ⁱ	6.69 ^{bc}	3.53 ^f	43.11 ^{fg}	79.2 ^e	85.60 ^e	75.16	12.13 ^{bc}	0.43 ^d	516 ^a	59.36 ^{de}	1.60 ^{abc}	1.80 ^a	101.67 ^c	2.20 ^{bc}
WH1025	11.40 ^h	6.89 ^{cde}	3.18 ^{bc}	36.49 ^{abc}	79.6 ^{ef}	82.60 ^d	70.75	11.66 ^a	0.25 ^{ab}	523 ^a	53.60 ^{ab}	1.00 ^a	0.93 ^a	159.90 ^d	1.23 ^a
HD3059	10.06 ^e	6.62 ^a	3.35 ^{de}	38.96 ^{cde}	76.8 ^b	78.93 ^c	70.22	12.06 ^{abc}	0.31 ^{bc}	657 ^b	58.70 ^{de}	1.53 ^{ab}	5.63 ^{bc}	30.00 ^a	1.90 ^{abc}
HD3086	9.06 ^c	6.84 ^{cde}	3.35 ^{de}	39.81 ^{de}	80.3 ^{fg}	78.70 ^{abc}	70.15	12.89 ^d	0.27 ^{ab}	604 ^b	58.10 ^{cd}	1.43 ^a	1.70 ^a	51.66 ^{ab}	1.63 ^{abc}
HD2967	10.70 ^g	6.32 ^a	3.46 ^{ef}	42.13 ^{efg}	78.0 ^{cd}	72.53 ^a	67.47	12.42 ^c	0.28 ^b	499 ^a	54.66 ^b	1.23 ^a	2.60 ^{ab}	60.00 ^{ab}	1.70 ^{abc}

Within a column, means followed by different alphabets differ significantly at $p \leq 0.05$.

*GM: Grain moisture, GL: Grain length, GD: Grain diameter, TKW: Thousand kernel weight, HLW: Hectoliter weight, KHI-Kernel hardness index, MC- Moisture content, AC: Ash content, FN: Falling number, WAC- Water absorption capacity, DDT: Dough development time, DST: Dough stability time, DS: Dough stability, EP: Energy at peak

**All parameters were measured in triplicate, except for milling yield, which was assessed once (n=1).

All rheological parameters were measured based on a flour moisture content of 14%.

Flour and gluten quality

The moisture content, ash content, protein content, SDS sedimentation volume, and falling number varied significantly among the studied wheat varieties, as presented in Tables 1 and 2. The moisture content of flour ranged from 11.66% for WH1025 to 14.59% for CBW38. Ash content also varied, with a minimum of 0.18% for HS562 and a maximum of 0.44% for DBW88. Protein content exhibited considerable variation, ranging from 7.92% to 13.22%. Wheat varieties with protein content below 10.5% included HI1531 and WH1025, while HD3059, HD2967, DBW88, HD3086, CBW38, C306, WH711, HS562, and HI1500 exhibited higher protein levels. The highest SDS sedimentation volume was recorded for CBW38 at 67 ml, while the lowest was observed for WH1025 at 30 ml. The falling number, which measures amylase activity, revealed lower values for C306 (480 s) and higher values for HD3059 (657 s), indicating varying degrees of enzymatic activity among the different wheat varieties.

The quantitative analysis of gluten was assessed through both wet and dry gluten content. Wet gluten content ranged from 17.95% for C306 to 28.91% for DBW88, while dry gluten content varied from 9.53% for WH1025 to 11.50% for DBW88. The gluten index (GI) serves as an indicator of the quality and strength of gluten proteins; weaker gluten passes easily through a sieve, whereas stronger, more elastic gluten resists passage through the Glutomatic sieve. Varieties HD3086 and DBW88 demonstrated higher gluten strength, while HI1500 exhibited the least strength. These findings align with those of Dewan *et al.* (2022a), who noted that DBW88 and its gluten fraction displayed strengthening properties, whereas HI1500 and its gluten fraction showed weakening characteristics. Devi *et al.* (2019) evaluated varieties HS490 and HD2967 for cookie making based on their Glu-1 score, SDS sedimentation volume, GI, wet gluten, and dry gluten content. Variety HS490 was characterized by a lower GI of 80%, while HD2967 exhibited a higher GI, indicating stronger gluten properties in the latter, in contrast to the stickier gluten of HS490.

The gliadin/glutenin ratio varied from 0.64 for HS562 to 1.44 for C306, indicating significant differences in the viscoelastic properties of gluten among the wheat varieties. The resistance/extension (R/E) ratio ranged from 0.75 to 4.22, with the highest value recorded for CBW38 and the lowest for WH711 and WH1025. A higher R/E ratio, along with a greater gluten index (GI) and a lower gliadin/glutenin ratio, suggests excellent gluten strength due to a higher proportion of polymeric proteins, which create a strong and dense network. Khatkar (2006) noted that the monomeric gliadins within the gluten network are primarily responsible for dough

Table 2. Gluten quality and HMWGS composition of different wheat varieties

Wheat variety	Gluten quantity and quality*										HMWGS composition			
	PC of flour (%)	SDSSV (ml)	WG (%)	DG (%)	GI	Glia %	Glu %	Glia/ Glu	R/E	Glu-D1	Glu-A1	Glu-B1	Glu-1 Score	
WH1025	9.93 ^b	30 ^a	18.33 ^a	9.53 ^a	97.01 ^{ef}	5.60 ^{gh}	3.93 ^a	1.42 ^h	0.75 ^a	2+12	2*	7	6	
C306	11.33 ^d	31 ^a	17.95 ^{ab}	9.63 ^a	95.89 ^{de}	5.83 ^h	3.80 ^a	1.44 ^h	1.06 ^a	2+12	Null	20	4	
DBW88	12.98 ^{fg}	66 ^g	28.91 ^e	11.50 ^d	97.66 ^f	4.60 ^{de}	6.9 ^g	0.67 ^{ab}	3.34 ^e	5+10	2*	17+18	10	
HD3086	12.20 ^e	64 ^{ef}	20.27 ^{abc}	9.80 ^b	97.66 ^f	4.40 ^{cd}	5.4 ^{cd}	0.77 ^{cd}	4.15 ^f	5+10	1	17+18	10	
HD2967	12.55 ^{ef}	66 ^g	22.37 ^c	9.89 ^b	94.80 ^d	4.76 ^{def}	5.13 ^c	0.94 ^e	2.51 ^{cd}	5+10	2*	17+18	10	
HI1500	10.53 ^c	47 ^{cd}	18.36 ^a	11.30 ^d	58.66 ^a	5.4 ^g	4.10 ^a	1.39 ^h	0.83 ^a	2+12	Null	20	4	
HI1531	7.92 ^a	43 ^a	22.23 ^c	9.66 ^a	75.88 ^b	4.90 ^{ef}	4.76 ^b	1.05 ^f	1.38 ^{ab}	2+12	2*	20	4	
HS562	10.61 ^c	64 ^{ef}	21.63 ^{bc}	10.46 ^c	94.90 ^d	3.70 ^{ab}	5.76 ^{ef}	0.64 ^{ab}	2.60 ^d	5+10	1	17+18	10	
CBW38	11.33 ^d	67 ^g	21.05 ^{bc}	9.83 ^b	97.15 ^{ef}	4.33 ^{cd}	5.50 ^{de}	0.71 ^{bc}	4.22 ^f	5+10	1	17+18	10	
HD3059	13.22 ^f	62 ^e	24.82 ^d	9.73 ^{ab}	94.89 ^d	4.60 ^{de}	5.13 ^c	0.85 ^d	1.91 ^c	5+10	2*	17+18	10	
WH711	12.33 ^e	45 ^{bc}	27.64 ^e	9.96 ^b	81.85 ^c	5.46 ^{gh}	4.50 ^b	1.27 ^g	0.75 ^a	2+12	2*	17+18	8	

Within a column, means followed by different alphabets differ significantly at $p \leq 0.05$.

*PC%: Protein content of flour (%), SDSSV: Sodium Dodecyl Sulphate Sedimentation Volume (ml), WG: Wet Gluten (%), DG: Dry Gluten (%), GI: Gluten Index, Glia(%): Gliadin(%), Glu(%): Glutenin(%), Glia/glu: Gliadin/Glutenin Ratio, R/E: Resistance/Extension ratio. All parameters were measured in triplicate.

viscosity and stretchability, while the polymeric glutenin fraction contributes to dough strength and elasticity. The qualitative parameter of gluten strength, represented by the R/E ratio, depends on the balance of elastic proteins (glutenins) and viscous proteins (gliadins). A higher percentage of glutenin enhances elasticity, resulting in a high R/E ratio, whereas an increased presence of monomeric gliadins may promote fluidity, leading to greater extensibility but reduced resistance to elasticity, thereby yielding a lower R/E ratio. Upon evaluating flour quality characteristics, varieties CBW38, DBW88, and HD3086 exhibited higher gluten strength, while HI1500, C306, and WH1025 were characterized by weaker gluten.

Dough rheological parameters

Rheological characterization of flour is influenced by its composition and is essential for predicting the efficient utilization of end products. The rheological parameters of various wheat varieties are summarized in Table 1. Water absorption capacity of the flours varied significantly, ranging from 52.70% to 60.26%. Dough development time (DDT) ranged from 1.00 min for WH1025 to 5.33 min for DBW88, while dough stability (DST) varied from 0.76 min for C306 to 7.93 min for DBW88. Notably, variety DBW88 exhibited strong dough rheology, characterized by a DDT of 5.33 min and a DST of 7.93 min. The energy required for the dough to reach peak height, termed peak energy (EP), varied from 1.23 Wh/kg for WH1025 to 7.32 Wh/kg for DBW88. The rheological characterization supports the strength of DBW88, which displayed the highest dough stability (7.93 min) and peak energy (7.32 Wh/kg). High values of dough stability indicate stronger flour (Moreira *et al.*, 2011). A decline in EP was observed when gliadin proteins were incorporated into the dough, suggesting that less energy was required to mix the dough due to the presence of more viscous proteins (Khatkar *et al.*, 2013). The results for C306 and WH1025, which exhibited weaker dough as indicated by lower values for DST, DDT, and EP, align with findings by Barak *et al.* (2014). Wheat varieties with a higher proportion of glutenin proteins tend to form stronger dough that requires more energy, in contrast to those with greater gliadin content, which form less stable dough. Consequently, weaker dough requires less energy to reach its peak, whereas stronger dough with higher glutenin content necessitates more energy. Dewan *et al.* (2022b) demonstrated that increasing the percentage of glutenins in strong varieties like DBW88 further enhances dough strength, reflected by increased values of DDT, DST, and EP. The variation in mixing properties among the selected wheat varieties is primarily driven by the quality and quantity of gluten proteins. Variations in the mixing properties of strong

and weak cultivars are attributed to differences in their gluten composition, while total protein content appears to play a minor role (Khatkar *et al.*, 1996). Thus, the rheological characterization indicates that DBW88 produces stronger dough, whereas C306 and WH1025 yield weaker dough.

High molecular weight glutenin subunits composition (HMW-GS) and gluten strength

Numerous studies have demonstrated that dough strength is closely linked to both the quantity and quality of high molecular weight glutenin subunits (HMW-GS). The composition of glutenins significantly influences the baking performance of wheat varieties (Veraverbeke and Delcour, 2002). The HMW-GS composition and their *Glu-1* scores for eleven wheat varieties are summarized in Table 2. Varieties HI1500 and C306 exhibited similar subunits, specifically 2+12 at *Glu-D1* and 20 at *Glu-B1*, with a null at *Glu-A1*. In contrast, variety HI1531 displayed a similar profile except for a 2* subunit at *Glu-A1*. Wheat varieties HD3059, HD2967, DBW88, and CBW38, characterized by subunits 17+18 and 5+10 at *Glu-B1* and *Glu-D1* respectively, exhibit the traits of “strong” wheat varieties, with higher dough stability values ranging from 2.56 to 7.63 min. Radovanovic *et al.* (2002) showed that the 5+10 subunit at *Glu-D1* positively influences mixing development time and peak energy—key rheological parameters used to classify wheat varieties based on gluten strength. The presence of an additional cysteine residue in the 5+10 subunit contributes to its superior performance (Wieser, 2007). Mohan and Gupta (2018) reported that the combination of HMW-GS 5+10, 2*, and 17+18 is indicative of strong gluten and dough strength, correlating with high quality. This combination was also identified in DBW88, whose dough and gluten strength were supported by both rheological assessments and quantitative and qualitative analyses of flour. Furthermore, microstructural and secondary structure analyses of gluten fortified with subunits 5+10 (*Glu-D1*), 2* (*Glu-A1*), and 17+18 (*Glu-B1*) revealed a strong, dense, and compact gluten network, highlighting its morphological functionality within the gluten matrix (Dewan and Khatkar, 2023). In contrast, variety HD3086, which expresses the subunits 5+10, 1, and 17+18, displayed weaker mixing properties, with DDT and DST values of 1.43 min and 1.70 min, respectively. This suggests that both the quantitative and qualitative effects of HMW-GS play critical roles in defining the strength characteristics of the dough for these varieties (Khatkar *et al.*, 1996).

Wheat varieties C306 and HI1500 exhibited weak dough characteristics, forming less stable dough with low dough stability values (<2.50 min). This deficiency can be attributed to the presence of the subunits 2+12

at *Glu-D1*, as demonstrated by Bakshi and Bhagwat (2016). Varieties exhibiting these subunits, along with a null allele at *Glu-A1* and subunit 20 at *Glu-B1*, were found to possess high gliadin content, which correlates with lower values for dough-strengthening factors. Supporting this, Karaduman *et al.* (2022) indicated that the combination of subunits 2+12 at *Glu-D1* and 20 at *Glu-B1* is associated with weak dough and inferior gluten quality. Additionally, the presence of a null allele at *Glu-A1* contributes to weak dough rheology and lower bread-making quality. Dewan and Khatkar (2023) revealed through scanning electron microscopy that the microstructural manifestations of dough weakening include the formation of large cavities and non-uniformity within the gluten network, particularly when subunits 2+12 at *Glu-D1*, the null allele at *Glu-A1*, and subunit 20 at *Glu-B1* are present. Consequently, specific subunits play a critical role in determining the strength and quality of gluten, significantly influencing dough rheology.

Principal component analysis

Principal component analysis (PCA) was conducted to elucidate the relationships among flour quality characteristics and identify factors influencing gluten strength. Three principal components were extracted: PC1, PC2, and PC3, which accounted for 41.54%, 20.78%, and 11.56% of the total variance, respectively. PC1 exhibited a positive correlation with SDS sedimentation volume, the resistance/extension (R/E) ratio, and the *Glu-1* score, while displaying a negative correlation with dough softening, gliadin content, and the gliadin/glutenin ratio (Fig. 1). These findings align with the positive correlation coefficients observed among various physicochemical and gluten quality parameters. PC2 was predominantly influenced by dough development time (DDT), dough stability (DST), dry gluten, wet gluten, glutenin percentage, and energy at peak (EP). In contrast, PC3 was primarily associated

with protein content and gluten index (GI). The loading factors for these components were significant, with SDS sedimentation volume (0.898), R/E ratio (0.885), and *Glu-1* score (0.748) being prominent in PC1. For PC2, the highest loading factors included DDT (0.955), EP (0.968), dry gluten (0.875), and wet gluten (0.580). In PC3, protein content (0.813) and GI (0.499) were also significant contributors to gluten and dough strength. Notably, several factors associated with the weakening of gluten and dough strength were identified through high negative loading factors in PC1, including dough stability (-0.826), gliadin content (-0.952), and the gliadin/glutenin ratio (-0.939). These insights highlight the complex interplay of flour quality characteristics in determining gluten strength.

Many researchers have utilized principal component analysis (PCA) to highlight the relationships among various wheat quality and quantity parameters in predicting end product quality. For instance, Panghal *et al.* (2019) reported that their PCA accounted for 63.35% of the original data variability, with PC1 primarily influenced by protein content, wet gluten, dry gluten, and *chapatti* scores. In this study, PC2 was defined by protein content and softness, highlighting the wheat quality parameters critical for chapatti quality. Similarly, Spina *et al.* (2021) demonstrated the significance of rheological parameters such as dough development time (DDT), farinograph stability, and peak dough height in relation to gluten strength and bread-making quality of durum wheat genotypes through PCA. The analysis revealed that gluten strength is positively influenced by both qualitative parameters, like SDS sedimentation volume and R/E ratio, and quantitative parameters, including protein content and glutenin percentage. Additionally, rheological parameters like DDT, dough stability (DST), and energy at peak (EP) were identified as key factors governing gluten strength. This evidence from PCA analysis reinforces the interconnectedness of these parameters in determining the quality of wheat and its end products.

Correlation among flour quality parameters and rheological parameters of wheat varieties

Correlations among grain, flour, and gluten quantities, as well as dough rheological parameters, were analyzed to identify predictors for gluten and dough strength (Table 3). Dough stability (DST) showed a strong positive correlation with dough development time (DDT) ($R^2 = 0.824$) and energy at peak (EP) ($R^2 = 0.795$), indicating that these parameters are effective indicators of dough strength. Notably, DDT was highly correlated with EP ($R^2 = 0.991$) at a 99% significance level, which aligns with findings by Dewan *et al.* (2022b), who assessed the functional impacts of gliadins and glutenins in various wheat varieties.

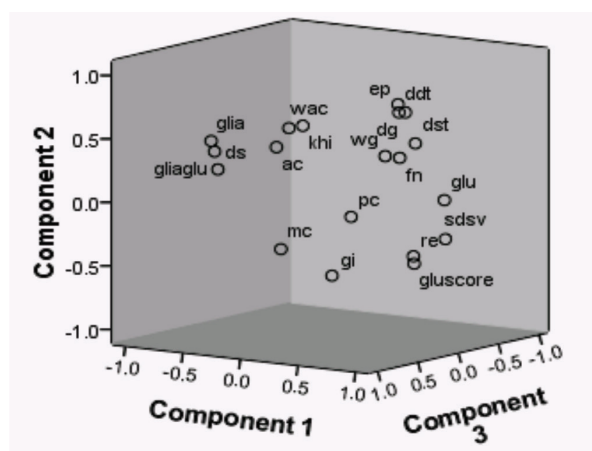


Fig. 1. Principal component analysis (PCA) for flour quality characteristics

Table 3. Correlation matrix of flour quality parameters

	Glu-1 score	PC	SDSSV	WG	DG	GI	Glia	Glu	Glia/Glu	R/E ratio	DDT	DST	DS	EP
Glu-1 score	1													
PC	0.689'	1												
SDSSV	0.860"	0.531	1											
WG	0.511	0.504	NS	1										
DG	0.666'	NS	NS	NS	1									
GI	0.632'	0.498	NS	0.504	NS	1								
Glia	-0.747"	NS	-0.686'	NS	NS	NS	1							
Glu	0.762"	NS	0.761"	0.636'	NS	0.504	-0.788"	1						
Glia/Glu	-0.826"	NS	-0.910"	NS	NS	NS	0.946"	-0.860"	1					
R/E ratio	0.746"	NS	0.824"	NS	NS	0.539	-0.764'	0.772"	-0.860"	1				
DDT	NS	NS	0.342	0.619'	0.805"	NS	-0.503	0.688'	-0.479	NS	1			
DST	NS	NS	0.543	0.727'	0.586'	NS	NS	0.735'	-0.643'	NS	0.824"	1		
DS	-0.674'	NS	-0.914"	NS	NS	NS	0.785"	-0.681'	0.807"	-0.659'	NS	-0.540	1	
EP	NS	NS	0.303	0.582	0.853"	NS	NS	0.632'	NS	NS	0.991"	0.795"	NS	1

"Significant at $p \leq 0.01$; Significant at $p \leq 0.05$

PC: Protein content, SDSSV: Sodium dodecyl sulphate sedimentation volume, WG: Wet Gluten, DG: Dry Gluten, GI: Gluten Index, Glia: Gliadin (%), Glu: Glutenin (%), Glia/Glu: Gliadin/ Glutenin Ratio, R/E ratio: Resistance/Extension, DDT: Dough Development Time, DST: Dough Stability, EP: Energy at Peak, NS: non-significant

Additionally, DDT exhibited strong positive correlations with dough stability (DS) ($R^2 = 0.97$) and peak energy (PE) ($R^2 = 0.99$), further corroborating the relationship between these parameters (Dewan *et al.*, 2022b). The gluten index (GI) and resistance/extension (R/E) ratio also demonstrated a positive correlation ($R^2 = 0.539$), highlighting their shared characteristics in predicting gluten strength. The SDS sedimentation volume and R/E ratio shared a robust positive correlation ($R^2 = 0.824$ at 99% significance), indicating that a greater swelling ability of glutenin contributes to stronger gluten and, consequently, superior dough properties. The *Glu-1* score showed high positive correlations with SDS sedimentation volume ($R^2 = 0.860$), glutenin percentage ($R^2 = 0.762$), and R/E ratio ($R^2 = 0.746$), suggesting that wheat varieties with higher *Glu-1* scores exhibit stronger dough rheology due to the influence of high molecular weight glutenin subunit (HMW-GS) composition. Furthermore, dry gluten content demonstrated strong relationships with DDT ($R^2 = 0.805$), EP ($R^2 = 0.853$), and DST ($R^2 = 0.586$), emphasizing the critical role of gluten percentage in dough rheology. The correlation of polymeric wheat proteins (glutenin percentage) with rheological parameters like R/E ratio ($R^2 = 0.772$), DDT ($R^2 = 0.688$), DST ($R^2 = 0.735$), and EP ($R^2 = 0.632$) illustrates the significant influence of glutenin content on the parameters defining gluten and dough strength. This analysis highlights the importance of both the quantity and quality of glutenin in determining the overall performance of wheat flour in baking applications.

Monomeric wheat proteins, particularly gliadins, are linked to the fluidity and viscosity of dough. The analysis revealed a negative correlation between gliadins and dough strengthening factors: glutenin percentage ($R^2 = -0.788$), resistance/extension (R/E) ratio ($R^2 = -0.764$), and dough development time (DDT) ($R^2 = -0.503$). This suggests that higher gliadin levels correspond with reduced dough strength. In contrast, gliadins were positively correlated with the gliadin/ glutenin ratio ($R^2 = 0.946$) and dough softening ($R^2 = 0.785$). These findings indicate that while gliadins enhance the extensibility and fluidity of the dough, they undermine the strength conferred by glutenins. This balance between gliadin and glutenin is essential for optimizing dough rheological properties.

The correlation analysis revealed that the gluten index (GI) and resistance/extension (R/E) ratio effectively predict gluten strength. Previous studies, such as Dhaka and Khatkar (2013), indicated a positive correlation ($R^2 = 0.766$) between the R/E ratio and SDS sedimentation volume, further supporting the relationship between these measures of gluten quality. Similarly, Zhang *et al.* (2008) found a significant correlation ($R^2 = 0.59$) between GI and SDS micro-sedimentation. SDS sedimentation volume consistently correlates positively with both GI

and R/E ratio, confirming its relevance as an indicator of gluten quality. However, alternative evaluation methods like alveograph and mixograph have also shown promising predictions for gluten strength compared to SDS sedimentation (Gaines *et al.*, 2006). Additionally, quantitative factors such as wet gluten, dry gluten, and glutenin content positively influenced dough rheological characteristics like dough development time (DDT) and dough stability (DST). Dangi *et al.* (2019) observed that incorporating glutenin increases DDT, DST, and energy at peak (EP), as it forms more disulfide bonds, leading to a robust dough structure. In contrast, gliadin content is negatively correlated with glutenin and R/E ratio, indicating its role in weakening dough strength. Increased gliadin levels are associated with a more open gluten structure, resulting in softer and weaker dough, which contributes to greater dough softening and diminished stability, as demonstrated by Khatkar *et al.* (2013). Thus, a higher gliadin content correlates with decreased gluten and dough strength, highlighting the balance between gliadin and glutenin in determining dough rheology.

Regression equation to predict gluten strength

When multiple independent factors are manipulated to predict a single dependent variable, multiple linear regression is the most suitable statistical tool (Yu *et al.*, 2018). Fidelis *et al.* (2017) found multiple linear regression to be particularly effective when the sample size exceeds the number of model variables. In this study, dough and gluten strength were predicted using a regression equation where dry gluten content, glutenin content, dough stability (DST), gluten index (GI), and the resistance/extension (R/E) ratio emerged as significant positive predictors. When SDS sedimentation volume was used as the dependent variable, the model achieved

an R^2 value of 0.970 (Table 4). The multiple linear regression analysis revealed the relative importance of parameters such as dough development time (DDT), energy at peak (EP), and DST, indicating that dough strength primarily depends on glutenin content, R/E ratio, and GI. All equations demonstrated a coefficient of determination (R^2) greater than 0.7, indicating that the developed models are well-suited for prediction.

Dough strength is primarily influenced by gluten protein, its composition and the nature of its network. The correlation between gluten network structure and aggregate size with dough strength has been well established (Lefebvre *et al.*, 2000). Polymeric wheat proteins, particularly glutenin, play a crucial role in forming a robust gluten network, as their molecular arrangement fosters numerous intramolecular and intermolecular disulfide bonds (Yazar *et al.*, 2017). This relationship is further supported by regression analysis, which showed that gluten index (GI), a measure of gluten strength, is primarily dependent on glutenin content, dough development time (DDT), and dough stability (DST), with an R^2 value of 0.771. Rheological traits such as DDT, energy at peak (EP), and DST exhibited strong correlations with glutenin content, GI, and the resistance/extension (R/E) ratio through linear regression equations. This analysis highlights the direct relationship between glutenin percentage and its quality with dough rheological and gluten quality parameters. Similarly, Kundu *et al.* (2017) and Panghal *et al.* (2019) demonstrated the ability to predict *chapatti* quality using various wheat flour characteristics, including SDS sedimentation volume, water absorption, protein content, wet gluten, and mineral content. Their regression models also achieved R^2 values greater than 0.7, indicating that these equations are best-fit for predicting quality outcomes.

Table 4. Regression equations for determining gluten strength

Gluten and Dough strength Factors	Prediction Equation*	R^2	R	$p \leq 0.05$
SDSV	$-48.257 + 2.36(R/E) + 0.861(DST) + 7.427(DG) + 6.434(Glu) + 0.120(GI) - 5.923(DDT) - 0.163(DS)$	0.970	0.985	0.026
DDT	$-11.453 - 0.010(DS) + 1.241(Glu) + 1.128(DG) + 0.049(R/E) + 0.001(GI) - 0.086(SDSV)$	0.907	0.952	0.046
EP	$-1.033 + 0.006(DST) + 0.170(Glu) - 0.24(GI) + 0.099(R/E) + 0.168(DG) + 1.125(DDT) + 0.101(DS) + 0.114(Glia/Glu)$	0.998	0.999	0.009
DST	$0.374 + 0.821(EP) + 0.418(PC) - 2.807(Glia/Glu) - 0.29(SDSV)$	0.790	0.889	0.03
Gluten Index	$53.002 + 1.831(R/E) + 0.369(DS) + 4.054(PC) - 42.298(Glia/Glu)$	0.771	0.878	0.040
R/E Ratio	$-5.458 + 0.006(DS) + 0.125(Glu) + 0.076(SDSV) + 0.027(GI)$	0.777	0.881	0.037

GI: Gluten Index, SDSV: Sodium Dodecyl Sulphate Sedimentation Volume, DG: Dry Gluten, Glu: Glutenin (%), DDT: Dough Development Time, DST: Dough Stability, EP: Energy Peak, R/E: Resistance/Extension Ratio, PC: Protein Content, Glia: Gliadin (%), Glia/Glu: Gliadin/Glutenin Ratio, DS: Dough Softening

CONCLUSION

In conclusion, this research has revealed the critical relationships between various qualitative and quantitative parameters of wheat varieties, providing valuable insights for predicting dough and gluten strength. The superior performance of the wheat variety 'DBW88' highlights the importance of selecting varieties with higher protein content, SDS sedimentation volume, and favourable gluten properties to enhance product quality. In contrast, the limitations observed in the 'HI1500' variety illustrate the potential pitfalls of weaker gluten networks.

Our findings emphasize the necessity for industry stakeholders to focus on specific parameters—such as the resistance/extension ratio, gluten index, and dough development time—as predictive indicators of gluten and dough strength. By leveraging these insights, producers can make informed decisions when selecting wheat varieties, ultimately leading to superior end products that meet consumer demands for quality in baked goods and other wheat-based foods. This research not only enhances our understanding of wheat variety performance but also serves as a foundation for further investigations aimed at optimizing wheat selection in agricultural and manufacturing processes.

Authors' contribution

Conceptualization and designing of the research work (BSK, AD); Execution of field/lab experiments and data collection (AD); Analysis of data and interpretation (AD, NC); Preparation of manuscript (AD, BSK, NC).

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