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Influence of gluten addition on rheological, pasting, thermal, textural properties and bread making quality of wheat varieties

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RESEARCH ARTICLE

Abstract

Gluten was incorporated to wheat flour to study its effect on rheological, pasting, thermal, textural and bread making quality. Addition of gluten resulted in an increase in water absorption capacity and dough development time of flour samples. A significant decline in peak viscosity, hot paste viscosity, breakdown, setback and final viscosity were observed with augmentation of gluten in wheat varieties. No remarkable difference was observed in pasting temperature with the addition of gluten. There was a noteworthy increase in peak temperature and decrease in enthalpies of wheat flours due to incorporation of gluten. Hardness, cohesiveness and adhesiveness of the flour gel lowered upon addition of gluten while the springiness showed an uptrend. It was concocted that the loaf volume of bread of variety WH 542 increased linearly with gluten incorporation up to a level of 15% and then decreased, while in variety C 306 with 18% gluten level maximum loaf volume was observed. Insights in dough rheology, pasting, thermal, textural and bread quality characteristics revealed that the addition of gluten enhanced the dough strength, peak temperature, springiness and bread making quality of weak variety C 306 more than the strong variety WH 542.

Keywords: gluten, rheological properties, pasting properties, thermal properties, textural properties, bread quality

1. Introduction

Gluten bestows the properties of elasticity and extensibility that are crucial for the functionality of wheat flours. It has been well acknowledged that with enhancement in total protein content of the flour there is an increase in gluten content (Curic *et al.*, 2001; Khatkar, 2006). It is important to mention here that the quantity of protein or gluten cannot be considered as a measure for gluten quality. Gluten quality is characterised by the degree of extensibility and elasticity. Scientists consider gluten as the functional part of dough which influences many product qualities.

The quantity and quality of gluten proteins largely determines the dough rheological characteristics of wheat flour (Dhaka *et al.*, 2012). The rheological properties of the gluten (the combination of its viscous, elastic and cohesive properties) are responsible for the bread making quality of wheat flour. It is widely accepted that gliadins confers viscous properties to gluten required for dough development whilst glutenin imparts strength and elasticity which is essential to hold gases produced during the process of fermentation (Khatkar and Schofield, 2002). The plastic properties of dough are acquired during the kneading process, when the gluten forms an increasingly expansible yet at the same time fragile network in the presence of variable amounts of water, depending upon flour quality. The gluten proteins are major determinant of the bread making potential of wheat varieties. They play an important role in subsequent stages of the bread making process and in the structure of the finished product: they are sufficiently expansible yet resilient enough to be able to stretch with the dough and trap the gases produced during fermentation. Besides, starch pasting properties also influence the bread baking quality especially bread volume and texture (Patel *et al.*, 2005). Excess of starch in wheat flour leads to sticky dough, low volume of bread and the proneness of bread to staling. On the contrary, if less starch component is there, it does not allow the formation of a continuous phase involved in gas wall of bread. Also, effect of gluten on energy of

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transformation and gelatinisation temperature were carried by Monhamed and Ryas-Durate (2003). They speculated that the increase of protein in the blend led to increase in the onset and peak temperatures in thermal analysis.

There is an increasing awareness for the relevance of gluten as an additive in baking industry to confer various functionalities. The incorporation of gluten into flour mixtures has the potential to improve textural properties. Gluten could also be used in baking industry to improve water holding capacity, to modify product's texture and volume. Addition of vital gluten increases the technological potential of wheat flours up to certain level of incorporation. Wheat gluten is not only mainly used as a natural additive for improvement of baking quality of flour in the food industry, but also one good bond and nutrient additive of animal feed in the feed industry.

In the present research, gluten was isolated from the wheat flours of varieties WH 542 and C 306 and it was incorporated up to 18% in the wheat flour of these varieties. Numerous studies have been carried out on the effect of gluten addition on rheological properties but very little literature is available on the influence of gluten addition on pasting, thermal and texture profile analysis. Thus, the research was aimed to study the influence of gluten addition on the rheological, pasting, thermal, textural properties and their relationship with the bread making quality.

2. Materials and methods

Materials

Grains of two different wheat varieties WH 542 and C 306 used in this study were obtained from Chaudhary Charan Singh Haryana Agriculture University, Hisar, India. These varieties were selected mainly on the basis of their difference in bread making potential. The grains were cleaned manually to remove soil particles, broken and foreign seeds. Grain length and breadth was measured by using a Vernier calliper. Thousand kernel weight (TKW) was determined by measuring the weight of 100 seeds and multiplying the weight obtained by a factor of 10. Hectolitre weight (HLW) was determined using Aqua TR (Chopin Technologies, Villeneuve-la-Garenne, France). The grains of individual varieties were milled on a Chopin laboratory mill (model CD1; Chopin Technologies) into flour after tempering.

Analyses of wheat flour

The flour samples of the wheat varieties were analysed for moisture, protein, ash, falling number, wet gluten, dry gluten and gluten index. These were determined according to standard AACC methods (AACC, 2000). The sodium dodecyl sulphate (SDS) sedimentation volumes of flour samples were estimated according to the method of Axford *et al.* (1978). Triplicate measurements were carried out for the chemical analysis and the results were averaged.

Gluten isolation and incorporation

Gluten was isolated from the defatted flours by Perten Glutomatic (Perten Instruments AB, Hägersten, Sweden) using 0.5M NaCl at 15 °C. The isolated gluten and the slurry obtained during washing were freeze dried and powdered uniformly to produce gluten with better functionality for bread making (Dua *et al.*, 2009). The dried gluten samples were analysed for protein, starch moisture, ash and lipid content. Gluten was incorporated at different levels (6, 9, 12, 15 and 18%) in the wheat flours in their respective varieties. As the wheat variety WH 542 contained 12.4% protein so the gluten content was adjusted to 6 and 9% by adding the dried and powdered slurry calculated on the basis of mass component balance. Similar process was followed for variety C 306 having 9.4% protein content.

Rheological analysis of gluten added flours by Mixolab

Changes in the dough rheological properties of the control flour were determined by Mixolab (Chopin Technologies). Key parameters derived from the Mixolab curve are water absorption (%) or the percentage of water required for the dough to produce a torque of 1.1±0.05 Nm, dough development (C1), protein weakening, i.e. decrease in dough consistency due to excessive mixing (C2), starch gelatinisation, i.e. starch granules swell and absorb water and amylose molecules leach out resulting in an increase in the viscosity (C3), amylase activity (C4), and retrogradation (C5).

Pasting properties of gluten added flour

The changes in the pasting characteristics of the control wheat flour of both wheat varieties were determined by Rapid Visco Analyzer-TecMaster (Perten Instruments AB) according to the AACC approved method 76-21 (AACC, 2000). The gluten was added to the base flour at 6, 9. 12, 15 and 18% level, respectively. The different parameters determined by the rapid visco analyser (RVA) were peak viscosity, breakdown, trough, setback, final viscosity, pasting temperature (°C) and peak time (min). All the parameters were expressed in rapid visco units (RVU).

Gluten addition and thermal properties

Calorimetric behaviour of wheat flour with added gluten of varieties WH 542 and C 306 was determined using TA instruments, Q_{10} DSC model (TA instruments, New Castle, DE, USA), using aluminium pans. Flour samples 3.0±0.2 mg of both varieties were weighed into aluminium pans, and covers were hermetically sealed into place. An empty, hermetically sealed aluminium pan was used as reference. Samples were analysed over a temperature range of 40 to 150 °C at a heating rate of 5 °C/min.

Instrumental texture profile analysis of gluten added flour gel

Flour gel obtained from RVA was studied using texture profile analysis (TPA). Flour gel texture properties were determined on the gels made from the RVA testing using a TA-XT2i Texture Analyzer (Stable Micro Systems, Godalming, UK) by a uniaxial compression test of two cycles (TPA). After RVA testing, the paddle was removed immediately, and the paste in the canister was covered by Para film (a semi-transparent, flexible, thermoplastic and highly water proof sheet material with water permeability of 0.88 g/m^2/24 h at 37.8 °C and 98% RH) and stored at room temperature for 24 h before testing. Samples were compressed to 75% of their original height. A plate-plate sensor system with a stainless probe SMSP/75 (Stable Microsystems, Godalming, UK) was used at a constant rate of 0.5 mm/sec. Parameters such as hardness, adhesiveness, cohesiveness and consistency were analysed. Hardness is the maximum force obtained during the first compression cycle. Adhesiveness is the negative area obtained during the first cycle. Cohesiveness was obtained as the ratio between the positive areas of the second cycle and the first cycle. Consistency is the sum of the positive areas of the first and the second cycles. All tests were performed in triplicate.

Bread making

The bread making performances of wheat flours were determined using the procedure described by Finney (Finney, 1984) with little modifications. The baking formula was: flour (100 g, 14% moisture basis), compressed yeast (5.3 g), salt (1.5 g), sugar (6.0 g), fat (3.0 g), malted barley flour (0.075 g) and ascorbic acid (100 mg/kg, flour basis). Salt, sugar, ascorbic acid and yeast were added in solution form. Yeast was added as a suspension, which was mixed well each time before dispensing. Doughs were mixed in a farinograph (Promylograph T6, 100 g capacity; Labortechnik Egger, Neumarkt, Austria). Water absorption and the development time were determined using the Chopin and protocol of Mixolab. Additional 2 ml water was added and mixing time of 1 min longer than Chopin and peak dough development time was used for baking.

After mixing, doughs were placed in bowls, and covered with a wet muslin cloth and fermented for 90 min at 35 °C and 98% RH. Doughs were moulded after 52, 77 and 90 min in dough moulder. After the final moulding, the dough was divided into four equal proportions and placed in lightly greased tins (internal dimensions for 30 g bread pan: bottom, 24.6×52.8 mm; top, 32.1×61.2 mm; height, 23.5 mm) and proved for 36 min at 35 °C and 98% RH. After adequate proving, doughs were baked for 13 min at 232 °C. After removing from the oven, loaves were placed on a wire grid for about 2 h for cooling.

Textural quality of bread

Loaf volume was measured by rapeseed displacement method. Crumb hardness was measured in a TA-XT2i Texture Analyzer (Stable Micro Systems) equipped with an aluminium 25 mm diameter cylindrical probe accordance with AACC method 74-09 (AACC, 2000). Slices of 2 cm thickness were compressed to 50% of their original height at a crosshead speed of 1 mm/s. The resulting peak force of compression was reported as crumb firmness. Three replicates from three different sets of baking were analysed and averaged.

Statistical analyses

Data was analysed using SPSS 16.0 software (SPSS Inc., Chicago, IL, USA) and Microsoft Office Excel 2007 (Microsoft Incorporation, Redmond, WA, USA). Means and standard error were derived with Microsoft Office Excel 2007 whereas correlation between various parameters were assessed by Pearson's test (significant levels at P<0.01 and P<0.05) in all cases using SPSS software.

3. Results and discussion

Grain and flour quality

TKW of the wheat varieties was determined. It was inferred from the data that the variety WH 542 had significantly higher TKW (43.2 g) than the variety C 306 (33.3 g). TKW is a useful tool for the assessment of the potential milling yield. Milling yield for variety WH 542 was 65.7% and for variety C 306 it was 59.8%. The kernel size contributes directly towards the improvement of grain yield as well as milling yield. It is reported that the wheat varieties possessing better grain weight offers great potential for higher milling yield and wide variation in grain weight can be exploited by the wheat breeders to improve this trait in the new genotypes. HLW depends on the shape, size and soundness of grains and should be more than 76 kg/l for industrial use. It was observed that HLW of both varieties did not vary significantly (Table 1).

The protein contents of the two varieties were 9.2% (C 306) and 12.4% (WH 542). Flours suitable for bread making are those made from hard wheat and generally have high protein content in the range of 11 to 14% (Ktenioudaki *et al.*, 2010). Protein content is a character determining water absorbing ability, dough stability, dough resistance and elasticity. Moreover, higher amount of good quality protein is required for gas retention and dough rise during fermentation or early stages of baking. WH 542 variety recorded higher value of SDS volume (49.5 ml)

Table 1. Physico-chemical analysis of wheat flour and gluten. The values are mean ± standard deviations of determinations made in triplicates.

Physico-chemical properties	Wheat variety	
	WH 542	C 306
Physical analysis of flour		
Thousand kernel weight (g)	33.3±0.5	43.2±0.3
Test weight (Aqua TR)	82.3±0.1	82.2 ±0.1
Milling yield (%)	65.7	59.8
Chemical analysis of flour		
Protein (%)	12.4±0.2	9.2±0.1
Falling number (sec)	509±4	454±6
Sedimentation volume (ml)	49.5±0.6	37.5±0.6
Wet gluten (%)	31.5 ±0.4	35.1 ±0.3
Dry gluten (%)	11.1±0.4	9.4±0.1
Gluten index (%)	80.8±0.2	65.1 ±0.2
Chemical analysis of gluten powder		
Protein (%)	78.4±0.1	75.7±0.2
Starch (%)	10.4±0.2	13.7±0.1
Moisture (%)	5.1±0.1	6.6±0.1
Ash (%)	0.6±0.1	0.8±0.1
Lipid (%)	4.5±0.1	3.9±0.2

and variety C 306 had a lower SDS volume of 37.5 ml. SDS sedimentation volume is one of the most important test used to discriminate wheat genotypes based on the quality and quantity of gluten proteins (Carter *et al.*, 1999). On the basis of SDS sedimentation test, wheat variety WH 542 could be classified as very good bread making variety and the variety C 306 could be classified as being poor bread making quality. It has gained wide acceptance as a useful, small-scale test in bread wheat breeding programmes to give a good indication of differences in both protein content and gluten quality, the two most important factors influencing bread baking quality (Axford *et al.*, 1978; Carter *et al.*, 1999; De Villiers and Laubscher, 1995).

The gluten proteins impart unique bread making properties to wheat. Wheat varieties varied considerably for their wet and dry gluten contents as shown in Table 1. The higher amount of 35.1% wet gluten was found in straight grade flour of wheat variety C 306 and the variety WH 542 contained 31.5% wet gluten. Wheat variety C 306 demonstrated less dry gluten content, i.e. 9.4%, whereas variety WH 542 reported a high value of 11.1%. Gluten Index provides information both on the wet gluten quantity in the tested sample of flour or semolina and on gluten strength expressed as the gluten index (mass fraction of gluten remaining on the sieve after centrifugation). Variety WH 542 depicted a higher gluten index of 80.8% and C 306 showed a value of 65.1%. A lower dry gluten yield was obtained from variety C 306 than the variety WH 542 and its gluten was sticky, crumbly and more difficult to separate from the starch components. This could be attributed to the low dry gluten content as well as low gluten index of variety C 306. The protein content, SDS volume and gluten index indicated that the variety WH 542 contained more 'good quality' protein than the variety C 306. On the basis of physico-chemical composition and gluten quality parameter results, the samples of the wheat varieties WH 542 and C 306 were considered suitable for this study, WH 542 representing good bread making quality and C 306 a poor bread making variety.

The composition of gluten is presented in Table 1. It was inferred from the data that the gluten derived from variety WH 542 contained 78.4% protein, 10.4% starch, 5.1% moisture, 0.6% ash and 4.5% lipid whereas, variety C 306 had 75.7% protein, 13.7% starch, 6.6% moisture, 0.8% ash and 3.9% lipid.

Effect of gluten addition on flour characteristics

While studying the effect of gluten incorporation on rheological and bread making quality, it is necessary to study the effect on SDS sedimentation volume and gluten index which are considered important factors in determining the quality of bread. Supplementation of gluten protein powder from 6 to 18% escalated the SDS sedimentation volume from 42.0 to 58.5 ml for variety WH 542 and it from 34.3 to 48.5 for variety C 306. Variation in SDS sedimentation volume reflects the variation in the ability of gluten to absorb water (which is supported by high water absorption value with upsurge of gluten in two varieties) as well as quantity and quality of gluten proteins. Gluten index is a major determinant of quantity of gluten proteins and gluten strength. With addition of gluten, gluten index in variety C 306 showed an upward trend from 62.8 to 72.7%. In variety WH 542, it first stepped up from 75.3 to 83.2% up to 15% of gluten supplementation and then declined (82.1%) as the concentration of gluten was increased to 18% in this variety (Table 2). Strength of the wheat flour dough of both the varieties improved substantially with increase in supplementation of gluten powder as indicated by gluten index. The strength characteristics of the dough as indicated by gluten index increased more for variety C 306 as compared to the variety WH 542 in response to the addition of dried gluten powder.

Effect of gluten incorporation on rheological characteristics

The determination of rheological properties of wheat flour dough is essential for the successful manufacturing of bread because they affect the behaviour of dough during mechanical handling, thereby influencing the quality of the finished product (Khatkar *et al.*, 2002a,b). These rheological properties of dough are water absorption, DDT, dough stability, time to break down, mixing tolerance index and elasticity on dough prepared with different incorporation level of gluten and presented in the Table 3. The Mixolab was used to study the rheological characteristics of flours of both varieties with 6, 9, 12, 15 and 18% gluten addition. Mixolab is an instrument which has the potential to assess the dough rheological properties as well as bread making quality at room and elevated temperature. Therefore, this instrument provides useful information on dough characteristics, α-amylase activity, starch and retrogradation properties. The results are displayed in Table 3.

Mixolab reports the mixing behaviour and dough strength of the flour exhibited by the parameters DDT and dough stability. Water absorption refers to the amount of water required to reach an optimum peak dough consistency of 1.1±0.05 Nm torque at C1. The data obtained (Table 3) showed that water absorption value increased from 56.7 to 66.2% and 61.7 to 67.6% on the addition of gluten in varieties WH 542 and C 306, respectively. The rise in water absorption capacity of dough may be due to ability of gluten to absorb more water in their interrelated network and interaction with starch granules. Higher water absorption capacity of dough represents desirable consistency in bread making. It can be interpreted from the results that incorporation of gluten linearly increased water absorption capacity of dough. These results were expected due structural modification in dough due to incorporation of gluten which allows more water absorption due to hydrogen bonding.

It was perceived from Table 3 that addition of gluten increased mixing time in both the varieties. Increase in dough stability and dough consistency maybe a consequence of reduction in the quantity of free water. Major proportion

Table 2. Effect of gluten addition on pasting properties of wheat varieties.[1]

Gluten level (%)	Peak viscosity (RVU)	Hot paste viscosity (RVU)	Break down viscosity (RVU)	Setback viscosity (RVU)	Final viscosity (RVU)	Pasting temp. (min)
Wheat variety WH 542						
6	2,681±3a	1,108±4a	1,537±3a	1,471±2a	3,081±1a	68.5±0.1a
9	2,594±5b	1,079±6b	1,529±2ab	1,459±6b	3,096±3b	68.6±0.2a
12	2,538±2c	1,053±5b	1,517±2b	1,411±4c	2,982±2c	68.6±0.1a
15	2,497±4d	1,013±3d	1,506±3bc	1,388±2d	2,954±1d	68.5±0.1a
18	2,483±3e	1,007±4d	1,493±1c	1,370±1e	2,941±2d	68.6±0.2a
CD[2]	6.54	8.32	4.28	6.43	3.59	N/A[3]
Wheat variety C 306						
6	2,756±2a	1,383±2a	1,863±2a	1,724±3a	3,541±4a	66.9±0.1a
9	2,715±6b	1,304±3b	1,845±2b	1,713±4ab	3,532±2a	67.0±0.1a
12	2,668±4c	1,277±2c	1,831±1c	1,687±6b	3,509±2b	67.0±0.2a
15	2,611±2d	1,198±4d	1,813±1d	1,673±2c	3,492±1bc	66.9±0.1a
18	2,524±5e	1,114±4e	1,802±2d	1,675±3c	3,437±2c	67.1±0.1a
CD[2]	7.59	5.77	3.08	7.09	4.44	N/A[3]

[1] The values are mean ± standard deviation of determinations made in triplicates. Values followed by different letters are significantly different at $P<0.05$.

[2] CD = critical difference at 5%.

[3] N/A = not applicable.

Table 3. Effect of gluten addition on dough rheological properties of wheat varieties assessed by Mixolab.[1]

Gluten level (%)	Water absorption (%)	DDT (min)[3]	Dough stability (min)	C2 (Nm)	C3 (Nm)	C4 (Nm)	C5 (Nm)
Wheat variety WH 542							
6	56.7±0.4a	2.0±0.2a	3.0±0.3a	0.4±0.1a	1.9±0.1b	1.9±0.1a	3.4±0.1e
9	60.1±0.2b	4.5±0.1b	4.5±0.1b	0.5±0.1b	2.0±0.0bc	1.7±0.0b	3.0±0.0d
12	62.8±0.4c	5.1±0.3bc	9.0±0.3c	0.5±0.0b	1.9±0.1b	1.6±0.1bc	2.7±0.0c
15	64.8±0.3d	5.5±0.2c	11.5±0.4d	0.6±0.1c	1.7±0.1a	1.5±0.1c	2.5±0.0b
18	66.2±0.2e	7.0±0.1d	11.0±0.1d	0.5±0.1b	1.6±0.0a	1.4±0.1cd	2.2±0.0a
CD[2]	0.58	0.36	0.49	N/A	0.14	0.17	0.08
Wheat variety C 306							
6	61.7±0.1a	3.0±0.1a	6.0±0.2a	0.6±0.1b	2.2±0.0a	1.5±0.0a	3.0±0.1a
9	62.0±0.3a	2.5±0.4a	9.5±0.1b	0.6±0.1b	1.9±0.1b	1.4±0.0b	2.8±0.1b
12	63.8±0.1b	6.0±0.2c	11.5±0.2c	0.7±0.0c	1.8±0.1b	1.3±0.1c	2.6±0.0c
15	66.3±0.4c	4.5±0.3b	13.0±0.3d	0.6±0.1b	1.8±0.0b	1.3±0.1c	2.5±0.0c
18	67.6±0.2d	5.0±0.1b	13.5±0.2d	0.5±0.0a	1.8±0.1b	1.2±0.1d	2.5±0.1c
CD[2]	0.46	0.46	0.39	N/A	0.14	0.14	0.14

[1] The values are mean ± standard deviation of determinations made in triplicates. Values followed by different letters are significantly different at $P<0.05$.
[2] CD = critical difference at 5%.
[3] DDT (dough development time) corresponds to C1 peak of Mixolab.

of free water of dough is used by gluten and starch for dough development. As the concentration of gluten is enhanced, it leads to the absorption of large amount of water (which is reflected by increase in water absorption values in Table 3) thereby increasing the dough consistency.

Stability of dough is the function of gluten development. The results revealed that addition of gluten intensified the gluten development, hence the stability of dough increased effectively with the supplementation of gluten. These results reflect that dough which contains high gluten concentration exhibited higher tolerance index to over mixing. The strength characteristics of the dough as indicated by dough development time and stability increased more for variety C 306, poor in bread making quality as compared to the good bread making variety WH 542 in response to the supplementation of dried gluten powder (Figure 1).

The differing proportions of added gluten and the varied water contents, and the interactions between them, resulted in distinguished effects on C2 which represents the minimum torque attained when the dough undergoes mixing as well as heating. It demonstrates the quality and stability of wheat protein network in response to gradual increase in temperature from 30 °C to 90 °C which results in protein weakening, i.e. drop in dough consistency due to

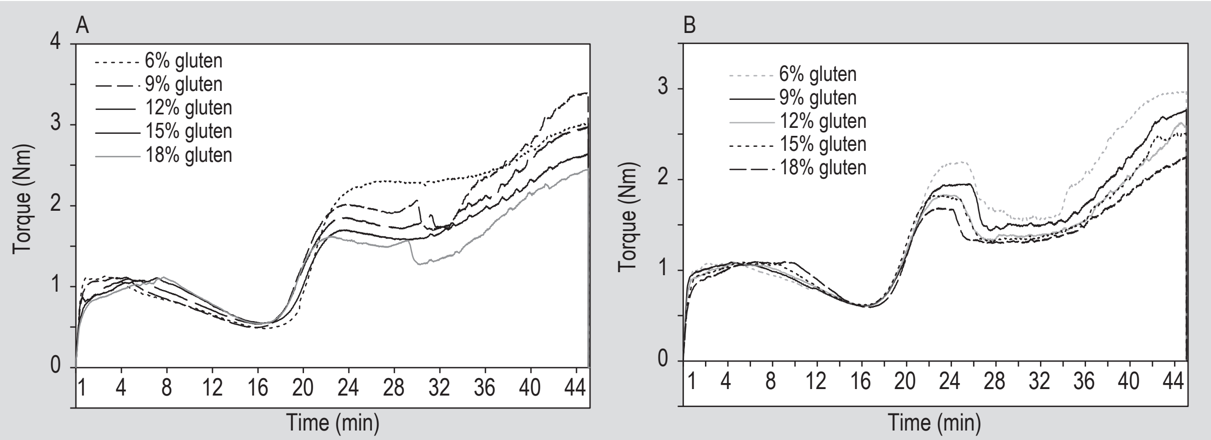

Figure 1. Effect of gluten incorporation on dough rheology of wheat varieties (A) WH 542 and (B) C 306.

excessive mixing under escalating temperature. In this case as the gluten proportion was increased, the proteins became more compact thus lowering the number of proteolytic enzymes attaching points thus leading to reduced dough softening. It was observed that with increase in the quantity of gluten an upward trend was witnessed up to 15% gluten level, thereafter, it decreased when the gluten level was increased from 15 to 18% in variety WH 542 and in variety C 306 increase was noticed till 12% incorporation level then it declined.

Starch granules absorb water; swell and amylose molecules leach out resulting in an rapid increase in the viscosity thus masking the protein weakening. It was evident from the results that C3 decreased as the gluten content increased in the two varieties. It may be due to the fact that the maximum swelling of the starch granules is adversely affected by the presence of higher gluten content which competes for the water along with the starch granules. Hence, decrease in starch proportion by gluten incorporation probably lead to reduction in C3.

C4 represents the torque exhibited by dough during mixing at a constant temperature of 90 °C thus exhibiting the stability of hot formed gel. In this stage of the Mixolab curve the dough temperature lies in a range over which starch is intensively degraded by α-amylase activation. Decrease in torque from C3 to C4 was observed in both varieties but a very steep decrease was observed in case of variety C 306 than variety WH 542. It was demonstrated that the wheat gel of variety WH 542 was stable post gelatinisation temperature and during hold period inferring better resistance to shear thinning of flour gel. It may be concluded from the above discussion that the stability of gel is dependent on the quantity of proteins in flour. However, the gel from the variety C 306 showed substantial drop in its consistency post gelatinisation temperature and during the hold period, reflecting higher enzymatic activity in flour of C 306 which is further supported by low value of falling number in C 306. In addition, the starch hydrolysis leads to an accumulation of hydrolysis products, especially low molecular-weight dextrin. Dextrin leads to decrease in starch gelling quantity that retains water and reduces dough consistency, and hence, a steeper decrease from C3 to C4 was observed.

In the last phase of Mixolab operation, the dough is subjected to decrease in temperature from 90 to 50 °C and then it is further held at 50 °C to allow recrystallisation and rearrangement of starch molecules. The maximum resistance offered by gel in terms of torque to the kneading arms of Mixolab due to increase in gel viscosity, when the temperature is kept constant at 50 °C is termed as C5. The viscosity gel is dependent on the starch content, amylose, amylopectin, amylose/amylopectin ratio. Viscosity of the flour gels increases due to the aggregation of the amylose molecules (Miles *et al.*, 1985). However, in this study C5 reduced remarkably in both varieties as with addition of gluten, the starch content of the dough decreased thus decreasing the viscosity and resistance offered by the gel to the mixing arms of Mixolab. It was observed that C5 in variety WH 542 lowered from 3.4 Nm to 2.2 Nm and in variety C 306 from 3.0 Nm to 2.5 Nm. It was deduced from the results that retrogradation (C5) is inversely related to the gluten content, i.e. as the gluten content increased, the C5 value of Mixolab decreased.

Effect of gluten addition on pasting properties

The rapid visco analyser specifies the viscosity of starch by measuring the resistance offered by slurry of flour and water to the stirring paddle. The pasting profile of the wheat flour incorporated with gluten are summarised in Table 4. Addition of gluten showed substantial decrease in peak viscosities in both varieties significantly. With the alteration of gluten level from 6 to 18%, the peak viscosity decreased from 2681 to 2483 RVU in variety WH 542 and from 2756 to 2524 RVU in variety C 306. Peak viscosity is an indicator of water binding capacity and ease with which starch granules are disintegrated and it is often correlated with final product quality (Ritika *et al.*, 2010). The observed decline in peak viscosity may be accredited to the competition among enhanced gluten level and starch components in a limited water system thus reducing the flour paste viscosity. The decrease in peak viscosity was more pronounced in variety C 306 than variety WH 542 (Figure 2). Peak viscosity is also affected by quantity and quality of starch in a particular variety. The breakdown viscosities differed considerably in the gluten incorporated flours of varieties WH 542 and C 306. A significant downtrend in breakdown viscosity was demonstrated with increase in the level of addition of gluten. Highest breakdown viscosity of 1863 RVU was achieved in variety C 306 with 6% gluten incorporation level. High value of breakdown is associated with high peak viscosity, which in turn, is related to the degree of swelling of the starch granules during heating (Ragee and Aal, 2006). Breakdown is caused by the disintegration of gelatinised starch granule structure during continued stirring and heating or paste stability during the holding period of viscosity test. It was perceived from the data that breakdown viscosity showed a decreasing trend with incorporation of gluten in both varieties. The flour of variety WH 542 depicted a lower breakdown as compared to variety C 306. This may be probably attributed to the shielding action of high protein content to breakdown. Gluten is known to form a fibrillar and globular structure in the gel which gets attached to the surface of starch granules and result in the formation of compound with the help of covalent bond. Additionally, starch granules preferably adsorb high molecular weight proteins with long fibrillar structure than the globular structures. Variety WH 542 had high quality glutenin subunits 5+10. Hence, it can be inferred that the

Table 4. Effect of gluten addition on thermal properties of wheat varieties.[1]

Gluten level (%)	Peak temperature (°C)	Enthalpy (J/g)
Wheat variety WH 542		
6	65.1±1.6a	16.1±3.2a
9	65.7±1.1a	15.7±2.1ab
12	66.4±2.3b	15.3±2.9b
15	70.5±1.2c	14.7±2.2c
18	72.8±1.8d	13.9±3.1d
CD[2]	3.07	N/A[3]
Wheat variety C 306		
6	57.8±2.1a	12.8±3.3a
9	58.0±1.3a	12.3±4.1a
12	59.7±1.5b	11.7±2.6b
15	60.1±1.2bc	11.2±1.7c
18	60.6±2.0c	11.3±2.4c
CD[2]	N/A	N/A

[1] The values are mean ± standard deviation of determinations made in triplicates. Values followed by different letters are significantly different at $P<0.05$.
[2] CD = critical difference at 5%.
[3] N/A = not applicable.

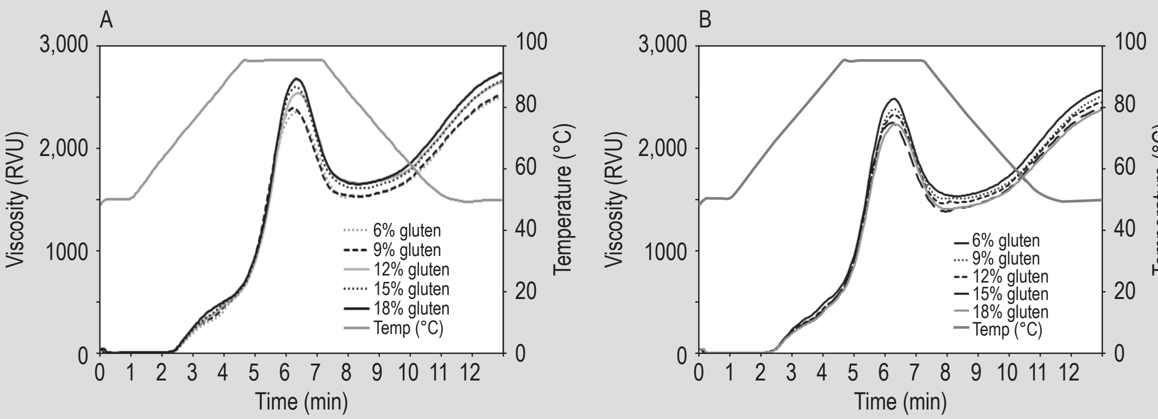

Figure 2. Effect of gluten addition on pasting properties of wheat varieties (A) WH 542 and (B) C 306.

gluten derived from this variety was stronger than that of C 306 wheat variety and hence had a high shielding effect. Setback reflects the retrogradation tendency of starch, which inhibits the increase in the minimum viscosity upon cooling (Zaidul *et al.*, 2007). High setback is associated with synergesis of gel and presents a quality defect in the products. Setback viscosity decreased with increase in the percentage of gluten added. The highest setback viscosity was reported by the flours of varieties WH 542 and C 306 with 6% gluten that is, 1471 and 1724 RVU, respectively. The final viscosities of the gluten incorporated flour reduced appreciably with an increase in the gluten content from 6% to 18%. The highest final viscosity was recorded for variety C 306 with 6% gluten (3541 RVU) and the lowest for variety WH 542 with 18% gluten (2941 RVU). Amylose, amylopectin, amylose/amylopectin ratio and starch content are major contributors to final viscosity. No remarkable difference was observed in pasting temperature with the addition of gluten signifying that pasting temperature is less relevant to the change in starch concentration due to gluten incorporation. Pasting temperature signifies the minimum temperature at which the viscosity increases during the heating process. The pasting properties of starch depend on the amylose content of the flour along with the amount of non-starchy components (protein and fat) and processing techniques. The low peak and breakdown viscosity of the starch in the flours could be correlated to the high protein content.

Effect of gluten addition on thermal properties

The thermal properties of wheat flour as influenced by gluten addition are presented in Table 2. There was a significant increase in peak temperature of wheat flour with incorporation of gluten. Increase in the levels of gluten up to 18% increased the peak temperatures of flours from 65.2 to 72.8 °C in variety WH 542 and from 57.8 to 60.6 °C in variety C 306 (Figure 3). In contrast, the enthalpies reduced from 16.1 to 13.9 J/g and 12.8 to 11.3 J/g in varieties WH 542 and C 306, respectively. The diverse peak temperatures and enthalpies among the blends could be attributed to the different chemical compositions, which largely determined the thermal properties of wheat flour. It was noted that the peak temperature and enthalpy were high in case of variety WH 542 (Table 2) presumably due to highly ordered structure of gluten. This in turn, enhances gas retention and controlled expansion for improved volume, uniformity and texture of bread. Furthermore, variety WH 542 possess 5+10 subunit which makes the gluten of this variety strong than the gluten of variety C 306 with subunit 2+12 which confers the gluten of this variety a weak gluten (Dhaka *et al.*, 2012). Consequently, the results obtained from thermal analysis reinforce the rheological behaviour of the two varieties based on glutenin subunit composition.

Effect of gluten incorporation on texture profile analysis of flour gel

Gluten addition in wheat flour of varieties WH 542 and C 306 affected the textural parameters of all flour gels stored at room temperature for 24 h (Table 5). Supplementation of gluten decreased the hardness of flour gels significantly from 65.7 to 54.9 g in variety WH 542 and 70.3 to 53.6 g in variety C 306. This decrease in hardness was more notable in variety C 306. This could be explained by the

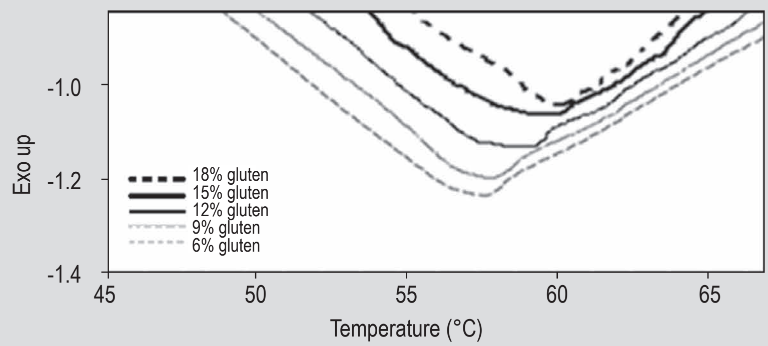

Figure 3. Effect of gluten incorporation on thermal properties of wheat variety C 306.

Table 5. Effect of gluten addition on textural properties of wheat varieties.[1,2]

Gluten level (%)	HD (g)	CO (ratio)	AD (g/s)	SP (ratio)
Wheat variety WH 542				
6	65.7±1.4a	0.41±0.01a	-38.6±0.6a	0.64±0.02a
9	61.3±0.9b	0.44±0.04ab	-37.3±0.4b	0.67±0.02ab
12	58.6±1.2c	0.48±0.03b	-34.9±2.3c	0.69±0.01ab
15	55.8±2.5d	0.52±0.05c	-31.4±1.8d	0.73±0.06b
18	54.9±1.5d	0.52±0.02c	-31.2±2.1d	0.72±0.02b
CD	2.94	0.06	3.02	0.06
Wheat variety C 306				
6	70.3±3.5a	0.35±0.01a	-47.6±2.6a	0.58±0.06a
9	67.7±2.3b	0.39±0.02b	-45.8±0.7b	0.60±0.02ab
12	62.6±2.9c	0.44±0.01c	-41.2±1.5c	0.63±0.02b
15	58.7±2.4d	0.49±0.03d	-38.3±1.8d	0.68±0.03c
18	53.6±1.6e	0.53±0.05e	-34.1±0.6e	0.70±0.01d
CD	4.82	0.05	2.98	0.06

[1] The values are mean ± standard deviation of determinations made in triplicates. Values followed by different letters are significantly different at $P<0.05$.
[2] HD = flour gel hardness; CO = flour gel cohesiveness; AD = flour gel adhesiveness; SP = springiness; CD = critical difference at 5%.

fact that gluten forms cross links with starch molecules thus hindering the reassociation of amylose by hydrogen bonds. Cohesiveness was a measure of the difficultly in breaking down the gel's internal structure. With increase in the amount of gluten, the concentration of starch lessened. During gel formation starch gets embedded in the gluten network. Gluten network is formed by strong inter and intra disulphide bonds which provide resistance to breakdown which leads to increase in cohesiveness in both varieties. This effect was more pronounced in variety C 306 as compared to WH 542. Another notable observation was that cohesiveness value of variety C 306 at 18% gluten level was almost similar to that of WH 542 at same gluten level indicating that the network formation showed more improvement in variety C 306 as the gluten level was hiked. Adhesiveness followed the similar trend with hardness. Adhesiveness is a surface property and depends on a combined effect of adhesive and cohesive forces. Addition of gluten decreased the adhesiveness of the gel as the gluten increases the elasticity of gel and hence prevents it adherence to the probe. The adhesiveness decreased from 38.6 to 31.2 g/s in variety WH 542. On the other hand, the decrease in adhesiveness in variety C 306 decreased from 47.6 to 34.1. Table 6 depicts that the springiness increased remarkably with addition of gluten, maximum springiness was 0.70 at 18% gluten level in variety C 306 whereas, variety WH 542 showed a peak value of 0.73 at 15% addition level.

Effect of gluten addition on bread quality

The mean values of loaf volume illustrated the effect of gluten addition on the volume of the bread loaves (Table 6). It has long been known that the gluten content of flour has a direct effect on the volume of bread produced with it, within wide protein range. The loaf volume (LV) of bread of variety WH 542 was found to increase linearly with incorporation of gluten up to 15% level and then decreased, when the gluten incorporation level was increased from 15 to 18% which may be endorsed to the extra strong nature of its gluten that prevented dough expansion during fermentation and the initial baking stage. In variety C 306, it was found that gluten supplementation caused increase in bread LV linearly and maximum volume was observed in case of sample containing 18% gluten level. It was evident from the results that the rise in LV with gluten incorporation level was variety specific with variety C 306 (r^2=0.976) showing more effect than variety WH 542 (r^2=0.937).

The addition of gluten increased loaf volume and reduced bread firmness. The addition of gluten intensifies the quantity of gluten in the dough, forming a gluten network much more arranged, which better holds all the other dough components. Because of this, the CO_2 retention is improved and the bread has a higher porosity. The increased protein content makes the bread loaf more elastic (Codina *et al.*, 2008). Similar results were also sought in this study. The optimum levels for bread firmness were obtained at higher levels of gluten addition in both varieties.

Table 6. Effect of gluten addition on sodium dodecyl sulphate (SDS) sedimentation volume, gluten index and bread making potential of wheat varieties.[1]

Gluten level (%)	SDS sedimentation volume (ml)	Gluten index (%)	Bread firmness (g)	Loaf volume (cm^3)
Wheat variety WH 542				
6	42.0±0.3a	75.3±1.9a	175.0±1.8e	78.0±1.3a
9	45.3±0.2b	78.7±2.2b	163.4±1.3d	80.0±0.4b
12	49.5±0.3c	80.8±1.9 c	158.2±1.6c	83.0±1.5c
15	53.6±0.2d	83.2±2.6e	150.3±1.2a	85.0±0.4d
18	58.5±0.3e	82.1±1.6d	155.7±0.6b	83.5±0.9c
CD[2]	0.45	3.81	2.51	1.85
Wheat variety C 306				
6	34.3±0.2a	62.8±2.8a	220.9±1.3d	68.0±0.9a
9	37.5±0.2b	65.1±2.7b	205.1±1.3c	70.0±0.4b
12	40.0±0.2c	68.6±2.2c	187.8±1.1b	77.0±0.4c
15	43.2±0.3d	70.6±2.5d	181.1±1.6a	80.0±0.9d
18	48.5±0.2e	72.7±2.3e	180.1±0.8a	84.3±0.8e
CD	0.41	4.63	2.53	1.32

[1] The values are mean ± standard deviation of determinations made in triplicates. Values followed by different letters are significantly different at P<0.05.
[2] CD = critical difference at 5%.

The results depicted in this experiment suggest that incorporation of gluten protein positively affects LV and bread firmness. It may also be concluded that the addition of gluten enhances the dough strength and bread making quality of weak variety C 306 more than the strong variety WH 542, which has been manifested by their dough rheology and bread quality parameters.

The result portrayed in this study strongly suggests that dry gluten can also be used as bread improver to overcome the poor bread making quality of some wheat varieties. Moreover, augmentation of gluten has added advantage in improving the texture, water holding capacity, volume of bread and also overall protein content of the bread.

4. Conclusions

The study endowed us an insight of the effect of gluten augmentation on bread quality. It bestowed useful predictions of technological quality of the wheat varieties investigated. The gluten addition substantially improved the dough rheological properties. The strength characteristics of the dough as indicated by dough development time and stability increased more for poor bread making variety C 306 as compared to the good bread making variety WH 542 in response to the supplementation of dried gluten. It can be concluded that gluten positively affect water absorption, dough stability, DDT and C2 and the bread quality characteristics. However, decrease in starch proportion by gluten incorporation probably lead to reduction in C3, C4 and C5 values. In this study, it was evident from the data obtained that wheat flour samples with different gluten levels led to a considerable variation in the pasting, thermal and textural properties. Baking tests showed that gluten incorporation had positive effect on the bread loaf volume however; the effect was more prominent in poor bread making variety C 306. Though, in commercial practice lower levels of addition may not yield the same orders of magnitude of change as in this study.

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