3.1 Instruments

Baking Oven (Model FG 156), Mono Equipments Ltd., U.K.
Chopin Aqua TR, Villeneuve, La Garenne, France
Chopin Mill (Model, CD 1), Villeneuve, La Garenne, France
Chopin Mixolab, Villeneuve, La Garenne, France
Chopin SDmatic, Villeneuve, La Garenne, France
Cooling Centrifuge (Model C-25), Remi Instruments, India.
Differential Scanning Calorimeter (Q600), TA instruments, USA
Dough Mixer (Model K5SS), Kitchen Aid, U.S.A.
Dough Sheeter (Model SF 600), Conti Bussolelengo, Italy
Electrophoresis Apparatus (Model AE-6220 and power supply model AE- 8150), Atto Corporation, Tokyo, Japan
Falling Number (Model 1100), Perten Instruments, Sweden
Freeze Dryer (Alpha-24LD Plus), Christ, Germany
Glutomatic, Perten Instruments, Sweden
Magnetic Stirrer, Spinot, India
Micro doughLab, Perten Instruments, Sweden
Noodles Cutting Machine, Imperia, Italy
Noodles Sheeting Machine, Imperia, Italy
3.2 Materials

3.2.1 Wheat Varieties

The pure samples of fifteen commercial wheat varieties were obtained from the Wheat Research Institutes/Stations across the country. These varieties were selected mainly on the basis of their wide physicochemical characteristics, protein quantity and quality, *Glu* 1 scores and product diversity. The wheat grains of all the cultivars were cleaned manually and stored at room temperature prior to milling. The grains of individual cultivars were milled on a Chopin Mill (Model, CD 1) into flour of different extraction rates after tempering for 24h. The soft wheat variety samples were tempered at 14.5% and hard wheat variety samples were tempered at 16.5% moisture content before milling. Extra 0.5% moisture was added 30 min before milling to facilitate the separation process. Flour extraction was calculated on a total product basis. All flour samples were stored at -20°C before their analysis.

**Table 3.1 Commercial wheat varieties obtained from various research stations**

<table>
<thead>
<tr>
<th>Wheat Variety</th>
<th>Procured From</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBW 38, NIAW 917, WH 1021, HUW 234, VL 892, PBW 590, C 306</td>
<td>Central State Farm, Hisar</td>
</tr>
<tr>
<td>WH 1025, PBW 550</td>
<td>HAU, Hisar</td>
</tr>
<tr>
<td>DBW 16, HI 8498, MACS-1967, HW 2004</td>
<td>DWR, Karnal</td>
</tr>
</tbody>
</table>
3.2.2 Chemicals

All chemicals were of analytical reagent grade. The chemicals used in the study were acrylamide, ammonium sulphate, L- ascorbic acid, boric acid, potassium sulphate anhydrous, selenium powder, sulphuric acid, hydrogen peroxide, sodium hydroxide, HCl, potassium iodide, sodium thiosulphate, sodium bicarbonate, sucrose, sodium carbonate, sodium chloride, bromophenol blue, coomassie brilliant blue (CBB) G-250, ethanol, lactic acid, 2-mercaptoethanol (2-ME), phosphoric acid, sodium dodecyl sulphate (SDS), sodium chloride, glycerol, N, N’methylene bisacrylamide (bisacrylamide), N, N, N’, N’-tetramethylenediamine (TEMED), methyl green, ferrous sulphate, trichloroacetic acid.

3.3 Methods

3.3.1 Physical Analysis of Grains

The grains were cleaned manually to remove soil particles, brokens and foreign seeds. General grain characteristics like 1000 kernel weight, hectoliter weight, kernel length and width of all the samples were determined. Thousand kernel weight (TKW) was determined by measuring the weight of 100 seeds and multiplying the weight obtained by a factor of 10. Hectoliter weight (HLW) was determined using Chopin Aqua TR (Villenueve, La Garenne, France). The kernel length and width of all the samples were determined using digital vernier caliper (Mitutogo Corporation, Model, CD-6” BS). The hardness of the kernels was estimated with the help of Single Kernel Characterization System.

3.3.2 Chemical Analysis

Moisture, protein (Kjeldhal N ×5.75) and ash content were determined by standard AACC methods (AACC, 2000). The fat content of the flours was determined by Solvent Extractor (Velp Scientifica, Italy). 2 g flour sample was taken in the thimble and 70 ml petroleum ether was taken in a pre-weighed extraction vessel. The extraction cycle was carried out for 2 hrs. At the end of the cycle the extraction vessel with the extracted fat from the flour sample was weighed and the fat percentage was calculated. The falling number values of all the flour samples (14% mb) were
determined by Falling Number apparatus. 7g of the flour was taken in a falling number tube and shook vigorously for 40 seconds to obtain homogenous suspension and kept in the falling number apparatus to obtain the falling number value. The sodium dodecyl sulphate (SDS) sedimentation volumes of flour samples were estimated according to the method of Axford et al., (1978). Flour (5g, 14% moisture basis) was added to water (50ml) in a cylinder, a stopclock was started and the material was dispersed by rapid shaking for 15 sec. The contents were re-shaken for 15 sec, at 2 min. and 4 min. immediately after the last shake. SDS- lactic acid reagent (50ml) was added, and mixed by inverting the cylinder four times before re-starting the clock from zero time. The SDS-lactic acid reagent was prepared by dissolving SDS (20g) in distilled water (1lt.) and then adding a stock diluted lactic acid solution (20ml; 1 part lactic acid plus 8 part distilled water by volume). Inversion (four times) was repeated at 2, 4 and 6 min before finally starting the clock once again from zero time. The contents of the cylinder were allowed to settle for 40 min before reading the sedimentation volume. Chopin SDmatic was used for determination of the damaged starch in the different wheat varieties. 1 g flour was weighed accurately and was placed in the spoon in the SDmatic. After six minutes of the SDmatic cycle, the damaged starch content of the flour sample was displayed on the screen. Solvent Retention Capacity profile (SRC) was obtained according to the AACC 56-11 method. Wheat flour samples (5 g) were suspended in each of 25 g of water, 500 g/l sucrose, 50 g/l sodium carbonate and 50 g/l lactic acid. The samples were hydrated for 20 min and centrifuged at 1000×g for 15 min. Each precipitate obtained was weighed and the SRC for each solvent was calculated using the following equation (Haynes et al., 2009):

\[
\% \text{ SRC} = \left\{ \left( \frac{\text{tube, stopper, gel wt.} - \text{tube, stopper}}{\text{flour weight}} \right) - 1 \right\} \left( \frac{86}{100 - \text{flour moisture}} \right) \times 100
\]

Alkaline water retention capacity (AWRC) was determined according to the AACC 56-10 method. All determinations were made in triplicate.

3.3.3 Gluten Isolation and Characterization

Gluten is the functional component of wheat protein. Its properties determine dough characteristics and influence end product quality. The Gluten quantity and quality affect the dough elasticity, gas retention, expansion properties and will largely influence the final baking quality. Furthermore, the ability to form non-sticky dough, to maintain the desired dough firmness
and to achieve constant pasta cooking characteristics is all influenced by the gluten properties. In the present study Glutomatic (Perten) was used to measure the Gluten Index as well as wet and dry gluten content. 10.0 g ± 0.01 g of flour was weighed and put into the Glutomatic wash chamber with an 88 micron polyester sieve. 4.8 ml of salt solution was added to the flour sample. Flour and the salt solution were mixed to form dough during 20 seconds. After termination of the mixing phase, the washing started automatically and continued for five minutes. Exactly 30 seconds after completion of washing, the undivided wet gluten piece was transferred to the special sieve cassette and centrifuged for one minute at 6000 ± 5 rpm in Centrifuge 2015. The fraction passed through the sieves was scraped off with a spatula and weighed. The fraction remaining on the sieve was collected and added to the balance. The total wet gluten weight was obtained. The total wet gluten piece was dried at 150°C for four minutes in the Glutork 2020. After drying, the gluten was weighed on the balance. The calculations were done as follows:

\[
\text{Gluten Index (GI)} = \frac{\text{Wet gluten remaining on sieve}}{\text{Total Gluten}} \times 100
\]

\[
\text{Wet Gluten Content (WGC)} = \text{Total wet gluten} \times 10
\]

\[
\text{Dry Gluten Content (DGC)} = \text{Dry gluten weight} \times 10
\]

The baking quality of the gluten was assessed by the gluten baking test. For this, 10 g of wet gluten was isolated from the fifteen wheat varieties and baked at 220°C for 20 min. The volume of the baked gluten was measured by the rapeseed displacement method.

3.3.4 Rheological Studies of Wheat Dough and Gluten

3.3.4.1 Rheological Properties of Dough

Dough rheological studies were conducted on wheat flour by Chopin Mixolab. The information obtained from the recorded curve of the Chopin S protocol included the percentage of water required for the dough to produce a torque of 1.1±0.07 Nm (water absorption, percent); the time to reach maximum torque at 30°C (dough development time, minutes); the elapsed time at which the torque produced is maintained at 1.1 Nm (stability, minutes); the difference between the maximum torque at 30°C and the ending torque after the holding time at 30°C (mechanical dough
weakening, Farinograph units (FU)), respectively was used to evaluate the rheological parameters of different flours.

3.3.4.2 Extensibility of Gluten

Uniaxial extensibility of gluten of different wheat varieties was assessed by the Kieffer dough and gluten extensibility rig developed by Stable Micro Systems for the TA-XT plus Texture Analyser. Gluten was extracted from the glutomatic and was rolled into a cylindrical shape and placed over three or four channels of the Teflon coated block. Prior to the placement of gluten, the Teflon-coated block was prepared by placing non adhesive Teflon strips which were coated with silicon oil in the channels. Once the gluten was placed in the Teflon-coated block, the upper half of the block was placed in position and tightly clamped, which distributed the gluten over three to four channels, to yield gluten strips of uniform geometry. The gluten was rested for 40 min at 25°C prior to the test. The gluten strips were then separated from the Teflon strips, positioned across the Kieffer rig dough holder, and immediately tested on the TA.XT plus at a hook speed of 3.3 mm/s and a trigger force of 1 g. The resistance to extension (g) and extensibility (mm) were determined in tension mode by recording the peak force and the distance at the maximum and the extension limit.

3.3.4.3 Dynamic Rheological Analysis

Dynamic oscillatory measurements were performed with a controlled stress rheometer (Anton Paar, MCR 301). In this rheometer, the upper plate is subjected to forced oscillation of known amplitude and frequency.
3.3.4.3.1 Sample Preparation for the Dynamic Rheological Measurements

For dynamic rheological measurements of the gluten, 10g dough samples were prepared from each cultivar and washed to get the wet gluten using the Perten Glutomatic. The glutens were rested for 30 min at room temperature before loading onto the rheometer.

3.3.4.3.2 The Rheometer (MCR 301) Experimental Conditions

The samples were placed carefully between the parallel plates (25mm and onto the rheometer and gap between plates (sample thickness) was adjusted to 1.0 mm. Excess gluten was trimmed off carefully with a razor blade, and a thin layer of low viscosity (<0.1% of the test material viscosity) silicon lubricant was applied to the exposed dough surfaces to prevent moisture loss. The samples were allowed to rest for 15 min on the rheometer to allow stresses including during sample handling to relax. The tests were conducted at 25°C.

3.3.5 Fractionation of Gluten into Gliadins and Glutenins

The wheat flours were defatted according to MacRitchie (1987). Flour (100g) was extracted with 200 mL of chloroform at room temperature and then filtered through filter paper. The extraction was repeated twice for a total of three extractions. The defatted flour was allowed to dry at room
temperature. Gluten was extracted from defatted flour samples by glutomatic and freeze dried. The freeze dried gluten samples were ground in a pestle mortar. The resulting freeze dried gluten powder was dissolved in 200 ml of 70% ethanol. The mixture was stirred on a magnetic stirrer for 3 h at 25°C followed by centrifugation for 30 min at 1000g at 4°C. Supernatant was collected and the pellet was again extracted with 70% ethanol. The supernatants were pooled and ethanol was removed from the gliadin extracts using rotary evaporator at 30°C. The gliadin and glutenin fractions, thus, obtained were freeze dried and powdered in pestle and mortar (Khatkar, 1996).

3.3.6 Effects of Addition of Gliadins and Glutenins on the Mixing Properties Using Micro Doughlab

Changes in the dough mixing properties of the base flour were determined by a 4g micro doughlab. The base flour (HW 2004) was supplemented with increasing amount of gliadin and glutenin fractions extracted from HW 2004, i.e. 2%, 4%, 6%, 8% and 10%, respectively. The gluten subfraction was added to the base flour and put into the mixing bowl of micro doughlab. The instrument allows the dry mixing of the flour for one minute followed by the addition of water as determined by the instrument to reach the 500 FU line in the chart. The mixing of the dough continued for 12 minutes for all the gliadin fortified flours. However, for the glutenin fortified flours, the mixing time was manually increased to 30 minutes as the dough became too strong and the departure time could not be determined in 12 minute cycle. The different mixing parameters determined by the micro doughlab were peak dough height (FU), arrival time (min), dough development time (min), dough stability (min), departure time (min), dough softening (FU), peak energy (Wh/Kg) and bandwidth at peak (FU), respectively. The determinations were made in triplicate.

3.3.7 Pasting properties of Gliadin and Glutenin Added Flours

The changes in the pasting characteristics of the base flour were determined by Rapid Visco Analyzer- TechMaster (Perten Instruments) according to the AACC approved method 76-21. The gliadin and glutenin fractions were added to the base flour at 2%, 4%, 6%, 8% and 10% level, respectively. Three RVA runs were conducted on each sample and the result were expressed as mean. The measured properties were pasting temperature: temperature of initial viscosity
increase; peak viscosity: maximum viscosity recorded during heating and holding cycles, usually occurs soon after heating cycle reaches 95°C; peak time: time required to reach peak; trough:

![Graph showing pasting properties obtained from Rapid visco analyzer](image)

**Figure 3.2** Graph showing pasting properties obtained from Rapid visco analyzer

minimum viscosity after peak; final viscosity: viscosity at test finish, corresponds to cool paste viscosity; breakdown: difference between peak and trough, indication of breakdown in viscosity of paste during 95°C holding period; and setback: difference between final viscosity and trough. All measurements were reported in Rapid Visco Units (RVU).

3.3.8 Texture Profile Analysis of Gliadin and Glutenin Added Dough

Dough samples prepared in the 4 g micro doughlab were studied using Texture Profile Analysis (TPA). Cylindrical samples of 2 cm diameter and height 1 cm were obtained from dough. Samples were compressed to 75% of their original height. A plate-plate sensor system with a stainless probe SMSP/75 was used at a constant rate of 0.5mm/sec. The texture of the dough was determined by a uniaxial compression test of two cycles (TPA) using TA-XT2i Texture Analyzer. Parameters such as hardness, adhesiveness, cohesiveness and consistency were analyzed. 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.
3.3.9 Gluten Recovery and Determination of Gluten Index from Gluten Obtained from Gliadin and Glutenin Added Dough

The dough with and without the addition of gliadin and glutenin fractions were prepared in micro doughlab and rested in water for 30 minutes. The dough was then washed under running tap water to remove the water soluble components such as starch. The weight of the resulting wet gluten was noted. The wet gluten was then centrifuged in gluten centrifuge to obtain the gluten index of the gliadin and glutenin added gluten.

3.3.10 Thermal Analysis of the Gliadin Added Gluten

The thermal analysis of control and gluten with addition of gliadins at 5% and 10% levels was carried out with differential scanning calorimetry (DSC) and thermo gravimetric analysis (TGA) techniques. For this, dough obtained after optimum mixing in the micro doughlab was washed under running water to wash away the starch and other water solubles and thus gluten was obtained. The gluten was freeze dried and powdered in pestle and mortar. DSC measurements were carried out according to Leon et al. (2003) using TA instruments, Q600 DSC model, USA. Powdered samples of freeze dried gliadin added gluten obtained were weighed 3.5 mg in aluminium pan and placed in the DSC instrument. An empty aluminium pan was considered as reference. All the measurements were performed at a heating rate of 10°C/min. The change in heat flow of different samples was analyzed over a temperature range of 30-130°C. TGA measurements of different samples (10 mg) were carried out in air using TA instrument, Q600 model at a heating rate of 10°C/min over a temperature range of 50-800°C.

3.3.11 Scanning Electron Microscopy Analysis

For microscopy, the freeze dried gluten samples were first fractured to expose interior structure then affixed to aluminium SEM stubs using either double sided tape for longitudinal sections and cross sections, respectively, at the base of each specimen (Lopez and Bushuk, 1982). Then the prepared specimens were coated with gold using a sputter coater to make the specimen conductive. These gold coated specimens were then analyzed in a Microtrac Semtrac Mini (Nikkiso, Tokyo, Japan) scanning electron microscope. The representative micrographs from all the samples were selected for illustration.
3.3.12 SDS-PAGE for Glutenins

SDS-PAGE was performed on Atto (Model, AE-6220) vertical electrophoresis cell (150×150×1mm) with a 12% polyacrylamide separating gel containing 1.35% bisacrylamide cross-linker according to the procedure of Laemmli (1970) with 2-mercaptoethanol. Flour (40mg) from different cultivars were suspended in SDS sample buffer (1.0ml) containing 62.5mM Tris buffer/HCl (pH 6.8), 2%(w/v) SDS, 10%(v/v) glycerol, 0.001%(w/v) bromophenol blue and with 5%(v/v) 2-mercaptoethanol. The flour buffer mixtures were vortexed mixed for 2min and allowed to stand at room temperature for 3h. The flour buffer mixtures were centrifuged, and the supernatants were heated for 3min in a boiling water bath. After cooling to room temperature, the samples (10µl) were loaded onto the gel. Two gels were run simultaneously for 5h at a constant current of 40mA.

3.3.12.1 Staining of Gels

The gels were stained overnight (~12hrs.) with coomassie brilliant blue (CBB) G-250. The staining solution was prepared from 80 ml of 0.1%(w/v), CBB G-250 in 2%(w/v), phosphoric acid 10%(w/v), ammonium sulphate and 20 ml methanol adjusted to a final volume of 100ml. After staining to the required sensitivity, the gels were briefly washed with 25%(v/v) methanol in distilled water and for stable storage transferred into 20%(w/v) ammonium sulphate solution.

3.3.13 Acid PAGE for Gliadins

Acid Poly Acrylamide Gel Electrophoresis (acid-PAGE) for gliadin composition of all wheat varieties was carried out according to the method of Lookhart et al. (1982) with some modifications as reported by Anjum (1991) and Guiraud et al., (1990). Gliadins were estimated from 250mg flour sample with 750µL 70%(v/v) ethanol for 1 hour at room temperature. The samples were centrifuged at 5500 rpm for 15 minutes. Five drops of glycerol were added to 350µL supernatants to increase the density of the protein solutions and one drop of 1% methyl green solution as a tracking dye was also added. The gel contained 6%(w/v) acrylamide, 0.25%(w/v) N,N-methylenebisacrylamide, 0.24%(w/v) ascorbic acid, 0.25%(v/v) of 0.1%(w/v) FeSO4.7H2O and 0.25%(w/v) sodium lactate. The pH was adjusted to 3.2 by the use of lactic acid. The gel solution was filtered and polymerized by adding 100µL of H2O2 per 100ml of gel solution (Lookhart et al. 1986). Combs of 3mm thickness were used. The gel solution was poured
immediately into the gel chambers already assembled. The gel was allowed to polymerize for 15 minutes. The running buffer solution for Acid PAGE contained 5.625 g sodium lactate dissolved in 4500 ml distilled water, and the pH was adjusted to 3.2 using lactic acid. Gliadin extract (15µL) was loaded in each 3mm thick slot. The temperature was set constant at 20º C. The electrophoresis was carried out at constant voltage of 500V for two and a half hours. The gels were removed from the glass plates and placed in plastic containers, and stained with a 300ml staining solution composed of 9 ml of 1% (w/v) Coomasie Brilliant Blue in ethanol and 61ml of 50% (v/v) trichloroacetic acid (TCA) for 4 hours. After staining, the gels were destained in a 300 ml solution containing 50% TCA and distilled water (Lookhart et al., 1986), and each gel was photographed.

3.3.14 Baking Test for Bread

The bread making performances of flours were determined using optimized baking tests following the procedure described by Finney (1984). For 30g bread making method the test baking formula was: flour (30g, 14% moisture basis), compressed yeast (1.59g), salt (0.45g), sugar (1.8g), fat (0.9g), malted barley flour (0.075g), ascorbic acid (100 ppm, flour basis). For the 100g bread making method, formulation amounts for the 30g method were multiplied by the factor 3.33. 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 prepared in the Farinograph mixer. After mixing, doughs were placed in bowls, and covered with a wet muslin cloth and fermented for 90 min at 30ºC and 98% R.H. doughs were punched after 52, 77 and 90 min in a machine moulder (Nagpal, New Delhi) by passing through a set of rollers with a gap setting of 9 mm. After the final punch, the doughs were placed in lightly greased tins. Dough’s were proved at 30ºC and 98% R.H.

The baking process was carried out for 25 min at 230ºC in baking oven. A water container was placed in the oven to provide adequate moisture conditions in the oven. Loaf volumes were measured by rapeseed displacement after cooling the loaves for 2h on a wire mesh. The bread from each cultivar was baked at least twice.

3.3.14.1 Textural Analysis of Bread (Texture analyzer, TA-XT 2i)
The bread firmness was determined using AACC (74-09) standard method. The texture analyzer TA-XT 2i was used with 25 mm cylindrical probe (P/36 R). The pre test speed, test speed, and post test speed was 1.0, 1.7 and 10.0 mm/sec, respectively with data acquisition rate of 250 pps.

3.3.15 Cookie Preparation

Cookies were prepared according to AACC Approved method 10-50D (2000) with slight modifications. The ingredients used were flour (225 g), sugar (130 g), shortening (64 g), dextrose solution (33 ml), sodium bicarbonate (1.6 g), ammonium bicarbonate (0.9g), sodium chloride (2.1 g) and distilled water (16 ml). The dough was sheeted to 10 mm thickness on a dough sheeter and cut into round shape with a cutter of 60 mm diameter. Baking was performed in baking oven at 205°C for 15 min. The diameter and thickness of six cookies was measured and the average was calculated. The spread ratio was calculated by dividing diameter (mm) with thickness (mm). Cookie preparation was done in triplicate.

3.3.15.1 Textural Analysis of Cookies

The texture of cookies was determined by Texture Analyzer (TA-XT 2i) in terms of the breaking force required to fracture the cookies. The probe used was Warner –Bratzler blade using 50 kg load cell. The test mode used was force in compression with pre test speed, test speed and post test speed of 1.5, 2.0 and 10.0 mm/sec, respectively.

3.3.16 Noodle Making

Flour (100g) was mixed with 35 ml of 6.25% sodium chloride in a mixer for 30 sec at low speed (60rpm) and then for 3 min at a medium speed (86rpm). The dough was then passed through the rolls of dough sheeting machine at 3mm gap. It was folded and passed through the rolls twice again. The dough sheet was rested for 1 h at ambient temperature (25°C) and then again rolled through the sheeting rolls three times at progressively smaller gap settings of 2.40 mm, 1.85 mm and 1.30 mm, respectively. The sheet was then cut into noodle strands by machine. Noodles were put into plastic bags and stored at 4°C for 24 h until being cooked. Noodles (10g) were cooked in 400 ml of boiling water for 5 min and subsequently rinsed in cold water to prevent the overcooking of the noodles.
3.3.16.1 Texture Profile Analysis of Cooked Noodles

Cooked noodle texture characteristics such as hardness, adhesiveness, springiness and cohesiveness were measured using Texture Profile Analysis (TPA) with a Texture Analyzer, TA-XT2i (Stable Micro Systems, Surrey, UK). The TPA was calculated from the areas of the force–time curves of two compressions using a flat-end cylindrical plunger (25-mm probe) descending to 70% of the original height of the noodles. Crosshead speeds of 4.0, 1.0 and 1.0 mm/s were used for pretest, test and post test settings, respectively. Five observations were made using two cooked noodle strands (2 cm long) placed side by side each time.

3.3.17 Statistical Analysis

Statistical analysis of the experimental data was performed by SPSS 16.0 software (SPSS Inc, Chicago) using analysis of variance (ANOVA), Pearson’s correlation coefficient and multiple linear regression. Best-fit linear regression model was determined using backward variable elimination.