ELECTROPHORETIC AND IMMUNOCHEMICAL CHARACTERIZATION OF PAKISTANI WHEATS IN RELATION TO CHAPATTI AND BREAD MAKING QUALITY

By

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NATIONAL INSTITUTE OF FOOD SCIENCE AND TECHNOLOGY
UNIVERSITY OF AGRICULTURE, FAISALABAD
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2009
Dedicated
to
my Beloved Parents
&
Uncles (Ashiq Ali Khan, Abdul Salam Khan)
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(Moazzam Rafiq Khan)
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ABSTRACT

Sixteen spring wheat varieties grown during the crop year 2005-06 were characterized for their physicochemical, biochemical, technological (bread and chapatti baking) and immunochemical (ELISA) properties. The physical characteristics such as thousand kernel weight and test weight varied significantly among the wheat varieties. The wheat variety C-273 possessed the highest test weight while the Kohinoor 83 yielded significantly the lowest test weight. The other chemical constituents like moisture, ash, crude protein, crude fat, SDS-sedimentation value, pelshenke value and falling number differed significantly among wheat varieties in both whole wheat flour and straight grade flour. Similarly, a significant variation occurred in the mineral profile like calcium, copper, iron, zinc and manganese among different wheat varieties. The whole wheat flour contained higher minerals content as compared to straight grade flour. The wheat flour of Pasban-90 variety got the maximum scores for bread and chapatti making quality and GA-02 got the lowest scores. In biochemical characterization high molecular weight glutenin subunit (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) were observed in different wheat varieties. The highest number of polypeptide bands for HMW-GS were identified in wheat varieties Pak-81 (80.1kDa to 103.6kDa), Pasban-90 (75.4kDa to 100.3kDa) and Iqbal-2000 (80.8kDa to 102.9kDa). The LMW-GS represented approximately 60% of the total wheat glutenin fractions of different wheat varieties and their molecular weight ranged from 32.3kDa to 67.4kDa. The antibody response assessed through animal modeling using rabbits as test animal showed significant variation in antibody response against HMG and LMG. The antibody response against HMG varied from 0.30 to 0.70 among different wheat varieties. The maximum antibody response against HMG was observed in wheat variety Iqbal-2000 (0.70) and for LMG, the maximum antibody response (0.90) was recorded in wheat variety MH-97. The lowest antibody response was exhibited by the LMG of wheat varieties i.e. Punjab-81, C-273 and FSD-85 which was 0.55, 0.55 and 0.58 respectively. Correlation studies showed significant relationship of HMG and LMG with different quality attributes and sensory parameters of chapatti and bread. A significant and positive correlation was observed between HMG antibody response with crude protein, farinographic water absorption, SDS-sedimentation value, wet gluten and sensoric attributes of both bread and chapatti like color, flavor, texture, taste, aroma and breakability. The LMG antibody response was negatively correlated with flour characteristics and sensorial attributes but with non-significant difference. The immunochemical studies may provide a single tool for the appraisal of the end use quality of wheat varieties.
Chapter 1

INTRODUCTION

Cereals are monocots belonging to the grass family (Gramineae) and 70% of the world’s farmland has been devoted to the cultivation of these crops. Wheat, corn, rice and barley are major cereal crops grown in the world. Wheat occupies an important position among cereal grains with respect to production and utilization. Wheat, rice, maize, sugarcane and cotton are principal crops grown in Pakistan. In Pakistan major cultivated area is devoted to wheat. Wheat is the cheapest source of calories, protein, and fiber in human nutrition. In Pakistan wheat was grown on an area of 8.49 million hectares which produced 23.52 million tonnes of grains during 2007-08 crop year (GOP, 2008). Pakistan has made a significant progress for increasing the grain yield per unit area through the introduction of new high yielding genotypes and new package of production technology.

Wheat is a staple food of Pakistani inhabitants and contributes more than 60% to the total daily requirements for the calories and proteins. In the world about 65% of wheat grain is used directly as a human food, 21% as a feed for animals, 8% as a seed, and the remaining 6% goes for other industrial applications. In Pakistan about 80% of the total wheat produced is used for the production of unleavened flat bread locally known as "chapatti" and its culinary variations like "tandoori roti", "naans", "prathas" and "poories". However, 20% of the rest wheat is used for production of other bakery products such as breads, cookies, cakes and pastries etc.

The quality of wheat is generally assessed on the basis of different physical, chemical, biochemical and rheological properties. The products prepared from wheat grains require different quality characteristics. The wheat flour’s ability to
be processed into different food products is mainly determined by the quantity and quality of gluten proteins (Weegels et al., 1996). The mature wheat grains contain 8% to 20% protein. The gluten proteins constitute up to 80% to 85% of total flour proteins and are responsible for imparting elasticity and extensibility character to the dough essential for functionality of wheat flours (Shewry et al., 1995). The main constituents of the gluten are gliadins and glutenins contributing major role towards the rheological and bread making properties of the wheat flour. The unique elastic and cohesive properties of wheat dough are mainly due to glutenin, which determines the end use quality of the baked products.

The glutenins comprising about 30-40% of the flour proteins possess complex of high molecular weight proteins consisting of a number of polypeptide subunits linked through covalent and non-covalent bonds. The glutenins exhibit a broad spectrum of molecular weights varying from 40 kDa to several millions. Two major classes of glutenin subunits have been identified in wheat endosperm, the high molecular weight (HMW) glutenins (80 -130 kDa) and the low molecular weight (LMW) glutenins (10-70 kDa) (Bietz and Walls, 1980). Though HMW glutenin subunits are present in minor quantity but play a major role to determine elasticity of the gluten (Payne et al., 1980).

Three to five HMW subunits have been found in common wheat varieties. High molecular weight glutenins (HMW-GS) subunits make the dough elastic and allow them to trap the gas bubbles produced by yeast during fermentation and give rise to loaf (Cornish et al., 2006). The HMW-GS are major determinant of the bread-making characteristics of dough, but the LMW-GS also play an important role with respect to dough resistance and extensibility (Cornish et al., 2001). Twice the amount of LMW-GS has been found to be necessary to obtain the same dough resistance as achieved with HMW-GS (Wieser and Kieffer, 2001). Although considerable information on the wheat protein composition in relation to bread making quality is available but limited work has been carried out on
wheat proteins, with respect to their HMW and LMW subunits composition in relation to chapatti making quality. The differences in chapatti making quality of wheats have been correlated with the quantities of glutenin and residue proteins and found that their quantities in equal proportion have resulted in good chapatti (Shurpalekar et al., 1976).

The quality of wheat is governed by the interaction of many constituents; therefore it is difficult to assess quality of wheat by any single test. Test baking is not always possible, especially for a large number of samples or where only small amounts of whole meal are available. Therefore, there is a dire need to develop a rapid, sensitive, single test to assess the chapatti making and bread making quality of Pakistani wheat varieties. The identification of food components has been mostly carried out in the last few years by different techniques. Chromatographic and electrophoretic techniques are considered to be very useful in identification of food components (Mayer, 2005) but these methods are not considered convenient for routine testing because they are relatively costly, time consuming, and complex to perform. Immunological assay has been reported to be used as an alternative method to reduce the test time and cost (Asensio et al., 2008).

The application of immunological methods to study and compare the cereal proteins from different species actually excludes the use of various chemical and rheological tests. Immunochemical quality assessment through immunological methods could provide a vital tool in measuring functionality and suitability of different Pakistani wheat varieties for chapatti and bread making quality.

Enzyme-Linked Immunosorbent Assay (ELISA) may be an effective tool for assessing chapatti and bread making qualities of different wheats. ELISA is an immunological technique involving an enzyme (a protein that catalyzes a biochemical reaction) to detect the presence of an antibody or an antigen in a test sample. Two most used variants of ELISA are the indirect and the sandwich
ELISA. The indirect ELISA utilizes two antibodies, one of which is specific to the antigen and the other of which is coupled to an enzyme. This second antibody gives the assay its “enzyme-linked” name, and will cause a chromogenic or fluorogenic substrate to produce a signal. The detection antibody can be coupled to an enzyme or can bind the conjugate (enzyme-linked antibody) that will produce the biochemical reaction (Goldsby et al., 2003). Both polyclonal and monoclonal antibodies (MAbs) can be used in ELISA techniques for identification of food components. Polyclonal antibodies offer a number of benefits such as recognition of a mixture of different epitopes of the antigens, more tolerance to small changes in the nature of antigen, like polymerization or slight denaturation and they are a preferred choice for detection of denatured proteins.

The food authenticity can be carried out through ELISA test which is used for the identification of meat and meat based products, fish and seafood, milk and dairy products, and other foods (Matsunaga et al., 1999; Liu et al., 2006). The use of ELISA has also been reported for the identification of LMW and HMW subunits of glutenins associated with bread-making quality of wheat flours (Brett et al., 1993). ELISA method has not still been reported for assessing chapatti and bread making quality of Pakistani wheat varieties. The wheat varieties developed in Pakistan need to be tested for the electrophoretic and immunochemical characteristics in order to assess their effect on the bread and chapatti making qualities.

Therefore, the present study was designed to assess the biochemical and immunochemical characteristics of wheat varieties grown in Pakistan in relation to the chapatti and bread making quality.
The objectives of the research study were:

- To evaluate wheat varieties grown in Pakistan for physico-chemical, HMW-GS and LMW-GS composition.
- To develop an indirect ELISA for HMW and LMW glutenin sub-units.
- To find out relationship of HMW and LMW glutenin sub-units and immunological test with chapatti and bread making quality.
Chapter 2

REVIEW OF LITERATURE

Wheat (*Triticum aestivum*) is ranked as a staple food for the people of all over the world. The varietal trials are common to develop new wheat varieties and maintaining the vigor of the old ones. Many tools are used to characterize wheat on the basis of their set criterion. The quality of cereal based products is often dependent on the quality and molecular chemistry of its proteins. ELISA represents domain of recent investigations mainly attempted to characterize wheat varieties on the basis of its antibody response. The research has been carried out on various quality attributes of wheat by using different techniques but the literature more pertinent to the present study is reviewed under the following headings:

2.1 Wheat quality

2.2 Factors affecting wheat quality

2.3 Physical characteristics

2.4 Chemical characteristics

2.5 Rheological properties

2.6 Wheat proteins

2.7 High molecular weight glutenin subunits (HMW-GS); relation to bread and chapatti quality

2.8 Low molecular weight glutenin subunits (LMW-GS); relation to bread and chapatti quality

2.9 Immunochemical characteristics
2.1 WHEAT QUALITY

The quality of wheat is affected by many intrinsic and extrinsic factors. The wheat quality is a result of the cumulative effects of soil, climate and seed stock in the wheat plant and its kernel component. The term wheat quality is a complex of many factors and cannot be defined in terms of a single attribute but depends on several milling, chemical, baking and rheological dough properties.

The quality of wheat has different meanings depending on its intended use and same applied when deciding the quality of wheat. The criteria of wheat quality varies with its varying usage and suitability of wheat for one specific purpose may not fulfil the demand or producing un-satisfactory results for another use (Halversion and Zeleney, 1988).

The wheat quality has been defined simply by Finney et al., (1987) as in terms of its suitability for a particular purpose or use. Wheat desirable for a specific use or purpose has good quality and that is not desirable has poor quality. Likewise, Kent and Evers (1994) have also explained in detail about different meanings of the wheat quality. They reported that wheat passes through many hands from field to table; all those who handle it are interested in the quality of cereals, but in different ways. The grower requires good cropping and high grain yields. The miller requires wheat grains of good milling quality-fit for storage, and capable of producing the maximum yield of flour suitable for a particular purpose. The baker requires wheat flour suitable for baking e.g., bread, biscuits or cakes. He also wants his flour to yield the maximum quantity of goods which meet rigid specifications, and therefore, requires consistency in the quality of raw material. The consumer reliance is set on sensorial attributes of the final product and demand for better palatability and good appearance in the baked goods with high nutritive value at reasonable price.

The requirements of wheat grain quality are different for the major baked products such as bread, pastries and cookies and also within each of the types of
these baked products. The overall bread making quality of wheat depends on several factors such as water absorption, loaf volume, internal and external loaf characteristics and tolerance to mixing and fermentation and these characteristics are correlated to the physical and chemical characteristics of wheat flour and dough (Misra and Gupta, 1995). The physical characters include grain appearance score, kernel hardness, vitreousness of kernel, kernel weight, test weight, kernel size and shape. The chemical characters include protein content, protein quality (gluten content), sedimentation test and other tests such as farinograph, extensograph, mixograph and alveograph which can asses the mixing or visco-elastic properties of the dough (Bushuk, 1985; Faridi, 1985; Menjivar, 1990). Other bread making quality tests like the Pelshenke dough ball test and the SDS (sodium dodecyl sulphate) sedimentation test can also provide valuable information about the baking quality of wheat.

Cornell and Hovelling (1998) were of the view that wheat quality could be usually assessed on the basis of the ability of the wheat flour to produce a high standard loaf of bread, (yeast leavened bread). They further reported that wheat quality is influenced both by genetic and agronomic practices and its assessment can be divided into tests based on physical and chemical criterion.

The important quality attributes for describing wheat quality are flour extraction rate, protein content, amylase activity and etc. which are affected by varieties, environments in which they are grown and the environment x genotype interactions (Souza et al., 2004).

A number of research workers have associated cooking quality of pasta with protein content, gluten composition and solubility, farinograph mixing characteristics, SDS-sedimentation volume and mixograph characteristics (Matsuo et al., 1982; Dexter et al., 1985). However, the kernel hardness and gluten proteins are also the two major determinants of the wheat quality (Tipples et al., 1994; Pena, 1997). Protein quality is again dependent upon glutenin and gliadin
contents and their respective proportionality (Singh et al., 1990; Sontag-Strohm et al., 1996). Molecular basis of glutenin sub-units is important in determining the wheat quality in terms of its end use (Southan and MacRitchie, 1999).

Cox et al. (1988) and Pates (1988) reported that in mid eightes a concern was generated in the baking industry regarding a decline in end use quality of wheat due to the improvement in grain yield achieved in hard red winter wheat. However, this concern was ruled out by Cox et al., (1989) who concluded in a research study that any decline and deterioration in the quality of hard red winter wheat perceived by the baking industry has been caused by the non genetic factors such as changes in environment, milling practices and commercial baking methods and formulations or a combination of these factors.

The wheat quality evaluation by the use of analytical methods may determine both the quality and quantity of wheat proteins using high-resolution techniques such as: SDS-PAGE (Payne et al., 1979, 1981), low pH PAGE (Damidaux et al., 1978; Kosmolak et al., 1980) and RP-HPLC (Bietz, 1983). However Tronsm et al., (2002) explicited that baking tests are more elaborative in explaining the end use of quality of wheat and among loaf volume is of major importance.

2.2 FACTORS AFFECTING WHEAT QUALITY

Variety is an important factor influencing the wheat grain quality; generally wheat is marketed according to the class where as each class consists of group of varieties with similar characteristics and is suited for similar purposes/ end use (Halverson and Zeleny, 1988). The wheat grain quality have been broadly classified in to two groups; physical characteristics such as vitreousness (correlated with hardness of grains, which indirectly is an index of protein and gluten content), color (two basic colors red or white used for wheat grading purposes), grain weight (function of grain size and density and more reliable to estimate the flour yield), grain size, and shape (closely related to grain weight and affect the flour yield), grain hardness (major factor for grain quality and
used in differentiating hard and soft classes of wheat) and chemical characteristics such as moisture content (effects keeping quality of wheat), protein content (major determinant of wheat quality, higher the protein higher the price of the wheat), amylase activity (negative effect on bread making quality), crude fiber and ash content (inversely related to the flour yield and are related to amount of bran in wheat grain). Other factors like class, environment i.e. climate, soil and cultural practices also affect the composition of the wheat grain (Gaines et al., 1996). These all quality factors are influenced by genetic makeup, environment and cropping patterns. Both genotype and environment greatly influenced Chinese steam bread quality (Liming et al., (1991). Peterson et al., (1992) also reported significant effect of genotype, environment, and interaction effects on the quality of all parameters. They concluded that environmental influences on end-use quality attributes should have an important consideration in cultivar improvement efforts toward enhancing marketing quality of hard red winter wheat. Millet and Pinthus (1983) observed the changes in weight, volume, water content and anatomical structure of grains for three heavy grained and three light grained cultivars of spring wheat throughout the period from grain set until ripening. They found that grain weight was significantly affected as a function of dry matter accumulation and increase in grain volume.

Lill and Purchase (1995) noted a larger influence of cultivars relative to the year for test weight and grain protein content. The variance ratio of cultivar to cultivar by year interaction indicated that cultivar effects accounted for most of the variability in grain protein yield, sedimentation volume, flour yield, falling number, mixograph dough development time, farinograph dough development time, farinograph stability and baking strength index. Susceptibility to interaction with year effects was observed for yield, starch yield, flour color, flour protein content and farinograph water absorption.
Faridi et al., (1990b) reported that individual farming practices also influence the quality of wheat and flour. Planting time of wheat can alter disease susceptibility, which in turn affects the wheat quality. If wheat is harvested at too high moisture content, incipient seed germination can result and may be detrimental to the quality of some cookies and crackers. Peltonen and Virtanen (1994) found that application of nitrogen fertilizer improved bread making quality of low molecular weight proteins *i.e.* gliadins.

The major storage proteins in wheat are the gliadins and glutenins. Gliadins are subdivided into α, β, and ω units and glutenins into low and high molecular weight sub units (LMW-GS and HMW-GS). (Shewry et al., 1989; Halford et al., 1992; Gupta and MacRitchie, 1994; Weegels et al., 1996; Martinant et al., 1998) also explained the genetic control and the relationships between gluten protein composition and quality characteristics.

Pechanek et al., (1997) reported that application of high amount of nitrogen led generally to a significant increase in total protein content of grain. As total protein content increased, the ratio of low molecular weight (LMW) to high molecular weight (HMW) glutenins decreased consistently, i.e., in all varieties, in both years and locations. Changes of LMW: HMW ratio showed a significant negative correlation to sedimentation value and bread loaf volume. These results suggested that ratio of HMW glutenin, especially x-type subunits, to total protein content could be the best early detectable parameter with high predictive value for bread making quality. Verbruggen et al., (2001) reported that addition and incorporation of HMW-GS increased maximum resistance (MR) and extensibility (EX) when LMW-GS and HMW-GS for dough extensibility were evaluated. The addition of glutenin subunits can be partially incorporated into the glutenin network in the presence of oxygen.

Overall, significant variation has been observed due to the effects of genotypes, environment and their interaction in different research investigations;
milling (Gaines et al., 1996), protein content (Peterson et al., 1992; Subda, 1991; Huebner et al., 1995; Bergman et al., 1998) and baking quality (Hou et al., 1996; Peterson et al., 1992; Gaines et al., 1996; Peterson et al., 1998). Gaines et al., (1996) have also reported a strong effect of environment on grain conditions, which ultimately influence the milling and baking characteristics of wheat flour. Blumenthal et al., (1991) attributed seasonal changes in wheat grain quality to be associated with high temperature during grain filling stage.

The improper handling at milling operations could also results in loss of wheat quality as errors such as sampling, incomplete removal of foreign material, inconsistent or inappropriate tempering, variation in ambient conditions, condition of milling equipment, improper mill settings, and inadequate standardization of procedures could results in valuable loss in wheat quality (Dexter and Tipples 1987).

Tempering at level of higher moisture contents could decrease the flour extraction rate and ash contents while improving the flour color (Butcher and Stenvert 1973, Hook et al., 1982a). The variation in setting appropriate moisture contents for milling depends on the type of wheat as hard wheats require higher tempering moisture and tempering time to reach prime milling conditions (Hook et al., 1982c, Stenvert and Kingswood 1977b). Habernicht et al., (2002) found negative effect on end use quality of hard red and hard white spring wheats contaminated with grain of contrasting classes. Crowley et al., (2000) described that the bread types could be distinguished by crumb grain characteristics. Sivri et al., (2001) concluded that pre harvest bug damage to wheat can cause significant losses in bread making quality which frequently occurs in most countries of Middle East.

Baking quality of wheat is primarily affected by storage proteins (i.e. gliadins and glutenins) and both qualitative and quantitative characteristics of
these proteins are considered when attempting to explain the quality variation observed among different wheat cultivars (Pomeranz, 1990).

2.3 PHYSICAL CHARACTERISTICS

Wheat is often assigned a numerical scale, which depends upon the results of certain tests comprising test weight, kernel weight, percentage of damaged kernels and the percentage of foreign matters and dockage which are usually used in wheat grading systems. The two important physical parameters are briefly reviewed here.

2.3.1 Test weight

Halverson and Zeleney (1988) demonstrated that physical tests and observations regarding wheat actually described some of the characteristics. The weight of wheat grains per unit volume or test weight is one of the simplest and most widely used criterions of wheat quality. Kernel weight is a function of both kernel size and kernel density. Though kernel size is an inheritance characteristic but it is also affected by growing conditions (Williams et al., 1986; Halverson and Zeleney, 1988).

Monsalve-Gonzalez and Pomeranz (1993) have reported that the test weight determines the milling properties such as flour yield and milling score. Hlynka and Bushuk (1959) found that kernel shape, and uniformity of kernel size and shape are important factors affecting test weight besides other important factor influencing the test weight is the density of the wheat grain. Shuey (1960) found positive correlation coefficient of 0.744 between test weight and flour yield. He was further of the view that though this relationship is significant but the test weight can not be considered a highly accurate or reliable predictor of flour yield. However, latter on Schuler et al., (1995) described that test weight was not correlated with flour yield, but was significantly correlated with flour protein content (r=0.54 P< 0.05) as was kernel density (r=0.49). Hook (1984) concluded that correlations between test weight and flour yield were poor. Marshall et al., (1986) found that the
relationship between test weight and milling yield was dependent on both the site and variety used, and therefore was not a reliable predictor of milling yield. There are conflicts in statements about the dependability of test weight to assess milling quality i.e. flour yield (Altaf et al., 1969; Hook, 1984; Finney et al., 1987). Donelson et al., (2002) rapidly measured the specific gravity (cm$^3$g$^{-1}$) of small (20 and 40g) samples of soft wheats and compared with bulk density (g cm$^{-3}$) measurements (micro test weights).

2.3.2 Thousand kernel weight

Thousand grain weight is the weight in grams of 1000 kernels of wheat. Since it is not volume based it is independent of some factors influencing bulk density, and may be preferred as a measure of grain quality. Wheat kernels can be classified according to grain weight as 15-25 g (very small), 26-35 g (small), 36-45 g (medium), 46-55 g (large) and over 55 g (very large) (William et al., 1986). The thousand kernel weight varied from 42.4 to 48.7g in 128 wheat varieties (Zenetti et al., 2001) while Anjum et al., (2002) found the kernel weight range from 31.43 to 37.28 g in different Pakistani wheat varieties. Thousand kernel weight is a component of grain yield along with spikes per unit area and number of kernels per spike. Kernel weight is determined by electronic seed counter and balances which are considered to be more reliable guide of flour yield than test weight. Kernel weight generally varies from 20- 45 grams per 1000 kernels, based on the type of wheat i.e. hard or soft grains. Schuler et al (1995) have shown that thousand kernel weight had no relation to milling qualities of soft red winter wheat. Baril (1992) reported that thousand kernel weight within a genotype was positively correlated with agronomic yield.

2.4 CHEMICAL CHARACTERISTICS

2.4.1 Moisture content

The moisture content is one of the most important factors for the determination of wheat grain quality. Moisture content is inversely related to the
dry matter of grain and has more effect on keeping quality of wheat as dry and sound wheat grains that can be kept for years when it is stored properly but wet wheat grains with higher moisture content may deteriorate faster in few days (Gooding and Davies, 1997). Storage of wheat grains with high or extremely low moisture contents often contributes towards losses in its quality. Usually, the moisture content of flour could vary from 11 to 15% depending upon the storage conditions and hygroscopic nature of the starch (Whitetlay, 1970). Various researchers have shown that the moisture content varied from 8.19-11.94 % (Ahmad et al., 2001; Butt et al., 2001; Hruskova and Famera, 2003). Cornell and Hoving (1998) have demonstrated that moisture contents of wheat are not only of economic significance but are also important with regard to the keeping qualities of wheat. They further stated that wheat of very low moisture is brittle and of high moisture content (>13.5%) has a tough character. The moisture content can be determined by using oven drying method (gravimetrically), but now more common method is to use a calibrated infra-red spectrophotometer (NIR) machine (Misra and Gupta, 1995; Gooding and Davies, 1997; Dowell et al., 2002).

2.4.2 Ash content

The purity of flour is assessed by the amount of ash in flour. Ideally the ash content in flour should range between 0.40-0.45%. A combination of high extraction and low ash is an indicator of efficient milling of wheat. The ash content may vary from 0.48-0.54% in wheat milled through short milling system by the Quadrumate Junior Mill. However, combination of 70% extraction flour with 0.48% ash would indicate efficient milling and good millability of the wheat. The Quadrumate can give an excellent 72-73% extraction of flour. The ash content ranged from 0.6 to 0.8% for extractions of 80% or more in Middle East flour (Williams et al., 1986). The ash content reported by various researchers varied from 0.27 to 0.40% (Yamamoto et al., 1996); 1.08 to 1.85 % (Ahmad et al., 2001; Butt et al., 2001).
2.4.3 Protein content

The protein content and kernel hardness has been reported to be the best suited classification tools to classify the wheats into hard red spring and hard red winter wheats (Slaughter et al., 1992). The dough containing more protein content expanded at a faster rate than those containing less protein contents, during proofing for expanded period. The differences in dough expansion rate are attributed to the effect of flour protein on dough extensibility (He and Hoseney, 1992).

The protein content is not only a factor assessing the end use property but it can also influence the baking properties of both hard wheat flour (Orth and Bushuk, 1972) and soft white wheat flour (Kaldy and Rubenthaler, 1987). Wheats having high protein content (hard wheat) get premium price in the market as they are useful in blending with low protein wheat (soft wheat) flours for bread production. Flours of low protein content wheats are useful in making other different products like cakes, cookies and biscuits (Hoseney et al., 1988). The protein content has been found to be correlated with the gluten content (Subda and Bishkupshi, 1979; Kulkarni et al., 1987; Anjum and Walker, 2000).

Butt et al., (1997) evaluated thirty wheat varieties and found that moisture content, crude protein, crude fibre and ash content differed significantly (P ≤ 0.01) among the wheat varieties. The protein, fiber and ash contents varied from 10.74 to 13.16%, 2.16 to 2.63% and 1.14 to 1.61%, respectively during 1993-94 crop years and for the crop year 1994-95 these varied between 10.82 to 13.23%, 2.09 to 2.98% and 1.03 to 1.64%, respectively

Ahmad (2001) found that the moisture, ash, crude protein, crude fat, crude fibre, nitrogen free extract, wet gluten and dry gluten ranged from 9.38 to 10.43%, 1.32 to 1.85%, 10.13 to 14.74%, 1.96 to 2.52%, 2.31 to 2.99%, 78.71 to 85.37%, 23.53 to 38.71% and 7.51 to 13.52%, respectively among wheat varieties. In Pakistan the protein content ranged from 10.43 to 14.74% in different wheat varieties grown
under identical conditions (Ahmad et al., 2001; Butt et al., 2004; Anjum et al., 2005).

2.4.4 Wet and Dry gluten

The gluten content is an important parameter in assessing the quality of wheat flour (Grabski et al., 1979; Kulkarni et al., 1987). The flour quality is mainly affected by the nature of the gluten and its various components. The gluten is formed due to the interaction of the glutenin and gliadin protein fractions, which is also associated with pentosans during dough formation (D Appolonia and Kim, 1976; Hoseney, 1986). The term ‘gluten’ refers to the proteins, because they play a key role in determining the unique baking quality of wheat by conferring water absorption capacity, cohesiveness, viscosity and elasticity on dough. The gluten, roughly comprising 78 to 85% of total wheat endosperm protein, is a very large complex composed mainly of polymeric (multiple polypeptide chains) and monomeric (single chain polypeptides) proteins known as glutenins and gliadins, respectively (Wieser et al., 2006). Zeleny and SDS-sedimentation tests are currently and most widely employed as rapid tests to screen early generation wheat lines for bread making quality (Weegels et al., 1996). The weak dough with an extensive gluten network is suitable for bread making (Pomeranz, 1988) whereas weak dough without an extensive gluten network is suitable for cookies and cakes production (Gaines, 1990).

The gluten has also been found to significantly affect the baking quality of wheat flour (Orth and Bushuk, 1972; Kaldy and Rubenthaler, 1987; Kent and Evers, 1994). The higher molecular weight glutenins exhibited positive correlation with excessive mixing time requirement (Huebner, 1970; Wall et al., 1971; Khan et al., 1989). The mixing strength is correlated with dough stability (Hamada et al., 1982; Bietz, 1986).

The gluten has a correlation with total protein, albumins and insoluble protein contents. The dough development time increased with increase in total
and true soluble pentosans and high activity of proteolytic enzyme. The dough stability also showed an increase with increase in gluten content and proteolytic enzyme activity.

The approach to wheat protein quality is based on considering potential end product. Gluten quality varies and is based on varietal characteristics. The wet and dry gluten contents vary widely among the wheat varieties (Paliwal and Singh, 1985). Paliwal and Singh (1985) reported variation in flour yield from 55.48 to 72.56%, wet gluten 12.77 to 44.06 %, ash 0.39 to 0.78 %, crude fibre 0.27 to 0.97 %, sedimentation value 17 to 35.3 ml, damaged starch 2.8 to 5.7 % and pelshenke value 82-133 minutes for different wheat varieties. Specific volume of breads prepared from 100 gm of white flour of different wheat varieties varied from 2.93 to 3.75 cc g⁻¹.

2.4.5 SDS-sedimentation test

The sedimentation test is a measure of the strength of wheat and depends on the degree of hydration of the proteins and their degree of oxidation. The addition of the mild detergent, sodium dodecyl sulphate (SDS) facilitates the hydration of proteins because the gluten molecules are associated with oil bound molecules. For this test ground flour sample is shaken in the presence of lactic acid and SDS (William et al., 1986). The proteins with good hydration capacity and good oxidation status are obtained from a stable suspension and the height of suspension has linear relationship with the strength and baking potential of the wheat. Sedimentation volume is a measure of baking quality of the flour protein (Zeleny, 1947). The higher the SDS value, the higher the potential baking strength of wheat flour (William et al., 1986). The SDS sedimentation value possesses the greatest potential as a screening test because of its small sample size, high thorough put, good correlation with loaf properties, growing sites and genetic differences in protein quality. Therefore SDS may be directly related to bread making potential (Blackman and Gill, 1980).
2.4.6 Falling number

Falling Number test measures the α-amylase activity. Falling Number test is applicable for flour (Hagberg, 1961). α-amylase is an inherent enzyme of wheat which converts starch into simple sugars (Bloskma, 1990). Falling Number value is critical for final product because there is direct relationship between α-amylase activity and finished product attributes e.g. bread crumb quality and loaf volume (Perten, 1964). α-amylase is an inherent enzyme of wheat which converts into simple sugars (Bloskma, 1990). Proper amount of α-amylase (FN=250) in the flour is desirable for proper baking to occur (Waldet, 1968). Falling Number value of greater than 250 is generally acceptable for bread making. Adequate α-amylase activity in flour results high volume bread with firm and soft texture (Muller, 1973). Flour having high α-amylase activity (low FN) requires less amount of water for mixing, softens the dough, weakens the bread structure and produces a soft sticky crumb having low loaf volume (Cauvain and Young, 2001). On the other hand, excessive amylase activity (low FN) converts more starch into dextrin and gummy substances during cooking and makes bread sticky and unattractive (Zeleny et al., 1963). Excessive α-amylase activity (low FN) results in the formation of darkened loaf crust as a result of sugar caramalization and sticky crumb structure which causes problems during slicing (Gooding and Davies, 1997). Millers prefer to avoid wheat with excessive α-amylase activity (low FN) (Muller, 1973). High α-amylase activity reduces the water holding capacity of the flour and weakens the bread crumb (Pyler, 1988). Anjum and Walker (2000) observed Falling number values exceeding 400 seconds and high starch paste viscosities as determined by RVA indicated low α-amylase activity were possessed by Pakistani wheat cultivars. Bread-baking quality was found to be better in some Pakistani wheat cultivars (Pak 81 and Faisalabad 85) than other wheat cultivars.
2.5 RHEOLOGICAL CHARACTERISTICS

The rheology of wheat flour dough is influenced by the wheat proteins. The physical dough properties, especially those coupled with dough baking properties are mainly assessed by the glutenin proteins (Fowler and De La Roche, 1975). The fitness of wheat flour for making products like chapattis, bread and biscuits depends largely on the particular rheological dough properties such as stability, extensibility, development time etc. The rheological properties of the dough are determined by farinograph, mixograph, extensograph etc (Austin and Ram, 1971).

The water absorption capacity is the most important physical parameter affecting the farinogram, and is a function of the wheat flour protein content and quality (Finney et al., 1987). The absorption is the amount of water required to counter the farinograph curve on the 500-Brabender Unit line for dough (Shuey, 1984). The flour with higher water absorption gives more favorable end products because it improves the texture and grain of the bread (Simon, 1987).

Anjum (1991) observed that some Pakistani wheats are more stable than U.S. wheats and farinographic characteristics of Pakistani wheat Barani 83 showed a significantly longer mixing time and stability over U.S wheat. Faisalabad 83 was found to have weak gluten quality characteristics as indicated by farinographic studies. The low protein wheats (less than 12%) require long mixing time and certain chemical agents particularly reducing agents lead to shorter the mixing time. The lower pH gives lower while high pH gives higher mixing time (Hoseney, 1986). D. Appolonia (1984) reported that farinograph curve characteristics for any given wheat cultivar changes from location to location. The weather and soil conditions affect the protein content and wheat quality and indirectly the shape of the farinographic curve. Direct correlation between flour protein and mixing strength does not always exist. Kunerth and D. Appolonia (1985) evaluated over 240 hard red spring wheats and reported little
or no relationship between wheat protein content and peak mixing time, dough stability and mixing tolerance index.

2.6 WHEAT PROTEINS

The wheat proteins are complex in nature but possess unique characteristics which are often attributed to them (Weegels et al., 1996). The most of technological properties related to dough making and baking are dependent upon the wheat proteins. The flour baking quality is also directly related to the type of proteins (Shewry and Halford, 2002).

The wheat contains four types of proteins that include albumins (soluble in water and dilute buffers), globulins (not soluble in water but soluble in saline solutions), prolamins (soluble in 70–90% ethanol), and glutenins (soluble in dilute acid or alkali). Most of the albumin proteins identified from proteomic analyses of mature grain endosperm of wheat (Singh et al., 2001) and barley (Hordeum vulgare L.) (Finnie et al., 2002) belong to a family of α-amylase/trypsin inhibitors. The gliadin and glutenin protein fractions consist of storage proteins and are defined as proteins that accumulate during the grain-filling period (Shewry and Halford, 2002).

The gliadins are heterogeneous mixtures of single chained polypeptides which are, in their native, soluble in aqueous alcohol. The gliadins can be separated into four groups, denoted as α, β and ω-gliadins, when fractionated through polyacrylamide gel electrophoresis at low pH. They are important in intramolecular disulfide bonds formation that also renders their stability against aggregate formation. Therefore, they are considered to be less critical for the bread making potential of wheat flour (Dubcovsky et al., 1997). The gliadin has a good extensibility but lacking in elasticity which is a fundamental requirement in dough making process (Cheftal et al., 1985). On the other hand, glutenins are large molecular weight proteins (several millions) which are multi-chained (Graveland et al., 1985). They are elastic in nature, blending of both these proteins
in the dough brings a specific elasticity and extensibility which can then be used in the processing of different flour products. Wall (1979) pointed out the relationship between gluten proteins and elasticity of dough which also determines the suitability of dough for bread making. The ratio of glutenin and gliadin influences the viscoelasticity of the gluten mass (Janssen et al., 1991) and is further used to correlate the quality of bread or chapatti prepared from different wheat cultivars (Ram and Nigam, 1981; Uthayakumaran et al., 1999).

MacRitchie (1980) delineated that the differences in baking quality can be attributed to the whole gluten protein or to a specific protein fraction. Graveland et al. (1982) reported that flour of good bread making variety Sicco possessed 45% glutenins and 11% glutelins compared with 38% glutenins and 18% glutelins for flour of poor bread making variety Tundra. Graybosch and Morris (1990) reported that correlations between end use quality characteristics (associated with substituted chromosomes) and endosperm storage proteins are reliable only when proteins of both gliadin and glutenin fraction are analysed. Bunce et al., (1985) isolated gluten by washing method and reported its composition on dry matter as gliadin 43%, glutenin 39% other proteins 4.4%, lipid 2.8%, sugars 2.1%, starch 6.4% with some cellulose and mineral matters.

The wheat quality is assessed by the molecular structure of the wheat storage proteins which, in turn, control the interactions of the proteins during the bread making process (Bushuk, 1998; Shewry et al, 1999). The bread making quality is significantly altered by variation in composition as well as protein content of wheat flour (Weegels et al., 1996; Lafiandra et al., 1999; Branlard et al., 2001).

Wang and Kovacs (2002) stated that molecular weight distribution of wheat proteins was primarily responsible for the viscoelastic properties of flour dough and furthermore high molecular weight glutenin (SDS insoluble protein) plays the major role. The glutenin proteins (polymeric proteins) are among the
largest protein molecules in nature (Wrigley, 1996) with molecular weight of glutenin polymers reaching over twenty million Daltons. These proteins are heterogenous mixtures of polymers formed by disulphide bonded linkages of polypeptides (Payne et al., 1979; Payne et al., 1985; Thompson et al., 1994).

Lawrence and Payne (1983) reported that glutenin fraction of hexaploid (Triticum aestivum L.) makes up approximately 40% of the total seed protein and this fraction plays an important role in determining the strength and elasticity of wheat flour dough because it contains protein aggregates of high molecular weight (up to several million) formed by the association of a number of constituent polypeptide chains.

Ohm and Chung (1999) concluded that gluten contents and hydration amounts had significant correlations with water absorption and have dominant role in bread making. Among the major gluten proteins, gliadin shows higher surface activity than glutenins under acidic conditions. Glutenins are likely to have been adsorbed more tightly than gliadins (Takeda et al., 2001).

Many attempts to reveal the structure of the gluten proteins have been carried out, although they have been troubled by the low solubility and lack of crystallinity of the proteins. The solubility properties of gluten proteins are determined by the primary structures of the individual proteins and their interactions by non-covalent forces (notably hydrogen bonds and hydrophobic interactions) (Belton et al., 1998) and by covalent disulphide bonds (Shewry et al., 2002). The whole protein structure is still far from being clear (Veraverbeke and Delcour, 2002). The mature wheat grains contain 8-20% proteins. The gluten proteins, the gliadins and glutenins, constitute up to 80-85% of total flour protein, and confer properties of elasticity and extensibility that are essential for functionality of wheat flours (Shewry et al., 1995). The gliadins and glutenins constitute each around 50% of the gluten proteins. Osborne (1907) was the first to classify wheat grain proteins on the basis of their solubility: albumins (soluble in
water), globulins (salt), gliadins (aqueous water) and glutenins (dilute acid or alkali). Due to findings of the Osborne fractions being heterogeneous and containing protein types overlapping each other, the methods of protein fractionation have been improved now a day (MacRitchie et al., 1990). Now the protein classifying system is based on biological characteristics of the proteins together with their chemical and genetic relationship, leading to different states of aggregation in dissociating solutions (Shewry and Tatham, 1990; Shewry et al., 1986). Thus, gliadins are a mixture of monomeric polypeptides and glutenins consist of polypeptides aggregated by disulphide bonds (Shewry and Tatham, 1990; Singh and MacRitchie, 2001a). The glutenin fraction is formed of a mixture of polymers, high-molecular weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS). The large glutenin polymers are stabilized by inter-chain disulphide bonds (Field et al., 1983).

2.7. HIGH MOLECULAR WEIGHT GLUTENIN SUBUNITS (HMW-GS); RELATION TO BREAD AND CHAPATTI QUALITY

The high molecular weight glutenin subunits (HMW-GS) account for 5-10% of the total protein (Payne, 1986). High molecular weight glutenin subunits (HMW-GS) are minor components in terms of quality; and are major determinants of gluten elasticity (Tatham et al., 1985). HMW-GS are closely associated with bread making quality (Bietz and Wall, 1973).

The HMW-GS have molecular weight ranging from 80-160,000 KDa as evident from SDS-PAGE (Payne et al., 1980). Their polypeptides with a molecular mass of less than 60,000 KDa containing glutenins were found to be large polymers linked by disulfide bonds or by non-covalent associations between LMW-GS and HMW-GS. The three to six HMW-GS (Margiotta et al., 1996; Payne and Corfield, 1979) are recognized in hexaploid wheat (Lew et al., 1992).

Bietz et al., (1975) conducted single kernel analysis of glutenin through SDS-PAGE and reported the five highest molecular weigh subunits (1:133000 mol wt;
2:104000 mol wt; 3:93000 mol wt; 4:86000 mol wt and 5:68000 mol wt) coded by chromosomes 1B (subunits 2 and 3), 1D (subunits 1 and 4), and 4D (subunit 5). They concluded that most of the tetraploid wheat lack glutenin subunits 1, 4 and 5 but only subunit 4 was consistently absent and in many varieties considerable variability also occurred. Hexaploid wheat varieties, however, were more uniform in glutenin subunit composition than tetraploid wheats. Payne and Lawrence (1983) reported that there was a considerable variation in the pattern of HMW glutenin subunits due to the presence of different alleles at each for three gene loci (Glu A1, Glu B1 and Glu D1) which control their synthesis.

High molecular weight glutenin sub units make dough elastic and allow them to trap the gas bubbles produced by yeast and to rise which are important in improving quality of final produce (Cornish et al., 2006). Cultivars of high bread making quality have high proportions of these high molecular glutenin polymers of proteins (Huebner and Wall 1976; Bottomely, 1982). Application of modern techniques like genetic engineering has been used to change the levels of these proteins and to check their utility recently field trials were conducted in Idaho and California (Bregitzer et al., 2006) and they concluded that bread-making performance has also been related to the glutenin polymer size distribution.

Yahata et al., (2006) quantified and observed significant correlation of HMW-GS in wheat mill streams with the SDS sedimentation volume and the mixing properties, which are respective indices of specific loaf volume and dough strength. Further work in this area has suggested the role of specific HMW subunit in contribution of glutenin to bread-making quality. Payne and others (1979) observed a direct relationship between bread making and proportion of subunit Glu-1Ax1. Anjum et al., (2000b) studied high molecular weight glutenin subunit composition of Pakistani hard white spring wheat grown at 3 locations for 2 years and found significant correlations between
certain HMW glutenin subunits and some quality attributes such as protein, farinograph dough development time, farinograph water absorption, loaf volume, and mixograph peak height (Anjum, 1991; Anjum et al., 2000a). Several studies have shown that amount and relative proportion of HMW subunit is an important factor in functional performance for bread-making quality (Kolster et al., 1992; Machylo et al., 1992b; Kolster and Vereijken, 1993).

The intrinsic viscosities of glutenins have shown to be related with bread making quality (Ewart, 1968). For the past 10 to 15 years, much of the emphasis was on defining the molecular basis of bread-making quality in relation of specific polypeptides of the gluten protein complex, especially HMW subunits of glutenin (Payne and others 1984a; MacRitchie and others 1990). In order to correlate the bread-making potential with HMW subunit composition, 2 statistical approaches have been used. Based on the SDS-sedimentation volume, Payne (1987b) assigned quality score to each subunit and then individual values were summed to calculate the total quality score for a wheat cultivar, with maximum score of 10. In another approach, Ng and Bushuk (1988) developed an equation for predicting the unit loaf volume, based on HMW subunit composition of a bread wheat cultivar.

Wieser and Kieffer (2001) demonstrated that correlations of the amount of gluten protein types of 14 cultivars of wheat flour determined on a micro-scale indicated that the maximum resistance of dough and gluten and the gluten index were strongly dependent on the quality of glutenin subunits (GS) in flour. Among HMW-GS, the contribution of X-type GS were more important than those of Y-type GS. The extensibility of dough and gluten was mainly dependent on the ratio of gliadin to total glutenin subunits, to HMW-GS and LMW-GS. Dough development time showed the highest correlation with total HMW-GS. Bread volume was influenced by the total amount of gluten protein more than by the amount of protein in different groups or of different types, probably because
of rather low range of flour protein contents (8.7-12.0%) within the set studied. Andrews and Skerritt (1996) found that dough strength characteristics (e.g. extensograph, maximum, resistance, farinograph, development time) have been dominantly correlated with HMW-GS content and HMW-GS composition.

Sontag-strjuuti (1997) studied the relationship between baking quality and HMW and LMW glutenin subunit and gliadin alleles on group 1 chromosomes in 75 spring wheats grown in Finland. They reported 80% of the spring wheats contained HMW glutenin subunit allele Glu-(5+10), which was associated with higher Pelsheinke test values than allele Glu-(2+12). Ivanov et al., (1998b) reported significant correlations between Glu-1 quality score and some qualitative features. HMW glutenin subunits 5+10 controlled by Glu-D1 locus demonstrated the highest effect on qualitative characteristics, followed by subunit 2* controlled by Glu-A1 locus in progenies of wheat crosses. Branlard and Dardevet (1985) determined protein quality in each of the bands of 70 wheat cultivars for HMW glutenin subunits composition by SDS-PAGE using densitometry and simply correlated with protein of each of HMW glutenin subunits and various flour quality characteristics, alveograph keeping in view strength, tenacity, swelling and extensibility. All of the characteristics were correlated with at least 4 subunits. Subunits 6 and 8 were the only subunits not correlated significantly with at least one of the characteristics. Subunits 17 and 18 remained favourably correlated significantly with swelling. They suggested that HMW glutenin subunits interact in producing the rheological characteristics of the dough and strength of wheat by can be improved by using wheat with bands positive for high strength and extensibility. Lorenzo et al., (1987) reported that HMW bands 5 and 10 in a homozygous state always were found to be correlated with higher loaf volumes than bands 3 and 12 or the heterozygous state bands 5 and 10+3 and 12, contributed by the D genome. They also concluded that the importance of individual subunits in the A and B genome depends on the interaction with the glutenin proteins.
Payne et al., (1988) evaluated wheat selections i.e. Shawnee and Ottawa by SDS-PAGE and concluded that the main reason for poorer bread making quality in Ottawa than Shawnee is that it does not contain a pair of HMW subunits of glutenin coded by genes on chromosome 1D. Later, Ammar et al., (2000) evaluated twenty seven durum wheat genotypes ands concluded that genotypes expressing high molecular weight glutenin subunits (HMW-GS) 6+8 exhibited better overall bread making quality compared with those expressing HMW-GS 7+8 or 20. Variation in the quality and type of high molecular weight glutenin subunits in a variety can strongly influence its bread making properties (Payne et al., 1988).

2.8. LOW MOLECULAR WEIGHT GLUTENIN SUBUNITS (LMW-GS); RELATION TO BREAD AND CHAPATTI QUALITY

Low-molecular-weight glutenin subunits (LMW-GS) are among the major components of wheat storage proteins, collectively known as prolamins because of their high content of the amino acids proline and glutamine. The LMW-GS have molecular weight ranging from 30-51,000 using SDS-PAGE (Payne et al., 1980). The LMW-GS most closely resemble γ-gliadins in sequence (Muller et al., 1998) and comprise about 20-30% of the total protein (Gupta et al., 1992) and 15-20 different LMW-GS proteins are recognized in 1 and 2D gels of hexaploid wheat (Lew et al., 1992). Despite their abundance, they have received much less research attention than the HMW-GS. This has been mainly due to the difficulty in identifying them in one-dimensional SDS-PAGE gels. The resolution of the problem, which was principally due to overlapping between LMW-GS and gliadins, was largely resolved when Singh and Shepherd (1988) developed a simplified two-step SDS-PAGE method. Earlier, Jackson et al., (1983), using a more complicated two-dimensional electrophoresis procedure, improved resolution and began systematic work on this group of subunits. More recently, Singh et al., (1991a) and Gupta and MacRitchie (1991) reported similar methods.
to analyze polymeric proteins after prior extraction of monomeric proteins with either 50% propan-1-ol or dimethyl sulfoxide (DMSO), respectively. Advances in the characterization of LMW-GS have also been enhanced by the production of wheat-rye translocation lines (single, double, and triple) (Gupta and Shepherd 1993), permitting the simplification of the electrophoretic pattern for closer study of the alleles of LMW-GS. RP-HPLC has also proved useful for the study of LMW-GS, showing that these proteins have higher hydrophobic surfaces than those from HMW-GS and comparable with the hydrophobic surfaces of gliadins. Recent improvements in capillary electrophoresis, as reported by Bean and Lookhart (2000), allow clear characterization of all glutenin subunits. The discovery of new sub-unit is still in progress as two new LMW-GS with molecular weights of $\approx 30-31,000$ Da (Glu-D4 locus) and 32,000 Da (Glu-D5 locus) were reported by Sreeramulu and Singh (1997). These glutenin subunits could be seen only in alkylated glutenin, and the one encoded at Glu-D5 locus has $\alpha$-type N-terminal sequence (Gianibelli, 1998). Formerly, Bunce et al. (1985) demonstrated that although SDS-PAGE was a convenient method for determining the relative molecular weight of cereal prolamins, however, the results should be treated with caution. In another research investigation, MacRitchie et al., (1991) reported that LMW proteins can be separated by gel permeation chromatography into two fractions with molecular weights around 44,000, 36,000.

Characterization of low molecular weight glutenin subunits (LMW-GS) that represent about 70% of glutenins and 20-30% of total proteins explicated their ability to form large aggregates that are related to dough strength (Melas et. al., 1994). However, the strength and resistance provided by LMW to the dough is half to that of HMW but still correlation coefficients for both of them are in a similar range within the family of glutenin proteins (Wieser and Kieffer, 2001). In contrary, Andrews and Skerritt (1996) found relationships between total glutenin subunit (LMW-GS), and dough extensibility with several sets of flours and they
found poor overall correlations between these proteins and other quality attributes of dough.

The products made from durum wheat; LMW-GS has a certain role to play for the end-use quality particular subunits encoded by loci present on chromosome 1B (Josephides et al., 1987). The best pasta making characteristics are associated with the presence of a specific allelic form of typical LMW-GS, named LMW-2 (Payne et al., 1984). This allele also seems to be important for determining bread making properties (Pena et al., 1994). Most commonly grown durum wheat cultivars have either the LMW-2/g-45 (plus vgliadin 35) or the LMW-1/g-42 (plus v-gliadins 33, 35 and 38) allelic forms, the latter being associated with poor quality pasta-making properties. LMW-2 also exerts a positive effect on gluten strength when present in hexaploid tritordeum (Alvarez et al., 1999). There are indications that the better quality associated with the presence of LMW-2 in durum wheat is mainly due to the fact that the subunits are more abundant than the LMW-1 subunits (Autran et al., 1987; D’Ovidio et al., 1992). Differences in the total amount of LMW-GS, associated with specific allelic forms, have also been reported to be an important cause of quality differences in bread wheat (Gupta and MacRitchie, 1994). The 42 KDa LMW-GS may also be present in good quality bread wheat (Masci et al., 2000b), but it is not associated with the LMW-2 group, which does not appear to occur in hexaploid wheat. Similarly, null alleles of LMW-GS have detrimental effects on these parameters (Benedettelli et al., 1992). Different allelic forms of LMW-GS seem to play different roles in determining different quality parameters (Luo et al., 2001). Thus the Glu-A3 alleles influence protein content, SDS sedimentation volume, and mixograph midline peak value of New Zealand wheat cultivars, whereas the Glu-D3 alleles do not have any influence on the SDS sedimentation. Lastly, studies have also demonstrated that g-42 and g-45 are only genetic markers for quality (Boggini and Pogna, 1989; Pogna et al., 1988).
2.9 IMMUNOCHEMICAL CHARACTERISTICS

The quality of wheat proteins can be judged by quantitative assessment of their molecular weights and studying their rheological behaviors along with end use quality. In the recent years, wheat breeders demand simple methods offering through assessment of wheat quality using small samples of grain, whole meal, or flour, because initial crosses within breeding programs may produce many thousands of progeny, each bearing only small amounts (500 mg-10 g) of grain. Large-scale rheological or other quality testing methods that require many grams of flour and are very labor-intensive cannot be used for wheat quality screening (Andrews et al., 1993; Skerritt, 1991).

Chromatographic and electrophoretic techniques have proved to be useful in food components identification (Mackie et al., 2000; Mayer, 2005; Berrini et al., 2006). Equipments of modern era like High Pressure Liquid Chromatography (HPLC), Mass Spectroscopy-Gas Chromatography (GC-MS) and Fourier Transfrom Infrared Radioscopy (FT-IR) are in common use in the developed nations. The time required to run each sample hinder its application on large scale (Skerritt, 1991, 1993). The tools of genetics can be used for the assessment of wheat quality and its relationship with end product quality. The basic antigen-antibody reaction provides a means for very sensitive and analytical procedures. Immunology has been increasingly recognized as a valuable analytical tool. The techniques probing antibodies productions in response to food intake are in progress (Asensio et al., 2008).

In number of research investigation, efforts were diverted in development of immunochemical assays; many techniques have also been developed (Gathumbi et al., 2003; Korde et al., 2003; Lipigorgoson et al., 2003) as qualitative or semi-quantitative and probably most useful for rapid screening. These types include radio-immunoassay and enzyme linked immuno-sorbent assay (ELISA). New and innovative approaches for rapid screening methods based on dip-
like kits and immunosensors based on transducer techniques such as surface plasmon resonance or the quartz-crystal microbalance, capable of simultaneous detection of different mycotoxins, have appeared in the most recent literature (van der Gaag et al., 2003). These techniques are important screening tools for the detection of drugs in biological fluids because of its pretreatment simplicity and high ability to treat many samples in a short time (Aoki et al., 1985; Aoki et al., 1990).

However, although they are of considerable value in certain instances, these methods are not convenient for routine sample analyses because they are relatively costly, time consuming, and complex to perform. Consequently, in the last years the identification of food products has been performed primarily by genetic and immunological techniques (Carrera et al., 1997; Matsunaga et al., 1999; Terzy et al., 2005; Liu et al., 2006). The Enzyme-Linked Immuno Sorbent Assay (ELISA) is the most widely used technique for regulatory purposes in detecting food authenticity because of its specificity, simplicity and sensitivity, among other advantages (Mackie, 1996). Indirect and the sandwich ELISA are two broader categories in such analysis. The detection antibody can be coupled to an enzyme or can bind the conjugate (enzyme-linked antibody) that will produce the biochemical reaction (Goldsby et al., 2003). ELISA may be used for qualitative and quantitative estimation of desired groups. Qualitative results provide a simple positive or negative result for a sample, however, the optical density or fluorescent units of the sample is interpolated into a standard curve in quantitative ELISA (Goldsby et al., 2003).

These techniques can be employed in cereals for detection of many physiologically important substances and their correlation. The approach has several advantages because large number of samples can be tested in shorter time with minimal sample volume (Lim et al., 1995; Lim et al., 1997). In order to carry out specific analyses, prerequisite for an ELISA is the availability of
amounts of antibodies i.e. monoclonal antibodies (MAb) and polyclonal antibodies (PAb). Both polyclonal and monoclonal antibodies (MAbs) can be used in ELISA methods for food components identification. PAb offer a number of benefits such as recognition of a mixture of different epitopes of the antigens and more tolerance to small changes in the nature of antigen (Harlow and Lane, 1999). In contrast, MAbs are a homogeneous population of antibodies produced by hybridoma technology that have defined biological activity, consistent specificity and their production is not limited (Goding, 1996). Both polyclonal antibodies and MAbs are used in the ELISA variants for food authentication that have been previously described (Harlow and Lane, 1999).

These immunoassays include utilization of MAb and PAb; their responses against specific proteins can be further split against storage (Glutenin/gliadin) and non-storage (albumins/globulins) proteins. The reactions which are in direct correlation with product quality are often attributed to glutenin sub-units like LMW-GS and HMW-GS thus their reaction with antibodies can be further used for quality assessment (Prabhasankar and Manohar, 2002). MAb are used for specific fraction of these protein molecules while PAb are used for predicting overall behavior of specific type of proteins. In this context, Mills et al. (2002) developed monoclonal antibodies (MAb) to wheat gluten proteins for use as diagnostics for screening of large numbers of wheat lines for their suitability for processing into bread and other products and as research tools for investigating protein structure and function (Skerritt, 1991; Andrews et al., 1993). Mills et al., (2000) further developed an ELISA for the prediction of dough strength using antibodies to high molecular weight glutenin subunits (HMW-GS) extracted from wheat flour samples. Similarly Skerritt (1991) observed strong relationship between MAb to HMW-GS containing extracts of flour samples. The relationship between antibody binding and the content of large glutenin polymers confirms allow detection of HMW-GS (Gupta et al., 1995). This effect has also been shown earlier in a detailed study using a number of MAbs (Skerritt, 1991). More
recently, Leszczyńska et al., (2008) estimated the usefulness of rabbit polyclonal anti-QQQPP peptide antibodies to determination of gluten proteins.

In sub-continent where chapatti is used as staple food, an indirect enzyme-linked immunosorbent assay (ELISA) has been developed for evaluation of chapatti-making quality of wheat varieties. Polyclonal antibodies against gliadin, low molecular weight glutenin (LMG), and high molecular weight glutenin (HMG) were developed and utilized in ELISA. Twenty-eight Indian wheat varieties were utilized in the ELISA. Out of these antibodies, an antigliadin antibody response was negatively correlated with farinograph water absorption (r ) -0.89 at P < 0.01), chapatti dough water absorption (r ) -0.91 at P < 0.01), overall chapatti sensory score (r ) -0.95 at P < 0.01), chapati puffed height score (r ) -0.95 at P < 0.01), and positively correlated with shear value of chapatti (r ) 0.76 at P < 0.01). Anti-LMG antibody response was not correlated with any of these parameters, whereas anti-HMG response positively correlated with chapati dough water absorption (r ) 0.44 at P < 0.05), farinograph water absorption (r ) 0.45 at P < 0.05), and overall chapati sensory score (r ) 0.44 at P < 0.05), and negatively correlated with shear value (r ) -0.38 at P < 0.05) and chapati puffed height (r ) -0.44 at P < 0.05). The results indicate that wheat varieties with good chapati-making quality were having less antigliadin antibody response (Prabhasankar and Manohar, 2002). The ability of simple antibody-based tests to assess and predict specific aspects of dough can be evaluated using ELISA that can be further used for the prediction of end use quality.

Wheat (Triticum aestivum), is ranked as a staple food of the people all over the world. It occupies an important position among cereal crops with respect to production and utilization. The varietals trials are common to develop new wheat varieties and maintaining the vigor of the old ones. Many tools are used to characterize wheat on the basis of their set criterion. The quality of wheat is generally assessed on the basis of different physical, chemical, biochemical and
rheological properties including milling yield, weight, protein, moisture and gluten. The wheat quality depends upon cultivar, climatic conditions and year and is expressed by a variety of physical and chemical tests such as test weight, kernel weight, moisture content, ash content, protein content, SDS etc.

The wheat flour’s ability to be processed into different food products is mainly determined by the contents and quality of gluten proteins. The main constituents of the gluten are gliadins and glutenins. The glutenins exhibit a broad spectrum of molecular weights varying from 40 kDa to several millions. Two major classes of glutenin subunits have been identified in wheat endosperm by SDS-PAGE, the high molecular weight (HMW) glutenins (80 -130 kDa) and the low molecular weight (LMW) glutenins (10-70 kDa). The HMW-GS are major determinant of the bread-making characteristics of dough, but the LMW-GS also play an important role related to dough resistance and extensibility.

Because the quality of wheat is governed by interaction of many constituents, it is difficult to judge quality by a single test. Immunochemical quality assessment through immunological methods has been increasingly recognized as a valuable analytical tool in measuring functionality and suitability of different wheat varieties for their end use. Enzyme-Linked Immunosorbent Assay (ELISA) may be an effective tool for assessing chapatti and bread making qualities of different wheats. The review of literature reported in different sections reflects that quality is a summation of various characteristics which is affected by genetic and non genetic factor.
Chapter 3

MATERIALS AND METHODS

3.1 COLLECTION OF WHEAT SAMPLES

Sixteen wheat varieties were collected from Wheat Research Institute, Faisalabad grown during the crop year 2005-06. The description of wheat varieties studied in the present study is shown in Table 3.1. The wheat varieties were evaluated for various physico-chemical characteristics, biochemical and immunochemical properties described as follows.

3.2 PHYSICAL TESTS

3.2.1 Thousand Kernel Weight

Thousand kernel weight of each variety was recorded in grams/1000 kernel. Representative sample (50g) of each wheat variety was drawn randomly and thousand-kernel weight was recorded by counting and weighing the clean, unbroken and sound kernels.

3.2.2 Test Weight

The test weight of each wheat variety was measured according to the procedure given in AACC (2000) method No.55-10. This test was carried out using a 1 liter vessel. The vessel was overfilled with grains, leveled and weighed on an electric balance. The test weight was expressed in Kg/hl.

3.3 MILLING

3.3.1 Whole Wheat Flour

The whole meal wheat flour of each wheat variety was prepared by grinding the wheat grains through Udy Cyclone Sample Mill (Seedburo Equipment Co., IL) fitted with 0.5 mm sieve.
### Table 3.1  Description of wheat varieties used in the study

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Variety</th>
<th>Parentage</th>
<th>Year of release</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>C-273</td>
<td>C209xC591</td>
<td>1957</td>
</tr>
<tr>
<td>2</td>
<td>Punjab-81</td>
<td>Inia/3/Son64/P4 106(E)//Son64</td>
<td>1981</td>
</tr>
<tr>
<td>3</td>
<td>Pak-81</td>
<td>Kviz-Buho 'S' x kal-Bb</td>
<td>1983</td>
</tr>
<tr>
<td>4</td>
<td>Kohinoor-83</td>
<td>Ore F1158/Fdl//Mexifen 'S'</td>
<td>1983</td>
</tr>
<tr>
<td>5</td>
<td>Faisalabad-83</td>
<td>Fury//Kal/Bb</td>
<td>1983</td>
</tr>
<tr>
<td>6</td>
<td>Faisalabad-85</td>
<td>Fury//Kal/Bb</td>
<td>1985</td>
</tr>
<tr>
<td>7</td>
<td>Punjab-85</td>
<td>Kviz/Trm/Bb/A   Ana</td>
<td>1985</td>
</tr>
<tr>
<td>8</td>
<td>Inqulab-91</td>
<td>WL711/CROWS</td>
<td>1991</td>
</tr>
<tr>
<td>12</td>
<td>MH-97</td>
<td>Attila</td>
<td>1997</td>
</tr>
<tr>
<td>13</td>
<td>Iqbal 2000</td>
<td>BURGUS/SORT-12-13//KAL/BB/3/PAK-81</td>
<td>2000</td>
</tr>
<tr>
<td>14</td>
<td>Manthar 03</td>
<td>KAUZ/ALSTAR</td>
<td>2000</td>
</tr>
<tr>
<td>15</td>
<td>Uqab 2000</td>
<td>CROW'S//NAC//BOW'S</td>
<td>2000</td>
</tr>
<tr>
<td>16</td>
<td>GA-02</td>
<td>DWL5023/SNB//SNB</td>
<td>2002</td>
</tr>
</tbody>
</table>
3.3.2 Straight Grade Flour

Two kg grains of each wheat variety was tempered to 16.0 % moisture level and allowed to equilibrate the moisture content in the wheat grains for 24 hrs at room temperature. The quantity of water required to temper the wheat grains of each wheat variety was calculated according to the expression given in AACC (2000) method No. 26-95. The tempered wheat grains were milled through a Brabender Quadrumate Senior Mill (C.W. Brabender Instruments, Inc.) The straight grade flour was prepared by blending the two flour fractions i.e. break roll flour and reduction roll flour.

3.4 CHEMICAL ANALYSIS

The whole meal wheat flour and straight grade flour of each wheat variety were tested for chemical characteristics as described below.

3.4.1 Moisture Content

The moisture content was determined in each flour sample by drying 3 g flour sample in an air forced draft oven at a temperature of 105± 5 °C till to constant weight. The procedure of AACC (2000) method No. 44-15 A was followed for the estimation of moisture content in each sample. The moisture content was calculated according to the following formula:

\[
\text{Moisture content (\%) = } \frac{\text{Weight of flour sample} - \text{Wt. of dried flour sample}}{\text{Weight of flour sample}} \times 100
\]

3.4.2 Crude Protein

The Kjeldhal’s method as described in AACC (2000) method No. 46-10 was used to determine the nitrogen content in each flour sample by digesting the
sample with concentrated H\textsubscript{2}SO\textsubscript{4} in the presence of catalysts. During digestion the organic compounds are oxidized and the nitrogen is converted to ammonium sulphate. In a distillation apparatus in an alkaline media the ammonia is liberated which is collected in a flask containing 4 \% boric acid solution using methyl red as an indicator. The nitrogen content in each sample was determined by titrating against 0.1N H\textsubscript{2}SO\textsubscript{4} solution and the crude protein percentage was calculated by multiplying the nitrogen percent with a conversion factor 5.7.

3.4.3 Crude Fat

The crude fat content in flour sample of each wheat variety was estimated by running dried samples through Soxhlet apparatus for 2-3 hours using petroleum ether as a solvent according to the procedure described in AACC (2000) method No.30-10.

3.4.4 Ash Content

The ash content in each flour sample was determined as a total inorganic matter by following the procedure given in AACC (2000) method No. 08-01.

Oven dried 5 g sample was charred on the burner and then ignited in a muffle furnace at a temperature of 550-600 °C for 5-6 hours or till grayish ash formed. The ash content was calculated according to following formula.

$$\text{Ash content \%} = \frac{\text{Weight of ash}}{\text{Weight of sample}} \times 100$$

3.4.5 Pelshenke Value

The Pelshenke value gives an estimate of the wheat gluten strength through gas production and retention capacity of the wheat carbohydrate/protein complex. The whole wheat meal flour of each wheat variety was tested for Pelshenke value according to AACC (2000) method No 56-50.

A dough ball was prepared using a suspension of baker's yeast in water. The dough ball was placed in about 120mL water at 30°C in water bath. By
fermentation CO\textsubscript{2} was produced within the dough ball. The gluten formed in the dough trapped the gas, which was retained and the ball floated to the surface. The gas pressure became too high with a passage of time and the dough ball broke up and fell to the bottom of the beaker. The difference between time of immersion and disintegration time of dough ball was recorded as Pelschenke value in minutes.

3.4.6 Wet and Dry Gluten

Wet and dry gluten contents in different flour samples were determined by hand washing method as detailed in AACC (2000) method No.38-10. A dough was made by adding 15mL of water to 25 g of flour in a bowl. The dough was allowed to stand for one hour and then kneaded gently under a stream of cold tap water, letting the washings passed through a fine sieve until all starch and soluble matter were removed. Starch removal was tested by squeezing a little water from the ball in to beaker and clear, cold water; cloudiness indicated that starch is still present. The ball was kept in cold water for an hour and water was squeezed with hands. Then the ball was placed in a tarred, flat-bottomed dish and weighed as moist gluten. To determine dry gluten the ball was dried in an air forced draft oven at temperature of 100 ± 5 °C to constant weight.

3.4.7 Sodium Dodecyl Sulfate (SDS)-Sedimentation Test

The SDS-Sedimentation test was performed for evaluating different wheat varieties according to the method described by Williams \textit{et al.}, (1986). In this test gluten is precipitated, value is determined and remaining all the material is solubilized by using suitable detergent. 3 g of flour was taken in a graduated cylinder of 100mL capacity. At 0 seconds 50 mL of indicator was added and shaked 15 times. Then it was shaken twice for 15 times after 100 and 200 seconds. At 300 seconds, 50 mL of SDS reagent was added and inverted the cylinder 4 times. Again inverted the cylinder 4 times at 400, 500 and 600 seconds. At 1500 seconds the volume was noted.
3.4.8 Falling Number

Falling number was measured in each flour sample by taking triplicate samples of 7 g each and 25 ml of distilled water (25°C) was added in tubes and run the samples in Falling Number apparatus, Model No. 1600 consisting dispenser No. 1025 (Perten Instruments, North America Inc., Reno, NV, USA) according to AACC (2000) method No 56-81B.

3.4.9 Minerals Content

The flour samples of each wheat variety were digested in di-acid mixture (3:1) of HCLO₄; HNO₃ at 180 °C for 2 hrs. The digested samples were tested for mineral contents i.e. Ca, Fe, Cu, Zn and Mn by using Atomic Absorption Spectrophotometer (A Analyst 100, Agilent, Norvalk, C.T., USA) in acetylene air flame at wavelengths: 422 nm, 248 nm, 325 nm, 214 nm and 279.5 nm, respectively. The procedure described in AOAC (1984) method No. 3.014-016 was adopted to determine and then calculating mineral content in different flour samples.

3.5 DOUGH RHEOLOGICAL PROPERTIES

The flour samples were evaluated for rheological properties by using Brabander Farinograph equipped with 50 g bowl capacity by following the procedure of AACC (2000) method No 54-21. The physical dough properties were derived from farinograms.

3.6 PROTEIN FRACTIONATION

The protein fractions i.e. high molecular weight (HMW) and low molecular weight (LMW) glutenin proteins from flour samples of each wheat variety were fractionated, using a modified sequential extraction standard methods developed by Curioni et al.( 2000). The schematic protein fractionation has been given in Appendix I.
3.7 SODIUM DODECYL SULPHATE POLYACRYLAMIDE GEL ELECTROPHORESIS (SDS-PAGE)

Each flour sample was run for SDS-PAGE analysis according to the basic method described by Laemmli (1970) and as modified by Singh and Shepherd (1985). The dried protein pellets were solubilized in 250 ul of a sample buffer. The detail of solutions prepared for running electrophoresis is given below;

- **30% Acrylamide solution**
  
  Acrylamide monomers (29.2g), 0.8g bisacrylamide were dissolved in deionized water and final volume was made 100mL with distilled water. The solution was filtered under vaccum using 0.45mm membrane and stored at 4°C in dark bottle.

- **Separating Gel Buffer (1.5 M, pH 8.8)**

  Tris base (18.20g) was dissolved in 80mL water, the pH was adjusted to 8.8 with HCl and volume (100mL) was made with distilled water.

- **Stacking Gel Buffer (0.5 M, 6.8 pH)**

  Tris base (6.1g) was dissolved in 80mL water; the pH was adjusted to 6.8 with HCl and volume made to 100 mL with distilled water.

- **10% Sodium Dodecyl Sulphate (SDS) Solution**

  SDS (2.0 g) was dissolved in 20mL of distilled water.

- **10% Ammonium Per Sulphate (APS) Solution**

  APS (0.1g) was dissolved in 1mL of distilled water.

- **Electrode Buffer**

  Lycine (14.4 g), 3.0 g tris base and 1g SDS were dissolved in distilled water to a final volume of 1000 mL.
• **Sample Buffer**

62.5 mM tris-HCl pH 6.8, 10% glycerol, 2% SDS, 0.002% bromophenol blue, 5% mercaptoethanol. (0.75g tris in 50 mL water having pH 6.8 adjusted with HCl, 10mL glycerol, 2 g SDS, 2 mg bromophenol blue, 5 mL mercaptoethanol were added to make 100 mL with distilled water.

• **Staining Solution**

Methanol (400mL), 100mL acetic acid was dissolved in 500mL distilled water. 1g Coomassie Blue R 250 was dissolved in 40mL water mixed and added to above solution. The final volume (1000 mL) was made with distilled water.

• **Destaining Solution**

Methanol (400mL) and 100mL acetic acid were added in distilled water to make final volume up to 1000mL.

• **Resolving Gel Solution (2 Gels)**

Lower gel buffer 1875μL, 30% acrylamide solution 2500 μL, 10% SDS 300 μL, distilled water 2800μL, 10% APS solution 50μL, N,N,N’N’ Tetramethylethylene diamine (TEMED) 5μL.

• **Stacking Gel Solution (2 Gels)**

Upper gel buffer 1250μL, 30% acrylamide Solution 875 μL, 10% SDS 200 μL, distilled water 2675μL, 10% APS solution 50μL, N,N,N’N’ Tetramethylethylene diamine (TEMED) 5μL.

The electrophoresis was carried out using a 10 % gel concentration (Laemmli, 1970). A 10-well, 0.75 mm comb was used in a Bio-Rad mini protein 3 system having gel size 8.3-7.3 cm. The samples were loaded at 10μL/lane. The gels were run at constant 70V for 3 hours. The gels were stained with 150mL
Coomassie Brilliant Blue (CBB) dye & destained with 100mL methanol, acetic acid water mixture destaining solution.

3.7.1 Interpretation of HMW and LMW Bands

The protein bands were imaged through gel documentation system (Syngene, Cambridge, UK) system. On first well of each gel, the proteins employed as the molecular weight (Daltons) markers ranging from 10-160 KDa (SDS-PAGE MW standards, Medium range, Cat # SM0671, Fermentas.

3.8 IMMUNOCHEMICAL STUDIES

3.8.1 Production of polyclonal antibodies

The immunization of rabbits with LMG and HMG was performed according to the method of Prabhasankar et al., (2002). New Zealand white rabbits were purchased from National Institute of Health, Islamabad and were injected with LMG and HMG. The initial immunization in three-time concentrated Freund’s complete adjuvant (250 ug LMG and HMG in 500 uL of 16 mM acetic acid per rabbit) was followed by two further immunizations of 150 ug LMG and HMG per rabbit in Freund’s incomplete adjuvant 2 and 4 weeks later. Doses were divided with half being given subcutaneously and half intradermally. Rabbits were rested for 2 months. These were given a booster of 100 uL intradermally. One week later, blood was collected; serum was separated and stored at -20 °C for further studies. Antibodies were purified from antiserum by using ammonium sulfate precipitation method and purified antibodies were stored in small aliquots along with 10% bovine serum albumin (BSA) and 0.01% sodium azide (Mc Kinney and Parkinson, 1987).

3.8.2 Development of Indirect ELISA for LMG and HMG

Indirect Enzyme Linked Immunosorbant assay (ELISA) method was developed for quantification of LMG and HMG according to the method of Skerritt (1985). Indirect ELISA method was developed by using anti HMG and
anti LMG antibodies and commercially available anti-rabbit IgG-Alkaline Phosphatase Conjugate as enzyme tracer. The optimal dilutions of antibody and enzyme conjugate were 1:15 000 and 1:10 000, respectively. The antibody responses for different wheat varieties were recorded. Accordingly, 200 uL of protein (LMG and HMG) extracts from different wheat varieties were diluted to the concentration of 650 ng with carbonate buffer pH 9.6; 50 mmol/L was added to the wells of micro titer plates and incubated for 2 h at 37 °C or overnight at 4 °C. After washing the plates three times with wash buffer (PBS-containing 0.5 g/L Tween 20), the empty sites were blocked with a 200 uL solution of gelatin in phosphate buffer saline (PBS). The plates were then incubated for 1 h at 37 °C and washed again with wash buffer. This was followed by the addition of LMG and HMG antibodies, incubation for 1 h at 37 °C, followed by addition of anti-rabbit IgGAKP and incubation of the plates at 37 °C for 1 h. The reading was recorded on ELISA reader at 405nm.

3.9 PREPARATION OF BREAD

The breads were prepared according to the AACC (2000) straight dough method No 10-10B. The ingredients were mixed for 5-10 minutes in a Hobart A-200 Mixer to form dough and allowed to ferment at 30 °C and 75% R.H. for 180 minutes. First and second punches were made after 120 and 150 minutes, respectively. The dough was molded and panned into 100 g test pans, and final proofing was done for 45 minutes at 95 °F (35 °C) and 85% R.H. The bread was baked at 232 °C for 13 minutes.

The sensory scores for external characteristics (volume, crust color, symmetry, evenness of bake, break and shred) and for internal characteristics (grain, crumb color, aroma, taste, mastication and texture) were recorded for each loaf assigned by a panel of trained judges according to the bread score method developed by the American Institute of Baking and reported by Matz (1960). The detail is given in Appendix II.
3.10 PREPARATION OF CHAPATTIS

The chapattis were prepared from whole meal wheat flour of each wheat variety according to the method developed by Haridas Rao et al., (1986). The dough for chapatti was made by mixing 200 g of whole meal wheat flour with predetermined quantity of water for 3 minutes and allowed to rest for 30 minutes. A dough piece weighing 80 g was rolled on a sheet of 2 mm thickness with a wooden roller pin on a specially designed wooden platform and cut into circle of 17 cm diameter. The chapattis were baked on thermostatically controlled hot plate at a temperature of 210 °C for 1.5 minutes.

Sensory evaluation of chapattis was carried out for color, flavor, taste and texture using hedonic scale by a panel of trained judges. The detailed evaluation performa is given in Appendix-III.

3.11 STATISTICAL ANALYSIS

The data obtained for each parameter were analyzed through Statistical Package Co-Stat-2003 (Cohort v-6.1). The experiment was performed using completely randomized design and the analysis of variance was applied to determine the level of significance. Moreover, Duncan multiple range test (DMRt) was applied to explore the significant ranges in different cultivars for their various quality attributes. Correlation matrix was generated to check the interdependence of variables over each other with special reference to immuno-chemical properties of cultivars.
Chapter 4

RESULTS AND DISCUSSION

Sixteen spring wheat varieties grown during the crop year 2005-06 in Pakistan were selected for the present studies to characterize for physicochemical, biochemical, technological (bread and chapatti baking) and immunochemical (ELISA) properties. The data obtained for all the studied quality attributes were statistically analyzed and the results are interpreted and discussed in the following sections.

4.1 PHYSICAL CHARACTERISTICS OF WHEAT VARIETIES

4.1.1 Thousand Kernel Weight

The statistical results pertaining to thousand kernel weight of different wheat varieties have been presented in Table 4.1. It is obvious from the statistical results that the 1000 kernel weight differed significantly among different wheat varieties.

The results presented in Table 4.2 indicated that 1000 kernel weight varied between 25.98 and 40g among different wheat varieties. Significantly the highest 1000 kernel weight (40.00 g) was exhibited by the grains of wheat variety Uqab 2000 followed by Punjab 81 (39.25 g), while significantly the lowest 1000 kernel weight (25.98 g) was yielded by the grains of wheat variety GA-02.

The wheat varieties C-273, Faisalabad 83, Faisalabad 85, Inqlab 91, Iqbal 2000, Pak-81, Pasban 90 and Punjab 96 were found statistically at par with respect to their 1000 kernel weight which was recorded for these wheat varieties 34.29, 34.60, 34.1, 35.71, 34.76, 34.85, 34.19 and 35.19 g, respectively. The wheat
varieties Kohinoor 83, Kohistan 97, Manthar 03 and MH-97 possessed relatively lower but statistically similar 1000 kernel weight. The wheat variety GA-02
# Table 4.1  Mean squares for 1000 kernel weight and test weight of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1000 K wt</td>
<td>Test wt</td>
<td></td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>46.81592**</td>
<td>28.662488**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>1.6228771</td>
<td>7.815</td>
<td></td>
</tr>
</tbody>
</table>

** Significant at P ≤ 0.01
<table>
<thead>
<tr>
<th>Varieties</th>
<th>1000 K wt (g)</th>
<th>Test wt (Kg/hL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>34.29b</td>
<td>78.00a</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>34.60b</td>
<td>70.30cde</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>34.17b</td>
<td>75.45abc</td>
</tr>
<tr>
<td>GA-02</td>
<td>25.98e</td>
<td>69.98cde</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>35.71b</td>
<td>77.02ab</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>34.76b</td>
<td>76.90ab</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>28.99d</td>
<td>66.47e</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>28.42d</td>
<td>72.48bcd</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>28.28d</td>
<td>73.90abcd</td>
</tr>
<tr>
<td>MH-97</td>
<td>30.21cd</td>
<td>69.91de</td>
</tr>
<tr>
<td>Pak-81</td>
<td>34.85b</td>
<td>72.49bcd</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>34.19b</td>
<td>73.96abcd</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>39.25a</td>
<td>70.00cde</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>31.89c</td>
<td>72.47bcd</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>35.87b</td>
<td>73.00abcd</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>40.00a</td>
<td>74.00abcd</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from one another.
Possessed significantly the lowest 1000 kernel weight as compared to other tested wheat varieties. The present study revealed existence of wide variation with respect to 1000 kernel weight among different wheat varieties. However, the results pertaining to 1000 kernel weight in the present study are similar to the earlier findings of Ahmad (2001) and Randhawa et al., (2002) who reported 1000 kernel weight ranges from 28.00 to 50.00 g for different wheat varieties grown in Pakistan. The results are also supported by the study of Pasha (2006) who found variation in 1000 kernel weight from 24.28 to 52.7 g among different Pakistani wheat varieties grown during two different crop years. Anjum et al. (2002) also observed 1000 kernel weight of different Pakistani wheat varieties exceeding 30 g which also supports to the findings of the present study in which 11 varieties out of 16 exceeded the 1000 kernel weight to 30g. The previous work of Williams et al. (1986) also indicated that kernel weight and size are not only genetically controlled but also affected by growth conditions. Thus the variation observed in the present study may be attributed to the non genetic factors including growth conditions prevalent to wheat varieties.

The 1000 kernel weight is a useful tool used for assessment of the potential milling yield i.e flour yield. The kernel size contributes directly towards the improvement of grain yield as well as milling yield. The present study suggests that all the wheat varieties possessing better grain weight i.e 30g offer great potential for better milling yield. The present study also indicated wide variation in grain weight which can be exploited by the wheat breeders to improve this trait i.e grain weight in the new genotypes. It is obvious that wheat varieties Uqab 2000, Punjab 81, Punjab 96 and Inqlab 91, possessing relatively higher grain weights (above 35 g/thousand kernels) may be explored by different stakeholders for improvement in grain yield and better flour extraction during milling, respectively.
4.1.2 Test Weight

The statistical results with respect to test weight of different wheat varieties shown in Table 4.1 indicated existence of highly significant differences in test weights of different wheat varieties.

The results presented in Table 4.2 indicated that significantly the highest test weight was yielded by the wheat variety C-273 (78.00 kg/hL) followed by Iqbal 2000 (76.90 kg/hL). The grains of Kohinoor 83 possessed significantly the lowest test weight (66.47 kg/hL). The wheat varieties C-273, Faisalabad 85, Inqlab 91 and Iqbal 2000 exhibited test weight more than 75 kg/hL. However, the wheat varieties GA-02, Kohinoor 83, MH-97, Faisalabad 83 and Punjab 81 possessed the lowest test weight which was less than 70 kg/hL.

The test weight per unit volume is considered to be one of the most important physical criteria in all wheat grading systems. The flour yield is the most important technical and economic factor in case of milling (Posner and Hibbas, 1999) and it is generally employed as a rough indicator of the flour yield during roller flour milling. The test weight is dependent on both grain size and shape. There is a rapid decrease in milling flour yield with decrease in the test weight. The results of the present study are in consistent with the earlier findings of other researchers Zahoor, (2003), Anjum et al. (2002) and Randhawa et al., (2002) who reported variations from 68.30 to 81.00 kg/hl in different Pakistani wheat varieties. Martin et al. (2001) found test weight ranging from 66.20 to 80.20 kg/ hl in 130 hard red spring wheat recombinant inbred lines grown at different locations of USA. The differences of present results in the test weight to those reported by U.S researchers may be attributed to the differences in climatic conditions, cropping practices and genetic makeup of the varieties tested in two different studies.
4.2 CHEMICAL CHARACTERISTICS OF WHEAT VARIETIES

4.2.1 Moisture content

The results regarding statistical analysis for whole wheat flour (WWF) and straight grade flours (SGF) of different wheat varieties have been presented in Table 4.3. The results showed that the moisture content in whole wheat flour and straight grade flour differed significantly among wheat varieties.

The moisture content ranged from 9.7 to 11.65 % and 11.43 to 13.42 % between the whole wheat flours and straight grade flours, respectively in different wheat varieties (Table 4.4). The moisture content in whole wheat flours was found relatively lower than the straight grade flour of the respective wheat varieties.

The highest moisture content (11.65%) was recorded in whole wheat flour of wheat variety Punjab 96 followed by Uqab 2000 (11.49%), Kohinoor 83 (11.49%) and Punjab 81 (11.46) but these wheat varieties possessed non significant differences for moisture content. It is also obvious from the results (Table 4.4) that significantly the highest moisture content (13.42%) was found in the SGF of wheat variety Punjab 96 while wheat variety Manthar 03 yielded the lowest moisture content (11.43%). The wheat varieties Inqlab 91, Manthar 03 and Pak-81 were statistically identical with respect to the moisture contents in SGF.

The higher moisture content in SGF as compared to WWF in the present study may be ascribed to the fact that SGF was obtained from the wheat grains tempered to 15.5% moisture content before their milling which is the reason of higher level of moisture in the SG flour. The moisture content is not only of economic significance but is important with respect to the keeping qualities of wheat grains and flour. The wheat grains with moisture content below 12 percent can be stored for an extended period. The wheat grains with very low moisture content is brittle but with high moisture content (>13.5%) possess a tough character (Cornell and Hoveling 1998). Pomeranz and Williams (1990) described
that the moisture content is an important factor for the measurement of wheat kernel texture. The present results with respect to moisture content of SGF of wheat varieties fall within the limits reported by Whiteley (1970) who found variation in SGF from 11 to 15 % depending upon the storage conditions and hygroscopic nature of wheat starch. The variation found in the moisture content of different wheat varieties might be attributed to genetic factor and climatic factors. The present findings are also in consistent with the findings of different workers (Slaughter et al., 1992) and (Mahmood, 2004) who held that moisture content is dependent both on genetic make up of varieties and climatic factors as well as agronomic conditions experienced by the wheat varieties during growth period.

4.2.2 Ash content

The statistical analysis pertaining to ash content of different wheat flours presented in Table 4.3 indicated that the ash content in whole wheat flour and straight grade flour differed significantly among different wheat varieties.

It is obvious form the results given in Table 4.5 that the ash contents in WWF and SGF ranged from 1.20 to 1.67% and from 0.38 to 0.63%, respectively among different wheat varieties.

The data showed that significantly the highest ash content (1.67%) was recorded in whole wheat flour of wheat variety Manthar 03 followed by Inqlab 91 (1.64%), Pak 81 (1.57%) and Punjab 85 (1.56%). The whole wheat flour of wheat varieties Iqbal 2000, Kohinoor 83 and Kohistan 97 possessed statistically similar ash content which was 1.47, 1.44 and 1.54%, respectively in these wheat varieties. It is also evident that significantly the highest ash content (0.63%) was found in the SGF of Manthar 03 followed by Inqlab 91 (0.60%), Kohistan 97 (0.58%), Pak 81 (0.59% ), Punjab 81 (0.57% ) and Punjab 85 (0.58% ).
Table 4.3  Mean squares for moisture and ash content of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Moisture</th>
<th></th>
<th>Ash</th>
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<tr>
<td></td>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td>WWF</td>
<td>SGF</td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.178851**</td>
<td>1.1672988**</td>
<td>0.0536172**</td>
<td>0.014141**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.1711917</td>
<td>0.3226312</td>
<td>0.0032917</td>
<td>0.0010729</td>
</tr>
</tbody>
</table>

** Significant at P≤0.01

WWF  Whole wheat flour
SGF  Straight grade flour
<table>
<thead>
<tr>
<th>Varieties</th>
<th>Moisture (%)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
</tr>
<tr>
<td>C-273</td>
<td>11.12abcd</td>
<td>12.92abc</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>10.61cdefg</td>
<td>12.30bcdef</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>11.49ab</td>
<td>13.17ab</td>
</tr>
<tr>
<td>GA-02</td>
<td>10.92abcde</td>
<td>12.67abcde</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>10.08fgh</td>
<td>11.83def</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>11.32abc</td>
<td>13.01abc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>11.49ab</td>
<td>13.25ab</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>10.50defg</td>
<td>12.26bcdef</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>9.71h</td>
<td>11.43f</td>
</tr>
<tr>
<td>MH-97</td>
<td>10.70bcdef</td>
<td>12.40abcd</td>
</tr>
<tr>
<td>Pak-81</td>
<td>9.86gh</td>
<td>11.60ef</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>10.87abcde</td>
<td>12.74abcd</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>11.46ab</td>
<td>13.18ab</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>10.32efgh</td>
<td>12.01cdef</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>11.65a</td>
<td>13.42a</td>
</tr>
<tr>
<td><strong>Uqab 2000</strong></td>
<td>11.49ab</td>
<td>13.09abc</td>
</tr>
<tr>
<td>--------------</td>
<td>---------</td>
<td>----------</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

WWF  Whole wheat flour
SGF  Straight grade flour
Significantly the lowest ash content (0.38%) was yielded by SGF of wheat variety MH-97 followed by Punjab 96 (0.41%). The wheat varieties Faisalabad 83, Faisalabad 85, GA-02, Inqalab 91, Iqbal 2000, Kohistan 97, Pak-81, Punjab 81, Punjab 85 and Uqab 2000 were found to be statistically similar with respect to ash content of SGF.

The concentration of mineral contents present in a given product is generally represented by the ash content. The presence of higher ash content indirectly reflects the availability of more amounts of minerals (Ibrahim, 1981). The ash content is also one of the best indicators of flour yield; hence the wheats with lower content of ash may have more endosperm and ultimately yield good flour extraction (William et al., 1986). The environmental conditions and stages of wheat grain maturation may affect the ash content. The differences observed in the ash content in the present study among wheat varieties may be ascribed to differences in wheat genotypes and environmental conditions. The ash content of wheat varieties has been reported to be influenced by genetic as well as non-genetic factors like soil, climatic conditions and use of fertilizer etc (Kent and Evers, 1994; Bushuk et al., 1969). The results of the present study are in close agreement to the earlier findings reported by Butt et al. (2001), Ahmad (2001), Pasha (2006) and Randhawa et al., (2002) who found variation in ash content of WWF from 1.08 to 1.85 %. The ash content in SGF of different Pakistani wheat varieties has been found to vary from 0.37 to 0.58 % (Afzal, 2004). The ash content in WWF found higher as compared to SGF in the present study is due to higher bran portion present in the WWF which to removed during the milling process for the production of SGF. The wheat varieties containing less ash content in whole wheat flour (1.33%, 1.23%, and 1.20%) may possess higher proportion of endosperm.
<table>
<thead>
<tr>
<th>Varieties</th>
<th>Ash (%)</th>
<th>WWF</th>
<th>SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>1.35ef</td>
<td>0.47de</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>1.47cd</td>
<td>0.54bc</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>1.48cd</td>
<td>0.54bc</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>1.53cd</td>
<td>0.56bc</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>1.64ab</td>
<td>0.60ab</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>1.47cd</td>
<td>0.55bc</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>1.44de</td>
<td>0.51cd</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>1.54bcd</td>
<td>0.58ab</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>1.67a</td>
<td>0.63a</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>1.20g</td>
<td>0.38f</td>
<td></td>
</tr>
<tr>
<td>Pak-81</td>
<td>1.57abc</td>
<td>0.59ab</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>1.33f</td>
<td>0.46de</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>1.54bcd</td>
<td>0.57abc</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>1.56bc</td>
<td>0.58ab</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>1.23g</td>
<td>0.41ef</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>1.53cd</td>
<td>0.55bc</td>
<td></td>
</tr>
</tbody>
</table>

Table 4.5 Ash content of whole and straight grade flours of different Pakistani spring wheat varieties
Means carrying same letters in a column are not significantly different from each other

WWF    Whole wheat flour
SGF    Straight grade flour
4.2.3 Crude protein

The statistical results for crude protein content of whole wheat flour (WWF) and straight grade flours (SGF) of different wheat varieties have been presented in Table 4.6. The results showed highly significant differences in the protein content of both whole wheat flour and straight grade flour of different wheat varieties.

The results given in Table 4.7 also indicated that the protein content ranged from 10.44 to 12.72 % and 10.01 to 11.60 % between the whole wheat flours and straight grade wheat flours, respectively among different wheat varieties. The whole wheat flour exhibited higher content of protein than the respective straight grade flour.

The highest protein content (12.72%) was found in the whole wheat flour sample of wheat varieties Iqbal 2000 followed by MH-97 (12.34%), Pasban 90 (12.21%), Uqab 2000 (12.07%) and Inqlab 91 (11.86%). The wheat varieties C-273 and Faisalabad 85 yielded statistically similar protein contents. It is also obvious from the results (Table 4.7) that significantly the highest protein content was found in the SGF of Iqbal 2000, Pasban 90 and Uqab 2000 wheat varieties and the lowest protein content (10.01%) was observed in SGF of wheat variety Faisalabad 83. The wheat varieties Manthar 03, GA-02, Kohistan 97, C-273, Faisalabad 83, Faisalabad 85, Kohinoor 83, Punjab 81 and Punjab 85 were found to possess lower protein content but all these wheat varieties possess statistically similar contents of protein.

The protein content was found higher in WWF as compared to SGF in the present study may be due to the fact that the whole wheat flours contain substantial amount of bran which is rich in proteins and contributes towards higher level of protein in whole wheat flour. The results of the present study are in consistent with the results
reported by Anjum et al. (2005) who reported variation in protein content from 9.68 to 13.45% among Pakistani wheat varieties. The results are also supported by the studies of Randhawa et al., (2002), Ahmad (2001) and Mahmood (2004) who found variation from 9.71% to 15.42% in protein content of different wheats. The protein content is an important criterion for assessing wheat quality. The differences in protein content among different wheat varieties could be related to the studies of Kent and Evers, (1994), Gupta et al.(1993), Schofield and Subda (1991) who stated that quantity of protein depends on genotype, environment and the growing conditions. It is evident from the results that wheat varieties Iqbal 2000, Pasban 90, MH-97, Inqlab 91 and Uqab 2000 possessing higher contents of protein are more suitable for bread and chapatti purposes. These wheat varieties may also be used by the wheat breeders in their breeding programme for the development of high protein content in new wheat varieties. The variation in protein content among different wheat varieties offers potential for a specific use by the different stakeholders.

4.2.4 Crude fat

It is obvious from the statistical results shown in Table 4.8 that highly significant variation existed among different wheat varieties with respect to crude fat content.

It is obvious from the results given in Table 4.8 that fat content in SGF of different wheat varieties ranged from 0.51 to 0.75% while in WWF of different wheat varieties it ranged from 0.92 to 1.37%.

It is also evident from results that significantly the highest fat content (1.37%) was found in whole wheat flour of MH-97 wheat variety followed by Punjab 96 (1.36%), Inqlab 91 (1.34%), Faisalabad 83 (1.33%), Punjab 85 (1.32%), C-
273 (1.30%) and Uqab 2000 (1.30%) and these varieties possessed non significant
differences for fat content. The wheat varieties Iqbal 2000, Pak-81 and Pasban 90
### Table 4.6 Mean squares for crude protein and crude fat content of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Crude protein</th>
<th></th>
<th>Crude fat</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td>WWF</td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.1363911**</td>
<td>0.8172467**</td>
<td>0.0708599**</td>
<td>0.0181817**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.2746563</td>
<td>0.2332938</td>
<td>0.002175</td>
<td>8.9375</td>
</tr>
</tbody>
</table>

** Significant at P ≤ 0.01

WWF Whole wheat flour
SGF Straight grade flour
Table 4.7 Crude protein content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Crude protein (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>C-273</td>
<td>11.64bcde</td>
<td>10.50bcde</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>10.44g</td>
<td>10.01e</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>11.68bcde</td>
<td>10.46bcde</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>10.60fg</td>
<td>10.45bcde</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>11.86abcde</td>
<td>11.30ab</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>12.72a</td>
<td>11.60a</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>10.99efg</td>
<td>10.23de</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>11.57bcdef</td>
<td>10.46bcde</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>11.39bcdefg</td>
<td>10.38bcde</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>12.34ab</td>
<td>11.20abc</td>
<td></td>
</tr>
<tr>
<td>Pak-81</td>
<td>11.32cdefg</td>
<td>11.03abcd</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>12.21abc</td>
<td>11.51a</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>11.10defg</td>
<td>10.33cde</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>11.29cdefg</td>
<td>10.85abcde</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>11.83abcde</td>
<td>10.25de</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>12.07abcd</td>
<td>11.45a</td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWF</td>
<td>Whole wheat flour</td>
</tr>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
</tr>
<tr>
<td>Varieties</td>
<td>Crude fat (%)</td>
</tr>
<tr>
<td>---------------------</td>
<td>---------------</td>
</tr>
<tr>
<td></td>
<td>WWF</td>
</tr>
<tr>
<td>C-273</td>
<td>1.30abc</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>0.92f</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>1.33ab</td>
</tr>
<tr>
<td>GA-02</td>
<td>1.00def</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>1.34ab</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>1.23c</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>0.97ef</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>1.02de</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>1.07d</td>
</tr>
<tr>
<td>MH-97</td>
<td>1.37a</td>
</tr>
<tr>
<td>Pak-81</td>
<td>1.22c</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>1.23c</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>1.27bc</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>1.32ab</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>1.36a</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>1.30abc</td>
</tr>
</tbody>
</table>
Means carrying same letters in a column are not significantly different from each other

WWF   Whole wheat flour
SGF   Straight grade flour
got statistically similar values for fat content in their whole wheat flours which were 1.23, 1.22 and 1.23%, respectively. The results presented in Table 4.8 also indicated that significantly the highest fat content (0.74%) was found in the SGF of Faisalabad 83 followed by Inqlab 91 (0.74%), Faisalabad 85 (0.73%), Punjab 85 (0.72%) and Uqab 2000 (0.70%). However, significantly the lowest fat content (0.51%) was found in SGF of MH-97 wheat variety.

The fat content is not only a good source of energy but also plays significant role in controlling the quality and shelf life of bread. The fat has been reported to influence the baking quality of flour probably due to its surfactant effects and interaction with wheat proteins (Matz, 1972). The results with respect to fat content in the present study are similar to the earlier studies of Afzal (2004) who reported variation in fat content from 0.86 to 0.98% in some Pakistani spring wheat varieties. These results are further supported by the findings of Ijaz (2001) who concluded that varietal differences (genotypes) exhibited significant effects on the fat content of different flours. The whole wheat flour possessed higher fat content than straight grade flour in the present study which might be due to the wheat germ portion which is remained present in case of WWF while it is removed from SGF during milling process.

4.2.5 Wet gluten

The results pertaining to wet gluten of different wheat varieties presented in Table 4.10 and their analysis of variance has been shown in Table 4.9. It is evident from the statistical results that wet gluten content differed significantly among different wheat varieties.

The wet gluten ranged significantly from 25.62 to 37.00 % in whole wheat flour of different wheat varieties. The wheat varieties C 273, Faisalabad 85, Iqbal 2000, Pasban 90, Faisalabad 83 and Punjab 96 possessed higher contents of wet gluten but with non significant differences with one another. The wet gluten content was observed lower in whole wheat flour of Kohistan 97, Kohinoor 83,
MH-97 and Punjab 81 wheat varieties. The wheat varieties Punjab 85, GA-02 and Uqab 2000 showed non significant differences in wet gluten content. The wet gluten ranged significantly from 28.47 to 41.09 % in SGF of different wheat varieties. Significantly the highest wet gluten (41.09%) was found in SGF of variety Faisalabad 85 followed by Pasban 90 (39.80%), Iqbal 2000 (38.83%), Faisalabad 83 (38.47%) and Punjab 96 (38.31%). However, significantly the lowest (28.47%) wet gluten content was found in SGF of Kohinoor 83 and Punjab 81 (31.28%). The wheat varieties Punjab 85, Uqab 2000 and GA-02 exhibited statistically similar amount of wet gluten.

The present study showed higher gluten content in SGF as compared to WWF which might be due to the fact that WWF flour has more extraction rate as compared to SGF and as the extraction rate decreases, the protein content decreases and consequently the gluten content is decreased.

Ahmad (2001) carried out research on Pakistani wheat varieties and reported significant differences in amount of wet gluten of different Pakistani wheat varieties which supports to significant variation in wet gluten among wheat varieties found in the present study. The results are also in consistent with the findings of Miralbes (2003) who found variation from 15.6 to 39.3 % in wet gluten of different wheat varieties. Paliwal and Singh (1985) also observed wet gluten range in 12.77 to 44.06% in Uttar Paradesh wheat varieties. The variation in wet gluten among the wheat varieties found in the present study may be attributed to the differences in genotypes and the environmental conditions like temperature and rainfall as reported by Wrigley et al., (1982). The results are supported by the findings reported by Lin et al. (2003) and Randhawa et al., (2002) who also found similar ranges for wet gluten of different wheats.
Table 4.9  Mean squares for wet gluten and dry gluten contents of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>Df</th>
<th>Wet gluten</th>
<th>Dry gluten</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WWF</td>
<td>SGF</td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>40.965208**</td>
<td>37.995931**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>2.0458938</td>
<td>2.7109771</td>
</tr>
</tbody>
</table>

** Significant at P≤0.01

WWF  Whole wheat flour
SGF  Straight grade flour
Table 4.10  Wet gluten content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Wet gluten (%)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>C-273</td>
<td>35.18ab</td>
<td>37.10bcd</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>34.46ab</td>
<td>38.47abc</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>37.00a</td>
<td>41.09a</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>30.35cd</td>
<td>34.35de</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>34.40ab</td>
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<td>Iqbal 2000</td>
<td>34.50ab</td>
<td>38.83abc</td>
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<td>Kohinoor 83</td>
<td>26.85ef</td>
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<td>Kohistan 97</td>
<td>25.62f</td>
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<td>Manthar 03</td>
<td>29.14de</td>
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<td>MH-97</td>
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<td>31.00fg</td>
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<tr>
<td>Pak-81</td>
<td>32.87bc</td>
<td>36.98bcd</td>
<td></td>
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<tr>
<td>Pasban 90</td>
<td>36.40a</td>
<td>39.80ab</td>
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<tr>
<td>Punjab 81</td>
<td>27.28ef</td>
<td>31.28fg</td>
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<td>Punjab 85</td>
<td>31.19cd</td>
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<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>34.39ab</td>
<td>38.31abc</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>31.13cd</td>
<td>35.18de</td>
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</table>

Means carrying same letters in a column are not significantly different from each other.
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWF</td>
<td>Whole wheat flour</td>
</tr>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
</tr>
</tbody>
</table>
4.2.6 Dry gluten

The statistical analysis regarding dry gluten content of both WWF and SGF given in Table 4.9 showed significant differences in dry gluten content of SGF and WWF among the tested wheat varieties.

The dry gluten content of different wheat varieties have been presented in Table 4.11. The dry gluten content in SGF of different wheat varieties ranged from 10.40 to 13.80% while it varied from 9.04 to 12.89% in WWF of different wheat varieties.

The dry gluten in whole wheat flour was found significantly the highest in wheat variety Faisalabad 85 (12.89%) followed by C-273 (12.51%) but both possessed non significant difference for this parameter. The wheat varieties Inqalab 91, Kohistan 97, MH-97, Pasban 90 and Punjab 81 were found to be statistically at par but placed at bottom with respect to dry gluten content in their whole wheat flours which was 9.75, 9.73, 9.35, 9.04 and 9.57%, respectively. It is obvious from the results that significantly the highest dry gluten content (12.89%) was found in the SGF of Faisalabad 85 followed by C-273 (13.60%) while the lowest dry gluten content (10.40%) was recorded in SGF of wheat variety MH-97 followed by Punjab 81 (10.43%). The wheat varieties GA-02, Inqalab 91, Kohinoor 83, Manthar 03, Pasban 90 and Punjab 81 were found to be statistically at par regarding dry gluten contents in SGF.

These results are comparable with the findings of Anjum and Walker (2000) who have reported significant variation in dry gluten contents among different Pakistani wheat varieties, which ranged from 8.88 to 10.09%. The gluten content of the protein determines the flour quality and has significant impact on bread making quality (Kent and Evers, 1994). The gluten content of different
<table>
<thead>
<tr>
<th>Varieties</th>
<th>WWF</th>
<th>SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>12.51ab</td>
<td>13.60ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>11.80bc</td>
<td>12.80c</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>12.89a</td>
<td>13.80a</td>
</tr>
<tr>
<td>GA-02</td>
<td>11.50cd</td>
<td>11.20efg</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>9.75fg</td>
<td>10.95efg</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>10.01ef</td>
<td>12.90bc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>11.89bc</td>
<td>10.49fg</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>9.73fg</td>
<td>12.16cd</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>11.48cd</td>
<td>11.05efg</td>
</tr>
<tr>
<td>MH-97</td>
<td>9.35fg</td>
<td>10.40g</td>
</tr>
<tr>
<td>Pak-81</td>
<td>11.27cd</td>
<td>12.33cd</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>9.04g</td>
<td>11.28ef</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>9.57fg</td>
<td>10.43fg</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>10.73de</td>
<td>11.73de</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>11.29cd</td>
<td>12.77c</td>
</tr>
<tr>
<td></td>
<td>Uqab 2000</td>
<td>10.62de</td>
</tr>
<tr>
<td>--------</td>
<td>-----------</td>
<td>---------</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.

- **WWF**: Whole wheat flour
- **SGF**: Straight grade flour
Pakistani wheat varieties has been found similar to those who observed differences among different wheat varieties ranging from 7-17% (Randhawa et al., 2002; Lin et al., 2003 and Pasha et al., 2007). In the present study the dry gluten content in SGF was found higher than their respective WWF. This difference may be ascribed to the fact that in case of SGF removal of bran portion results in the increase in the gluten forming proteins of resultant flour. The wheat varieties Pasban 90, Inqlab 91, Iqbal 2000, Faisalabad 85, C-273 and Faisalabad 83 possessing higher wet and dry gluten content may have better potential for bread making and should be used to explicit their potential in the development of new varieties by the wheat scientists.

4.2.7 Pelshenke value

The statistical results regarding Pelshenke value of both WWF and SGF given in Table 4.12 indicated highly significant effect of wheat varieties on Pelshenke value of whole wheat flour and straight grade flour.

The Pelshenke value ranged from 48 to 220 minutes in WWF while it ranged from 50.85 to 223 minutes in SGF of different wheat varieties Table 4.13.

It is also evident from the results that significantly maximum Pelshenke value (220 minutes) was measured in whole wheat flour sample of Pasban 90 wheat variety followed by C-273 (145.02 minutes). The whole wheat flour of wheat varieties Punjab 96, Punjab81, MH-97, GA-02, Kohinoor 83, Kohistan 97, Punjab 85 and Manthar 03 possessed statistically similar Pelshenke value. The Pelshenke value was found significantly the highest in the SGF of Pasban 90 (223 minutes) followed by C-273 (150.12 minutes) and Inqlab 91 (107.25 minutes). The Pelshenke value (52.03 minutes) was measured significantly lowest in SGF wheat variety Kohistan 97 (Table 4.13). The wheat varieties, Punjab 81, Punjab 96, MH-97, GA-02 and Kohistan 97 possessed statistically identical Pelshenke values for their SGF.

The Pelshenke test is also known as wheat meal fermentation time test and is one of the most important tests used by the wheat scientists to evaluate
Table 4.12  Mean squares for Pleshenke value, SDS value and Falling number contents of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Pleshenke value WWF</th>
<th>Pleshenke value SGF</th>
<th>SDS value WWF</th>
<th>SDS value SGF</th>
<th>Falling number WWF</th>
<th>Falling number SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>15</td>
<td>6581.8262**</td>
<td>6741.3203**</td>
<td>9.724883**</td>
<td>8.6190128**</td>
<td>22161**</td>
<td>31775.567**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>12.483823</td>
<td>13.035785</td>
<td>1.0741333</td>
<td>1.2126229</td>
<td>915.25953</td>
<td>719.37926</td>
</tr>
</tbody>
</table>

** Significant at P≤0.01

WWF  Whole wheat flour
SGF  Straight grade flour
Table 4.13  Pleshenke value of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Pleshenke value (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
</tr>
<tr>
<td>C-273</td>
<td>145.02b</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>48.00g</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>62.30e</td>
</tr>
<tr>
<td>GA-02</td>
<td>51.07fg</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>105.00c</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>96.10d</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>50.03fg</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>50.00fg</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>48.90fg</td>
</tr>
<tr>
<td>MH-97</td>
<td>53.50fg</td>
</tr>
<tr>
<td>Pak-81</td>
<td>91.95d</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>220.00a</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>54.05fg</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>49.00fg</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>54.88f</td>
</tr>
<tr>
<td>WWF</td>
<td>Whole wheat flour</td>
</tr>
<tr>
<td>---------</td>
<td>-------------------</td>
</tr>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
their breeding material for the assessment of gluten strength. The wheat flour quality primarily depends on the gluten strength which is capable of retaining gas and to produce soft, well aerated baked products. The wheats possessing higher gluten contents results in higher Pelshenke value. Williams et al. (1986) have described that the Pelshenke value less than 60 minutes reflects poor or soft gluten quality and the wheats with Pelshenke value ranging from 60-120 minutes are regarded as medium strong gluten while wheat with the Pelshenke values exceeding 120 minutes may exhibit strong gluten quality. The results regarding Pelshenke value obtained in the present study are in close agreement with the earlier findings of Randhawa et al., (2002) who found Pelshenke values varying from 108-185 minutes among different Pakistani wheat varieties. The results reported by Zahoor (2003) also support the present findings who reported that Pelshenke value was significantly affected due to differences in varieties. The wheat variety Pasban 90 may have strong gluten proteins and should be used for exploit this trait in new genotypes.

4.2.8 SDS-Sedimentation Value

The SDS-Sedimentation value in both type of flours i.e. WWF and SGF differed significantly among different wheat varieties.

The SDS-Sedimentation value of different wheat varieties presented in Table 4.14 indicated that SDS-Sedimentation value in WWF ranged from 20.00 to 26.70 mL while it ranged from 21.48 to 28.14 mL in SGF of different wheat varieties.

The results further indicated that significantly the highest SDS-Sedimentation value (26.70 mL) was recorded in whole wheat flour samples of Inqlab 91 wheat variety followed by Iqbal 2000 (25.09 mL) and Pak 81 (25.00mL). The WWF of wheat varieties C-273, Kohinoor 83, Kohistan 97, Manthar 03 and Punjab 81 was found statistically at par with respect to SDS-Sedimentation value. It is also obvious from the results presented in the Table.
Table 4.14  SDS sedimentation value of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>SDS (mL)</th>
<th>WWF</th>
<th>SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>21.23ef</td>
<td>23.03ef</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>23.15bcde</td>
<td>24.64bcde</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>22.00de</td>
<td>23.47def</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>23.32bcd</td>
<td>24.82bcde</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>26.70a</td>
<td>28.14a</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>25.09ab</td>
<td>25.67bc</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>21.32ef</td>
<td>22.83ef</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>21.30ef</td>
<td>22.82ef</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>20.00f</td>
<td>21.48f</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>22.33cde</td>
<td>23.99cde</td>
<td></td>
</tr>
<tr>
<td>Pak-81</td>
<td>25.00ab</td>
<td>26.48ab</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>24.40b</td>
<td>25.82bc</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>21.31ef</td>
<td>22.82ef</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>23.32bcd</td>
<td>24.81bcde</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>22.00de</td>
<td>23.49def</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>24.17bc</td>
<td>25.4bcd</td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

- WWF  Whole wheat flour
- SGF  Straight grade flour
4.14 that SGF of Inqlab 91 possessed significantly the highest SDS-Sedimentation value (28.14 mL) while SGF of wheat variety Manthar 03 exhibited the lowest SDS-Sedimentation Value (21.48 mL). The SDS-Sedimentation value in SGF of wheat varieties C-273, Faisalabad 85, Kohinoor 83, Kohistan 97, Manthar 03, Punjab 81 and Punjab 96 did not differ significantly with respect to one another.

The SDS-sedimentation value is an estimate of the protein strength relating to the wheat quality which depends on the degree of hydration and degree of oxidation of proteins in the wheat. More SDS-sedimentation volume (more than 30 ml) indicates more strength of protein (Williams et al. 1986) and will be better for bread. The results of the present study are in conformity to the earlier findings of Pasha et al., (2007) who reported that SDS sedimentation value of Pakistani wheats ranged from 18.83 to 36.0 mL. Coskuner and Karababa (2005) and Deng et al. (2005) also reported the range of SDS-sedimentation value from 22.5 to 28.5 ml and 42.5 to 59.6 mL, respectively. The differences in the present results might be due to the differences in genetic makeup and variation in growth conditions of wheat varieties tested in different studies. It may be concluded from the present investigations that the wheat varieties on the basis of SDS-sedimentation value may be regarded as medium strong proteins. The wheat variety Inqlab 91 possessing the highest sedimentation value may be used by different stakeholders for their intended purposes.

### 4.2.9 Falling number

The results regarding mean squares for falling number of whole wheat flour (WWF) and straight grade flours (SGF) of different wheat varieties presented in Table 4.12 indicated that the falling number differed significantly in both type of flours among different wheat varieties.

The falling number ranged from 529 to 828 seconds and 400.50 to 775.50 seconds between the straight grade flour (SGF) and whole wheat flour (WWF), respectively of different wheat varieties (Table 4.15). The falling number in
Table 4.15  Falling number of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>WWF</th>
<th>SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>657.00bc</td>
<td>749.00bc</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>535.00f</td>
<td>548.00h</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>656.50bcd</td>
<td>754.00b</td>
</tr>
<tr>
<td>GA-02</td>
<td>643.50bcd</td>
<td>727.50bcd</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>450.00hi</td>
<td>670.00e</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>400.50j</td>
<td>694.50cde</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>630.00bcde</td>
<td>665.50e</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>676.00b</td>
<td>732.00bcd</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>775.50a</td>
<td>828.00a</td>
</tr>
<tr>
<td>MH-97</td>
<td>580.55e</td>
<td>607.50fg</td>
</tr>
<tr>
<td>Pak-81</td>
<td>600.55de</td>
<td>692.00de</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>425.00ij</td>
<td>573.00gh</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>610.50cde</td>
<td>699.00bcde</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>500.00fg</td>
<td>529.50h</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>600.50de</td>
<td>643.00ef</td>
</tr>
<tr>
<td>Variety</td>
<td>Mean 1</td>
<td>Mean 2</td>
</tr>
<tr>
<td>---------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>480.00gh</td>
<td>547.50h</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.

- **WWF**  Whole wheat flour
- **SGF**  Straight grade flour
straight grade flour of different wheat varieties was found higher than that of falling number of whole wheat flours of respective wheat varieties. The results further delineated that significantly the highest falling number 828 seconds was recorded in straight grade flour of Manthar 03 wheat variety followed by Faisalabad 85 (754 seconds) and C-273 (749 seconds), Kohistan 97 (732 seconds) and Punjab 81 (699 seconds). The SGF of wheat varieties Faisalabad 83, Uqab 2000 and Punjab 85 exhibited non significant differences in the falling number. It is also evident from the results (Table 4.15) that significantly the highest falling number (775.50 seconds) was recorded in WWF of Manthar 03 wheat variety while the lowest falling number (400.50 seconds) was recorded in WWF of wheat variety Iqbal 2000. The falling number in WWF of wheat varieties Pak 81 and Punjab 96 did not differ significantly.

The falling number is an indicator of $\alpha$-amylase activity in wheat flour. The falling number values for wheat varieties exceeding 400 seconds had very low or no alpha amylase activity. Mailhot and Patton (1988) reported that all types of bread flours should possess falling number values between 200-300 seconds. The results of the present study are in line with the findings of Anjum (1991), Butt (1997), Wahab (2005), Pasha et al., (2007) and Zahoor (2003) who have also demonstrated that Pakistani wheats are low in amylase activity as indicated by their falling numbers exceeding 400 sec. The present study also showed that WWF possessed lower falling number as compared to SGF. This might be due to the reason that WWF containing bran portion have more $\alpha$ amylase activity which results in decreasing the falling number as this explanation has been supported by the findings of Every et al. (2002) who held that presence of more $\alpha$ amylase in flours results in decreasing its falling number.
4.3 MINERAL CONTENT OF DIFFERENT WHEAT VARIETIES

4.3.1 Zinc

The statistical results for zinc content in whole wheat flour (WWF) and straight grade flours (SGF) of different wheat varieties have been presented in Table 4.16. It is obvious from these statistical results that the zinc content both in WWF and SGF was affected significantly by the wheat varieties.

The zinc content ranged from 1.55 to 3.40 mg/100g and 0.89 to 2.10 mg/100g between whole wheat and straight grade flours, respectively of different wheat varieties. The zinc content was found higher in WWF than the straight grade flours (Table 4.17).

It is evident from the results that significantly the highest zinc content (3.40 mg/100g) was recorded in whole wheat flour of Iqbal 2000 wheat variety followed by MH-97 (3.03 mg/100g). The wheat varieties Faisalabad 85, GA-02, Kohistan 97, Manthar 03 and Punjab 81 possessed statistically similar content of zinc in their WWF. The results in (Table4.17) indicated that significantly the highest zinc content (2.10 mg/100g) was measured in the SGF of Iqbal 2000 while the lowest zinc content (0.89 mg/100g) was observed in SGF of Pasban 90 and Punjab 96. The zinc contents in SGF of wheat varieties C-273, Faisalabad 83 and Punjab 85 did not differ significantly with one another.

The results of the present study regarding zinc content of different wheat varieties are in line to the earlier findings of Wahab (2003) who reported that zinc content ranged from 31.35 to 33.52 ppm in wheat varieties cultivated in Sarhad province. The studies of Anjum et al. (2002) also support the present results as the concentration of zinc was found by them ranged 9.0 to 81ppm in some Pakistani spring wheat varieties. However, slight variation between the results of present and earlier studies might be due to differences in genetic makeup of wheat varieties and agro climatic factors experienced by the varieties tested in different studies. In the present study the zinc content in whole wheat flour of wheats was
Table 4.16  Mean squares for zinc and iron contents of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Zinc WWF</th>
<th>Zinc SGF</th>
<th>Iron WWF</th>
<th>Iron SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>15</td>
<td>0.8507988**</td>
<td>0.5768467**</td>
<td>1.3561443**</td>
<td>0.3078083**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.0064146</td>
<td>0.0042333</td>
<td>0.0282542</td>
<td>0.0111396</td>
</tr>
</tbody>
</table>

** Significant at P≤0.01

WWF  Whole wheat flour
SGF  Straight grade flour
Table 4.17  Zinc content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Zinc (mg/100g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>C-273</td>
<td>1.64g</td>
<td>1.10gh</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>1.89f</td>
<td>1.20fg</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>2.30c</td>
<td>1.80c</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>2.40c</td>
<td>2.00ab</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>2.12e</td>
<td>1.82c</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>3.40a</td>
<td>2.10a</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>1.87f</td>
<td>1.24ef</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>2.30c</td>
<td>1.03h</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>2.28cd</td>
<td>2.00ab</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>3.03b</td>
<td>1.95b</td>
<td></td>
</tr>
<tr>
<td>Pak-81</td>
<td>1.67g</td>
<td>1.01h</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>1.29h</td>
<td>0.89i</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>2.30c</td>
<td>1.50d</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>1.93f</td>
<td>1.11gh</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>1.55g</td>
<td>0.90i</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>2.15de</td>
<td>1.32e</td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

WWF  Whole wheat flour
SGF  Straight grade flour
found higher than SGF which might be related to the presence of bran portion in whole wheat flour which is rich in minerals than the rest of grain portion.

Zinc is an essential component of enzymes that participate in the synthesis as well as metabolism of carbohydrates, lipids, proteins, nucleic acids and other nutrients. It also stabilizes muscular structure, cellular components and membrane playing an essential role in polynucleotide transcription and immune system. Dietary deficiency of zinc in human causes growth retardation, delayed sexual and bone maturation, skin lesions, impaired appetite, increased susceptibility to infections (Hambidge, 1987). The higher zinc contents in the WWF of wheats can be explored as an added benefit over the SGF produced from the same wheats. The wheat varieties showing relatively higher zinc content (3.40mg/100g, 3.03mg/100g) should also be given more emphasize for further commercial exploitation as it is one of the deficient micronutrient in the world and Pakistan has no exception to it. The wheat varieties with higher zinc content may help to overcome this problem.

4.3.2 Iron

The iron content in WWF and SGF varied significantly among different wheat varieties (Table 4.16). The iron content in WWF of different wheat varieties ranged from 3.38 to 5.56 mg/100g while it ranged from 1.98 to 3.02 mg/100g in SGF of different wheat varieties (Table 4.18).

The iron content was recorded significantly the highest in whole wheat flour samples of Inqlab 91 wheat variety (5.56 mg/100g) followed by Iqbal 2000 (5.30 mg/100g) but with non significant differences for iron content between two varieties. The iron content of wheat varieties Manthar 03, MH-97, Pasban 90 and Punjab 85 did not differ significantly. It is also obvious from the results that significantly the highest iron content was found in SGF of Inqlab 91 (3.02 mg/100g) followed by Iqbal 2000 (2.90 mg/100g) and Punjab 96 (2.27 mg/100g). The iron content was found significantly lowest (1.98 mg/100g) in SGF of wheat
variety MH-97. The iron content did not differ significantly in SGF of wheat varieties Faisalabad 85, GA-02, Kohinoor 83, Kohistan 97, Pak-81, Manthar 03, MH 97 and Pasban 90.

The wheat flour containing higher amount of iron content is beneficial to the population suffering with anemia which may help to alleviate iron deficiency anemia (IDA). Iron deficiency anemia reduces a person’s ability to perform physically demanding tasks and anemic labourers have demonstrated productivity (Basta et al., 1990). The results of the present study with respect to iron content fall within the ranges reported by MacGrath (1995) who found iron content ranging from 12.07 to 73.62 ppm in UK wheat samples. Ragaee et al. (2006) have also reported iron content 13.20 ppm in hard wheats and 13.9 ppm in soft wheats. The results in this study are also supported by the work done by Pasha (2006), Anjum (2002) and Wahab (2005) who reported significant differences in iron content among wheat varieties. Iron deficiency anemia is one of the identified micronutrient deficient in all over the world including Pakistan. The most vulnerable segment of the population suffering from IDA is children below 5 years, pregnant and lactating women. Therefore the wheat varieties possessing higher content of iron such as Inqlab 91, C-273 and Iqbal 2000 should be explored for consumption which can help to reduce the deficiency of this micronutrient in our country. It may also be advocated to take whole wheat flour as compared to straight grade flour since WWF contained significantly higher amount of iron as compared to SGF. The wheat varieties identified in the present study may also be exploited for development of new wheat varieties with improved iron content. This approach of biofortification seems more feasible to overcome this IDA problem in Pakistan.
Table 4.18  Iron content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Iron (mg/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
</tr>
<tr>
<td>C-273</td>
<td>5.04bc</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>3.71ef</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>4.29d</td>
</tr>
<tr>
<td>GA-02</td>
<td>4.80c</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>5.56a</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>5.30ab</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>4.20d</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>4.19d</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>3.60efg</td>
</tr>
<tr>
<td>MH-97</td>
<td>3.63efg</td>
</tr>
<tr>
<td>Pak-81</td>
<td>3.85e</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>3.38g</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>4.75c</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>3.51fg</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>4.25d</td>
</tr>
<tr>
<td><strong>Uqab 2000</strong></td>
<td><strong>3.78ef</strong></td>
</tr>
<tr>
<td>----------------</td>
<td>----------------</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.

- **WWF**    Whole wheat flour
- **SGF**    Straight grade flour
4.3.3 Copper

The mean squares shown in Table 4.19 indicated highly significant differences in the copper content for both type of flours i.e. WWF and SGF of different wheat varieties. The copper content in whole wheat flour of different wheat varieties varied significantly from 0.23 to 1.08 mg/100g (Table 4.20). Significantly the highest copper content (1.08 mg/100g) was found in WWF of Manthar 03 while significantly the lowest copper content was observed in the WWF of Pak 81 and Pasban 90. The wheat varieties showed similar trend of significance level for copper content with respect to their straight grade flours. The copper content in SGF of different wheat varieties varied significantly from 0.11 to 0.25 mg/100g. The copper content in SGF of wheat varieties Kohinoor 83, Kohistan 97, Punjab 85 and Uqab 2000 was not found statistically different.

Copper is an essential nutrient for the maintenance of normal hemoglobin status and it is also a part of many enzyme systems. The results of present study are in close agreement with the findings of earlier studies of Anjum et al. (2002) who held that copper content in whole wheat flour of some Pakistani wheats ranged from 10.00 to 17.50 ppm. Wahab (2003) found variation from 9.72 to 11.01 ppm in copper content of different Pakistani wheats. Lopez et al. (2003) reported 1.2 ppm copper content in whole wheat flour. Similarly Pasha (2006) found significant variation in copper content among different Pakistani wheat varieties. Significant variation in the wheat varieties tested in the present study indicated that wheat varieties containing higher concentration in WWF such as Manthar 03, C-273, Punjab 96 and Uqab 2000 should be used as a source of high copper content in the biofortification programmes to be taken up for the improvement of copper content in new varieties.
### Table 4.19  Mean squares for copper, manganese and calcium contents of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Cu</th>
<th>Mn</th>
<th>Ca</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td>WWF</td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>0.2153899**</td>
<td>0.0063654**</td>
<td>2.67288**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>5.75e-4</td>
<td>5.8333e-5</td>
<td>0.0298083</td>
</tr>
</tbody>
</table>

**Significant at P≤0.01

WWF  Whole wheat flour
SGF  Straight grade flour
Table 4.20  Copper content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Copper (mg/100g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>C-273</td>
<td>0.90b</td>
<td>0.25a</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>0.33h</td>
<td>0.11fg</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>0.58f</td>
<td>0.15d</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>0.80c</td>
<td>0.20b</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>0.34h</td>
<td>0.13e</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>0.69de</td>
<td>0.15d</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>0.23i</td>
<td>0.17c</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>0.35h</td>
<td>0.17c</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>1.08a</td>
<td>0.25a</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>0.44g</td>
<td>0.15d</td>
<td></td>
</tr>
<tr>
<td>Pak-81</td>
<td>0.21ij</td>
<td>0.10g</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>0.17j</td>
<td>0.12ef</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>0.34h</td>
<td>0.11fg</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>0.61f</td>
<td>0.17c</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>0.71d</td>
<td>0.20b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Uqab 2000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-----------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>WWF</td>
<td>Whole wheat flour</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other
4.3.4 Manganese

The analysis of variance regarding the manganese content in whole wheat flour (WWF) and straight grade flours (SGF) of different wheat varieties have been presented in Table 4.19. It is evident from the statistical results that the manganese content differed significantly among WWF and SGF of different wheat varieties.

The results depicted in Table 4.21 showed that the manganese content ranged significantly from 2.30 to 5.50 mg/100g and 0.81 to 1.97 mg/100g between whole wheat flour and straight grade wheat flour of different wheat varieties, respectively. The whole wheat flour possessed higher manganese content as compared to straight grade flour of respective wheat variety.

The results showed significantly the highest amount of manganese content (5.50 mg/100g) in whole wheat flour of wheat variety Iqbal 2000 followed by Faisalabad 85 (5.45 mg/100g), Kohinoor 83 (5.40 mg/100g) and C-273 (5.21mg/100g). The wheat varieties C-273, Faisalabad 85, Iqbal 2000, Kohinoor 83 and Punjab 96 were found to be at top and wheat varieties Faisalabad 83, Kohistan 97, Manthar 03, Pasban 90 and Punjab 85 were ranked at the bottom with respect to manganese content in their WWF. It is also evident from the results (Table 4.21) that significantly the highest manganese content was measured in the SGF of Faisalabad 85 (1.97 mg/100g) and Iqbal 2000 (1.88mg/100g) while the lowest manganese content (0.81 mg/100g) was yielded by the SGF of wheat variety Faisalabad 83. The manganese content in SGF of wheat varieties Kohistan 97, Manthar 03 and MH-97 was found non significantly different with one another.
Table 4.21  Manganese content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mn (mg/100g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>C-273</td>
<td>5.21ab</td>
<td>1.81bc</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>2.30i</td>
<td>0.81h</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>5.45a</td>
<td>1.97a</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>4.98bc</td>
<td>1.72cde</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>5.03bc</td>
<td>1.73cde</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>5.50a</td>
<td>1.88ab</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>5.40a</td>
<td>1.79bcd</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>3.78f</td>
<td>1.34f</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>4.18e</td>
<td>1.44f</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>3.74f</td>
<td>1.34f</td>
<td></td>
</tr>
<tr>
<td>Pak-81</td>
<td>4.80cde</td>
<td>1.60e</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>3.40g</td>
<td>1.20g</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>5.05bc</td>
<td>1.67de</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>3.11h</td>
<td>1.12g</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>4.81cd</td>
<td>1.70cde</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------</td>
<td>--------</td>
<td>--------</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>4.52d</td>
<td>1.64e</td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>WWF</td>
<td>Whole wheat flour</td>
</tr>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
</tr>
</tbody>
</table>
Pasha (2006) reported variation from 24.22 to 57.54 ppm in Pakistani wheats which well supports to the present results pertaining to managanese content in WWF of different wheat varieties. McGrath (1995) also found concentration of manganese in different wheat samples ranging from 9.93 to 40.88 ppm which also confirms the present results. Wahab (2003) and Anjum et al. (2002) reported significant varietal differences for the manganese content of different wheats. The present study also revealed that manganese content was found higher in WWF as compared to SGF of different wheat varieties which can be attributed to the presence of bran in WWF.

4.3.5 Calcium

The statistical analysis regarding calcium content of whole wheat flour and straight grade flours of different wheat varieties presented in Table 4.19 indicated that calcium content was significantly affected due to differences in wheat varieties.

The calcium content ranged significantly from 38.01 to 63.52 mg/100g in WWF of different wheat varieties (Table 4.22). The wheat varieties C 273, Pak-81 and Inqlab 91 possessed significantly higher calcium content. The calcium content was found significantly lower in WWF of wheat varieties GA-02 and Kohistan 97 whereas the calcium content in WWF of wheat varieties Faisalabad 85, Kohinoor 83, MH-97, Punjab 85 and Uqab 2000 differed non significantly with one an other for this attribute. The results further showed that the calcium content varied from 17.90 to 30.81 mg/100g in SGF of different wheat varieties. Significantly the highest calcium content was recorded in SGF of wheat variety C-273 (30.81 mg/100g) followed by Pak-81(28.77 mg/100g) and Inqlab 91(27.11
Table 4.22  Calcium content of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Calcium (mg/100g)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
<td>SGF</td>
<td></td>
</tr>
<tr>
<td>C-273</td>
<td>63.52a</td>
<td>30.81a</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>44.20ghi</td>
<td>21.02gh</td>
<td></td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>49.63ef</td>
<td>24.01ef</td>
<td></td>
</tr>
<tr>
<td>GA-02</td>
<td>38.60j</td>
<td>17.90i</td>
<td></td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>57.02bc</td>
<td>27.11bc</td>
<td></td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>52.52de</td>
<td>25.06de</td>
<td></td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>48.72f</td>
<td>23.15f</td>
<td></td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>38.01j</td>
<td>18.31i</td>
<td></td>
</tr>
<tr>
<td>Manthar 03</td>
<td>42.50i</td>
<td>20.13h</td>
<td></td>
</tr>
<tr>
<td>MH-97</td>
<td>47.62f</td>
<td>22.75fg</td>
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<tr>
<td>Pak-81</td>
<td>60.17b</td>
<td>28.77b</td>
<td></td>
</tr>
<tr>
<td>Pasban 90</td>
<td>43.21hi</td>
<td>20.70h</td>
<td></td>
</tr>
<tr>
<td>Punjab 81</td>
<td>53.60d</td>
<td>6.30cd</td>
<td></td>
</tr>
<tr>
<td>Punjab 85</td>
<td>46.37fgh</td>
<td>22.68fg</td>
<td></td>
</tr>
<tr>
<td>Punjab 96</td>
<td>55.35cd</td>
<td>26.15cd</td>
<td></td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>47.08fg</td>
<td>22.94f</td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other
<table>
<thead>
<tr>
<th>WWF</th>
<th>Whole wheat flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
</tr>
</tbody>
</table>
mg/100g) and the lowest calcium content was found in the SGF of GA-02 and Kohistan 97.

The results regarding the calcium content in straight grade wheat flours are supported by the findings of Ragaee et al. (2006) who reported 159.50 mg/kg calcium content in hard wheats and 202.2 mg/kg in soft wheats. Lopez et al. (2003) have reported 348.0 mg/kg calcium content in whole wheat flour. Hussain (1985) reported that whole wheat flour contained 430 mg/kg calcium. Wahab (2003) and Pasha (2006) also found calcium content in the same ranges as observed in the present studies. The slight differences in the results may be attributed to the differences in genetic make up of varieties and climatic as well as agronomic conditions of the varieties tested in different studies.

With respect to mineral content in WWF and SGF of different wheat varieties, it is evident that though significant variation existed among wheat varieties but the concentration of minerals was found higher in WWF as compared to SGF. It may be concluded that use of WWF over SGF will provide more minerals in the diet. The wheat possessing higher concentration of these micronutrients should be explored for commercial exploitation in biofortification programmes to alleviate the micronutrient deficiencies prevailing in our country.

4.4 FARINOGRAPHIC CHARACTERISTICS OF WHEAT VARIETIES

The farinograph is a sensitive instrument which provides two informations, the absorption or the amount of water required for dough to reach a definite consistency and secondly a general profile of the mixing behavior of the dough. The flour from strong wheat varieties possessed the ability to absorb and retain larger amounts of water (Pyler, 1988)

The mean squares regarding physical dough properties such as water absorption (WA), dough development time (DDT) and dough stability time (DST) derived from farinograms of WWF and SGF of different wheat varieties
Table 4.23  Mean squares for water absorption, dough development time and dough stability time of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
<th></th>
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<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>WA</td>
<td>DDT</td>
<td>DST</td>
<td>WWF</td>
<td>SGF</td>
<td>WWF</td>
<td>SGF</td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>36.18115**</td>
<td>7.094226**</td>
<td>6.812839**</td>
<td>7.683173**</td>
<td>17.18307**</td>
<td>17.07286**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>6.0101146</td>
<td>6.7830125</td>
<td>0.0139354</td>
<td>0.05105</td>
<td>0.0432708</td>
<td>0.1017292</td>
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</tr>
</tbody>
</table>

** Significant at P≤0.01

WWF    Whole wheat flour
SGF    Straight grade flour
Table 4.24  Water absorption of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Water absorption (%)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
</tr>
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<td>Faisalabad 83</td>
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<td>65.41bcdef</td>
</tr>
<tr>
<td>GA-02</td>
<td>59.42gh</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>67.37abcd</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>71.23a</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>61.54fgh</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>64.79cdef</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>63.78cdefg</td>
</tr>
<tr>
<td>MH-97</td>
<td>69.56ab</td>
</tr>
<tr>
<td>Pak-81</td>
<td>63.39defg</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>66.42bcde</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>62.16efgh</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>63.22defg</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>66.25bcdef</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>68.38abc</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

- **WWF**  Whole wheat flour
- **SGF**  Straight grade flour
<table>
<thead>
<tr>
<th>Varieties</th>
<th>Dough development time (minutes)</th>
<th>WWF</th>
<th>SGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td></td>
<td>3.45d</td>
<td>4.95c</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td></td>
<td>1.50k</td>
<td>2.75f</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td></td>
<td>2.80f</td>
<td>4.20d</td>
</tr>
<tr>
<td>GA-02</td>
<td></td>
<td>2.25i</td>
<td>3.65e</td>
</tr>
<tr>
<td>Inqalab 91</td>
<td></td>
<td>3.45d</td>
<td>4.95c</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td></td>
<td>2.50gh</td>
<td>4.00de</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td></td>
<td>7.70a</td>
<td>9.20a</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td></td>
<td>3.75c</td>
<td>5.00c</td>
</tr>
<tr>
<td>Manthar 03</td>
<td></td>
<td>2.35hi</td>
<td>3.85de</td>
</tr>
<tr>
<td>MH-97</td>
<td></td>
<td>2.37hi</td>
<td>3.70e</td>
</tr>
<tr>
<td>Pak-81</td>
<td></td>
<td>3.25de</td>
<td>4.70c</td>
</tr>
<tr>
<td>Pasban 90</td>
<td></td>
<td>5.45b</td>
<td>6.95b</td>
</tr>
<tr>
<td>Punjab 81</td>
<td></td>
<td>1.75j</td>
<td>2.45f</td>
</tr>
<tr>
<td>Punjab 85</td>
<td></td>
<td>3.45d</td>
<td>4.95c</td>
</tr>
<tr>
<td>Punjab 96</td>
<td></td>
<td>3.20e</td>
<td>4.70c</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td><strong>Uqab 2000</strong></td>
<td><strong>2.60g</strong></td>
<td><strong>4.00de</strong></td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

- **WWF**  Whole wheat flour
- **SGF**  Straight grade flour
Table 4.26  Dough stability time of whole and straight grade flours of different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Dough stability time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WWF</td>
</tr>
<tr>
<td>C-273</td>
<td>7.15c</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>3.85hi</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>3.70i</td>
</tr>
<tr>
<td>GA-02</td>
<td>3.50i</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>6.15e</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>5.85e</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>11.40a</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>5.85e</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>4.10h</td>
</tr>
<tr>
<td>MH-97</td>
<td>3.67i</td>
</tr>
<tr>
<td>Pak-81</td>
<td>5.45f</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>10.20b</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>2.65j</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>6.60d</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>4.65g</td>
</tr>
<tr>
<td></td>
<td>4.85g</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td><strong>Uqab 2000</strong></td>
<td></td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other

<table>
<thead>
<tr>
<th>WWF</th>
<th>Whole wheat flour</th>
</tr>
</thead>
<tbody>
<tr>
<td>SGF</td>
<td>Straight grade flour</td>
</tr>
</tbody>
</table>
have been shown in Table 4.23. It is evident from the results that physical dough properties derived from farinograms differed significantly as a function of varietal differences.

The results regarding WA, DDT, and DST presented in Tables 4.24, 4.25, and 4.26 showed existence of wide and significant variation among the WWF and SGF of different wheat varieties. The WA in WWF of different varieties was found significantly the highest in wheat variety Iqbal 2000 (71.23%), MH 97 (69.56%) and Uqab 2000 (68.38%) while significantly the lowest WA was given by the WWF of Faisalabad 83, GA 02, Kohinoor 83 and Punjab 81 but with non-significant differences. The SGF of wheat varieties Iqbal 2000, Pak-81, Kohinoor 83, Kohistan 97 and Uqab 2000 showed maximum but statistically similar level of water absorption (WA). The wheat variety Faisalabad 83 showed minimum value of WA.

The dough development time in WWF and SGF of different wheat varieties ranged significantly from 1.50 to 7.70 minutes and 2.75 to 9.20 minutes, respectively. The DDT was recorded significantly the highest in WWF and SGF of wheat variety Kohinoor 83 and significantly the lowest DDT was recorded in the WWF of Faisalabad 83 (1.50 min.) and SGF of Punjab 81 (2.45 min.). The SGF of wheat varieties C-273, Inqlab 91, Kohistan 97, Pak-81, Punjab 85 and Punjab 96 showed non-significant differences in DDT.

Similarly, the dough stability in WWF and SGF of different wheat varieties showed significant variation. The highest DST (11.40 min.) was observed in WWF of Kohinoor 83 while the lowest (2.65 min.) DST was recorded in WWF of wheat variety Punjab 81. The DST ranged from 3.75 to 12.45 min in SGF of different wheat varieties. The results further substantiated that wheat varieties Faisalabad 83, Faisalabad 85, GA 02 and MH-97 were found to be statistically identical with respect to DST of SGF.
The quality of end product largely depends upon the dough rheology taking place during the processing of different constituents (Lindahl, 1990). The rheological properties of dough are affected by the arrangement and interaction of components (especially proteins) and the structure of materials (Bushuk, 1985). The rheological and mechanical properties of the dough exert great impact on the overall quality of baked products (Bloksha and Bushuk, 1988). The nature and amount of ingredients, mixing time and beating conditions are also responsible for the quality of batter which finally determines the quality of the baked product (Baixauli et al., 2007). The quality and quantity of the proteins influence the water absorption capacity of the dough (Finney, 1984). The results of the farinographic studies derived in the present study are in consistent with the earlier findings of Wahab (2004) who reported that WA, DDT and DST in WWF of different wheat varieties grown in NWFP ranged from 54.9 to 63.7%, 2.8 to 8.6 min. and 6.98 to 19 min., respectively. The present results are also in line with the results of Anjum (1991), Butt et al. (1997) and Rehman et al. (2002) who also found similar results for these dough properties. However, the results of present study are slightly different from the ranges reported by Prabhasankar et al. (2002) who concluded that WA and DDT of some Indian wheat varieties ranged from 72.5% to 81.0% and 5.0 to 7.0 minutes, respectively. The differences in these results may be due to the variation in agro climatic factors, genetic makeup and protein contents of Pakistani and Indian wheat varieties tested in different studies.

The results in the present study indicated that WWF of different wheat varieties exhibited higher water absorption and lower dough development time as well as dough stability time compared to SGF of respective wheat varieties. The variation in water absorption in WWF may ascribed to the fact that WWF contains bran portion which is rich in cellulosic and hemicellulosic material considered to be responsible for holding more water. Similarly the bran portion in WWF contributes towards diluting gluten content and also the particle size
and sharp edges of the bran interfere during mixing which may cause in declining the dough development time and dough stability in WWF as compared to SGF. The present study suggests that physical dough properties studied for different wheat varieties provide ample information to scientists and bakers for selection of variety suitable for intended use.

4.5 SENSORY CHARACTERISTICS OF BREAD

4.5.1 Loaf volume

The mean squares for scores assigned to loaf volume of breads prepared from SGF of different wheat varieties have been presented in Table 4.27. The results indicated a significant variation in loaf volume scores of breads as a function of varietals differences.

It is evident from Table 4.28 that significantly the highest scores (7.80) were assigned to loaf volume of breads prepared from flour of Pak-81 followed by Pasban 90 (7.50), MH 97, Inqlab 91, Punjab 85, Iqbal 2000 and C-273 with non significant differences to one another. Significantly the lowest scores (6.00) for loaf volume were given by the panelist to the breads prepared from wheat variety Manthar 03 and GA 02. The breads prepared from wheat varieties C-273, Faisalabad 85, Inqlab 91, Iqbal 2000, Kohinoor 83, MH-97, Pasban 90, Punjab 85, Punjab 96 and Uqab 2000 got lower scores for loaf volume but with non significant differences.

4.5.2 Crust color

The analysis of variance for scores given to crust color of breads showed existence of significant differences in scores assigned to crust color of breads prepared from different wheat varieties (Table 4.27).

The scores for crust color of breads presented in (Table4.28) indicated that significantly the highest scores were given to crust color of the breads prepared
Table 4.27  Mean squares for loaf volume and crust color of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.0755086**</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.3149037**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.1718181</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1141281</td>
</tr>
</tbody>
</table>

** Significant at P≤0.01
Table 4.28  Loaf volume and crust color scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Loaf volume</th>
<th>Crust color</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>7.35abc</td>
<td>6.20a</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>6.80cd</td>
<td>5.80abcd</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>7.25abc</td>
<td>5.70abcd</td>
</tr>
<tr>
<td>GA-02</td>
<td>6.25de</td>
<td>5.35d</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>7.40abc</td>
<td>5.90abc</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>7.33abc</td>
<td>5.90abc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>7.00bc</td>
<td>5.45cd</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>6.81cd</td>
<td>5.81abcd</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>6.00e</td>
<td>5.60bcd</td>
</tr>
<tr>
<td>MH-97</td>
<td>7.32abc</td>
<td>5.74abcd</td>
</tr>
<tr>
<td>Pak-81</td>
<td>7.80a</td>
<td>6.20a</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>7.50ab</td>
<td>6.08ab</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>6.80cd</td>
<td>5.50cd</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>7.40abc</td>
<td>5.80abcd</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>7.00bc</td>
<td>5.55cd</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>7.20abc</td>
<td>5.70abcd</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other
from wheat varieties C-273, Pak-81, Iqbal 2000, Inqalab 91, Kohistan 97, Pasban 90 and Uqab 2000 but all these wheat varieties possessed non significant differences for this sensory parameter of bread. The lowest scores (5.35) were given to crust color of breads prepared from wheat variety GA-02, whereas the wheat varieties Faisalabad 83, Faisalabad 85, Inqalab 91, Iqbal 2000, Kohinoor 83, Kohistan 97, Manthar 03 and MH-97, Punjab 81, Punjab 85, Punjab 96 and Uqab 2000 were ranked statistically at the same level with respect to crust color by the judges.

4.5.3 Bread symmetry

The results pertaining to statistical analysis of scores assigned to symmetry of breads prepared from different wheat varieties have been presented in Table 4.29. It is evident from the results that highly significant affect on scores assigned to symmetry of breads existed by the different wheat varieties.

It is also obvious from the results (Table 4.30) that scores assigned to symmetry of breads ranged from 2.30 to 1.90 scores for different wheat varieties. The wheat varieties Pak-81 and Pasban 90 were ranked at top (2.30) for symmetry by the judges, whereas the wheat variety Punjab 96 was ranked at the bottom with respect to symmetry scores.

4.5.4 Evenness of bake

The data regarding mean squares for scores assigned to evenness of bake of breads prepared from SGF of different varieties presented in Table 4.29 indicted that scores given to evenness of bake of breads were significantly affected as a function of varietals differences.

It is evident from the results in Table 4.30 that scores assigned to evenness of bake to different breads varied significantly from 2.04 to 2.29 scores among different wheat varieties. Significantly the highest scores for this attribute were assigned to breads prepared from MH-97 wheat variety while the lowest scores were assigned to the breads prepared from variety Kohinoor 83. All the tested
Table 4.29  Mean squares for bread symmetry and evenness of bake of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bread symmetry</td>
<td>Evenness of bake</td>
<td></td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>0.0612813**</td>
<td>0.0369426*</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.0160869</td>
<td>0.016615</td>
<td></td>
</tr>
</tbody>
</table>

** Significant at P≤0.01
Table 4.30  Bread symmetry and evenness of bake scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Bread symmetry</th>
<th>Evenness of bake</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>2.25abc</td>
<td>2.27ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>2.15abcd</td>
<td>2.10abc</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>2.25abc</td>
<td>2.27ab</td>
</tr>
<tr>
<td>GA-02</td>
<td>2.08cd</td>
<td>2.25ab</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>2.17abcd</td>
<td>2.25ab</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>2.17abcd</td>
<td>2.19abc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>2.17abcd</td>
<td>2.04c</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>2.15abcd</td>
<td>2.20abc</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>2.10bcd</td>
<td>2.22abc</td>
</tr>
<tr>
<td>MH-97</td>
<td>2.29ab</td>
<td>2.29a</td>
</tr>
<tr>
<td>Pak-81</td>
<td>2.30a</td>
<td>2.27ab</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>2.30a</td>
<td>2.27ab</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>2.17abcd</td>
<td>2.08bc</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>2.25abc</td>
<td>2.25ab</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>1.90e</td>
<td>2.08bc</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>2.00de</td>
<td>2.10abc</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
Table 4.31  Mean squares for character of crust and break and shred of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Character of crust</th>
<th>Break and shred</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>15</td>
<td>0.0307306*</td>
<td>0.0322033*</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.0163356</td>
<td>0.0166563</td>
</tr>
</tbody>
</table>

** Significant at P ≤ 0.01
Table 4.32  Character of crust and break and shred scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Character of crust</th>
<th>Break and shred</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>2.25ab</td>
<td>2.20a</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>2.10bc</td>
<td>2.15ab</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>2.25ab</td>
<td>2.26a</td>
</tr>
<tr>
<td>GA-02</td>
<td>2.20abc</td>
<td>2.28a</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>2.22abc</td>
<td>2.23a</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>2.19abc</td>
<td>2.16ab</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>2.17abc</td>
<td>2.15ab</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>2.20abc</td>
<td>2.25a</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>2.20abc</td>
<td>2.23a</td>
</tr>
<tr>
<td>MH-97</td>
<td>2.24abc</td>
<td>2.29a</td>
</tr>
<tr>
<td>Pak-81</td>
<td>2.33a</td>
<td>2.25a</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>2.22abc</td>
<td>2.25a</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>2.10bc</td>
<td>2.00b</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>2.25ab</td>
<td>2.25a</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>2.05c</td>
<td>2.10ab</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>2.05c</td>
<td>2.10ab</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other
wheat varieties except Kohinoor 83, MH-97 fell statistically in the same group with respect to the scores assigned to evenness of bake.

**4.5.5 Character of crust**

The scores given to character of crust of breads prepared from SGF of different wheat varieties differed significantly among different wheat varieties (Table 4.31). The results presented in Table 4.32 indicated that breads prepared from straight grade flour of different wheat varieties got significantly different scores for character of crust (Table 4.32). The breads prepared from the flour of Pak-81 wheat variety got significantly the highest scores (2.33) while the lowest scores (2.05) for character of crust were given to the breads prepared from wheat varieties Punjab 96 and Uqab 2000. All the other 13 wheat varieties except Pak-81, Punjab 96 and Uqab 2000 were found to be statistically at par with respect to the scores assigned to character of crust of breads.

**4.5.6 Break and shred**

The analysis of variance for scores assigned to break and shred of breads prepared from different wheat varieties presented in Table 4.31 showed that differences in wheat varieties significantly affected the scores given to break and shred of breads.

The results in (Table 4.32) indicated that scores assigned to break and shred of breads of different wheat varieties ranged from 2.29 to 2.00 scores. The wheat variety MH-97 got maximum scores (2.29) by the judges, whereas the wheat variety Punjab 81 was ranked at the bottom by judges with respect to scores assigned to break and shred of breads. With exception of Punjab 81 all the other tested wheat varieties fell statistically in the same group with respect of this sensory attribute of bread.
<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Mastication</td>
<td>Texture</td>
<td></td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.779157**</td>
<td>2.011347**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.1723531</td>
<td>0.4164125</td>
<td></td>
</tr>
</tbody>
</table>

** Significant at $P \leq 0.01$
Table 4.34  Mastication and texture scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Mastication</th>
<th>Texture</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>7.84ab</td>
<td>11.48ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>6.60fg</td>
<td>10.04de</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>7.20cde</td>
<td>11.32b</td>
</tr>
<tr>
<td>GA-02</td>
<td>6.24gh</td>
<td>9.64e</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>6.84ef</td>
<td>11.18bc</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>6.80efg</td>
<td>11.16bc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>7.02def</td>
<td>10.84bcd</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>7.45bcd</td>
<td>11.06bc</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>7.44bcd</td>
<td>10.64bcd</td>
</tr>
<tr>
<td>MH-97</td>
<td>7.36bcde</td>
<td>11.01bc</td>
</tr>
<tr>
<td>Pak-81</td>
<td>7.64abc</td>
<td>10.96bcd</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>8.04a</td>
<td>12.28a</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>5.92h</td>
<td>10.24cde</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>7.20cde</td>
<td>11.52ab</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>6.44fgh</td>
<td>10.60bcd</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>6.48fgh</td>
<td>10.56bcd</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other
4.5.7 Mastication

The results pertaining to analysis of variance for the scores assigned to mastication of breads prepared from different wheat varieties have been presented in Table 4.33. The results indicated that scores assigned to mastication of bread were affected significantly by the wheat varieties.

The scores assigned to mastication of breads prepared from SGF of different varieties ranged from 5.92 to 8.04 scores (Table 4.34). The wheat varieties Pasban 90 and C-273 got significantly the highest scores whereas the wheat varieties Punjab 81, GA 02, Punjab 96 were ranked at bottom by judges for mastication of bread.

4.5.8 Texture

The scores assigned to texture of breads prepared from SGF of different wheat varieties were significantly affected as function of varietals differences (Table 4.33).

The results showed that scores assigned to texture of different breads varied from 9.64 to 12.28 scores among different wheat varieties. Significantly the highest scores were assigned to texture of breads prepared from Pasban 90 and Punjab 85 while the lowest scores were given to the breads prepared from wheat varieties GA-02, Punjab 81, Faisalabad 83 by the judges.

4.5.9 Grain

The analysis of variance for scores assigned to grain of breads prepared from different wheat varieties presented in Table 4.35 showed highly significant effect of wheat varieties on the scores assigned to grain of different breads.

It is obvious from the results (Table 4.36) that scores given to grain of breads prepared from SGF of different wheat varieties ranged from 5.80 to 7.60
Table 4.35  Mean squares for grain and crumb color of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Grain</td>
<td>Crumb color</td>
<td></td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.591147**</td>
<td>1.6114072**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.1715263</td>
<td>0.1640381</td>
<td></td>
</tr>
</tbody>
</table>

** Significant at P ≤ 0.01
Table 4.36 Grain and crumb color scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Grain</th>
<th>Crumb color</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>7.00a</td>
<td>7.00bcd</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>7.00a</td>
<td>7.10abc</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>7.20a</td>
<td>6.80cde</td>
</tr>
<tr>
<td>GA-02</td>
<td>5.80c</td>
<td>5.60g</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>7.40a</td>
<td>7.50ab</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>7.50a</td>
<td>7.60a</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>7.20a</td>
<td>7.20abc</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>7.41a</td>
<td>7.41ab</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>6.40b</td>
<td>6.45de</td>
</tr>
<tr>
<td>MH-97</td>
<td>7.52a</td>
<td>6.71cde</td>
</tr>
<tr>
<td>Pak-81</td>
<td>7.40a</td>
<td>6.40ef</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>7.40a</td>
<td>6.80cde</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>6.00bc</td>
<td>7.25abc</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>7.60a</td>
<td>7.40ab</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>6.40b</td>
<td>5.90fg</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>7.00a</td>
<td>7.00bcd</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
scores. All tested wheat varieties except GA 02, Manthar 03 and Punjab 81 were placed statistically in the same group with respect to scores assigned to grain.

4.5.10 Crumb color

The scores assigned to crumb color of breads differed significantly among different wheat varieties (Table 4.35).

The results presented in Table 4.36 revealed that scores for crumb color of bread prepared from different straight grade flours ranged from 5.60 to 7.60 scores. Maximum scores were given to crumb color of bread prepared from Iqbal 2000 and bread for GA 02 got lower scores for crumb color. The wheat varieties Faisalabad 83, Inqlab 91, Iqbal 2000, Kohinoor 83, Kohistan 97, Punjab 81 and Punjab 85 got statistically similar scores for crumb color to their breads.

4.5.11 Taste

The results pertaining to analysis of variance for scores given to taste of breads prepared from SGF of different wheats revealed that difference in wheat varieties exhibited significant effect on the scores assigned to taste of breads (Table 4.37).

The results in Table 4.38 indicated that the breads prepared from wheat variety Pak-81 got the maximum scores (11.66) whereas minimum scores (9.64) for taste were assigned to breads prepared from wheat variety Punjab 81. The wheat varieties C-273, Faisalabad 83, Faisalabad 85, Inqlab 91, Kohinoor 83, Kohistan 97, Manthar 03, MH-97, Paban 90, Punjab 85 and Uqab 2000 were ranked statistically at the same level for this sensory attribute of bread.

4.5.12 Aroma

The results regarding analysis of variance for scores assigned to aroma of breads prepared from different wheat varieties presented in Table 4.37 showed highly significant effect of wheat varieties on the aroma scores given to breads.
Table 4.37  Mean squares for taste, aroma and total score of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Taste</td>
</tr>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.6828077**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.4111044</td>
</tr>
</tbody>
</table>

** Significant at P≤0.01
Table 4.38  Taste and aroma scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Taste</th>
<th>Aroma</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>11.40ab</td>
<td>7.60abc</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>10.52bcde</td>
<td>7.00cdef</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>11.26abc</td>
<td>7.30bcde</td>
</tr>
<tr>
<td>GA-02</td>
<td>10.08de</td>
<td>6.40f</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>10.48bcde</td>
<td>7.30bcde</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>11.61a</td>
<td>7.60abc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>10.84abcd</td>
<td>6.50f</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>11.30abc</td>
<td>7.01cdef</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>11.32abc</td>
<td>6.70ef</td>
</tr>
<tr>
<td>MH-97</td>
<td>11.26abc</td>
<td>7.37bcd</td>
</tr>
<tr>
<td>Pak-81</td>
<td>11.66a</td>
<td>7.70ab</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>11.44ab</td>
<td>8.00a</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>9.64e</td>
<td>6.60f</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>11.04abc</td>
<td>7.40bcd</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>10.40cde</td>
<td>7.00cdef</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>10.84abcd</td>
<td>6.80def</td>
</tr>
</tbody>
</table>
Means carrying same letters in a column are not significantly different from each other
Table 4.39  Total scores of breads prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Total scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>74.72ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>69.30bcde</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>72.78abcd</td>
</tr>
<tr>
<td>GA-02</td>
<td>64.06e</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>73.07abcd</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>74.14ab</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>70.53abcd</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>73.00abcd</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>69.15bcde</td>
</tr>
<tr>
<td>MH-97</td>
<td>73.36abc</td>
</tr>
<tr>
<td>Pak-81</td>
<td>74.85ab</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>76.02a</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>66.90de</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>74.35ab</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>67.28cde</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>69.90abcde</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
It is evident from the results (Table 4.38) that scores given to aroma ranged from 6.40 to 8.00 scores among breads prepared from SGF of different varieties. The highest scores were given to bread prepared from wheat varietyied Pasban 90 and Iqbal 2000. The bread for wheat variety GA-02 was ranked at the bottom by judges with respect to scores given aroma of breads. The wheat varieties C-273, Faisalabad 85, Inqlab 91, Iqbal 2000, MH-97, Pak-81, and Punjab 85 did not differ to a significant level for this sensory parameter.

### 4.5.13 Total scores

The statistical results regarding total scores assigned to breads prepared from SGF of different wheat varieties presented in Table 4.37 showed total scores differed significantly as a function of varietals differences.

Significantly the highest total scores were assigned to breads prepared from flour of Pasban 90 followed by Pak 81 (74.85), C-273 (74.72) Punjab 85 (74.35), Iqbal 2000 (74.14) and Inqlab 91 (73.07), wheat varieties while significantly the lowest total scores (64.06) were given to the breads prepared from wheat variety GA-02. The wheat varieties Faisalabad 83, Faisalabad 85, Kohinoor 83, Kohistan 97, Manthar 03, Punjab 81, Punjab 85 and Uqab 2000 were found to fell statistically in the same group with respect to total scores assigned to breads.

**Discussion**

Wheat flour, water and yeast are the major ingredients in pan breads (Osuji, 2006 and Klopfenstein and Hoseney, 1995). Pomeranz (1990) has emphasized important role in baking performance of the quality and quantity of wheat proteins. The quality of breads based on sensoric attributes is described by appearance, aroma, texture, flavor and salt (Lawless and Heyman, 1999; Meilgard *et al.*, 2007). The present study showed that all the sensory attributes were significantly differed among different wheat varieties. The differences in
the scores assigned to different attributes may be ascribed to the differences in chemical composition of wheat varieties especially the crude protein, wet and dry gluten content as has been observed in the previous sections. The results of the present study regarding difference in scores assigned to various characters of breads prepared from different varieties are also supported by the earlier findings of (Zahoor) 2003 and Butt (1997) who reported that sensory attributes of breads are significantly affected by wheat varieties. They concluded that genetic factors, damaged starch and alpha amylase activity of flours may have significant effect on sensory attributes of breads prepared for different wheat cultivars. Therefore the differences in bread quality attributes may mainly be due to differences in genetic make up. Several others researchers like Stewart (1977), Preston et al. 1992, Bushuk and Wrigley (1974), Graybosch et al. (1993) and Booth and Melvin (1979) also reported that difference in chemical composition such as protein and sugar contents of wheat varieties significantly affects the sensory attributes of breads. In the present study existence of significant variation in sensory parameters of breads prepared from different wheat varieties suggested that wheat varieties possessing higher scores such as Pasban 90, Iqbal 2000, C-273, Pak 81, Punjab 85 and Inqlab 91 should be used by the bakers. In bread purposes this information is useful to the wheat scientists to exploit the potential of these good bread quality wheats in their future breeding programmes.
4.6 SENSORY CHARACTERISTICS OF CHAPATTIS

Chapatti is a staple diet for the people of Pakistan. Any food product must give pleasure and satisfaction to the consumer if it is to be a part of their eating habits. Sensory attributes are very important towards the liking or disliking of the chapattis. The consumers prefer chapattis with light brown and creamy colour possessing soft enough texture to fold it for making the desired scoop. The chapattis prepared from the WWF of different wheat varieties were presented to a panel of six judges and evaluated for sensory parameters like color, flavor, texture, feel to touch, foldability, breakability and total scores. The results for sensory characteristics of chapattis are discussed here under.

4.6.1 Color

The statistical results regarding scores assigned to colour of chapattis prepared from WWF of different wheat varieties presented in Table 4.40 indicated a significant effect of wheat variety on scores given to color of chapattis. The chapattis prepared from flour of Pasban 90 variety got significantly the highest (8.40) scores while the lowest scores (6.20) for color were given to the chapattis prepared from variety MH-97 (Table 4.41). All chapattis from the other 14 wheat varieties out of total 16 wheat varieties were found to fell statistically in same group with respect to scores assigned to color.

4.6.2 Flavor

The results pertaining to analysis of variance for scores given to flavor of chapattis prepared from WWF of different wheat indicated that difference in wheat varieties exhibited significant effect on scores assigned to flavor of chapattis (Table 4.40).

The mean scores assigned to flavor of chapattis presented in Table 4.41 showed that the chapattis prepared from wheat variety Pasban 90 got the highest scores followed by chapattis from wheat varieties C-273, Inqlab 91, Iqbal 2000, Pak-
Table 4.40  Mean squares for colour and flavour of chapattis prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>$df$</th>
<th>Color</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Varieties</td>
<td>15</td>
<td>1.1408273**</td>
<td>2.2336317**</td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.1958169</td>
<td>0.1528281</td>
</tr>
</tbody>
</table>

** Significant at $P \leq 0.01$
Table 4.41 Color and flavor scores of chapattis prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Color</th>
<th>Flavor</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>8.00ab</td>
<td>7.60ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>7.60b</td>
<td>6.60de</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>7.60b</td>
<td>6.80cd</td>
</tr>
<tr>
<td>GA-02</td>
<td>7.60b</td>
<td>6.20ef</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>8.00ab</td>
<td>7.20bc</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>8.00ab</td>
<td>7.20bc</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>8.00ab</td>
<td>6.60de</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>8.00ab</td>
<td>6.60de</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>7.60b</td>
<td>6.40de</td>
</tr>
<tr>
<td>MH-97</td>
<td>6.20c</td>
<td>6.60de</td>
</tr>
<tr>
<td>Pak-81</td>
<td>7.40b</td>
<td>7.20bc</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>8.40a</td>
<td>8.00a</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>7.60b</td>
<td>5.80f8</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>7.57b</td>
<td>7.17bc</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>7.40b</td>
<td>5.40g</td>
</tr>
<tr>
<td>Ulqab 2000</td>
<td>7.60b</td>
<td>7.40b</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
81, Punjab 85 and Uqab 2000 but wheat varieties possessed non significant differences for this sensory parameter of chapattis. Significantly the lowest scores (5.40) were assigned to flavor of chapattis prepared from wheat variety Punjab 96. The chapattis from wheat varieties Faisalabad 83, Faisalabad 85, Kohinoor 83, Kohistan 97, Manthar 03 and MH-97 got statistically similar scores for flavor by judges.

### 4.6.3 Texture

The data regarding analysis of variance for the scores assigned to texture of chapattis prepared from different wheat varieties have been presented in Table 4.42. It is evident from the results that scores given to the texture of chapattis differed significantly due to differences in wheat varieties.

It is obvious from the results (Table 4.43) that scores assigned to texture of chapattis prepared from WWF ranged from 5.20 to 7.40 scores among different wheat varieties. The chapattis of wheat varieties Iqbal 2000, Pak 81, Inqlab 91, C-273 and Pasban 90 got significantly the highest scores but with non significant differences. The chapattis of wheat variety GA-02 was ranked at the bottom by judges with respect to scores assigned to texture of chapattis.

### 4.6.4 Feel to touch

The statistical analysis pertaining to scores assigned to feel to touch of chapattis prepared from WWF of different wheat varieties showed significant variation in scores assigned to feel to touch due to wheat varieties (Table 4.42).

The scores assigned to feel to touch of chapattis presented in Table 4.43 indicated that the chapattis prepared from wheat variety Iqbal 2000 and Pak-81 got significantly the highest scores (8.40) followed by the chapattis prepared from wheat varieties C-273, MH 97 and Punjab 85. The lowest scores (6.40) for feel to touch were given to the chapattis prepared from wheat variety Faisalabad 83. The scores given to feel to touch of chapattis prepared from wheat varieties C-273, Inqlab 91,
Table 4.42 Mean squares for texture and feel to touch of chapattis prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Texture</td>
<td>Feel to touch</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Variety</td>
<td>Error</td>
<td></td>
</tr>
<tr>
<td>Varities</td>
<td>15</td>
<td>1.7583193**</td>
<td>1.5264613**</td>
<td></td>
</tr>
<tr>
<td>Error</td>
<td>32</td>
<td>0.1477556</td>
<td>0.1959056</td>
<td></td>
</tr>
</tbody>
</table>

** Significant at P≤0.01
Table 4.43  Texture and feel to touch scores of chapattis prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Texture</th>
<th>Feel to touch</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-273</td>
<td>7.20a</td>
<td>8.00ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>6.40b</td>
<td>6.40d</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>7.20a</td>
<td>7.20c</td>
</tr>
<tr>
<td>GA-02</td>
<td>5.20d</td>
<td>6.60d</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>7.20a</td>
<td>7.40bc</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>7.40a</td>
<td>8.40a</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>6.40b</td>
<td>7.60bc</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>6.60b</td>
<td>7.60bc</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>6.40b</td>
<td>7.60bc</td>
</tr>
<tr>
<td>MH-97</td>
<td>6.60b</td>
<td>8.00ab</td>
</tr>
<tr>
<td>Pak-81</td>
<td>7.40a</td>
<td>8.40a</td>
</tr>
<tr>
<td>Pasban 90</td>
<td>6.60b</td>
<td>7.60bc</td>
</tr>
<tr>
<td>Punjab 81</td>
<td>6.20bc</td>
<td>7.40bc</td>
</tr>
<tr>
<td>Punjab 85</td>
<td>6.57b</td>
<td>7.97ab</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>5.80c</td>
<td>7.20c</td>
</tr>
<tr>
<td>Ulqab 2000</td>
<td>6.40b</td>
<td>7.40bc</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
Kohinoor 83, Kohistan 97, Manthar 03, MH-97, Pasban 90, Punjab 81, Punjab 85 and Uqab 2000 were found statistically at par for this sensory parameter.

4.6.5 Foldability

The scores assigned to foldability of chapattis prepared from WWF of different wheat varieties differed significantly as a function of varietal differences (Table 4.44). The results presented in Table 4.45 revealed that chapattis prepared from whole wheat flour of different varieties got significantly different scores for foldability (Table 4.45). The scores to foldability of chapattis were given significantly the highest to chapattis prepared from wheat varieties Pasban 90, MH-97 and Pak-81 and Inqlab 91 but these varieties differed non significantly for this sensory parameter. The lowest scores (5.20) for foldability were given to the chapattis prepared from variety GA-02. The chapattis from wheat varieties C-273, Faisalabad 83, Faisalabad 85, Inqlab 91, Iqbal 2000, Kohinoor 83, Kohistan 97 and Manthar 03 got statistically similar scores to foldability of chapattis.

4.6.6 Breakability

The data regarding mean squares for scores assigned to breakability of chapattis prepared from WWF of different wheat varieties have been presented in Table 4.44.

The statistical results indicted that scores of breakability assigned by the judges to chapattis differed significantly among the wheat varieties.

The scores assigned to breakability of chapattis showed that scores assigned to breakability of different chapattis varied from 4.80 to 6.60 scores among different wheat varieties. The highest scores were assigned to breakability of chapattis prepared from Pasban 90 wheat variety while the lowest scores were assigned to the chapattis prepared from wheat variety GA-02 (Table 4.45).
<table>
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<th>SOV</th>
<th>df</th>
<th>Mean Squares</th>
<th></th>
<th></th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Foldability</td>
<td>Breakability</td>
<td>Total score</td>
<td></td>
</tr>
<tr>
<td><strong>Varieties</strong></td>
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<td><strong>1.4004732</strong></td>
<td><strong>30.845091</strong></td>
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<tr>
<td><strong>Error</strong></td>
<td>32</td>
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<td><strong>0.1118875</strong></td>
<td><strong>5.4661737</strong></td>
<td></td>
</tr>
</tbody>
</table>

**Significant at P≤0.01**
Table 4.45  Foldability and breakability scores of chapattis prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Foldability</th>
<th>Breakability</th>
</tr>
</thead>
<tbody>
<tr>
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<td>6.20ab</td>
</tr>
<tr>
<td>Faisalabad 83</td>
<td>5.80bc</td>
<td>5.40cd</td>
</tr>
<tr>
<td>Faisalabad 85</td>
<td>5.80bc</td>
<td>6.20ab</td>
</tr>
<tr>
<td>GA-02</td>
<td>5.20d</td>
<td>4.80e</td>
</tr>
<tr>
<td>Inqlab 91</td>
<td>6.20ab</td>
<td>6.40a</td>
</tr>
<tr>
<td>Iqbal 2000</td>
<td>5.80bc</td>
<td>6.40a</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>6.20ab</td>
<td>5.40cd</td>
</tr>
<tr>
<td>Kohistan 97</td>
<td>6.20ab</td>
<td>5.80bc</td>
</tr>
<tr>
<td>Manthar 03</td>
<td>5.80bc</td>
<td>5.40cd</td>
</tr>
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<td>Pak-81</td>
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<td>5.78bc</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>5.40cd</td>
<td>5.20de</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>5.40cd</td>
<td>5.40cd</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other
Table 4.46  Total scores of chapattis prepared from different Pakistani spring wheat varieties

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Total scores</th>
</tr>
</thead>
<tbody>
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<tr>
<td>GA-02</td>
<td>35.60f</td>
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<tr>
<td>Inqlab 91</td>
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</tr>
<tr>
<td>Iqbal 2000</td>
<td>43.20a</td>
</tr>
<tr>
<td>Kohinoor 83</td>
<td>40.20abcd</td>
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<tr>
<td>Kohistan 97</td>
<td>40.80abcd</td>
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<tr>
<td>Manthar 03</td>
<td>39.20bcde</td>
</tr>
<tr>
<td>MH-97</td>
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<td>Pak-81</td>
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<td>Punjab 85</td>
<td>41.43abc</td>
</tr>
<tr>
<td>Punjab 96</td>
<td>36.40ef</td>
</tr>
<tr>
<td>Uqab 2000</td>
<td>39.60bcde</td>
</tr>
</tbody>
</table>

Means carrying same letters in a column are not significantly different from each other.
4.6.7 Total scores

The statistical results pertaining to total scores assigned to chapattis prepared from different wheat varieties presented in Table 4.44 showed significant effect of wheat varieties on this sensory attribute.

It is evident from the results (Table 4.46) that total scores assigned to chapattis prepared from WWF of different wheat varieties ranged from 35.60 to 43.60 scores. The wheat varieties Pasban 90, Iqbal 2000, C-273, Pak 81 and Inqlab 91 got significantly the highest total scores by judges but with non significant differences with one another. The wheat variety GA-02 was ranked at the bottom by the judges regarding total scores. The wheat varieties Faisalabad 83, GA-02, Punjab 85 and Punjab 96 were placed at the same group with respect to total scores given to chapattis.

Discussion

The chapatti making quality of wheat is mainly dependent on protein. Qarooni et al. (1988) described that flour with protein content ranging from 10-12% is more suitable for the production of Arabic bread Shaikh et al., (2007) and Haridas et al., (1986) reported that the most important parameters of chapatti quality are color, texture and flavor and are evaluated as greater pliability, soft texture, and light creamish brown colour with small brown spots, fully puffed, slight chewiness and baked wheatish aroma. The sensory attributes of chapattis studied during the present study are significantly affected due to differences of wheat varieties. The results are in conformity with the findings of Anjum et al., (2008) and Ahmad et al., (2007) who found significant variation in sensory attributes of chapattis due to differences in wheat varieties. The results are also in line with earlier findings of various research workers (Butt 1997, Ahmad 2001) who found similar results while testing different Pakistani wheats. Farvili et al. (1997) concluded that optimum tanoor bread quality was produced from moderate protein flour (11.2%). Yamazaki and Lamb (1961), Murphy and Austin (1963) and
Ahmad (2001) reported that variation in quality characteristics based on sensory evaluation regarding breakability among the wheat varieties may be due to differences in wheat varieties, their protein content and quality.

The present results provide useful information to the processors, consumers and scientists regarding wheat varieties for production of chapattis. In Pakistan wheat is mostly used for preparation of chapattis and thus the wheat varieties Pasban 90, Iqbal 2000, Inqlab 91 and C-273 possessing higher total scores may be preferred by the end users. These wheat varieties should also be used as source for developing new wheat varieties with improved chapatti quality.
4.7. ELECTROPHORETIC CHARACTERIZATION OF WHEAT VARIETIES

The high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunits (LMW-GS) fractions in different wheat varieties were determined through SDS-polyacrylamide gel electrophoresis (PAGE) and the results interpreted from electrophoretograms are shown in figures 1, 2 as well as in Tables 4.47 and 4.48. Two major classes of glutenin polypeptides have been identified in different wheat varieties which were designated as HMW-GS and LMW-GS. These two classes of glutenin polypeptides occur in wheat flour as crossed-linked proteins resulting from inter-polypeptide disulphide link-ages as reported by Luo et al., (2001). These HMW and LMW subunits were identified by comparing with the standards of molecular weight sub units run in the same gel.

The polypeptides detected in the present study can be divided in to two main molecular weight regions. The polypeptides present at 40 kDa to 70 kDa are known low molecular weight glutenin subunits (LMW-GS) while those which are present in the region above 70 kDa are high molecular weight glutenin subunits (HMW-GS) as already reported by Beitz and Wall, 1972). It is evident from the electrophorogram that more number of polypeptides were present in the region falling under low molecular weight glutenin subunits.

The electrophorogram showed the presence of two electrophoretic bands in the region of 33kDa and 36kDa in all the tested wheat varieties. One polypeptide of LMW-GS in the region around 45kDa was also present in all wheat varieties except Pasban 90 in which it was absent. The electrophorogram also indicated the presence of electrophoretic band around the region 54kDa with exception of Kohinoor 83 in all other wheat varieties. With respect to high molecular weight glutenin subunits (HMW-GS), the polypeptides were present in the region around 80kDa in all wheat varieties except wheat varieties Punjab.
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<th>70kDa</th>
<th>55kDa</th>
<th>45kDa</th>
<th>35kDa</th>
<th>25 kDa</th>
</tr>
</thead>
</table>

M = Marker, 1 = Inqlab-91, 2 = Punjab-85, 3 = MH-97, 4 = Pasban-90, 5 = Manthar-03, 6 = GA-02, 7 = Pak-81, 8 = Faisalabad-85

**Fig 1.** Presence of HMW-GS and LMW-GS in different wheat varieties by SDS-PAGE

**Fig 2.** Presence of HMW-GS and LMW-GS in different wheat varieties by SDS-PAGE
<table>
<thead>
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<th>Band #</th>
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<th>M.wt (kDa)</th>
<th>Intensity</th>
<th>M.wt (kDa)</th>
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<th>M.wt (kDa)</th>
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</table>

HMW-GS = High molecular weight glutenin subunits
LMW-GS = Low molecular weight glutenin subunits
Table 4.48  Presence of HMW-GS and LMW-GS in different wheat varieties

<table>
<thead>
<tr>
<th>Band #</th>
<th>Sample Name</th>
<th>C-273 M.wt (kDa)</th>
<th>Iqbal-2000 M.wt (kDa)</th>
<th>Kohinoor-83 M.wt (kDa)</th>
<th>Uqab-2000 M.wt (kDa)</th>
<th>Punjab-96 M.wt (kDa)</th>
<th>Punjab-81 M.wt (kDa)</th>
<th>Kohistan-97 M.wt (kDa)</th>
<th>Faisalabad-83 M.wt (kDa)</th>
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</table>
HMW-GS = High molecular weight glutenin subunits
LMW-GS = Low molecular weight glutenin subunits
85, Manthar 03, Faisalabad 85 and Punjab 96 in which it was missed. The electrophoretic bands in the region of 90kDa – 92kDa were also identified in all the wheat varieties except Kohistan 97, Iqbal 2000 and Manthar 03. It is obvious from the electrophoretic patterns that there was a variation in regions of molecular weight of glutenin subunits with differences in their intensities in different wheat varieties. The SDS-PAGE patterns of molecular weight of glutenin subunits of different wheat varieties given in tables 4.47 and 4.48 showed the presence of glutenin subunits in the range of 32kDa to 103.6kDa. It is obvious from the results that the highest molecular weight glutenin subunit (103.6 kDa) was found in wheat variety Pak 81 and the lowest molecular weight subunit (32.7kDa) was observed in wheat variety Iqbal 2000. A wide variation with respect to high molecular weight subunits in different wheat varieties occurred. The highest numbers of HMW-GS polypeptide bands were identified in wheat varieties Pak-81 (ranging at different intervals of 103.6 kDa, 99.2 kDa, 87.5 kDa and 80.1 kDa), Pasban-90 (100.3 kDa, 92.9 kDa, 80.7 kDa and 75.4 kDa) and Iqbal-2000 (102.9 kDa, 98.8 kDa, 87.1 kDa and 80.8 kDa). MacRitchie (1992) has reported that glutenin polymers are made up of high molecular weight (100kDa) and low molecular weight (31-45kDa) glutenin subunits linked together by disulfides bonds which support to the present electrophoretic bands influenced for LMW-GS. However, Gianibelli et al. (2001) reported range from 70 kDa to 130 kDa for HMW-GS and results for HMW-GS identified in present study fall within these ranges. Payne (1987) has reported that the gene coding for HMW-GS subunits were located on the long arms of chromosomes 1A, 1B and 1D at the Glu-A1, Glu-B1 and Glu-D1 loci, respectively. However, the gene coding for LMW-GS occur on the short arms of group-1 chromosomes at the Glu-A3, Glu-B3 and Glu-D3 loci which are tightly linked to the Gli-1 locus (Ponga et al., 1990; Luo et al., 2001). A large contribution towards dough properties have been reported due to HMW-GS coded by Glu-D1 (Anjum et al., 2000b and khan, 2001).
It is well known that HMW-GS strongly contributes to the rheological properties of dough. The presence of particular subunits has also been associated with superior bread making quality. The reason of this relationship is still unclear but may have importance due to cysteine aminoacid which affects the degree of cross linking and to the structural regularity, thus affecting the elasticity of the molecule (Shewry et al., 1992).

The low molecular weight glutenin subunits (LMW-GS) represented approximately 60% (Bietz and Wall, 1973; Zhang et al., 2004) of the total wheat glutenin fractions of different wheat varieties, and their molecular weight ranged from 32.3 kDa to 67.4 kDa (Bietz and Wall, 1973). The LMW-GS in wheat varieties Inqlab-91 ranged from 33.9 kDa to 66.9 kDa, Pak-81 (3.0 kDa to 66.9 kDa), Pasban-90 (33.4 kDa to 66.1 kDa) GA-02 (33.4 kDa to 66.1 kDa), Punjab-85 (33.4 kDa to 66.1 kDa), MH-97 (33.0 kDa to 66.1 kDa), Faisalabad-85 (33.4 kDa to 66.1 kDa), Manthar-03 (33.4 kDa to 66.1 kDa), C-273 (33.4 kDa to 66.3 kDa), Iqbal-2000 (32.7 kDa to 65.9 kDa), Kohinoor-83 (32.9 kDa to 65.6 kDa) Uqbab-2000 (32.9 kDa to 66.6 kDa), Punjab-96 (33.0 kDa to 67.0 kDa), Punjab-81 (32.8 kDa to 66.3 kDa), Kohistan-97 (33.5 kDa to 66.9 kDa) and Faisalabad-83 (32.3 kDa to 67.4 kDa).

The variation observed in electrophoretic bands with respect to LMW-GS in the present study is in concordance with the findings of Bietz and Wall (1972) and Gianibelli et al., (2001) who also reported range of low molecular weight glutenin subunits (LMW-GS) from 10 kDa to 70 kDa. The wheat varieties tested in the present study possessed LMW-GS in the upper limits reported by the above researchers and it may be due to genetic make up of varieties tested in different studies.

The LMW-GS accounts for 60-70% of the total glutenins comprising a number of individual components which can be classified into three groups (B, C and D) on the basis of their electrophoretic pattern on SDS-PAGE (Jackson et al.,
The major group, the B-subunits comprises basic proteins, whereas the minor C-subunits have a wide range of isoelectric points varying from slightly acidic to highly basic. The B group corresponds to proteins ranging in molecular weight from 40 kDa to 50 kDa while the C-subunits range from 30 kDa to 40 kDa. The remaining group, the D-subunits, has slower mobilities than the B-and C-subunits and form one of the most-acidic groups of proteins in the endosperm. It is evident from the results that two polypeptides were present in all wheat varieties in C group. The wheat varieties Iqbal 2000, Kohinoor 83, Punjab 81, Faisalabad 83, Inqlab 91, MH 97, Pasban 90, Pak 81 and Faisalabad 85 possessed two polypeptide band corresponding B group. One polypeptide band was identified in wheat varieties Punjab 85, Manthar 03, GA 02, C-273, Uqab 2000, Punjab 96 and Kohistan 97 corresponding to the B group.

Kasarda (1989) suggested on the basis of structural characteristics of the B, C and D groups, the existence of two functional groups of LMW-GS. One group, which includes the majority of the B-type sub-units designated as molecular weights of B-subunits of different wheat varieties may act as chain extender of the growing polymers because of their ability to form two inter-molecular disulphide bonds. The second group, which includes most of the C and D-type LMW-GS designated in different studied wheat varieties, may act as chain terminators of the growing polymer, having only one cysteine available to form an inter-molecular disulphide bond (Ovidio and Masci, 2004).

The LMW-GS may have a pronounced effect in determining the physical dough properties of flour during bread making (Gupta et al. 1989; Gupta and McRitchie, 1994) and pasta making (Ruiz and Carrillo, 1995). The wheat varieties Pak-81, Punjab-85, MH-97, Faisalabad-85, Punjab-81, Punjab-96, Iqbal-2000, Pasban-90 and Inqlab-91 contained more bands of LMW-GS which may have good physical dough properties of flour during bread making as reported by Gupta and McRitchie, 1994.
In the present study the wheat varieties Punjab-81, Iqbal-2000, Inqlab-91, Pasban-90, MH-97 and Pak-81 showed the presence of LMW-GS of ~42 kDa which play only a major role in determining the quality characteristics. LMW-GS ~42 kDa was also isolated and studied by Masci et al., (1998) who reported that this ~42 kDa subunit as a linear chain extender (Lew et al., 1992) capable of enhancing chain length during glutenin polymer formation. If 42 kDa subunit in indeed linear chain extender it might be in accordance with the correlation of this type of subunit with good quality. Masci et al., (2000) also described the presence of LMW-GS 42 kDa indicating the good quality of bread wheat. In the present study the wheat varieties mentioned above have shown presence of this LMW-GS 42 kDa which indicates that these wheat varieties might possess good quality of bread. The LMW-GS with molecular weight ~32 kDa (Glu-D5 locus) have been identified in wheat varieties Iqbal-2000, Kohinoor-83 and Punjab-81. The LMW-GS approx 32 kDa (Glu-D5 locus) in different wheat varieties have been reported by Sreeramulu and Singh (1997). These glutenin subunits could be seen only in alkylated glutenin, and the one encoded at Glu-D5 locus has an α-type N-terminal sequence. The genes encoding (Glu-D5) is located on chromosome 7D as reported by Gianibelli et al., (2001). This study suggests that the role of LMW-GS and HMW-GS in relation of end use quality further investigated.

4.8 IMMUNOCHEMICAL CHARACTERIZATION OF WHEAT VARIETIES

In generally the quality of wheat for bread and chapatti is assessed by chemical, rheological and baking tests. The quality of wheat is governed by the interaction of many constituents; hence it is difficult to evaluate quality by any single test. The rheological or other quality tests used for wheat quality screening require large flour sample and are very labor-intensive (Andrew et al., 1993). Immunoassay has been recognized as a valuable analytical tool for screening of
good quality wheats. The technique probing antibodies productions in response to food intake are in progress (Asensio et al., 2008). In number of research investigations, efforts have been diverted in the development of immunochemical assays; many techniques have been developed as qualitative or semi-quantitative and probably most useful for rapid screening. Therefore in the present study an Enzyme Linked Immunosorbant Assay (ELISA) was developed for assessing the suitability of different Pakistani wheat varieties with respect to their end use quality such as chapatti and bread.

The data pertaining to antibody response evaluated through animal modeling using rabbits as test animal depicted wide variation among different wheat varieties through antibody responses against high molecular weight glutenins (HMG) and low molecular weight glutenins (LMG). The antibody response against HMG varied from 0.300 to 0.70 among different wheat varieties. The maximum antibody response against HMG was yielded by wheat variety Iqbal-2000 (0.70) followed by Pasban 90 (0.65) and Inqlab-91 (0.65). The wheat varieties such as Kohistan 97 and Pak-81 exhibited the identical antibody response of 0.600 against HMG. The wheat varieties Kohinoor 83 and Manthar 03 also showed similar antibody response of 0.500 and the wheat varieties MH-97, Punjab-85 and Uqab-2000 showed similar antibody response of 0.460 against HMG. However, the wheat varieties Punjab-96, GA-02 and Punjab-81 exhibited the lowest antibody response against HMG which was 0.300, 0.350 and 0.380, respectively, as compared to other tested wheat varieties (Figure 3).
Figure 3: Antibody response of high molecular weight glutenin
Figure 4: Antibody response of low molecular weight glutenin
It is evident from Figure 4 that wide variation existed among wheat varieties with respect to the antibody response against low molecular glutenin (LMG). Maximum antibody response of 0.900 was recorded against LMG for MH-97 followed by FSD-83 (0.85). The wheat varieties Punjab-96 and Punjab-85 possessed identical antibody response of 0.80. The antibody response against LMG was similar for wheat varieties Uqab-2000, Manthar-03 and Pasban-90. The wheat varieties Kohistan-97 and Pak-81 exhibited same value for antibody response of 0.70, whereas the antibody response of GA-02, Kohinoor-83 and Iqbal 2000 was also similar 0.650 against LMG. The lowest antibody response against LMG was observed in wheat varieties i.e. Punjab-81, C-273 and FSD-85 which was 0.55, 0.55 and 0.58, respectively.

The present study showed higher antibody responses against LMG as compared to HMG for different wheat varieties. The present results are in consistent with the earlier findings of Prabhasanker and Manohar, (2002) who developed an indirect ELISA for evaluating chapatti making quality of some Indian wheat varieties and responses against anti-LMG antibodies and anti-HMG antibodies varying from 0.307-0.703 and 0.303-0.710, respectively. They also reported that anti-HMG response was positively correlated with overall chapatti sensory score. Li et al 2008 suggested that polyclonal antibodies could be used for rapid prediction and screening of wheat quality. Mills et al (2002) also developed monoclonal antibodies to wheat gluten proteins for use as diagnostic for screening of large number of wheat lines for their suitability for processing in to bread. The present results for antibody responses against HMG fall within the ranges reported by Prabhasanker and Manohar, (2002) but the antibody response against LMG was higher then reported by these researchers. This variation may be due to differences in the genetic make up of wheat varieties used in these
studies. Existence of wide variation with respect to antibody responses of HMG and LMG indicates differences in the end quality of these wheat varieties.

4.9 CORRELATION STUDIES BETWEEN ANTIBODY RESPONSES AGAINST HMG AND LMG WITH DIFFERENT QUALITY ATTRIBUTES

Correlation matrix given in Table 4.49 showed a significant relationship of HMG and LMG with different quality attributes of whole wheat flour and sensory parameters of chapatti. It is obvious from the results that a significant and positive correlation existed between HMG antibody response and crude protein content \((r = 0.51)\), farinographic water absorption \((r = 0.61)\). The SDS-sedimentation value \((r = 0.44)\) and wet gluten \((r = 0.37)\) were also positively correlated with antibody responses against HMG. The HMG antibody response showed a highly significant positive relationship with flavor \((r = 0.76)\), breakability \((r = 0.87)\), texture \((r = 0.81)\) and total chapatti scores \((r = 0.91)\). The folding-ability \((r = 0.62)\), feel to touch \((r = 0.58)\) and color of chapatti \((r = 0.49)\) also showed linear relationship with antibody response against HMG. The antibody response against LMG showed non-significant correlation with flour characteristics and chapatti scores with the exception of existence of negative correlation with that of color of chapatti \((r = -0.52)\) and overall chapatti scores \((r = -0.42)\).

The correlation studies were also carried out of straight grade flour and sensory attributes of bread with antibody responses to HMG and LMG (Table 4.50). The antibody response against HMG showed significant and positive relationship with protein content of flour \((r = 0.60)\), pleshenke value \((r = 0.61)\) and farinographic water absorption \((r = 0.50)\). A positive correlation existed between wet and dry gluten with that of HMG antibody response. A significant and positive relationship of bread sensory attributes with antibody response against HMG was also observed. There was a significant correlation of HMG
with mastication \((r = 0.64)\), texture \((r = 0.69)\), taste \((r = 0.72)\), aroma \((r = 0.68)\),
grain \((r = 0.71)\) and total scores \((r = 0.83)\). The loaf volume also showed
relationship with that of HMW antibody response \((r = 0.51)\). It is obvious from
the correlation studies that LMG antibody response was negatively and non
significantly correlated with flour characteristics and sensory attributes of
breads.

The results regarding correlation studies in the present study are in close
agreement with the findings of Prabhasanker and Manohar, (2002) who
developed an indirect ELISA for evaluating chapatti making quality of some
Indian wheat varieties. They found that anti-HMG response was positively
correlated with farinograph water absorption and overall chapatti sensory scores
whereas anti-LMG was negatively correlated with most of the quality attributes
studied by them. The present results are further supported by (Xi Yun and Fang,
1998) who found that antibodies produced against HMG was positively
correlated with protein content, wet and dry gluten and bread volume as has
been observed in the present study. Skerrit (1991a) reported an assay to predict
strength related properties of wheat dough using antibodies against high
molecular weight glutenin subunits (HMW-GS) and concluded existence of high
correlations between antibody binding and dough strength parameters. Andrew
et al. (1993) investigated correlations between antibody binding and dough
strength parameters by using antibodies specific for low molecular weight
glutelin subunits (LMW-GS) and found similar kind of relationship among some
quality parameters. The present study suggests that use of antibodies response
against HMG offers good tool for predicting quality of wheat for suitability to
bread making and chapatti making quality.
Table 4.49 Correlation coefficients of HMG and LMG antibody responses with quality attributes in different wheat varieties

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HMG antibody response</th>
<th>LMG antibody response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein WWF</td>
<td>0.51</td>
<td>0.08</td>
</tr>
<tr>
<td>Wet Gluten WWF</td>
<td>0.37</td>
<td>-0.03</td>
</tr>
<tr>
<td>Dry Gluten</td>
<td>0.18</td>
<td>-0.19</td>
</tr>
<tr>
<td>SDS-sedimentation value WWF</td>
<td>0.44</td>
<td>0.04</td>
</tr>
<tr>
<td>Pleschenke value WWF</td>
<td>0.61</td>
<td>-0.18</td>
</tr>
<tr>
<td>Water absorption WWF</td>
<td>0.48</td>
<td>0.05</td>
</tr>
<tr>
<td>Chapatti Flavor</td>
<td>0.76</td>
<td>-0.07</td>
</tr>
<tr>
<td>Chapatti Breakability</td>
<td>0.87</td>
<td>-0.20</td>
</tr>
<tr>
<td>Chapatti foldability</td>
<td>0.62</td>
<td>0.19</td>
</tr>
<tr>
<td>Chapatti .feel to touch</td>
<td>0.58</td>
<td>-0.07</td>
</tr>
<tr>
<td>Chapatti Texture</td>
<td>0.81</td>
<td>-0.26</td>
</tr>
<tr>
<td>Chapatti color</td>
<td>0.49</td>
<td>-0.52</td>
</tr>
<tr>
<td>Chapatti total Score</td>
<td>0.91</td>
<td>-0.21</td>
</tr>
</tbody>
</table>
Table 4.50. Correlation coefficients of HMG and LMG antibody responses with quality attributes in different wheat varieties

<table>
<thead>
<tr>
<th>Parameters</th>
<th>HMG antibody response</th>
<th>LMG antibody response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude Protein</td>
<td>0.60</td>
<td>0.11</td>
</tr>
<tr>
<td>Wet Gluten</td>
<td>0.28</td>
<td>-0.12</td>
</tr>
<tr>
<td>Dry Gluten</td>
<td>0.26</td>
<td>-0.20</td>
</tr>
<tr>
<td>Pleshenke</td>
<td>0.61</td>
<td>-0.18</td>
</tr>
<tr>
<td>SDS sedimentation value</td>
<td>0.41</td>
<td>0.05</td>
</tr>
<tr>
<td>Water absorption</td>
<td>0.50</td>
<td>0.16</td>
</tr>
<tr>
<td>Mastication of bread</td>
<td>0.64</td>
<td>0.09</td>
</tr>
<tr>
<td>Texture of bread</td>
<td>0.69</td>
<td>-0.06</td>
</tr>
<tr>
<td>Taste of bread</td>
<td>0.72</td>
<td>0.12</td>
</tr>
<tr>
<td>Aroma of bread</td>
<td>0.68</td>
<td>0.07</td>
</tr>
<tr>
<td>Crumb Color of bread</td>
<td>0.56</td>
<td>-0.21</td>
</tr>
<tr>
<td>Grain of bread</td>
<td>0.71</td>
<td>0.22</td>
</tr>
<tr>
<td>Crust Color of bread</td>
<td>0.73</td>
<td>-0.07</td>
</tr>
<tr>
<td>Loaf Volume bread</td>
<td>0.51</td>
<td>-0.04</td>
</tr>
<tr>
<td>Symmetry of form of bread</td>
<td>0.58</td>
<td>-0.16</td>
</tr>
<tr>
<td>Break and Shred of bread</td>
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<td>0.15</td>
</tr>
<tr>
<td>Crust Character of bread</td>
<td>0.58</td>
<td>-0.24</td>
</tr>
<tr>
<td>Evenness of bake of bread</td>
<td>0.48</td>
<td>-0.08</td>
</tr>
<tr>
<td>Total score of bread</td>
<td>0.83</td>
<td>-0.01</td>
</tr>
</tbody>
</table>
Chapter 5

SUMMARY

Sixteen spring wheat varieties grown during the crop year 2005-06 in Pakistan were selected to characterize for physicochemical, biochemical, technological (bread and chapatti baking) and immunochemical (ELISA) properties.

The physical characteristics such as thousand kernel weight and test weight differed significantly among the wheat varieties. Thousand kernel weight varied between 40 and 25.98 g and wheat variety C-273 possessed the highest test weight (78.00 kg/hL). The wheat variety Kohinoor 83 yielded significantly the lowest test weight (66.47 kg/hL).

The chemical parameters such as moisture content, ash content, crude protein, crude fat, SDS sedimentation value, Pelshenke value, and falling number differed significantly for both whole wheat flour (WWF) and straight grade flour (SGF) among different wheat varieties. The moisture content ranged significantly from 9.7 to 11.65 % and 11.43 to 13.42 % between the whole wheat flour and straight grade flour, respectively in different wheat varieties. The highest moisture content (13.42%) was found in SGF of wheat variety Punjab 96 while wheat variety Manthar 03 possessed the lowest moisture content (11.43%) in SGF. The ash content in WWF and SGF of different wheat varieties ranged significantly from 1.20 to 1.67% and from 0.38 to 0.63%, respectively.

The protein content ranged from 10.44 to 12.72 % and 10.01 to 11.60 % between the whole wheat flours and straight grade flours, respectively among different wheat varieties. The protein content was found higher in WWF than the respective, straight grade flour. The highest protein content (12.72%) was found in whole wheat flour sample of wheat variety Iqbal 2000. Significantly the
highest protein content (11.60%) was found in the SGF of wheat variety Iqbal 2000 while the lowest protein content (10.01%) was observed in SGF of wheat variety Faisalabad 83. The fat content ranged from 0.51 to 0.75% and from 0.92 to 1.37% in SGF and WWF, respectively of different wheat varieties. The wet gluten varied from 25.62 to 37.00 % in whole wheat flour of different wheat varieties. The wheat varieties C 273, Faisalabad 85, Iqbal 2000, and Pasban 90 contained the highest wet gluten contents. The gluten content was found lower in whole wheat flour of wheat varieties Kohistan 97, Kohinoor 83 and MH-97. Significantly the highest wet gluten (41.09%) was found in SGF of Faisalabad 85 while the lowest (28.47%) wet gluten content was found in SGF of Kohinoor 83. The dry gluten content of both WWF and SGF showed significant variation in dry gluten content between SGF and WWF of different wheat varieties. The dry gluten content ranged from 10.40 to 13.80% and from 9.04 to 12.89% between SGF and WWF, respectively of different wheat varieties. The Pelshenke value ranged from 48 to 220 minutes and from 50.85 to 223 minutes in WWF and SGF of different wheat varieties. The SDS-Sedimentation Value ranged from 20.00 to 26.70 mL and from 21.48 to 28.14 mL in WWF and SGF, respectively of different wheat varieties. The SGF contained significantly the higher SDS-Sedimentation Values as compared to WWF. The falling number differed significantly in SGF and WWF of different wheat varieties and ranged from 529 to 828 seconds and 400.50 to 775.50 seconds between the straight grade flour (SGF) and whole wheat flour (WWF), respectively. Straight grade flour of different wheat varieties possessed higher falling number than whole wheat flour of different wheat varieties. The physical dough parameters derived from farinograms differed significantly as a function of wheat, varietals differences. The water absorption (WA) in WWF of different varieties was found significantly the highest (71.23%) in wheat variety Iqbal 2000 while the wheat variety Faisalabad 83 possessed significantly the lowest WA (58.46%). The WA was higher in SGF of wheat varieties Iqbal 2000 and Pak-81 and lower in SGF of wheat variety Faisalabad 83. The dough development time
varied from 1.50 to 7.70 minutes and 2.75 to 9.20 minutes, respectively in WWF and SGF of different wheat varieties. The dough stability in WWF and SGF of different wheat varieties showed wide variation. The highest (11.40 min.) DST was observed in WWF of Kohinoor 83 while the lowest (2.65 min.) DST was recorded in WWF of wheat variety Punjab 81.

The minerals such as calcium, copper, iron, zinc and manganese content differed significantly among different wheat varieties. The zinc content ranged significantly from 1.55 to 3.40 mg/100g and 0.89 to 2.10 mg/100g in whole wheat and straight grade flours, respectively of different wheat varieties. The iron content was significantly the highest (5.56 mg/100g) in whole wheat flour samples of Inqlab 91, while the lowest iron content (1.98 mg/100g) was found in SGF of wheat variety MH-97. The copper content varied from 0.23 to 1.08 mg/100g in WWF and from 0.11 to 0.25 mg/100g in SGF of different wheat varieties. The manganese content ranged from 2.30 to 5.50 mg/100g and 0.81 to 1.97 mg/100g between whole wheat flour and straight grade flour of different wheat varieties, respectively. The calcium content in SGF varied from 17.90 to 30.81 mg/100g and significantly the highest calcium content (30.81 mg/100g) was found in SGF of C-273 and the lowest calcium content (17.90 mg/100g) was found in the SGF of GA-02. The mineral content was higher in whole wheat flour as compared to respective straight grade flour.

The sensoric attributes of breads varied significantly among different wheat varieties. The highest total scores (76.02) were assigned to breads prepared from flour of Pasban 90 while significantly the lowest total scores (64.06) were given to the breads prepared from wheat variety GA-02. The total scores assigned to chapattis prepared from WWF of different wheat varieties ranged from 35.60 to 43.60 scores. The wheat variety Pasban 90 got significantly the highest total scores whereas the wheat variety GA-02 was ranked at the bottom with respect to total scores of chapattis.
The high molecular weight glutenin subunits (HMW-GS) and low molecular weight glutenin subunit (LMW-GS) fractions of different wheat varieties measured through SDS-polyacrylamide gel electrophoresis (PAGE) showed two electrophoretic bands in the region of 33kDa and 36kDa in all the wheat varieties. LMW-GS in the region around 45kDa was also present in all wheat varieties except Pasban 90 in which it was absent. With exception to Kohinoor 83, all the tested wheat varieties possessed LMW-GS around 54kDa. With respect to high molecular weight glutenin subunits (HMW-GS), the polypeptides in the region around 80kDa was present in all wheat varieties except of wheat varieties Punjab 85, Manthar 03, Faisalabad 85 and Punjab 96 in which it was missed. HMW-GS in the region 90kDa – 92kDa was identified in all wheat varieties except Kohistan 97, Iqbal 2000 and Manthar 03. The highest number of polypeptide bands for HMW-GS were identified in wheat varieties Pak-81 (ranged at different intervals of 103.6 kDa, 99.2 kDa, 87.5 kDa and 80.1 kDa), Pasban-90 (100.3 kDa, 92.9 kDa, 80.7 kDa and 75.4 kDa) and Iqbal-2000 (102.9 kDa, 98.8 kDa, 87.1 kDa and 80.8 kDa).

The low molecular weight glutenin subunits (LMW-GS) represented approximately 60% of the total wheat glutenin fraction of different wheat varieties, and their molecular weights ranged from 32.3 kDa to 67.4 kDa. The LMW-GS present in wheat varieties Inqlab-91 ranged from 33.9 kDa to 66.9 kDa, Pak-81 (33.0 kDa to 66.9 kDa), Pasban-90 (33.4 kDa to 66.1 kDa) GA-02 (33.4 kDa to 66.1 kDa), Punjab-85 (33.4 kDa to 66.1 kDa), MH-97 (33.0 kDa to 66.1 kDa), Faisalabad-85 (33.4 kDa to 66.1 kDa), Manthar-03 (33.4 kDa to 66.1 kDa), C-273 (33.4 kDa to 66.3 kDa), Iqbal-2000 (32.7 kDa to 65.9 kDa), Kohinoor-83 (32.9 kDa to 65.6 kDa) Uqbab-2000 (32.9 kDa to 66.6 kDa), Punjab-96 (33.0 kDa to 67.0 kDa), Punjab-81 (32.8 kDa to 66.3 kDa), Kohistan-97 (33.5 kDa to 66.9 kDa) and Faisalabad-83 (32.3 kDa to 67.4 kDa).
The antibody response assessed through animal modeling by using rabbits as test animals showed significant variation in antibody response towards high molecular weight glutenin (HMG) and low molecular weight glutenin (LMG) of different wheat varieties. The antibody response against HMG varied from 0.300 to 0.70 among different wheat varieties. The antibody response against HMG was recorded maximum in wheat variety Iqbal-2000 (0.70) followed by Pasban 90 (0.65) and Inqlab-91 (0.65). The wheat varieties such as Kohistan 97 and Pak-81 exhibited identical antibody response of 0.600 against HMG. The wheat varieties Kohinoor 83 and Manthar 03 also showed similar antibody response of 0.500 and the wheat varieties MH-97, Punjab-85 and Uqab-2000 showed similar antibody response of 0.460 against HMG. However, the wheat varieties Punjab-96, GA-02 and Punjab-81 exhibited the lowest antibody response against HMG which was 0.300, 0.350 and 0.380, respectively, as compared to other tested wheat varieties.

Significant variation existed among wheat varieties with respect to antibody response against low molecular glutenin (LMG). Maximum antibody response of 0.900 was recorded against LMG for MH-97 followed by FSD-83 (0.85). The wheat varieties Punjab-96 and Punjab-85 showed identical antibody response of 0.80. The antibody response was also found similar for wheat varieties Uqab-2000, Manthar-03 and Pasban-90 against LMG. The wheat varieties Kohistan-97 and Pak-81 also gave similar antibody response of 0.70, whereas GA-02, Kohinoor-83 and Iqbal gave similar antibody response of 0.650 against LMG. The lowest antibody response was exhibited by the LMG of wheat varieties i.e. Punjab-81, C-273 and FSD-85 which was 0.55, 0.55 and 0.58, respectively. The antibody response against LMG was higher as compared to HMG of different wheat varieties.

Correlation studies showed significant relationship of HMG and LMG with different quality attributes of whole wheat flour and sensory parameters of
chapatti. A significant and positive correlation existed between HMG antibody response and crude protein (r = 0.51), farinographic water absorption (r = 0.61). The SDS- sedimentation value (r = 0.44) and wet gluten (r = 0.37) also correlated positively with antibody responses against HMG. The HMG antibody response showed a highly positive relationship with flavor (r = 0.76), breakability (r = 0.87), texture (r = 0.81) and total chapatti scores (r = 0.91). The folding-ability (r = 0.62), feel to touch (r = 0.58) and color of chapatti (r = 0.49) also showed significant correlation with antibody response against HMG. The antibody response against LMG showed non-significant correlation with different flour characteristics and chapatti scores with the exception of existence of negative correlation with that of chapatti color (r = -0.52) and overall chapatti scores (r = -0.42).

A positive correlation of wet and dry gluten with that of HMG antibody response was also observed. A significant and positive relationship of bread sensory attributes with antibody response against HMG was observed. The mastication (r = 0.64), texture (r = 0.69), taste (r = 0.72), aroma (r = 0.68), grain (r = 0.71) and total score (r = 0.83) showed highly significant association with antibody response against HMG. The loaf volume correlated linearly with that of HMW antibody response (r = 0.51). The LMG antibody response was negatively correlated with flour characteristics and sensorial attributes with non significant level. The information obtained from the present study on the characterization of wheat varieties is useful for traders, millers and bakers for their intended uses. The results of the immunochemical studies can also serve as a single tool for the assessment of the end use quality of wheat varieties.
Conclusions

• The thousand kernel weight and test weight differed significantly among different wheat varieties.

• The chemical characteristics like crude protein, ash, crude fat, SDS-sedimentation value, wet gluten and Pelshenke value differed significantly among the wheat varieties.

• The mineral contents like, calcium, iron, zinc and copper significantly differed among the wheat varieties.

• The chemical constituents like protein, crude fat, ash and minerals were found higher in whole wheat flour than straight grade flour of the respective wheat varieties.

• Significant variation existed among the wheat varieties with respect to chapatti and bread making quality.

• The low molecular weight glutenin sub units ranged from 32.3 kDa to 67.4 kDa.

• The range of HMW – GS in wheat varieties was recorded between 74.6 kDa to 103.6 kDa in different wheat varieties.

• The wheat varieties having more number of HMW-GS bands are good for bread and chapatti making quality.

• Anti – LMG antibody response is negatively correlated to total scores of breads and chapattis.

• Anti- HMG antibody response is positively correlated to total scores of breads and chapattis.

• Anti- HMG antibody can be used as a tool for screening wheats for good quality chapattis and breads.
Recommendations

- The physical and chemical quality parameters were significantly different among wheat varieties, thus the information obtained in this study can be useful for millers and bakers for the selection of suitable variety for their intended uses.

- ELISA is a reliable method and can be used as single tool for the wheat quality assessment therefore it should be developed for all wheat varieties.

- A research project should be conducted to check the antibody response of gliadin; one of the gluten forming proteins with respect to end use quality.
LITERATURE CITED


associated with the loss of low Mr or high Mr glutenin subunits. J. Cereal Sci. 21:103–116.

Gupta, R.B. and F. MacRitchie. 1994. Allelic variation at glutenin subunit and Gliadin Loci-Clu-1, Glu-3 and Gli-1 of common wheats. II- Biochemical


rheology and baked product texture. H.Faridi and JM Faubion, eds Avi: NY.

Miralbes, C. 2003. Prediction chemical composition and alveograph parameters 
on wheat by near-infrared transmittance spectroscopy. J. Agric. Food 
Chem. 51:6335-6339.

Directorate of Wheat Research, Karnal, Haryana, India. Technical Bulletin 
3: 4- 10.

growth habitat on compositional, milling, and baking characteristics of 

bonds of adjacent cysteine residues in low molecular weight subunits of 

2005. Evaluation of two commercial lateral-flow test kits for detection of 

Ng, P.K.W. and W. Bushuk. 1988. Statistical relationships between high 
molecular weight subunits of glutenin and bread-making quality of 

Ohm, J.B. and O.K. Chung. 1999. Gluten, pasting, and mixograph parameters of 
76(5):606-613.

Osborne, T.B. 1907. The protein of the wheat kernel. Publication No. 84. Carnegie Institute: Washington, DC.


related lines of wheat that have contrasting breadmaking quality. J. Cereal Sci. 8:285-288.


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APPENDIX I

Schematic representation of protein fractionation

2.5g Sample + 10ml Tris HCl (50mmol, pH 8.8)

\[ \downarrow \]

Stirring (4°C for 1 hr)

\[ \downarrow \]

Centrifuge (20000g, 20min, 4°C)

\[ \downarrow \]

Supernatant discarded

\[ \downarrow \]

Repeat

\[ \downarrow \]

Water wash

\[ \downarrow \]

Pellet + 10ml 75% ethyl alcohol

\[ \downarrow \]

Vortex

\[ \downarrow \]

Stirring (4°C for 1 hr)

\[ \downarrow \]

Centrifuge (20000g, 20min, 4°C)

\[ \downarrow \]

Supernatant discarded (gliadin)

\[ \downarrow \]

Water wash

\[ \downarrow \]

Pellet + 10ml SDS buffer

\[ \downarrow \]

Vortex

\[ \downarrow \]

Stirring (4°C for 1 hr)

\[ \downarrow \]

Centrifuge (20000g, 20min, 4°C)

\[ \downarrow \]

Pellet (HMW GS)  Supernatant (LMW GS)
# APPENDIX II

## Score card for sensory evaluation of breads

**Name of Judge __________________________**  Evaluation after ______ Days

<table>
<thead>
<tr>
<th>External Characteristics</th>
<th>Max.Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Loaf Volume</td>
<td>10</td>
</tr>
<tr>
<td>Color of Crust</td>
<td>8</td>
</tr>
<tr>
<td>Symmetry of form</td>
<td>3</td>
</tr>
<tr>
<td>Evenness of bake</td>
<td>3</td>
</tr>
<tr>
<td>Crust Character</td>
<td>3</td>
</tr>
<tr>
<td>Break and shred</td>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Internal Characteristic</th>
<th>Max.Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain</td>
<td>10</td>
</tr>
<tr>
<td>Color of crumb</td>
<td>10</td>
</tr>
<tr>
<td>Aroma</td>
<td>10</td>
</tr>
<tr>
<td>Mastication</td>
<td>10</td>
</tr>
<tr>
<td>Taste</td>
<td>15</td>
</tr>
<tr>
<td>Texture</td>
<td>15</td>
</tr>
</tbody>
</table>

Signature: _______________________
Date: _______________________

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clxxxvii
APPENDIX III
Score card for sensory evaluation of chapattis

Directions

Please take these samples one by one and evaluate them for the following parameters on hedonic scale as given at the end of form. It is very important to rinse mouth thoroughly with clean water after evaluation of each sample.

Name of the Judge ---------------------------
Age -------------------------------
Sex -------------------------------
Signature ------------------------------- Date ------------------------

<table>
<thead>
<tr>
<th>Attribute</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colour</td>
<td></td>
</tr>
<tr>
<td>Flavour</td>
<td></td>
</tr>
<tr>
<td>Texture</td>
<td></td>
</tr>
<tr>
<td>Feel to touch</td>
<td></td>
</tr>
<tr>
<td>Foldability</td>
<td></td>
</tr>
<tr>
<td>Breakability</td>
<td></td>
</tr>
</tbody>
</table>

**Hedonic scale**

9  Like extremely
8  Like very much
7  Like moderately
6  Like slightly
5  Neither like nor dislike
4  Dislike slightly
3  Dislike moderately
2  Dislike very much
1  Dislike extremely