

# Grain Hardness: A Major Determinant of Wheat Quality

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Wheat quality, a complex term, depends upon intentional use for unambiguous products. The foremost determinants of wheat quality are endosperm texture (grain hardness), protein content and gluten strength. Endosperm texture in wheat is the single most important and defining quality characteristic, as it facilitates wheat classification and affects milling, baking and end-use quality. Various techniques used for grain hardness measurement are classified into diverse groups according to grinding, crushing and abrasion. The most extensively used methods for texture measurement are PSI, NIR hardness, SKCS, pearling index, SDS-PAGE and PCR markers. Friabilin is a 15 kDa endosperm specific protein associated with starch granules of wheat grain and is unswervingly related to grain softness. Chemically, it is a concoction of different polypeptides, primarily puroindolines; Pin a and Pin b. Hardness (Ha) locus of chromosome 5DS makes the distinction between soft and hard classes of wheat. Some additional modifying genes are also present which contribute to the disparity within wheat classes. Numerous allelic mutations in Pin have been reported and their relation to end product quality has been established. This treatise elaborates the consequence of grain hardness in wheat eminence.

*Key Words:* wheat quality, grain hardness, puroindolines

## INTRODUCTION

Wheat is inimitable among all the cereal grains grown in many parts of the world as a staple food, and forms the basis for numerous food products. The term wheat quality is intricate and is complicated to articulate in terms of a single property. Numerous parameters like milling, chemical, baking and rheological dough properties influence the wheat quality. Wheat quality is a produce of overall contribution of seed stock, effects of soil, climate, and kernel components. It may also be defined in terms of its suitability for a particular purpose or use (Finney et al., 1987). Variety is an imperative factor that manipulates grain quality. Generally, wheat is marketed according to the class and each class consists of a group of varieties with similar characteristics apt for similar purposes and end-use (Halverson and Zeleny, 1988).

Factors that persuade wheat quality have been broadly classified into two groups: physical and chemical characteristics. Grain vitreousness, color, weight,

shape and hardness are some essential physical characteristics, which influence wheat grain quality (Gaines et al., 1996) while chemical characteristics include protein content, SDS-sedimentation value and gluten strength, etc.

The kernel texture is one of the most important characteristics for milling and baking quality of wheat. Wheat is classified into soft, medium soft, hard, medium hard and extra hard on the basis of kernel hardness (Kent and Evers, 1994; Hansen and Poll, 1997). This categorization forms the fundamental basis for differentiating the world trade of wheat grain. Growers, millers and bakers also entail this classification for their intended end-use (Morris, 2002). The major determinants of softness and hardness are particle size index (MacRitchie, 1980; Jolly et al., 1996; Delwiche, 2000; Osborne et al., 2001), energy required for grinding a selected weight of sample (Kosmolak, 1978), pearling value (Kramer and Albrecht, 1948; Beard and Poehlman, 1954; Chung et al., 1977) and near infrared reflectance (Wetzel, 1984; Williams and Sobering, 1986; Delwiche, 1993; Manely et al., 1996).

Soft wheat kernels are easy to be fractured, which results in production of large number of intact starch granules and fine flour having less damage to starch. Flour with coarser texture is produced by hard wheat, having broken granules of starch fracture planes and higher levels of starch damage with more power consumption in the flour mill. Hard wheat is more suitable

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for those breads which are leavened by yeast because broken starch granules, that is damaged starch, absorb more water, while it is good to use flour of soft wheat for cookies, cakes and pastries due to less protein and starch damage (Morris and Rose, 1996). Other parameters that ultimately influence hardness are density of grain (Gasiorowski and Poliszko, 1977) and its vitreousness (Simmonds, 1974). Sodium carbonate retention capacity, which is one of the solvent retention capacity tests included in AACC Approved Method 56-11 (Gaines, 2000) primarily measures damaged starch.

The interaction between carbohydrates and proteins strongly influence the processing quality of flour and is closely related with the hardness of endosperm (Preston, 1998). There are two main types of protein fraction which are associated tightly with starch granules, storage proteins and starch granule-associated proteins. Proteins which remain adsorbed to the starch granules surface even after extraction of starch are glutenins and gliadins, also called grain storage protein. As the starch granule-associated proteins (SGAPs) are tightly bound to the surface or integral starch component, so these proteins are biologically different from plant storage proteins (Goldner and Boyer, 1989; Skerritt et al., 1990; Skerritt and Hill, 1992; Baldwin, 2001).

The SGAPs have been divided into two groups on the basis of molecular weights: low molecular weight proteins which are termed as 'surface' SGAPs; and proteins with higher molecular weight which are called 'internal' granule-associated starch proteins (Baldwin, 2001). The proteins of more interest for scientists are the 15 kDa 'group of polypeptides' in which the major sub-group is puroindoline and is also termed as friabilin. It has been indicated by the SDS-PAGE that the friabilin band is prominent in soft wheat varieties; wheat of hard character; has a faint band, while it is totally lacking in durum wheat (Greenwell and Schofield, 1986; Schofield and Greenwell, 1987).

The discovery of friabilin, a starch granule protein, which linked with the texture and quality of wheat grain, formed the biochemical basis for assessment of kernel texture. The protein complex friabilin regulates adhesion degree of starch granules to the protein matrix and this factor is of great importance as it tells about the hardness (Beecher et al., 2002; Hogg et al., 2004). Two main components are present in friabilin: Pin a and Pin b puroindoline (Turner et al., 1999; Wanjugi et al., 2007a). The puroindolines have five disulfide bonds with tryptophan-rich domains, hence these proteins were named owing to the unique tryptophan-rich region, which has an indole ring (*puros* means wheat and *indoline* from indole ring of tryptophan) (Gautier et al., 1994; Dubreil et al., 1997). The puroindolines are categorized as lipid-binding proteins (Dubreil et al., 1998) and grain softness is controlled by these proteins having various transgenic changes (Giroux and Morris, 1998).

The variation in kernel texture (hardness or softness) is inherited and controlled by a single locus referred to as Hardness (*Ha*), which comprises three genes (Pin a, Pin b and Gsp-1) within a region of about 82,000 bp (Chantret et al., 2005). The *Ha* locus resides on chromosome 5Ds (Mattern et al., 1973; Doekes and Belderok, 1976; Bhave and Morris, 2008b). The hard wheats possess the recessive or mutated form (*ha*) while soft wheats have the prominent or wild type form (*Ha*) (Gazza et al., 2005; Bhave and Morris, 2008a). The durum wheats lack the D-genome and represent a harder class of wheats (Jolly et al., 1993; Morris et al., 1994). In wheat grain, the accumulation of friabilin is dependent on the softness/hardness genes *Ha/ha* (Schofield and Greenwell, 1987). It is now a well-known fact that both Pin a and Pin b genes have various different alleles in hexaploid wheats. To date, 17 Pin a and 25 Pin b alleles have been reported in common wheat and related species (Morris and Bhave, 2008). The variation in Pin function significantly affects milling and end product quality characteristics in wheat (Hogg et al., 2005; Wanjugi et al., 2007b; Martin et al., 2008; Feiz et al., 2009).

## GRAIN HARDNESS

Grain hardness is used as a grading factor to determine the type of wheat (Morris, 2002). It is a key determinant for classification of wheat and end product quality (Campbell et al., 1999). Grain hardness is important for the flour industry because it has significant impacts on milling, baking and qualities of wheat (Bettge et al., 1995). The texture of endosperm influences certain physical properties, for example the tempering requirements, flour particle size, flour density, starch damage, water absorption and milling yield (Martin et al., 2001, 2007; Cane et al., 2004; Chen et al., 2007). This indicates the suitability of specific flours for different products. Flour color is also an important quality characteristic with utmost importance for specific products like noodles. Significant relationship between flour color and hardness was depicted by Nagamine et al. (2003); however, Konopka et al. (2005) did not find any relationship. The endosperm texture is also imperative to the wheat growers as generally hard wheat fetch higher prices due to protein content differences. Hard wheat consist of relatively uniform and large particle size one as compared to soft wheat, in which the cell contents fracture leaving the starch granules intact and result in wide particle size distribution (Hoseney et al., 1988; Pomeranz, et al., 1988). Rheological properties of the dough are also influenced by grain texture (Martinant et al., 1998; Branlard et al., 2001). The most important physical difference between the endosperm of hard and soft wheats is that soft wheat

contains attached starch granules with protein matrix surrounding these granules (Simmonds et al., 1973).

Less damaged starch is produced by kernels of soft wheat. These kernels easily break down yielding fine powder-like flour having less damaged starch. Hard kernels are difficult to crush and grind, and produce coarser-textured flour with higher levels of starch damage (Jolly et al., 1993; Ikeda et al., 2005). The damaged starch has a higher water absorption capacity (Brites et al., 2008) and is more readily hydrolyzed by  $\alpha$ -amylase. It is more difficult to reduce the particle size of hard wheat flour because hard wheat tends to fracture along the lines of cell boundaries and thus produces flour with higher values of mean particle size than that of flours of soft wheat (Pomeranz, 1988).

The flour products of soft wheat are baked to low moisture contents. There are two main characteristics; increased water absorption capacity and substrate availability, are not desirable in flours of soft wheat. It is better to use hard wheat for making those pan breads leavened by yeast, as the high levels of damaged starch granules in these flours absorb more water, while soft wheat flour is used for cookies, cakes, pastries and confections (Morris and Rose, 1996).

Hard and soft wheats are macroscopically similar, as far as morphology of grains is concerned. A-type (large lenticular) and B-type (spherical) starch granules are present in mature grains of wheat when the observations are carried out at light microscopic level. The nonstarch fractions of polysaccharide, which are membrane associated, and variation in grain texture are among soft wheat samples but no similar effect is found in 'hard' wheat samples (Bettge and Morris, 2000). Grain hardness evaluated by NIR has found significant correlation with starch damage, medium diameter and Rapid Visco-Analyzer setback (Brites et al., 2008). The wheat hardness is controlled genetically and certain environmental factors can modify it, for example moisture, lipids and contents of pentosan (Glenn et al., 1991).

Friabilin is an endosperm specific, starch associated fraction of cereal grain protein. It was named friabilin to highlight the fact that soft wheats are more friable than hard wheats (Greenwell and Schofield, 1989; Morrison et al., 1992). Subsequently it was also called grain softness protein (GSP) (Jolly et al., 1993). The discovery of friabilin provided a biochemical basis for the distinction between hard and soft wheat. Its presence was perfectly correlated with the qualitative level of endosperm hardness. This friabilin endosperm softness relationship was confirmed in hundreds of genotypes (Greenwell and Schofield, 1986; Morris et al., 1994). On the basis of molecular weights there are two groups of starch granule associated proteins: low molecular weight proteins (Mw of ~5, 8, 15, 19 and 30 kDa) and higher molecular weight proteins (Mw of ~60, 77, 86, 95 and 149 kDa). Low molecular weight proteins are termed as 'surface' granule associated proteins (Baldwin, 2001). The

important and useful indicator which is measurement of friabilins of starch or endosperm makes the biochemical basis of grain softness (Bettge et al., 1995) and markers for the texture of endosperm are may be endosperm lipids (Morrison et al., 1984, 1989; Greenblatt et al., 1995).

Friabilin is linked with kernel softness (Greenwell and Schofield, 1986; Morris et al., 1994; Bettge et al., 1995; Matus-Cadiz et al., 2008). The hard and soft wheat endosperms physically differ from each other due to adhesive strength among starch granules and protein matrix which surrounds it (Simmonds et al., 1973). It was noticed that the adhesive strength was independent of environmental conditions during growth and other polypeptides associated with starch granule (Jolly et al., 1993). When the friabilins are present the grains exhibit more softness, hence the softness of grains varies in a positive way with the presence of friabilin. The bound polar lipids mediate the occurrence of friabilin on the surface of starch granule (Greenblatt et al., 1995). The friabilin on the surface of water washed starch is localized however there exist a phenomena that partitioned the related lipid binding properties of friabilin and isolation procedure of starch (Malouf et al., 1992). It was shown that among fractionated flours (starch, gluten and water solubles) friabilin partition toward the gluten and starch fraction but not to water solubles (Jolly et al., 1990, 1996; Jolly, 1991; Rahman et al., 1991, 1994).

Patterns followed by the occurrence of bound glycol and phospholipids at the surface of wheat starch granule is the same as followed by friabilin (soft wheat contains high levels than that of hard wheat) (Greenblatt et al., 1995), and it is believed that these kinds of lipids play a vital role in the friabilin/puroindoline polypeptides to granule surface association in wheat of soft varieties (Oda and Schofield, 1997). The friabilin protein is composed of a family of 13 closely related proteins found on water washed wheat starch and it is not a single protein (Morris et al., 1994). The membrane-bound proteins, Puroindoline a and b, are two main components of friabilin (Rahman et al., 1994; Oda, 1994). These components of friabilin correspond to the basic cystein-rich proteins Pin a and b (Gautier et al., 1994). Puroindolines are considered as unique proteins due to their respective domains which are rich in tryptophan, which have an apparent high affinity for binding lipids (Wilde et al., 1993; Dubreil et al., 1997; Bhave and Morris, 2008a).

Puroindolines are basic cystein-rich polypeptides and mean value of their molecular weight is 12.8 kDa. Puroindolines contain a unique domain rich in amphiphilic tryptophan (Blochet et al., 1993). Functional or wild state of both puroindolines gives soft textured grains. The hard texture of wheat grains is due to lack of puroindolines as in case of durum wheat. The puroindolines bind lipids and this property is important in



processing of cereals. The destabilization of foams (by oil globules) is prevented by puroindolines in bread. A finer crumb structure of bread can be formed by addition of small amounts of puroindolines in breads while making bread doughs. Certain physical properties of bread dough-like tenacity and extensibility are also affected by puroindolines and on its addition in dough, ultimately the texture of baked products altered. Destabilization of foams by neutral and polar lipids can be restored in beer processing by using puroindolines (Clark et al., 1994). The chromosome 5D genetically controls friabilin (Jolly et al., 1993; Morris et al., 1994). Domain of tryptophan with different character is present in genes of Pin a and b (Gautier et al., 1994) and for this domain it is considered that it is an active site for binding of those lipids which are mostly present on starch granule surface (Marion et al., 1994; Greenblatt et al., 1995). The soft character of grains required the presence of genes Pin a and b in chromosome. Pin a and Pin b have 55% similarity at level of cDNA (Gautier et al., 1994). Hardness of the grains can also be developed by alteration in genes either Pin a or b, when these genes undergo to the mutation.

Softness of the kernel is genetically controlled trait and it depends on friabilin concentration in the grain and this concentration is also related to the gene Ha for softness locus on the 5D chromosome (Sourdille et al., 1996; Tranquilli et al., 1999). In hexaploid wheat it is carried on the short arm of chromosome 5D. The puroindolines represent the basis molecular genetic of the hardness locus on chromosome 5DS and the soft (Ha) and hard (ha) alleles present in hexaploid wheat varieties (Morris, 2002; Igrejas et al., 2002; Turnbull et al., 2003).

The mutations in Pin a and b is present in all hard-textured wheats (Giroux and Morris, 1997). Variations in Pin a or b can modify grain hardness significantly and affect end-use quality (Morris, 2002; Turnbull and Rahman, 2002). Cultivars with soft grain possess wild type alleles (Gazza et al., 2005). The wild type Pin a-D1a sequence is complemented by the Pin a-D1b allele of hard wheats, which represents a soft trait (Martin et al., 2006). The amino acid glycine is changed into serine in tryptophan-rich domain related to Pin b (allele Pin b-D1b). It is assumed that it is able to increase water absorption and lipid binding properties of Pin b. In other words when there is no pin (allele Pin a-D1b) grain hardness will be increased (Giroux and Morris, 1997, 1998). The lack of puroindoline b is often obtained for spring cultivars (Dubreil et al., 1994). The allelic forms of Pin b, pinB-D1b and pinB-D1c, have been most commonly found in many new cultivars (Lillemo and Morris, 2000; Morris et al., 2001). Linkage studies with Pin detected RFLPs and hardness (Sourdille et al., 1996), the Gly-46 versus Ser-46 puroindoline b sequence (Giroux and Morris, 1997) and hardness (Campbell et al., 1999, 2001) have been reported. The most

common mutations of hard texture in common wheat are associated with certain Pin gene; deletion of gene Pin a also known as null allele (Pin a-D1) and Pin b-D1b allele in addition to some other point mutations in different germplasm (Bhave and Morris, 2008a). The tightly linked genes are puroindoline a (Pin a-D1a) and puroindoline b (Pin b-D1a). These are cloned and determined from the locus of Ha (Giroux and Morris, 1997, 1998; Tranquilli et al., 1999), that are involved in controlling the grain texture in wheat. The lines having Pin a deletion showed lower PSI values, decreased milling yield and increased water absorption (Cane et al., 2004; Gale, 2005). Swan et al. (2006) described Pin b as a greater limiting factor than Pin a for grain softness in transgenic soft wheats. It is reported that 17 Pin a (Pin a-D1a-q) and 25 Pin b (Pin b-D1a 17-w, aa, ab) alleles are common in wheat and its related species (Morris and Bhave, 2008).

### Factors Affecting Grain Hardness

Grain hardness is normally influenced by various environmental, physical and chemical factors like kernel protein, vitreousness of grain, kernel size, water-soluble pentosans, moisture content and lipid content (Anjum and Walker, 1991; Turnbull and Rahman, 2002).

#### *Protein Content*

Wheat having high protein content tends to be hard, have strong gluten and produce good quality bread. Wheat of low protein content tends to be soft, have weak gluten and produce small loaves of inferior crumb structure (Bushuk, 1998; Tipples et al., 1994), but produce better quality cookies. The higher protein content and density are exhibited only by vitreous kernels than that of those kernels which are starchy or mealy, as air pockets account for low density (Sharp, 1927). The protein content and kernel hardness relationship exhibits that 1particle size index (PSI) increases with increasing protein content in some cultivars while in some other cultivars it was opposite (Symes, 1965). Significant positive correlation of grain protein content with SKCS hardness ( $r=0.26$ ) and NIR hardness ( $r=0.45$ ) was reported by Groos et al. (2004). The total protein content showed nonsignificant correlation with kernel hardness (Miller et al., 1984), while in some individual varieties no correlation was found (Pomeranz et al., 1985). Environmental factors are able to affect composition of grain protein but this protein composition and concentration are genetically controlled parameters (Graybosch et al., 1996; Huebner et al., 1997; Zhu and Khan, 2001). Different scientists have reported grain hardness and protein relationship has presented in Table 1.

**Table 1.** Relationship of wheat hardness and protein (Adapted from Galande, 2002).

Method(s)	Results	References
Cracking	No relation	Newton et al. (1927)
Particle size	No relation	Worzella (1942)
Particle size	uninfluenced by protein	Berg (1947)
Particle size	influenced by protein	Fajerson (1950)
Particle size	Protein effect varies among varieties	Symes (1961)
PSI, starch damage	No relation	Williams (1967)
Particle size	No relation	Symes (1969)
Wheat hardness index	Relation with protein per square meter of flour and protein	Greenaway (1969)
Microscopy of endosperm particles	Protein particles for hard (unlike soft) wheat compact and hard to disrupt	Seckinger and Wolf (1970)
Penetrometer	No varietal differences	Barlow et al. (1973)
Pearling resistance, PSI	Negative relation for single cultivar	Moss et al. (1973)
PSI (protein by dye binding)	Very low relation affected by variety and environment	Trupp (1976)
Time to produce a fixed volume of ground wheat	Positive relation with protein content	Stenvert and Kingswood (1977)
Starch damage, particle size	Optimum hardness and starch damage related to minimum protein	Moss (1978)
Miscellaneous	No relation	Obuchowski and Bushuk (1980)
Work required to grind	No relation	Miller et al. (1981)
Time to grind, work to grind, particle size, NIR	No relation	Miller et al. (1982)
Near-infrared spectroscopy (NIR)	Positive relation	Sourdille et al. (1996)
Miscellaneous	Positive relation	Bushuk (1998)
NIR	Significant correlation	Giroux et al. (2000)
NIR, SKCS	Positive correlation for NIR, Negative for SKCS	Martin et al. (2001)
NIR	Positive correlation with protein content, Negatively with Pin a	Igrejas et al. (2002)
SKCS	Positive significant correlation with NIR protein ( $r = 0.43$ )	Wanjugi et al. (2007b)
NIR	Positive with NIR protein ( $r = 0.34$ )	Bordes et al. (2008)
SKCS, Pin b content	Significant positive correlation	Gazza et al. (2008)
Pearling value, near-infrared spectroscopy (PSI)	No relation with crude protein	Pasha et al. (2009a)
NIR hardness	No relation with NIR protein	Pasha et al. (2009b)

The most important component of wheat grain is protein (8–15% on weight basis), which governs end-use quality (Weegels et al., 1996). Quality characteristics of wheat grains can be determined by examining structure of wheat. The wheat storage protein molecular structure not only affects protein interaction during various steps of bread making but also affects final product quality (Bushuk, 1998; Shewry et al., 1999). Bread making quality is significantly altered by variation in composition as well as protein content of flour (Lafiandra et al., 1999; Branlard et al., 2001). The composition of wheat flour affects flour sedimentation value and this value is related to protein content, wheat hardness and pan volume and hearth characters. Protein contents influence both the volume and zeleny value so that, a stronger correlation of bread loaf volume and zeleny sedimentation volume was found compared to SDS sedimentation volume (Shewry and Tatham, 2000).

Osborne (1907) carried out systematic study for the development of proteins of cereal seed and this

development is based on solubility and extraction parameters. There are four different groups of protein albumins which are soluble in water and dilute buffers, globulins which are soluble in saline solutions only prolamins, which are 70–90% soluble in ethanol and dilute acid or alkali-soluble glutelins. Fifth fraction was added by Chen and Bushuk (1970) to the original four proteins separated from Osborne's procedure. The mature grain endosperm proteomic analyses of wheat can provide the information about albumin proteins (Singh et al., 2001). Barley (*Hordeum vulgare* L.) is a member of a family of enzyme,  $\alpha$ -amylase or trypsin inhibitors, and it has dual storage roles (Finnie et al., 2002). The gliadins and glutenins are storage proteins accumulated during the grain-filling period (Shewry and Halford, 2002).

The gluten proteins, gliadins and glutenins (polymeric proteins) account for 80% of the total grain proteins (Shewry and Tatham, 1997) and these are considered as among the largest protein molecules in nature (Wrigley, 1996) with a molecular weight of glutenin

polymers reaching over 20 mDa. These proteins are heterogeneous mixtures of polymers formed by disulphide bonded linkages of polypeptides (Payne et al., 1979, 1985; Thompson et al., 1994) and are responsible for viscosity and extensibility of dough. High molecular weight glutenin subunits (HMW-GS) are present in minor quantity; but are major contributor to visco-elastic properties of wheat (Tatham et al., 1985; Gianibelli et al., 2001). HMW-GS are closely related with bread quality as compare to low molecular weight glutenin subunits (LMW-GS), one-third of total seed proteins (Bietz and Wall, 1973). The LMW-GS showed the capability to form large aggregates that are related to dough strength (D'Ovidio and Masci, 2004). Significant correlations between different quality parameters and wet and dry glutes have been reported (Pasha et al., 2007).

In addition to these functionally important proteins, there are some starch associated endosperm proteins, which also play a significant role in grain texture and end product quality of wheat.

#### *Moisture Content*

Moisture content play an important role in measuring the wheat kernel texture (Pomeranz and Williams, 1990) and has a very strong effect on grinding time particularly for soft wheats (Williams and Sobering, 1988). All methods used for measuring wheat kernel texture have been reported to be affected by kernel moisture content (Newton et al., 1927). Moisture content (6–18%) showed highly positive correlation with PSI for soft wheat cultivars (Obuchowski and Bushuk, 1980; Yamazaki and Donelson, 1983).

#### *Kernel Size*

The correlation between kernel size and hardness has shown varying results. Particle size index and kernel weight are strongly correlated for hard cultivars while negatively correlated for soft cultivars (Williams and Sobering, 1984). The grinding time and PSI increased as kernel size decreased, whereas PSI and NIR reflectance are directly correlated to kernel size (Pomeranz and Afework, 1984). However, kernel size may have a small (Williams et al., 1987) or direct effect (Pomeranz, 1988) on hardness.

#### *Kernel Vitreousness*

A continuous protein in hard wheat kernels physically traps starch granules and produces hardness (Stenvert and Kingswood, 1977). Easy separation of starch granules and proteins is possible due to discontinuous structure, containing many air spaces, of soft wheat. The environment affects the degree of continuity of matrix and the wheat hardness is genetically controlled. A soft variety of wheat produces vitreous kernels when grown

under optimum environmental conditions, but kernels remain soft. On the other hand, many hard types of wheat are opaque (Hoseney, 1987; Hoseney et al., 1988).

#### *Pentosans*

Dough water balance is maintained by pentosans as 6–10 times more water can be absorbed by pentosans on the basis of weight. The quality of pentosan is more important than the quantity in determining different hardness levels. Wheat hardness is directly related to both higher soluble and insoluble fractions of pentosans (Hong et al., 1989). Grain hardness was affected by the amount of pentosans within soft wheats much more than in hard wheats and this variation may not be controlled by the *Ha* locus (Bettge and Morris, 2000). The level of water-soluble pentosans was strongly affected by environmental conditions, while hardness was found to be cultivar dependent (Hong et al., 1989).

#### *Lipids*

Lipids present in different endosperm membranes like aleurone layers and starch granules of all cereals including wheat. The true lipids are located inside the starch granules and the starch surface lipids are located on the surface of starch granules. Similarly the nonstarch lipids are not associated with starch (Turnbull and Rahman, 2002). In wheat and barley the surface lipids are mostly free fatty acids found in amounts correlated with starch granule surface area. The lysophospholipids are true lipids which are correlated with amylose content (Morrison and Gadan, 1987). The decreasing amount of free polar lipids is strongly correlated with increasing hardness in some wheat cultivars (Morrison et al., 1989). The lipids play a very important role in different processes such as milling, dough mixing, bread making and staling. The hexane extractable free lipids were found to be of a higher quantity in harder wheat cultivars than in soft cultivars (Panozzo et al., 1993).

#### **Methods of Measuring Grain Hardness**

The endosperm texture or the relative hardness or softness of a grain can be defined as a measure of the resistance to deformation based on the Single Kernel Characterization System (SKCS). It measures the force required to crush individual grains of a sample between two surfaces, taking into account the weight, diameter and moisture of the grain. Historically, the first mechanical means of measuring wheat grain texture was developed around 1908 (Roberts, 1910) and it determined the force required to crush the individual kernel's strength. The methods used for measuring the grain hardness may be categorized into four major groups on the basis of grinding, crushing, abrasion or indentation by stylus (Anjum and Walker, 1991). There are some other

methods as well which include the weight measurement requires kernel crushing (Cobb, 1897), and the distribution of granule by the process of sieving and grinding (Cutler and Brinson, 1935; Symes, 1961; Williams et al., 1998), the required energy for grinding an exact amount of sample (Kosmolak, 1978), resistance to pearling (Kramer and Albrecht, 1948; Beard and Poehlman, 1954; Chung et al., 1977) and near infrared reflectance analysis (Saurer, 1978; Wetzel, 1984; Williams and Sobering, 1986). Anjum and Walker (2000) used Kansas State University hardness tester, based on the kernel shearing principle, along with pearling value and NIR hardness to measure grain hardness in Pakistani wheat varieties and found all hardness methods were affected by cultivar, growth location and years.

More indirect definitions of grain texture refer to the manner in which grain breaks down to meal or flour and how that meal or flour behaves during processing. The methods that are used to measure the texture of wheat grain quantify the textural phenotype of bulk grain lots or individual kernels. These hardness methods provide a discrete numerical separation of qualitative classes of soft and hard wheat. The standardization of texture methods was used to facilitate the exchange of information (Williams and Sobering, 1986; Morris et al., 1999; Gaines et al., 1996; AACC, 2000). The most commonly used texture methods are PSI, NIR reflectance (Williams et al., 1986) and SKCS (Osborne et al., 2001). The methods of texture measurements may be grouped according to grind, crush, abrade or indent the sample, but none of the methods measure a fundamental material property (MacRitchie, 1980).

The SKCS is well developed system for evaluating the individual wheat kernels quality characteristics (Osborne et al., 1997; Sissons et al., 2000; Martin et al., 2001; Sayaslan et al., 2005). The SKCS provided the best discriminating measure of genetically different wheat based on hardness (Morris et al., 1999; Chen et al., 2007; Wang et al., 2008; Feiz et al., 2009). SKCS hardness was significantly and positively correlated with grain protein, ash content and mixograph water absorption in a set of homozygous recombinant inbred wheat lines (Wanjugi et al., 2007b). Break flour yield, flour yield and mixing score are positively correlated with grain hardness while flour ash is negatively correlated.

There are two most wide spread approaches to texture measurement that rely on differences in granularity (particle size index) of meals or flours after grinding or milling. The first method, PSI, quantifies granularity by sifting the ground or milled material and expressing the proportion of material that passes through a sieve of defined aperture (Worzella and Cutler, 1939). Consequently, a higher number indicates softer texture (due to the lower particle size distribution of soft wheat meals). The second method, NIR reflectance, provides an indirect assessment of particle size through the

optical reflectance of ground flour samples (Martin et al., 2001). Although the measurement of grain texture has been studied and characterized at a material property level (Glenn et al., 1991; Delwiche, 2000), it is still predominantly assessed empirically using either the granularity (particle size distribution) of meal produced by grinding, or the force/fracture characteristics of individual kernels observed while crushing. Significant correlation, that is  $r=0.94$  and  $0.71$  between SKCS and NIR hardness was observed in wheats by Giroux and Morris (1997) and Groos et al. (2004), respectively.

Some parameters of ground products like vitreous character (Simmonds, 1974), grain density (Gasiorowski and Poliszko, 1977), and the proportion of damaged starch can measure hardness indirectly (FMBRA, 1976). Different properties of grains can be measured by using all these methods, which can also produce certain chances of variation also in the results and these methods provide a slightly different phenotype (Morris et al., 1999). In methods based on PSI of wheat and its flour, two steps of Brabender hardness tester tell average energy input and particle size, which categorized the wheat in the proper classes and order but these two steps are more time consuming and less sensitive. The wheat classes are ranked in a different way by pearling resistance index. Lai et al. (1985) Three methods; a continuous, automated, single kernel hardness tester (CASK-Hat) were used which measured the stress-strain relationship. Although the positive correlation exists among methods like power to grind, particle size index and NIR reflectance, the relation between the PSI and NIR reflectance was very high (Miller et al., 1984). The soft and hard wheats may be differentiated by the use of rapid, single kernel wheat hardness tester with 80% classification reliability (Eckhoff et al., 1988).

The wheat classification evaluated by individual kernel texture analysis has 90–95% reliability (Gaines, 1986). A technique for analyzing the emitted sounds during rupture of kernels showed 80% reliability and it can give individual kernel hardness (Slaughter, 1989). A1-10 (hard-soft) hardness level was established by Mattern (1988) viewed crushed grains under a dissecting microscope, and a strong correlation coefficient of greater than 0.90 with PSI and NIR reflectance has been observed. The tablet technique was used for measuring the possible factors of hardness in wheat and sorghum by Malouf (1989) and Lawton (1989).

SDS-PAGE is one the most widely adapted techniques to determine the presence or absence of friabilin proteins, the protein responsible for grain softness or hardness (Giroux and Morris, 1997; Martin et al., 2006). The variation in puroindoline (Pin a and b) may be determined using gene specific polymerase chain reaction (PCR) markers (primers) which are currently being used as an efficient and reliable approach for the determination of Pin a and b allelic variations (Lilemmo and Morris, 2000; Morris et al., 2001; Cane



et al., 2004; Ikeda et al., 2005; Xia et al., 2005; Chen et al., 2006, 2007; Pickering and Bhave, 2007; Wang et al., 2008; Gazza et al., 2008; Morris and Bhave, 2008; Feiz et al., 2009). It is easily applicable for analyzing leaf material from the field before harvesting the desired genotype lines (Eagles et al., 2001; Gale, 2005; Gupta et al., 1999).

## FINAL REMARKS

Wheat is the most broadly grown crop in the world and its economical significance for human kind is matched only by rice. Wheat belongs to the tribe Triticeae comprising some 300 species classified into 22 genera including several other important crops (barley, rye and triticale) and a number of important forage species. Wheat quality depends upon cultivar, climatic conditions, year and process of harvest, and storage conditions. It is expressed by a variety of physical and chemical traits such as test weight, kernel weight, PSI, moisture content, ash content, protein content and SDS sedimentation.

The major determinants of wheat quality are endosperm texture and protein content. Endosperm texture has a profound effect on milling, baking and end-use quality. A varietal character, endosperm hardness, is also influenced by environment. It is controlled by the hardness (*Ha*) locus on the short arm of 5D chromosome. Grain hardness is mainly influenced by various physical and chemical factors like protein, vitreousness, kernel size, water-soluble pentosans, moisture content and lipids.

Different methods have been used for measuring grain hardness but PSI, NIR hardness, pearling value, SKCS, SDS-PAGE, and PCR markers are the most important in this respect. Protein (quality and quantity) is the most important component of wheat grain and is a major determinant of end-use quality. Wheat quality is also assessed by the molecular structure of wheat storage proteins. Wheat grain hardness appears to be determined by a degree of adhesion between starch granules and protein matrix. It is regulated by a protein called friabilin that is isolated from starch granules. Friabilin provides a biochemical basis for the distinction between hard and soft wheats. Grain softness is a genetically controlled phenomenon with friabilin accumulation in the kernel while it is independent of prevailing environmental conditions during growth. Friabilin is composed of two main components: Pin *a* and *b* which are membrane bound and basic cysteine-rich proteins. Significant research has been carried out recently on the allelic variation of Pin in relation to chemical and end product quality. The review of literature reported here indicates that quality is a summation of various characteristics, and is affected by genetic and nongenetic factors.

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