AN ABSTRACT OF THE THESIS OF

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Attempts to develop dual-purpose durum wheat cultivars for both pasta and bread-making have been unsuccessful. To better understand this limitation, thirty durum genotypes, selected based on their diverse geographical origin, and five bread wheat cultivars were compared as to their flour mixing properties, dough physical characteristics and baking performance. The polymeric composition of gluten protein was determined using SE-HPLC of unreduced flour protein extracts and the size-distribution of the gluten polymer was estimated by determining the SDS-unextractable polymer (macro-polymer) content. Durum genotypes were characterized by a lower bread-making quality compared to bread wheats, in spite of higher total flour protein and gluten polymer contents. This was due to a weaker gluten and lack of dough extensibility. The stronger gluten characteristics of bread wheats were associated with a greater ability to form macro-polymers. However, two durum cultivars had a higher macro-polymer content than many of the bread wheats, suggesting that an additional property, unrelated to size-distribution of the gluten, contributes to the greater loaf volumes observed for bread wheats. Considerable variability for most quality attributes was observed among durum genotypes. Gluten strength and dough extensibility were the most important factors associated with superior baking performance. These two parameters were not inter-related. Durum genotypes expressing LMWG-1 had the weakest gluten and the poorest baking performance. This allele contributed less protein to the glutenin fraction and the sub-units produced exhibited a reduced ability to form macro-polymers. Among the durum

genotypes expressing LMWG-2, those carrying HMWG-(6+8) were characterized by a better baking quality than genotypes carrying HMWG-(7+8) or HMWG-20. Genotypes carrying HMWG-20 were characterized by a comparatively weaker gluten and a lower macro-polymer content. The better baking performance of genotypes expressing HMWG-(6+8) relative to that of genotypes carrying HMWG-(7+8) was attributed to the greater dough extensibility characterizing the former group, but not to differences in gluten strength-related parameters. There was no indication of differences in the gluten polymer's size-distribution between the two groups.

Gluten Protein Polymeric Composition and Allelic Variation as Related to Bread-Making Quality in Durum Wheat (*Triticum turgidum L. Var. Durum*)

By

Karim Ammar

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I. Introduction

Durum wheat represents about 8 % of the wheat produced worldwide (Bozzini, 1988). In countries around the Mediterranean basin, 50 to 90% of the wheat produced is durum (Bozzini, 1988). The end-product uses include numerous types of pasta products, cous-cous, bulgur and other local foods. Approximately 24 % of the durum wheat produced worldwide, and up to 70-90 % in some Middle-Eastern countries, is used in households or small bakeries to bake many types of local breads (Quaglia, 1988). Its use in commercial baking industries has been restricted because its gluten was considered weak and not suitable for bread-making. This was supported by the results from studies comparing the bread-making quality and gluten characteristics of old durum wheat cultivars to those of the leading bread wheats (Boyacioglu and D'Appolonia, 1994). The demonstration that major determinants of gluten strength and baking quality were located on chromosome 1D (Welsh and Hehn, 1964; Schmidt et al., 1966; Morris et al., 1966, 1968) which is absent in durum wheat, provided genetic evidence that further supported the perception that durum wheat was not suitable for bread-making. However, efforts to develop durum wheats with stronger gluten to improve their pasta-making quality has resulted in this perception being challenged. Some of the more recently developed durum cultivars have been shown to approach the hexaploid wheats in their bread-making performance (Dexter et al., 1981; Josephides, 1982; Quick and Crawford, 1983). Also, the potential for further genetic improvement has been evidenced by the substantial variability in bread-making properties and gluten strength observed within sets of durum wheat cultivars from Canada (Dexter et al., 1981) and Italy (Boggini et al., 1988; Boggini and Pogna, 1989). In countries where durum wheat is a major cereal crop, greater interest

is now given to improving the bread-making potential of durum wheat, while preserving its pasta-making quality. The development of such durum cultivars would provide farmers with an alternative outlet for their crop in years of over-production. In addition, it should increase the commercial value of clear flours, which represent approximately 8-12 % of the milled grain produced by durum wheat mills as a by-product of the semolina production process (Basanik, 1981). It should also alleviate the dependency of some durum producing-countries (in North-Africa and the Middle-East) on bread wheat imports to satisfy their increasing demand for flour. Finally, it should promote the development or expansion of a large-scale baking industry in countries where durum wheat is already commonly used for bread-making.

Three general approaches have been suggested to improve the bread-making quality of durum wheat. The first is the transfer of the genes responsible for good bread-making quality in bread wheats to durums. As previously noted, these genes correspond to the locus Glu-D1 which is located on chromosome 1D. This can be accomplished via inter-specific hybridization and selection. Since chromosome 1D does not have a homologue in durum wheats, extensive cytogenetic manipulations would be required to induce exchanges between non-homologous chromosomes and identify the durum types carrying a translocated segment from chromosome 1D. Recently, this approach has been successfully implemented to develop durum wheat types carrying the allele from Glu-D1 coding for the High Molecular Weight (HMW) glutenin sub-units 5+10, which is associated with good bread-making quality in bread wheat (Ceolini et al., 1993; Lukashewski, personal communication). An alternative way to transfer the Glu-D1 locus to durum wheat would be to directly transform existing cultivars with the corresponding cloned genes. This was made possible by the cloning of these genes, including those coding for HMW glutenin sub-units 5+10 (Anderson et al., 1985) and the recent development of biolistic (gene gun) methods for transformation of wheat immature embryos (Weeks et al., 1993; Vasil et al., 1993, Nehra et al., 1994; Becker et al., 1994). These methods have recently shown potential for practical use as they were successfully implemented to develop transgenic bread wheats expressing trans-genes coding for HMW glutenin sub-units, at normal levels (Blechl and Anderson, 1996; Alpeter et al.; 1996).

The third approach to improve durum wheat bread-making quality is to capitalize on the existing variability for gluten characteristics present in the durum germplasm through crossing and subsequent selection. The potential genetic progress that could be achieved through selection is largely unknown because of the lack of extensive research to identify superior genotypes from different breeding programs. Most studies investigated collections of durum wheats representing only a rather limited germplasm base (Dexter et al., 1981, Boggini et al., 1988, Boggini and Pogna, 1989). Several studies did not include bread wheats as checks for comparison (Boggini et al., 1988, Boggini and Pogna, 1989, Peña et al., 1994), therefore, quality parameters that are most critical to the improvement of the bread-making quality of durum flour have not been clearly identified. Furthermore, the relationship between bread-making quality and gluten protein composition at glutenin locus Glu-B1 has been investigated only in a set of Italian (Boggini et al., 1988, Boggini and Pogna, 1989) and Mexican genotypes (Peña et al., 1994). Validation of these relationships for a wider array of genotypes is needed if the HMW glutenin alleles are to be used, in a reliable manner, as markers in selecting for better bread-making quality. Finally, the biochemical basis underlying the differences in bread-making quality (at the molecular level) between bread and durum wheat is largely uncharacterized.

In this context, a comprehensive study of the bread-making quality of selected durum wheats and its underlying biochemical basis was warranted. Results from such a study would contribute to the knowledge regarding the differences at the molecular level between the gluten complexes of bread and durum wheat. This knowledge could lead to a better assessment of the potential of durum wheat for bread-making and allow a more rational breeding effort.

The specific objectives of the present study were fourfold:

1- Evaluate the mixing properties, the dough physical characteristics and the baking performance of flour samples produced from a set of wheat genotypes including five bread wheats used for comparison. The durum wheats differed in their geographical origin and represented a wide array of the genotypic variability present in the spring durum germplasm.

2- Identify durum wheat genotypes with the best bread-making quality. Identify critical quality parameters and corresponding testing methods that could be used in a breeding effort to improve the bread-making of durum wheat through hybridization and selection.

3- Investigate the relationship between bread-making quality parameters and allelic composition at the glutenin loci Glu-B3 and especially Glu-B1, to assess the reliability of glutenin markers-assisted selection for improved bread-making quality.

Investigate the molecular nature of differences between bread and durum wheat, and among durum wheats with different allelic compositions, in terms of the polymeric structure of their gluten network, using Size-Exclusion High Performance Liquid Chromatography. An attempt to explain differences in bread-making quality by investigating parameters such as the proportion in total protein or amount in the flour of polymeric protein (total or SDS-insoluble) is addressed.

II. Review of the literature

II.1. The transformation of wheat into bread

Wheat is the most important crop produced, consumed and traded worldwide (Oleson, 1994). Aside from being adapted to a wide range of environments, the wheat grain produces numerous products that are highly palatable to humans. Wheat products are the staple food for 35 % of the world's population. The uniqueness of wheat, among all cereals, resides in its flour which, upon mixing with water, produces a cohesive dough that can retain the gases produced during fermentation thereby producing a leavened product of desirable attributes (Hoseney, 1994 b).

Bread, the most popular wheat product, has been a staple food for humans throughout the recorded history. It comes in a wide array of shapes, sizes, color, textures and tastes. Considerable variation also exists in the ingredients used in making bread. Only four are required, however, including flour and water which produce the bread structure, yeast which is responsible for leavening the bread through a fermentation process , and salt, which is added for taste and to promote a better interaction between the protein during dough development.

Regardless of the ingredients used and the attributes desired, wheat flours are transformed into bread through a process consisting of three main steps, namely dough formation, leavening of the latter during fermentation and stabilization and drying of the structure during baking.

II.1.1. Dough formation

A dough is formed by mixing flour and water. Three goals are achieved by mixing. They include the production of a macroscopically homogeneous system, development of a protein network into a three-dimensional structure with gas retention capacity, and the incorporation of air cells into the dough (Bloksma, 1990 a; Eliasson and Larsson, 1993 a). Bernardin and Kasarda (1973) reported that, upon contact with water, the gluten protein fibrils are formed and extend into the surrounding water. Gluten proteins are hydrated rather than dissolved (Eliasson and Larsson, 1993 a). The friction occurring during mixing between the flour particles and the mixer surfaces on one hand, and between neighboring flour particles on the other hand, wears away the hydrated surfaces, thereby continuously exposing new, unhydrated surfaces (Faubion and Hoseney, 1990; Hoseney, 1994 b). Before mixing, the dry gluten is referred to as a "glassy polymer" and undergoes "glassy transition" upon hydration and mixing to yield an amorphous mass in which the component protein are better able to interact and form a dough (Hoseney et al., 1986). A dough is optimally mixed or developed when all the protein and starch are hydrated (Hoseney, 1994 b). The evolution from a discontinuous system (flour particles) to a continuous one (protein-starch matrix) can be explained at the molecular level by the cross-linking of protein molecules originating from different flour particles through thiol-disulfide interchanges between the various thiol groups (-SH) occurring on the molecule's surface and the disulfides bonds (-S-S-) linking polypeptides within the same flour particle (Bloksma and Bushuk, 1988).

The second purpose of mixing is to incorporate air into the dough to produce gas nuclei that will subsequently expand during fermentation to form the gas cells. Since the early 1940s, it has been known that yeast fermentation does not create new gas cells. Rather, these develop from air nuclei incorporated by occlusion into the continuous dough phase during mixing (Baker et al., 1941; Baker et al., 1946). At the end of mixing, the diameter of the gas nuclei ranges from 10 to 100 μm and their number is estimated to vary between 10^{11} to 10^{13} nuclei per m^3 of dough (Bloksma, 1981).

The atmosphere's nitrogen is believed to be responsible for initiating gas cells while oxygen and carbon dioxide dissolve readily in the dough's aqueous phase.

II.1.2. Fermentation and expansion of the gas cells

Leavening of a dough is required to obtain a porous bread product. It is achieved by the entrapment of carbon dioxide in the gluten-starch matrix. Yeast fermentation is but one of the mechanisms that produce carbon dioxide in a dough. Yeast fermentation is used in the making of most breads, both as a source of carbon dioxide and to produce a desirable texture, aroma and flavor in the finished product (Eliasson and Larsson, 1993 b). Upon hydration, yeast cells are activated and metabolize the fermentable sugars to produce ethanol and carbon dioxide. After saturating the dough's aqueous phase, the carbon dioxide diffuses in the gaseous phase and the resulting pressure inside the cells provides the driving force for their expansion. Gas retention is achieved by two consecutive mechanisms. First, gas cells are embedded in a continuous protein-starch matrix which stretches under the excess pressure produced by the release of carbon dioxide. The ability of the matrix to be stretched into thin membranes is determined by the visco-elastic properties of the gluten proteins (Bloksma and Bushuk, 1988; Bloksma, 1990 a; Hoseney, 1994 a). At this stage, the response of the dough is determined largely by the extensibility of the bulk dough phase (Bloksma, 1990 a; Gan et al., 1995). Second, as the gas cells expand and tensile stress increases, the protein-starch matrix ruptures and the integrity of the gas cells as well as their gas retention capacity is ensured by a thin, lamellar liquid film lining the inner surface of the cell. This "liquid film" theory has been proposed by MacRitchie (1976) and later supported by Scanning Electron Microscopic analysis (Gan et al., 1990). More recently, the factors affecting the stability of the liquid film were reviewed by Gan et al. (1995): Surface active components, presumably water soluble pentosans and polar lipids, are believed to play a role in stabilizing the liquid film once the protein-starch membrane is ruptured.

At this advanced stage of fermentation the physical properties of a wheat dough approach those of a foam (Bloksma, 1990 a; Gan et al., 1995).

II.1.3. Baking

The primary function of baking is to stabilize and dry the whole structure to yield a product that can be consumed. Several phenomena take place during baking which transform a foam-like dough with a discontinuous gas phase imbedded in a continuous bulk dough phase into a sponge-like loaf with continuous gas phase and a dry, discontinuous solid phase. As the temperature inside the dough rises, the yeast cells become more active and produce more carbon dioxide. This additional expansion, referred to as "oven spring" stops when the temperature reaches approximately 60°C, which is the yeast's point of thermal inactivation (Kulp, 1988). Further expansion is achieved when the liquid phase evaporates and gases expand in the cells as a result of increased temperature (Bloksma, 1990 a; Hoseney, 1994 b). Oven spring is stopped by the onset of starch gelatinization which occurs at approximately 65 °C (Hoseney, 1994 b) and continues until the end of baking. Gelatinization is accompanied by the swelling of the starch granules due to the absorption of free water present in the system. It has also been shown that water absorbed on the surface of the gluten protein is transferred to the starch granules, further contributing to their swelling (Eliasson and Larsson, 1993 b; Kulp, 1988). Starch gelatinization is believed to be the cause of the dramatic increase in dough viscosity observed at temperatures above 60 °C, which, in turn, results in the increase of tensile stress on the thin gas cell walls causing the weakest cells to rupture (Bloksma, 1990 a; Eliasson and Larsson, 1993 b; Gan et al., 1995). The extent of increase in viscosity is related to the amount of starch in the flour and, if starch is removed, no increase in viscosity is observed (Dreese et al., 1988). The gas-continuous structure of the bread crumb starts to form around 72 °C (Hoseney, 1994 b). Ultimately, the dough looses its gas retention capacity and becomes sponge-like.

II.2. Rheological properties and physical testing of wheat doughs

II.2.1. Definition

Rheology describes the relationship of the stress on a material, its deformation or strain, and time." (Bloksma, 1990 a). Hoseney (1994 a) defines rheology as "...the study of how materials deform, flow or fail when force is applied...". Knowledge of the rheological properties of wheat doughs is critical for two reasons. First, because they determine the dough's handling characteristics in the bakery, and secondly because they affect the quality of the end product.

II.2.2. Fundamental versus empirical approaches

The approaches used in the study of the rheology of wheat doughs have been classified as fundamental or empirical (Bloksma and Bushuk, 1988; Menjivar, 1990). Fundamental measurements, also referred to as rheometric, describe well-defined, simple physical properties and are obtained from experiments conducted under well-defined conditions. Fundamental parameters, expressed in SI units, are independent from the instrument used or the geometry of test dough-piece, which makes them useful for characterizing the intrinsic physical characteristic of doughs. Consequently, they are suitable for use in process and engineering calculations as well as to test and/or validate hypotheses regarding the structure / property relationships in wheat doughs (Bloksma and Bushuk, 1988; Menjivar, 1990; Hoseney, 1994 a). However, extensive fundamental knowledge about the basic rheology of doughs is still lacking (Spies, 1990) and the great majority of the research uses empirical rather than fundamental approaches (Faubion and Hoseney, 1990; Spies, 1990). This refers to methods that employ physical dough-testing instruments such as farinographs, mixographs, extensographs and alveographs which are

widely used in the milling and bread-making industries. Because the geometry of the test dough-pieces used in such instruments is complex and the stress applied cannot be quantified, the empirical methods do not yield parameters that describe the fundamental rheological properties of doughs, but measure properties that have been empirically shown to correlate with one or more bread-making quality parameters (Bloksma and Bushuk, 1988; Menjivar, 1990; Hoseney, 1994 a). Furthermore, these instruments cannot discriminate between viscous and elastic deformations which are both characteristic of wheat doughs. Wheat doughs are referred to as visco-elastic, meaning that they exhibit both viscous flow and elastic recovery (Bloksma and Bushuk, 1988; Eliasson and Larsson, 1993 a; Hoseney, 1994 a). Viscous flow designates the inability of a material to recover its original shape or size once the stress applied on it is released, while elastic recovery indicates the capacity to do so (Hoseney, 1994 a). Despite the fact that some dough-testing instruments are designed to imitate one or more steps of the bread-making process, the conditions prevailing during all of these tests are different from those observed during bread-making, particularly in terms of duration of the process, deformation rates and stress applied, and formulation of the dough components. This led Bloksma (1990 b) to state that physical dough-testing instruments do not measure characteristics that are intrinsically related to baking performance, rather, they measure some other properties that correlate with some unknown property, the latter directly affecting baking performance.

Despite these apparent shortcomings, all of the authors cited above agree that the empirical approaches to dough rheology have been, and still are, useful in characterizing the handling properties of wheat doughs. This view was recently supported by the results reported by Janssen et al. (1996) who found good agreement and complementarity between fundamental rheological measurements and parameters obtained from load-extension instruments, namely the extensigraph and alveograph. This was true despite the large differences in applied strain, deformation rates and deformation modes.

II.2.3. Characteristics and uses of physical dough-testing instruments

Of the many dough testing instruments used in research and in the bread-making industry, four appear to be predominant, namely, the mixograph, the farinograph, the extensigraph and the alveograph. The first two are referred to as recording dough mixers and yield information on the changes in rheological properties of doughs during mixing. They record the resistance to mixing over time. The latter two belong to the class of load-extension instruments which stretch a dough piece until it breaks, while measuring the force used versus the resulting extension.

II.2.3.1. The mixograph

The mixograph (National Manufacturing Co., Lincoln, Nebraska) is a small, high speed recording dough-mixer. Its mixing action is provided by four vertical planetary pins revolving at constant speed about three stationary pins in the bottom of the mixing bowl. The first version, described in 1933 by Swanson and Working, used a 400 gram mixing bowl. It was updated in 1939 by a machine using a 35 gram mixing bowl. Finney and Shogren (1972) further reduced the amount of flour needed by developing a mixograph with a 10 gram bowl. The increasing force required for the pins to rotate through the developing water-flour dough causes the mixing bowl to rotate against a spring. The instrument records the torque on the mixing bowl (Kunerth and D'Appolonia, 1985). The vertical axis of a mixogram measures the dough's resistance to the extension caused by the pull-fold-repull action of the mixing pins. The horizontal axis is related to time. The width of the trace is related to the cohesiveness and elasticity of the dough (AACC method 54-40 A, 1995; Faubion and Hoseney, 1990).

Since researchers first started using the mixograph, it was reported that the parameters measured, as well as the general pattern of the curves depended on the wheat

cultivar and, for a single cultivar the curve was affected by the protein content and water absorption of the flour (Swanson, 1941; Johnson et al., 1943; Finney and Shogren, 1972). The relationship between mixogram pattern and the amount of water added is the basis for the mixographic estimation of the flour's water absorption (Kunerth and D'Appolonia, 1985). A subjective evaluation of the mixogram's appearance (in term of its "spikyness" or "slackness") provides a basis for adjusting the amount of water added. The mixograph water absorption is thus defined as the amount of water added to the flour to yield an "optimally" looking mixogram (Shogren and Finney, 1972). The reliability of this approach is highly dependent on the experience of the operator (Spies, 1990).

Researchers have also relied on the mixograph to study the effect of certain additives on the rheology of wheat doughs, including oxidants, reducing agents, fats and different enzymes. In bakeries, mixographs are used mainly to test the uniformity of the mixing characteristics and water absorption of different batches of flour (Hoseney, 1985; Faubion and Hoseney, 1990; Eliasson and Larsson, 1993; Spies, 1990).

The main advantage of mixographs is the speed of the analysis, its relative simplicity and the small amount of flour required for each test. Further simplification of the testing process was described by Sibbit and Harris (1945) and Bruinsma et al. (1978) who used ground wheat instead of flour. These properties make mixographic analysis particularly amenable to early quality evaluation of breeding lines. In fact, mixographs have been used extensively in breeding programs to provide general information on the mixing properties (dough development time and tolerance to mixing) of breeding lines and the "strength" of their flours. However, because of the limitations described below, evaluations should rely primarily on the general shape and pattern of the curves rather than on specific values measured (Shuey, 1975).

Mixing properties can be translated into several numerical parameters (Johnson et al. 1943; Shuey, 1975; Kunerth and D'Appolonia, 1985) including the following: Peak time, or the time (in minutes or seconds) at which the curve reaches its maximum height. It corresponds to the optimum mixing time or time for optimum dough development (Hoseney, 1985; Faubion and Hoseney, 1990). Peak Height, or height (in centimeters) of the center of the curve from the baseline at the time of maximum height, provides an

indication of flour "strength". Tolerance to overmixing is assessed by several parameters including the height of the curve at a specific time after the peak, the angle between the ascending and descending portion of the curve. The area under the curve is a comprehensive parameter reflecting both flour "strength" and tolerance to overmixing.

The many attempts to correlate mixogram parameters with end-product quality parameters such as loaf volume have not been particularly successful. Johnson et al. (1943) first reported the lack of significant association between mixogram peak height and loaf volume when the effect of flour protein content on both parameters was accounted for. Later studies did not report any stronger relationships; The published correlation coefficients range from 0.35 to 0.50 and, in most cases, were not statistically significant (Baker and Campbell, 1971; Fowler and De La Roche, 1975; Branlard et al, 1991; Souza et al., 1993). The same general trend is observed for the relationship between loaf volume and mixogram peak time. Non significant correlation coefficients between both parameters were reported by Baker and Campbell (1971), Khan et al. (1989) and Souza et al. (1993). When significant correlation was reported, the magnitude of the coefficient was low (Fowler and De La Roche, 1975; Branlard et al, 1991). In most studies, flour protein content explained more of the variability in loaf volume than did either mixogram peak time or peak height, confirming the conclusion of Johnson et al. (1943) regarding the lack of predicting power of mixogram parameters. Furthermore, broad-sense heritability estimates reported for mixogram peak height and peak time are low suggesting that selection for these parameters in early generations is not warranted (Baker and Campbell, 1971; Branlard et al., 1991).

Recently, a direct-drive mixogram requiring only 2 grams of flour has been described by Rath et al. (1990), extending the potential use of mixographic analysis to single plants from segregating breeding populations. Gras and O'Brien (1992) analyzed 2-gram flour samples from F2 plants and their F3 progenies from three different crosses and reported a wide range in mixogram parameters with an acceptable error associated with their determination. Contrary to previous reports (Baker and Campbell, 1971; Branlard et al, 1991) they obtained medium to high heritability estimates for peak time using offspring/parent regression analysis. However, they report a low heritability estimate for

peak height. Also, the 2-gram mixograph proved to be most valuable for studying the functional properties of single purified polypeptides corresponding to the high molecular weight glutenin sub-units (Bekes et al, 1994 a; Bekes et al., 1994 b).

II.2.3.2. The farinograph

Introduced in the early 1930s, The Brabender Farinograph (Brabender OHG, Germany) is now the most universally used dough-testing instrument (Shuey, 1975). Mixing is provided by two sigma-type blades rotating in opposite direction from each other, at different speeds (Kunerth and D'Appolonia, 1985). The resistance to mixing of a water flour dough is transmitted from the blades to a dynamometer connected to a scale and lever system which moves a tracing pen. The farinograph mixing bowl (300 or 50 grams versions are available) is hollow allowing for water circulation, thereby ensuring operation at a constant temperature. The kneading type mixing action of farinographs is gentler than the harsher pull-fold repull mixing pattern in mixograms (Kunerth and D'Appolonia, 1985; Spies, 1990). Nevertheless, both instruments stress the dough beyond its elastic limits (Shuey, 1975; Menjivar, 1990).

Several numerical parameters (AACC method 54-21, 1995) can be measured on farinograms similarly to those taken from mixograms, including peak time or dough development time and a number of parameters indicating the tolerance of the dough to overmixing (Mixing tolerance index, stability, arrival and departure times, etc.).

The main advantage of the farinograph over the mixograph, aside from temperature control, is that water absorption can be determined without a subjective evaluation of the curve's appearance. In fact, farinograph water absorption is defined as the amount of water added to a flour, on a 14 % moisture basis, in order to produce a dough with an arbitrarily selected consistency. This is achieved when the curve's maximum reaches a pre-defined line on the farinogram, usually 500 Brabender units or BU (Shuey, 1975; Bloksma and Bushuk, 1988). Nevertheless, farinograph absorption is not

always an infallible predictor of the optimum baking absorption, especially when the flours tested differ to a great extent in their protein and starch damage levels (Spies, 1990).

A major disadvantage of the farinograph is the relatively large amount of flour needed to perform a test. This probably precluded its extensive use in breeding programs, particularly in the early stages of the breeding process. Also, farinograms suffer the same limitations as mixograms in terms of the usefulness of the numerical parameters they yield and the power of these parameters to predict bread loaf volume. Studies using different sets of cultivars/lines have resulted in correlation coefficients between farinograph dough development time and loaf volume ranging from non significant to highly significant but low in magnitude (Baker et al., 1971; Fowler and De La Roche, 1975; Campbell et al, 1987; Cressey et al., 1987; Branlard et al., 1991; Preston and Lukow, 1992; Slaughter et al., 1992). However, Orth et al. (1972), reported a relatively substantial association between the two parameters ($r = 0.64$). A similar trend can be observed for the association between loaf volume and farinograph Mixing Tolerance Index (Branlard et al. 1991; Preston and Lukow, 1992; Slaughter et al., 1992). However, both Orth et al. (1972) and Fowler and De La Roche (1975) reported medium high correlation coefficients ($r = -0.79$ and $r = -0.72$, respectively) between the two traits.

II.2.3.3. The extensigraph

Introduced in 1936 to complement the farinograph, the Brabender extensigraph measures the force required to stretch a cylindrical piece of water-salt-flour dough prepared according to a defined protocol (AACC method 54-10, 1995). The dough is usually mixed to maximum consistency in a farinograph prior to being molded and allowed to rest at constant temperature for variable periods of time, depending on the protocol used (Shuey, 1975; Bloksma and Bushuk, 1988). The stretching is performed by a hook moving downward at constant speed through the middle of the dough piece, transmitting the force to a dynamometer through a series of balances and levers. The resulting

extensigram is a plot of the force (in extensigraph units) versus time. Because the speed of the hook is constant, time can be equated to extension (Bloksma and Bushuk, 1988).

The parameters commonly determined from extensigrams are: R_{max} or the maximum resistance to stretching corresponds to the maximum height of the curve (in Brabender Units). It is one measure of the flour's strength (Hoseney, 1994 a). The ratio E/R_{max} provides an estimation of the balance between the elastic and viscous component of the dough's rheological properties. E designates the length of the curve (in centimeters) from the beginning of stretching to the point of rupture of the dough piece. It measures the dough's extensibility. The area under the curve is considered another measure of the flour's strength.

Because of the relatively long time required to run a single test, extensigraph have been used more extensively in research applications rather than in quality control in bakeries (Spies, 1990). This instrument has been used to investigate the effect of numerous factors on dough strength and elasticity (Hoseney, 1994 a) including oxidants such as bromate or iodate (Bloksma and Bushuk, 1988). Because the same piece of dough can be stretched and remolded several times, extensographs can be used to simulate and monitor the structural relaxation of the dough during fermentation.

The few correlation studies available report non significant to weak associations between loaf volume and extensibility or R_{max} (Baker et al., 1971; Cressey et al., 1987; Campbell et al., 1987).

II.2.3.4. The alveograph

The alveograph (Chopin, s.a. - Tripette et Renault, Villeneuve la Garenne, France) was invented in 1920 by Marcel Chopin. It measures the pressure required to blow a bubble in a sheeted piece of dough. The test piece is a water-salt-flour dough which is mixed, sheeted, cut and allowed to rest according to a defined protocol (AACC method 54-30, 1995). Unlike the extensigraph, which uses a dough piece produced by adding the

amount of water required to produce a dough with maximum consistency, the alveograph usually tests dough pieces with a fixed water content corresponding to 50 % of the flour weight on a 15 % moisture basis, regardless of the absorption capacity of the flour tested (Faridi and Rasper, 1987). Flours with different levels of damaged starch can have markedly different hydration requirements, which, in turn, affects the alveographic measurements. Chen and D'Appolonia (1985) reported that using dough with constant consistency, as determined by the farinograph, yields alveographic parameters that correlate better with bread making quality in Hard Red Spring wheats. This was not the case for Soft White Winter wheats which have much lower starch damage levels (Rasper et al., 1986). In most studies involving alveographic analysis of durum wheat flours, a variable amount of water was used to adjust for the presumed high and more variable levels of starch damage resulting from the milling of the harder durum kernels (Peña et al., 1994; Dexter et al., 1994; Ciaffi et al., 1995).

Regardless of the amount of water added, the following parameters are most commonly determined from alveograms (Faridi and Rasper, 1987; Bloksma and Bushuk, 1988; Hoseney, 1994 a): The over-pressure P corresponds to the maximum height (in centimeters) of the curve, multiplied by a factor of 1.1. It is related to the "tenacity" of the dough, that is to its resistance to deformation. The average length L (in millimeters) of the curve corresponds to the abscissa of the average curve at the point of rupture of the bubble. It is a measure of the dough's extensibility. The P/L ratio, also referred to as the configuration ratio, is a characteristic of the alveogram's shape and provides information on the visco-elastic balance of the dough. The deformation energy, W, is derived by multiplying the surface under the curve by a constant factor. It corresponds to the energy (in 10^{-7} Joules) required to inflate the dough bubble until it ruptures. W is related to the dough strength and is the single, most extensively used parameter in a number of European countries, particularly France.

The main advantage of the alveograph over the extensigraph resides in the type of deformation generated. While the extensigraph stretches the dough in only one direction (uni-axial deformation) at a constant rate, the alveograph expands the dough in all directions (bi-axial deformation) at a deformation rate that varies as the bubble grows.

The latter mode of stretching is a more faithful simulation of the expansion occurring during fermentation and oven rise (Faridi et al., 1987; Spies, 1990; Hoseney, 1994 a).

The studies investigating the association between loaf volume and alveogram parameters yielded different and often contradictory results. Shogren and Finney (1962) reported correlation coefficients between loaf volume and extensibility of different samples within the same cultivar ranging from 0.70 to 0.84. The area under the curve was found to correlate less with loaf volume, with coefficients varying from 0.38 to 0.57. Khattak et al (1974), did not find a significant or substantial association between loaf volume and any of the alveogram parameters using a set of 13 Hard Red Spring wheats and concluded that the alveograph was not suitable for the evaluation of cultivars for this market class. Using a modified method (constant consistency dough), Chen and D'Appolonia (1985) reported a significant negative correlation ($r = -0.67$) between loaf volume and dough tenacity (P). The correlations with other parameters were not significant. In a study involving 15 hard wheats, Bettge et al. (1989) concluded that dough extensibility (L) correlated best with loaf volume ($r = 0.90$), and thereby was the best predictor of the latter. They also reported that loaf volume was not significantly associated with tenacity ($r = -0.48$), but was significantly correlated with the deformation energy W ($r = 0.68$). High correlation coefficients were reported by Addo et al. (1990) between loaf volume and L ($r = 0.90$) or W ($r = 0.97$). The latter parameters were also found to correlate with loaf volume in a study published by Branlard et al. (1991), albeit to a lesser extent ($r = 0.60$ for L, $r = 0.62$ for W). In durum wheat, a significant correlation was reported by Peña et al. (1994) between bread loaf volume and W.

II.2.4. Rheological requirements for good bread-making quality

A review of the processes occurring during bread-making reveals that the most critical requirement for the production of an acceptable loaf of bread is the ability of the gluten-starch matrix to stretch during fermentation and baking to allow for the expansion

of the gas cells and sufficient oven rise (Bloksma, 1990a, 1990 b; Gan et al., 1995). This leads to the conclusion that the main rheological property required for good bread-making performance is dough extensibility, i.e., its aptitude to deform or flow without loosing its cohesiveness, which in turn, depends on the extensibility of the gluten protein network (Bloksma 1990 a). Recently, Janssen et al. (1996), confirmed that a minimum extensibility was the most important rheological requirement for the production of a high loaf volume and a fine crumb texture.

Another prerequisite is sufficient viscosity of the dough to prevent the ascent of the gas cells to the dough's surface. As this condition is verified in any normal dough, it does not constitute a critical factor (Bloksma, 1990 a; 1990 b). While acknowledging the well demonstrated partially elastic response of wheat doughs, Bloksma (1990 a) reports that elasticity was never satisfactorily demonstrated to constitute a requirement for good bread-making performance. In fact the volume elasticity exhibited by doughs during fermentation and oven rise is due to the occluded gas and does not constitute a property of the dough phase per se.

II.3. The proteins of wheat

II.3.1. Importance in bread-making

The production of a well-risen loaf of bread with desirable texture and taste is the result of complex interactions between all the flour components and all the ingredients used during mixing, fermentation and baking. While all the flour components can affect the quality of the end-product, researchers are unanimous in regarding the gluten proteins as the single most important component that determines the bread-making performance of a flour. This predominant role is illustrated by the results of Finney and Barmore (1948) which show a strong linear relationship between the loaf volume and the protein content

of different flour samples from a same wheat cultivar. This quantitative, "within cultivar" relationship was consistently observed for over 13 lines varying greatly in bread-making quality. This relationship was subsequently confirmed by several other studies including those performed by Fifield (1950) and Bushuk (1985). However, it is not yet known how a greater quantity of gluten can result in a greater loaf volume (Hoseney, 1994 a). Also, it is believed that the genetic potential of a wheat line, in terms of its bread-making potential, resides mostly in the quality of its gluten proteins rather than on their quantity. This qualitative difference is illustrated by the large differences in the slope of the regression lines relating loaf volume to protein content for different cultivars (Finney and Barmore, 1948; Hoseney and Finney, 1971; Bushuk, 1985). Direct evidence for the predominant role of gluten protein in determining the bread-making potential of wheat flours was provided by the results of the fractionation-reconstitution experiments of Finney (1943). He was able to fractionate flours into gluten, starch and water-soluble fractions without affecting their functionality and properties as demonstrated by the nearly identical loaf volume of reconstituted and original flours. Results obtained from inter-changing the various fractions from cultivars differing greatly in bread-making quality revealed that bread-making performance was determined almost entirely by the gluten fraction. These results were confirmed by several subsequent fractionation-reconstitution experiments including those by Hoseney et al. (1969 a, 1969 b), MacRitchie (1978) and Booth and Melvin (1979).

II.3.2. Major classes of proteins in the wheat kernel

First described by the Italian scientist Beccari as early as 1728 (translated by Bailey, 1941), gluten is the first protein to be purified from a plant source (Hoseney, 1994 a), well before the word "protein" was proposed by Mulder and Berzelius in 1838 (Wrigley and Bietz, 1988). The first experimental evidence for the heterogeneity of gluten was given by Einhof in 1810 (cited in Bailey, 1944) who fractionated gluten using alcohol

solvents. In 1820, Taddei (cited in Bailey, 1944) assigned the name "gliadin" to the alcohol soluble fraction of gluten and called the insoluble fraction "zimome", which later became "glutenin". However, the first systematic study of the wheat grain proteins was published by Osborne in 1907. Today, his classifications and fractionation scheme remains largely in use in the studies of the wheat proteins. Based on their solubility in different solvents, he distinguished four major classes of proteins, namely, the water-soluble albumins, the salt-soluble globulins, the alcohol-soluble gliadin and the glutenin which are soluble (or dispersible) in dilute acid or alkali solutions. The Osborne solubility fractions correspond to groups of protein that differ in overall composition and properties (Kasarda et al., 1976).

Both albumins and globulins are not part of the gluten complex. In fact, they are solubilized and/or washed away into the water soluble fraction upon gluten isolation. They are consequently called either "non-gluten" or "soluble" proteins. Together, they make up between 18 % and 30 % of the total grain protein depending on the method of extraction and estimation (Eliasson and Larsson, 1993 c). They consist of numerous metabolic enzymes and hydrolytic enzymes synthesized during seed development (amylases, proteases,...) to be used to provide nutrients for the future embryo (Wrigley and Bietz, 1988; Eliasson and Larsson, 1993 c). Over 100 polypeptides could be detected by RP-HPLC of salt extracts (Bietz, 1983) and up to 160 components could be identified using two-dimensional gel electrophoresis of the same extracts (Lei and Reeck, 1986 a, 1986 b). Most of these polypeptides have a molecular weight less than 40 kilo Daltons (kDa) as shown by SDS-PAGE of salt extracts and considerable overlap has been found between the two classes depending on the extraction procedures (Wrigley and Bietz, 1988).

II.3.3. The gluten proteins

Gluten proteins represent the main storage proteins in the wheat endosperm where their only physiological function is to provide a source of nitrogen usable by the developing embryo (Kasarda et al., 1976, Shewry et al., 1986). Osborne (1907) divided the gluten protein into gliadin and glutenin and speculated that "the glutenin probably forms the nucleus to which the gliadin adheres and thus binds the gluten protein into a coherent elastic mass." A more recent view which better describes the complexity of gluten is given by Eliasson and Larsson (1993 c) who state that "gluten can be viewed as a protein mixture with a continuous molecular weight distribution from about 30,000 to perhaps 20 million". The compositional complexity of gluten makes the study of its structure and functionality a challenging task as outlined by Bietz (1985): "the fractionation and characterization of cereal proteins may be among the most difficult problems in biochemistry: these proteins are heterogeneous, have unusual solubility properties and have marked tendencies to aggregate both covalently and non-covalently." Gluten proteins are characterized by an unusually high content in glutamine (over 30 %) and proline.

II.3.3.1. Classification and properties

Defined by Osborne (1907) as the protein fraction soluble in aqueous alcohol solutions (70 % ethanol or 50 % propanol are most commonly used), gliadin represents the prolamine fraction of the wheat grain. It is homologous to the hordein of barley, the secalin of rye and the zein of maize. Gliadin is a highly heterogeneous fraction consisting of many monomeric polypeptides. Early attempts to further fractionate gliadin used free boundary electrophoresis in aluminum lactate buffer at pH 3.2 (Jones et al., 1959) and resulted in the sub-division of gliadin into four mobility groups designated as α-, β- , γ-,

and ω-gliadin, with the α-gliadin migrating the closest to the cathode. This nomenclature remains in use to date, after being adapted to starch gel electrophoresis (Woychick et al., 1961) and to polyacrylamide gel electrophoresis or PAGE (Lee, 1963). Improved PAGE separation methods at pH 3.1, referred to as Acid-PAGE or A-PAGE, are able to resolve up to 30 bands in some wheat cultivars (Bushuk and Zillman, 1978; Lookhart et al., 1982; Khan et al., 1985; Branlard et al., 1990). Further resolution was possible by the development of two-dimensional techniques which yielded up to 46 components from ethanol extracts (Wrigley and Shepherd, 1973; Lafiandra and Kasarda, 1985). The application of reversed phase-HPLC, or RP-HPLC, to the analysis of gliadin allowed the identification of more than 80 components (Bietz, 1986; Bietz and Simpson, 1992). As determined by SDS-PAGE of reduced gliadin extracts, the molecular weights of gliadin components vary between 30 and 40 kDa for the α-, β-, and γ- gliadin and between 60 to 80 kDa for the ω-gliadin (Kasarda et al., 1976; Wall, 1979).

Glutenin was initially defined by Osborne (1907) as the protein insoluble in water or salt or alcohol, but soluble in dilute alkali or acid solutions. Since then, many glutenin preparations have been described, as reviewed by Kasarda et al. (1976) who refer to glutenin as "... the least soluble half (or even smaller fraction) of the total flour protein". More recently, Bietz (1985), Eliasson and Larsson (1993 c), and Weegles et al. (1996) defined glutenin as the residual protein fraction remaining after extracting the albumins, globulins and gliadin. This latter definition differs from that of Osborne in that it designates as glutenin both acid-soluble and acid-insoluble fractions. Glutenin is also partially soluble in highly concentrated denaturants such as urea (Pomeranz, 1965), Detergent solutions such as sodium dodecyl sulfate or SDS (Danno et al., 1974; Graveland et al., 1979). The polymeric structure of native glutenin was demonstrated by Pence and Olcott (1952) who showed that reducing agents (sulfites) were responsible for the loss of glutenin's visco-elastic properties and viscosity in solution. Later, Nielsen et al. (1962) reported a dramatic decrease in glutenin's molecular weight after treatment with sulfites. These results established that native glutenin is a high molecular weight polymer made of sub-units linked together through disulfide bonds (covalent bonds). Its maximum molecular weight is difficult to estimate as the largest polymers are also the least soluble,

thereby the most difficult to analyze (Eliasson and Larsson, 1993 c). However, molecular weights of up to 20 million (20,000 kDa) have been suggested from the results of size exclusion liquid chromatography (Huebner and Wall, 1976; Bietz, 1985; Graveland et al., 1985). The unextractable fraction of the glutenin, which consist of the largest polymers has been referred to as "residue protein" (Orth and Bushuk, 1972) or "gel protein" (Mecham et al., 1962; Mecham et al., 1972; Graveland et al., 1979). More recently, this fraction has been designated as "glutenin macro-polymer" (Weegles et al., 1996).

When denaturing agents such as SDS or urea are used in combination with a reducing agent (which break disulfide bonds) such as 2-β-mercaptoethanol or dithioerithreol (DTT), near complete extraction and solubilization of the glutenin can be achieved (Danno et al., 1974) through the destruction of its polymeric structure. This extraction procedure, in combination with the development of SDS-PAGE fractionation protocols permitted the analysis and characterization of the glutenin components or sub-units (Bietz and Wall, 1972; Orth and Bushuk, 1973). Jackson et al. (1983) were able to identify up to 19 individual components in a single cultivar. Glutenin polypeptides have been sub-divided into high molecular weight (HMW) and low molecular weight (LMW) sub-units according to their apparent size in SDS-PAGE gels (Huebner and Wall., 1976; Lawrence and Shepherd, 1980). High molecular weight sub-units were characterized by apparent molecular weights ranging from 95 to 136 kDa, whereas LMW sub-units exhibited molecular weights varying from 35 to 51 kDa as determined by SDS-PAGE (Payne and Corfield, 1979). However, these values are over-estimates of the actual molecular weights of the sub-units, particularly for the HMW components (Ng and Bushuk, 1989). In fact, more accurate estimates, ranging from 63 to 88 kDa, have been calculated from the deduced amino-acid sequences obtained from sequencing the cloned genes of six different HMW sub-units (Shewry et al., 1989).

II.3.3.2. Synthesis and cellular localization of gluten proteins

The continuous protein matrix observed in the mature endosperm of wheat is the result of the coalescing and fusion of discrete protein bodies in which storage proteins are aggregated during grain maturation (Wrigley and Bietz, 1988; Evers and Bechtel, 1988). Isolated protein bodies are characterized by the same storage protein profile as that of a mature grain (Miflin et al., 1980; Payne et al., 1986).

Storage proteins are synthesized on the polysomes associated with the rough endoplasmic reticulum in which they are subsequently sequestered (Miflin et al., 1983). Storage proteins aggregate into protein bodies either inside the lumen of the endoplasmic reticulum or in the vesicles associated with the Golgi apparatus (Campbell et al., 1981; Bechtel and Gaines, 1982 a; Miflin et al, 1983; Rubin et al., 1992). In the first case, protein bodies are transported to the vacuole directly from the endoplasmic reticulum (Rubin, 1992) or via the Golgi apparatus (Campbell et al., 1981; Rubin, 1992). Rubin et al. (1992) presented evidence for the existence of two types of protein bodies differing in their density upon centrifugation in density gradients. They report the presence of HMW glutenins in the dense bodies only, whereas gliadins are present in both dense and light bodies.

Although protein bodies have been detected (Bechtel et al., 1982 b) and radio-labeled gliadin polypeptides were present as early as 6 days after flowering (Greene et al., 1985), the rate of storage protein synthesis increases dramatically only around 12 days post-flowering (Greene, 1983; Greene et al., 1985). At this stage, endosperm cells seem to become committed primarily to a storage activity as suggested by the subsequent decrease in non-storage protein levels in maturing grains (Galtiero et al., 1987; Wrigley and Bietz, 1988). Also, most of the gliadin and glutenin components can be detected electrophoretically between 10 and 12 days after flowering (Bushuk and Wrigley, 1971; Mecham et al., 1981; Galtiero et al., 1987; Greene et al, 1985). The simultaneous expression of most of the gene families involved suggests that the genes are under the control of a small number of regulatory elements and that the subsequent differences

observed in the expression of storage protein genes are mainly quantitative rather than qualitative (Greene et al., 1985).

II.3.3.3. Genetics and allelic composition of gluten proteins

Because storage proteins are destined to be broken down into peptides and amino-acids, mutations in the genes encoding them are not detrimental to their function. This explains the considerable heterogeneity found among storage proteins (Eliasson and Larsson, 1993 c). Gliadin polypeptides are encoded by genes displaying multiple allelism located on chromosomes from homeologous groups 1 and 6 in hexaploid wheat (Wrigley and Shepherd, 1973; Mecham et al., 1978; Brown and Flavell, 1981). These studies involving the electrophoretic analysis of various aneuploid stocks revealed that all ω-gliadin as well as most of the γ-gliadin and a few β-gliadin polypeptides were encoded by genes located distally on the short arm of chromosome from group 1 (1A, 1B, 1D) whereas genes located on chromosomes from group 6 were responsible for encoding the others. Gliadin polypeptides are inherited as discrete blocks (alleles) within which no recombination occurs (Wrigley and Shepherd, 1973; Mecham et al., 1978; Brown and Flavell, 1981; Sosinov and Poperelya, 1980; Metakovski et al., 1984). Similar observations were reported regarding the mode of inheritance and chromosomal location for durum wheat (Daminaux et al., 1980; Joppa et al., 1983; Bebyakin and Kumarov, 1981, cited in MacRitchie et al., 1990). Genes on chromosomes from group 1 were shown to be arranged in single complex loci designated as Gli-A1, Gli-B1 and Gli-D1 (Payne et al., 1984). Genes on chromosomes from group 6 are believed to be associated in tightly linked clusters designated as Gli-A2, Gli-B2 and Gli-D2 (Sosinov and Poperelya, 1980). Recently, a catalogue of all the gliadin alleles identified to date was published (Metakovsky, 1991) based on the analysis of 360 wheat cultivars and 45 crosses. A total of 111 gliadin alleles mapping to the 6 gliadin loci was compiled.

Glutenin sub-units are encoded by genes organized in tightly linked clusters, displaying multiple allelism and mapping to chromosomes from homeologous group 1 in both hexaploid and tetraploid wheats (Orth and Bushuk, 1974; Bietz et al., 1975; Brown et al., 1979; Lawrence and Shepherd, 1980; Payne et al., 1981 a; Lawrence and Shepherd, 1981). Loci harboring the HMW glutenin genes have been designated (Payne and Lawrence, 1983) as Glu-A1 (coding for 0 or 1 sub-units), Glu-B1 (coding for 1 or 2 sub-units) and Glu-D1 (coding for 2 sub-units), and have been mapped to the long arms of chromosomes 1A, 1B and 1D respectively, at genetic distances of 7.6, 9.2 and 10.1 cM from their respective centromeres (Payne et al., 1982). However, Singh and Shepherd (1988 a) reported higher recombination frequencies between Glu-B1, Glu-D1 and their respective centromeres (25.5 % and 27.5 %, respectively). In a survey of 195 bread wheat cultivars, Payne et al. (1981 a) identified some 20 HMW sub-units including 3 encoded at Glu-A1, 11 at Glu-B1 and 6 at Glu-D1. Ng and Bushuk (1989) detected 3 additional sub-units encoded at the Glu-B1 locus and one more at the Glu-D1 locus, for a total of 24 sub-units. A total of 18 sub-units, 7 from Glu-A1 and 11 from Glu-B1, were identified in a collection of 502 durum wheats from 23 countries by Branlard et al. (1989). However, a very limited variability was detected within the collection analyzed as 83.5 % of the cultivars analyzed had the null allele on Glu-A1 (no bands expressed), and 85.7 % had one of three alleles (6+8 or 7+8 or 20) on Glu-B1, with the remainder of the alleles existing at very low frequencies.

Low molecular weight sub-units have been shown to be under the control of gene clusters mapping to the short arm of chromosomes from homeologous group 1 (Jackson et al., 1983; Shepherd, 1988). Due to the difficulty in separating LMW glutenin sub-units from the overlapping gliadin, genetic mapping of the former was difficult to implement (Payne, 1987; Shepherd, 1988) and it was initially thought that these genes were within the Gli-1 loci which encode some of the gliadin components. It is now known that genes coding for the LMW glutenins are located in different loci, designated as Glu-3 (McIntosh et al, 1989) and which are closely linked to the Gli-1 loci on the respective short arms of chromosomes from group 1 (Singh and Shepherd, 1988 b). The development of electrophoretic procedures allowing visualization of the LMW glutenin bands without the

overlapping gliadin (Gupta and Shepherd, 1988; Gupta and Shepherd, 1990; Gupta and MacRitchie, 1991; Singh et al., 1991) greatly facilitated the mapping of genes coding for these proteins as well as the identification of the various alleles expressed. In fact, Gupta and Shepherd (1990) analyzed a collection of 222 wheats from 22 countries and identified 6 alleles from Glu-A3, 9 from Glu-B3 and 5 from Glu-D3.

II.3.3.4. Structure and conformation of gluten proteins

The study of complete amino-acid sequences deduced from the cloned genes coding for various wheat gluten proteins reveals the existence of a common structure, namely the presence of a central, repetitive region of variable length flanked by non-repetitive N-terminal and C-terminal domains (Wrigley and Bietz, 1988; Shewry et al, 1989; Tatham et al., 1990). Different polypeptides vary in the length of their 3 domains, in the repeat motif within their central region and, most importantly, in the location of the cysteine residues along the molecule (Tatham et al., 1990; Scofield, 1994).

II.3.3.4.1. Gliadin polypeptides

The main structural characteristic of the gliadin polypeptides is their monomeric nature. Cysteine residues found in α-, β-, and γ- gliadins are involved primarily in intra-molecular disulfide bonds as shown by the lack of great difference between native and reduced proteins in their migration during SDS-PAGE (Kasarda et al., 1976; Wall, 1979; Shewry et al., 1986; Wrigley and Bietz, 1988). Most of the cysteine residues are found in the relatively large C-terminal domain (Tatham et al., 1990). Results from circular dichroism spectroscopic analysis of gliadin (Tatham and Shewry, 1985) predict that α-, β-, and γ- gliadins contain 30 to 35 % α-helix and 10 to 20 % β-sheets motifs. These conformations are stabilized primarily by strong hydrogen bonding and, to a lesser extent

by intra-molecular disulfide bridges. In contrast, ω-gliadin is practically devoid of cysteine residues (Kasarda et al., 1976; Wall, 1979), and thereby lacks the ability to form disulfide bonds. In this case, strong hydrophobic interactions are believed to impart to ω-gliadin its conformation, which consist of many β-turns interspersed with random coil motifs (Tatham and Shewry, 1985). All of the studies on the conformation of gliadin (reviewed by Tatham et al., 1990 and Schofield, 1994) allow for the prediction of the shape of the different gliadin molecules. While a compact, globular shape is the most likely for α- and β-gliadins, a more extended structure seem to characterize the γ-gliadin. The ω-gliadin is thought to have a rod-shaped structure.

II.3.3.4.2. Glutenin sub-units

To date, genes coding for 9 HMW glutenin sub-units have been cloned (Shewry et al., 1989), including all 6 sub-units from cultivar Cheyenne (Halford et al., 1987; Anderson and Greene, 1989; Anderson et al., 1989; Forde et al., 1985), sub-units 2 from cultivar Yamhill (Sugiyama et al., 1985) and sub-units 12 from cultivar Chinese Spring (Thompson et al., 1985). In contrast to gliadin polypeptides, most of the cysteine residues found in the HMW glutenin sub-units are located in the N-terminal domain (3 to 5 residues) whereas only one residue is present in the C-terminal. However, an additional single cysteine residue is present towards the C-terminus, within the repetitive domain of sub-units 9, 10 and 12 domain (Shewry et al., 1989).

Two complete sequences are available for the LMW glutenin sub-units (Okita, 1985; Colot et al., 1989). The available data suggests a gene structure that is related to that of α-, β- and γ-gliadin in terms of the length and repeat motif of the repetitive domain as well as for the distribution and location of the cysteine residues, most of these being in the C-terminal region (Tatham et al., 1990).

This similarity is also evident in the conformation of the LMW glutenin sub-units which resembles that of α-, β- and γ-gliadin, as evidenced by circular dichroism

spectroscopy (Tatham et al., 1987). The authors reported a mixture of α-helix (34 % to 37 %) and β-sheet (18 % to 24 %) conformations. In contrast, HMW glutenin sub-units are thought to be rod-shaped molecules containing α-helical structures within their N- and C- termini while their repetitive domain forms regularly repeated β-turns (Tatham et al., 1985). Based on hydrodynamic studies (study of the change in intrinsic viscosity in solutions) and spectroscopic analysis of a purified HMW glutenin sub-units from durum wheat cultivar Bidi 17, Field et al. (1987) suggested that the β-turns present in the repetitive domains form a loose spiral structure similar to that formed by pentapeptides from elastin. The similarity of the two polypeptides and the well demonstrated elastic properties of elastin (a protein found in connective tissue of animals) led the authors to suggest that the β-turns-mediated spiral structure of HMW glutenin sub-units might be intrinsically elastic. Direct physical evidence for the presence of this structure and its similarity to elastin was recently provided by scanning tunneling microscopic analysis of the purified polypeptide mentioned above (Miles et al., 1991).

II.3.3.4.3. Structural models for the glutenin complex

The existence of native glutenin as a high molecular weight polymer consisting of disulfide-linked sub-units has been established more than 40 years ago. However, and despite the detailed structural data available for the glutenin sub-units, little data exists that allows for the elucidation of the structure of the native glutenin polymer. Thus many models have been proposed which describe its hypothetical structure and attempt to explain its functionality.

One of the earliest models was proposed by Ewart (1968) and subsequently modified and expanded by Ewart (1979). It suggests a linear structure for native glutenin which is presumably formed by the concatenation of polypeptides, linked head-to-tail by disulfide bonds, in random order. Based on the study of the kinetics of viscosity loss of gluten dispersions upon addition of reducing agents, Ewart (1979) concludes that no

branching is involved in polymeric glutenin and that two adjacent sub-units are linked head-to-tail by a single disulfide bridge. Since his first report, Ewart (1968) suggests that glutenin sub-units have a conformation that promotes elastic deformation when a shearing force is applied. This accounts for the elastic behavior of wheat doughs. The intrinsic elasticity of glutenin sub-units has, since then, been confirmed by strong experimental evidence discussed above (Field et al., 1987; Miles et al., 1991). Ewart also postulated that mechanical scission of disulfide bridges will occur when extension exceeds the elastics limit of the sub-units, resulting in viscous flow. The original strength of the complex could be recovered by the formation of new disulfide bonds through thiol-disulfide interchange reactions.

Another linear model was proposed by Kasarda et al. (1976), which stipulates an overall linear structure in which globular sub-units are stabilized by intra-molecular disulfide bonds in a conformation that promotes their aggregation, through secondary forces, into linear microfibrils which, in turn, aggregate lengthwise into macrofibrils. This model was dismissed because it does not account for the elastic strength of the glutenin complex, nor does it agree with the results from experiments involving the re-oxidation of glutenin sub-units in solution (Beckwith and Wall, 1966; Ewart, 1979; Ewart, 1990).

Kasarda (1989), proposed yet another linear model which acknowledges the predominant role of inter-molecular disulfide bonds. In this model, glutenin forms sheeted structures in which HMW sub-units are not linked together directly, but through stacks of LMW sub-units to which they are linked by disulfide bonds. The stacks are made of a number of LMW sub-units linked to each other, tail-to-tail, by disulfide bridges at their C-termini.

Alternative models have been proposed which involve a more or less branched structure (Bietz and Huebner, 1980; Bietz and Wall, 1980). Ewart (1990) dismissed these models presumably because a branched glutenin complex could not be oriented, thereby could not be "work-hardened", and doughs would not need development by mixing. Also, these models are not consistent with the microscopic observations of Bernardin and Kasarda (1973) who described the formation of fibrillar structures upon hydration of gluten.

Finally, Graveland et al. (1985) proposed a model in which glutenin consist of repeats of a composite unit structure assembled from 3 HMW and 12 LMW sub-units. This model is based on the electrophoretic and solubility analyses of size exclusion chromatography fractions of SDS extracts from wheat flour. The backbone of the unit structure is linear and consists of the 3 HMW sub-units linked together, head-to-tail, by disulfide bonds. The middle sub-unit has four disulfide-linked clusters, each made of 3 folded LMW sub-units linked together by disulfide bonds. This model implies the existence of specific recognition sites which have not been identified. Also the size of the branches is believed to be too large to be accommodated on a single HMW polypeptide (Ewart, 1990).

II.3.3.5. Functionality of gluten proteins: Molecular basis of bread-making quality

II.3.3.5.1. Gliadin versus glutenin

All the knowledge accumulated on the structure of gliadin polypeptides comes short of providing a clear understanding of the biochemical basis of its functionality, that is, how its components interact together and with the other gluten components to influence the bread-making performance of a flour (MacRitchie et al., 1990; Eliasson and Larsson, 1993 c; Schofield, 1994). Because of its viscous properties and the monomeric nature of its components, gliadin is believed to act as a "plasticizer" of the glutenin complex which, alone, would be too "stiff" to allow adequate cell expansion during bread-making (Wall, 1979; Ewart, 1990; Eliasson and Larsson, 1993 c). This view is supported by experimental results reported by MacRitchie (1987) which show that a gliadin rich fraction decreases the mixing requirements of a base flour. However, fractionation-reconstitution experiments aimed at demonstrating the role of different gluten protein fractions in bread-making yielded different and sometimes contradictory

results. Studies conducted by Hoseney et al. (1969 a), Hoseney et al. (1969 b) and Finney et al. (1982) provided evidence for a major role played by the gliadin-rich fractions in the determination of loaf volume, while the glutenin-rich fractions controlled the mixing requirements of the flour. The gliadin fractions used were the 70 % ethanol-soluble proteins or the dilute acetic acid-soluble proteins (Hoseney et al., 1969 a) or a supernatant from ultracentrifugation of acetic acid extracts (Hoseney et al., 1969 b; Finney et al., 1982). A different conclusion was reached by MacRitchie (1978) whose results suggested the acetic acid-soluble fraction, or gliadin-rich fraction, had only a minor effect on loaf volume compared to the major effect provided by the glutenin-rich fraction (acetic acid-insoluble protein). After fractionating gluten into 10 fractions with increasing concentrations of dilute hydrochloric acid and adding the freeze-dried fraction to a base flour, MacRitchie (1987) concluded that the gliadin-rich fractions affected loaf volume only marginally, decreasing it slightly, while the glutenin-rich fractions were responsible for a dramatic increase in loaf volumes. By comparing the fractionation schemes of Hoseney et al. (1969 b) and MacRitchie (1978), Chakraborty and Khan (1988 a) attributed these conflicting results to the compositional differences of the different fraction tested. After duplicating these two experiments with different cultivars, Chakraborty and Khan (1988 b) concluded that the glutenin fraction, not the gliadin, is mostly responsible for the differences in loaf volumes observed between cultivars. This view is supported by the results of correlation studies conducted by Branlard and Dardevet (1985) which failed to detect any substantial association between single gliadin components and functional tests parameters such as alveograph parameters and sedimentation values. Campbell et al. (1987) and Cressey et al. (1987) studied the association between bread-making quality and gluten protein composition in a set of 71 bread wheats with different genetic backgrounds and in a set of 60 advanced breeding lines, respectively. They reported that the presence of some gliadin bands was associated with increased dough resistance to stretching. They also identified several other gliadin components that seemed to be associated with other quality traits including loaf volume. However, in both studies, the major portion of the differences in bread-making quality was associated with variation in the allelic composition at loci coding for the high molecular weight glutenin sub-units. Again, this suggests a

relatively minor effect of the gliadin polypeptides on the bread-making quality of wheat flours. Thus, the major part of the research on the functionality of gluten proteins deals with the role played by the glutenin fraction

The direct experimental evidence provided by fractionation-reconstitution discussed above is supported by a wealth of indirect evidence provided by experiments aimed at establishing statistical relationships either between the quantity of different glutenin fractions and bread-making quality parameters (quantitative approach), or between the latter and the presence of specific glutenin sub-units or alleles (qualitative approach).

II.3.3.5.2. Qualitative relationship between bread making quality and glutenin composition

Bread-making quality was first related to the expression of a single glutenin allele by the work of Payne et al. (1979, 1981 b). Their first report (Payne et al., 1979) illustrates a strong association (r=0.72) between the amount of the Glu-A1 encoded HMW glutenin sub-units 1 and SDS-sedimentation volume, in a segregating population of 60 F2 plants from a cross between two British lines differing in their bread-making quality. A second study (Payne et al., 1981 b) based on the analysis of progenies from six crosses confirmed this association (qualitatively) and established an additional relationship between SDS-sedimentation volume and the allelic composition at the Glu-D1 locus. In the latter case, lines expressing the allele corresponding to HMW sub-units 5+10 had a markedly higher SDS-sedimentation volume than those expressing the alternative allele corresponding to sub-units 2+12. Also, the results suggest that both loci affect SDS-sedimentation volume in an additive fashion.

These findings stimulated a great deal of research and were subsequently confirmed and extended by numerous studies involving different wheat collections from many countries as well as recombinant inbred lines and segregating populations from a number of crosses. Some of these investigations are discussed below.

Moonen et al. (1982) studied the relationship between the allelic composition at the HMW glutenin loci and SDS-sedimentation volume, loaf volume and gel protein content (SDS-insoluble protein) of flour samples from 60 European wheat cultivars as well as 36 F_6 progenies from a cross between cultivars Atlas 66 and Atys. They reported that the presence of allele 5+10 (versus 2+12) was associated with a significantly higher SDS-sedimentation volume, a greater loaf volume and proportion of gel protein. However, HMW glutenin sub-units 1 was not associated with better quality.

Using 70 bread wheats from 14 countries, Branlard and Dardevet (1985) reported that alleles 2* (Glu-A1), 5+10 (Glu-D1), 7+9 (Glu-B1) were associated with better dough strength (measured by the alveograph W and the Zeleny sedimentation test) and greater tenacity (measured by alveograph P) whereas alleles 1 (Glu-A1), 13+16 or 17+18 (Glu-B1) were associated with higher extensibility (measured by the alveograph L). Despite the statistical significance of these relationships, the amount of variability in quality parameters accounted for by the presence of a single glutenin band was very small. Furthermore, when multiple regression analysis was used to predict quality traits from sub-unit composition, it was found that only 44.6 %, 36.8 % and 23.0 % of the variability in alveograph W (work), G (swelling index) and P (tenacity), respectively, could be accounted for by models using 6 to 8 HMW sub-units as predictor variables. The substantial unexplained variability remaining after fitting these models was attributed by the authors to the effects of differences in gliadin composition.

Results from two identical studies performed using baking and extensigraph data from a collection of 71 wheat cultivars from 23 different countries (Campbell et al., 1987) and a set of 60 recombinant inbred lines from the New Zealand breeding program (Cressey et al., 1987) associated the presence of sub-units 5+10 with higher dough resistance loaf volume and work input. No proteins were found to correlate with extensibility in the first study whereas, sub-units 7+9 (Glu-B1) were associated with greater extensibility and sub-units 5+10 were associated with low extensibility in the second study. However, only the significance of the correlations and not the magnitudes were reported in both of these studies. Also, as discussed in the previous sections, gliadin components were also correlated, to a lesser extent, with quality parameters.

Khan et al. (1989) studied the relationship between bread-making parameters and the allelic composition at Glu-A1 (2* vs. 1 or null) and Glu-B1 (7+8 vs. 7+9) in a collection of 44 cultivars grown in North-Dakota (43 of them expressed allele 5+10 at the Glu-D1 locus). The presence of sub-units 2* was associated with a higher wet gluten content and lower mixing time when compared to sub-units 1 or null allele. About 15 % of the variability in mixing time could be explained by the presence of sub-units 2*. Allele 7+9 was found to be associated with higher loaf volume and gluten content than allele 7+8. However, only 12 % and 8 % of the variability in loaf volume and mixing time respectively, could be accounted for by the presence of sub-units 9.

Payne et al. (1987) summarized the relationship between bread-making quality and HMW sub-units composition by ranking the alleles expressed at the three Glu-1 loci according to their known or presumed contribution to bread-making quality and assigning them scores from 1 to 4. For the Glu-A1 locus, alleles 1 and 2* are assigned 3 points and the null allele 1 point. For the Glu-B1 locus, alleles 17+18 and 7+8 are given 3 points, 7+9 are assigned 2 points and 7 or 6+8 are given 1 point. For the Glu-D1, allele 5+10 are assigned 4 points whereas alleles 2+12 or 3+12 are given 2 points. A comprehensive Glu-1 score (varying from 3 to 10) is then obtained by summing the scores for the individual loci. In a set of 84 British cultivars grown for two years and divided into 4 groups according to their bread-making quality (determined by independent evaluations) , it was found that the Glu-1 score could explain 47 % to 60 % of the variation in quality ranking (Payne et al., 1987). Allelic composition at Glu-A1, Glu-B1 and Glu-D1 accounted for 28.4 %, 7.7 % and 23.6 %, respectively, of the variability in quality. A similar study involving 67 cultivars grown in Canada (Lukow et al., 1989) resulted in 59 % to 62 % of the variability in bread-making quality ranking accounted for by the Glu-1 score. Using data collected from a set of 33 cultivars grown in Spain, Payne et al. (1988) reported that the Glu-1 score could account for 53 % and 78 % of the variability in Zeleny sedimentation volume and Alveograph W, respectively.

While these studies, as well as others, clearly demonstrate the important functional role played by specific HMW sub-units, a critical review of the results (Weegles et al., 1996) stresses the relatively small proportion of the variability in quality that can be

explained by HMW glutenin sub-units composition. This is particularly true for loaf volume (Hamer et al., 1992; Andrews et al., 1994). Prediction models based on HMW sub-units composition were not able to account for more than 30 to 34 % of the variability in loaf volume within a collection of 184 wheat samples (Hamer et al., 1992). In the latter study, the prediction power of the model fell to 0.005 % when a validation attempt was made using 84 other samples grown during the following year. According to Weegles et al. (1996), the lack of predictive power characterizing the electrophoretic scoring systems based on HMW sub-units composition precludes their use in predicting quality in the context of a milling or baking industry. Nevertheless, the authors acknowledge the usefulness of some HMW glutenin alleles (2* or 5+10 for example) in a breeding context, as markers for quality in early generations. However, the superiority of allele 5+10 over 2+12 can, in some cases be over-emphasized as it often depends on the presence of other sub-units encoded by GLU-A1 and Glu-B1. In fact, Sutton (1991) reports that the wheat cultivar with the best bread-making performance in New Zealand, namely Otane, expresses allele 2+12 at Glu-D1. Also, significant epistatic (inter-locus) effects between the three HMW glutenin loci in the expression of quality traits have been reported (Carillo et al., 1990; Dong et al., 1991; Rousset et al., 1992; Nieto-Taladriz et al., 1994) confirming that the effect on quality of some sub-units depends partly on the presence of other sub-units coded by the other genomes. These results suggest that fixation within a breeding population of the 3 HMW glutenin loci with alleles presumed to be most favorable on the basis of their additive effects might lead to the elimination of good quality lines which superiority is due to epistatic interactions. Also, fixation of one allele that is thought to be ideal for one quality parameter (7+8 and mixing time) will eliminate an alternative allele that might be ideal for another quality parameter (17+18 and loaf volume) (Dong et al., 1991).

Aside from the epistatic effects between loci coding for the HMW sub-units, all the genetics studies cited above report a substantial proportion of the genetic variance for the quality traits measured that is not due to any of the HMW glutenin genes. Although differences in gliadin composition might be involved (Campbell et al., 1987; Cressey et al., 1987), variation at loci coding for the low molecular weight (LMW) glutenin is now

believed to be more important (Gupta and Shepherd, 1988; Gupta et al., 1989; Gupta and MacRitchie, 1991; Pogna et al., 1988). Low MW glutenin sub-units are approximately 2.5 fold more abundant than the HMW glutenin sub-units in the wheat endosperm (Gupta et al., 1994). However, the full range of allelic variation at loci coding for LMW glutenin sub-units, as well as their relationship with quality, was elucidated only recently thanks to the development of electrophoretic procedures allowing the SDS-PAGE analysis of LMW glutenin bands without the overlapping gliadins (Singh and Shepherd, 1988; Gupta and Shepherd, 1990, Gupta and MacRitchie, 1991; Singh et al., 1991). Gupta et al. (1989), using data from 56 F_2-derived F_6 progenies from a single cross, reported that variation at the Glu-A3 locus (LMW) had a greater effect on dough resistance and extensibility than that at Glu-A1 (HMW), and that the effects on these traits appeared to be additive. Gupta and Shepherd (1988) reported that allelic variation at Glu-A3 resulted in significant differences in extensigraph characteristics between two biotypes of each of cultivars Condor and Gamenya. Nieto-Talabriz et al. (1994) did not detect any significant differences in alveograph parameters between F_6 progenies carrying contrasting alleles at each of Glu-A3 and Glu-D3. However, they reported significant epistatic effects between Glu-1 (HMW) and Glu-3 (LMW) which affected both tenacity and extensibility. Gupta et al. (1994) studied the effects on extensigraph parameters of the allelic composition at the Glu-1 and Glu-3 loci in a population of 74 F_3-derived F_7 progenies of a single cross between parental lines carrying contrasting alleles at the 6 glutenin loci. Two of the three loci coding for LMW glutenin sub-units, namely Glu-A3 and Glu-B3, were shown to affect dough maximum resistance. However, HMW glutenin loci Glu-B1 and Glu-D1 had a more pronounced effect on this parameter. Effects of alleles at both groups of loci were mostly additive, accounting for 80 % of the variability in dough maximum resistance. Epistatic interactions significantly contributed to the variation in this parameter. Extensibility was affected mostly by variation at the Glu-D1 and, to a lesser extent, at Glu-D3.

A comprehensive review of the effect on quality of the individual alleles coding for LMW glutenin sub-units is not yet available. However, the studies cited above strongly

suggest a significant functional role for these proteins which constitute, from a quantitative standpoint, the most important portion of the glutenin complex.

II.3.3.5.3. Quantitative relationship between bread making quality and glutenin composition

The relationship between the proportion of glutenin in flour, particularly that of unextractable glutenin, and quality parameters have been well documented by several authors. Studying 26 wheat lines grown at 4 locations, Orth and Bushuk (1972) reported a strong association between the amount of acetic acid-unextractable glutenin or "residue protein" and loaf volume (r=0.87) as well as with dough development time (r=0.67). Mecham et al. (1972) found that long dough development times were associated with higher proportions of "gel" protein which represents an acetic acid-insoluble glutenin fraction and is similar to the residue protein of Orth and Bushuk (1972). Huebner et al. (1976) separated total gluten extracts by gel filtration chromatography and reported that flours with strong mixing properties were those having a high proportion of "glutenin I", which corresponds to the glutenin fraction with the highest molecular weight. Another "gel protein" fraction corresponding to the SDS-insoluble gel layer produced upon ultracentrifugation of flour suspensions in 1.5 % SDS solutions was described by Moonen et al. (1982). It consisted mostly of glutenin and its amount was highly correlated with loaf volume (r=0.87).

More recently, procedures using size-exclusion HPLC (SE-HPLC) were developed that effectively separated the polymeric gluten fraction from the monomeric gluten and non-gluten proteins thereby providing a reliable and high through-put option to quantify each fraction (Lundt and MacRitchie, 1989; Dachkevitch and Autran, 1989; Singh et al., 1990 a, 1990 b; Batey et al., 1991; Graybosh et al., 1993; Gupta et al., 1995). These methods usually involved the sonication of flour/SDS-buffer suspensions to ensure quasi complete extraction of the unreduced gluten proteins, which is a prerequisite for an accurate quantification. Sonication reduces the size of the largest polymer enough to

solubilize them but without destroying their polymeric nature (Singh et al., 1990 a, 1990 b). Alternatively, sonication can be omitted and the protein present in the unextracted fraction can be quantified by measuring the nitrogen present in the residue after extraction (Dachkevitch and Autran, 1989; El Haddad et al., 1995). In any case, these separations yield chromatograms that can be divided into three peaks (or groups of peaks) which correspond to the polymeric proteins, the monomeric gliadins and the non-gluten proteins, respectively. The polymeric peaks have been shown to consist primarily of glutenin (Lundt and MacRitchie, 1989; Singh et al., 1990 a; Batey et al., 1991; Graybosh et al., 1993) and can therefore be used to estimate the amount or proportion of glutenin in total protein or in flour. Also, SDS-unextractable glutenin, corresponding to the "residue" or "gel" protein described above, can be quantified by SE-HPLC analysis of residues from previous extraction with SDS-buffer (Gupta et al., 1993; Gupta et al., 1995).

Lundt and MacRitchie (1989) used SE-HPLC to characterize the polymeric composition of 10 fractions prepared by sequential fractionation in dilute hydrochloric acid (MacRitchie, 1985) of flours from two cultivars differing in their bread-making quality. When these fractions were used to fortify a base flour and their respective effects on loaf volume and mixing requirements were measured, the authors observed that the fractions that most positively affected quality parameters were those characterized by a high proportion of glutenin. Also, the cultivar with better bread-making performances had consistently greater proportions of glutenin than the one with poor quality. Singh et al. (1990 b) reported highly significant correlations between the percent glutenin in total protein and loaf volume ($r=0.72$), dough development time ($r=0.84$), extensigraph dough resistance ($r=0.84$) and extensibility ($r=0.84$). The absolute amount of glutenin in the flour tested was also highly correlated with extensibility ($r=0.76$) and dough development time ($r=0.89$). Gupta et al. (1993) used SE-HPLC to investigate the association between the amount/size distribution of polymeric protein and quality parameters in four different sets of wheat lines grown at two locations. They concluded that the proportion of polymeric protein (glutenin) either in total protein or in flour was not consistently nor substantially associated with dough strength or loaf volume. However, a strong association between these traits and the proportion of SDS-unextractable polymer (either in total protein or in

flour) was consistently observed in all the sets analyzed. Lee and al. (1995) used SE-HPLC to study the biochemical basis underlying the quality defects associated with the 1B/1R translocation. The authors observed that detrimental effects on quality were associated with a reduced proportion of glutenin in the flour from lines carrying the translocation.

The functional role played by specific sub-units or groups of sub-units is evidenced by a number of studies relating their amount or proportion to bread-making quality parameters. Some of these studies involved the use of reversed phase-HPLC or RP-HPLC of reduced, partially purified glutenin extracts, whereby the individual polypeptides are separated according to their surface hydrophobicity (Bietz, 1985, 1986). Peaks corresponding to single sub-units are obtained and characterized, usually by SDS-PAGE (Marchylo et al., 1989; Sutton, 1991; Weegles et al., 1995). Sutton et al. (1989) used RP-HPLC to quantify some unidentified glutenin fraction eluting in the two earliest peaks and attempted to use the areas under these peaks to predict loaf volume and bake scores. A model using the two peak areas as dependent variables was developed that explained 72 % of the variability in loaf volume in a set of 140 flour samples. However, when the same approach was used with a set of 243 samples from lines with very different origins and grown at several locations, the proportion of the variability in loaf volume explained by the model decreased to 33 % (Sutton et al., 1990). Despite supporting the functional role played by glutenin, this result stresses the need for adequate validation of any predictive models using the amount of glutenin sub-units as predictor variables (Weegles et al., 1996). Later, Sutton (1991) reported a quantitative RP-HPLC analysis of the HMW glutenin fractions of two cultivars differing in their bread-making quality and observed that the cultivar with the better quality was characterized by a higher absolute amount of HMW glutenin (as determined by summing the areas under the peaks corresponding to individual sub-units) as well as a greater proportion of HMW glutenin in the total protein. The two cultivars had similar protein contents. Andrews et al. (1994) reported a substantial correlation between the amount of HMW or LMW (as determined by RP-HPLC) and either of farinograph dough development time or extensigraph maximum resistance. These quantitative parameters (amounts of HMW and LMW glutenin) were

better correlated with quality traits than qualitative scores (HMW scores) based on the presence or absence of specific bands. Extensibility tended to be associated with the amount of LMW glutenin in the flour.

II.3.3.5.4. The molecular basis of bread-making quality

Gluten extensibility, that is its ability to deform and stretch without loosing its cohesiveness, is the most important rheological requirement for good bread-making performance (Bloksma, 1990 a, 1990 b; Janssen et al., 1996). Despite having its relevance questioned by Bloksma (1990 a), gluten elasticity is also believed to play an important role in gluten functionality (Shewry et al., 1989; Shewry et al., 1992; Scofield, 1994). Both extensibility and elasticity require long polymer chains with some degree of covalent cross-linking (Shewry et al., 1989; Hoseney et al., 1994 a). Extensibility is enhanced by the presence of large molecules because these can slide further along each other without loss of cohesiveness (Bloksma, 1990 b). Elasticity is positively affected by the degree of cross-linking within polymers. It is then apparent that the ability of glutenin sub-units to form large size polymers (or macro-polymer as designated by Weegles et al., 1996) is critical to gluten functionality in bread-making.

Experimental evidence supporting this relationship was discussed in the previous section. Additional evidence is provided by the work of Popineau et al. (1994) showing that deletion of Glu-A1 and Glu-D1 (2 of the 3 loci coding for HMW glutenin sub-units) from cultivar Sicco resulted in a decrease in gluten visco-elasticity apparently due to a lower content in large glutenin polymers. Furthermore, Gupta et al. (1995) reported that the loss of one or all loci coding for either the LMW (Glu-3 Loci) or the HMW (Glu-1 loci) glutenin sub-units resulted in a decrease of both total and unextractable polymeric protein, which was paralleled by a decrease in dough strength, extensibility and mixing time requirements. Their work also demonstrated that both HMW and LMW glutenin sub-units can independently form a macro-polymer when present alone in the gluten of

Glu-3-null or Glu-1-null lines, respectively. However, when both groups of sub-units are present together, the amount of macro-polymer produced is much higher than the sum of the amounts produced when only one group of sub-units is expressed. Finally, the authors reported that the loss of HMW glutenin sub-units reduced the macro-polymer formation to a much greater extent than did the loss of LMW sub-units (on an equal weight basis).

The molecular basis for the effect of specific glutenin alleles is only recently beginning to be elucidated thanks to experiments in which allelic composition is related to macro-polymer formation. Gupta and MacRitchie (1994) reported that, within a population of 74 recombinant inbred lines from a single cross between parents differing in their allelic composition at six glutenin loci, lines carrying allele 5+10 produced a significantly higher proportion of SDS-unextractable polymer or macro-polymer than lines expressing allele 2+12. So did lines carrying sub-units 17+18 when compared to those expressing sub-units 20. No differences were found in the amount of total polymer between the contrasting classes. Also, the macro-polymer content accounted for all of the main and interaction effects of the alleles on dough characteristics. Furthermore, contrasting alleles at Glu-D1 and Glu-B1 produced similar amounts of protein as measured by RP-HPLC of reduced, partially purified glutenin (for example, there was as much protein corresponding to 5+10 than that corresponding to 2+12). Taken together, these results demonstrate that the allelic effects of the HMW glutenin sub-units involved in this study are due to their intrinsic ability to form large size polymers and not to any quantitative differences. This conclusion was confirmed by Popineau et al. (1994) who reported that the substitution of HMW sub-units 5+10 from cultivar Sicco by sub-units 2+12 changed neither the total polymer content nor the relative amounts of sub-units present in the flour, but did decrease the macro-polymer content as well as the gluten visco-elasticity.

Consequently, it becomes apparent that HMW glutenin sub-units influence gluten functionality indirectly, via their ability to promote the formation of glutenin macro-polymer. In turn , the macro-polymer-forming capacity of a sub-unit depends on the number and distribution of its disulfide bond-forming cysteine residues. It has been suggested that the presumed positive effect of sub-units 5, from the allelic pair 5+10

(Lafiandra et al., 1993) on quality is due to its better polymerizing capacity resulting from the presence of an additional cysteine residue at position 97 which contains a serine in sub-units 2, from the allelic pair 2+12 (Greene et al., 1988; Shewry et al., 1989). A greater number of cysteine residues in specific positions within the molecule is expected to promote cross-linking, thereby increasing elasticity. Gluten elasticity could also be affected by differences in the presumed intrinsic elasticity of the β-spiral characteristic of the central repetitive domain of HMW glutenin sub-units (Miles et al., 1991). Goldsborough et al. (1989) suggested that small differences between sub-units 10 and 12 in their amino-acid sequences in the central domain could result in a more regular β-turns pattern for sub-units 10; This might enhance its intrinsic elasticity resulting in better gluten visco-elastic properties. However, this proposition still requires conclusive evidence for the intrinsic elasticity of the β-helix conformation (Shewry et al., 1989). Furthermore, quality differences among the pairs 2+12 and 5+10 are thought to be determined by differences between sub-units 5 and 2 rather than 10 and 12 (Lafiandra et al., 1993).

High MW glutenin sub-units encoded by Glu-A1 can positively influence gluten functionality via quantitative effects. In fact, it has been shown that the presence of either sub-units 1 or 2*, compared to the expression of the null allele, results in a higher proportion of HMW glutenin (Seilmeier et al., 1991-cited in Shewry et al., 1992; Halford et al., 1992) which in turn affects the production of glutenin macro-polymer as well as quality parameters (Gupta et al., 1995).

The effect of LMW glutenin sub-units on gluten polymer formation and thereby on its functionality has been clearly demonstrated (Gupta et al., 1995). Low MW glutenin sub-units are more numerous than HMW sub-units but only 2 sequences are available to date (Okita, 1985; Colot et al., 1989). Many of the cysteine residues are located centrally within the molecule in the unique C-terminal region (Tatham et al., 1990). It is not known whether all of them participate in inter-molecular disulfide bonds or some contribute to intra-molecular bonds (Kasarda, 1990). The lack of comprehensive sequence information precludes suggesting any hypothesis regarding qualitative differences at the molecular level between LMW glutenin sub-units that could explain the allelic effects on dough

properties. In contrast, quantitative differences have been observed between contrasting LMW glutenin alleles from both Glu-A1 and Glu-D1 which appeared to be associated with differences in glutenin macro-polymer formation and dough maximum resistance, suggesting that differences in gluten functionality are also due to differences in the amounts of protein produced by contrasting LMW glutenin alleles (Gupta et al., 1994). However, the authors concluded that qualitative differences in the molecular structure of LMW glutenin sub-units affecting macro-polymer formation cannot be ruled out.

II.4. Durum wheat for bread-making

II.4.1. Durum wheat production and uses

Durum wheat is grown on an average of 30 million hectares, representing about 8 % of the area planted in wheat worldwide (Bozzini, 1988). An average of 30 million metric tons was produced annually between 1989 and 1992 (International Wheat Council, London, January 1992) corresponding to 5 % of the total annual wheat production. The importance of durum wheat is far greater than these statistics might reveal as its production is concentrated in relatively small areas, located mainly in developing countries for which durum products are often the main staple foods (Abdallah et al., 1992). Six countries, namely, India, Syria, Turkey, Algeria, Morocco and Tunisia account for 83 % of the durum-planted area in the developing world (Wheat Facts and Trends. CIMMYT, 1987-1988). The United States, Canada and Italy are the major producers among the industrialized countries (Bozzini, 1988). From a geographical standpoint, more than half of the durum-planted area is located around the Mediterranean, including North Africa, Southern Europe and the Middle East (Liu et al., 1996; Bozzini et al., 1988). Durum Wheat is the major cereal crop in several countries in terms of area planted, including Italy (53 %), Jordan (95 %), Syria (70 %), Algeria (65 %), Tunisia (90 %) and Ethiopia (90 %)

(Bozzini et al., 1988). It is also the major crop in terms of the percentage of wheat produced in Italy (50 %), Algeria (75 %), Tunisia (75 %) and Ethiopia (70 %) (Abdallah et al., 1992).

Durum wheat is used in the production of pasta products (28 % worldwide), cous-cous (16 %), various types of local breads (24 %) and other products (32 %) including bulgur, various porridges and desserts (Quaglia, 1988). The relative importance of the different products made from durum wheat depends on the country. In Europe and the Americas, the major part of the durum wheat is used for the production of pasta (Quaglia, 1988; Boyacioglu and D'Appolonia, 1994 d). Cous-cous is the major durum-derived product in Tunisia (50 %) and Saudi Arabia (80 %), whereas durum wheat finds its major use in the baking of various types of breads in Jordan (95 %), Morocco (85 %), Cyprus (90 %), Turkey (60 %) and Afghanistan (60 %). This is the case, albeit to a lower extent, for Italy and Greece (Quaglia, 1988).

In most cases, durum wheat breads are made in households or small bakeries. Durum wheat flours have been, and still are, perceived as having unsuitable gluten characteristics for their use in commercial bread-making industries. The development in several countries of new cultivars with stronger gluten followed by the increase in the research efforts devoted to the study of durum wheat for bread-making led several authors to challenge that perception (Quick and Crawford, 1983; Boggini, 1985; Boggini et al., 1988; Boggini and Pogna, 1989; Dexter et al., 1994; Boyacioglu and D'Appolonia, 1994 a, 1994 b).

A review of the number of reports published during the last 15 years that dealt with the use of durum wheat for bread-making clearly illustrate the renewed interest for the subject (Feillet et al., 1996). In fact, the number of reports increased from six during the decade of 1980 to 1990, to 25 for the past five years (1990 to 1995).

II.4.2. Potential uses of a "dual purpose" durum wheat

The development of a dual purpose durum wheat, that is a cultivar with suitable bread-making as well as pasta-making quality is considered to be advantageous in many ways. Bakers as well as consumers could take advantage of the longer shelf life and better staling characteristics of bread made from durum flours (Quick and Crawford, 1983; Boggini, 1985; Boyacioglu and D'Appolonia, 1994 c; Boyacioglu and D'Appolonia, 1994 d and references therein).

It has been reported that many people around the world prefer the yellow colored, fine and uniformly structured crumb, as well as the characteristic taste and smell of durum wheat breads over those of breads made from common wheat flours (Boyacioglu and D'Appolonia, 1994 d and references therein; Liu et al., 1996). Even in cases where such preferences do not prevail, it is believed that providing the customer with an additional bread type characterized by an alternate flavor and color might be advantageous (Quick and Crawford, 1983; Boggini, 1985). The use of durum wheat by the bread-making industry should also benefit wheat growers by offering an alternate market for their crop, which would be particularly useful in years of high durum wheat production or in cases of bread wheat shortages (Quick and Crawford, 1983; Boggini, 1985).

In many countries, durum wheat is the major cereal crop because of its better adaptation to the environment, cultural traditions and/or better market value. The availability in those countries of durum cultivars with good bread-making quality would decrease their dependence on bread wheat imports to satisfy their often increasing demand for flour. These durums could be used alone or blended with bread wheat. Boggini (1985) indicates that such durum wheats are sought as a replacement for the imported hard red wheat used in blends to improve the bread-making quality of Italian bread wheats. Several authors have reported that durum wheat flour can be used in blends to improve the bread-making quality of ordinary or poor quality bread wheat flours (Harris et al., 1952; Prabhavati et al., 1976; Boggini and Pogna, 1990; Boyacioglu and

D'Appolonia, 1994 b). These reports indicate that the improving effect depends largely on the quality and proportion of durum flour blended.

The process of milling durum wheat into semolina yields 8 to 12 % of "clear flour" or "residue flour" (Basanik, 1981). Durum wheat cultivars with better bread-making quality are likely to produce a clear flour that could be used in making bread, thereby increasing its commercial value and resulting in added value for the millers (Boyacioglu and D'Appolonia, 1994 d, and references therein).

In countries where most of the durum wheat is already used in bread-making, the availability of cultivars with superior industrial quality should promote the development of larger scale, commercial bread-making industries.

Finally, it has been reported that gliadin peptides from durum wheat are much less toxic to people affected by gluten intolerance (celiac disease) that those from bread wheat. It is possible that such people could consume bread made entirely from durum wheat without health risks (Boyacioglu and D'Appolonia, 1994 d, and references therein).

II.4.3. Comparison between the bread-making quality of durum and bread wheat and variability within the durum germplasm.

The earliest investigations undertaken from the early 1920's to the late 1970's (Reviewed by Boyacioglu and D'Appolonia, 1994 d) clearly demonstrate the unsuitability for bread-making of old durum cultivars. These produced a weak gluten resulting in poor dough handling characteristics and inferior baking performance. Their use in commercial bread-making was therefore dismissed, except as minor components in blends with bread wheat flours.

In the early 1980's, the release of durum cultivars with stronger gluten, aimed at improving their pasta-making potential, provided researchers with new material to evaluate. Dexter et al. (1981) compared the gluten strength, mixing properties and baking characteristics of 22 durum and 38 bread wheats grown in Canada. Their results confirmed that durum wheats, as a group, were characterized by a weaker gluten, shorter

mixing times, as well as lower baking strength indices (BSI) and loaf volumes. However, much variability was observed for all these parameters among the durum wheats tested and some of these were considered to have a fair baking quality as indicated by mixing times, BSI and loaf volumes which approached those of bread wheats. They suggested that no fundamental difference existed in the behavior of the two classes during bread-making, that is, durum wheats exhibited the properties of weak bread wheats. Josephides et al. (1982) reported that cultivar Vic (strong gluten) produced an acceptable loaf of bread with a volume only 10 % lower than that of a hard red spring check. Quick and Crawford (1983) found that the stronger gluten durum cultivars such as Edmore and Vic had markedly better dough handling characteristics and baking properties than the weak gluten cultivars such as Ward or Wakooma. In fact, the former type nearly equaled the Hard Red Spring check in overall baking evaluation. The bread-making quality of cultivars Vic and Lloyd was also compared to that of weak gluten cultivars Rugby and Cando and to that of Hard Red Spring cultivar Waldron by Holm (1985). She reported that Vic and Lloyd did not perform consistently as well as the bread wheat Waldron, but they were clearly superior to Rugby and Cando.

Later, a series of reports were published describing the evaluation of the bread-making quality of a number of Italian durum wheat cultivars (Boggini, 1985; Boggini et al., 1988; Boggini and Pogna, 1989). Results clearly indicated that the most critical parameter for better bread-making characteristics was gluten strength. Total protein content was not consistently associated with loaf volume ($r= 0.14$ to $r=0.70$) over three years of testing (Boggini and Pogna., 1989). Practically none of the mixogram parameters were found to correlate with loaf volume, which was moderately associated with farinograph dough development time and with dough stability. In contrast, the correlation between loaf volume and gluten strength was highly significant and consistent over three years of testing. Although no bread wheat checks were included in these evaluations for comparison, they clearly illustrate the wide range of variability in bread-making quality parameters, including loaf volume, observed within the Italian durum germplasm. Pasqui et al. (1991) reported that loaf volume depended on the rheological properties of the dough as measured by the alveograph, particularly on W (an indication of gluten strength)

and on the P/L ratio (tenacity/extensibility, which is an estimate of the visco-elastic balance of a dough). Their results, especially the reported trend towards a negative association between W and loaf volume, are in disagreement with most of the reports dealing with either durum or bread wheat. It is to be noted that only five cultivars, at two protein levels were tested, which represent a limited sample set.

Recently, Peña et al. (1994) reported a highly significant association between loaf volume and gluten strength as measured by either the SDS- sedimentation test (r=0.82) or by the alveograph W (r=0.62), in a set of 26 durum from the CIMMYT program, grown in Mexico. Again, a considerable variability in loaf volume (410-740 cc), sedimentation volume (4.0-19.5 ml) or W (51-468) was observed in this set of lines.

II.4.4. Genetic control of gluten strength and its relationship to bread-making quality in durum wheat

The importance of the D genome, in particular that of chromosome 1D, in terms of its contribution to baking quality and gluten strength in hexaploid wheat (2n = 6x = 42 = AABBDD) has been clearly demonstrated by numerous studies. Direct evidence comes from investigations involving the evaluation of genetic stocks either lacking or possessing an additional 1D chromosome, or having the original 1D chromosome substituted by that of another line differing in quality (Welsh and Hehn, 1964; Schmidt et al., 1966; Morris et al., 1966, 1968; Rogers et al., 1988). More recently, the importance of specific genes mapping to chromosome 1D was demonstrated through the evaluation of lines deficient in a number of high and low molecular weight glutenin genes (Gupta et al., 1990; Lafiandra et al., 1993; Popineau et al., 1994; Gupta et al., 1995). A third line of evidence consists of the numerous correlation studies which indicated that variation for baking quality and gluten strength, either within a collection of lines or within a population of recombinant lines from a cross, was most consistently associated with variation in the allelic composition at the Glu-D1 locus located on chromosome 1D (see references cited in section III-3-5-b). In light of this relationship, the comparatively inferior baking

performance and weaker gluten of durum wheats (2n = 4x = 28 = AABB) has been attributed to the absence of the D genome (Quick and Crawford, 1983; Boggini et al., 1989; Peña et al., 1994; Ciaffi et al., 1995; Liu et al., 1996). However, a number of studies have also shown a significant effect on gluten strength of the genes located on chromosome 1B in hexaploid wheat (Nieto-Taladriz et al., 1994; Gupta and MacRitchie, 1994; Gupta et al, 1994; Peña et al., 1995; Gupta et al, 1995). In durum wheat, genes located on chromosome 1B have major effects on gluten strength and therefore on bread-making quality. This relationship was first established when gliadin components (γ-gliadin 42 and 45) whose presence was strongly associated with gluten strength (Daminaux et al., 1978, Kosmolak et al., 1980) were mapped to the Gli-B1 locus on chromosome 1B (Daminaux et al., 1980; Joppa et al., 1983). These gliadin alleles were later shown to be merely markers that co-segregated with LMW glutenin alleles from the tightly linked Glu-B3 locus which turned out to be the protein responsible for the 1B-determined variation in gluten strength (Pogna et al., 1988). The relationship between allelic variation at Glu-B3 and bread-making quality in durum wheat was confirmed by several reports (Boggini et al., 1988; Boggini and Pogna, 1989; Peña et al., 1994). Another locus on chromosome 1B was shown to influence bread-making quality in durum wheat, namely Glu-B1, coding for the HMW glutenin sub-units. In fact, Boggini et al. (1988) and Boggini and Pogna (1989) reported that certain alleles from Glu-B1 appeared to be associated with better bread-making quality in durum wheats from Italy. Of the 3 most frequent HMW glutenin alleles identified in durum wheat, 7+8 appeared to impart the best bread-making characteristics, followed by 20 and 6+8. These results were confirmed by Peña et al. (1994) in a collection of 26 durum wheats from the CIMMYT program. In this latter study, lines carrying 6+8 had slightly, but not significantly, better bread-making properties than those expressing allele 20.

Direct evidence for the major contribution to gluten strength and baking performance of genes on chromosome 1B of durum wheat was first provided by the work of Josephides et al. (1987). Using a Langdon substitution line nullisomic for 1B and disomic for 1D (from hexaploid wheat cv. Chinese Spring), they were able to replace chromosome 1B from Langdon (weak gluten) with that of Kharkof-5 or Edmore (both

strong gluten cultivars). Results from the quality evaluation of these lines demonstrated that the 1B substitution lines had the gluten characteristics of the donor parent rather than those of the recurrent parent Langdon. Similar results were reported by Liu et al. (1994 a, 1994 b) who evaluated the mixing properties and the proportion of glutenin in segregants from a cross involving Langdon 1D(1A) and Langdon carrying chromosome 1B from cultivar Edmore. Their results suggested that the presence of 1B from Edmore resulted in an increased proportion of glutenin and glutenin / gliadin ratio (as measured by SE-HPLC) and in a rise in dough mixing time and peak height. When chromosome 1D was also present, the effect of both chromosomes on the parameters measured was additive (Liu et al., 1994 a). Lines with 1B from Edmore had a proportion of glutenin similar to that of medium strength hexaploid wheats (Liu et al., 1994 b).

Although the great majority of durum wheats worldwide carry a null allele at locus Glu-A1 on chromosome 1A, few cultivars do express one of the 8 identified HMW glutenin variants of either sub-units 1 or 2* (Branlard et al., 1989). Because the expression of a non-null allele at Glu-A1 has been associated with an increased proportion of HMW glutenin in flours from hexaploid wheats (Sutton, 1991; Halford et al., 1992), it is believed to contribute a positive effect on bread-making quality (Shewry et al., 1992). Recent results from an evaluation of the baking performance and rheological properties of durum wheats expressing a non-null allele at Glu-A1 seem to support this belief (Boggini et al., 1995). They reported that lines carrying the LMW-2 allele at Glu-B1 (associated with strong gluten) and a non-null allele at Glu-A1 had better gluten characteristics and produced larger bread loaves than control lines carrying null alleles at Glu-A1. They also suggested that the production of additional HMW glutenin sub-units influenced quality via an increase in dough extensibility.

II.4.5. Approaches to improve the bread-making quality of durum wheat

Several approaches have been suggested to develop durum wheat cultivars with a bread-making quality similar to that of hexaploid wheats, the final objective being its use in commercial bread-making industries. The most straightforward approach capitalized on the substantial variability available within the durum wheat germplasm (Dexter et al., 1981; Boggini et al., 1988; Boggini and Pogna, 1989; Peña et al., 1994) and attempted to produce recombinant lines with an improved bread-making quality through hybridization and selection for high gluten strength. As a result of this classical strategy, whose primary goal was to improve the pasta-making quality of old durum cultivars, some durum wheats have been released with a baking performance that approached that of hexaploid wheats (Dexter et al., 1981; Josephides, 1982; Quick and Crawford, 1983; Holm, 1985). However, despite this undeniable improvement, durum wheats continue to be generally inferior to hexaploid wheats in their bread-making quality and thereby are not yet able to successfully compete with these on a commercial scale. Recent results reported by Boggini et al. (1995) revealed that the expression of either HMW glutenin sub-units 1 or 2* (or a variant of these) was associated with greater dough extensibility and better baking characteristics. These findings, if confirmed, raise the possibility for further improvement of the bread-making quality of durum wheat by making use (in crosses or as such) of the relatively few known durum lines expressing a non-null allele on chromosome 1A.

A second approach has been reported by Ciaffi et al. (1995), which capitalizes on the positive effects on gluten strength of a Glu-A1 allele from wild emmer wheat, Triticum dicoccoides ($2n = 4x = 28 = AABB$). Expression of this allele results in the production of 2 protein sub-units (one x-type and one y-type) as opposed to all other Glu-A1 alleles from either durum or bread wheat which produce a single sub-unit (x-type). Evaluation of the bread-making quality of 13 F_7 recombinants from a double cross, segregating for the dicoccoides allele, revealed that the presence of this allele, in combination with allele LMW-2 of Glu-B3, was associated with better baking performances and alveographic

characteristics. One such line performed as well as bread wheat cv. Centauro which was evaluated as a check.

A final strategy, consisting in introgressing favorable alleles from Glu-D1 of hexaploid wheat, is expected to lead to the most significant improvement in the bread-making quality of durum wheat. This transfer has been advocated by several authors, including Kaltsikes et al. (1968), Quick and Crawford (1983), Boggini (1985), Liu et al. (1994 a, 1994 b), Boggini et al. (1995) and Ceolini et al. (1995). Experimental results from the work of Josephides et al. (1987) have shown that potential for substantial improvement exists as a result of such a transfer. In fact, they have demonstrated that Langdon durum could produce a strong gluten resulting in high loaf volume when its 1B chromosome was replaced by chromosome 1D from Chinese Spring. Furthermore, in a population segregating for chromosome 1D from Chinese Spring, its presence was associated with stronger mixing properties and greater proportion of glutenin in the grain (Liu et al., 1994 a, 1994 b). This positive effect was observed despite the fact that chromosome 1D of Chinese Spring carries the allele coding for HMW glutenin sub-units 2+12 which are associated with weaker gluten and poor bread-making quality in hexaploid wheats. Therefore, it is believed that an even more spectacular improvement in bread-making quality should be achieved if the allele coding for HMW glutenin sub-units 5+10 is transferred to durum wheat. Also, despite the ability of chromosome 1D to substitute in a stable fashion its homeologous counterparts from durum wheats (1A or 1B) and become part of its genome (Joppa and Williams, 1988), the transfer of Glu-D1 via the translocation of a small segment of chromosome 1D seems to be more suitable in terms of preserving the identity of the recurrent durum parent. This approach has been successfully implemented by Lukaszewski and Curtis (1992) who were able to transfer the Glu-D1 allele coding for HMW glutenin 5+10 to hexaploid triticale via a 1D/1R translocation. They subsequently transferred this locus to chromosome 1A of the same triticale through homoelogous recombination (Lukaszewski and Curtis, 1994). The successful transfer via translocation of a chromosome segment carrying the Glu-D1 allele coding for HMW glutenin 5+10 from bread-wheat Torim 73 to durum wheat was reported by Ceolini et al. (1995). This was achieved by disabling the homologous pairing mechanism using the

hexaploid (Chinese Spring ph1b) and tetraploid (Capelli ph1c) mutants lacking the genes controlling homologous pairing. Ultimately, tetraploid (2n = 28) individuals homozygous for the Glu-D1 allele producing HMW glutenin sub-units 5+10 were identified. This approach does not allow to target the translocation to any particular A or B chromosome, however.

III. Materials and Methods

III.1. Plant material

Thirty spring durum wheat cultivars and/or breeding lines were selected to represent a wide array of the genetic backgrounds. Five bread wheat cultivars were also included in the study for comparison. These included two adapted cultivars with good bread-making quality (Hard Red Spring cv. "McKay" and Hard White Spring cv. "Klasic") and three Hard White Spring cultivars released in Tunisia which are characterized by a wide range of bread-making quality ("Florence Aurore", "Byrsa" and "Tanit").

III.2. Experimental design and growing conditions

The thirty five genotypes were grown in 5 x 1.5 meters plots, arranged in a Randomized Complete Block Design with two replications, during the springs of 1993 and 1994. The experimental site was the "Rugg's" farm near Pendleton, Oregon. The soil type at this site is a coarse silty typic haploxeroll. Planting dates were April 15th and March 30th in 1993 and 1994, respectively. All entries were sown at a seeding rate of 120 grams of seed per plot. Fertilization was performed according to the common agronomic practices in the area: 78 kg/ha nitrogen, 22 kg/ha of phosphorus, 11 kg/ha sulfur and 1.7 kg/ha Boron were applied in the fall. Additional nitrogen (11 kg/ha) and sulfur (11 kg/ha) were broadcast during flowering. Plots were irrigated once during grain filling. The rainfall at the site was 241 and 142 millimeters from March 1st to July 31st in 1993 and 1994, respectively.

III.3. Kernel characteristics

Plots were harvested with a plot combine and the seed cleaned to eliminate all contaminating material including shrunken and broken kernels.

Test weight (TWT) was obtained by weighing 1 liter of clean seed from each plot. Randomly sampled aliquots were used to determine the weight of one thousand kernels (TKW). These were subsequently ground in a UDY-Cyclone mill equipped with a 1 mm opening size screen and stored in moisture-proof plastic containers.

Ground wheat samples were used to determine total grain protein content (GRPROT) using a Technicon 450 Near Infrared Reflectance (NIR) spectrometer calibrated using wheat samples whose protein content (percent nitrogen x 5.70) was determined by direct measurement of the nitrogen content. Different calibrations were performed for durum and bread wheats, which resulted in the protein determination being based upon different equations.

Grain hardness was also determined by NIR and the calibration was performed using hardness data of the calibration sample set provided by the Federal Grain Inspection Service (F.I.G.S.).

III.4. Milling conditions

Prior to milling, durum wheat samples were tempered to 17 % moisture and bread wheat samples to 15.5 %, for 24 to 36 hours. Tempering was performed in 1 liter bottles (two bottles per sample) containing 450 grams of seed and inverted with a mechanical rotor for two hours upon addition of the appropriate amount of water calculated after measuring the moisture content of 2 aliquots (450 grams, each) of seed with a Dickey John moisture meter.

Tempered seed was milled in a Quadrumat Senior mill according to the manufacturer's instructions (Brabender Gmbh. Duisburg, Germany). The feed gate opening - through which the kernels are fed to the break rolls- was kept at a setting of 2. The middlings stream feeding the reduction rolls was run for 3.5 minutes. This stream was subsequently collected, diverted into a plastic container and added to the "shorts" fraction. Once the reduction step was interrupted, sifters were allowed to proceed for 10 minutes. The bran and "shorts" fractions were discarded and the break and reduction flours were collected and weighed separately and then mixed thoroughly to yield a straight flour on which all of the subsequent analyses were performed. Flour samples were stored in moisture-proof plastic bags or containers.

III.5. Flour protein content determination

Since most physical dough-testing methods are heavily influenced by the protein content of the flour tested, it was important to obtain an accurate estimation of this parameter. This was achieved using a direct method of nitrogen content determination. The Dumas combustion method was used in this experiment. Percent nitrogen (by weight) was determined on approximately 0.25 g of flour using a LECO nitrogen analyzer according to A.A.C.C. method 46-30 (A.A.C.C. Approved Methods, 9th edition, 1995). Percent protein content was calculated as percent nitrogen x 5.70, and reported on a 14 % moisture basis. Moisture content was determined using an air-oven method (method 44-15A, A.A.C.C. Approved Methods, 9th edition, 1995).

III.6. SDS-Sedimentation test

Gluten strength was evaluated by measuring the height (in mm) of the sediment resulting from performing a Sodium Dodecyl Sulfate-sedimentation test on 1 g of ground wheat according to Dick and Quick (1983). The test tubes used were checked for inner-diameter uniformity with water to ascertain that height was an accurate measure of sediment volume. Sediment height was read at 20 minutes.

III.7. Mixing properties

Mixing properties were studied with a mixograph equipped with a 10 gram bowl according to A.A.C.C. method 54-40 A (A.A.C.C. Approved Methods, 9th edition, 1995) with a spring setting of 11. Enough distilled water was added to 10 grams of flour (on a 14 % moisture basis) to result in a mixogram with optimum appearance, as judged by the amplitude and width of the trace. That particular amount of water was considered an estimator of the flour's mixograph water absorption (MABS). The mixing parameters measured were time-to-peak (TTP, in minutes) or time to reach the trace's maximum height, peak height (PHT, in cm), and height of the curve after 7 minutes (H7M, in cm) which is considered a measure of tolerance to over-mixing.

III.8. Dough physical testing

The physical properties, or rheological characteristics, of the dough pieces from each bread wheat sample were studied by alveographic analysis performed according to A.A.C.C. method 54-30 A (A.A.C.C. Approved methods, 9th edition, 1995) on a Chopin Alveograph. The same procedure was used for durum flours, except that the volume of

sodium chloride solution added to the flour was increased by 8-12 % of that recommended in the original method. This was done to compensate for the typically greater water absorption resulting from high levels of starch damage occurring upon milling the much harder durum wheat grain (Peña et al. 1994; Dexter et al., 1994; Peña, personal communication; D'Appolonia, personal communication). Consequently, the alveographic test was performed for durum wheat samples at 55-59 % water addition (on a 15 % moisture basis), rather than at 50% as in the original method. The resulting alveograms were used to determine the over-pressure P (in mm) as an indicator of the dough tenacity or resistance to deformation, the abscissa L (in mm) at the point of bubble rupture which measures dough extensibility and the deformation energy W (in 10^{-4} Joules) required to inflate the dough-bubble until it ruptures. The configuration ratio P/L was also calculated as an indication of the rheological balance of the dough.

III.9. Baking performance evaluation

Baking performance was evaluated by performing a bake test using 100-g of flour (14 % moisture basis) according to the straight-dough method used at the USDA-ARS Western Wheat Quality Laboratory. Optimum bake water absorption (BABS, in ml) and mixing time (BMT, in minutes) were those resulting in a dough with optimum handling characteristics as judged by three expert bakers. Loaf volume (LVOL, in cc) was determined by rape-seed displacement on fresh loaves. A subjective crumb score (1 for excellent to 9 for very poor) was assigned to each loaf by the bakers to describe the suitability of the crumb structure.

III.10. Determination of allelic composition at glutenin loci

III.10.1. Electrophoretic analysis of total protein extracts

Allelic composition at glutenin loci was determined by Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of total reduced protein extracts, according to the discontinuous buffer system of Laemmli (1970). Flour samples (20 mg) were suspended in 1 ml of an extraction buffer containing 0.063 M tris-Cl (pH 6.80), 2 % (w/v) SDS, 20 % (v/v) glycerol, 5 % (v/v) 2-β-mercaptoethanol and 0.02 % (w/v) bromophenol blue, for 1 hour with intermittent vortexing and then placed in a boiling water bath for 5 minutes. After cooling, extracts were clarified by centrifugation at 8,800 g for 15 minutes. Clear supernatants (10 µl) were used for electrophoresis in polyacrylamide gels (0.15 x 16 x 14 cm) using a vertical dual-gel electrophoresis apparatus (SE-600 from Hoeffer). The separating gel was made of 12 % (w/v) acrylamide + bis-acrylamide (1.5 % cross-linker), 0.375 M tris-Cl (pH 8.80), 0.1 % (w/v) SDS and polymerized with 0.033 % (w/v) ammonium persulfate and 0.5 % (v/v) TEMED. Stacking gels were made of 4 % (w/v) acrylamide + bis-acrylamide (2.7 % cross-linker), 0.125 M tris-Cl (pH 6.80), 0.1 % (w/v) SDS and polymerized with 0.05 % (w/v) ammonium persulfate and 0.1 % (v/v) TEMED. Electrophoresis was performed at 40 milli-amperes (mA) per gel for approximately 4 hours. Gels were then stained without fixing in a colloidal solution of coomassie brilliant blue G-250 according to Neuhoff et al. (1988) for 24-30 hours and rinsed in 25 % Methanol for 1 hour.

The nomenclature used is that of Payne and Lawrence (1983) for the High Molecular Weight (HMW) glutenin alleles and that of Payne et al. (1984) for the Low Molecular Weight (LMW) glutenin alleles.

III.10.2. Analysis of purified glutenin proteins

The analysis of total protein extracts allowed the determination of the allelic composition at both glutenin loci. A second analysis of purified glutenin proteins was performed using an adaptation of the method of Singh et al. (1991), to ascertain the identity of the LMW glutenin alleles without the presence of overlapping gliadins and other soluble polypeptides present in the total extracts. Flour samples (20 mg) were extracted twice with 70 % ethanol (30 minutes, each) and supernatants containing mostly gliadin were discarded. Glutenins were extracted from the pellet by sonicating the suspension in 50 % 1-propanol/0.08 M tris-Cl (pH 8.0) containing 0.1% (w/v) freshly mixed Dithioerithreol (DTT) as a reducing agent. Sonication was done with an ULTRASONIC sonicator (model W-10) equipped with a 3 mm probe at an output of 8-10 Watts, for 30 seconds. Reduced glutenins were alkylated with 4-vinylpyridine to prevent re-formation of the disulfide bonds. Proteins were then complexed with SDS in a 0.08 M tris-Cl (pH 8.0) sample buffer containing 2% (w/v) SDS, 40% (w/v) glycerol, 0.02% (w/v) Bromophenol blue. Aliquots of 10 µl were loaded onto a SDS-PAGE gel and the remainder of the procedure was as described for total protein extracts analysis.

III.11. Size Exclusion High Pressure Liquid Chromatography (SE-HPLC) analysis of flour proteins

III.11.1. Optimized protocol

Unreduced, total flour protein was extracted by suspending 20 mg of flour in 1 ml of an extraction buffer made of 0.05 M sodium phosphate (pH 6.95) containing 0.5% (w/v) SDS and sonicating the suspension for 30 seconds using an ULTRASONIC sonicator (model W-10) equipped with a 3 mm probe, at maximum power (8-10 Watts

output). After clarification of the extracts by centrifugation at 8,800 g for 20 minutes, supernatants were filtered through a low protein-binding, polyvinyledene difloride (PVDF) syringe filter with a pore size of 0.45 μm (Durapore membrane from Millipore).

SDS-insoluble polymeric protein was extracted in the same manner except that the starting material consisted of a residue (pellet) from a prior extraction (30 minutes with constant shaking) in the same extraction buffer, without sonication (Gupta and Mac Ritchie, 1993).

Size-exclusion HPLC analysis was performed essentially according to the procedure of Batey et al. (1991), using a computer controlled Beckman System Gold HPLC apparatus equipped with a variable wavelength UV-Visible detector. The column used was a stainless-steel Waters Protein-Pak 300SW size-exclusion column (300 x 7.8 mm) packed with diol-coated silica beads (10 μm diameter) having an average pore size of 300 Å. The mobile phase was a 50 % acetonitrile in HPLC grade deionized water with 0.1% (v/v) trifluoroacetic acid, circulated at a constant flow rate of 0.5 ml/minute, at room temperature (24-26 °C). Eluting proteins were detected at a wavelength of 214 nm.

Data analysis and integration of the areas under the chromatographic peaks were performed automatically by the software running the system. Chromatograms were divided into three main peaks, I, II, and III corresponding to the polymeric protein -P- (mostly glutenin), the monomeric proteins - M- (mostly gliadin) and the non-gluten soluble proteins - NG-(albumins + globulins), respectively, in decreasing order of molecular size (Singh et al., 1990 a; 1990 b; Batey et al., 1991). The parameters determined directly from the chromatograms were the areas under the three main peaks (APF, AMF, ANGF), the total area under the chromatogram (ATOT), and the area corresponding to the SDS-insoluble polymeric protein peak (AINSF). All these parameters are expressed in absorbance units per minute per milligram of flour. Computed parameters included the proportion of total protein represented by each of peak I, II, and the peak corresponding to the SDS-insoluble polymeric fraction designated respectively by %PTP, %MTP, %INSTP. The percent protein in the flour from each of these fractions, designated by %PF, %MF and %INSF, was calculated by multiplying the proportion in total protein by the corresponding flour protein content.

III.11.2. Preliminary validation experiments

III.11.2.1. Estimation of the loss of protein due to filtration

Protein contents in extracts from cultivar WPB 881, before and after filtration, were determined by the bicinchonic acid protein quantitation assay (Smith et al., 1985) using a kit from Pierce, according to the manufacturer's instructions. Determinations were made in triplicate.

III.11.2.2. Estimation of protein recovery

Ten flour samples were selected to represent a wide range of protein content, gluten strength and glutenin composition. Total protein content (percent nitrogen x 5.70) of these samples was determined in triplicate on 0.2 g sub-samples by the Dumas combustion method using a LECO nitrogen analyzer as described above. For each flour sample, residues (pellets) from 15 extractions (each resulting from extracting 20 mg of flour in 1 ml of extraction buffer) were oven-dried, bulked and pulverized to yield approximately 0.2 g of dry material. The procedure was conducted for two sonication treatments corresponding to a duration of either 15 or 30 seconds, aiming at optimizing the sonication time for maximum protein extraction. The protein content (percent nitrogen x 5.70) of the bulked residues was determined as for the flour samples. The amount of protein recovered upon extraction was calculated by subtracting the protein left in the residue from the flour's total protein content, on a dry weight basis.

III.11.2.3. Confirmation of chromatographic peaks identity

Peak composition was investigated by manually collecting the fractions corresponding to peak 1, 2 , and 3, eluting from 8.4 to 13.0, 15.0 to 21.0, and 23.0 to 27.0 minutes, respectively. Duplicate fractions from protein extracts from six durum wheat and two bread wheat flours were dried under vacuum in a SPEEDVAC centrifugal evaporator. Proteins were re-suspended in 100 µl of sample buffer (0.063 M tris-Cl (pH 6.80), 2 % (w/v) SDS, 20 % (v/v) glycerol, and 0.02 % (w/v) bromophenol blue) and incubated at 65 °C for 30 minutes. For one of the replicates, proteins were reduced by adding 2-β-mercaptoethanol to a final concentration of 5% (v/v) prior to incubation. Both reduced and unreduced protein fractions were analyzed by SDS-PAGE as described above, except that 20 µl were loaded onto the gel.

III.12. Statistical Analysis

All the statistical analysis were performed using the SAS computer software (The SAS Institute, Cary, NC). Analysis of variance for all the traits was performed using first data from both years taken together and then for each year, separately.

Three sets of comparisons were made. First, durum wheats as a group, were compared to the bread wheat checks. Second, durum wheats expressing allele LMWG-1 at the low molecular weight glutenin locus Glu-B3 were compared to durum wheats expressing the alternate allele, LMWG-2. Third, within the group of durum wheats expressing LMWG-2 (the most common in the modern durum germplasm), a three way comparison was made between lines carrying alleles HMWG-(6+8), HMWG-(7+8) and HMWG-(20) at the high molecular weight glutenin locus Glu-B1. The significance of the differences between group means was tested using a Student t-test.

Possible association between traits within the set of durum wheat cultivars/lines were investigated by computing Pearson's simple correlation coefficients for means from each year, separately.

IV. Experimental Results

The bread-making potential of durum wheat was investigated by evaluating potentially relevant quality parameters in thirty durum and five common wheat genotypes grown in a duplicated Randomized Complete Block Design experiment conducted at the "Rugg's" farm, near Pendleton-Oregon, during the springs of 1993 and 1994.

For each measured or computed parameter, three sets of comparisons were made. First, durum genotypes were compared to the five common wheats. Second, within the durum set, genotypes carrying the LMWG-1 allele at the Low Molecular Weight Glutenin locus Glu-B3 were compared to those carrying the contrasting allele, LMWG-2. Finally, among genotypes from the latter group, a three way comparison was made between genotypes expressing alleles HMWG-(6+8), HMWG-(7+8) and HMWG-(20), at the High Molecular Weight Glutenin locus Glu-B1.

Group means, ranges, analysis of variance and simple correlation coefficients between selected traits are presented in the following sections. First, results pertaining to grain characteristics and milling performance are presented. Second results describing the mixing properties of the flours tested are summarized. A third section summarizes the results from testing the dough physical characteristics. Then, results for parameters directly related to baking performance are presented. Finally, data from SE-HPLC analysis of flour gluten proteins, evaluating the gluten's polymeric composition, is summarized.

IV.1. Allelic Composition at two Glutenin Loci

Electrophoretic analysis of total flour protein extracts and purified glutenin extracts resulted in the unambiguous characterization of the genotypes evaluated in this study in terms of their allelic composition at the two glutenin loci always expressed (non-

null alleles) in durum wheats. These are the Glu-B1 locus, located on the long arm of chromosome 1B and coding for the High Molecular Weight Glutenin (HMWG) sub-units, and the Glu-B3 locus, mapping to the short arm of the same chromosome and encoding the Low Molecular Weight Glutenin (LMWG) sub-units. All of the durum genotypes tested carried the null allele on Glu-A1 (chromosome 1A). Twenty seven genotypes expressed the LMWG-2 allele at Glu-B3. Among this group, eleven genotypes carried allele HMWG-(6+8), nine were characterized by the presence of HMWG-(7+8) and the remaining seven genotypes expressed allele HMWG-20. Three cultivars expressed allele LMWG-1, two of them also carried HMWG-(7+8), and one expressed HMWG-(6+8) at Glu-B1. Common wheat genotypes were also analyzed, but the effect of their allelic composition on bread-making quality was not investigated in this study. The allelic composition at glutenin loci of all the genotypes included in the present study is shown in Table 1. A photograph from an SDS-PAGE separation of total protein and purified glutenin extracts from genotypes representing the different combinations of HMW and LMW glutenin alleles evaluated is shown in Figure 1.

IV.2. Grain characteristics and milling parameters

Grain characteristics such as test weight, weight of one thousand kernels, grain protein content and hardness, are believed to influence milling performance. Significant differences between lines were observed for all four traits, as evidenced by the highly significant mean squares for genotypes, shown in Table 2. Combined analysis of variance for both years revealed a significant difference between years for all four traits as well as highly significant genotype by year interactions. Both common and durum wheats genotypes were characterized by a slightly lower kernel weight and higher protein content in 1994 as compared to 1993. The opposite was true for test weight which was greater for both wheat groups in 1993. As seen in Table 3, durum wheat genotypes did not differ from common wheats in their test weights, in both years.

Table 1: Origin and allelic composition at different glutenin loci for 30 durum and five common wheat genotypes included in the present study.

		LMWG subunits	*HMWG subunits*		
GENOTYPE	**Origin**	**Glu-B3**	**Glu-B1**	**Glu-A1**	**Glu-D1**
Durum Wheats:					
Creso	Italy	2	6+8	null	n/a
D86741	North-Dakota	2	6+8	null	n/a
D88450	North-Dakota	2	6+8	null	n/a
Lloyd	North-Dakota	2	6+8	null	n/a
Mondur	France	2	6+8	null	n/a
Monroe	North-Dakota	2	6+8	null	n/a
Quilafen	Chile	2	6+8	null	n/a
Renville	North-Dakota	2	6+8	null	n/a
Valdur	France	2	6+8	null	n/a
Vic	North-Dakota	2	6+8	null	n/a
WPB 881	Arizona	2	6+8	null	n/a
Altar 84	Mexico	2	7+8	null	n/a
Chen'S'	Mexico	2	7+8	null	n/a
OR 4910045	Oregon	2	7+8	null	n/a
OR 918122	Oregon	2	7+8	null	n/a
Razzak	Tunisia	2	7+8	null	n/a
SULA//WLS/DWL.5023	Mexico	2	7+8	null	n/a
Valgerardo	Italy	2	7+8	null	n/a
Valnova	Italy	2	7+8	null	n/a
ZY1980	Mexico	2	7+8	null	n/a
Ambral	France	2	20	null	n/a
Brindur	France	2	20	null	n/a
Capelli	Italy	2	20	null	n/a
Carcomun'S'	Mexico	2	20	null	n/a
OR 4910060	Oregon	2	20	null	n/a
Valfiora	Italy	2	20	null	n/a
Valgiorgio	Italy	2	20	null	n/a
Ward	North-Dakota	1	6+8	null	n/a
Karim	Tunisia	1	7+8	null	n/a
Sham 1	Syria	1	7+8	null	n/a
Common Wheats:					
Klasic	California	n.d.	17+18	1	5+10
Florence-Aurore	Tunisia	n.d.	7+9	1	5+10
McKay	Idaho	n.d.	7+9	2*	5+10
Tanit	Tunisia	n.d.	17+18	2*	2+12
Byrsa	Tunisia	n.d.	7+9	2*	5+10

n.d.: Not determined *n/a: Not applicable*

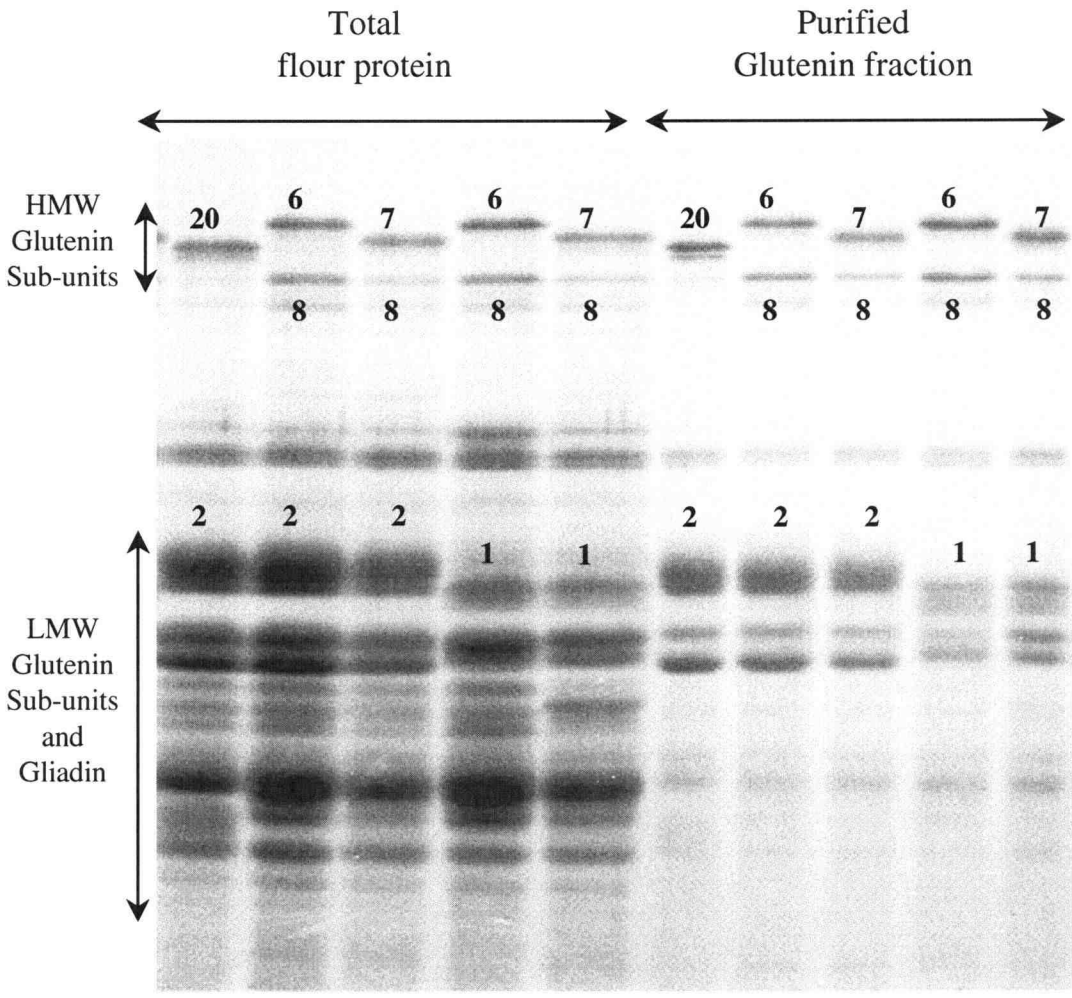

Figure 1: **SDS-PAGE separation of total flour protein and purified glutenin extracts, illustrating all the allelic combinations represented in the present study.**

Table 2: **Observed mean squares for Test Weight, Thousand Kernel Weight, Grain Protein Content and Hardness computed for 30 durum and five common wheat genotypes, grown in a duplicated randomized complete block design, at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean Squares			
Year	Source of Variation	df	Test Weight	Thousand Kernel Weight	Protein Content	Grain Hardness
1993	Genotype	34	483.2**	23.5**	2.18**	1022.9**
	Blocks	1	72.0*	6.9	0.05	182.4
	Error	34	17.2	2.8	0.15	53.2
	C.V. (%)		***0.55***	***4.20***	***3.38***	***6.40***
1994	Genotype	34	1029.2**	50.4**	2.35**	710.4**
	Blocks	1	145.7	16.2*	1.40	33.5
	Error	34	62.3	2.4	0.48	61.7
	C.V. (%)		***1.02***	***3.96***	***5.26***	***7.08***
Both	Genotype	34	1186.2**	64.4**	3.5**	1618.5**
Years	Year	1	25569.0**	27.0**	94.6**	304.9*
	Blocks(year)	2	108.9	11.6*	0.7	107.9
	Genotype*Year	34	326.2**	9.5**	1.0**	114.8**
	Error	68	39.8	2.58	0.3	57.4
	C.V. (%)		***0.83***	***4.08***	***4.55***	***6.74***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

Table 3: **Means and ranges for grain characteristics, including Test Weight, Thousand Kernel Weight, Grain Protein Content and Hardness computed for 30 durum and five common wheat genotypes grown at the Rugg's site, near Pendleton, Oregon in 1993 and 1994.**

Year	Wheat Group	Number of Lines	Test Weight (g/l)	Thousand Kernel Weight (g)	Protein Content (%, as is)	Grain Hardness (a.u.)
1993	Durum	30	**748** [a]	**40.1**[a]	**11.3** [a]	**122** [a]
			696-776	*33.0-52.0*	*9.6-13.5*	*91-149*
	Common	5	**752** [a]	**38.2** [a]	**13.0** [b]	**64** [b]
			728-770	*34.0-46.0*	*12.1-14.0*	*44-80*
1994	Durum	30	**774** [a]	**39.7** [a]	**13.05** [a]	**118** [a]
			732-817	*30.6-49.1*	*11.1-15.9*	*98-145*
	Common	5	**785** [a]	**34.5** [b]	**14.22** [b]	**71**[b]
			762-821	*29.3-41.9*	*12.7-15.6*	*47-94*
Both Years	Durum	30	**761** [a]	**39.9** [a]	**12.2** [a]	**120** [a]
			696-817	*30.6-52.0*	*9.6-15.9*	*91-149*
	Common	5	**768** [a]	**36.4** [b]	**13.6** [b]	**67** [b]
			728-821	*29.3-46.0*	*12.1-15.6*	*44-94*

Grain protein content and hardness were determined by NIR. Hardness is expressed in arbitrary units.
Means with the same letter are not significantly different at the 0.05 level.

Common wheats were characterized by a lower average kernel weight, in 1994, however. They had significantly higher grain protein content, as measured indirectly by NIR, than durum wheats, which, on the other hand, were characterized by a much harder grain texture. It is apparent, from the present set of lines, that common and durum wheats belong to different, non-overlapping, classes of kernel textures. The hardest common wheat tested was softer than the softest durum wheat.

The milling yields (percent of dry, clean wheat) ranged from 63.3 to 75.8% for common wheats and from 50.2 to 66.8 % for durum wheats. Table 4 shows that the latter group was characterized by a significantly lower flour yield than the former, in both years. However, the most striking difference between the two groups resided in how much of the total flour was break flour or reduction flour. Common wheats produced more break flour (34.1-45.5%) than reduction flour (22.5-34.7%). These percentages were percent by weight of clean dry wheat. In contrast, most of the flour produced by durum wheats was in the form of reduction flour (36.5-48.2 %), with break flour accounting only for 11.9 to 28.0 % of clean dry wheat. Genotypic differences for the three milling traits were highly significant in both years, as shown in Table 5. So were the effects of years and genotype by year interactions. However, no significant differences in terms of milling parameters were found among durum wheats, between genotypes grouped according to their allelic composition at the two glutenin loci (Table 4). An exception to this trend was that genotypes expressing HMWG-(6+8) at the Glu-B1 locus produced, on average, slightly more break flour than the other two groups.

Of the four grain characteristics measured on the thirty durum wheat genotypes, test weight was the only one that correlated in a substantial fashion with milling parameters, in particular with flour yield (Table 6). Its association with percent break flour was not consistent over years, and when significant, its magnitude was low. Thousand kernel weight was found to correlate substantially only to percent reduction flour. Grain protein content was negatively associated with flour yield, and percent break and reduction flour. However, this correlation was not consistent over years in the case of percent break or reduction flour.

Table 4: **Means and ranges for milling parameters, including Percent Break Flour, Percent Reduction Flour, and Total Flour Yield, computed for flour samples from 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

Groups		Percent Break Flour		Percent Reduction Flour		Total Flour Yield	
	N	1993	1994	1993	1994	1993	1994
Common		**41.1** [a]	**39.0** [a]	**26.5** [a]	**31.8** [a]	**67.6** [a]	**70.8** [a]
	5	*38.7-45.5*	*34.1-45.0*	*22.5-28.9*	*27.9-34.7*	*63.7-70.3*	*68-8-75.8*
Durum		**19.4** [b]	**17.0** [b]	**43.4** [b]	**44.0** [b]	**62.8** [b]	**61.0** [b]
	30	*13.1-28.0*	*11.9-22.8*	*36.5-47.7*	*39.3-48.2*	*50.2-66.8*	*53.9-66.6*
LMW-1		**18.4** [a]	**16.4** [a]	**44.7** [a]	**45.1** [a]	**63.1** [a]	**61.5** [a]
	3	*15.9-21.3*	*12.2-19.1*	*43.1-46.6*	*42.1-47.0*	*59.5-66.6*	*54.3-63.4*
LMW-2		**19.5** [a]	**17.1** [a]	**43.2** [a]	**43.8** [a]	**62.7** [a]	**60.9** [a]
	27	*13.1-28.0*	*11.9-22.8*	*36.5-47.7*	*39.3-48.2*	*50.2-66.8*	*53.9-66.6*
HMW 6+8		**20.9** [a]	**18.3** [a]	**42.7** [a]	**43.0** [a]	**63.5** [a]	**61.3** [a]
	11	*17.3-28.0*	*14.8-22.8*	*37.7-45.7*	*39.3-47.0*	*61.4-66.6*	*54.3-64.5*
HMW 7+8		**18.8** [b]	**16.4** [b]	**43.5** [a]	**44.4** [a]	**62.4** [a]	**60.8** [a]
	9	*13.1-25.6*	*11.9-22.8*	*36.5-47.7*	*40.2-48.2*	*50.2-66.8*	*53.9-64.7*
HMW 20		**18.2** [b]	**16.0** [b]	**43.8** [a]	**44.5** [a]	**62.0** [a]	**60.5** [a]
	7	*14.5-26.0*	*12.4-22.6*	*38.7-47.0*	*39.8-47.2*	*58.6-66.8*	*55.8-66.6*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level).
Comparisons are made separately for each year.

Table 5: **Observed mean squares for Percent Break Flour, Percent Reduction Flour, and Total Flour Yield, computed for 30 durum and five common wheat genotypes grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean Squares		
Year	Source of Variation	df	Break Flour	Reduction Flour	Flour Yield
1993	Genotype	34	133.4**	81.6**	18.1**
	Blocks	1	0.1	55.8**	50.6**
	Error	34	3.1	1.1	2.5
	C.V. (%)		***7.82***	***2.57***	***2.49***
1994	Genotype	34	134.7**	47.3**	35.9**
	Blocks	1	0.2	0.8	0.2
	Error	34	3.1	0.5	3.3
	C.V. (%)		***8.71***	***1.62***	***2.92***
Both Years	Genotype	34	262.8**	122.2**	43.4**
	Year	1	190.9**	55.8**	41.3**
	Blocks(year)	2	0.2	28.3**	25.4**
	Genotype*Year	34	5.3*	6.7**	10.6**
	Error	68	3.1	0.8	2.9
	C.V. (%)		***8.24***	***2.14***	***2.71***

**, ** : Significant at the 0.05, 0.01 probability levels, respectively.*

Table 6: **Phenotypic correlation coefficients between means of grain characteristics and milling parameters computed for 30 durum wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

	Year	Test Weight	Thousand Kernel Weight	Grain Protein Content	Grain Hardness	% Break Flour	% Reduction Flour
Test Weight							
Thousand Kernel Weight	*1993*	0.19 ^{ns}					
	1994	0.53**					
Grain Protein Content	*1993*	-0.58**	0.19 ^{ns}				
	1994	-0.79**	-0.34 ^{ns}				
Grain Hardness	*1993*	0.17 ^{ns}	0.41*	0.35 ^{ns}			
	1994	-0.16 ^{ns}	0.20 ^{ns}	0.49**			
% Break Flour	*1993*	0.37*	-0.21 ^{ns}	-0.61**	-0.33 ^{ns}		
	1994	0.05 ^{ns}	-0.43*	-0.23 ^{ns}	-0.49**		
% Reduction Flour	*1993*	0.39*	0.44*	0.08 ^{ns}	0.49**	-0.49**	
	1994	0.74**	0.77**	-0.46**	0.17 ^{ns}	-0.44*	
Flour Yield	*1993*	0.73**	0.15 ^{ns}	-0.59**	0.06 ^{ns}	0.67**	0.32 ^{ns}
	1994	0.71**	0.25 ^{ns}	-0.65**	-0.33 ^{ns}	0.59**	0.45*

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

^{ns}: *not significant.*

Grain hardness appeared to be associated only marginally with percent break (negatively) or reduction flour (positively), and not in a consistent fashion over years.

IV.3. Flour mixing properties

Mixing properties of the flours were determined using a mixograph, on 10 g samples (on a 14 % moisture basis). A sample of the variability in mixograms patterns observed in the present study is illustrated in Figure 2, which shows the traces obtained for two common and six durum wheat flours.

Genotypic differences for the most commonly used mixogram parameters were highly significant, in both years, as shown in Table 7. The difference between years was not significant for height at 7 minutes (H7M, an indication of the tolerance to over-mixing) but genotype by year interactions were. The opposite was true for time to peak (TTP) for which no significant genotype by year interactions were detected, in spite of significant differences between years. All the components of variation were significant for Peak Height (PHT).

Means and ranges for the different groups of genotypes compared, are presented in Table 8. In spite of the differences in mixograms pattern (Figure 2), the only numerical parameter to differentiate between common wheat and durum wheat genotypes was TTP. On average, more time was required for common wheat flours to reach maximum dough development than for durum flours.

Durum genotypes expressing LMWG-1 were characterized by shorter TTP and lower H7M than those carrying LMWG-2; They did not significantly differ in PHT, however. Among the latter group, genotypes expressing HMWG-(7+8) reached optimum dough development slightly later than those carrying either of the alternative alleles. However, the delay over the mean TTP of lines expressing HMWG-(6+8) was statistically significant only in 1994. The other two parameters did not differentiate further between the three groups.

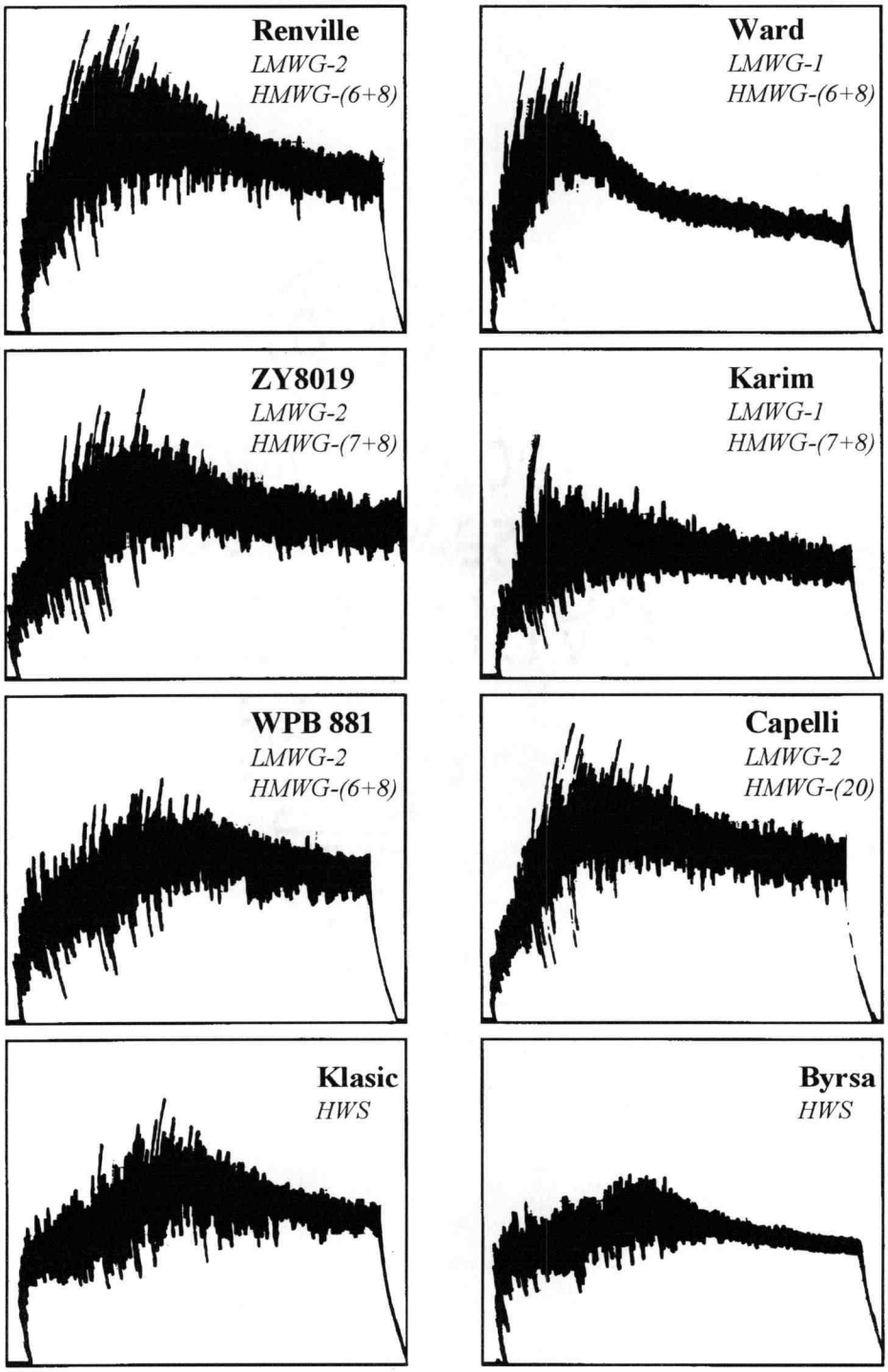

Figure 2: **Mixograms for six durum and two bread wheat (HWS) flours illustrating the variability in mixogram patterns observed in the present study.**

Table 7: **Observed mean squares for Time to Peak, Peak height and Height at 7 minutes, computed for 30 durum and five common wheat genotypes grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean Squares		
Year	Source of Variation	df	Time to Peak	Peak Height	Height at 7 minutes
1993	Genotype	34	0.76**	0.51**	0.40**
	Blocks	1	0.23	0.02	0.05
	Error	34	0.11	0.04	0.04
	C.V. (%)		***11.96***	***3.91***	***4.98***
1994	Genotype	34	0.57**	0.56**	0.32**
	Blocks	1	0.34	1.24**	0.43**
	Error	34	0.11	0.08	0.04
	C.V. (%)		***13.32***	***5.59***	***4.66***
Both Years	Genotype	34	1.18**	0.88**	0.62**
	Year	1	2.23**	0.35*	$0.7\ E^{-5}$
	Blocks(year)	2	0.29	0.63**	0.24**
	Genotype*Year	34	0.15	0.19**	0.1**
	Error	68	0.11	0.06	0.04
	C.V. (%)		***12.61***	***4.84***	***4.83***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

Table 8: **Means and ranges for selected mixogram parameters, including Time to Peak, Peak Height, and Height at 7 minutes, computed for flour samples from 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

Groups		Time to Peak (minutes)		Peak Height (cm)		Height at 7 min. (cm)	
	N	**1993**	**1994**	**1993**	**1994**	**1993**	**1994**
Common		**3.3** [a]	**3.1** [a]	**5.3** [a]	**5.1** [a]	**4.0** [a]	**4.3** [a]
	5	*2.5-4.0*	*1.6-4.0*	*4.8-5.9*	*4.5-5.6*	*3.2-4.5*	*3.7-4.5*
Durum Wheats		**2.7** [b]	**2.4** [b]	**5.0** [a]	**5.1** [a]	**4.2** [a]	**4.1** [a]
	30	*1.5-4.5*	*1.5-3.5*	*3.9-6.4*	*3.6-6.2*	*3.0-5.1*	*3.1-5.0*
LMW-1		**2.1** [a]	**1.9** [a]	**4.9** [a]	**4.8** [a]	**3.6** [a]	**3.4** [a]
	3	*2.0-2.5*	*1.6-2.6*	*4.5-5.2*	*4.1-5.7*	*3.0-4.1*	*3.2-3.6*
LMW-2		**2.7** [b]	**2.5** [b]	**5.0** [a]	**5.1** [a]	**4.2** [b]	**4.2** [b]
	27	*1.5-4.5*	*1.5-3.5*	*3.9-6.4*	*3.6-6.2*	*3.1-5.1*	*3.1-5.0*
HMW 6+8		**2.8** [a]	**2.4** [a]	**4.8** [a]	**5.1** [a]	**4.2** [a]	**4.2** [a]
	11	*2.0-4.1*	*1.5-3.1*	*4.0-5.7*	*4.4-6.0*	*3.1-5.0*	*3.6-4.8*
HMW 7+8		**3.0** [a]	**2.8** [b]	**5.0** [a]	**4.9** [a]	**4.3** [a]	**4.2** [a]
	9	*2.0-4.5*	*1.9-3.5*	*3.9-6.4*	*3.6-6.0*	*3.8-5.1*	*3.1-5.0*
HMW 20		**2.2** [b]	**2.1** [a]	**5.1** [a]	**5.5** [b]	**4.2** [a]	**4.3** [a]
	7	*1.5-2.7*	*1.6-2.5*	*4.6-6.0*	*4.8-6.2*	*3.8-5.0*	*4.0-4.8*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level).
Comparisons are made separately for each year.

It appeared, however, that genotypes expressing HMWG-20 tend to have, on average, a slightly higher peak height than those of the two other groups. This difference was significant only in 1994.

IV.4. Dough physical characteristics

The physical characteristics of doughs produced by the genotypes evaluated were studied using a Chopin Alveograph. Results from such an analysis can be used to make inferences on the rheological properties of flour doughs. The parameters measured from the alveograms include an estimator of dough tenacity (P), a measure of its extensibility (L), and an estimation of the energy required to inflate a bubble in the dough piece until rupture, or deformation energy (W). An additional parameter, the configuration ratio (P/L) was computed and considered an indicator of how balanced the rheological characteristics of the dough were. A sample of the variability in alveographic patterns observed in the present study is illustrated in Figure 3, which shows the traces obtained for two common and six durum wheat flours.

Highly significant genotypic differences were detected for all alveographic parameters in both years, as shown in Table 9. Combined analysis of variance for the two years indicates highly significant differences between years for all parameters. Genotype by year interactions were also highly significant. However, as shown in Table 10, means of the different groups compared were generally similar in both years.

Durum wheat doughs were generally more tenacious than common wheat doughs as indicated by a significantly higher P in 1993. The difference was not significant in 1994. However, even in 1994, the range was broader and extended much further towards high tenacity in the case of durum wheats. In contrast, bread wheat doughs were significantly more extensible than durum wheat dough (approximately 30% lower L, on average). These differences resulted in a significantly greater configuration ratio for durum wheat genotypes.

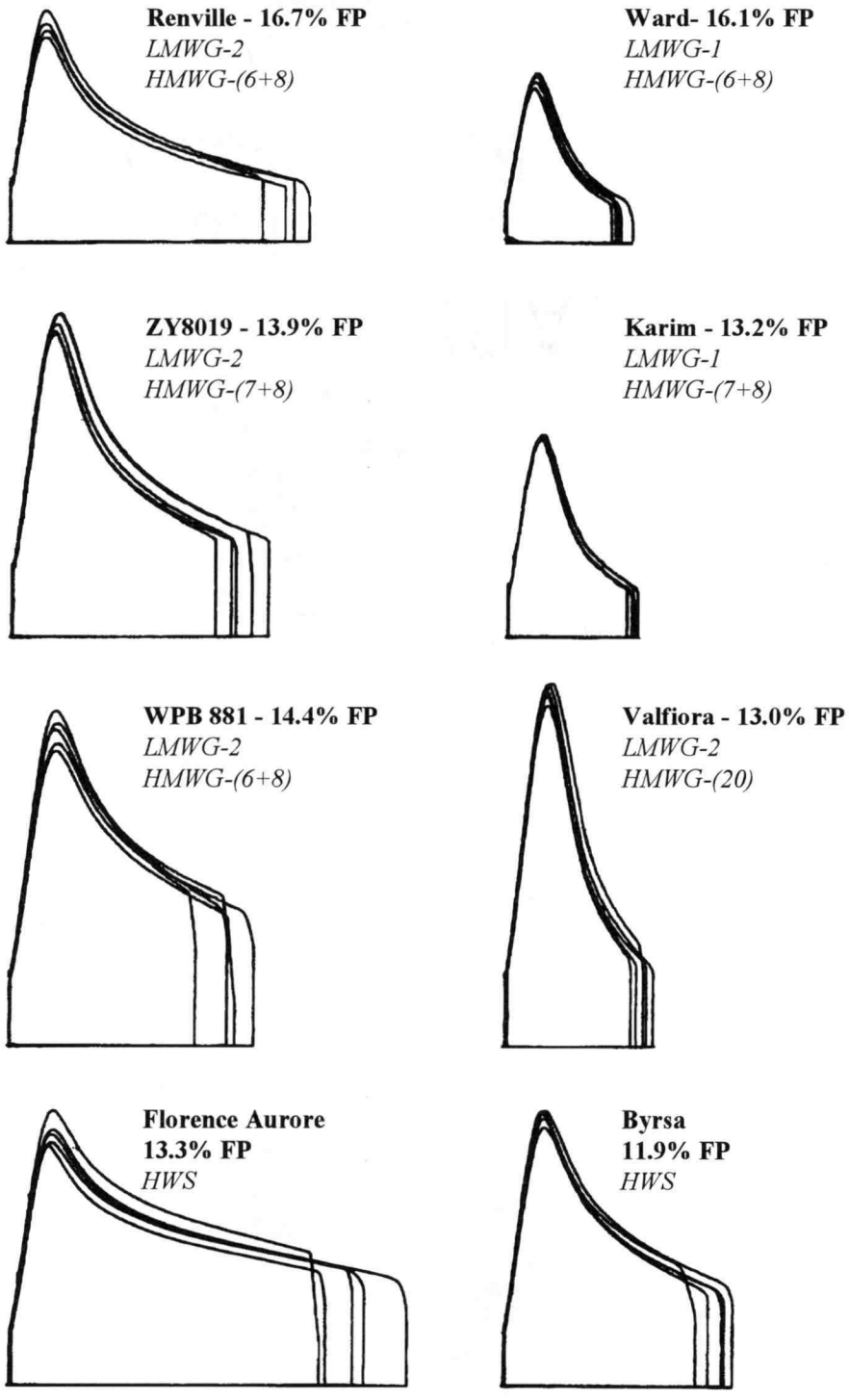

Figure 3: **Alveographic traces for six durum and two bread wheat (HWS) flours illustrating the variability in alveogram patterns observed in the present study.**

Table 9: **Observed mean squares for, Tenacity, Extensibility, Configuration ratio and Deformation Energy, computed for 30 durum and five common wheat genotypes grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean Squares			
Year	**Source of Variation**	**df**	**Tenacity P**	**Extensibility L**	**Configuration Ratio P/L**	**Deformation Energy W**
1993	Genotype	34	1650.1**	623.5**	1.30**	11453.4**
	Blocks	1	372.6	2.8	0.29	1355.2
	Error	34	102.9	31.8	0.08	434.9
	C.V. (%)		***10.20***	***8.81***	***15.60***	***10.00***
1994	Genotype	34	1355.1**	628.6**	1.05**	9447.2**
	Blocks	1	21.5	147.2*	0.24	246.7
	Error	34	35.5	26.9	0.04	397.7
	C.V. (%)		***6.75***	***7.85***	***12.92***	***10.46***
Both Years	Genotype	34	2786.9**	1186.1**	2.19**	19688.1**
	Year	1	4418.4**	146.9*	2.25**	11394.1**
	Blocks(year)	2	107.5	54.6	$1.1E^{-3}$	1379.1
	Genotype*Year	34	218.3**	65.9**	0.17**	1212.5**
	Error	68	72.4	30.3	0.06	413.5
	C.V. (%)		***9.06***	***8.46***	***15.48***	***10.19***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

Table 10: **Means and ranges for alveograph parameters, including Tenacity, Extensibility, Configuration Ratio, and Deformation Energy, computed for flours from 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

Groups		Tenacity P (mm)		Extensibility L (mm)		Configuration Ratio P/L		Deformation Energy W (10^{-4} Joules)	
	N	1993	1994	1993	1994	1993	1994	1993	1994
Bread Wheats		**81.9** [a]	**82.8** [a]	**91.0** [a]	**91.6** [a]	**0.9** [a]	**0.9** [a]	**271.9** [a]	**279.2** [a]
	5	*61-99*	*68-97*	*57-107*	*65-109*	*0.6-1.6*	*0.7-1.5*	*195-398*	*235-380*
Durum Wheats		**102.4** [b]	**89.1** [a]	**59.6** [b]	**61.9** [b]	**1.9** [b]	**1.6** [b]	**198.1** [b]	**175.8** [b]
	30	*55-161*	*44-156*	*35-92*	*33-92*	*0.7-4.0*	*0.7-3.9*	*70-366*	*58-317*
LMW-1		**82.4** [a]	**60.9** [a]	**40.6** [a]	**40.1** [a]	**2.2** [a]	**1.5** [a]	**111.3** [a]	**81.4** [a]
	3	*57-119*	*47-70*	*34-48*	*34-52*	*1.4-3.4*	*1.2-1.9*	*70-160*	*58-95*
LMW-2		**104.6** [a]	**92.3** [b]	**61.7** [b]	**64.3** [b]	**1.8** [a]	**1.6** [a]	**207.7** [b]	**186.3** [b]
	27	*55-161*	*44-156*	*36-92*	*33-92*	*0.7-4.0*	*0.7-3.9*	*100-366*	*64-317*
HMW 6+8		**88.5** [a]	**78.2** [a]	**69.2** [a]	**72.4** [a]	**1.4** [a]	**1.1** [a]	**193.3** [a]	**173.7** [a]
	11	*55-147*	*55-125*	*43-92*	*47-92*	*0.8-2.9*	*0.7-2.6*	*100-366*	*115-278*
HMW 7+8		**123.0** [b]	**106.7** [b]	**55.8** [b]	**58.0** [b]	**2.3** [b]	**2.0** [b]	**244.1** [b]	**214.0** [b]
	9	*66-160*	*44-156*	*36-71*	*33-76*	*1.0-4.0*	*0.7-3.9*	*109-345*	*64-317*
HMW 20		**106.3** [b]	**95.9** [b]	**57.4** [b]	**59.6** [b]	**2.0** [b]	**1.7** [b]	**183.6** [a]	**170.6** [a]
	7	*58-161*	*59-123*	*47-91*	*34-78*	*0.7-3.1*	*0.8-3.5*	*108-314*	*108-243*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level). Comparisons are made separately for each year.

The differences in configuration ratio approached a twofold magnitude between the means of the two groups. Whereas the maximum configuration ratio recorded for bread wheats was 1.6, some durum wheats had a configuration ratio of 4.0. In spite of a high tenacity component, durum wheat doughs were characterized by a significantly lower deformation energy, indicating the development of an overall "weaker" gluten.

Durum wheat genotypes expressing LMWG-2 were characterized by more tenacious, yet significantly more extensible doughs than those expressing LMWG-1. This translated into a significantly greater deformation energy for the former group, indicating the development of a relatively "stronger" gluten. This difference is clearly illustrated in Figure 3 with two examples of flours with similar protein contents and the same allelic composition at Glub-B1, but having contrasting alleles at Glu-B3. Cultivar Renville is compared to Ward, and ZY8019 is contrasted with Karim. However, differences in configuration ratio were not consistent over years and were not statistically significant.

Also illustrated in Table 10 is the relative superiority of genotypes expressing HMWG-(6+8) over those expressing either alternative alleles at the same locus, in terms of the rheological balance of the dough. In fact, genotypes expressing HMWG-(6+8) produced doughs that were, on average, significantly less tenacious and more extensible. This resulted in a configuration ratio approaching that of the bread wheat doughs tested in this study, indicating a better balance in the dough rheological properties of this group of durum wheats. No significant differences were apparent between the two other HMWG groups. However, genotypes expressing HMWG-(7+8) were characterized by a significantly higher W than genotypes belonging to the other two groups. This apparent higher gluten strength seems, therefore, to result from a higher tenacity component rather than from a higher dough extensibility. This is confirmed by the high configuration ratio characterizing this group of lines.

IV.5. Flour protein content, gluten strength and baking performance

Bread-making quality was ultimately determined by performing an optimized bake test and measuring the volume of the resulting loaves. Results for other important baking parameters are also described below, as well as those for flour protein content and gluten strength, as measured by the SDS-micro-sedimentation test. Genotypic differences were highly significant for all traits in both years, as shown in Table 11. The differences between years were also significant for all traits. Genotype by year interactions were not significant for bake water absorption or bake mixing time. The growing conditions prevailing in 1994 resulted in flours with higher protein content, greater water absorption, slightly shorter mixing times and a usually greater average loaf volume, except in the case of bread wheats. Monthly average minimum and maximum temperatures were similar during both seasons except during the month of July which includes most of the grain filling period. The average maximum temperature in July was substantially higher in 1994. Also, the number of days with maximum temperatures greater than 35°C was much greater in 1994 (22 days) as compared to 1993 (4 days). Finally, total rainfall was much lower in 1994 than in 1993. A two-fold difference was observed between the June-July (from flowering to grain maturation) rainfalls in both years.

As seen in Tables 12 and 13, bread wheats developed a stronger gluten than durum wheats as shown by their almost two-fold higher SDS-sedimentation height, in both years. Common wheat flours also required, on average, less water for optimum dough development as shown by a significantly lower bake water absorption, compared to that of durum wheat flours. With a few exceptions, durum flours reached optimum development slightly faster than common wheat flours. However, the difference was not statistically significant. A major difference in average loaf volume was observed between the two flour types. In spite of a significantly lower average flour protein content, bread wheat flours produced loaves that were, on average, 30% larger than those produced by durum wheat flours. However, a considerable variability in loaf volume was observed among the durum genotypes, as shown by the wide range recorded for this trait, in both years.

Table 11: **Observed mean squares for Flour Protein, Sedimentation height, Bake Water Absorption, Bake Mixing Time, Loaf Volume and Crumb Score, computed for 30 durum and five common wheat genotypes, grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean Squares					
Year	Source of Variation	df	Flour Protein	Sedimentation Height	Water Absorption	Mixing Time	Loaf Volume	Crumb Score
1993	Genotype	34	1.76**	5.50**	13.30**	1.81**	59633.2**	6.39**
	Blocks	1	0.06	0.25**	0.01	0.42	515.7	0.36
	Error	34	0.24	0.03	2.16	0.26	1383.4	0.45
	C.V. (%)		***3.91***	***4.30***	***2.23***	***13.94***	***5.79***	***8.39***
1994	Genotype	34	3.36**	3.27**	14.86**	1.13**	53803.7**	9.49**
	Blocks	1	0.80	0.03	35.00**	0.87*	7000.0*	0.23
	Error	34	0.46	0.08	1.53	0.17	1400.0	0.79
	C.V. (%)		***5.05***	***7.43***	***1.82***	***11.95***	***5.42***	***12.57***
Both Years	Genotype	34	3.69**	8.45**	25.92**	2.69**	110215.8**	14.64**
	Year	1	28.89**	1.19**	151.63**	2.02**	79682.9**	28.35**
	Blocks(year)	2	0.43	0.14	17.50**	0.64	3757.9	0.29
	Genotype*Year	34	1.43**	0.32**	2.24	0.24	3221.1**	1.25**
	Error	68	0.35	0.06	1.85	0.21	1391.7	0.62
	C.V. (%)		***4.56***	***6.00***	***2.03***	***13.06***	***5.60***	***10.46***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

Table 12: Means and ranges for Flour Protein Content, Sedimentation Height, computed for 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci wheat genotypes) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.

Groups	*N*	Percent Flour Protein (on 14% m.b.) 1993	Percent Flour Protein (on 14% m.b.) 1994	Sedimentation Height (cm) 1993	Sedimentation Height (cm) 1994
Common		**11.9** [a]	**12.4** [a]	**7.5** [a]	**6.4** [a]
	5	*10.6-12.9*	*11.5-13.4*	*5.5-8.5*	*4.4-8.4*
Durum		**12.5** [b]	**13.5** [b]	**3.5** [b]	**3.5** [b]
	30	*10.9-15.6*	*11.0-16.8*	*2.0-5.4*	*1.9-4.7*
LMW-1		**12.8** [a]	**13.5** [a]	**2.15** [a]	**2.3** [a]
	3	*12.0-13.9*	*11.6-16.2*	*2.0-2.4*	*1.9-2.6*
LMW-2		**12.5** [a]	**13.5** [a]	**3.7** [b]	**3.6** [b]
	27	*10.9-15.6*	*11.0-16.8*	*2.2-5.4*	*2.2-4.7*
HMW 6+8		**12.4** [a]	**14.1** [a]	**4.1** [a]	**3.8** [a]
	11	*10.9-14.7*	*12.2-16.8*	*3.0-5.4*	*2.7-4.7*
HMW 7+8		**12.7** [a]	**13.1** [b]	**3.9** [a]	**3.7** [ab]
	9	*11.0-15.6*	*11.0-15.1*	*2.5-4.7*	*2.2-4.2*
HMW 20		**12.5** [a]	**13.2** [b]	**2.8** [b]	**3.3** [b]
	7	*10.9-13.8*	*11.4-15.0*	*2.2-4.0*	*2.6-4.2*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level).
Comparisons are made separately for each year.

Table 13: **Means and ranges for baking quality parameters, including Water Absorption, Mixing Time, Loaf Volume and Crumb Score, computed for 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci wheat genotypes) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

Groups		Bake Water Absorption (ml)		Bake Mixing Time (minutes)		Loaf Volume (cc)		Bread Crumb Score (1-9 scale)	
	N	1993	1994	1993	1994	1993	1994	1993	1994
Common		**63.9** [a]	**65.4** [a]	**4.1** [a]	**3.9** [a]	**968** [a]	**965** [a]	**4.2** [a]	**3.4** [a]
	5	*60-68*	*63-70*	*2.8-6.0*	*2.8-5.1*	*800-1100*	*825-1110*	*2-8*	*2-6*
Durum		**66.3** [b]	**68.4** [b]	**3.6** [a]	**3.3** [a]	**589** [b]	**645** [b]	**8.6** [b]	**7.7** [b]
	30	*59-71*	*61-73*	*2.1-8.6*	*2.0-6.4*	*390-800*	*365-935*	*6-9*	*4-9*
LMW-1		**61.3** [a]	**63.6** [a]	**2.7** [a]	**2.6** [a]	**460** [a]	**475** [a]	**9.0**	**9.0**
	3	*59-64*	*61-66*	*2.1-3.3*	*2.0-3.3*	*390-520*	*365-610*	*9-9*	*9-9*
LMW-2		**66.8** [b]	**69.0** [b]	**3.7** [b]	**3.4** [b]	**603** [b]	**664** [b]	**8.5**	**7.5**
	27	*63-71*	*63-73*	*2.3-8.6*	*2.2-6.4*	*390-800*	*405-935*	*6-9*	*4-9*
HMW 6+8		**66.9** [a]	**68.5** [a]	**3.5** [a]	**3.3** [a]	**672** [a]	**740** [a]	**8.3** [a]	**6.6** [a]
	11	*64-70*	*63-72*	*2.8-4.7*	*2.4-5.4*	*535-800*	*565-935*	*6-9*	*4-9*
HMW 7+8		**67.1** [a]	**69.2** [a]	**4.4** [b]	**3.9** [b]	**589** [b]	**619** [b]	**8.6** [a]	**7.8** [b]
	9	*63-71*	*63-73*	*2.3-8.6*	*2.2-6.4*	*390-800*	*405-810*	*6-9*	*4-9*
HMW 20		**66.4** [a]	**69.4** [a]	**3.1** [a]	**3.0** [a]	**513** [c]	**601** [b]	**8.9** [a]	**8.6** [b]
	7	*63-71*	*65-73*	*2.4-3.9*	*2.3-3.6*	*405-660*	*490-730*	*7-9*	*6-9*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level). Comparisons are made separately for each year.

This is also illustrated in Figure 4, which shows loaf mid-sections from five durum (bottom) and five bread (top) wheat genotypes. Some durum flours produced loaves that approached or even, as in 1994, exceeded those made by the poorest common wheat flours. This was the case for flours from durum wheat cultivars W.P.B. 881 and Renville, as shown in Table 14. Cultivar W.P.B. 881 performed the best overall, followed by Vic.

The crumb structure for loaves baked from durum wheat flours was generally unsatisfactory, as indicated by the high (closer to 9) crumb score. Crumb cell walls usually collapsed during baking and oven-spring preventing the production of a high-volume loaf with an optimum crumb structure.

Among the durum wheats, genotypes expressing LMWG-1 performed the poorest. Flours from these genotypes were characterized by a significantly lower water absorption, and reached their optimum development sooner than those from genotypes expressing allele LMWG-2. The weaker gluten network developed by genotypes carrying LMWG-1, as shown by their significantly lower sedimentation height, resulted in the production of the smallest loaves, with the worst structure. It is important to note that no differences were observed in protein content between the two groups.

Among the twenty seven durum genotypes carrying LMWG-2, those also expressing HMWG-(6+8) produced larger loaves than genotypes expressing either of the alternative alleles at the same locus. This overall superiority was consistent over years. In fact, when genotypes were ranked according to their average loaf volume (mean of four observations) as in Table 14, it was apparent that, out of the eight best performing genotypes (greater than 700 cc), seven carried allele HMWG-(6+8). Genotypes carrying HMWG-(7+8) exhibited, on average, greater loaf volumes than those carrying HMWG-20.

Differences in average flour protein content between HMWG allelic groups were mostly not significant, except for genotypes expressing HMWG-(6+8), which were characterized by a significantly higher protein content than the other two groups, in 1994 only. The difference in sedimentation height between genotypes carrying HMWG-(6+8) and those expressing HMWG-(7+8) was not significant. However, these were found to develop, on average, a stronger gluten than lines expressing HMWG-20.

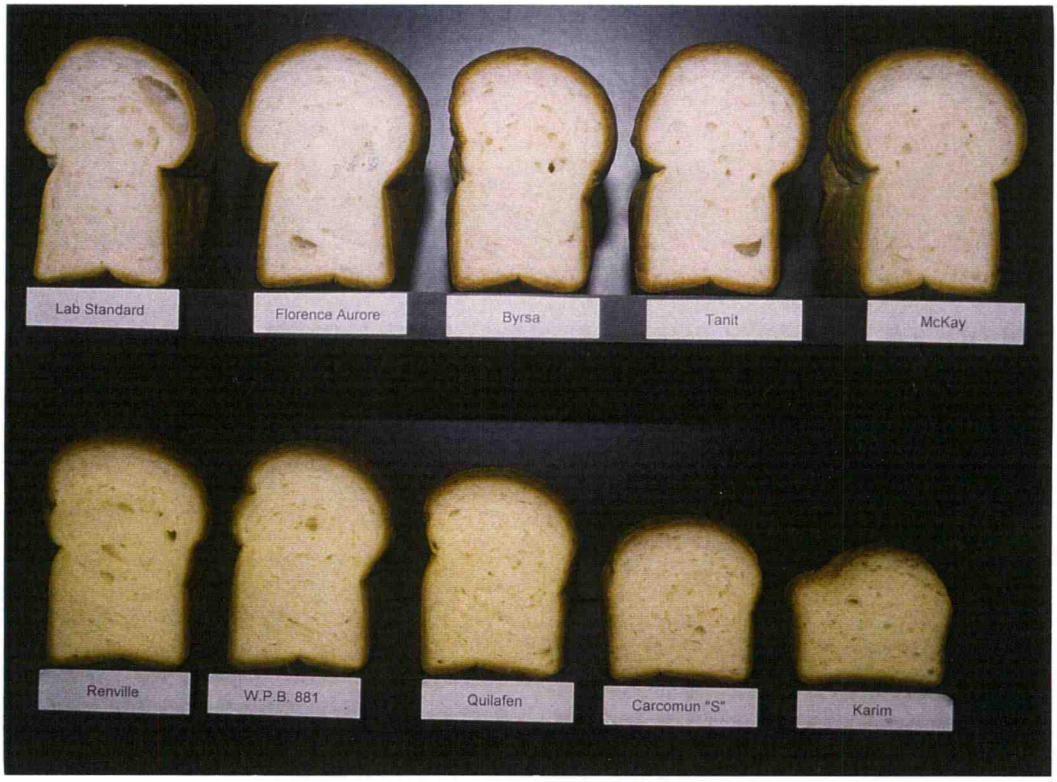

Figure 4: **Sample of the variability in loaf volume observed in four common (Top) and five durum (Bottom) wheats. At the top left, WWQL bread standard.**

Table 14: Flour protein content and loaf volume for 30 durum and five bread wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.

		Alleles at Glutenin locus		Flour protein content (%, 14 % m.b.)			Bread loaf volume (cc)		
Rank	Genotype	*Glu-B1*	*Glu-B3*	*1993*	*1994*	**Mean**	*1993*	*1994*	**Mean**
1	WPB 881	6+8	2	14.3	14.4	**14.4**	758	875	**816**
2	Vic	6+8	2	13.4	15.8	**14.6**	780	808	**794**
3	ZY1980	7+8	2	15.4	13.9	**14.7**	795	768	**781**
4	Renville	6+8	2	12.9	16.7	**14.8**	703	860	**781**
5	Mondur	6+8	2	11.7	12.9	**12.3**	733	755	**744**
6	Valdur	6+8	2	13.1	12.4	**12.8**	713	765	**739**
7	Monroe	6+8	2	12.5	15.3	**13.9**	690	768	**729**
8	Quilafen	6+8	2	12.1	13.6	**12.8**	680	745	**713**
9	OR 4910045	7+8	2	13.2	14.3	**13.7**	623	763	**693**
10	Valnova	7+8	2	13.0	14.6	**13.8**	668	698	**683**
11	Capelli	20	2	13.6	14.5	**14.1**	640	715	**678**
12	D86741	6+8	2	11.5	13.3	**12.4**	623	668	**645**
13	Altar 84	7+8	2	11.4	13.4	**12.4**	603	655	**629**
14	Brindur	20	2	12.4	14.5	**13.4**	613	643	**628**
15	Lloyd	6+8	2	11.9	13.8	**12.9**	608	635	**621**
16	Valgerardo	7+8	2	12.2	12.4	**12.3**	615	603	**609**
17	D88450	6+8	2	12.1	13.8	**12.9**	558	655	**606**
18	Ambral	20	2	11.8	13.0	**12.4**	518	680	**599**
19	Creso	6+8	2	11.1	12.5	**11.8**	545	608	**576**
20	OR 918122	7+8	2	12.3	12.5	**12.4**	573	563	**568**
21	SULA//WLS/DWL.5023	7+8	2	12.8	13.4	**13.1**	535	590	**563**
22	Valgiorgio	20	2	12.9	13.3	**13.1**	488	583	**535**
23	Ward	6+8	1	13.0	16.1	**14.6**	490	580	**535**
24	OR 4910060	20	2	11.6	12.0	**11.8**	450	545	**498**
25	Carcomun'S'	20	2	11.6	12.4	**12.0**	450	545	**498**
26	Sham 1	7+8	1	12.0	12.3	**12.1**	488	473	**480**
27	Razzak	7+8	2	12.4	11.3	**11.8**	498	450	**474**
28	Valfiora	20	2	13.4	13.0	**13.2**	433	495	**464**
29	Chen'S'	7+8	2	11.7	12.2	**12.0**	395	490	**443**
30	Karim	7+8	1	13.2	12.0	**12.6**	403	373	**388**
B-1	Klasic	n/a	n/a	12.4	13.1	**12.7**	1088	1075	**1081**
B-2	Florence-Aurore	n/a	n/a	12.7	13.3	**13.0**	963	1025	**994**
B-3	McKay	n/a	n/a	10.8	11.9	**11.3**	950	963	**956**
B-4	Tanit	n/a	n/a	11.8	12.1	**11.9**	970	908	**939**
B-5	Byrsa	n/a	n/a	11.7	11.9	**11.8**	870	853	**861**

Flours from genotypes carrying HMWG-(7+8) generally reached optimum dough development later than those from genotypes carrying the alternative HMWG alleles. No difference in average bake water absorption was apparent between the three groups.

IV.6. Correlation between selected quality traits

The association among the different quality traits measured by different tests was investigated by computing Pearson's correlation coefficients on the parameters means for the 30 durum wheat genotypes included in the present study. These coefficients are shown in Table 15. Loaf volume was significantly correlated with both protein quantity (total flour protein content) and protein quality (as estimated by the SDS-sedimentation test) in both years. Strong evidence for the independence of gluten strength from protein quantity is shown by the non-significant correlation between sedimentation height and protein content, in both years. Surprisingly, protein quantity was moderately correlated with the alveograph deformation energy W, which constitutes another measure of gluten strength. However, this correlation was significant in 1993 only and cannot be considered to be strong evidence for a consistent association between the two traits. Protein quantity seemed neither to be consistently, nor substantially associated with any of the other alveograph parameters. In contrast, the correlation between protein content and maximum dough resistance to mixing (as measured by mixogram peak height) was highly significant in both years. This was not the case for the other two mixogram parameters. Dough development time was positively associated with gluten strength as indicated by the highly significant correlation coefficient between sedimentation height and either bake mixing time, or mixogram time to peak. This association was consistent over years but its extent was moderate. Flours from genotypes with stronger gluten tended to have higher water absorption as shown by the consistent and significant correlation between bake water absorption and sedimentation height. The latter did not seem to be correlated with the alveograph parameters to a great extent, aside from W.

Table 15: **Phenotypic correlation coefficients between means of selected quality parameters and mean Flour Protein, Sedimentation Height and Loaf Volume computed for 30 durum wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

	Flour Protein		Sedimentation Height		Loaf Volume	
	1993	1994	1993	1994	1993	1994
Sedimentation Height	0.08 ^{ns}	0.07 ^{ns}			0.64**	0.64**
Loaf Volume	0.42*	0.67**	0.64**	0.64**		
Bake Mixing Time	-0.06 ^{ns}	-0.29 ^{ns}	0.56**	0.57**	-0.05 ^{ns}	0.04 ^{ns}
Bake Water Absorption	0.49**	0.28 ^{ns}	0.66**	0.66**	0.65**	0.54**
Mixogram Parameters						
Time to Peak	-0.00 ^{ns}	-0.27 ^{ns}	0.63**	0.50**	0.31 ^{ns}	0.02 ^{ns}
Peak Height	0.77**	0.68**	0.06 ^{ns}	0.12 ^{ns}	0.51**	0.54**
Height at 7 min.	0.56**	0.32 ^{ns}	0.58**	0.70**	0.54**	0.62**
Alveograph Parameters						
Tenacity	0.37*	-0.21 ^{ns}	0.40*	0.46*	-0.02 ^{ns}	-0.05 ^{ns}
Extensibility	0.17 ^{ns}	0.42*	0.36 ^{ns}	0.50**	0.80**	0.79**
P/L	0.12 ^{ns}	-0.38*	0.08 ^{ns}	0.06 ^{ns}	-0.49**	0.46*
Deformation Energy	0.57**	0.09 ^{ns}	0.65**	0.74**	0.47**	0.42*

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

^{ns}: *not significant.*

Both Sedimentation height and W are different measures of the same attribute, namely, gluten strength, and the correlation between them was expected. Another estimator of gluten strength would be the tolerance to over-mixing as measured by the height of the mixogram trace after 7 minutes of mixing. The latter was also associated with sedimentation height. All of the mixogram and alveogram parameters, with the exception of time to peak and dough tenacity, tended to show some moderate to weak association with loaf volume.

The most important result however, resides in the strong and consistent association between loaf volume and dough extensibility. Flours producing more extensible doughs tended to produce larger bread loaves. This relationship appeared to be stronger than that between loaf volume and gluten strength. As previously stated, the latter was not associated with dough extensibility.

IV.7. SE-HPLC analysis

IV.7.1. Preliminary validation experiments

The validity of SE-HPLC analysis to quantify the different protein fractions depends on two main factors. First, complete (or close to complete) extraction and solubilization of the gluten protein must be achieved. This was attempted by subjecting flour samples suspended in a phosphate buffer in the presence of 0.5 % SDS to ultra-sonic waves, which reduces the size of the gluten polymer enough to make it soluble while preserving its polymeric nature, thereby allowing the chromatographic quantification of such extracts upon size fractionation (Singh et al., 1990 a). Second, the polymeric protein fraction must be effectively separated from the monomeric fraction during chromatography. A preliminary experiment was undertaken to confirm the validity of these assumptions under the conditions of the present study. The protein recovered by

sonication of flour suspensions in extraction buffer was determined by subtracting the protein content of the extraction residue from that of the original flour. Both protein determinations were made using the same nitrogen analyzer, according to the Dumas combustion method. Results for ten flour samples, selected to represent the whole range of gluten strength and gluten protein composition occurring in this study, are shown in Table 16. The protein recovered by extraction ranged from 90.7 to 94.4% when suspensions were sonicated for 15 seconds, and from 92.8 to 96.4% when the sonication time was 30 seconds. Under the latter condition, an average of 94.8% of the flour protein was extracted. The percent protein extracted did not appear to be associated with gluten strength. Samples from the weak gluten cultivar like Ward had the second lowest protein recovery and relatively lower than WPB 881, the cultivar with the strongest gluten. Therefore, it can be assumed that even the most insoluble polymer (the largest) was extracted. Protein recovery observed in the present study was similar to those reported by Singh et al. (1990 b) and Lee et al. (1995). It is to be noted that Singh et al. (1990 b) used the bincinchonic acid protein determination assay (Smith et al., 1985) to quantify the protein in extracts. This spectrophotometric assay is known to be subject to protein-to-protein variability as it uses bovine serum albumin (BSA) as a reference to construct a standard curve which is used to infer the unknown concentration of an aqueous protein solution. In this experiment, this method resulted in a significant under-estimation of the protein content of gluten extracts (results not shown), probably due to the large compositional differences between the protein used as standard (BSA) and the wheat gluten proteins. It was felt that a more accurate estimation of the protein recovery could be obtained by subtracting nitrogen content of the extraction residues from that of the original flour, using the same determination method for both. The same approach was used by Pasaribu et al. (1992) with similar results. In summary, the assumption that the totality of the gluten protein was extracted under the conditions of the present study was validated.

Table 16: **Percent protein (% nitrogen x 5.70, on a dry weight basis) extracted in 0.05M sodium phosphate buffer (pH 6.95) containing 0.5% (w/v) SDS, using two sonication times, from 10 durum wheat flour samples.**

	Protein Content (% Nitrogen x 5.70, on a dry weight basis)				
		15 second sonication		30 seconds sonication	
Genotype	*In Flour* [a]	*In Residue* [b]	*Extracted* [c]	*In Residue* [b]	*Extracted* [c]
Altar 84	15.639	1.023	93.5	0.816	94.8
WPB 881	16.632	1.274	92.3	0.972	94.2
Karim	13.488	0.856	93.7	0.514	96.2
Vic	19.339	1.789	90.7	1.399	92.8
Ambral	14.369	0.806	94.4	0.551	96.2
AzMS 1	16.583	1.080	93.5	0.915	94.5
Valnova	17.453	1.506	91.4	1.002	94.3
Ward	18.495	1.657	91.0	1.237	93.3
Creso	14.111	0.900	93.6	0.703	95.0
Capelli	16.443	1.058	93.6	0.595	96.4
Average			**92.8** (90.7-94.4)		**94.8** (92.8-96.4)

[a]: *Average of three determinations, each on approximately 0.2 g. Of flour.*

[b]: *Based on one determination on approximately 0.2 g. of residue produced by 15 extractions (20 mg of flour each).*

[c]: *% protein extracted = [(% in flour - % in residue) / % in flour] x 100.*

Also, since protein extracts had to be filtered through a 0.45 μm filter to protect the column from any particulate matter that remained in suspension after clarification of the extracts, it was necessary to confirm that no loss of protein occurred during the filtration process. A PVDF filtration membrane (Durapore, from Millipore) was chosen for its low-protein binding properties. Confirmation of such properties was provided by the results of the second preliminary experiment designed to estimate the eventual loss of protein from extracts due to filtration prior to SE-HPLC fractionation. These results are shown in Table 17. The protein content determined, using the bicinchonic acid protein quantitation assay (Smith et al., 1985), before and after filtration on three different extracts from cultivar WPB 881, were not significantly different. In fact, the difference was not statistically different from zero indicating that the loss of protein due to filtration could be neglected.

The third preliminary experiment was designed to confirm the identity of the different SE-HPLC peaks considered to quantify the different protein fractions. Electrophoretic analysis of the eluting fractions corresponding to peaks I and II confirmed that these corresponded to the polymeric (mostly glutenin) and monomeric (mostly gliadin) fractions of the total gluten protein, respectively. These results are illustrated in Figure 5, showing the SDS-PAGE separation of unreduced (top) and reduced (bottom) protein fractions corresponding to each peak, for three durum wheat cultivars. The polymeric nature of the proteins corresponding to peak I is evidenced by the presence of a smear in the top of the gel under non-reducing conditions. This smear represents the unbroken gluten polymer (no reducing agent was included in the extraction media) which is too large to migrate within the pores of the polyacrylamide gel. When this fraction was reduced with β-mercaptoethanol, thereby breaking the disulfide bonds prior to SDS-PAGE, bands corresponding to the high and low molecular weight glutenin sub-units were apparent, without any indication of the presence of bands corresponding to gliadin polypeptides. Unreduced fractions corresponding to peak II entered the gel readily and migrated to a distance that was characteristic of unreduced gliadin, thereby demonstrating their monomeric nature. When reduced, this fraction yielded an SDS-PAGE pattern typical of the gliadin proteins.

Table 17: Estimation of the loss of protein from total flour protein extracts from durum wheat cultivar WPB 881 due to filtration thru a 0.45 μm PVFD membrane prior to SE-HPLC analysis.

	*Protein concentration in mg/ml**		
	Before Filtration	**After Filtration**	**Difference**
Extract 1	1.369	1.412	-0.043
Extract 2	1.422	1.409	0.013
Extract 3	1.462	1.361	0.101
Average	**1.418**	**1.394**	**0.024****

* : *Determined using the bicinchonic acid protein quantitation assay*

**: *Difference was not statistically different from 0. T= 0.56, and the probability of T<t was 0.63.*

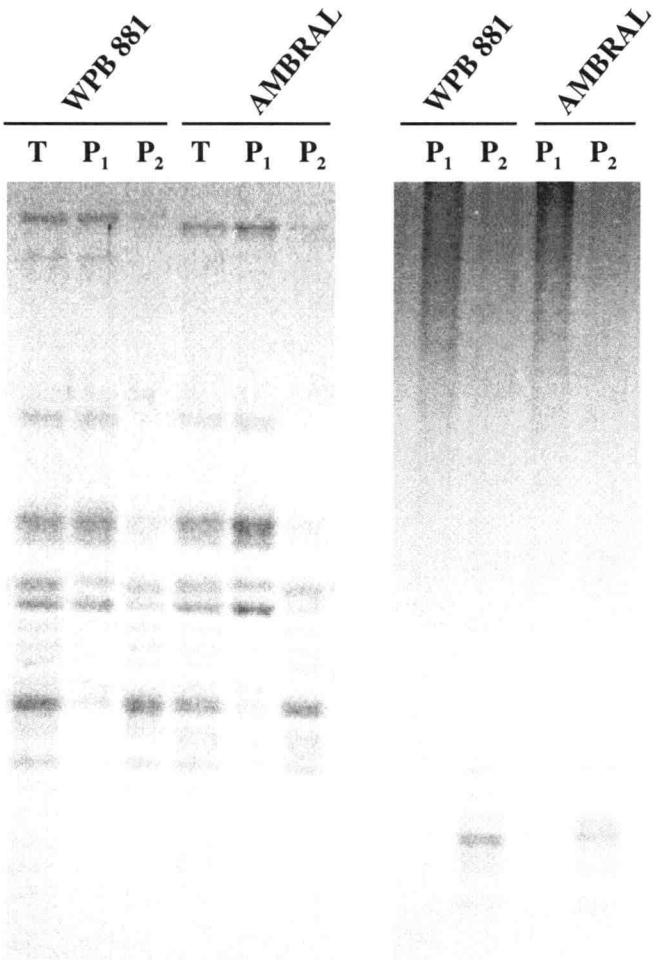

Figure 5: **SDS-PAGE analysis of protein fractions collected from peaks I (P_1) and II (P_2) of a SE-HPLC fractionation of total unreduced gluten protein extracts from two flour samples.**
Left: reduced Right: unreduced

Faint bands, corresponding to the HMW glutenin sub-units, were also barely visible in this fraction and could have originated from fraction overlap after the separation, during the fraction collection phase. Judging from the faint staining, their amount appeared to be minimal, however.

IV.7.2. SE-HPLC analysis: Results

SE-HPLC was used to investigate the differences in polymeric composition of the gluten protein produced by the different groups of genotypes tested. Three types of parameters were considered. First, the apparent amount of protein, expressed in absorbance units/minute/mg flour, corresponding to each SE-HPLC fraction, was considered. Then, the different fractions expressed in terms of percent of the total protein or percent of flour, were examined. Representative chromatograms from the SE-HPLC fractionation of total and SDS-insoluble gluten protein extracts from cultivar W.P.B. 881, illustrating the main peak areas upon which the results were based, are shown in Figure 6.

As shown in Tables 18, 19, and 20, genotypic differences were significant in both years for all the SE-HPLC parameters considered; Differences between years were also significant. Genotype by year interactions were highly significant for all the parameters, as well. Figure 7 illustrates a sample of the variability observed among chromatograms corresponding to the SE-HPLC fractionation of total and SDS-insoluble gluten protein extracts from four durum and two bread wheat genotypes.

As seen in Table 21, none of the parameters expressing amounts of the different protein fractions in the flour significantly and consistently differentiated between flours from bread wheats and those from durum wheats. The total area under the chromatograms (ATOT) used as reference to calculate all other parameters did not vary between the two groups. The total area under a chromatogram is an indirect measure of total protein content. The amount of SDS-insoluble protein was higher in bread wheat flours but the difference was significant only in 1993.

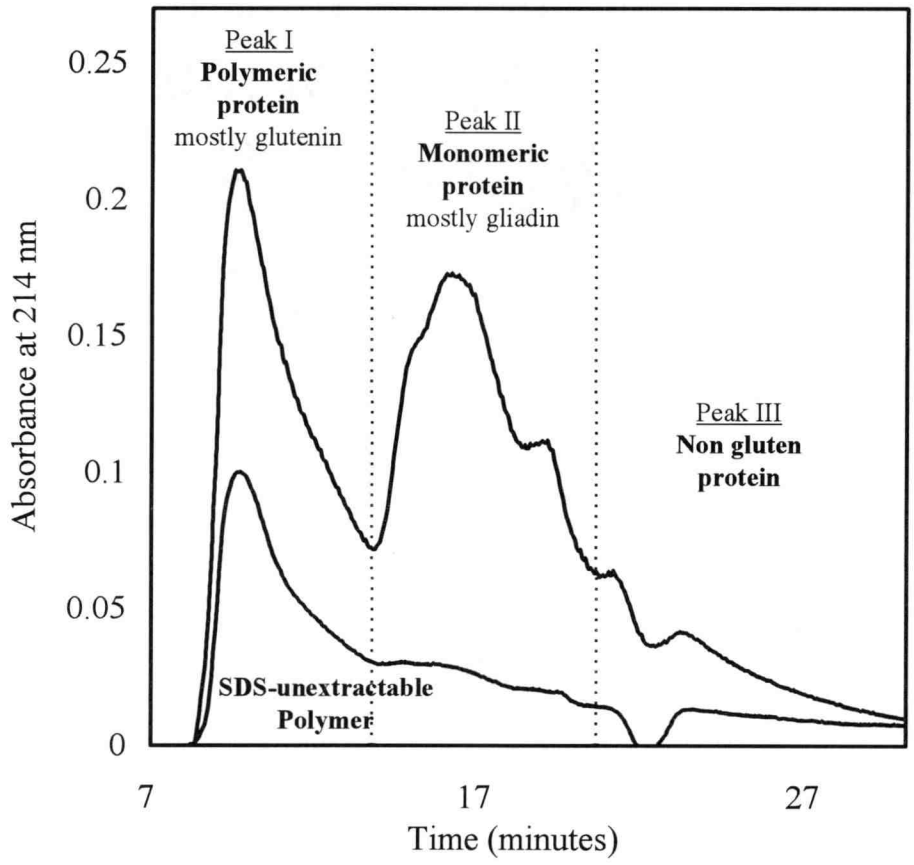

Figure 6: **Chromatographic trace from the SE-HPLC analysis of unreduced, total and SDS-unextractable gluten protein extracts from cultivar WPB 881**

Table 18: **Observed mean squares for areas under SE-HPLC peaks I, II, Total, and Polymeric/monomeric gluten protein ratio, computed for 30 durum and five common wheat genotypes, grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean squares			
Year	Source of Variation	df	I (APF)	II (AMF)	Total (ATOT)	Monomeric Ratio
1993	Genotype	34	18.6**	57.1**	112.2**	0.01**
	Blocks	1	15.4	19.6	71.2	$0.5E^{-3}$
	Error	34	4.4	9.1	25.1	$0.1E^{-2}$
	C.V. (%)		***7.36***	***6.97***	***6.42***	***5.52***
1994	Genotype	34	19.7**	41.4**	84.9**	0.01**
	Blocks	1	2.8	9.2	20.8	$0.1E^{-3}$
	Error	34	1.3	3.4	9.3	$0.8E^{-3}$
	C.V. (%)		***3.85***	***4.40***	***3.99***	***2.15***
Both	Genotype	34	28.6**	75.9**	1363.6**	0.02**
Years	Year	1	20.2**	74.7**	95.9*	0.06**
	Blocks(year)	2	9.1*	14.4	46.0	$0.3E^{-3}$
	Genotype*Year	34	9.7**	22.6**	60.4**	$0.2E^{-2}$**
	Error	68	2.8	6.2	17.2	$0.3E^{-3}$
	C.V. (%)		***5.83***	***5.88***	***5.37***	***4.10***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

Table 19: **Observed mean squares for the percentages of Polymeric, Monomeric protein fractions in total protein and in flour, computed for 30 durum and five common wheat genotypes, grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean squares			
Year	**Source of Variation**	**df**	**%PTP**	**%PF**	**% MTP**	**%MF**
1993	Genotype	34	15.6**	0.66**	14.5**	1.6**
	Blocks	1	2.1	0.26	0.05	0.2
	Error	34	1.8	0.09	2.0	0.2
	C.V. (%)		***3.66***	***5.85***	***2.56***	***6.24***
1994	Genotype	34	15.2**	0.44**	15.9**	0.98**
	Blocks	1	0.01	0.02	0.5	0.10
	Error	34	0.3	0.04	0.2	0.11
	C.V. (%)		***1.31***	***3.96***	***0.89***	***4.70***
Both	Genotype	34	28.6**	0.90**	28.6**	1.20**
Years	Year	1	108.2**	0.50**	14.0**	12.61**
	Blocks(year)	2	1.0	0.14	0.3	0.16
	Genotype*Year	34	2.2**	0.20**	1.8*	0.63**
	Error	68	1.0	0.06	1.1	0.16
	C.V. (%)		***2.70***	***5.02***	***1.93***	***5.59***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

Table 20: **Observed mean squares for the area under the peak corresponding to the SDS-Insoluble protein fractions, the percent in total protein and in flour, computed for 30 durum and five common wheat genotypes, grown in a duplicated randomized complete block design at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

			Mean squares		
Year	**Source of Variation**	**df**	**AINSF**	**%INSTP**	**%INSF**
1993	Genotype	34	20.6**	38.3**	0.67**
	Blocks	1	4.3	0.14	$0.4E^{-3}$
	Error	34	3.1	2.3	0.05
	C.V. (%)		***17.7***	***9.99***	***11.79***
1994	Genotype	34	26.4**	28.2**	0.58**
	Blocks	1	0.5	1.8	0.07
	Error	34	1.3	2.8	0.07
	C.V. (%)		***9.81***	***13.29***	***15.54***
Both	Genotype	34	40.7**	59.4**	1.08**
Years	Year	1	91.6**	208.3**	1.26**
	Blocks(year)	2	2.4	1.0	0.03
	Genotype*Year	34	6.4**	7.1**	0.17**
	Error	68	2.2	2.6	0.06
	C.V. (%)		***13.77***	***11.51***	***13.62***

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

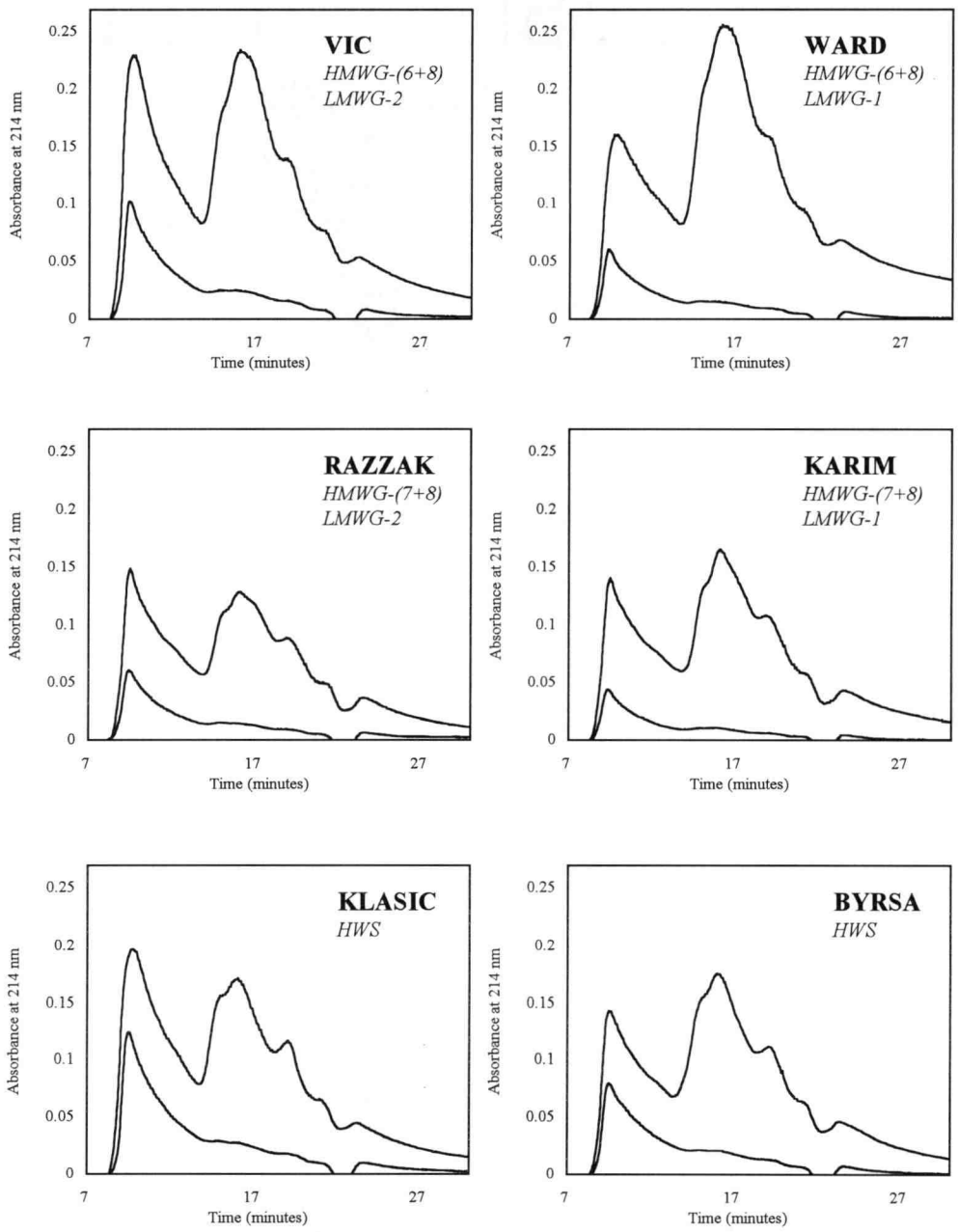

Figure 7: Chromatographic traces from SE-HPLC analysis of total (upper curves, three main peaks) and SDS-unextratable (lower curves, one main peak) observed for four durum and two bread wheats (HWS).

Table 21: **Means and ranges for areas under SE-HPLC peaks, corresponding to the amount of Polymeric (peak I), Monomeric (peak II), Total protein (ATOT) and polymeric/monomeric protein ratio computed for flour samples produced by 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci wheat genotypes) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

Groups		Area under peak (Absorbance Units / minute / mg flour)							
		I (APF)		II (AMF)		Total (ATOT)		Peak I/Peak II (APF/AMF)	
	N	1993	1994	1993	1994	1993	1994	1993	1994
Common		**28.7** [a]	**26.1** [a]	**42.4** [a]	**43.7** [a]	**76.1** [a]	**76.2** [a]	**0.68** [a]	**0.60** [a]
	5	*22.7-33.6*	*18.9-31.7*	*36.5-46.5*	*39.6-48.6*	*69.2-85.3*	*67.4-82.6*	*0.50-0.79*	*0.44-0.72*
Durum		**29.2** [a]	**28.8** [a]	**41.6** [a]	**43.1** [a]	**76.4** [a]	**78.3** [a]	**0.71** [a]	**0.68** [a]
	30	*22.9-36.1*	*22.2-35.6*	*32.9-51.9*	*32.7-62.3*	*61.2-90.9*	*62.7-99.4*	*0.47-0.86*	*0.45-0.79*
LMW-1		**25.6** [a]	**24.6** [a]	**48.7** [a]	**48.9** [a]	**79.9** [a]	**80.4** [a]	**0.53** [a]	**0.51** [a]
	3	*24.1-28.2*	*22.2-28.2*	*44.6-51.9*	*39.5-62.3*	*75.6-84.3*	*67.6-97.9*	*0.47-0.58*	*0.45-0.56*
LMW-2		**29.7** [b]	**29.3** [b]	**40.8** [b]	**42.4** [a]	**76.0** [a]	**78.1** [a]	**0.72** [b]	**0.69** [b]
	27	*22.9-36.1*	*23.3-35.6*	*32.9-50.3*	*32.7-56.9*	*61.2-90.9*	*62.7-99.4*	*0.62-0.86*	*0.59-0.79*
HMW 6+8		**29.3** [a]	**30.5** [a]	**40.1** [a]	**44.7** [a]	**75.0** [a]	**81.8** [a]	**0.73** [a]	**0.69** [a]
	11	*25.4-36.1*	*25.2-35.6*	*34.5-48.7*	*37.3-56.9*	*65.1-90.9*	*69.5-99.4*	*0.63-0.79*	*0.60-0.76*
HMW 7+8		**29.5** [a]	**27.8** [b]	**41.5** [a]	**41.0** [b]	**76.4** [a]	**75.1** [b]	**0.71** [a]	**0.68** [a]
	9	*22.9-35.8*	*23.3-32.6*	*32.9-50.3*	*33.3-48.1*	*61.2-90.2*	*62.7-87.5*	*0.62-0.82*	*0.59-0.74*
HMW 20		**30.5** [a]	**29.2** [a]	**41.0** [a]	**40.7** [b]	**77.1** [a]	**76.1** [b]	**0.75** [a]	**0.72** [b]
	7	*26.5-35.2*	*25.6-32.4*	*34.9-48.2*	*32.7-47.7*	*66.5-89.3*	*63.3-86.9*	*0.68-0.86*	*0.66-0.79*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level). Comparisons are made separately for each year.

No differences between these two groups were found in the polymer/monomer ratio, which is considered to estimate the glutenin/gliadin ratio (Table 21). Durum wheat genotypes appeared to have a higher proportion of polymer in their total protein than did bread wheats (Table 22). This difference was significant only in 1994, however. The proportion of polymer in the flour was, on average, significantly higher for durum wheats, in both years. Conversely, durum wheats had a slightly lower proportion of monomer in their total protein. These two fractions are inversely related. The most striking difference between the two groups resided in the proportion of their total protein represented by SDS-insoluble polymers (Table 23). A similar difference was apparent for the percent SDS-insoluble protein in flour.

Significant differences were observed between durum wheat genotypes expressing LMWG-1 and those carrying the contrasting allele, for all the SE-HPLC parameters considered, with the exception of ATOT. Data consistently indicated that, for a same total protein level, genotypes carrying LMWG-1 produced, significantly less polymeric protein (apparent amounts) than those carrying LMWG-2 (Table 21). They were also characterized by a significantly lower proportion of polymeric protein and a higher proportion of monomeric protein, both in total protein and in flour (Table 22). This resulted in a significantly lower polymer to monomer -or glutenin/gliadin- ratio. In fact, as shown in Table 21, the ranges in polymer/monomer ratios observed for the two groups did not overlap, in both years. Differences in the parameters related to the SDS-insoluble fraction were even more pronounced, especially in 1994. As shown in Table 23, the difference between the two groups in average proportion of SDS-insoluble protein was close to two-fold.

Among the genotypes carrying LMWG-2, the differences in SE-HPLC parameters between groups expressing different HMWG alleles were not as pronounced. In fact these were seldom consistently significant over the two years, as shown in Tables 21, 22 and 23. In 1994, genotypes carrying HMWG-(6+8) were characterized by a higher average amount of polymer in their flour than those expressing HMWG-(7+8). However this coincided with these lines having a significantly higher total protein content (ATOT) also.

Table 22: **Means and ranges for the percentages of Polymeric, Monomeric protein fraction in total protein and in flour, computed from the results of SE-HPLC analysis of extracts from flour samples produced by 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci wheat genotypes) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

		Percent in Total Protein				Percent in Flour			
		Polymer (%PTP)		Monomer (%MTP)		Polymer (%PF)		Monomer (%MF)	
	N	1993	1994	1993	1994	1993	1994	1993	1994
Common		**37.7** [a]	**34.0** [a]	**55.8** [a]	**57.4** [a]	**4.6** [a]	**4.3** [a]	**6.8** [a]	**7.3** [a]
	5	*31.1-41.3*	*27.9-38.4*	*52.2-62.1*	*53.5-63.6*	*3.5-5.1*	*3.3-5.2*	*5.7-7.7*	*6.5-8.6*
Durum		**38.3** [a]	**36.9** [b]	**54.4** [a]	**54.8** [b]	**4.9** [b]	**5.1** [b]	**7.0** [a]	**7.6** [a]
	30	*29.6-43.2*	*28.9-40.7*	*50.5-63.5*	*51.3-63.7*	*3.9-6.0*	*3.8-6.2*	*5.8-9.2*	*5.9-10.6*
LMW-1		**32.1** [a]	**30.8** [a]	**60.9** [a]	**60.5** [a]	**4.2** [a]	**4.2** [a]	**8.0** [a]	**8.4** [a]
	3	*29.6-34.1*	*28.9-32.8*	*59.0-63.5*	*58.4-63.7*	*3.9-4.8*	*3.8-4.8*	*7.3-8.6*	*7.1-10.6*
LMW-2		**39.0** [b]	**37.5** [b]	**53.6** [b]	**54.2** [b]	**5.0** [b]	**5.2** [b]	**6.9** [b]	**7.5** [a]
	27	*35.6-43.2*	*33.8-40.7*	*50.5-57.6*	*51.3-57.4*	*4.2-6.0*	*4.2-6.2*	*5.8-9.2*	*5.9-9.9*
HMW 6+8		**39.0** [a]	**37.4** [ab]	**53.5** [a]	**54.4** [ab]	**5.0** [a]	**5.4** [a]	**6.8** [a]	**7.9** [a]
	11	*35.8-40.7*	*34.8-39.3*	*51.5-56.4*	*52.0-57.4*	*4.2-6.0*	*4.7-6.2*	*5.9-8.1*	*6.7-9.9*
HMW 7+8		**38.6** [a]	**37.0** [a]	**54.2** [a]	**54.6** [a]	**5.0** [a]	**5.0** [b]	**7.1** [a]	**7.3** [a]
	9	*35.6-41.7*	*33.8-38.7*	*51.0-57.6*	*52.4-57.2*	*4.2-5.7*	*4.2-5.8*	*6.0-9.2*	*5.9-8.5*
HMW 20		**39.4** [a]	**38.4** [b]	**53.1** [a]	**53.4** [b]	**5.0** [a]	**5.2** [ab]	**6.8** [a]	**7.3** [a]
	7	*37.2-43.2*	*36.5-40.7*	*50.5-55.0*	*51.3-55.5*	*4.5-5.7*	*4.5-5.8*	*5.8-7.6*	*6.2-8.4*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level). Comparisons are made separately for each year.

Table 23: **Means and ranges for the parameters related to the SDS-Insoluble protein fraction, computed from the results of SE-HPLC analysis of extracts from flour samples produced by 30 durum (grouped according to their allelic composition at Glu-B3 and Glu-B1 glutenin loci wheat genotypes) and five common wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

		Amount (A.U./min/mg) (AINS)		Percent of total protein (%INSTP)		Percent in Flour (%INSF)	
	N	**1993**	**1994**	**1993**	**1994**	**1993**	**1994**
Common		**16.4** [a]	**12.9** [a]	**21.5** [a]	**16.7** [a]	**2.6** [a]	**2.1** [a]
	5	*9.6-20.5*	*7.1-17.9*	*13.2-24.6*	*10.5-21.6*	*1.5-3.1*	*1.3-2.9*
Durum		**10.8** [b]	**9.5** [a]	**14.1** [b]	**12.0** [b]	**1.8** [b]	**1.7** [b]
	30	*5.3-18.0*	*4.4-16.6*	*6.8-22.0*	*5.5-20.2*	*0.8-3.3*	*0.7-3.0*
LMW-1		**8.3** [a]	**5.5** [a]	**10.4** [a]	**6.7** [a]	**1.4** [a]	**0.9** [a]
	3	*5.4-11.3*	*4.4-8.3*	*6.8-13.7*	*5.4-9.0*	*0.8-1.9*	*0.7-1.5*
LMW-2		**11.0** [b]	**9.9** [b]	**14.5** [b]	**12.6** [b]	**1.9** [b]	**1.8** [b]
	27	*5.3-18.0*	*5.0-16.6*	*6.9-22.0*	*6.6-20.2*	*0.9-3.3*	*0.9-3.0*
HMW 6+8		**11.2** [a]	**11.3** [a]	**14.9** [a]	**13.7** [a]	**1.9** [a]	**2.0** [a]
	11	*7.3-18.0*	*7.2-16.6*	*9.8-22.0*	*9.4-20.2*	*1.2-3.3*	*1.3-3.0*
HMW 7+8		**12.5** [a]	**10.2** [a]	**16.4** [a]	**13.5** [a]	**2.1** [a]	**1.8** [a]
	9	*8.0-17.3*	*6.0-14.2*	*10.0-21.3*	*8.8-18.3*	*1.3-2.7*	*1.1-2.5*
HMW 20		**8.9** [b]	**7.4** [b]	**11.4** [b]	**9.7** [b]	**1.5** [b]	**1.3** [b]
	7	*5.3-12.6*	*5.0-13.2*	*6.9-14.8*	*6.6-16.1*	*0.9-2.1*	*0.9-2.2*

Means with the same letter, for each comparison, are not significantly different (at the 0.05 level). Comparisons are made separately for each year.

Results shown in Table 21 do not provide strong evidence for any difference in the polymer/monomer ratio between the three groups. It was apparent, however, that genotypes expressing HMWG-20 were characterized by a significantly lower average proportion of SDS-insoluble protein compared to that of genotypes carrying either alternative HMWG alleles, even in 1994 where the former produced a significantly higher proportion of total polymer in total protein.

IV.8. Correlation between Selected SE-HPLC parameters and quality traits

The association of selected quality traits and SE-HPLC parameters related to the polymeric protein composition of the gluten was investigated by simple correlation analysis using the means for 30 durum genotypes. The polymeric composition was described either in terms of the proportion of polymer in total protein (%PTP or %INSTP) or the resulting amount of polymer in the flour (%PF or %INSF). The correlation between quality traits and the polymer/monomer ratio was also considered. Results are shown in Table 24.

The correlation coefficients between quality traits and SE-HPLC parameters related to SDS-insoluble protein were generally greater then those between quality traits and SE-HPLC parameters related to total protein, indicating that the former protein fraction was more critical to bread-making quality. An exception to this general trend was observed for mixogram peak height (maximum resistance to mixing) which appeared to be moderately correlated with parameters describing the amount of total polymeric protein in flour but not with those related to SDS-insoluble protein. Another general trend shown in Table 24 is that the correlation coefficients obtained in 1994 were generally greater than those computed for 1993. In many cases, non-significant correlations from the first year were found to be significant in the second. In most of these cases however, the statistical significance was not matched by a substantial increase in the magnitude of the correlation coefficients which remained close or less than 0.50.

Table 24: **Phenotypic correlation coefficients between means of selected quality attributes and means of parameters pertaining to the polymeric gluten protein fraction, obtained from SE-HPLC analysis of total and SDS-insoluble protein extracts, computed for 30 durum wheat genotypes, grown at the Rugg's site, near Pendleton-Oregon, in 1993 and 1994.**

	%PTP Percent Polymer in Total Protein		**%PF** Percent Polymer in Flour		**Polymer / Monomer Ratio**		**%INSTP** SDS-ins. Polymer in Total Protein		**%ISNF** SDS-ins. Polymer in Flour	
	1993	**1994**	**1993**	**1994**	**1993**	**1994**	**1993**	**1994**	**1993**	**1994**
Sedimentation Height	0.52**	0.70**	0.46*	0.55**	0.51**	0.68**	0.71**	0.74**	0.66**	0.68**
Loaf Volume	0.13 ns	0.24 ns	0.44*	0.80**	0.11 ns	0.18 ns	0.31 ns	0.61**	0.42*	0.75**
Mixing Time	0.42*	0.47**	0.27 ns	0.07 ns	0.41*	0.48**	0.62**	0.60**	0.53**	0.46*
Water Absorption	0.51**	0.54**	0.79**	0.65**	0.49**	0.50**	0.41*	0.56**	0.52**	0.58**
Mixogram Parameters										
Time to Peak	0.16 ns	0.41*	0.14 ns	0.04 ns	0.13 ns	0.42*	0.57**	0.47**	0.51**	0.35 ns
Peak Height	-0.19 ns	-0.01 ns	0.48**	0.62**	-0.21 ns	-0.07 ns	-0.04 ns	0.04 ns	0.18 ns	0.23 ns
Height at 7 min.	0.31 ns	0.54**	0.70**	0.67**	0.29 ns	0.48**	0.47**	0.52**	0.59**	0.57**
Alveograph Parameters										
Tenacity	0.25 ns	0.44*	0.49**	0.10 ns	0.23 ns	0.43*	0.47**	0.43*	0.53**	0.32 ns
Extensibility	0.29 ns	0.31 ns	0.36 ns	0.62**	0.27 ns	0.26 ns	0.04 ns	0.34 ns	0.10 ns	0.44*
P/L	0.05 ns	0.18 ns	0.13 ns	-0.24 ns	0.05 ns	0.19 ns	0.31 ns	0.12 ns	0.30 ns	-0.01 ns
Deformation Energy	0.31 ns	0.52**	0.70**	0.45*	0.28 ns	0.48**	0.61**	0.72**	0.72**	0.67**

*, ** : *Significant at the 0.05, 0.01 probability levels, respectively.*

ns: *not significant*

Two important exceptions were observed, namely, the association between dough extensibility and %PF (r=0.62 in 1994) as well as that between loaf volume and %INSTP (r=0.61 in 1994).

Quality parameters measuring gluten strength were usually associated with the presence of more polymeric gluten protein. Sedimentation height appeared to correlate better with the proportion of polymer in total protein than with the amount of polymer in flour. This was the case for both total and SDS-insoluble polymers. It was also the quality parameter that correlated the best with the polymer/monomer ratio. In contrast, the other measures of gluten strength (W and H7M) appeared to be associated to a greater extent to the amount of total polymer in flour and to a lesser extent to the proportion of polymer in total protein. Both SDS-insoluble parameters correlated with the deformation energy in a similar fashion. The same was true for tolerance to over-mixing (H7M), but the association was weaker than in the case of deformation energy.

There was no significant association between loaf volume and the proportion of polymer in total protein or polymer/monomer ratio. The strength of the relationship between loaf volume and the other SE-HPLC parameters considered lacked consistency over years. Greater amounts of total and SDS-insoluble polymer present in the flour were associated with the production of larger bread loaves. This association was much more pronounced in 1994. The proportion of SDS-insoluble polymer, expressed as a percent of total protein, was positively associated with loaf volume only in 1994.

In general, no substantial associations were consistently observed between SE-HPLC parameters and alveograph parameters with the exception, previously described, of the relationship with the deformation energy.

Dough development time, as measured either by bake mixing time or mixogram TTP, appeared to be moderately associated with the proportion of polymer in total protein, and to a greater extent with that corresponding to the SDS-insoluble fraction. The amount of total polymer in flour was not correlated with dough development time. Water absorption was positively correlated with all the SE-HPLC parameters. The extent of these associations was greater with parameters related to total amount of polymer in flour (%PF or %INSF).

V. Discussion

The development of durum wheat cultivars with strong gluten and superior pasta-making quality is challenging the perception that durum flour is unsuitable for commercial bread-making. Recent studies have investigated the bread-making quality of such cultivars and renewed interest among cereal chemists and durum wheat breeders for the potential use of durum wheat flour in commercial bread-making operations (Dexter et al., 1981; Quick and Crawford, 1983; Boggini et al., 1988; Boggini and Pogna, 1989; Peña et al., 1994). The genotypic variability in baking performance reported in these studies suggested that further improvement in overall bread-making quality of durum wheat might be possible through inter-crossing of superior genotypes and subsequent selection, in spite of the absence of the most important loci associated with bread-making quality in hexaploid wheat (genes on chromosome 1D).

However, the quality attributes that are most critical for the improvement of the bread-making quality of durum wheat flour are not completely identified and the relationship between them is not well characterized. Extensive research to identify superior genotypes originating from different breeding programs, and to characterize the basis of their superiority, is lacking. Furthermore, the few studies that investigated the relationship between bread-making quality and gluten protein composition at major gluten protein loci, namely those conducted in Italy (Boggini and Pogna, 1989) and at the International Maize and Wheat Improvement Center-Mexico (Peña et al., 1994), involved sets of local genotypes representing a limited germplasm base. Consequently, more studies are needed to assess the use of glutenin alleles as markers in selecting for improved bread-making quality within the durum wheat germplasm. Finally, the molecular basis, in terms of the polymeric composition of the gluten complex, underlying the differences in bread-making quality between durum and bread wheat flours, as well as among durum wheats, remains largely unknown.

A comprehensive study was undertaken to address these issues. The first part of the investigation involved the evaluation of the mixing properties, dough physical characteristics and baking performance of thirty durum and five bread wheat genotypes. The second part consisted of an analysis of the polymeric composition of the gluten complex of each genotype, using Size-Exclusion HPLC (SE-HPLC).

V.1. Bread-making quality of durum wheat: Important attributes and relationship with gluten protein allelic composition.

V.1.1. Quality attributes underlying the difference in baking performance between durum and bread wheat

Durum genotypes were selected to represent a wide range of the variability in agronomic traits and gluten characteristics present in the spring durum wheat germplasm. Diversity in geographical origin was sought and cultivars and/or lines were chosen from most of the important durum growing areas worldwide. Among the five common wheat included in this study, four were characterized by good bread-making quality. The remaining check, namely Byrsa, had questionable gluten characteristics and baking performance for a bread wheat, and was used to compare durum wheat performances to that of a common wheat with marginal quality. It was felt that the inclusion of bread wheat checks grown and analyzed as part of the same experiment was appropriate, not only to identify the main "defects" that characterized durum flours, but also to estimate the magnitude of the genetic progress that has to be achieved to breed durum genotypes with acceptable overall bread-making quality.

V.1.1.1. Differences in grain characteristics and milling performance

The present study was not designed to accurately evaluate the milling yield potential of each line, as conditions were not optimized individually for each cultivar or for each class of wheat. Nevertheless, some relevant trends were apparent regarding the milling performance of the two wheat classes. Durum wheat yielded less total flour than common wheats. This can be explained in part by the fact that the break and reduction rolls specifications were designed and optimized, in terms of their spacing and corrugations, for milling the much softer and often smaller common wheat grain. Another reason might be that, because most of the durum flour was produced by the reduction rolls, more opportunities existed for losses in the mill stream. Unlike bread wheats which produced more break flour than reduction flour, durum grains produced more reduction than break flour. This can be explained by the much harder texture of the durum kernel which is the result of stronger interactions between protein matrix and starch granules. The first passage through the break rolls mostly yields a coarse grind (middlings) that needed further reduction to produce endosperm particles that could be considered flour. Despite the fact that both flour types were obtained with the same set of sieves, durum flours felt coarser to the touch than bread wheat flours. Passage through the same set of sieves results in the same maximum particle size for all flour types, but does not always produce the same particle size distribution. A particle size distribution that is skewed toward smaller sizes is the likely explanation for the finer texture of common flours. The importance of this observation stems from the impact it might have on water absorption. Finer particles provide a greater surface area to be hydrated upon mixing and they are likely to result in higher water absorption in common wheat flours. This was not the case in the present study, however. On the contrary, durum flours were characterized by a higher water absorption than bread wheat flours. A major factor affecting water absorption is the proportion of damaged starch. Although starch damage was not quantified in this study, it is presumed that the milling of the much harder durum grain resulted in a significantly higher proportion of damaged starch granules in durum flours,

which would explain the higher water absorption (Quaglia, 1988; Boyacioglu and D'Appolonia, 1994 a; Dexter et al., 1994). The increased incidence of starch damage in durum flours was also evidenced by the additional requirement for sodium chloride solution in order to produce a dough piece that is hydrated enough to be inflated during the alveograph analysis. Moderate levels of starch damage is beneficial as it increases water absorption and the gassing power of the dough, but in excess it can adversely affect baking performance (Dexter et al., 1994).

Conflicting results were observed when comparing the average protein content in the grain or in the flour of the two wheat classes. Whereas common wheats had a significantly higher grain protein content than durum wheats, the latter were characterized by a higher average flour protein content. Grain protein content was determined indirectly using NIR, whereas flour protein content was obtained by a direct measurement of the percent nitrogen in the flour according to the Dumas combustion method. Comparing the protein content of the two wheat classes based on NIR measurements is not reliable, because two different calibration equations were used, each one valid only for determining the protein content in samples of the specific wheat class. Also, a major factor affecting the accuracy and precision of NIR measurements is particle size. As discussed earlier, it is most likely that durum ground-wheat samples were much coarser than bread wheat samples even if both were ground through a 1 mm opening in the same grinder. On the other hand, the accuracy of the combustion method does not depend on any calibration procedure. Therefore it is considered herein as a more accurate estimator of protein content.

V.1.1.2. Differences in bread-making quality

As a class, durum wheats were characterized by an inferior overall bread-making quality when compared to the bread wheats. This general observation is in agreement with results from all the studies that have compared the baking performances of the two

wheat classes (Dexter et al., 1981; Quick and Crawford, 1983; Holm, 1985; Bakhsi and Baines, 1987; Boyacioglu and D'Appolonia, 1994 a).

Baking performance in general, and loaf volume in particular, depend partly on the protein quantity present in the flour. A very strong association between loaf volume and flour protein content, within different flour samples of the same genotype, was first demonstrated by the work of Finney and Barmore (1948) and subsequently confirmed by several authors (Fifield et al., 1950; Hoseney and Finney, 1971; Bushuk, 1985). Several reports suggested that a similar relationship existed even when sets of different bread wheat genotypes were considered (Johnson et al., 1943; Baker and Campbell, 1971; Bettge et al., 1989; Khan et al., 1989; Souza et al., 1993). The strength of the association varied according to the set of genotypes tested and was often moderate to weak. Similar results were reported for durum wheat flours (Boggini and Pogna, 1989; Peña et al., 1994). Protein content was not consistently associated with loaf volume in the present study. However, regardless of its magnitude or consistence, this relationship could not explain the differences in baking performance observed in the present study between bread and durum wheats, as the latter group was characterized by a higher average protein content and still produced markedly smaller bread loaves. In fact, it was often reported that durum wheat flour, or semolina, had a slightly higher protein content than common wheat flour (Quick and Crawford, 1983; Finney et al., 1987; Feillet, 1988; Quaglia, 1988; Boyacioglu and D'Appolonia, 1994; Liu et al., 1996). But in all cases where the baking performances were compared, durum wheats were always inferior to bread wheats. This strongly indicates that factors other that protein quantity were involved in determining the bread-making quality of the two wheat classes. These factors can be designated collectively as "protein quality".

Protein quality is a very general and quite complex concept that is related to the ability of the flour proteins to develop a gluten network that imparts suitable physical properties to the resulting dough, and facilitates the production of a large loaf of bread with a desirable crumb texture. Dough handling properties and the production of a well-risen and porous bread loaf are two inter-related aspects of overall bread-making quality. They will be discussed successively in the following sections.

A flour dough is considered to have suitable physical properties if it exhibits a balanced visco-elastic response. Such a rheological attribute results in adequate handling properties in the bakery during the mixing, proofing, dividing and molding steps. The balance between viscous (flow upon application of stress) and elastic (recovery of the original shape upon relaxation of an applied stress) properties of a dough is usually described as "dough strength" (MacRitchie et al., 1990). Since the rheological response of flour doughs is determined primarily by the gluten protein fraction (Finney, 1943; Hoseney et al., 1969 a, 1969 b; MacRitchie, 1978), "dough strength" is equivalent to "gluten strength". In the present study, the quality attribute aside from loaf volume that better differentiated between durum and bread wheats was gluten strength. The two wheat species could be classified into practically discrete classes according to their sedimentation volumes, which is one of the most commonly used estimator of gluten strength. The only overlap in sedimentation volume was observed for cultivar WPB 881, which was the durum genotype with the strongest gluten and was characterized by a sedimentation volume that approached or, as seen in 1994, exceeded that of the weaker bread wheat check, Byrsa. Another gluten strength-related parameter, namely the deformation energy determined from alveographic analysis of a developed dough, was substantially greater for bread wheats. However, in the case of deformation energy, some overlap between the ranges of the two classes was observed.

The lower average loaf volume observed for durum genotypes can be partly explained by their relatively weaker gluten characteristics. Bread-making quality, and in particular loaf volume, is known to increase as gluten strength increases. Published correlation coefficients between loaf volume and sedimentation volume were typically greater than 0.60 and varied between 0.50 and 0.88 for bread wheats (Orth et al., 1972, Moonen et al., 1982; Campbell et al., 1987; Cressey et al., 1987; Branlard et al., 1991; Hamer et al., 1992). Similar correlations were observed within sets of durum wheats (Boggini and Pogna, 1989; Peña et al., 1994). Also, it was observed in several studies evaluating the baking performance of a few genotypes that durums with stronger gluten had better baking performance than those with weak gluten (Quick and Crawford, 1983; Holm, 1985). Dexter et al. (1981) tested twenty two durum and thirty eight bread wheats

grown in Canada and found that gluten strength was the main factor contributing to baking performance. They concluded that there was no "...fundamental difference in behavior between the two classes of wheats..." and that ... "durum wheats performed exactly as weak wheats would be expected to....". This consistent association led several other authors to attribute the better baking performance of some durum genotypes to their increased gluten strength (Quick and Crawford, 1983; Holm, 1985; Boggini and Pogna, 1989; Peña et al., 1994). This conclusion could certainly be drawn in light of the highly significant association between gluten strength (sedimentation volume and deformation energy) and loaf volume observed among the thirty durum genotypes tested in the present study. Also, the shorter average dough development time observed for durum wheat flours was consistent with their weaker gluten. Dough development time, as measured by either bake, mixogram or farinogram mixing times, is considered to be a strength-related trait (Dexter et al., 1981; Gupta, 1994). Both bake mixing time and mixogram time to peak were significantly associated with gluten strength in this study. The "qualitative" nature of strength-related parameters was stressed by their independence from flour protein content.

It is then apparent that the lack of sufficient gluten strength was still a major defect characterizing even the modern durum cultivars with relatively stronger gluten. However, only 41% of the variability in loaf volume in any year could be explained by the variation in sedimentation volume. The variation in deformation energy accounted for 22% of the variation in loaf volume. Also, it is important to point-out that a relatively narrow range of sedimentation values (approximately 2 cm) corresponded to a wide range in loaf volumes (570 cc). Finally, many cultivars characterized by relatively high sedimentation values performed poorly in the baking test. These considerations strongly suggest that a factor other than gluten strength also affected the baking performance of durum wheats. The consistently strong association between loaf volume and alveogram parameter L suggested that dough extensibility was such a factor. In fact, dough extensibility was the parameter that correlated best with loaf volume for the set of durum wheats included in the present study. As a class, durum wheats produced doughs that were on average 32-34 % less extensible than bread wheats. This difference was highly significant.

The lack of extensibility of durum wheat doughs has been mentioned in several reports but its incidence within the durum wheat germplasm and the magnitude of the effect it might have on baking performance have not been addressed in a satisfactory manner. The physical properties of durum wheat dough were studied in great detail and compared to those of bread wheat dough by Boyacioglu and D'Appolonia (1994 a). Using both alveograph and extensigraph, they reported that dough from durum wheat flour was more tenacious and less extensible than dough from bread wheat. However, the material they have studied consisted of different commercial flour types collected from the millstreams of durum and bread wheat mills. As most commercial flours, these probably originated from a mixture of different genotypes. Therefore, it was not possible to make any inferences regarding the association between dough properties and baking performance among genotypes, based on their results.

The few studies involving the alveographic analyses of a number of durum wheat genotypes concentrated primarily on the association between baking performance and alveograph parameters other than dough extensibility. These were the gluten strength-related parameter W and the configuration ratio indicating the balance between tenacity and extensibility (Pasqui et al., 1991; Peña et al., 1994; Boggini et al., 1995). The specific relationship between dough extensibility and baking performance was not addressed in any of these studies. As mentioned by Peña et al. (1994) regarding their specific study, one likely reason for not addressing this issue was the limited variability for dough extensibility observed in the populations evaluated.

The need for specifically addressing dough extensibility can be appreciated if one considers the rheology of the bread-making process, as done by Bloksma (1990 a, 1990 b). Once a dough is optimally mixed, the gluten complex is fully developed and all of the gas cell nuclei are formed by occlusion of air. From this point on, the production of a well-risen bread loaf with a porous crumb structure depends primarily on the ability of the gas-cell walls (protein-starch matrix) to stretch and maintain their integrity long enough to retain the gases produced during fermentation and oven-spring (additional increase in volume at the beginning of baking). In turn, the ability of the protein-starch matrix to extend without rupturing depends on the extensibility of the gluten protein complex.

These considerations lead Bloksma (1990 b) to conclude that the most striking rheological requirement for the production of an adequate bread loaf was dough extensibility. This conclusion was supported experimentally by a study on the rheological properties of bread wheat flour doughs using both empirical testing methods (extensigraph and alveograph) and fundamental rheometric measurements (Janssen et al., 1996). Among other rheological parameters, the bi-axial dough extensibility - as the one measured by the alveograph - was required to exceed a minimum level in order to produce of a well-risen loaf of bread with a fine crumb structure. Additional empirical evidence for the importance of dough extensibility is provided by several studies reporting a strong relationship between loaf volume and the alveograph L parameter (Shogren et al., 1962; Bettge et al., 1989; Addo et al., 1990; Branlard et al., 1991). Bettge et al. (1989) observed such an association in both soft (58 genotypes) and hard wheat (15 genotypes) and found that the variation in the alveograph L parameter was able to explain 71% and 81%, respectively, of the variability in loaf volume. Therefore, dough extensibility was found to be the best predictor of loaf volume.

Within the set of durum genotypes evaluated in the present study, 62-64 % of the variation in loaf volume could be explained by variation in dough extensibility. Visual inspection of the crumb structure of the bread loaves baked from most durums suggested the premature collapse of the crumb cell walls during fermentation and/or oven-spring. That translated into high crumb scores for most durum genotypes (high designates poor structure). It appears that the gluten fibrils forming the crumb cell walls were not able to stretch sufficiently without rupturing, thereby failing to ensure the gas-retention ability of the bulk dough phase.

Another important aspect apparent in the present study was the considerable variability for most quality related parameters, observed within the set of durum genotypes evaluated. This was also the case for sets of genotypes tested by other researchers (Boggini and Pogna, 1989; Peña et al., 1994). It was hypothesized that this variability could be explained, in part at least, by the variation in allelic composition at the two major glutenin loci always expressed in durum wheat, namely Glu-B1 and Glu-B3. This relationship is addressed in the following sections.

V.1.2. Relationship between bread-making quality and allelic composition at the glutenin loci

V.1.2.1. Allelic composition at Glu-B3

Among the thirty durum genotypes, only three cultivars were chosen that expressed the LMWG-1 allele at the Glu-B3 locus, coding for the LMW glutenin sub-units. This proportion is clearly not representative of the frequency of this allele in the durum wheat germplasm. However, it is being selected against in most breeding programs as its presence (detected also by the expression of the linked γ-gliadin 42 allele at Gli-B1) is consistently associated with very poor gluten characteristics, (Daminaux et al., 1978; Kosmolak et al., 1980; DuCros et al., 1982; Pogna et al., 1988; Boggini and Pogna, 1989; Peña et al., 1994). Consequently, the allelic frequency of LMWG-1 is expected to dramatically decrease within the modern pool of durum wheat cultivars. Two cultivars expressing this allele were included as they still are widely grown in their respective country of origin. These were cultivar Karim (Tunisia) and Sham 1 (Syria). The third cultivar carrying LMWG-1 was Ward (North-Dakota). The inferior bread-making quality of these cultivars was expected and their inclusion in the study was aimed mainly at trying to identify the molecular basis underlying the weakness of their gluten, which will be addressed in the second part of this discussion.

Genotypes expressing LMWG-1 produced compact bread loaves with very low volume, and the poorest crumb structure. In fact, not much leavening was observed for these flours during fermentation and oven-spring. This was observed in spite of the lack of significant difference between the average flour protein content of the two groups, which suggested that the differences in bread-making quality between the two groups of durums were mostly the result of differences in protein "quality". Indeed, these three cultivars were characterized by a much weaker gluten, as indicated by significantly lower values for all the strength-related parameters. Differences in sedimentation volumes as well as in deformation energy were striking. In fact, a two-fold difference was observed

between the average deformation energy values of the two groups. Consistent with their much weaker gluten characteristics, are significantly shorter dough development times and lower tolerance to over-mixing. Dough handling properties characteristic of the three genotypes expressing LMWG-1 were consequently very poor as they exhibited a predominantly viscous behavior. Dough physical properties such as tenacity and extensibility were also lower; however, at this level of gluten strength, these values were relatively meaningless.

Similar results were reported by Boggini et al. (1989) and Peña et al. (1994) with different sets of cultivars which included a greater number of genotypes carrying the LWMG-1 alleles than the present study.

V.1.2.2. Allelic composition at Glu-B1

In terms of the relationship between bread-making quality and gluten protein composition, the present study was mostly concerned with the allelic composition at the Glu-B1 locus, coding for the HMW glutenin sub-units. Three of the most frequent alleles were represented, namely HMWG-(6+8), HMWG-(7+8) and HMWG-(20). These alleles were present at a combined frequency greater than 85%, within a collection of 502 durum wheat genotypes from 23 countries (Branlard et al., 1989). The relative frequencies reported in that study for the three alleles were 30.7%, 30.2%, and 39.1%, respectively. In the present study, these were 36.7%, 33.0%, and 23.3%, respectively. It is then apparent that HMWG-(6+8) and HMWG-(7+8) were present at similar frequencies as in the collection characterized by Branlard et al. (1989), whereas genotypes carrying HMWG-20 were somewhat under-represented.

As opposed to the variation in allelic composition at Glu-B3 which is known to result in dramatic differences in gluten strength, the variation in HMW glutenin sub-units encoded by Glu-B1 has not been shown to yield large and consistent differences in gluten characteristics. Studies involving large numbers of cultivars and breeding lines failed to

provide strong evidence for associations between gluten strength or pasta-making quality parameters and allelic composition at Glu-B1 (Autran, 1981; Vallega, 1986; DuCros, 1987, Autran and Galtiero, 1989). Even when the correlation coefficients reported were highly significant, their magnitude was usually low.

However, studies conducted in Italy (Boggini et al., 1988; Boggini and Pogna, 1989) and Mexico (Peña et al., 1994) suggested that the allelic composition at Glu-B1 might affect bread-making quality of durum wheat flours. These studies have identified allele HMWG-(7+8) as being associated with better baking performance and gluten characteristics than alleles HMWG-(20) or HMWG-(6+8). This is obviously in disagreement with the results of the present study which strongly indicates that, among durum wheats expressing LMWG-2, genotypes also carrying allele HMWG-(6+8) had a significantly better overall baking performance, especially loaf volume, than genotypes expressing either HMWG-(7+8) or HMWG-(20). In fact, of the eight best performing genotypes in the present set, seven expressed HMWG-(6+8). The better bread-making quality of genotypes expressing HMWG-(6+8) over those carrying HMWG-(7+8) could not be attributed to differences in either flour protein content or gluten strength. Whereas sedimentation volume differences were not significant, genotypes carrying HMWG-(7+8) were characterized by a higher average deformation energy and dough development time. This is consistent with the results reported by Peña et al. (1994). However, it was evident that this apparent indication of a greater strength was due primarily to the high tenacity and lack of extensibility characteristic of flours from genotypes with HMWG-(7+8), which resulted in a substantially greater configuration ratio. It did not result in higher loaf volume, however. On the other hand, it is apparent that the greater average loaf volume observed for genotypes carrying HMWG-(6+8) was associated with the significantly greater average dough extensibility characteristic of these genotypes. This is consistent with the high correlation coefficients between loaf volume and dough extensibility discussed in the previous sections, and suggests that at similar levels of gluten strength, HMWG-(6+8) imparts greater extensibility to the gluten complex, which in turn results in the better baking performance. This is also consistent with the better crumb structure that distinguished most strong gluten genotypes expressing HMWG-(6+8).

The discrepancy between the results of the present study and that reported by Peña et al. (1994) are probably due to the rather limited germplasm base represented by the genotypes they have analyzed, as suggested by the limited variability in dough extensibility observed. Studies conducted in Italy by Boggini et al. (1988) and Boggini and Pogna (1989) did not include alveographic dough testing, and no data on the dough extensibility was made available for the set of cultivars they have evaluated. Nevertheless, as these were all Italian cultivars, it is likely that the genotypic variability for most traits was not as great as that observed in our study. It is relevant to mention that the genotypes that produced the most extensible dough were those originating either from North-Dakota (Vic, Lloyd, Renville, Monroe) or France (Mondur, Valdur, Brindur). Most of these, except Brindur, carried HMWG-(6+8) and were not included in the two studies mentioned above.

In summary, results from the present study strongly suggested that the main factors contributing to bread-baking performance of durum wheat, and differentiating these from bread wheats, are gluten strength and dough extensibility. Whereas the effect of gluten strength has been well documented, this study is, to our knowledge, the first to report such an important role for dough extensibility among a set of durum wheat genotypes. Our ability to identify such a role was most likely the result of including genotypes representing a wide germplasm base. These results also suggested that gluten strength (either estimated by sedimentation volume or deformation energy) and dough extensibility were essentially independent from each other. Consequently, these two properties of the gluten protein complex are expected to have a different basis, at the molecular level. The molecular basis underlying differences in bread-making quality is addressed in the second part of this discussion.

V.2. Relationship between gluten quality characteristics and the its polymeric composition. Insights on the molecular basis of bread-making quality.

The study of the molecular mechanisms by which gluten proteins might influence both gluten strength and extensibility, and ultimately bread-making performance, should result in a better understanding of how the gluten complex of the different durum groups and bread wheats differ, and how these differences at the molecular level might explain the variation in bread-making quality discussed in the above sections.

The importance and functionality of gluten protein resides primarily in the ability of the glutenin sub-units to form inter-molecular disulfide bonds between cysteine residues from different polypeptides, yielding a polymer with a variable molecular weight distribution. Therefore, it logical to hypothesize that one likely molecular basis for variation in bread-making quality resides in the quantitative difference among genotypes with respect to the ability to form a polymeric structure.

The second likely basis for variation in bread-making quality might be the genotypic difference in the molecular size distribution within the gluten polymer. The molecular weight distribution characteristic of the gluten polymer is not known nor is it possible to determine with the techniques presently available (Kasarda, 1990; Gupta et al., 1993). That which is known is that molecular weights ranging from more than one hundred up to 20 million Dalton are possible (Huebner and Wall, 1976; Bietz, 1985; Graveland et al., 1985). Nevertheless, a method based on the inverse relationship between molecular size and extractability in SDS has been developed by Gupta et al. (1993) to estimate the relative size distribution of the polymeric protein in wheat gluten. Because the smaller-sized polymers are readily extracted without sonication in buffer containing 0.5% SDS, the protein left in the residue of such extractions will consist mainly of the largest - most insoluble - polymers. These can then be extracted by sonication and quantified using the same SE-HPLC fractionation as for the total protein. The resulting proportion of SDS unextractable protein in total protein is considered a measure of the polymer size distribution (Gupta et al., 1993; MacRitchie and Gupta, 1993).

These hypothesis were tested by analyzing the polymeric protein composition of flour samples from all the genotypes included in the study, using SE-HPLC of unreduced protein extracts. The assumptions underlying the SE-HPLC method used (Singh et al., 1990a, 1990b, as modified by Baley et al., 1991), namely, the close-to-complete extraction of gluten proteins and the effective separation of the polymeric fraction from the monomeric proteins, were validated under the conditions of the present study in preliminary experiments.

V.2.1. Variation in the quantity of total polymeric protein

Traits such as dough extensibility and loaf volume are believed to be associated with the total amount of polymer in the flour (Singh et al., 1990 b; Gupta et al., 1992; Gupta et al., 1993; MacRitchie and Gupta, 1993; Gupta, 1994). Among the thirty durum genotypes evaluated in the present study, it was indeed apparent that loaf volume and dough extensibility correlated best with the total amount of polymer in the flour. These associations were weak (non significant in the case of extensibility) in 1993, but rather substantial in 1994.

However, the relationship between total amount of polymer in the flour and dough extensibility/loaf volume could not be extended to include the bread wheats. There was indication that durum wheats, as a group, had a slightly higher proportion of polymeric protein as well as a higher polymer/monomer ratio than bread wheats. These differences were seldom statistically significant. However, when differences in flour protein content are accounted for by considering the total polymer in flour, significant differences were observed between the two flour types. If the association between total polymer and either dough extensibility or loaf volume was valid across species, some durum wheats should have produced a more extensible dough and greater loaf volume than bread wheats, as they had a greater average amount of total polymer. This was evidently not the case. This result suggests that the molecular basis underlying differences in extensibility and loaf

volume between durum and bread wheats is not a greater ability of the latter to form a polymeric network.

Similarly, it is apparent that differences in either dough extensibility or loaf volume among the durum genotypes expressing LMWG-2 could not be attributed to variation in total polymer content. The range observed for the proportion of total polymer in protein and in flour was narrow, indicating a limited variability in the genotype's overall ability to form a polymeric structure.

In contrast, dramatic differences in the total polymer in protein or in flour were observed between durum genotypes expressing LMWG-1 and those carrying LMWG-2. In fact, genotypes from each group could be assigned to non-overlapping classes according to the two parameters related to total polymeric protein. Because the main difference between these two groups of genotypes resided in their allelic composition at Glu-B3, our results suggest that the expression of allele LMWG-1 results in a significantly lower proportion of glutenin in the protein and flour.

The much larger magnitude of the effect associated with variation in the LMW glutenin alleles compared to that resulting from variation in the HMW glutenin composition can be explained by the fact that LMW glutenin sub-units are present in large excess over the HMW sub-units (Huebner and Bietz, 1985; Kasarda, 1990). Consequently, any molecular feature that reduces the polymerizing ability of the former would have a greater impact on the production of total polymer, than if such a feature occurred in a HMW glutenin sub-unit.

In the first part of this discussion, it was stated that differences in the better baking performance of durum genotypes expressing HMWG-(6+8) compared to that of genotypes carrying HMWG-(7+8) was due to the higher dough extensibility of the former and not to any variation in gluten strength. Size-exclusion HPLC analysis of either total or SDS-unextractable (discussed in the next section) failed to detect any difference in the gluten polymeric structure that could provide an explanation for the difference in dough extensibility observed between the two groups of durum wheats. Therefore, molecular mechanisms other than those related to the amount of polymer or its size distribution are likely to be responsible for the difference in dough extensibility between the two groups.

It is important to remember that the functionality of gluten proteins does not depend exclusively on disulfide bond formation. Secondary forces are also involved in ensuring the continuity of the gluten network. The generally accepted structural model describing the glutenin polymer, as proposed by Ewart (1969, 1978, 1990), states that glutenin sub-units are linked head-to-tail by disulfide bonds, in random order, forming linear (unbranched) polymers and that the continuity of the network depends on the non-covalent cross-links (hydrogen bonds and hydrophobic interactions) as well as chain entanglement between adjacent polymers (Bloksma, 1990 a). If extensibility depends on the ability of each gluten polymer (each concatenations of disulfide-linked glutenin sub-units) to slide along each other's side without breaking the continuity of the gluten network (Bloksma, 1990 a), it is conceivable that sub-units (6+8) might exhibit some molecular feature that promotes better secondary interactions between the gluten fibrils than sub-units (7+8), thereby ensuring the integrity of the gluten complex over a wider range of stress. This hypothesis can only be confirmed by the examination of the amino-acid sequences, which are not yet available, of the polypeptides involved. In any case, our results suggest that some mechanism that is intrinsic to the sub-units, but unrelated to their polymerizing ability should be at the origin of the difference in extensibility and baking performance observed between genotypes expressing HMWG-(6+8) and those carrying HMWG-(7+8).

V.2.2. Variation in the size distribution of the polymeric protein

It has been shown that gluten strength- related traits (mixing time, resistance to stretching, sedimentation volumes and deformation energy W) are associated with the size distribution of the polymeric protein rather than with its proportion in total protein or in flour (Gupta et al., 1993; Gupta, 1994). Whereas some studies have reported a strong correlation between percent total polymer and strength-related attributes (Singh et al., 1990 b; Gupta et al, 1992), Gupta et al. (1993) have found that these associations were

not consistent over a wide range of genotypes and environments. In the latter study, the proportion of SDS-unextractable protein, that is the relative size distribution of the glutenin polymer, was found to be a better indicator of gluten strength. This was indeed observed within the set of thirty durum genotypes evaluated in the present study. The gluten strength-related parameters, particularly sedimentation volume and deformation energy tended to be associated with the relative size distribution of the total protein (that is the percent SDS-unextractable polymer in total protein) or with the amount of SDS-unextractable polymer in the flour.

Although the method used in the present study was recently published (Gupta et al., 1993), the relationship between the proportion/amount of insoluble (or largest glutenin polymers) and bread-making quality attributes is well documented. The SDS-unextractable protein fraction quantified in the present study is equivalent to the "acetic acid-insoluble glutenin" or "residue protein" first described by Orth and Bushuk (1972), the "gel protein" of Mecham et al. (1972), the "glutenin I" fraction isolated by low pressure gel permeation chromatography by Huebner et al. (1976) or to the SDS-insoluble "gel protein" isolated by ultra-centrifugation of flour suspensions in SDS by Moonen et al. (1982). It also corresponds to the polymeric fraction designated as "glutenin macro-polymer" by Weegels et al. (1996). While all these methods were successful in detecting a strong relationship between the insoluble protein fraction and bread-making quality attributes, they were time-consuming and labor-intensive, and thereby not amenable to the routine analysis of large sample sets. Size-exclusion HPLC fractionation of sonicated SDS-unextractable protein extracts provides a very simple, high throughput test for estimating proportion or amount of the glutenin polymers that effectively contribute to dough strength related traits and ultimately to baking performance.

Most importantly, SE-HPLC parameters related to the size distribution of the polymeric protein clearly differentiated between bread and durum wheats. The proportion of large polymers (macro-polymers) in the total protein were, on average, markedly higher for bread wheats. Also, in spite of a slightly lower average flour protein content, bread wheats had a significantly higher amount of SDS-unextractable polymers in the flour. The

greater ability of the glutenin sub-units from bread wheat to form these macro-polymers can therefore account for the greater strength of their gluten network.

The weak gluten cultivars expressing LMWG-1 produced significantly less macro-polymer than the relatively stronger gluten genotypes expressing LMWG-2. This suggested that the polypeptides encoded by LMWG-1 have a reduced ability to form large-sized polymers. This deficiency could be due to a reduced cysteine content (residue involved in disulfide bond formation in wheat) or a molecular configuration of the sub-units that make the existing cysteine residues unavailable for inter-molecular disulfide bond formation. The former is not a likely explanation as LMW glutenin sub-units from hexaploid wheat are classified as sulfur-rich proteins, i.e., they have a high cysteine content (Shewry et al., 1986). Although no data are available for durum wheats, it is most likely that the LMW glutenin from durum wheat is similarly rich in cysteine because of the close sequence homology observed between homologous genomes (B genome). On the other hand, it has been shown that not all cysteine residues are available for inter-molecular disulfide bond formation (Graveland et al., 1985; Graveland et al., 1985; Kasarda, 1990). Consequently, a more likely basis for the reduced polymerizing ability of LMWG-1 sub-units would be a primary and/or secondary structure favoring the formation of intra-molecular bonds, thereby preventing most cysteine to participate in inter-molecular disulfide bond formation (Graveland et al., 1985; Kasarda, 1990).

The relationship between gluten strength and glutenin polymer size distribution is consistent with the structural model describing the glutenin polymer, as proposed by Ewart (1969, 1978, 1990), in which glutenin sub-units are linked head-to-tail by disulfide bonds, in random order, forming a linear (unbranched) polymer. Since the cohesion of the gluten complex depends partly on chain entanglement between adjacent polymers (Bloksma, 1990 a), the presence of more macro-polymers increases opportunities for secondary cross-links and entanglements, which results in increased resistance to deformation or strength of the overall network. This is also supported by studies of the molecular parameters affecting the tensile strength of synthetic polymers (polystyrene for example) which have demonstrated that only polymers with a molecular weight larger than

a threshold value effectively participate in chain entanglements, thereby contributing to tensile strength (Bersted and Anderson, 1990; Gupta et al, 1993).

A significant overlap was observed between bread wheats and durum wheats with respect to the proportion of SDS-unextractable polymer in protein and in flour. Several durum genotypes that were characterized by a gluten complex containing more large-sized polymers than some bread wheats produced smaller loaves. This demonstrates that several durum genotypes are expressing gluten proteins that have intrinsically adequate polymerizing ability, and at relative levels that promote the adequate formation of macro-polymer. This suggests that the inability of these genotypes to produce higher volume bread loaves is not related to a lesser ability to form a macro-polymer, but to some other property, not associated with the strength of the gluten complex. Also, the superior baking performance of durum genotypes expressing HMWG-(6+8)/LMWG-2 over those carrying HMWG-(7+8)/LMWG-2 could not be explained by a difference in gluten polymer size distribution parameters between the two groups, which is consistent with the lack of difference in sedimentation volume.

In summary, SE-HPLC analysis indicated that there was no intrinsic deficiency in the ability of durum wheat to produce a gluten polymer, except in genotypes expressing LMWG-1. It was partly able to attribute the weaker gluten characteristics and baking performance of durum wheats to their overall reduced ability to form large polymers (macro-polymers). However it failed to provide an explanation for differences in baking performance that were not associated with gluten strength related parameters, and was unable to provide a basis for the differences in baking performance between durum wheats expressing different HMWG alleles in combination with LMWG-2. These shortcoming are related to the fact that a major part of the differences just mentioned seem to be related to differences in gluten characteristics affecting dough extensibility which could not be addressed in terms of gluten protein polymeric composition or size distribution.

V.3. Relevance of the results of the present study for breeding durum wheats with improved bread-making quality.

One practical objective of the present study was to provide a framework for the planning of a breeding strategy directed towards developing durum wheat genotypes with improved overall bread-making quality. Whereas the need for increased gluten strength appeared to be important in light of our results, and as supported by several previously published studies (Dexter et al., 1981; Quick and Crawford, 1983; Holm et al., 1985; Boggini and Pogna, 1989; Peña et al., 1996), the present study is, to our knowledge, the first to clearly demonstrate the essential need for specifically addressing the improvement of durum dough extensibility characteristics. In order to be successful, any selection scheme should include selection for both stronger gluten and greater dough extensibility. Our results strongly indicate that there is very little likelihood of improving dough extensibility indirectly, by selecting for stronger gluten, as these attributes appeared to be independent from each other and have different basis at the molecular level.

Selection for gluten strength can be implemented by selecting for a greater glutenin macro-polymer content as estimated by SE-HPLC analysis. This analysis is a simple, high throughput test to estimate the quantity of gluten polymer that effectively contributes to dough strength. However, as this test is based on HPLC analysis, it requires more time and much more laboratory resources than procedures such as the SDS-sedimentation test. Size-exclusion-HPLC has greatly contributed to the better understanding of the gluten polymeric structure and the molecular basis underlying bread-making quality. However, it is our opinion that its use in the context of a breeding program cannot be justified because a much less costly and time-consuming procedure such as the SDS-sedimentation test can be as effective in selecting for increased gluten strength. Sedimentation volume, as determined by the micro-sedimentation test described by Dick and Quick (1983) which requires only small amounts of ground wheat (1 gram) and minimal laboratory resources, has been shown to be under the control of genes acting primarily in an additive fashion (Ammar and Kronstad, 1992). This makes it an ideal test to select for greater gluten

strength in the early stages of a breeding program, as early as in F_2 or F_3 segregating populations.

On the other hand, selection for increased dough extensibility is more challenging as simple, high-throughput testing procedures using small samples are not currently available. Our results indicate that SE-HPLC parameters were found to be associated mostly with strength-related attributes and failed to provide a reliable predictor of dough extensibility within the collection of durum genotypes evaluated. The currently used methods to determine dough extensibility require the use of either the extensigraph or the alveograph. Both methods are time-consuming and require large flour samples (250 grams minimum). Therefore they are not applicable for early generation selection. Other "scaled-down" methods based on the extensigraph principle have been recently developed to allow the estimation of dough extensibility using smaller amounts of flour. These are the micro-extensigraph (Brabender) and the TA.XT2 Texture Analyzer (Texture Technologies Corp.). Although only 30 to 50 g of flour (50-80 g of seed) are required for these tests, not enough data are available to validate the accuracy of the dough extensibility measurements obtained with these instruments. Conflicting results have been reported regarding the ability of the TA.TX2 texture meter to provide extensibility values that correlate well with those obtained from an extensigraph (Hou and Kruk, 1996; Schlichting et al., 1996). Furthermore, regardless of the method used to determine dough extensibility, there is no indication that this trait is highly heritable, which raises doubts about the potential effectiveness of any early generation selection scheme. Consequently, given the testing methods and the limited inheritance data currently available to breeders, rational selection for increased dough extensibility can only be implemented in the later stages of breeding, possibly on seed harvested from the first yield evaluations. It is important to point out that, at this stage, there is usually enough seed to perform a bake test which require much less flour than an alveographic or extensigraphic analysis. Selection for high loaf volume, which is after all the ultimate measure of bread-making quality, can be directly implemented at this stage, without need for testing the physical properties of the dough.

Regarding the use of gluten protein alleles as markers to aid in the selection of genotypes with good overall bread-making quality, our results suggest that genotypes carrying LMWG-1 at Glu-B3 are most likely to perform poorly and should therefore be selected against. Although cultivars expressing this allele are slowly being replaced around the world, some of them are likely to be used extensively as parents in crossing programs because of their excellent yielding ability and adaptability to a wide range of environments. Examples of such cultivars are the two included in the present study, namely, Karim and Sham 1, which are still sown on a substantial part of the acreage devoted to durum wheat in their respective countries. The use of these cultivars as parents does not constitute any major problem as there are no reports of negative association between their superior agronomic attributes and their poor quality. Plants or families from segregating populations generated from such crosses can be readily characterized by SDS-PAGE, and those found to carry LMWG-1 can therefore be discarded. Since LMWG-1 alleles confer such weak gluten characteristics, a micro-sedimentation test could be almost as effective as electrophoretic analysis in identifying the segregants carrying this allele.

The use of alleles corresponding to the HMW glutenin sub-units as markers in selection for improved bread-making quality is more problematic. Conclusions from earlier reports indicating that HMWG-(7+8) was associated with better bread-making quality (Boggini et al., 1988; Boggini and Pogna, 1989; Peña et al., 1994) were contradicted by our results which strongly suggest that HMWG-(6+8) was associated with greater extensibility and better overall baking performance. These discrepancies call for the cautious and critical interpretation of any association between the allelic composition at Glu-B1 and bread-making quality. Since these associations are obviously dependent on the set of genotypes evaluated, the allelic composition at Glu-B1 per-se cannot be considered as a reliable indication of the bread-making quality potential of a durum genotype.

Finally, the substantial genotypic variability for most quality attributes determining the overall bread-making quality observed among the durum genotypes evaluated in the present study and others, suggested that inter-crossing of superior (or complementary)

genotypes and subsequent selection are likely to result at least in some degree of improvement of the bread-making quality of durum wheat. However, the magnitude of this improvement might not be sufficient to result in the development of a durum genotype with a bread-making quality equivalent to that of a good quality common wheat. It is important to remember that several durum genotypes (WPB 881, Vic, Renville) were characterized by a significantly greater total protein content, proportion of total polymeric protein and a greater macro-polymer content than most of the bread wheats checks, but still produced smaller bread loaves. This suggested that, even in the presence of adequate quantity of macro-polymer, the gluten complexes of these cultivars were deficient in some "qualitative" property, that is, they lacked some component that was essential for the production of an extensible protein matrix with adequate gas retention capacity. Because of the limited genetic variability characterizing durum wheat in terms of alleles coding for both low and high molecular weight glutenin sub-units, it is unlikely to find such a component within the durum wheat germplasm. Also, the most frequent allelic combinations have already been tested for their relationship with bread-making quality. Consequently, it becomes apparent that the likelihood of achieving a "spectacular" improvement in bread-making quality using the allelic combinations indigenous to the durum wheat germplasm is small.

Other approaches involving the transfer of glutenin sub-units non-indigenous to durum wheat are likely to result in a more substantial improvement of its bread-making quality. Few attempts at such transfer have been reported. Liu et al. (1994 a; 1994 b) reported that the presence of the 1D chromosome from Chinese spring (carrying HMWG-2+12) in a segregating population was associated with stronger mixing properties and a greater proportion of glutenin. Ciaffi et al. (1995) reported the development of a line expressing two protein sub-units encoded by the Glu-A1 allele from Triticum dicoccoides that was characterized by a bread-making quality equivalent to that of the bread wheat check cv. Centauro. The most promising results however, are expected from the successful transfer, via translocation between homeologous chromosomes, into durum wheat of the HMWG-(5+10) that have been associated with good quality in hexaploid wheat (Ceolini et al., 1995; Lukaswevski, personal communication). To date, these are

just preliminary reports and the potential improvement in bread-making quality resulting from such a transfer has not yet been reported.

VI. Conclusions

The potential use of durum wheat for bread-making was investigated by evaluating the mixing properties, dough physical characteristics and baking performance of thirty durum and five bread wheat genotypes. Some of the mechanisms underlying bread-making quality at the molecular level were addressed through the analysis of the gluten protein polymeric composition using SE-HPLC. In light of the experimental results discussed in the above sections, the following conclusions could be drawn:

1- Substantial variability was observed among durum wheat genotypes for most quality-related parameters. Two attributes were identified as most critical for better baking performance in durum wheat, namely, gluten strength and dough extensibility. Therefore, any breeding effort directed towards improving the bread-making quality of durum wheat must include selection for greater gluten strength and dough extensibility.

2- The presence of low molecular weight sub-units encoded by allele LMWG-1 was associated with a greatly reduced glutenin and macro-polymer contents. This was believed to be the basis for the very weak gluten and poor baking performance characterizing genotypes expressing LMWG-1. Therefore, their presence should be selected against in any breeding effort aiming at improving the bread-making quality of durum wheat.

3- Among the durum genotypes carrying LMWG-2, the presence of high molecular weight sub-units 6+8 was associated with greater dough extensibility and better overall baking performance. This was not due to differences in gluten strength-related attributes but to some intrinsic property of sub-units 6+8, possibly a molecular configuration promoting favorable non-covalent cross-links (hydrophobic interactions and

hydrogen bonds), which resulted in a gluten complex with a greater ability to stretch without loss of cohesion.

4- The association between HMW glutenin alleles and bread-making quality attributes depends on the set of genotypes evaluated. Therefore, they cannot be considered as reliable markers to aid in the selection of durum genotypes with better bread-making quality.

5- The SE-HPLC procedure used in the present study failed to provide any reliable predictor of dough extensibility. Also, in spite of the valuable information that SE-HPLC has contributed to the better understanding of the gluten polymeric structure and the molecular basis underlying bread-making quality, its use in the context of a breeding program cannot be justified. Much less costly and time-consuming procedures such as the SDS-sedimentation test can be as effective in selecting for increased gluten strength.

6- As a group, durum wheats were characterized by a weaker gluten and produced a dough that lacked the extensibility required to produce a high volume bread loaf with adequate crumb structure. This difference could generally be attributed to the difference in size distribution of the gluten protein. Durum wheat's glutenin sub-units usually produced less macro-polymer (large size polymers).

7- Some durum genotypes, in particular those with the strongest gluten characteristics, produced more macro-polymer and smaller loaves than most bread wheats. It was concluded that a factor unrelated to gluten strength but affecting the properties of the gluten complex in a "qualitative" fashion must be lacking.

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APPENDICES

APPENDIX 1: Origin and brief description of the cultivars/lines tested in the present study.

Durum wheats:

Altar 84: (RUFF / FGO // MEXI75 /3/ SHWA, released in 1984). Black awned, high yielding semi-dwarf variety developed by the International Center for Maize and Wheat Improvement (CIMMYT) in Mexico, characterized by a large number of kernels per spike, a fair overall gluten quality and kernel pigment content. Its protein content is usually low for a durum. It is the leading durum wheat cultivar in Mexico, partly because of its tolerance to karnal bunt (*Tilletia indica)*.

Quilafen: (LD357E / 2*TC60, released in 1969). Black awned, high yielding line with pubescent glumes, developed in Chile. It is characterized by a good gluten quality and good grain color. It has an average protein content.

WPB 881: Private cultivar from Western Plant Breeders, released in California and Arizona in 1981. It has a poor yield potential in Eastern Oregon. It is characterized by a high protein content, excellent gluten quality, seed characteristics, especially yellow pigment content. It produces a high quality semolina which makes an excellent pasta product. It is considered the standard in durum wheat quality by many pasta manufacturers around the world.

ZY8019: Breeding line of unknown pedigree, probably out of the CIMMYT program. It is characterized by a poor yielding ability under Eastern Oregon conditions. Its gluten quality, protein content and kernel yellow pigment content are satisfactory.

Carcomun 'S': (SHWA / MEXI 75 // YAV, released in 1982). Cultivar developed by CIMMYT-Mexico. It is characterized by fair seed characteristics, except for kernel yellow pigment content. Gluten quality is questionable.

Chen 'S': (SHWA / YAV, developed in 1983). Black-awned breeding line developed by CIMMYT-Mexico, characterized by good yield potential under Eastern Oregon conditions, a wide adaptation and good yellow pigment content. Its gluten quality is questionable.

Karim: (JO / AA // FGO, released in 1977). Black awned, early, high yielding and widely adapted durum line selected in Tunisia from a cross made at CIMMYT. It is characterized by a rapid grain filling ability. Its gluten quality is very poor and the yellow pigment content of its kernels is usually unsatisfactory. It has a low protein content. It is nevertheless the leading cultivar in Tunisia as are a number of its sib lines.

Razzak: (JO / AA // FGO /3/ DM // 69 / 331, released in 1984). It is a Black awned, early, high yielding cultivar developed in Tunisia where it is replacing Karim. Its gluten characteristics are better than those of Karim but are still considered questionable.

Sham 1: (PLC / RUFF // GTA / RLT, released in 1985). Developed by ICARDA, it is a high yielding, widely grown cultivar in Syria. Its gluten quality is poor and its yellow pigment content is questionable.

OR 918122: (UVY162 / 61.30 // 73.44 / OVI65 /3/ BERK / OVI65 /4/ C.BUG1018 / BR180). A breeding line selected from a winter by spring cross made at CIMMYT. It has been kept in the Oregon State University spring durum wheat breeding program for its adaptation to the Eastern Oregon conditions and its good seed quality. Its gluten quality and yellow pigment content are fair.

OR 4910045: (Arizona Male Sterile Line). A breeding line introduced to the Oregon State University spring durum wheat program. It has been characterized by a good adaptation to the Eastern Oregon conditions and good gluten and seed characteristics.

OR 4910060: (Arizona Male Sterile Line). A breeding line introduced to the Oregon State University spring durum wheat program. It has been characterized by a good adaptation to the Eastern Oregon conditions and fair gluten and seed characteristics. Its grain color is excellent.

SULA // WLS / DWL5023: A breeding line selected from the 1991 Elite Durum Yield Trial, from a cross made in CIMMYT. It has a good yield potential under Eastern Oregon conditions.

Vic: A durum wheat cultivar released by the North-Dakota State breeding program in 1979. It is not adapted to the Eastern Oregon growing conditions because of its excessive plant height. It is however the standard for quality in the U.S. durum belt. Its grain characteristics, including yellow pigment content, as well as the quality of its gluten are considered excellent. It has a high protein content.

Ward: (LK /3/ LK357 // CI7780 / LD362 /4/ LDS, released in 1972). A cultivar out of the North-Dakota State breeding program. It exhibits a somewhat bronze-colored glumes and awns. It is as tall as Vic but is characterized by very poor gluten quality despite a high protein content.

Lloyd: Short-strawed cultivar released in 1983 by the North-Dakota State breeding program. It is characterized by a fair quality, both in terms of yellow pigment content and gluten strength.

Monroe: Tall, early cultivar released in 1988 by the North-Dakota State breeding program. It is characterized by a good pigment content and its gluten quality is fair to questionable.

Renville: Tall cultivar released in 1988 by the North-Dakota State breeding program. It is characterized by an even better overall quality than Vic.

D 88450: Short-strawed elite breeding line from the North-Dakota State breeding program.

D 86741: Short strawed elite breeding line from the North-Dakota State breeding program.

Capelli: Tall, early, black awned cultivar developed in Italy where it was the leading cultivar until the end of the 1960s. It was selected in 1915 from a Tunisian population of the landrace "Jennah Khortifa". It is characterized by a very hard kernel and was considered to have good pasta-making quality in Italy.

Creso: Short, black awned, widely adaptable Italian cultivar released in 1974, and has dominated the durum wheat acreage during the 1970s and 1980s. It has overall acceptable quality characteristics, and is considered to have good to excellent pasta-making quality by the Italian pasta industry.

Valgiorgio: Short, early, black awned, Italian cultivar released in the early 1980s.

Valgerardo: Short, early, black awned, Italian cultivar released in the early 1980s. It is considered to have an acceptable pasta-making quality by the Italian pasta industry.

Valfiora: Short, early, black awned, Italian cultivar released the early 1980s.

Valnova: Short, black awned, Italian cultivar released in 1975. It is considered to have good to excellent pasta-making quality by the Italian pasta industry.

Brindur: Short, black awned, high yielding private cultivar developed in France. It is characterized by a large number of spikes per unit area resulting is relatively small kernels. It has an exceptionally high yellow pigment content and fair gluten quality.

Mondur: Tall, high yielding private cultivar developed in France. Characterized by an exceptionally high yellow pigment content, it also has good seed characteristics and a fair gluten quality.

Valdur: Tall, high yielding private cultivar developed in France. Same overall characteristics as Mondur.

Ambral: (D76018 / VALDUR) Short strawed, black awned, high yielding cultivar released in France and Italy. Its quality characteristics are fair.

Bread wheats:

Mac Kay: A Hard Red Spring, semi-dwarf cultivar released in Idaho in 1981. It has satisfactory milling and baking quality and is adapted to the Pacific Northwest.

Klasic: A Hard White Spring, short stature cultivar from Northrup King, released in 1980. It is a very early line with excellent grain characteristics and good milling and baking quality.

Florence Aurore: Old French Hard White spring cultivar, grown extensively in North Africa for its gluten strength and superior quality. It is early maturing, tall and awnless. It is characterized by high test weight and protein content.

Tanit: (TZPP /3/ PATO // INIA66'S' / NAPO /4/ 7C, released in 1980). A Hard White Spring line from a CIMMYT cross selected in Tunisia. It has a good yield potential and is characterized by a fair milling and baking quality.

Byrsa: A Hard White Spring line from a CIMMYT cross selected in Tunisia. It has a good yield potential but is characterized by a questionable gluten quality.

APPENDIX 2: **Summary of the weather data collected at the Pendleton Experimental Station, near the testing site, during the 1993 and 1994 seasons.**

1993

Month	Average Temperature (°C) Maximum	Minimum	Days with Temp. >35°C	Rainfall (mm)
January	0.2	-9.0	0	61.7
February	2.4	-6.0	0	26.4
March	10.1	-0.5	0	58.9
April	16.1	3.4	0	78.7
May	23.1	8.2	1	40.1
June	24.4	9.5	4	51.1
July	26.2	10.7	2	11.9
August	28.6	9.6	8	66.0

Total rainfall from January 1st to July 31st: 328.9 mm
Total rainfall from March 1st to July 31st: 245.4 mm

1994

Month	Average Temperature (°C) Maximum	Minimum	Days with Temp. >35°C	Rainfall (mm)
January	9.6	0.2	0	60.5
February	5.4	-3.2	0	42.4
March	14.7	-0.3	0	13.2
April	18.5	4.3	0	28.7
May	22.1	6.9	0	73.2
June	25.7	8.1	3	19.1
July	33.4	11.7	22	8.4
August	31.4	10.3	11	1.8

Total rainfall from January 1st to July 31st: 240.8 mm
Total rainfall from March 1st to July 31st: 142.5 mm

Shaded area correspond to the growing season from planting to harvest.

APPENDIX 3: **Filters and equations used to determine grain protein content (in durum wheat samples) and grain hardness by NIR using a Technicon 450.**

Grain protein Content:

Filter number	NIR wavelength	Computed Regression Coefficient
F_{00}	none	4.129
F_{10}	2180 nm	484.207
F_{14}	2100 nm	-319.762
F_{20}	1680 nm	-212.389

Number of samples used for calibration: 29
R-Squared for the fitted model: 93.81%
Standard error of estimate: 0.428
Estimated prediction error: 0.433

Grain Hardness:

Filter number	NIR wavelength	Computed Regression Coefficient
F_{00}	none	-316.750
F_{06}	2230 nm	2430.736
F_{20}	1680 nm	-2464.082

Number of samples used for calibration: 41
R-Squared for the fitted model: 98.04%
Standard error of estimate: 5.304
Estimated prediction error: 5.398

The filters selected were those recommended by the Federal Grain Inspection Service (F.G.I.S)

APPENDIX 4: Procedure used to perform the SDS micro-sedimentation test on ground wheat (adapted form Dick and Quick, 1983).

Stock solutions:

- SDS stock (2% w/v): 40 g SDS dissolved in 2 liters of distilled water.
- Lactic acid Stock: 10 ml of 85% lactic acid syrup mixed with 80 ml of distilled water

Working sedimentation reagent: 1 part lactic acid stock, 48 parts SDS stock (prepared fresh every day).

1- One gram of ground wheat (UDY-Cyclone mill, 1 mm opening size screen) was weighed into 150 x 16 mm borosilicate test tubes. Tubes were previously checked for inner diameter uniformity to ensure that height is an accurate indication of volume.

2- Tubes were grouped in sets of seven and attached with a rubber band so they make an hexagonal bundle that can be handled uniformly as one unit. These were placed horizontally on the bench and tapped lightly to expose as much of the ground wheat as possible thereby facilitating its suspension in water.

3- Four ml of distilled water were added to the tubes and the wheat was suspended by vortexing the bundles at high speed for 10 seconds.

4- Tubes were left to stand on the lab bench for 5 minutes, after which they were vortexed for 5 seconds.

5- Tubes were left to stand on the lab bench for an additional 5 minutes, after which they were vortexed for five seconds.

6- Twelve ml of a 1:48 lactic acid stock: SDS stock were immediately added to the suspensions, and the tubes were inverted 10 times.

7- Tubes were left to stand on the lab bench for 20 minutes, after which the height (in mm) of the sediment was measured with a ruler.