THE UNIVERSITY OF MANITOBA

RELATIONSHIP BETWEEN HIGH MOLECULAR WEIGHT SUBUNITS OF GLUTENIN AND BREADMAKING QUALITY OF CANADIAN GROWN WHEATS

by

Perry Kwok Wong Ng

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A thesis submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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TO MY PARENTS

Whoever loves discipline loves knowledge,
but he who hates reproof is stupid.

Proverbs 12:1 (RSV)

For the moment all discipline seems painful
rather than pleasant; later it yields the
peaceful fruit of righteousness to those
who have been trained by it.

Hebrews 12:11 (RSV)

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Preparing this thesis was clearly an experience of discipline. This thesis with its data, its ideas, its methods and theories may become forgotten or outdated, yet confrontation with such attributes as personal integrity, devotion to work, and struggle and joy in it leave a lasting impression.

ABSTRACT

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RELATIONSHIP BETWEEN HIGH MOLECULAR WEIGHT SUBUNITS OF GLUTENIN AND BREADMAKING QUALITY OF CANADIAN GROWN WHEATS.

Major Professor: Dr. Walter Bushuk.

Study of relationships between glutenin subunit composition and breadmaking quality requires a precise practical procedure for estimation of molecular weights (MWs) of the subunits. A modified method of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) was developed to improve the resolution of high molecular weight (HMW) glutenin subunits by using a combination of (a) high total concentration of acrylamide and bisacrylamide, (b) low concentration of cross-links between bisacrylamide and acrylamide, (c) low constant current, and (d) long running time.

Molecular weights of glutenin subunits of the wheat cultivar (cv.) Marquis were estimated by the modified SDS-PAGE using characterized proteins from other sources as MW markers. The Marquis subunits were then used as secondary standard reference proteins for determination of subunit MWs of other wheat varieties. Statistical analysis of replicate data for cv. Neepawa showed that the precision of the proposed method for estimating MWs was better than 1.5%. The main advantages of this proposed procedure are: (a) the reference proteins are handled in exactly the same way as the unknown wheat proteins, (b) the reference proteins are similar to the unknown proteins in chemical and physical properties, (c) the entire range of MWs is covered by a large number of reference proteins, and (d) the source of reference proteins

is inexpensive.

The relationship between HMW subunits of glutenin and breadmaking quality was investigated using the data for 26 varieties of the 1983 Uniform Quality Nursery (UQN). Preliminary study showed no differences in the HMW subunit patterns of wholewheat meal, flour, or extracted glutenin. Accordingly, flour was used as the starting material for routine analysis of wheat varieties by SDS-PAGE. Molecular mass in kilodaltons (kD) was adopted to identify each subunit for nomenclature and statistical analysis purposes.

Thirteen different HMW glutenin subunits, with MWs in the range from 90.0 to 147.4 kD, were identified from the SDS-PAGE patterns of the 26 varieties. Each variety had four or five of these HMW subunits. High quality varieties had a predominance of specific subunits. Subunits 128.1 and 91.6 were significantly correlated to good breadmaking quality whereas subunit 90.0 was significantly correlated to poor breadmaking quality.

Stepwise multiple regression analysis was used to develop seven prediction equations for breadmaking criteria, dough development time, mixing tolerance index, extensibility (E), maximum resistance (R), ratio of R and E, baking strength index, and loaf volume per unit protein, based on presence or absence of specific HMW glutenin subunits. The prediction equations were tested with data for a separate set of eight varieties of the 1985 Bread Wheat Cooperative Test. The equation for ULV using eight HMW glutenin subunits in the MW range from 96.3 to 147.4 ($r^2 = 0.669$; $P < 0.01$) gave predicted ULV values that agreed with actual values within a 95% confidence limit. The prediction equations had high predictive power for all quality parameters

except E, which indicated that factors other than glutenin contribute substantially to E.

The findings of the present study would be useful in developing an early-generation test for screening varieties in breeding programs, and in further research on the contribution of HMW glutenin subunits to breadmaking quality.

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I. INTRODUCTION

Wheat is the world's largest crop (Canada Grains Council, 1986) and provides almost 20% of the total food calories and proteins for the human race (Bushuk, 1982). For wheat exporting countries, such as Australia, Canada and the U.S.A., it contributes substantially to the gross national product. The major class of wheat is used in the form of bread; breadmaking quality is the key criterion of the commercial value of the crop.

It is generally accepted that it is the properties of the proteins present in wheat flour that dictate the wheat's suitability for processing into bread. These properties are collectively responsible for the so-called "protein quality" for breadmaking. It is now well established that glutenin, a fraction of flour protein according to the Osborne (1907) classification, contributes significantly to protein quality and is mainly responsible for the intervarietal differences in breadmaking potential (Orth and Bushuk, 1973b; Payne et al., 1980b). Glutenin comprises about 45% of the total endosperm protein and is made up of at least 15 polypeptide subunits obtained after reduction of its disulfide bonds (Khan and Bushuk, 1978). In order to assist the plant breeder in the development of new wheat varieties of improved breadmaking quality, it is essential to understand much more of the biochemistry and genetics of glutenin proteins and their subunits. Recently, attention has become focused on the relationship between high molecular weight (HMW) subunits of glutenin and breadmaking quality (Payne et al., 1980b).

The objective of this thesis project is to investigate further and in greater detail possible inter-relationship(s) between the com-

position of the HMW glutenin subunits and breadmaking quality of a number of wheat varieties grown in Western Canada. The research plan comprises the following sub-projects:

(1) Determination of the breadmaking quality of 26 1983 Uniform Quality Nursery (UQN) Canadian grown varieties with a broad range in quality.

(2) Development of a practical procedure for separating HMW glutenin subunits using polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE).

(3) Development of an appropriate nomenclature for HMW glutenin subunits.

(4) Investigation of the relationships between certain HMW glutenin subunits and technological criteria for the 26 UQN varieties.

(5) Development of prediction equations using the HMW glutenin subunit composition to predict certain technological parameters.

(6) Testing of the predictive power of the developed prediction equations by using selected wheat cultivars.

The results of this study are presented and discussed in this thesis.

II. LITERATURE REVIEW

A. Introduction

The main objective of this thesis project is to extend knowledge about the relationships between glutenins [more specifically high molecular weight (HMW) glutenin subunits] and breadmaking quality of bread wheats. Therefore, this literature review will cover in some detail those publications that are pertinent to the subject of glutenins and breadmaking quality, including such areas as the classification and preparative procedures of glutenin, and its structure. Since the main purpose of this study is to obtain information on the role of glutenin in breadmaking quality, the literature on the role of glutenin, and especially the HMW subunits of glutenin in breadmaking quality will receive special emphasis.

B. Definition and Preparative Procedures of Glutenin

The classical scientific fractionation of wheat proteins was first reported by Osborne (1907). He classified wheat proteins into four major fractions based on their differential solubility by sequential solvent extraction:

1. albumins---extractable in water;
2. globulins---extractable in saline;
3. gliadins---extractable in 70% to 90% aqueous alcohol;
4. glutenins---extractable in dilute aqueous acid or alkali.

The Osborne-type extraction for preparing glutenin, however, has some drawbacks since it is known that not all the glutenin is extracted by dilute acid or alkali (Kasarda et al., 1976). Because of

this, many different preparations have been suggested in the literature for preparing glutenin. The following will review some of the most commonly used preparative methods.

Jones et al. (1959) developed two methods for preparing glutenin. For both methods, they first defatted flour with water-saturated n-butanol and then prepared a gluten ball by washing the dough in 0.1% NaCl solution. In one of the methods, they then dispersed the washed gluten in 70% (v/v) ethanol to solubilize the gliadins. The suspension was then centrifuged and the residual protein was defined as glutenin. Their second method involved dispersing the washed gluten in 0.01 M acetic acid and then adding ethanol to make the dispersion 70% (v/v) in ethanol. The pH of the dispersion was then adjusted to 6.5 with 2 N NaOH. The protein that precipitated at 0° to 4°C and separated by centrifugation was defined as glutenin.

Woychik et al. (1964) prepared glutenin by a procedure similar to the second method of Jones et al. (1959). They dispersed wet gluten in 0.01 N acetic acid, then added ethanol to make the dispersion 70% (v/v) in ethanol and adjusted the pH to 6.7 with 1 N NaOH. The precipitate was separated by centrifugation, redissolved in dilute acetic acid and reprecipitated. The final precipitate was their glutenin.

Ewart (1972a) developed a procedure for preparing glutenin that was similar to the first method of Jones et al. (1959). He classified as glutenin the total residue after sequential extraction of flour with 0.04 M NaCl and 70% ethanol.

Meredith and Wren (1966) extracted the protein of flour using the strongly-dissociative AUC solvent (0.1 M acetic acid, 3 M urea, and

0.01 M cetyltrimethylammonium bromide); 95% of the total protein of flour was extracted by this solvent. They then separated the flour proteins in the AUC extract by gel chromatography on a 2.3 x 43 cm column of Sephadex G-200, and obtained four major peaks which they classified on the basis of elution volume. Proteins that eluted at 85 ml, at 85 to 145 ml, at 145 to 180 ml, and at 180 ml and beyond were considered to be, respectively, glutenins, gliadins, albumins, and non-protein substances.

Shogren et al. (1969) solubilized gluten proteins in 0.005 N lactic acid and precipitated glutenins by the addition of 0.1 N Na_2CO_3. They reported that, as the pH increased, the solubility of glutenins decreased.

Huebner (1970) used 0.2 M acetic acid to chromatograph proteins in 0.1 M acetic acid extracts of wheat flour on Sephadex G-100. He reported that three distinct peaks and an intermediate cut between peaks one and two were obtained. The four fractions corresponded to glutenins, ω-gliadins, gliadins, and albumins or globulins, respectively.

Orth and Bushuk (1973a) prepared their glutenin by pH precipitation and purified it with ion exchanger. They dispersed gluten in AUC, centrifuged the mixture, and decanted the supernatant. The supernatant was then mixed with ethanol to 70% (v/v) and its pH was adjusted to 6.4 by 1 N NaOH. The resulting precipitate (glutenin) was allowed to settle overnight at 2°C and then was separated by centrifugation. The precipitated glutenin was dispersed in 0.01 M acetic acid, dialyzed against distilled water for 5 days and then lyophilized to give the so-called "crude glutenin". The crude glutenin (0.2 g) was puri-

fied by dispersion into 50 ml AUC solvent containing 0.1 g of sulfoethyl-Sephadex ion-exchanger and centrifuged. The supernatant was dialyzed against water and lyophilized to yield "purified glutenin". The yield of "purified glutenin" was about 10% of the total protein.

Wasik and Bushuk (1974) published a preparation procedure for glutenin based on stepwise precipitations with ammonium sulfate from AUC extracts of flour. The precipitate obtained from the first addition of ammonium sulfate was dissolved in AUC and re-precipitated with a second equal addition of ammonium sulfate to yield "purified glutenin". The yield from this method was higher than the yield of Orth and Bushuk (1973a); 27% versus 10%, respectively.

Danno et al. (1974) compared the effectiveness of several solubilizing agents for flour proteins. The most effective agents were sodium dodecyl sulfate (SDS) and AUC. They reported 79% extraction of protein from flour with 0.5% SDS at pH 6.8 and only 72% extraction with AUC. They also reported that adding ß-mercaptoethanol or mercuric chloride to the 0.5% SDS solution resulted in almost complete extraction of proteins.

Bietz and Wall (1975) solubilized glutenins (residue proteins after salt and alcohol extractions) in several solvents: (a) acetic acid, (b) acetic acid-mercuric chloride, and (c) acetic acid-ß-mercaptoethanol. The fractions from the acetic acid and acetic acid-mercuric chloride extracts were further purified by the pH precipitation outlined by Jones et al. (1959). Bietz and Wall (1975) concluded that a series of solvents was required for a complete extraction of glutenins and that a pH precipitation should be used for preparation of purified glutenins.

More recently, Graveland et al. (1979 and 1982) developed a novel procedure for extracting glutenins from wheat flour. They used 1.5% SDS to separate flour proteins into SDS-soluble and SDS-insoluble fractions. The SDS-insoluble fraction was resuspended in water and separated into water-insoluble proteins (gel proteins or glutenins I), starch and glycoprotein.

By adding 70% ethanol to the SDS soluble fraction and chromatographing the supernatant, glutenins III, gliadins and albumins were obtained. The residue was dispersed in water resulting in a supernatant consisting of pentosans and globulins, and a second residue. This residue was extracted with 5 M urea to yield a supernatant designated glutenins II and a third residue which, with the aid of SDS and chromatography, separated into glutelins I, II, III and IV.

As can be seen the term "glutenin" has been used to refer to quite different groups of wheat proteins than that first described by Osborne (1907). Perhaps, as Lasztity (1984) pointed out, the names of the researchers who developed the method of preparation of glutenin should be used as a suffix (e.g., "glutenin prepared by the Orth and Bushuk method") and only the protein prepared according to Osborne's (1907) procedure should be called "glutenin".

C. Studies of the Structure of Glutenin

Numerous physico-chemical studies have been carried out to elucidate the structure of glutenin and its relationship to functionality in breadmaking. Wu and Dimler (1963a, b) reported that a very high proportion of glutamine was present in the amino acid compositions of glutenin---about one in every three amino acids. The presence of very

large amounts of glutamine suggests that there is most likely hydrogen bonding between polypeptides. On the other hand, there were relatively low proportions of amino acids in glutenin capable of carrying a net positive charge at acidic pH (e.g., arginine, lysine, and histidine). Thus, ionic interactions between polypeptides are unlikely.

Wu and Dimler (1964) investigated the structural properties of glutenin by using viscosity, sedimentation velocity ultracentrifugation, ultraviolet difference spectra, and optical rotatory dispersion studies. Based on their findings, they concluded that glutenin molecules were highly asymmetric. Subsequently Wu and Cluskey (1965) reported that glutenin had a low α-helix content in urea solution but a somewhat higher content in hydrochloric acid solution. Cluskey and Wu (1966), using the same technique (optical rotatory dispersion) on gliadin and glutenin in aluminum-lactate buffer, found that gliadin had a higher proportion of α-helix than did glutenin. Addition of urea produced a decrease in α-helicity of glutenin. They postulated that the secondary structure of glutenin was a mixture of random coil and α-helix, with the random coil being the predominant structure.

Pence and Olcott (1952) were possibly the first group to suggest that glutenin was made up of polypeptide chains and joined together by disulfide bonds. This conclusion was based on results of their experimental investigation of the effect of reducing agents on the viscosity of gluten.

It is known that addition of reducing agents increases the solubility of glutenin. Beckwith and Wall (1966) investigated redox reactions of glutenin, and observed a drastic decrease in viscosity right after addition of a reducing agent. This was followed, upon subsequent

standing, by a slight increase in viscosity. The initial decrease in viscosity was attributed to the cleavage of inter-polypeptide disulfide bonds and the slight increase was attributed to either unfolding of polypeptide chains after slower reduction of intra-polypeptide disulfide bonds or to non-covalent aggregation of the products. Oxidation of reduced glutenin at a concentration of 5% (w/v) gave a product that was similar to native glutenin in viscosity, molecular weight, and elasticity. Based on these findings, Beckwith and Wall (1966) concluded that it is essential for intra- and inter-polypeptide disulfide bonds to be present in an appropriate ratio in order to produce the visco-elastic properties of glutenin necessary for optimum functionality in the breadmaking process.

Ewart (1972a) and Stevens (1973) attempted to use specific chemical reagents to differentiate between inter- and intra-subunit disulfide bonds present in glutenin. Their results indicated that a large number of inter-subunit disulfide bonds are present in glutenin. However, Kasarda et al. (1976) pointed out that the interpretation of Ewart (1972a) and Stevens (1973) results was based on the assumption that inter-subunit disulfide bonds were reactive and that intra-subunit disulfide bonds were not reactive; this may not be the case.

Dalek-Zawistowska et al. (1975) conducted a very novel experiment. They dispersed gluten in acetic acid and subjected it to gel filtration chromatography on Sephadex G-200. They obtained two peaks, glutenin (excluded) and gliadin (included) peaks. Rechromatography of the glutenin peak again yielded two peaks, the second peak eluting at the same position as the gliadin peak in the first chromatography. The amino acid composition of the two "gliadin" fractions, however,

were different. When the protein from the gliadin peak obtained from the glutenin peak by rechromatography was radioactively labelled and incubated with unlabelled proteins from the glutenin peak and subjected to gel filtration chromatography again, they found radioactivity in the glutenin peak. On the basis of their results, they postulated that in solution glutenins existed in an equilibrium between a high molecular weight protein and low molecular weight proteins by way of aggregation and disaggregation.

More recently, Schofield and Baianu (1982) used cross-polarisation carbon-13 nuclear magnetic resonance (NMR) techniques to study wheat protein structures. In their experiments, a glutenin-enriched fraction gave sharp resonance peaks in the region of the NMR spectrum corresponding to aliphatic and aromatic amino acid side chains. The gliadin-enriched fraction gave broad peaks in this region. On the basis of this evidence, they suggested that the polypeptides in the gliadin-enriched fraction are much more tightly folded than those in the glutenin-enriched fraction. Further, they postulated that a much greater level of hydrophobic interactions exists between side chains in the gliadin-enriched fraction than in the glutenin-enriched fraction. Their findings indicated that hydrophobic interactions may not be that important in glutenin quaternary structure as some models have suggested. (Models of the structure of glutenin proteins will be discussed in the latter part of this section.)

Apart from studying glutenin structure, a number of researchers have carried out investigations on the molecular weight of glutenin and its subunits.

Jones et al. (1961) used the analytical ultracentrifuge to obtain

a weight-average molecular mass of glutenin which was 2 to 3 million daltons. Their preparations contained small molecules with molecular mass of about 50,000 daltons. They dissolved glutenin in highly dissociating solvents such as aluminum lactate and guanidine thiocyanate, and obtained very stable molecular weights in these solvents. They concluded that the large molecules were not aggregated particles but were true molecules.

Bietz and Wall (1972) studied the molecular weight distribution of components of reduced gliadin and glutenin proteins using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). They found that glutenin was comprised of about 15 subunits with molecular masses ranging from 11,000 to 133,000 daltons. Orth and Bushuk (1973b) reported approximately the same number of subunits for reduced glutenin of bread wheats determined by SDS-PAGE.

Bietz and Wall (1973) reported additional information on glutenin subunits. They found that 62% of reduced and alkylated glutenin was soluble in 70% ethanol. This fraction, analyzed by SDS-PAGE, consisted mainly of gliadin-like subunits with molecular masses of 44,000 and 36,000 daltons. However, the amino acid compositions of these subunits were definitely different from those of gliadin proteins.

In addition to the physico-chemical studies of glutenin, a number of studies on its ultrastructure by electron microscopy have been reported. Seckinger and Wolf (1970) obtained transmission electron micrographs of particles and surface dispersions of proteins from both hard and soft wheats. Particle diameters of 20 to 80 Angstroms were obtained from a purified glutenin fraction, representing molecular masses between 17,000 and 216,000 daltons. The glutenin fraction, how-

ever, did not form discrete particles.

The transmission electron microscope was used by Simmonds (1972) to demonstrate the presence of residual structures such as endoplasmic reticulum and amyloplast membranes in mature endosperm cells. He concluded that a proportion of the glutenin fraction originated from the endoplasmic reticulum. This interesting speculation has not been confirmed. The remainder of the glutenin was thought to originate from (a) the high molecular weight protein fraction in the endosperm of developing and mature wheat grain (Bushuk and Wrigley, 1971), and (b) the association of protein and lipid molecules when water was added to flour during dough formation (Simmonds and Wrigley, 1972).

Graham et al. (1962), Buttrose (1963), and Jennings et al. (1963) demonstrated by using transmission electron microscopy that storage proteins in wheat endosperm were deposited in protein bodies within lipoprotein membranes. The number and size of these protein bodies increased throughout grain development. This increase corresponded with an increase in the amount of acetic acid soluble glutenin (Jennings et al., 1963; Jennings and Morton, 1963a,b; Jennings, 1968). The protein bodies fuse together during grain desiccation so that it has not been possible to isolate discrete protein bodies from wheat flour.

Bernardin and Kasarda (1973a, b) used scanning and transmission electron microscopy to study wheat endosperm proteins and observed that storage proteins (gliadins and glutenins) were deposited in a laminar form in protein bodies. Upon hydration, the laminar structure was converted to a sheeted structure. When subjected to stress, as in dough mixing, these sheets ruptured forming fibrillar webs of proteins

which consisted of fibrils ranging in diameter from fifty to several thousand Angstrom units.

Although numerous studies have focused on glutenin structure, it is still not possible to assign a definite molecular structure to this protein. A number of different models have been proposed for glutenin based on the data available in the literature. The following section will review some of the more popular models.

The most common view of the structure of glutenin is that it consists of subunits cross-linked by inter-polypeptide disulfide bonds resulting in a high molecular mass of up to millions of daltons. However, one may ask whether the inter- or intra-polypeptide disulfide bonds are the major bonds that contribute to the high molecular weight of glutenin, or whether some other interactions/bonds are also involved.

Ewart (1968) presented the first model of glutenin molecules which was based on results obtained from the analysis of the number of disulfide bonds in glutenin in his earlier study (Redman and Ewart, 1967a, b). He proposed that glutenin was a linear molecule that consisted of polypeptide chains attached one to another by inter-polypeptide chain disulfide bonds. Ewart (1972a) was unsuccessful in explaining the rheological properties of dough based on the linear model. He later (Ewart, 1972b) proposed a more complex concatenated structure for glutenin. In this model, extensively folded polypeptide chains are joined by two inter-chain disulfide bonds to each of the neighboring polypeptides. A further modification of the concatenated structure of glutenin was proposed by Greenwood and Ewart (1975). This modification included unbranched molecules with a variable number of

polypeptide chains---not necessarily of the same type---joined together by disulfide bonds to form huge molecules with a linear-unbranched configuration. Greenwood and Ewart (1975) postulated that the elastic characteristic of glutenin arises from strong secondary forces at regions of interaction called nodes. This hypothesis was subsequently modified (Ewart, 1977) by reducing emphasis on these regions of interaction and increasing emphasis on a concept of sequential buildup of secondary forces to account for the unique elasticity in dough. In a recent report, Ewart (1979) reconciled this model of glutenin with his earlier linear model (for glutenin) on the assumption that one disulfide bond, not two, joins adjacent chains.

Contrarily, Kasarda et al. (1976) proposed that there are no inter-polypeptide disulfide bonds in glutenin, but that all of the disulfide bonds are the intra-polypeptide type. Secondary forces, such as hydrogen bonds and ionic and hydrophobic interactions, play a major role in forming insoluble glutenin. The authors pointed out that intra-polypeptide disulfide bonds constrain the subunits of glutenin into a specific conformation necessary for the aggregation. This specific conformation allows specific secondary forces to interact strongly between adjacent subunits to give the functional aggregate. The subunits have a globular shape and interact with each other to give fibril structures. This view was based on the observation of the reversible aggregation of A-gliadin, depending on pH and ionic strength, to form particles with molecular masses of several million daltons (Bernardin et al., 1967; Kasarda et al., 1967).

Another model was proposed by Lasztity et al. (1970 and 1972, cited in Lasztity, 1984). In this model, which is basically the same

as that of Ewart (1979), polypeptide chains are joined by one disulfide bond and the molecules essentially have a linear structure.

Recently, a slightly modified model was advanced by Graveland et al. (1985). They classified wheat glutenins into five molecular forms, namely glutenins I, IIa, IIb, IIIa and IIIb. Glutenin I is SDS-insoluble with an apparent molecular weight of 2×10^7 or more and is formed from SDS-soluble glutenins IIa and IIb, which are linked through constituent high molecular weight (HMW) A subunits. Glutenins IIa and IIb have average molecular weights of 8×10^5 and several million, respectively. Glutenin IIa comprises three HMW A subunits and four glutenin IIIa molecules, whereas glutenin IIb is a linear polymer of glutenin IIa molecules. Glutenins IIIa and IIIb have average molecular weights of 1.2×10^5 and 4.8×10^5, respectively. Glutenin IIIa consists of low molecular weight subunits (B and C), whereas glutenin IIIb is formed from four glutenin IIIa molecules linked by disulfide bonds. In this model, HMW A subunits act as a backbone linked by head-to-tail interchain disulfide bonds to form a linear chain for the structure of glutenins I and II.

A model for so-called functional glutenin was proposed by Khan and Bushuk (1978 and 1979) and Bushuk et al. (1980). In this model, the glutenin complex is formed by both inter- and intra-polypeptide disulfide bonds, and secondary forces such as hydrogen bonds and ionic and hydrophobic interactions. Based on their investigations, the authors postulated that two types of molecules are involved in forming the complex, namely glutenin I (subunits with molecular mass of 68,000 daltons or less) and glutenin II (subunits with higher than 68,000 daltons). Glutenins I and II are joined by at least one inter-polypep-

tide disulfide bond. The glutenin I subunits are held by secondary forces through protein-protein interactions, protein-lipid interactions and protein-carbohydrate interactions. These subunits account for the viscous flow. On the other hand, the glutenin II subunits contribute the elasticity in dough through disulfide cross-linkages between polypeptide-chains.

D. Glutenin and Breadmaking Quality

Since the turn of the century, it has been common knowledge that high protein wheats produced better bread (i.e., higher loaf volume). It was shown by Aitken and Geddes (1938 and 1939) that the breadmaking quality factor was in the gluten. The next major milestone was the work of Finney and Barmore (1948) who showed that breadmaking potential depended directly on protein content.

Protein content itself is a product of various factors acting in concert. Given the same environmental conditions, some cultivars have the genetic capacity to produce grain with higher protein content than other cultivars. And for grain of any given cultivar, the protein content depends strongly on environmental conditions during growth. Additionally, for most wheat cultivars, although not all, higher protein content is normally correlated with lower yield.

Finney and Barmore (1948) showed that loaf volume (in an optimized baking test) and protein content had a linear relationship within the limits of protein content encountered, i.e., 8% to 18%. When loaf volume was plotted against protein content for samples of the same or similar wheat varieties, the slope of the regression line varied among varieties. Varieties that had high breadmaking potential

were characterized by high slope values. This work was confirmed by Bushuk et al. (1969).

Since the first report of the linear relationship between loaf volume and protein content by Finney and Barmore (1948), many cereal chemists have devoted considerable effort to the search for the fundamental reasons for the variation in the slope of the regression line for the relationship between loaf volume and protein content among wheat varieties. This variation of the slope reflects the so-called "protein quality" of wheat. This protein quality is primarily a genotypic trait of a variety.

One of the first reports on glutenin and breadmaking quality was from Pomeranz (1965). He found that poor breadmaking cultivars contained a greater proportion of proteins extractable with 3 M urea solution (albumins, globulins, gliadins, and soluble glutenins) than did good breadmaking cultivars, or conversely, that the good breadmaking cultivars contained a higher proportion of urea-unextractable proteins (mostly insoluble glutenins). These results were later confirmed by baking studies on reconstituted flours (Shogren et al., 1969).

Hoseney et al. (1969) studied the role of various protein fractions in breadmaking quality. They fractionated gliadin and glutenin with 70% ethanol from gluten. Experiments on reconstituted flours showed that glutenin fractions governed the mixing requirement of a wheat flour and that gliadin fractions controlled the loaf-volume potential of a wheat flour. It should be pointed out, however, that Hoseney et al. (1969) used two samples for their reconstituted experiments, one pure flour from a single variety and one mixed flour from many composited varieties. Thus, their findings are inconclusive inso-

far as interpreting differences in breadmaking quality in terms of intervarietal differences in gliadin proteins.

Huebner (1970) studied the salting-out (precipitation) response of glutenins from eleven wheat cultivars with different qualities of breadmaking. The salting-out response suggested that the protein quality was related to the sensitivity of its glutenin to changes in ionic strength. Glutenins from good breadmaking cultivars had steep precipitation curves. Huebner (1970) suggested that glutenins of good bread quality cultivars had a greater proportion of high molecular weight glutenins and were therefore more easily salted out of solution.

Lee and MacRitchie (1971) used the mixograph and the alveograph to study the effect of flour protein fractions on rheological properties of dough. Flour was sequentially extracted with water, 2 M urea, 4 M urea and 0.1 N NaOH. When the fraction extracted by 2 M urea (gliadins and soluble glutenins) was added to a base flour, the mixing stability was decreased and doughs were weaker and more extensible. On the other hand, the fractions extracted with 4 M urea and NaOH (mainly glutenins) increased mixing stability and produced stronger doughs.

The first comprehensive study of the relationship between protein fractions obtained by the Osborne solubility fractionation and breadmaking quality was reported by Orth and Bushuk (1972) and Orth et al. (1972). The flour proteins of 26 wheat cultivars of diverse baking quality grown in four locations in Western Canada were fractionated. They found that loaf volume (used as an index of breadmaking quality) was inversely related to the proportion of the acetic acid soluble glutenin and directly related to the proportion of the insoluble glutenin (residue protein). Furthermore, these relationships were

not dependent on environment. Thus they suggested that these relationships could be considered to be one of the inherited properties (intervarietal characteristics) determining breadmaking quality. Orth and Bushuk (1972) concluded that, at constant protein content, glutenin was responsible for the differences in breadmaking quality between varieties of bread wheat.

Orth et al. (1973) used scanning electron microscopy (SEM) to demonstrate the relationship between the ultrastructure of glutenin and breadmaking quality. They observed that freeze-dried glutenin from two hard red spring wheat cultivars and a synthetic hexaploid (all of good breadmaking quality) consisted of fibrous structures with long and thick strands. In contrast, glutenin from two poor breadmaking quality flours, durum wheat and rye, consisted of broad ribbon-like and short rod-like structures, respectively. Glutenin from an intermediate breadmaking quality flour, a triticale derived from durum and rye parents, consisted of both ribbon- and rod-like structures. Further work, however, is required to determine the exact relationship between molecular structure and ultrastructure of glutenin as seen in the SEM, and their relationship to breadmaking quality.

Huebner and Wall (1976) used agarose gel filtration columns (Sepharose 2B and 4B) to fractionate glutenin from cultivars of diverse breadmaking quality into two fractions, I and II. They found that fraction I was a high molecular weight (HMW) fraction and fraction II a low molecular weight (LMW) fraction of glutenin. The glutenin I and II fractions differed only slightly in amino acid composition and in SDS-PAGE band patterns of their subunits. However, the ratio of glutenin I to glutenin II was generally higher for good breadmaking culti-

vars. These observations also agreed with Orth and Bushuk (1972) who found that the good breadmaking cultivars contained the highest amounts of unextracted protein (insoluble glutenin or residue protein). Huebner and Wall (1976) postulated that a sufficient total amount of protein and suitable proportions of the two glutenin fractions were essential for a good baking flour.

Arakawa and Yonezawa (1975) and Arakawa et al. (1977) studied the relation between flour quality and aggregation behavior of glutenin. They found that the aggregation behavior of glutenin depended on its polypeptide composition. They used gel filtration (Sephadex G-100) to fractionate gluten proteins into glutenins and gliadins. Glutenins were further fractionated on a Sephadex G-150 column to obtain three fractions, I, II and III. They found that strong mixing flour was rich in fraction II and gave a high value of T_{10}/C (where T_{10} was the turbidity at 10 minutes of reaction time and C was the protein concentration). In contrast, the weak mixing flour was found to be poor in fraction II and had a low value of T_{10}/C.

Booth and Melvin (1979) studied the effect on breadmaking quality of interchanging flour components of a Canadian hard red spring wheat (good breadmaking quality) with those of a European wheat (e.g., "Maris Huntsman", poor breadmaking quality). They found that the key component which controls the breadmaking quality was in the gluten, but not in the starch or the water-soluble fraction. "Maris Huntsman" had a much lower proportion of protein insoluble in lactic acid solution (residue protein) than did the Canadian wheat. This finding agrees, in general, with the report by Orth and Bushuk (1972). Booth and Melvin (1979) found no improvement in loaf volume when protein

levels were increased with residue protein obtained from "Maris Huntsman". Another study done on good and poor quality Australian wheats gave similar results (MacRitchie, 1978).

E. Studies of High Molecular Weight (HMW) Subunits of Glutenin and Breadmaking Quality

Apart from showing that glutenin is an important protein component in breadmaking, a great deal of research has focused on a more precise issue, namely the relationship between the subunit composition of glutenin and breadmaking quality. Bietz and Wall (1972) used SDS-PAGE to show some quantitative differences in the subunit composition of reduced glutenin from good and poor breadmaking varieties. However, the actual relationship between these differences and breadmaking quality was not clear. Similarly, results reported by Orth and Bushuk (1973b) indicated there was no apparent relationship between the SDS-PAGE band patterns of the reduced glutenin and breadmaking quality; wheats with essentially the same SDS-PAGE patterns had substantially different breadmaking qualities. These negative findings may be due to the fact that the SDS-PAGE technique was only in its early stages of development.

Subsequently, a great amount of work was done in the Plant Breeding Institute of Cambridge, U.K. showing a very strong relationship between the composition of HMW glutenin subunits and breadmaking quality.

Payne et al. (1979) found two HMW glutenin subunits present in the variety "Maris Widgeon" (good breadmaking quality) but not in the variety "Maris Ranger" (unsuitable for breadmaking) by SDS-PAGE.

These two varieties were crossed, and after several generations a progeny of good breadmaking quality was selected and named "Maris Freeman". Only one of the two HMW glutenin subunits present in "Maris Widgeon" was found in "Maris Freeman". This HMW glutenin subunit was termed glutenin subunit 1 which had a molecular mass of about 145,000 daltons. In the same study, Payne et al. (1979) screened 67 varieties for the presence of glutenin subunit 1 and found it in 31% of the varieties. When the relative amount of glutenin subunit 1 of these varieties was correlated to their breadmaking quality according to the SDS-sedimentation test (Axford et al., 1978), they found a significant correlation between the two ($r = 0.72$; $p < 0.001$). Similar results were also reported by Burnouf and Bouriquet (1980). They found the breadmaking quality of a stock of 47 genetically related varieties was correlated with two HMW subunits of glutenin (122,000 and 108,000 daltons).

In another study, Payne and Corfield (1979) fractionated 12 glutenin subunits with molecular masses ranging from about 31,000 to 136,000 daltons. They divided these subunits into three groups, A, B and C. Group A subunits had approximate molecular masses of 136,000, 127,000, 106,000, and 95,000; group B, 51,000, 46,800, 43,900, and 42,000; and group C, 35,500, 34,500, 33,000, and 31,500 daltons. It seemed the elasticity of glutenin was due to at least these 12 subunits.

In subsequent studies (Payne et al., 1980a), 12 HMW subunits were resolved from 7 varieties and had nominal molecular masses of between 95,000 and 145,000 daltons. They numbered these HMW subunits from 1 to 12. Then a year later, Payne et al. (1981a) found a few more

subunits from other varieties with molecular weights between those of subunits 6 to 9. They numbered these new subunits 13 to 16. In their studies (Payne et al., 1980b, 1981a), they determined that (a) subunit 1 was coded for by genes on chromosome 1A and (b) subunits 5 and 10 were coded for by 1D. Furthermore, subunits 5 and 10 had a stronger relationship to breadmaking quality than subunit 1. When all three subunits were present, the mean sedimentation volume was 66.1 ml; those with only subunit 1 and lacking subunits 5 and 10 had a mean volume of 48.7 ml; and those with subunits 5 and 10 and lacking subunit 1 had a mean volume of 51.8 ml. These differences were significant ($p < 0.001$) and indicated that an additive effect may play a role.

Recently, Branlard and Dardevet (1985) carried out a more extensive study on the relationship between breadmaking qualities and HMW subunits from 70 wheat cultivars. They used simple correlations to find the relationships between HMW subunits and several flour quality characteristics: the Chopin Alveograph strength (W), tenacity (P), swelling (G) and extensibility (L), the Zeleny sedimentation volume (Z) and the Pelshenke swelling time (Pe). They used the nomenclature of Payne et al. (1980a, 1981b) to assign numbers to the subunits. All these characteristics were correlated with at least 4 subunits, excluding subunits 6 and 8. These two subunits were not correlated significantly with any of the characteristics. They found that characteristics W, P, Z and Pe were positively correlated to subunits 5 and 10, and negatively to subunits 2 and 12. Subunit 9 was correlated positively to W, P, and Z; subunits 1 and 2* were correlated positively with G and W, respectively. Furthermore, they used a stepwise regression analysis to predict each of the flour quality characteris-

tics in terms of HMW glutenin subunits. They reported that 40% of the variation in W, 41% of the variation in Z and 46% of the variation in Pe was explained by eight, seven and six subunits, respectively.

The review of the literature presented above clearly indicates that HMW glutenin subunits have certain definite relationships to specific flour quality characteristics. The ultimate goal of the previous studies performed was to improve wheat quality, namely to improve the breadmaking quality. The present study was designed to extend that work by adding more precise information about the relationships between HMW glutenin subunits of wheats grown in Western Canada and their breadmaking qualities.

III. MATERIALS AND METHODS

A. Materials

1. Wheat Samples

The main set of wheat samples used for this study was the 1983 Uniform Quality Nursery (UQN) with 26 varieties. Their pedigrees and origins are presented in Table 1. The 1983 UQN was grown at four locations in Western Canada: the Lethbridge, Regina, Saskatoon and Swift Current Research Stations, Agriculture Canada, and samples were provided by Dr. A.B. Campbell of the Winnipeg Research Station, Agriculture Canada.

Grain of the Canadian hard red spring cultivar (cv.) Marquis was from the I.C.C. (International Association for Cereal Science and Technology) Standard Sample (for wheat variety identification by gliadin electrophoresis) maintained in the Department of Plant Science, University of Manitoba. Grain of the British cv. Holdfast was provided by Dr. P. Payne of the Plant Breeding Institute, Cambridge; of the German cv. Diplomat by Dr. B. Fretzdorff of the Federal Research Institute for Cereal and Potato Processing, Detmold, FRG; and of the Australian cv. Halberd by Dr. C.W. Wrigley of the CSIRO Wheat Research Unit, Sydney, Australia.

Five wheat samples of the 1985 Central Bread Wheat Cooperative Test were provided by the Grain Research Laboratory, Canadian Grain Commission. These were cvs. Marquis, Neepawa, Sinton, Benito and Columbus. Three wheat samples of the 1985 Western Bread Wheat Cooperative Test were provided by the Winnipeg Research Station, Agriculture Canada. These were cvs. Katepwa, Leader and Lancer. Pedigrees and

Table 1. 1983 Uniform Quality Nursery varieties

No.	Variety	Cross/Pedigree and Origin
1	Cypress	Rescue/Chinook, Canada
2	Neepawa	Thatcher*7/Frontana//Thatcher*6/ Kenya Farmer/3/Thatcher*2// Frontana/Thatcher, Canada
3	Kenya 321.BT.1.B.1	Australia 45C5/Kenya 117A, Kenya
4	Columbus	Neepawa*6/RL 4137, Canada
5	Cook	WW31/Timgalen, Australia
6	Tobari/Romany	Tobari 66=Tezanos Pintos Precoz/ Sonora 64A, Mexico Romany=Colotana 261//51/ Yaktana 54A, Kenya
7	Neelkant sib	HD1220/KAL*3//NAC, Mexico
8	Veery[1] #4	CM33027-F-12M-1Y-10M-1Y-3M-1Y-0M, Mexico
9	Veery[1] #5	CM33027-F-15M-500Y-0M, Mexico
10	Nacozari 76	TZPP/PL//7C, Mexico
11	Veery[1] #1	CM33027-F-8M-1Y-8M-1Y-2M-0Y, Mexico
12	Veery[1] #2	CM33027-F-12M-1Y-4M-2Y-2M-0Y, Mexico
13	Veery[1] #3	CM33027-F-12M-1Y-6M-0Y, Mexico
14	Bobwhite sib	AU//KAL/BB/3/WOP"S", Mexico
15	Hork-Ymh x Kal-Bb	Hopps/Robin//Kalyan, Mexico
16	Veery[1] sib	Mexico

[1]Veery KVZ/BUHO"S"//KAL//BB, Mexico
KVZ = Kavkaz, USSR
BUHO = SR/3/LR 64/Inia 66//Inia 66//BB, Mexico
KAL = Kalyansona, India
BB = Bluebird, Mexico

Table 1. Cont.

No.	Variety	Cross/Pedigree and Origin
17	----	Gll-AustII61-157//Cno/No/3/ Rm "S", Mexico
18	Pavon 76	VCM//CNO"S"/7C/3/KAL/BB, Mexico
19	Oxley	Penjamo 62/Gabo 56*4//Tezanos Pintos Precoz/Nainari 60/4/ Lerma Rojo*2//Norin 10/Brevor 14/ 3/Andes*3, Australia
20	Olympic	Baldwin/Quadrat, Australia
21	Halberd	Scimitar/Kenya C6042//Bobin/3/ Insignia-49, Australia
22	Condor	Carstens VI/?/Dankowes/Carstens V /?/Wjatka, Germany
23	Chile	Unknown, Tunisia
24	HY 334	Pitic 62/ACEF-125//S 5713-232, Canada
25	IAS 5	Unknown, Brazil
26	SUN 43A	Unknown, Australia

origins of these two sets of samples are presented in Table 2.

Four varieties of the 1981 UQN, cvs. Tesopaco, Neepawa, James and Glenlea, used in the study of differences between high molecular weight (HMW) subunits of glutenin of varieties of widely different baking quality, were provided as flour by Dr. A.B. Campbell of the Winnipeg Research Station, Agriculture Canada.

2. Reagents and Chemicals

Primary standard reference proteins (Table 3), glycine, Tris [tris(hydroxymethyl)aminomethane], glycerol, potassium hydroxide, Coomassie Brilliant Blue G-250 and Coomassie Brillant Blue R-250 were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A.. Acrylamide, bisacrylamide (N,N'-methylene-bisacrylamide) and sodium dodecyl sulfate (SDS) were of electrophoretic grade and were obtained from Bio-Rad, Richmond, California, U.S.A. All other chemicals used were of analytical reagent grade. Distilled deionized water was used in all experiments.

B. Preparation of Composite Samples and Milling

Samples of each variety of the 1983 UQN from the four locations were blended to form a uniform single sample after checking the purity of each sample by electrophoresis. Four hundred g of grain from each location was used to give a composite of 1600 g. The grain of variety 5 (cv. Cook) from the Regina location was discarded because it was a mixture of several varieties (see IV-A). Accordingly, 533.3 g from each of the other three locations of variety 5 were used to give a total of 1600 g.

For milling, the wheat was tempered to 15.5% moisture content by

Table 2. Cultivars from the 1985 Bread Wheat Cooperative Test

Cultivar	Pedigree and Origin
Marquis	Hard Red Calcutta/Red Fife, Canada
Neepawa	Thatcher*7/Frontana//Thatcher*6/Kenya Farmer /3/Thatcher*2//Frontana/Thatcher, Canada
Sinton	Manitou/3/Thatcher*6/Kenya Farmer//Lee*6/ Kenya Farmer, Canada
Benito	Neepawa/3/RL4255*4//Manitou/C17090, Canada
Columbus	Neepawa*6/RL4137, Canada
Katepwa	Neepawa*6/RL2938/3/Neepawa*6//C.I.8154/2* Froncor, Canada (RL2938=Lee*2/Kenya Farmer)
Leader	Fortuna/Chris, Canada
Lancer	Turkey/Cheyenne//Hope/2*Cheyenne, U.S.A.

Table 3. Primary standard reference proteins

Protein	MW x 10^{-3}
carbonic anhydrase	29.0
albumin, egg	45.0
albumin, bovine	66.0
phosphorylase B	97.4
ß-galactosidase	116.0
myosin	205.0

blending in appropriate amounts of water and allowing to stand for 24 hr at 21°C. The grain of the UQN samples was milled into straight-grade flour on a Buhler pneumatic laboratory mill. The flour was rebolted through a 70 GG (236μ) screen and blended before use. Grain of Diplomat, Halberd, Holdfast and the varieties of the Cooperative Test were milled on a Brabender Quadromat Junior mill because of the small sample size. Wholewheat meal samples were prepared on a Udy Cyclone mill (Udy Analyzer Co., Colorado, U.S.A.).

C. Polyacrylamide Gel Electrophoresis (pH 3.1)

Polyacrylamide gel electrophoresis (PAGE) was done on a vertical apparatus as described by Sapirstein (1984). The procedure was that of Sapirstein and Bushuk (1985), with some minor modifications, based on the original procedure of Bushuk and Zillman (1978).

1. Gliadin Extraction

For electrophoresis, 10 g of grain was ground on the Udy Cyclone mill. This amount was considered to be a representative sample of the larger sample available. Then 100 mg of each ground product was transferred into a 1.5 ml microcentrifuge tube and extracted with 200 μl of 70% ethanol by mixing briefly on a Vortex mixer. The mixture was left to stand at room temperature for at least 15 min. The contents were then centrifuged for 2 min at 10,000 x g at room temperature using a tabletop Beckman Microfuge B centrifuge. After centrifugation, an aliquot of the clear supernatant (100 μl) was diluted with 2 times its volume of extract dilution solution and stored in a sealed vial in a refrigerator until used. The extract dilution solution consisted of electrode buffer (see the following section, III-C-2) containing

40% w/v sucrose and 0.5% w/v methyl green dye.

2. Electrophoresis

The apparatus constructed in the laboratory according to Sapirstein (1984) uses 100 ml of gel solution to form a slab 15x20x0.3 cm (WxHxT). Detailed recipes for the gel solution and electrode buffer are presented in Table 4.

The gel solution comprised 0.25% w/v aluminium lactate adjusted to pH 3.1 with lactic acid (electrode buffer), 6% w/v acrylamide, 0.3% w/v bisacrylamide, 0.1% w/v L-ascorbic acid, and 0.0015% w/v ferrous sulfate heptahydrate. The gel was polymerized by addition of 0.015% v/v hydrogen peroxide. After polymerization, the apparatus was oriented in the vertical position and tank reservoirs were each filled with 250 ml of electrode buffer.

Gliadin extracts (10 µl/slot) were applied (11 slots/gel slab) with a micro-pipette. The loaded gel was subjected to electrophoresis for approximately 4 hr at a constant current of 60 mA and at 20°C. Electrophoresis was terminated when the second marker dye band of methyl green migrated to the end of the gel.

3. Gel Staining and Photography

Gels were stained overnight in a gel staining solution containing 1% w/v Coomassie Brilliant Blue R-250 (dissolved in 10 ml of 95% ethanol and filtered) in 240 ml of 12% (w/v) trichloracetic acid (TCA) with gentle shaking on a Junior Orbit shaker. The gels were destained for approximately 4 hr in 12% TCA with gentle shaking. The gels were then photographed using Kodak Technical Pan 2415 film. Prints were developed with HC110 developer used at a dilution of one part devel-

Table 4. Recipes for gel and electrode buffer solutions for PAGE

	Amount required for 100 ml final volume
Gel solution	
Acrylamide	6.0 g
Bisacrylamide	0.3 g
L-ascorbic acid	0.1 g
Ferrous sulfate heptahydrate	0.0015 g
Hydrogen peroxide[1], 3%	0.5 ml
Electrode buffer solution	
Aluminium lactate	0.25 g
Lactic acid	to pH 3.1

[1]Practical grade (3%) of hydrogen peroxide was purchased at a local pharmacy.

oper to 9 parts water to obtain a medium degree of contrast.

D. Preparation of Glutenin

Glutenin was prepared by the differential solubility method of Payne et al. (1979), with some minor modifications. Eight g of whole-wheat meal were defatted with 200 ml of n-hexane by stirring for 1 hr at room temperature. The suspension was then filtered on a Buchner funnel through Whatman No. 1 filter paper. The residue was re-suspended with another volume (200 ml) of n-hexane for 1 hr and filtered as above. The final residue was air-dried at room temperature until solvent odor was no longer detectable (overnight).

To remove the soluble proteins, the dried residue was stirred with 400 ml of 0.5 M NaCl for 1.5 hr at 4°C, and centrifuged at 30,100 x g for 20 min at 4°C. The pellet was re-suspended in 400 ml of distilled deionized water at 4°C and re-centrifugated as above. Gliadin was extracted from the residue with 400 ml of 70% (v/v) ethanol at 4°C with stirring for 4 hr at 4°C. The mixture was centrifuged at 30,100 x g for 20 min at 4°C. The pellet was re-dispersed into another 400 ml of 70% (v/v) ethanol and re-centrifuged as above. The final pellet was frozen and freeze-dried. The freeze-dried product (hereafter referred to as extracted glutenin) was stored at -20°C for future use.

E. Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE[1] was done on an LKB 2001 Electrophoresis Unit. The pro-

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[1] A practical routine procedure is presented in Appendix 1.

cedure was that of Payne et al. (1979 and 1980a), with some minor modifications, based on the original procedure of Laemmli (1970).

1. Glutenin Solutions for SDS-PAGE

Glutenin solutions for SDS-PAGE were prepared by suspending 40 mg of wholewheat meal or flour, or 6 mg of extracted glutenin (see section III-D) in 1 ml buffer solution. The buffer solution comprised 0.063 M Tris/HCl at pH 6.8, 2% (w/v) SDS, 5% (v/v) ß-mercaptoethanol, 10% (v/v) glycerol, and 0.01% (w/v) Pyronin Y. Each mixture was allowed to stand at room temperature for 2 hr with occasional shaking on a Vortex mixer, then heated for 2.5 min in a boiling water bath and allowed to cool to room temperature. The suspension was allowed to settle and an aliquot of the clear top layer was used as the experimental glutenin protein extract.

A solution of reference proteins (Table 3) was prepared similarly by dissolving 3 mg of the protein mixture in 1 ml of the buffer.

In the early part of this study, the amount of protein solution that was loaded into each slot was 15.0 µl for extracts of wholewheat meal and flour, 10.0 µl for extracted glutenin, and 5.0 µl for the reference proteins. Subsequently, it was found that slightly better resolution was obtained by adjusting the volume of extract according to the original protein content of the wheat. Accordingly, for routine analysis by SDS-PAGE, 7.5 µl was the extract volume for wheats grown in Canada and 10.0 µl for cvs. Diplomat, Halberd, and Holdfast.

Furthermore, preliminary experiments (see section IV-B) showed that the glutenin patterns of each variety from wholewheat meal, flour and the extracted glutenin preparation were qualitatively identical

(see section IV-B). Accordingly, glutenin solutions for SDS-PAGE were routinely prepared from flour.

2. Electrophoresis

The LKB 2001 Electrophoresis Unit can accommodate 2 gels for each run. The size of each gel is 14x16x0.15 cm (WxHxT).

The separating gel solution comprised 0.375 M Tris/HCl buffer solution at pH 8.8 and 17.33% (w/v) acrylamide and bisacrylamide. The concentration of bisacrylamide was 0.45% of the total acrylamide and bisacrylamide. The gel solution was deaerated, then 0.1% (w/v) SDS was added, followed by addition of 0.025% (w/v) ammonium persulfate and 0.05% (v/v) TEMED (N,N,N',N'tetramethyl ethylene diamine). The mixture was immediately poured into the electrophoresis unit to a height of 14 cm and allowed to polymerize.

The stacking gel solution contained 0.125 M Tris/HCl buffer solution at pH 6.8 and 3.03% (w/v) acrylamide and bisacrylamide. The concentration of bisacrylamide was 1.43% of the total of acrylamide and bisacrylamide. The gel solution was deaerated, 0.1% (w/v) SDS was then added, followed by addition of 0.0375% (w/v) ammonium persulfate and 0.075% (v/v) TEMED, then immediately poured into the electrophoresis apparatus to a height of 1 cm; the 15-place slot former was inserted and the solution allowed to polymerize.

Electrode buffer solution (pH 8.3) comprised 0.025 M Tris, 0.192 M glycine and 0.1% (w/v) SDS. The gel, usually loaded with 13 samples (outer slots were not used), was subjected to electrophoresis for 22 hr at 20°C at a constant current of 5 mA (per gel) for the first 2 hr, followed by 18 hr at 10 mA and finally for 2 hr at 15 mA.

The electrode buffer was circulated continuously during electrophoresis. After 22 hr, the tracking dye, Pyronin Y, runs off the gel. It was included in the sample extract solution to facilitate addition of the otherwise colorless solution to the slot.

3. Gel Staining and Photography

Gel staining solution was prepared according to Blakesley and Boezi (1977). The solution was prepared as follows: One g of Coomassie Brilliant Blue G-250 was dissolved in 500 ml H_2O; this was then mixed with 500 ml of 2 N H_2SO_4 (54.5 ml conc. H_2SO_4 in 1000 ml H_2O). The mixture was allowed to stand for at least 4 hr and then filtered through Whatman No. 1 filter paper; 110 ml of 10 N KOH was added to the filtrate. Finally TCA was added to a final concentration of 12% (w/v). The resulting solution was filtered through Whatman No. 1 filter paper and stored in a dark bottle.

Gels were stained overnight with gentle shaking. The gels were then rinsed with distilled deionized water, and photographed immediately through an orange filter using Kodak Technical Pan 2415 film. Prints were developed with HC110 developer used at a dilution of one part developer to 9 parts water to obtain a medium degree of contrast.

F. Estimation of Relative Molecular Weight for High Molecular Weight (HMW) Subunits of Glutenin from SDS-PAGE

The procedure for estimating the molecular weights (MWs) of glutenin subunits of wheat samples was according to the original method of Weber and Osborn (1969) with some modifications. The relative mobilities (Rm) of the primary standard reference protein bands were calculated using the mobility of the smallest protein (carbonic

anhydrase) as the front marker (instead of the tracking dye band as in the standard procedure). Mobilities were determined from the photograph of the stained gel. This obviated errors, due to shrinkage or swelling of the gels, in mobilities measured directly on the gels. Rm was calculated as follows:

protein migration (cm)/carbonic anhydrase migration (cm).

Two different relationships were examined to obtain the MW vs. mobility calibration curve of the primary standard reference proteins (see section IV-E-1). In addition to the commonly-used plot of log MW vs. Rm, a plot of log MW vs. log Rm was examined and found to give a better regression line than the commonly-used relationship.

On the basis of the experience gained in this study, it was decided that the subunits of a reference wheat variety would be more suitable as MW markers than the proteins from other sources. The glutenin subunit of MW 34,600 of the historical wheat variety Marquis was adopted as the front marker for calculating Rm values (see IV-E-2), and for estimating MWs of the HMW glutenin subunits of all wheat varieties studied in this thesis project. The experimental mobilities were measured from the photograph of the stained gel. Rm was then calculated as follows:

protein migration (cm)/migration of subunit 34,600 (cm).

A calibration curve based on the Marquis glutenin marker proteins was derived from the relationship of log MW and log Rm for 13 major glutenin subunits. In the analysis of experimental samples, three slots (two outer and centre) were used for the reference protein (cv. Marquis) to check for uniformity of the gel. The mobilities of the subunits in the centre slot were used to develop the regression

equation for estimating MWs of the subunits of experimental varieties analyzed in a particular gel.

G. Technological Tests

1. Protein Content

Total nitrogen of flour or wholewheat meal was determined by the macro-Kjeldahl method (method 46-12, AACC 1983). Protein content was obtained by multiplying the nitrogen content by the conversion factor 5.7 according to Tkachuk (1969).

2. Test Weight

Test weight was determined on a dockage-free sample using an Ohaus Test Weight Apparatus with a 0.5 liter container. The total weight in kg of two measured 0.5 liter (i.e., 1 liter) containers of grain was multiplied by 100 to obtain the equivalent kilograms per hectoliter. The average of three measurements was reported on an "as is" moisture basis.

3. Grain Hardness

The hardness of a grain was determined by the grinding time test according to Kosmolak (1978). The grinding time was the time required to grind 5 g of wheat to meal in a burr mill. The harder the grain, the shorter the grinding time in seconds.

4. Moisture Content of Grain and Flour

The moisture content of whole grain samples was determined according to the AACC standard method (method 44-11, AACC 1983), using an electronic moisture meter (Canadian Aviation Electronics Ltd. model

CAE 919). The moisture content of the flour was determined according to the AACC standard method (method 44-15A, AACC 1983) using a Brabender Rapid Moisture Tester.

5. Ash Content of Flour

Ash content of flour was determined according to the AACC standard method (method 08-01, AACC 1983) in which the flour was incinerated at 560°C.

6. Sedimentation Value

Sedimentation test value was determined according to the AACC standard method (method 56-60, AACC 1983).

7. Falling Number Value

The Falling Number Value was determined according to the AACC standard method (method 56-81B, AACC 1983) using the single sample apparatus from the Falling Number Co. of Sweden.

8. Amylograph Test

Amylograms were obtained according to the AACC standard method for 65 g of flour (method 22-10, AACC 1983) using a Brabender Visco-amylograph.

9. Wet Gluten Content

Flour wet gluten content was determined according to the I.C.C. standard method 137 (ICC, 1982) using the Falling Number Co. Glutomatic 2100.

10. Farinograph Test

Farinograms were obtained according to the AACC standard method

(method 54-21, AACC 1983) using 50 g of flour.

11. Extensograph Test

Extensograms were obtained according to Holas and Tipples (1978) using a Brabender Extensograph. The doughs were mixed in the farinograph mixer to the farinograph dough development time. Doughs were stretched at 45 min and 135 min; data will be reported only for the 135 min curves. Areas under the curves were measured with a polar compensation planimeter (Sokkiska Ltd. of Tokyo).

12. Baking Test

a. Remix Baking Test

The remix baking test was according to Kilborn and Tipples (1981) for 100 g of flour. Volumes of the resulting loaves were measured using a pup-loaf volumeter (National Mfg. Co.).

b. Baking Strength Index

The baking strength index was determined according to Tipples and Kilborn (1974).

H. Statistical Analyses

Where required, data were analyzed by standard statistical methods. A Student t-test for comparing differences between two mean values was used according to Mendenhall (1979).

For the statistical comparison of glutenin subunit patterns, 1 and 0 were used to indicate presence and absence of a subunit, respectively. [Data designated as 1 and 0 are considered as indicator or binary variables (Neter and Wasserman, 1974).] Correlation analyses

between technological data, between HMW glutenin subunits, and between technological data and HMW glutenin subunits were carried out. The analyses were executed on the University of Manitoba's Amdahl 5870 computer system using the Statistical Analysis System (SAS, 1985) program package with procedure CORR (correlation analysis).

To generate a prediction equation of a dependent variable (a technological parameter) using independent variables (HMW glutenin subunits), the STEPWISE (stepwise multiple regression) procedure from SAS (1985) was used with the maximum r^2 improvement option to select the best set of independent variables for the prediction equation. Using this particular prediction equation with the selected independent variables to predict another set of data, the REG (linear regression) procedure from SAS (1985) was used with the option of 95% confidence limit for an individual predicted value.

IV. RESULTS AND DISCUSSION

Results of this study are presented and discussed in nine sections. Section A deals with results of experiments in which polyacrylamide gel electrophoresis (PAGE) of gliadin proteins was used to check the identity and purity of the samples of the 26 varieties of the 1983 Uniform Quality Nursery (UQN) grown at four locations. Section B reports results of experiments with polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) designed and carried out to determine if varietal patterns are affected by the nature of the starting material (wholewheat meal, flour, or extracted glutenin) used to prepare the protein solution for electrophoresis. Results of experiments carried out to check the effect, if any, of the location of growth on SDS-PAGE patterns are presented in section C.

Section D deals with results of technological tests made on varietal samples obtained by compositing the grain from different locations. Test values presented in this section were subsequently used for statistical correlations with high molecular weight (HMW) glutenin subunit composition. Sections E and F deal with details of routine SDS-PAGE method and nomenclature. Results of the determination of HMW glutenin subunit composition for the 26 UQN varieties are presented in section G. The data presented in this section form the main part of the thesis project.

Results of statistical analyses of HMW glutenin subunit patterns and technological test data are presented and discussed in section H. The final section, I, presents prediction equations, based on HMW glutenin subunit composition, for predicting breadmaking quality. The prediction equations are tested using appropriate data for eight

varieties of the 1985 Bread Wheat Cooperative Test.

A. Identification of Grain Samples of Each Variety from Different Locations by Polyacrylamide Gel Electrophoresis

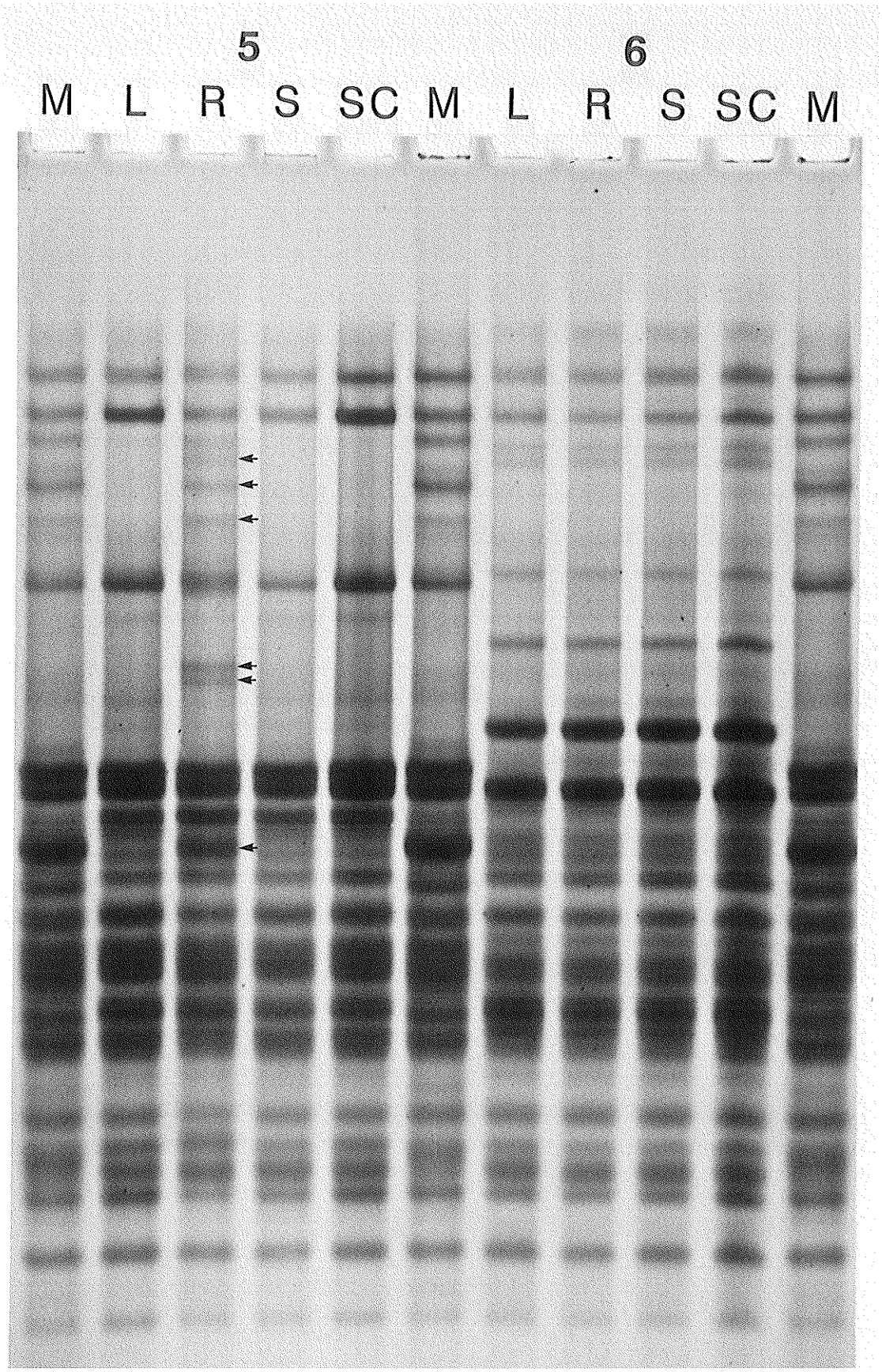
In order to have sufficient grain for the entire study, it was necessary to combine the samples from the four locations for each variety. Before this could be done, it was necessary to verify the identity of each location sample. It is known from past experience that samples may be admixed or misslabeled during harvesting and packaging for shipment to the laboratory.

Accordingly each of the 104 grain samples of the UQN (26 varieties grown at four locations) was analyzed by PAGE according to Sapirstein and Bushuk (1985). PAGE has recently been adopted by the International Association for Cereal Science and Technology (I.C.C.) as one of the two standard reference methods for identification of wheat varieties.

By way of example, Figure 1 shows results for two varieties, Cook (5) and Tobari/Romany (6). The PAGE electrophoregram for the Regina (R) sample of variety 5 showed that this sample contained grain of another, unknown variety (note arrows indicating foreign gliadins). Accordingly, this sample was not used in preparing the composite sample. The four location electrophoregrams for variety 6 were identical as were the patterns for the other 24 varieties (see Appendix II). The level of admixture (1 sample in 104) is not considered serious from the practical point of view. While this preliminary study involved a substantial amount of work, it was considered essential to ensure that each varietal composite was as pure as practically possible.

Figure 1. Gliadin electrophoregrams for samples of varieties 5 (Cook) and 6 (Tobari/Romany) from four locations, and the Marquis reference sample.

Letters represent M, Marquis; L, Lethbridge location; R, Regina; S, Saskatoon; SC, Swift Current. Foreign gliadins in sample R of variety 5 are indicated by arrows.


5
6
M L R S SC M L R S SC M

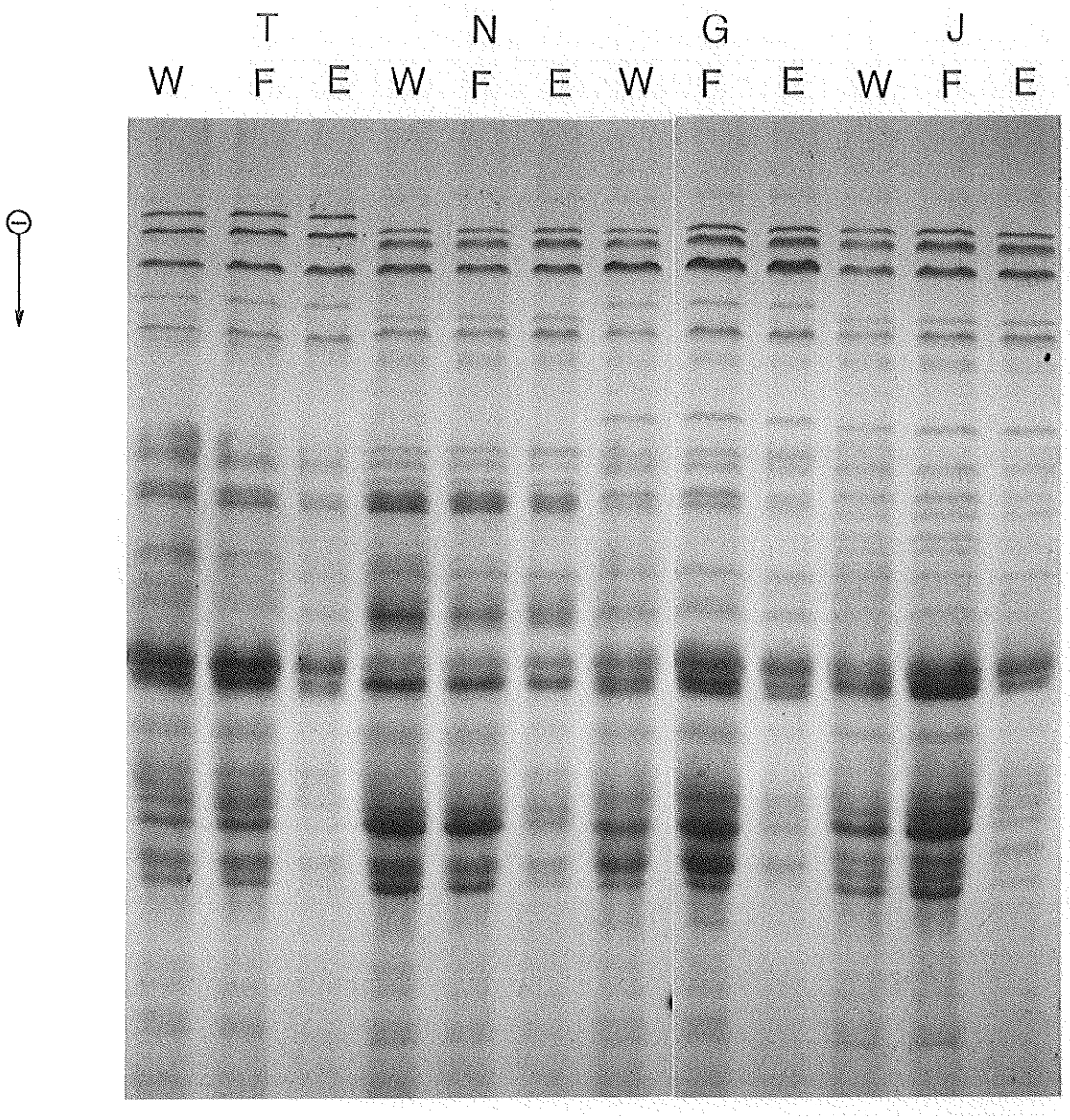
B. SDS-PAGE Patterns for Glutenins from Wholewheat Meal, Flour, and Extracted Glutenin

By way of preliminary experiments, it was necessary to determine the best starting material for preparing the glutenin solution for SDS-PAGE. Preparation of glutenin by the Osborne (1907) solubility procedure was considered impractical as a routine procedure for analyzing a large number of samples. Accordingly, experiments were carried out to develop a simpler, more rapid procedure.

Three different starting materials (wholewheat meal, flour, and extracted glutenin) were examined for four varieties of widely different breadmaking quality (Glenlea, James, Neepawa, and Tesopaco). Glenlea is a Canadian utility wheat with very strong dough mixing characteristics, Tesopaco is a soft wheat, James has medium quality and Neepawa possesses good breadmaking quality.

The modified SDS-PAGE procedure developed in the present study (high concentration of acrylamide and bisacrylamide, low degree of cross-linkaging, low constant current, and long running time) yielded high resolution of glutenin subunits, especially for the low mobility subunits (high molecular weight subunits). Results for the three starting materials of the four varieties are shown in Figure 2. No intravarietal differences were found in the HMW glutenin region (region of prime relevance in this study). Furthermore, the lanes for extracted glutenin (E) gave bands that are not as dense as the same bands in the other two lanes; some bands were missing in the high mobility region of the electrophoregram for this starting material. It appears that some protein components are lost in the preparation of extracted glutenin, but this loss did not affect the HMW glutenin

Figure 2. SDS-PAGE patterns of four varieties and three starting materials.
First row: T, Tesopaco; N, Neepawa; G, Glenlea; and J, James. Second row: W, wholewheat meal; F, flour; and E, extracted glutenin.


T
N
G
J
W F E W F E W F E W F E

subunit region.

The evidence presented here indicates that wholewheat meal or flour could be used directly for preparing the glutenin extract for SDS-PAGE without going through the tedious steps of fractionating glutenin. Accordingly, the remaining studies used flour as the starting material for all SDS-PAGE analyses.

C. Effect of Environment on SDS-PAGE Patterns

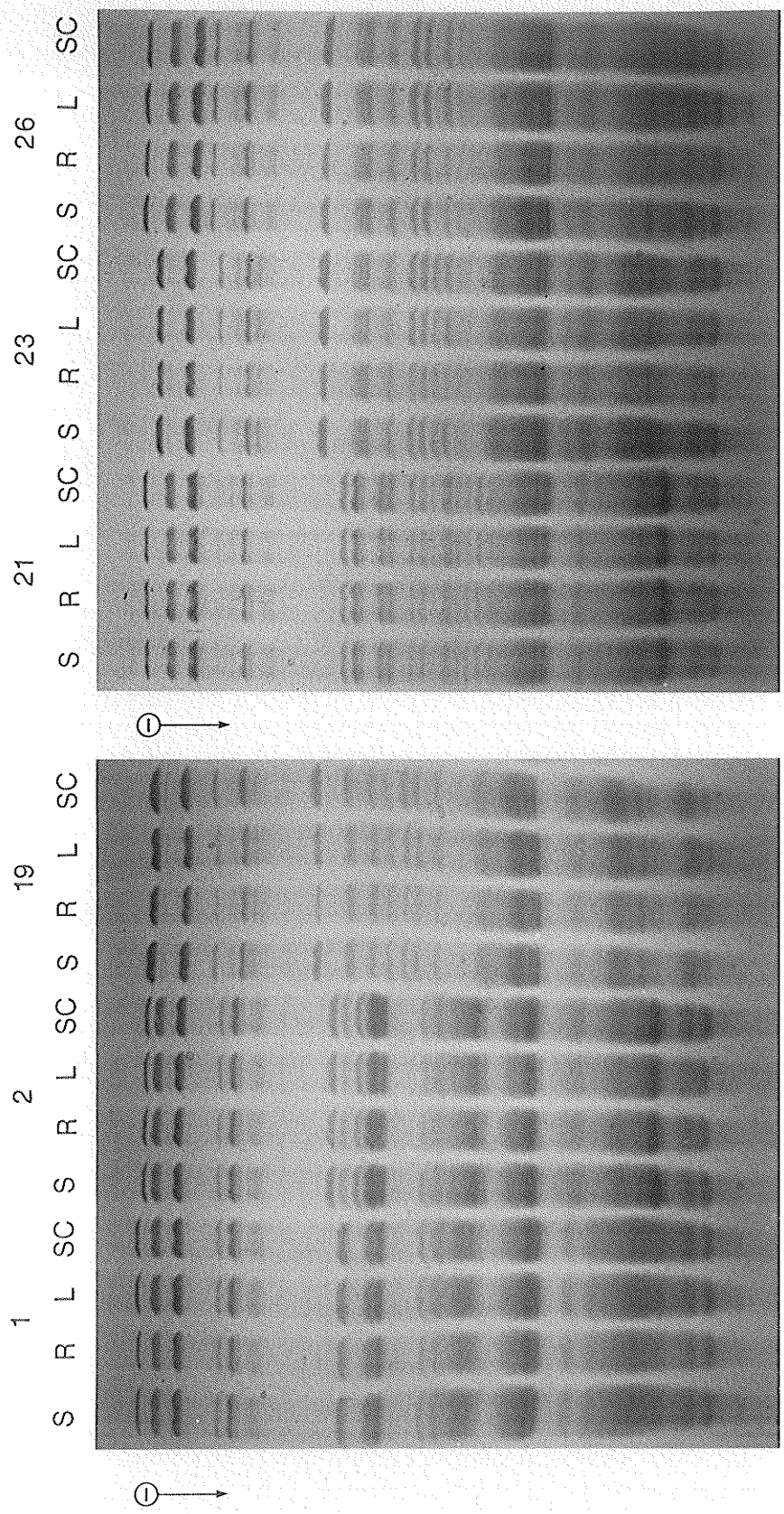
In section A of this chapter, it was confirmed that growth location did not affect the PAGE patterns of gliadin proteins. That is, the PAGE electrophoregram is a true genotypic fingerprint. For the purpose of the present study, it was necessary to confirm that the SDS-PAGE patterns of glutenin proteins were also genotype specific (not affected by growth environment).

Six varieties of the 1983 UQN were selected based on preliminary studies of their baking quality (not presented here). Three of these six varieties were hard white wheats (with baking quality decreasing in the order no. 26 > no. 19 > no. 21), one was a soft white wheat (no. 23) and two were hard red wheats (no. 1 and no. 2). Figure 3 shows the SDS-PAGE patterns of these six varieties grown at four locations. No intravarietal differences were observed. Accordingly, it was concluded that glutenin subunit patterns are genetically controlled and are not affected by environmental conditions. These findings are in general agreement with published information (Orth and Bushuk, 1973b; Payne et al., 1984).

Based on the results for the six varieties presented in this section, it is reasonably safe to presume the same conclusion would apply

Figure 3. SDS-PAGE patterns of samples of six varieties, each grown at four locations.

Top row: variety numbers (see Table 1). Second row (Locations): S, Saskatoon; R, Regina; L, Lethbridge; and SC, Swift Current.


S R L SC S R L SC S R L SC S R L SC
1 2 19 21 23 26
I
I

to the other 20 varieties in the UQN. Accordingly, each sample of the 1983 UQN varieties was composited from the four locations as described in section B of chapter III. The remaining studies in this thesis project were done using the composited samples of the 1983 UQN varieties.

D. Technological Tests

This section presents and discusses, where necessary, results of a wide variety of so-called technological tests that are used in the assessment of breadmaking quality of wheat. These tests were done for two main reasons. First, it was essential to ascertain that the varieties selected for the study did indeed span the presumed wide range of breadmaking quality. Second, SDS-PAGE patterns were statistically correlated with characteristics (technological tests) related to breadmaking quality.

The technological test results are presented in four sections. Section 1 deals with whole wheat grain, section 2 with flour, section 3 with the rheological characteristics of dough, and section 4 with baking.

The data for all the technological tests are tabulated in Table 5 and correlation coefficients between tests are presented in Table 6. Hereafter, discussion of these tests refers to these two tables. It should be pointed out that not all significant correlation coefficients are relevant to the present study. Thus, only those coefficients that are considered relevant are discussed.

1. Results of Tests on Whole Wheat Grain

Hectoliter weights (HW) for the 26 varieties ranged from

Table 5. Technological data[1] for the 26 varieties of the 1983 Uniform Quality Nursery[2]

No.	HW	GP	FN	FY	GT	FP	ASH	SV	AV	WG	FA	DT	MTI	E	R	R/E	A	LV	BSI	ULV
1	79.0	14.7	435	73.3	44	14.2	0.39	70	610	37.3	60.3	8.0	40	185	955	5.16	226	910	97	64.1
2	78.5	15.0	450	70.4	45	14.5	0.38	52	700	40.2	62.2	5.5	30	192	580	3.02	147	875	91	60.3
3	77.0	14.2	365	70.6	68	12.4	0.42	40	460	37.0	57.4	2.5	50	195	300	1.54	85	530	65	42.7
4	78.9	15.5	495	73.5	30	15.1	0.42	61	920	40.9	63.6	6.0	40	195	520	2.67	135	905	91	59.9
5	81.0	14.2	465	74.7	31	13.6	0.40	70	780	37.1	62.0	6.5	30	215	640	2.98	182	855	96	62.9
6	77.1	13.2	395	71.4	79	12.5	0.41	60	840	34.6	56.4	5.5	35	210	730	3.48	204	685	84	54.8
7	78.6	14.0	475	72.7	32	13.1	0.40	60	910	36.0	63.3	5.5	25	183	615	3.36	147	795	93	60.7
8	77.3	14.2	395	71.1	32	13.4	0.41	47	530	37.4	62.2	7.5	5	143	650	4.54	128	810	92	60.4
9	76.5	13.2	230	72.1	37	12.7	0.46	50	250	33.9	60.3	5.5	20	163	670	4.11	148	820	99	64.6
10	76.0	14.4	475	72.2	36	14.0	0.43	61	940	39.5	60.8	7.0	25	178	790	4.44	185	800	87	57.1
11	78.4	14.0	285	71.5	38	13.7	0.47	58	390	36.0	61.0	7.5	30	187	870	4.65	208	890	99	65.0
12	77.3	14.0	415	71.1	36	13.5	0.46	49	580	37.6	62.7	7.0	10	155	610	3.93	126	820	92	60.7
13	77.0	14.1	425	72.0	33	13.6	0.45	52	560	37.8	62.3	7.0	15	154	670	4.35	133	840	94	61.8
14	77.7	13.8	430	70.8	38	12.8	0.40	60	570	32.2	60.2	9.5	15	144	1050	7.29	188	820	98	64.1
15	76.3	13.8	415	73.7	30	12.9	0.43	67	690	34.5	63.4	11.0	20	163	1100	6.75	226	830	98	64.3
16	77.0	13.9	415	71.5	31	13.2	0.43	48	510	36.8	62.5	10.5	10	135	770	5.70	135	810	94	61.4
17	77.1	13.7	450	72.6	30	12.8	0.46	52	1060	34.5	60.4	5.5	20	162	630	3.89	135	805	96	62.9
18	79.8	13.9	480	72.1	27	12.8	0.42	70	1030	33.4	62.4	7.0	25	178	795	4.47	191	940	112	73.4
19	74.6	13.6	410	73.9	50	12.9	0.49	51	740	37.7	59.1	4.0	50	215	435	2.02	133	730	86	56.6
20	72.9	13.6	370	70.4	113	12.2	0.48	54	690	32.8	54.0	4.5	50	203	535	2.63	178	590	74	48.4
21	77.5	13.3	420	73.8	50	12.5	0.45	38	840	36.4	58.8	3.5	30	178	405	2.28	103	660	81	52.8
22	76.7	14.7	410	72.9	35	13.9	0.44	55	660	39.7	61.3	4.5	40	210	380	1.81	111	815	89	58.6
23	75.1	14.0	395	68.7	147	12.7	0.43	30	780	38.3	56.9	2.0	70	175	170	0.97	46	420	51	33.1
24	74.4	13.2	460	71.8	31	12.3	0.45	55	720	35.8	64.7	4.0	40	205	460	2.24	136	740	92	60.2
25	75.4	15.2	355	66.7	112	14.4	0.43	50	830	41.6	57.8	2.5	60	204	380	1.86	111	490	52	34.0
26	73.0	15.4	365	73.2	51	15.0	0.45	72	590	38.7	62.4	9.5	30	212	910	4.29	252	975	98	65.0

[1]HW=hectoliter weight (kg/hl); GP=grain protein (14% m.b.; N x 5.7%); FN=Falling Number (sec; 14% m.b.); FY=flour yield (%); GT=grinding time (sec.; m.b. "as is"); FP=flour protein (14% m.b.; N x 5.7%); ASH=ash (%; 14% m.b.); SV=Sedimentation Value (cc); AV=Amylograph viscosity (B.U.); WG=wet gluten (%); FA=Farinograph absorption (%); DT=Farinograph development time (min); MTI=Farinograph mixing tolerance index (B.U.); E=extensibility (mm); R=maximum resistance (B.U.); R/E=ratio of R and E; A=area under the curve (cm^2); LV=remix loaf volume (cc); BSI=remix baking strength index; ULV=remix loaf volume per unit protein (14% m.b.).
[2]Variety no. corresponds to variety no. listed in Table 1.

Table 6. Correlation coefficients[1] between technological data[2] for the 1983 UQN varieties (n=26)

	HW	GP	FN	FY	GT	FP	ASH	SV	AV	WG	FA	DT	MTI	E	R	R/E	A	LV	BSI	ULV
HW	1																			
GP	097	1																		
FN	347	188	1																	
FY	298	-109	315	1																
GT	-491*	021	-306	-679***	1															
FP	169	916***	145	062	-183	1														
ASH	-603**	-352	-437*	061	114	-271	1													
SV	266	271	281	493*	-472*	384	-257	1												
AV	139	091	731***	113	066	031	-165	182	1											
WG	-017	763***	162	-150	113	757***	-155	-147	109	1										
FA	363	228	397*	444*	-802***	389*	-213	408*	-002	175	1									
DT	200	100	099	371	-574**	259	-187	602**	-236	-254	523**	1								
MTI	-354	183	-104	-347	764***	-024	111	-258	210	311	-582**	-725***	1							
E	-191	241	018	123	288	181	084	256	260	299	-256	-485*	688***	1						
R	219	0	021	337	-507**	172	-209	724***	-167	-410*	362	910***	-622***	-350	1					
R/E	232	-091	016	223	-520**	059	-205	503**	-242	-450*	380	922***	-741***	-631***	940***	1				
A	077	083	-006	401*	-344	237	-116	878***	-044	-329	201	705***	-323	124	870***	658***	1			
LV	425*	237	229	631***	-832***	465*	-157	720***	-075	-062	731***	728***	-664***	-154	693***	603**	644***	1		
BSI	423*	-110	198	664***	-869***	119	-071	661***	-079	-376	674***	713***	-747***	-258	715***	665***	624***	932***	1	
ULV	419*	-087	200	665***	-869***	140	-073	667***	-083	-358	679***	716***	-742***	-249	716***	662***	629***	940***	1***	1

[1]Decimals omitted, except for those with perfect correlations (i.e., r = -1 or 1).
[2]As defined in Table 5.
*, **, *** significantly correlated at 5%, 1%, 0.1%, respectively.

72.9 kg per hl to 81.0 kg per hl. The value for the working standard variety Neepawa (No. 2) was 78.5 kg per hl. According to guidelines for the Bread Wheat Cooperative Test, varieties with hectoliter weights that are 3.0 kg or more higher than the value for Neepawa are rated as excellent in terms of this characteristic. Other ratings are: very good, +1.6 to 2.9 kg; good, 0 to +1.5 kg; and poor, -2.0 kg or lower. The data of Table 5 show that the UQN varieties cover a wide range of hectoliter weights from poor to very good compared with Neepawa. This is consistent with the original objective of having a set of experimental samples that covered a broad range of characteristics related to baking quality.

Protein content (GP) values covered a fairly narrow range (13.2% - 15.5%), again supporting the suitability of the samples for the study. Protein content of wheat depends strongly on environmental factors (CIGI, 1983). In a study such as this, where the emphasis is on intervarietal differences, it is preferable to largely minimize or completely eliminate environmental effects. The range of protein content for the samples in Table 5 is consistent with published data on inheritance of protein content in wheat (CIGI, 1983).

Falling Number (FN) and flour yield (FY) values showed normal variability. These characteristics are not directly relevant to glutenin subunit composition but the Falling Number Value is relevant to breadmaking in that it is a measure of the α-amylase activity in the grain. The range of FN values in Table 5 (230 sec - 495 sec) indicates that the grain samples had not suffered any significant damage due to pre-harvest sprouting (i.e., high α-amylase activity or low FN values).

Grain hardness values measured by grinding time (GT) indicate that the samples cover the normal range of hardness from soft to hard. Consistent with common knowledge, the results of this study showed that flour yield (from the Buhler experimental mill) is highly significantly correlated with grain hardness ($r = -0.679^{***}$).

Flour protein content (FP) was in general about 0.7% lower than grain protein content (GP) due to the removal of the aleurone layer and germ portion of the wheat kernel. The ash content of flour is also used as an index of milling quality. In general, minerals (main component of flour ash) are concentrated in the aleurone layer of the wheat kernel. During milling, the aleurone layer together with nucellar epidermis, seed coat and pericarp form a by-product which the miller calls bran (Hoseney, 1986). Flour with a high ash content normally is presumed to contain an abnormally high amount of fine bran particles. Commercial hard wheat flours of straight grade generally have ash contents ranging from 0.41 to 0.49% (Ziegler and Greer, 1971). Although ash content does not necessarily affect the breadmaking performance of a flour per se, it does affect the consumer acceptability of the bread. Flour with high ash content produces a loaf of darker crumb color (Pratt, 1971). In the samples used in the present study, the ash content ranged from 0.38 to 0.49%. Flours with ash content in this range would be quite acceptable from a commercial point of view.

2. Results of Technological Tests on Flours

The sedimentation test was developed by Zeleny (1947) for estimating the so-called "baking strength" of wheats. The test measures

the volume of the sediment of a flour that has been allowed to swell in aqueous lactic acid and then to settle for a given length of time. Generally, the values fall in the range of 10 to 20 cc for very weak wheats to 70 cc or more for very strong wheats. The sedimentation values for the samples in this study (from 30 to 72 cc) covered a wide range of baking strength or quality. Sedimentation values (SV) depend on several factors, the main ones being protein content and protein quality. Fowler and De La Roche (1975) reported a highly significant correlation between sedimentation value and protein content for a group of similar varieties in the Bread Wheat Cooperative Test. On the other hand, Orth et al. (1972) did not find any correlation between these two variables for a group of widely different varieties. The correlation obtained in the present study (Table 6) was similar to that obtained by Orth et al. (1972). Nevertheless, the sedimentation value was highly significantly correlated with loaf volume ($r = 0.720^{***}$).

Amylograph viscosity (AV) values, another test for α-amylase activity, were consistent with Falling Number Values, again indicating that the samples had not suffered sprouting damage. Wet gluten content of flour is strongly related to its protein content. But, in so-called high quality bread flours more of the flour protein is recovered as gluten. As expected wet gluten content was highly significantly correlated with flour protein content ($r = 0.757^{***}$). However, it did not correlate well with baking strength index (BSI) ($r = -0.376$) nor with remix loaf volume (LV) ($r = -0.062$). Thus, wet gluten content appears to be a good indicator of protein content or quantity, but not necessarily a predictor of breadmaking quality for the particular

group of varieties used in this study.

3. Rheological Tests

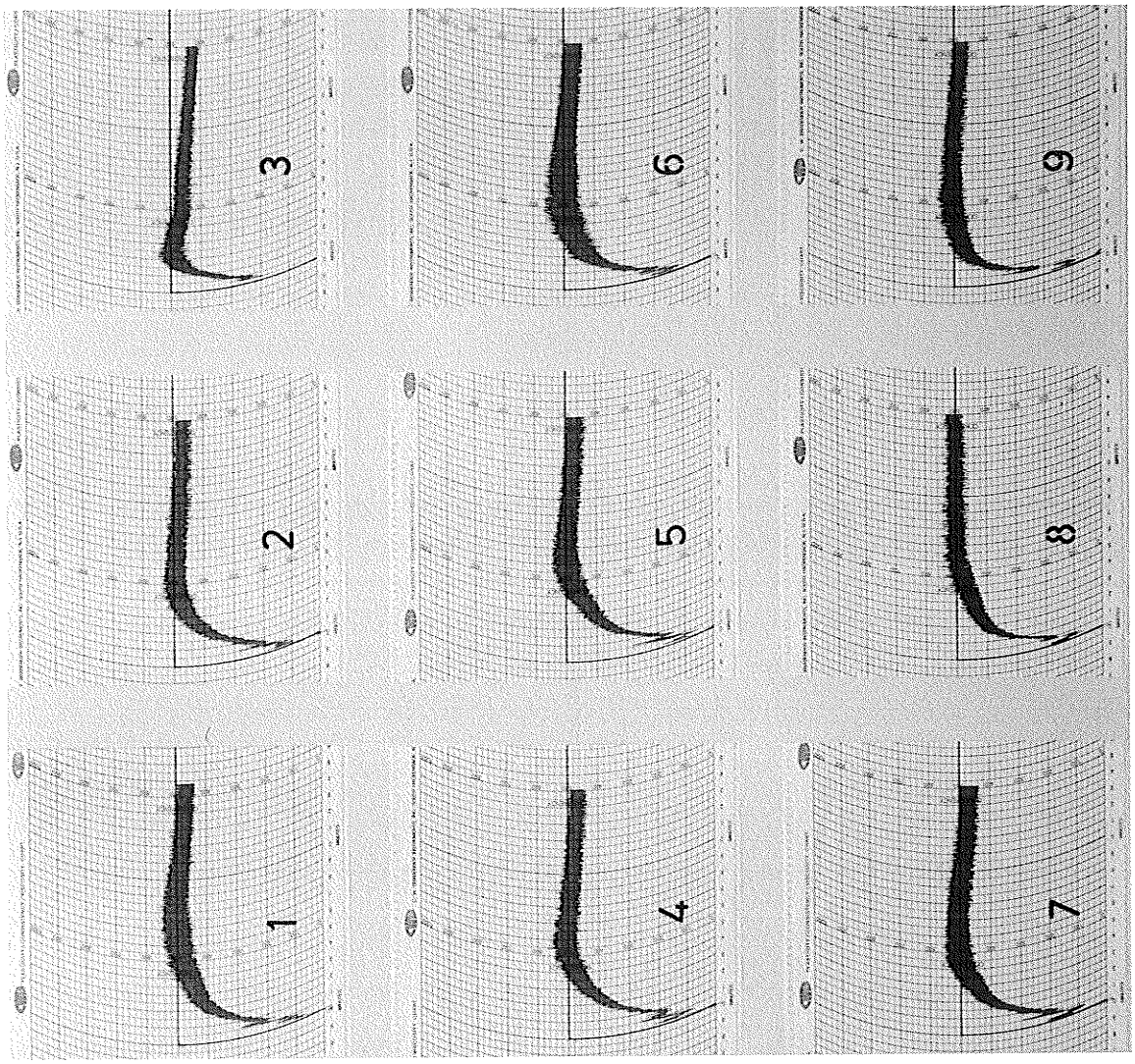
The farinograph test is used widely for evaluating the dough mixing properties and water absorption of wheat flour. A broad range of farinogram types is represented by the 26 samples used (Figures 4 - 6).

Several different parameters derived from the farinogram are used as indices of flour quality. Dough development time (DT) is the mixing time from the first addition of water to the maximum consistency. DT is an index of the "strength" of the dough; the longer the DT, the stronger the dough. The range of DT values for the 26 varieties was from 2.0 to 11.0 min, which is considerably greater than that encountered for commercial bread flours (4.0 - 7.0 min). Of the various technological tests discussed so far DT was most strongly correlated with Sedimentation value ($r = 0.602^{**}$).

Another flour quality parameter derived from the farinogram is the mixing tolerance index (MTI). It is the difference, in Brabender Units (B.U.), between the top of the curve at maximum consistency and the top of the curve 5 min after the maximum. This parameter gives an indication of the ability of the dough to withstand overmixing. Doughs with a high MTI must be mixed precisely, while those with a low MTI have a good tolerance to mixing and can be overmixed without causing major problems. MTI is related to the "strength" of the dough; in this study the correlation coefficient between MTI and DT was $r = -0.725^{***}$. The range of the MTI values for the 26 varieties in Table 5 was 5 to 70 B.U..

Figure 4. Farinograms of varieties 1 to 9.

Curve no. corresponds to variety no. listed in Table 1.


1
2
3
4
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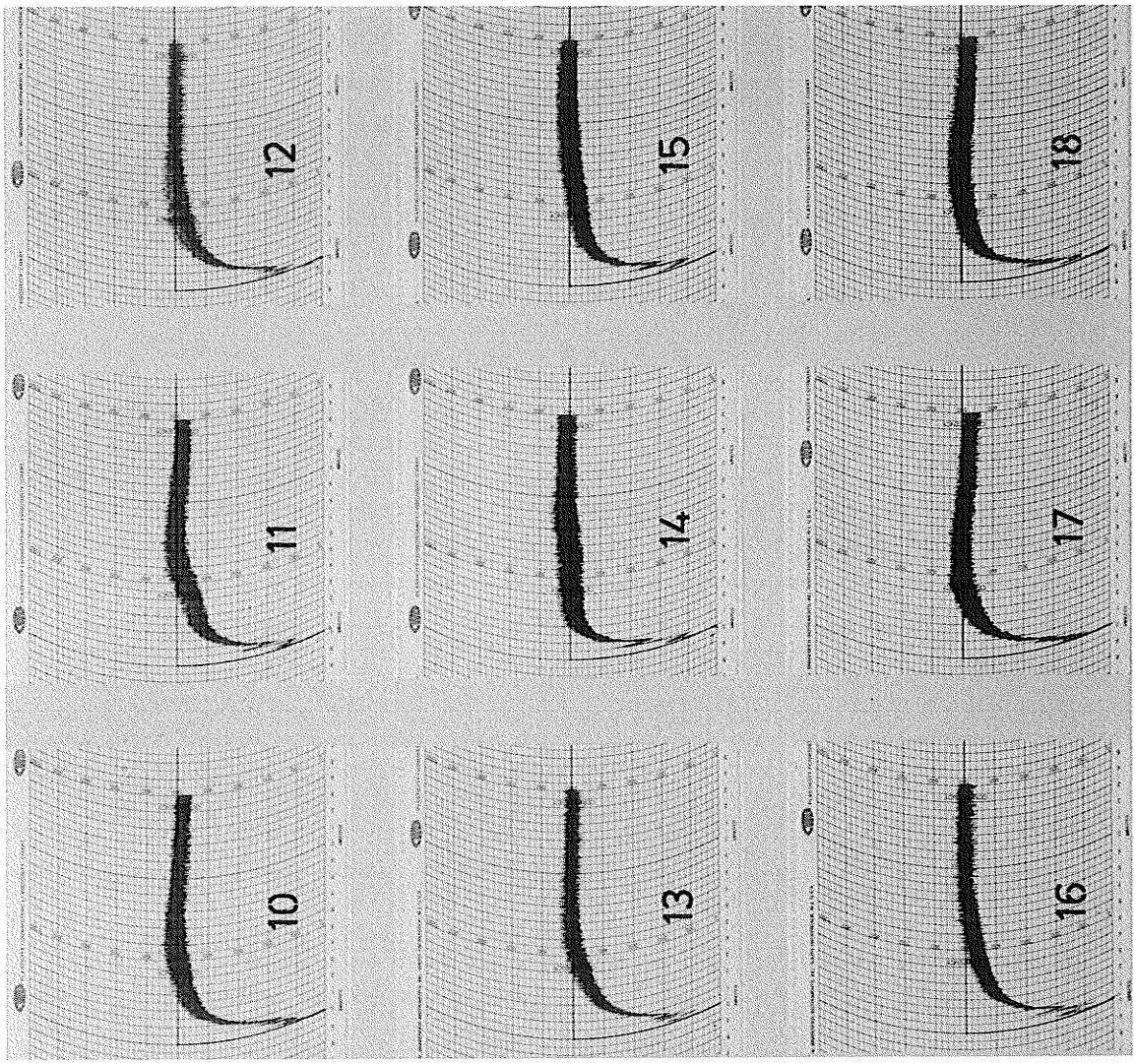
6
7
8
9

Figure 5. Farinograms of varieties 10 to 18.

Curve no. corresponds to variety no. listed in Table 1.


10
11
12
13
14
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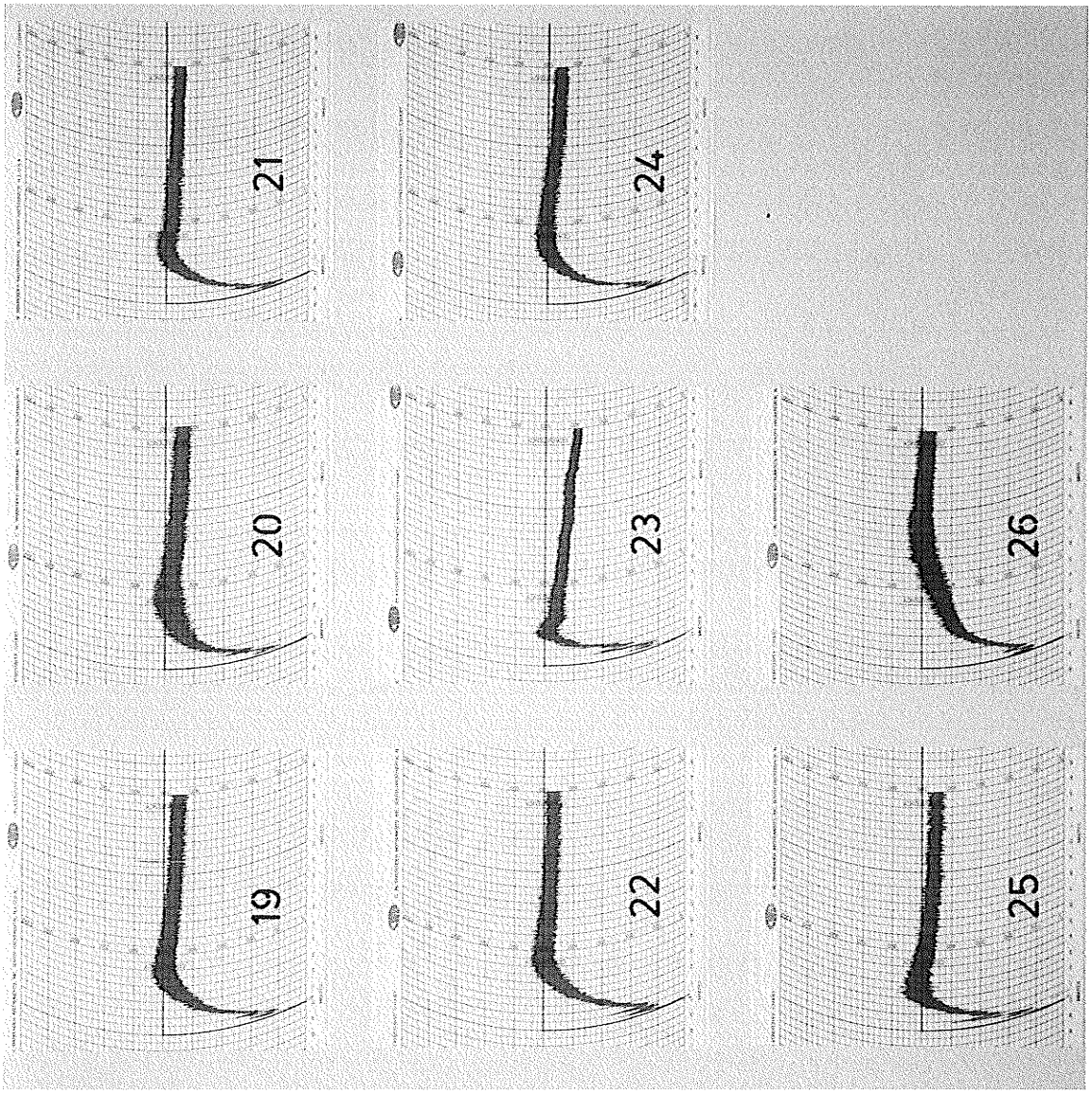
18

Figure 6. Farinograms of varieties 19 to 26.

Curve no. corresponds to variety no. listed in Table 1.


19
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Farinograph absorption (FA) is the amount of water that must be added in the Farinograph test to give a dough with a maximum consistency at 500 B.U. The varieties used in this study covered a wide range of FA (54.0 to 64.7%). FA was correlated with flour protein ($r = 0.389^{*}$) and highly significantly correlated with remix loaf volume ($r = 0.731^{***}$). These observations are generally consistent with published data (Fowler and De La Roche, 1975).

The extensograph test is used to measure the physical state of bread dough during the period between mixing and baking. The instrument measures the resistance of a specially shaped piece of dough to extension, and its extensibility. These parameters are obtained directly from the extensograph curve (extensogram). The maximum resistance (R) is the height of the curve at its highest point in Brabender Units (B.U.) and the extensibility (E) is the length of the curve in millimeters from the start of stretching until the dough breaks. The area under the curve (A) is related to the energy needed to stretch a piece of dough to the breaking point. A dough with the required A value for optimum baking performance must also have the right balance between R and E (Shuey, 1975). This is expressed in terms of the R/E ratio, with doughs of strong wheat varieties having a high R/E value and doughs of weak varieties a low value. Extensograms for the 26 varieties (Figures 7 - 9) gave a wide range of R, E, A and R/E values (Table 5).

Of the various statistical correlations that were calculated (Table 6) the following were significant: GT versus FY ($r = -0.679^{***}$); FP versus GP ($r = 0.916^{***}$); AV versus FN ($r = 0.731^{***}$); WG versus FP ($r = 0.757^{***}$) DT versus SV

Figure 7. Extensograms of varieties 1 to 9.

Curve no. corresponds to variety no. listed in Table 1.

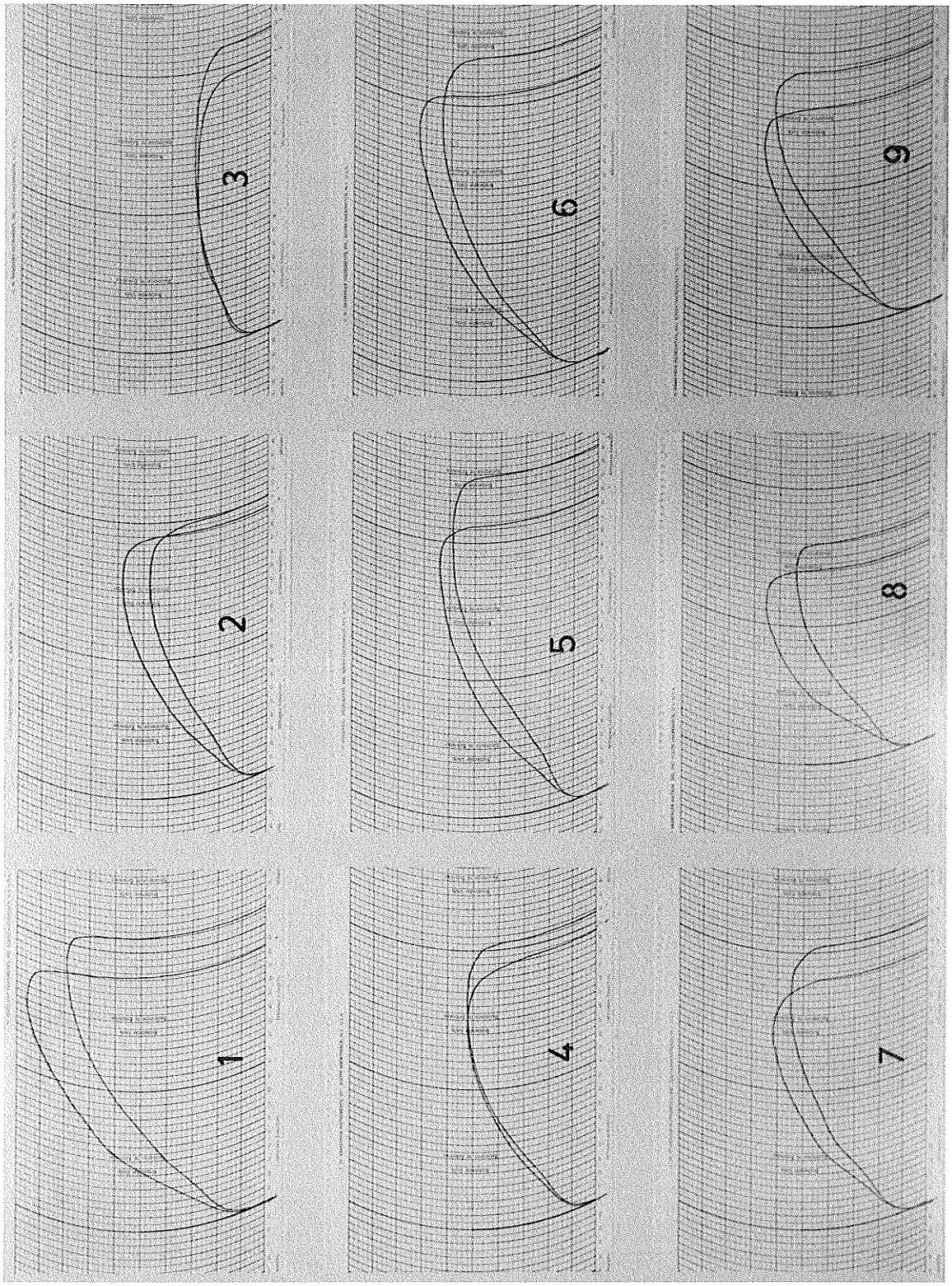
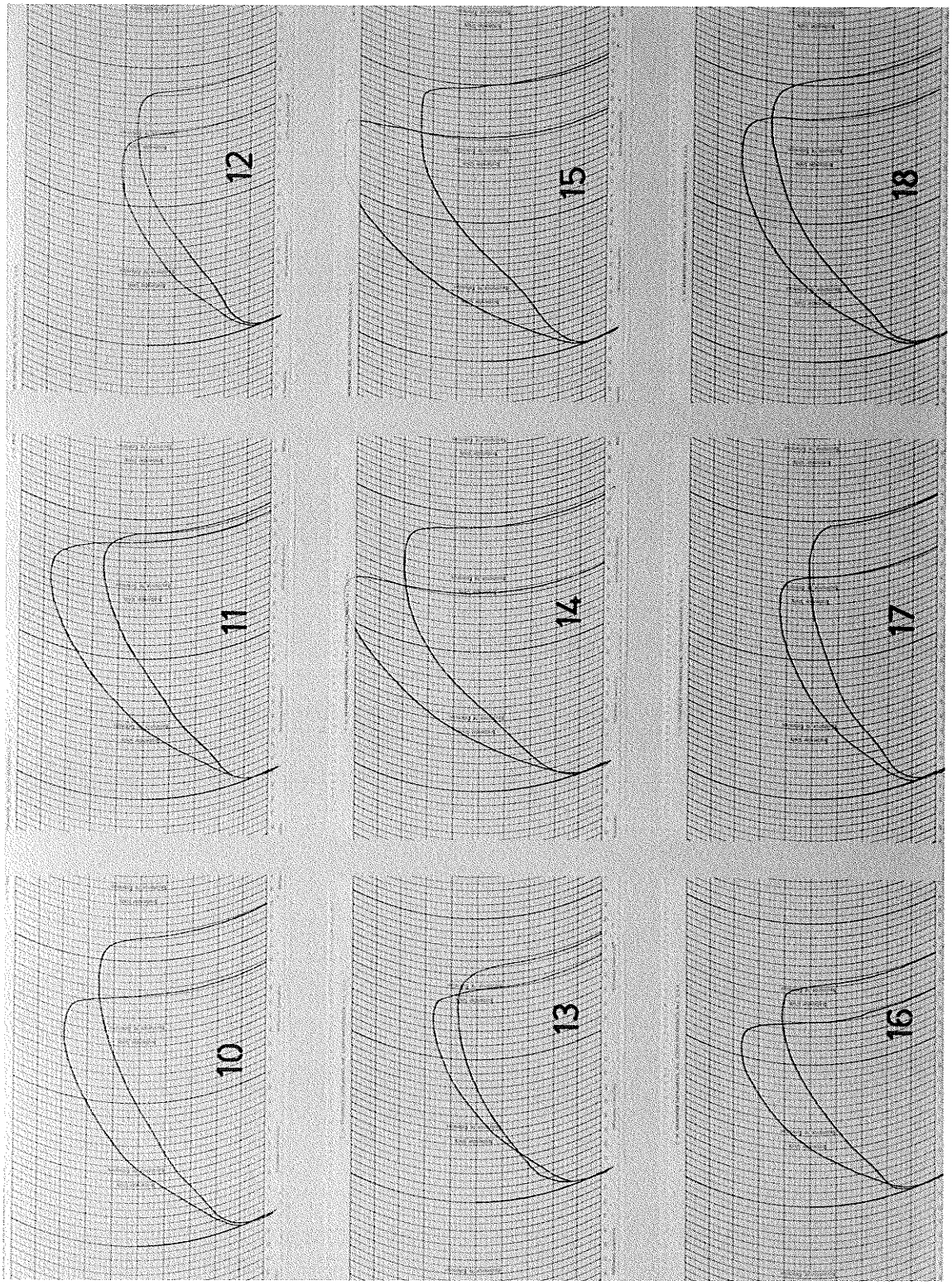

3
6
9
2
5
8
1
4
7

Figure 8. Extensograms of varieties 10 to 18.

Curve no. corresponds to variety no. listed in Table 1.


12
15
18
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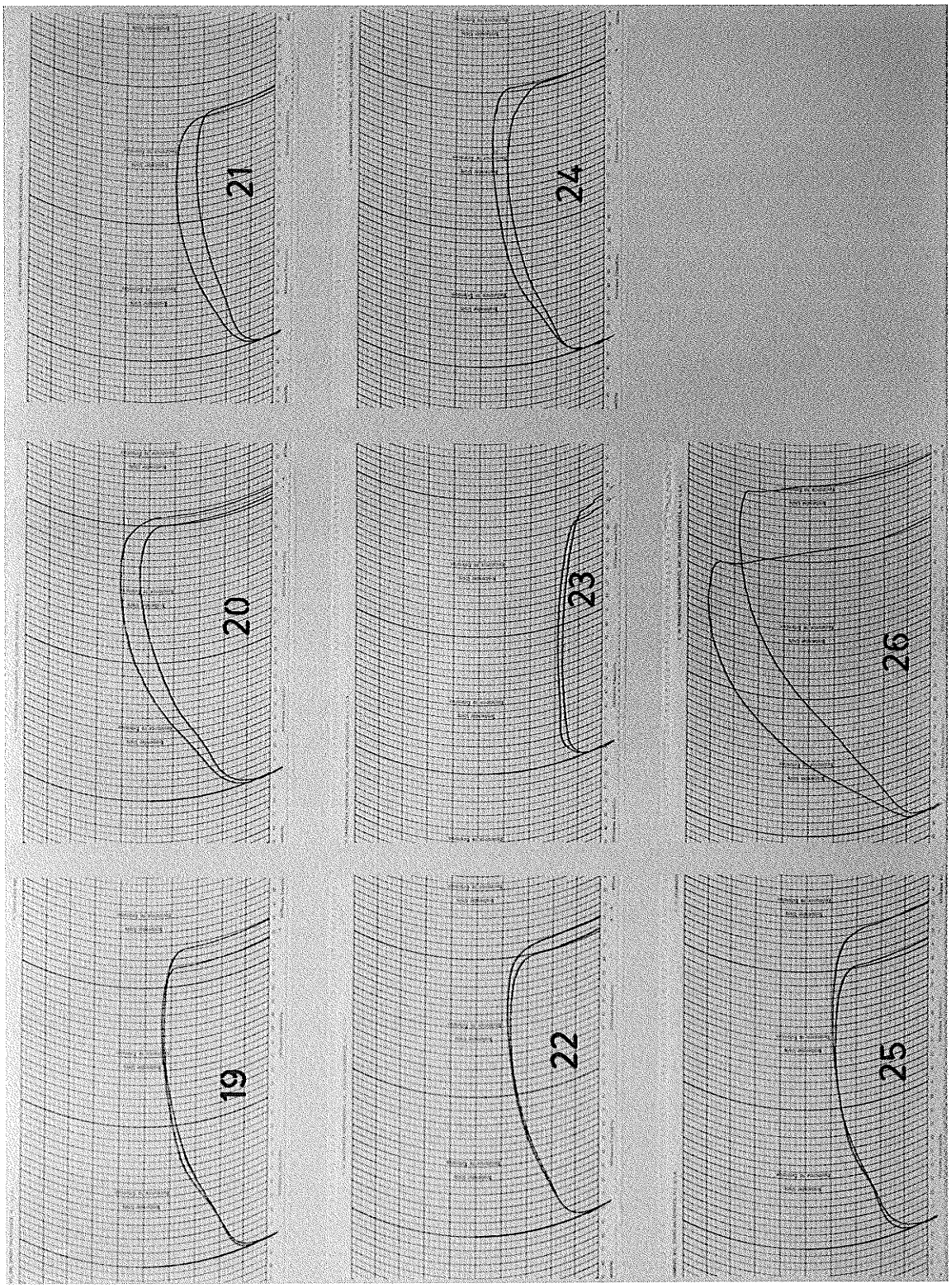
14
17
10
13
16

Figure 9. Extensograms of varieties 19 to 26.

Curve no. corresponds to variety no. listed in Table 1.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(r = 0.602**); DT versus FA (r = 0.523**); MTI versus FA (r = -0.582**); MTI versus DT (r = -0.725***); E versus DT (r = -0.485*); E versus MTI (r = 0.688***); R versus SV (r = 0.724***); R versus WG (r = -0.410*); R versus DT (r = 0.910***); R versus MTI (r = -0.622***); R/E versus SV (r = 0.503**); R/E versus WG (r = -0.450*); R/E versus DT (r = 0.922***); R/E versus MTI (r = -0.741***); R/E versus E (r = -0.631***); R/E versus R (r = 0.940***); A versus SV (r = 0.878***); A versus DT (r = 0.705***); A versus R (r = 0.870***); A versus R/E (r = 0.658***). Detailed discussion of these significant correlations is outside the subject of this thesis.

4. Baking Tests

The all-inclusive test of breadmaking quality is the baking test. The remix baking test is the one that is commonly used to assess Canadian bread wheats (Irvine and McMullan, 1960). Figures 10 to 12 are photographs of external and internal loaf characteristics for the 26 varieties investigated. The range of loaf volumes (LV) was 420 to 975 cc (Table 5).

Statistical analyses (Table 6) showed that LV is highly significantly correlated with SV (r = 0.720***); with FA (r = 0.731***); with DT (r = 0.728***); and with MTI (r = -0.664***).

It is well documented in the literature (Chapter II-D) that loaf volume is directly related to the protein content of the flour. In studying the contribution of protein quality to breadmaking quality, it is necessary to normalize the LV values to a constant protein content. This can be done in several ways. Orth and Bushuk (1972) used

Figure 10. Photographs of experimental loaves showing external and internal characteristics of varieties 1 to 9.
Loaf no. corresponds to variety no. listed in Table 1.

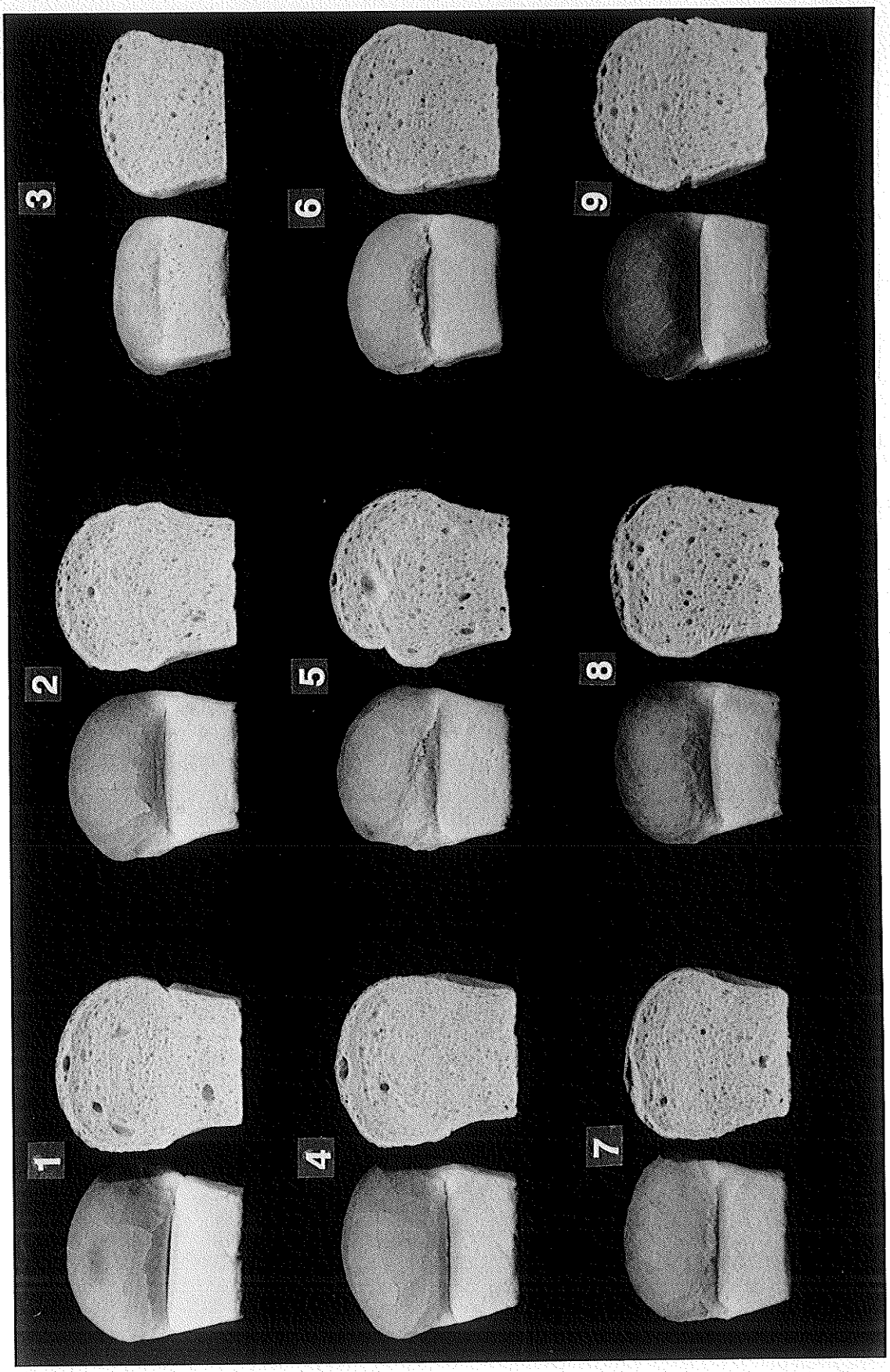
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Figure 11. Photographs of experimental loaves showing external and internal characteristics of varieties 10 to 18.
Loaf no. corresponds to variety no. listed in Table 1.

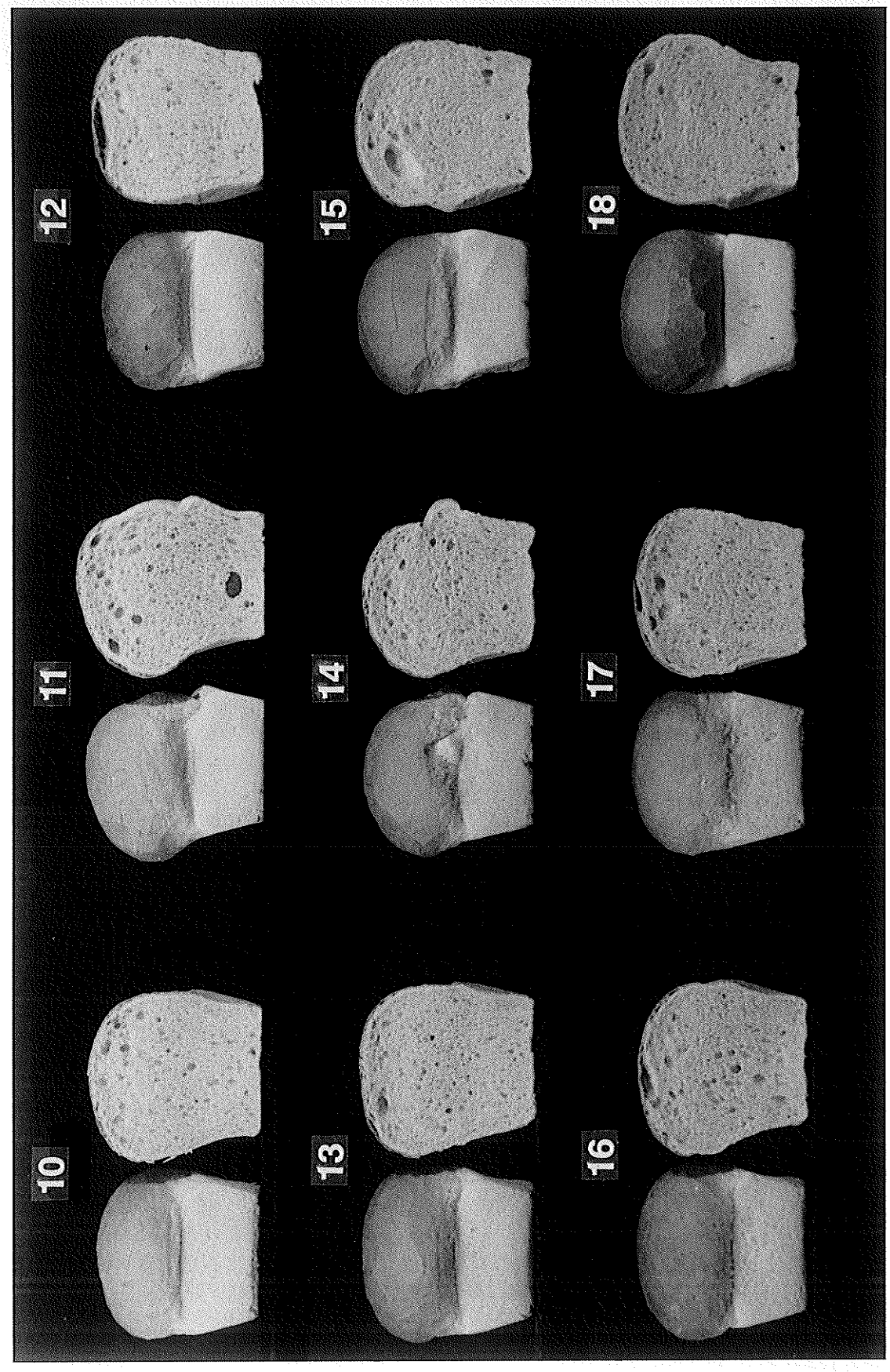
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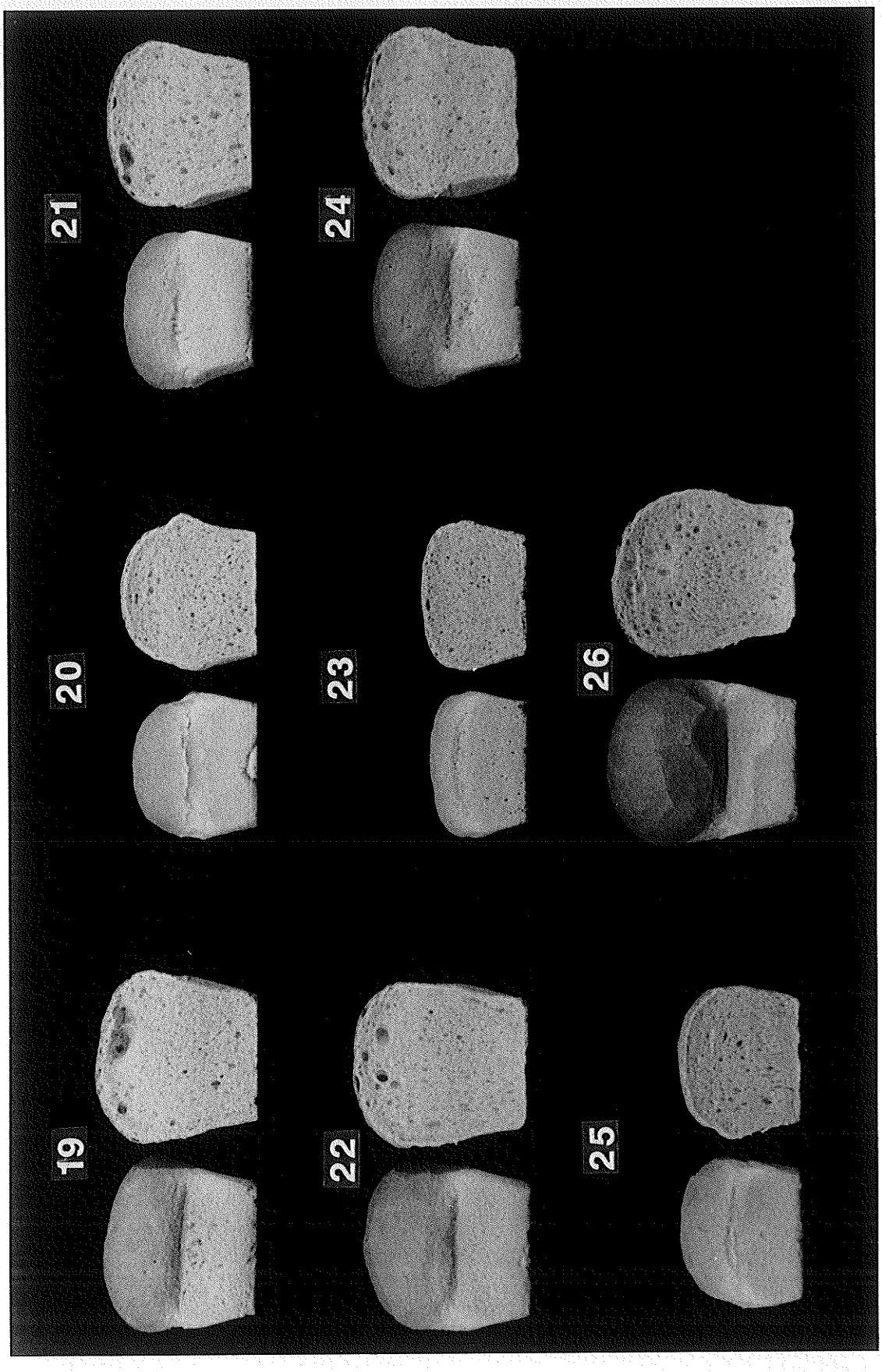
Figure 12. Photographs of experimental loaves showing external and internal characteristics of varieties 19 to 26.
Loaf no. corresponds to variety no. listed in Table 1.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the loaf volume per unit protein (ULV) and Tipples and Kilborn (1974) used the baking strength index (BSI). The two parameters are calculated as follows:

$$ULV = LV/(\% \text{ of flour protein})$$

$$BSI = (LV \times 100)/[(\% \text{ of flour protein} \times 70) - 58]$$

The equation for BSI was developed from a linear regression analysis of 116 samples for remix loaf volume vs. flour protein content (Tipples and Kilborn, 1974). The values 70 and 58 in the calculation are the slope and the intercept of the regression equation, respectively. BSI is expressed as a percent.

Statistical analyses (Table 6) showed that ULV and BSI are highly significantly correlated with extensograph maximum resistance (R) ($r = 0.716^{***}$ and 0.715^{***}, respectively). Maximum resistance in the extensograph test is related to the elastic modulus of dough which in turn depends on the amount and the nature of glutenin in the flour (Hoseney, 1986; Khan and Bushuk, 1978; Schofield and Booth, 1983). On the other hand, there is good evidence that dough extensibility depends more strongly on the gliadin than on the glutenin component. The indirect evidence for the interrelationship between glutenin and loaf volume presented above is consistent with published information (see chapter II-D) and accentuates the practical significance of this research.

Results of the technological tests presented and briefly discussed in this chapter show that the wheat varieties selected for this study cover a wide range of breadmaking quality. The range is considerably broader than that encountered for commercial flours used for bread production. It would appear that the varieties should be highly

suitable for the intended research objective---to investigate the relationship between HMW glutenin subunits and breadmaking quality.

E. Estimation of Relative Molecular Weights for HMW Subunits of Glutenin from SDS-PAGE

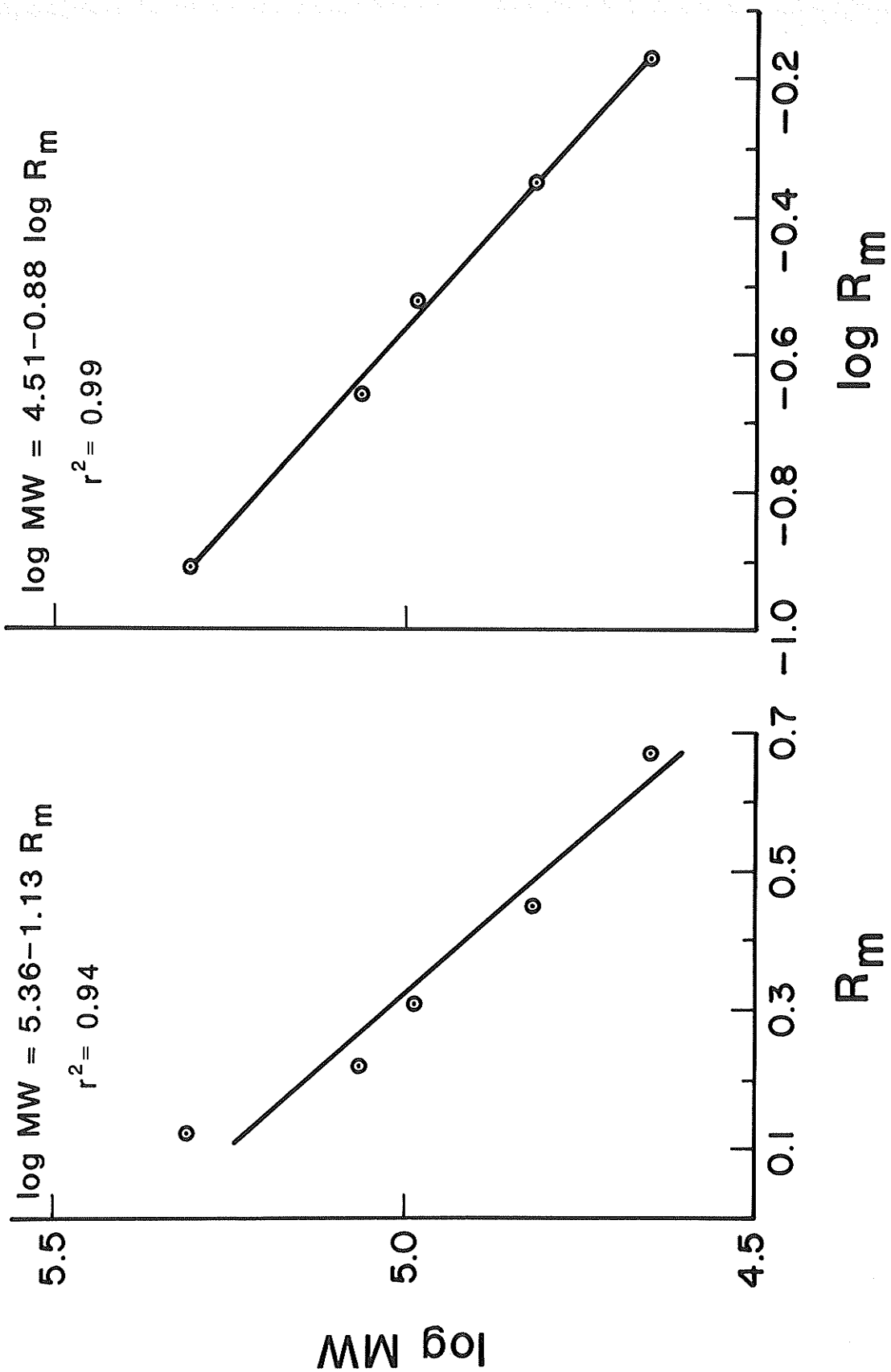
1. Primary Standard Reference Proteins

The final step in the preliminary part of this thesis project is the determination of the molecular weights (MWs) of glutenin subunits of the wheat variety Marquis. The glutenin of this variety will be used subsequently as the reference markers in the determination of subunit MWs of the experimental varieties.

Molecular weights of glutenin subunits of the wheat cv. Marquis were determined by the standard procedure using primary standard reference proteins of known MW to develop a primary calibration curve. Marquis was selected as the reference cultivar because of its extensive use in wheat breeding programs and because it is already used widely as the reference in cultivar identification by gliadin electrophoresis (Bushuk and Zillman, 1978; Sapirstein and Bushuk, 1985). It should be noted that the MWs that are obtained by SDS-PAGE are relative to the MWs of the markers. For this reason, the MW is sometime referred to as relative molecular mass (Bunce et al., 1985).

For the primary calibration curve, the relative mobility (Rm) of each of the primary standard reference proteins was calculated using the mobility of the smallest protein (carbonic anhydrase) as the front marker as described in chapter III section F. To obtain the MW vs. mobility calibration curve, two different relationships, log MW versus Rm and log MW versus log Rm were examined (Fig. 13). The regression

Figure 13. Relationship between log MW vs. Rm (relative mobility) and log MW vs. log Rm for primary reference proteins.


log MW = 5.36−1.13 R_m
r^2= 0.94
log MW = 4.51−0.88 log R_m
r^2= 0.99
log MW
5.5
5.0
4.5
0.1
0.3
0.5
0.7
−1.0
−0.8
−0.6
−0.4
−0.2
R_m
log R_m

line for the log MW vs. log Rm plot was slightly better than for the conventional plot (r^2 = 0.99 and r^2 = 0.94, respectively). Accordingly, the regression equation for log MW vs. log Rm was used to estimate the MWs of Marquis subunits (for subsequent use as the secondary standard MW reference) and of the subunits of other wheat cultivars. The purely mathematical treatment of log Rm did not introduce any additional error over that inherent in Rm since the MWs of subunits were calculated from the regression equation and not measured directly from the calibration curve.

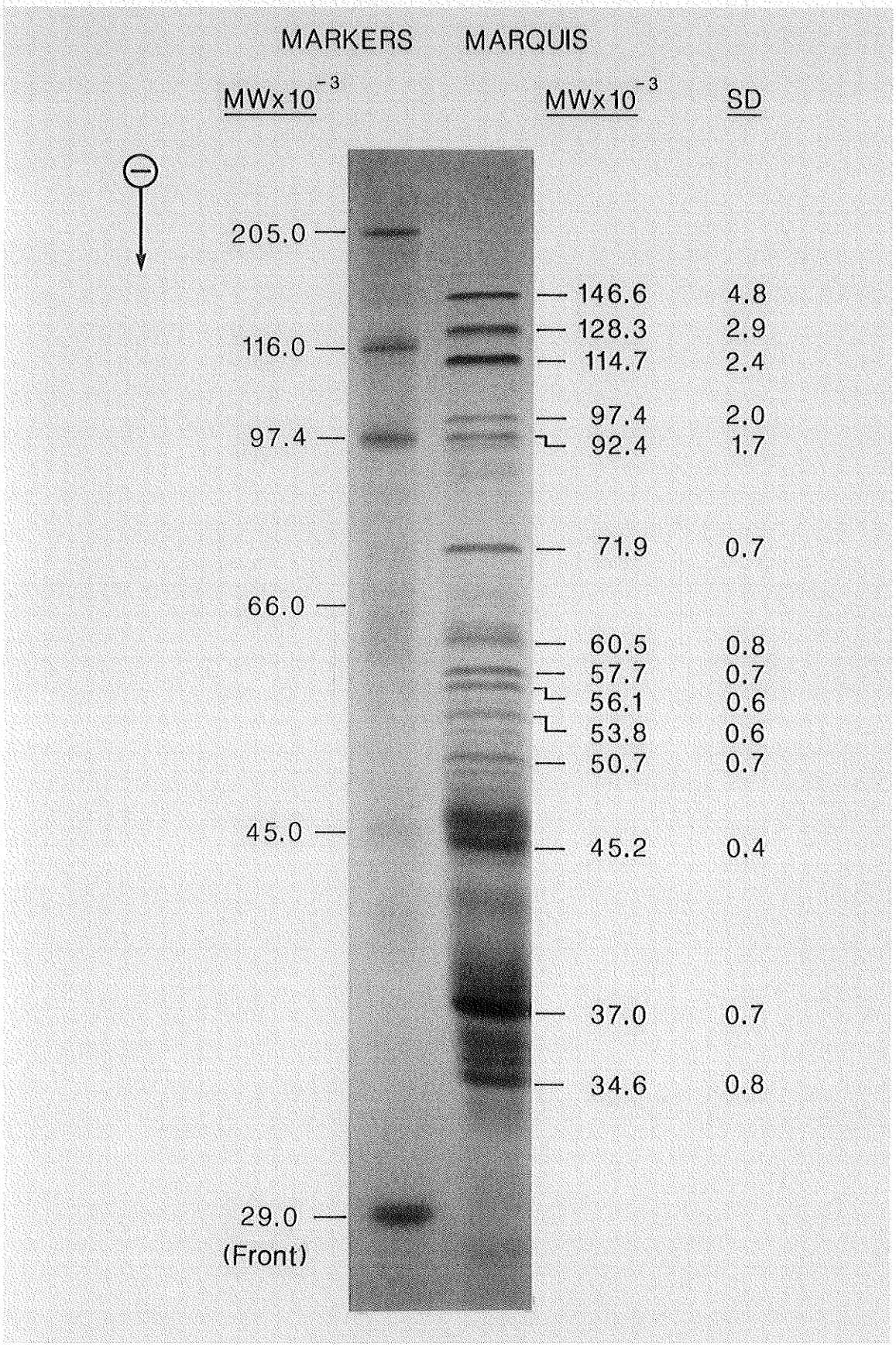
2. Secondary Standard Reference Proteins

Figure 14 shows SDS-PAGE patterns of cv. Marquis glutenin and the primary standard reference protein mixture. The major Marquis subunits, which will be used as the secondary standard reference proteins, are identified and their MWs indicated. The Rm values of the Marquis subunits were calculated relative to the mobility of carbonic anhydrase. The MWs indicated in the figure are average values of six separate runs. Standard deviations for each subunit are shown also. The range of precision for the results in Figure 14 is 0.88% to 3.27% (SD/mean x 100%).

For the secondary calibration curve, the Rm of each of the Marquis glutenin subunits (Fig. 14) was calculated using the mobility of the subunit of MW 34,600 as the front marker. If the front marker subunit is present in the experimental variety, it would serve as an additional check on the precision of the electrophoretic run. In fact this subunit was present in all varieties analyzed in the present study.

Figure 14. SDS-PAGE patterns for primary standard reference proteins and glutenin subunits of cv. Marquis. SD, standard deviation (n = 6).

Coincidentally, a cv. Marquis subunit had the same MW as the 97.4 kD primary reference protein even though it was at a distinctly different position on the electrophoregram. The MW of this subunit was the average of six separate experiments, each with its respective regression equation established from the primary reference proteins.


MARKERS
MARQUIS
MWx10 -3
MWx10 -3
SD
205.0
116.0
97.4
66.0
45.0
29.0
(Front)
146.6 4.8
128.3 2.9
114.7 2.4
97.4 2.0
92.4 1.7
71.9 0.7
60.5 0.8
57.7 0.7
56.1 0.6
53.8 0.6
50.7 0.7
45.2 0.4
37.0 0.7
34.6 0.8

3. Estimation of Relative Molecular Weight

Four separate analyses of Neepawa glutenin were made to check the reproducibility of subunit patterns and to determine the reproducibility of MW values by using the proposed method of using cv. Marquis subunits (as reference proteins) to obtain the working calibration curve. In each analysis, glutenins of cv. Marquis and the experimental wheat variety (cv. Neepawa) were run together. For each Marquis pattern, the Rm values were determined relative to the position of the 34,600 subunit (front marker) in the same pattern. Then, the regression equation for log MW vs. log Rm was determined for data of each analysis. This regression equation was then used to estimate the MWs of the HMW glutenin subunits of the experimental variety (i.e., Neepawa). The MWs for the experimental variety from the four separate runs were averaged (Table 7). The four MWs for each subunit so obtained differed by less than 1.5%.

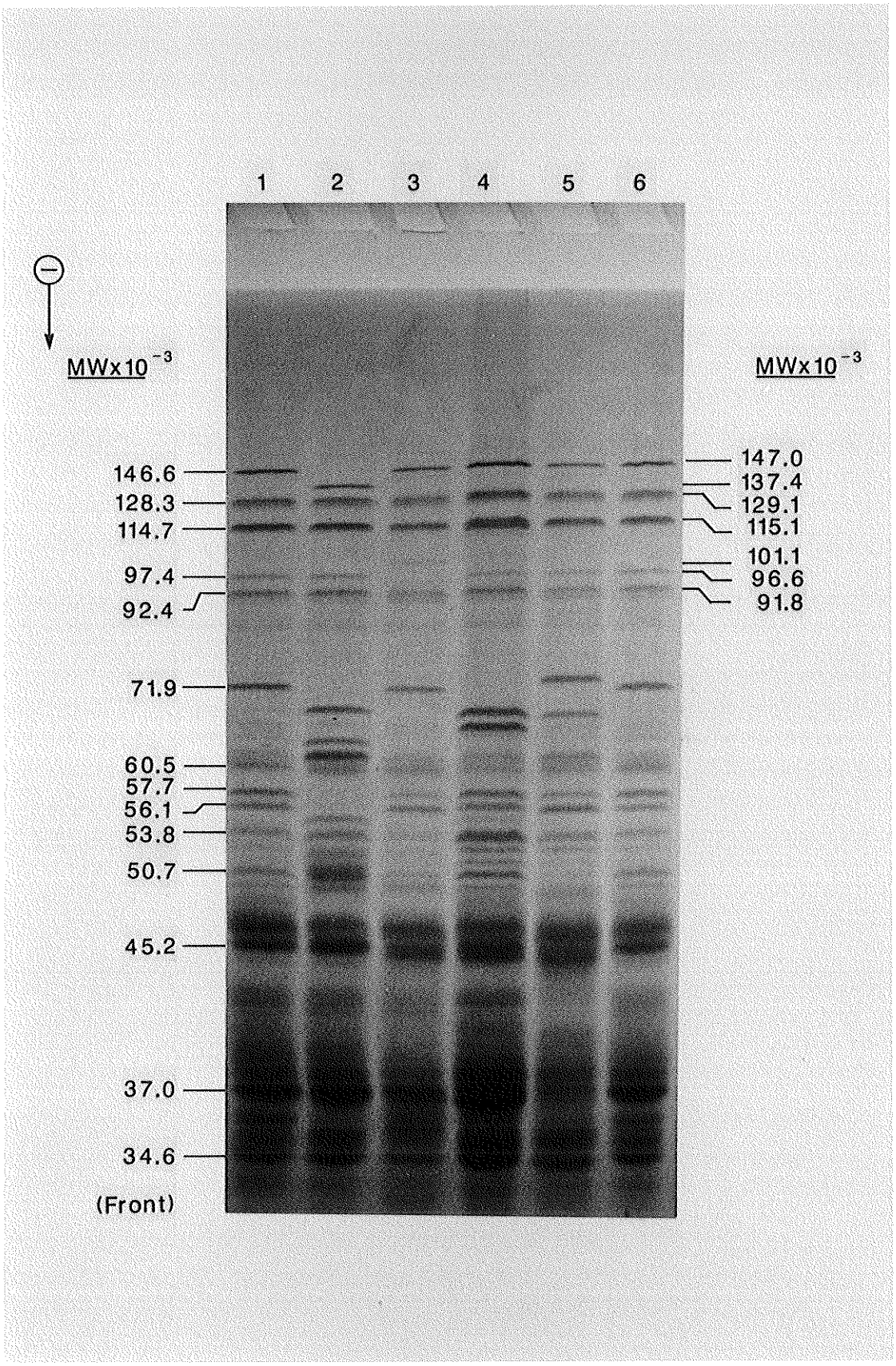
On the basis of these results, it was concluded that the cv. Marquis glutenin subunit pattern can be used with sufficient precision to warrant its use as a reference for determination of MWs of "glutenin" subunits of other wheat cultivars.

The glutenin subunits of Marquis are being proposed as the standard reference proteins for determining the MWs of glutenin subunits of other wheat cultivars. In laboratories in other countries, local cultivars can be used as standards after calibration against cv. Marquis. Figure 15 shows the SDS-PAGE patterns of the Australian cv. Halberd, British cv. Holdfast, German cv. Diplomat, and Canadian cv. Neepawa. The MWs of their HMW glutenin subunits were determined using Marquis as the reference and are indicated on the right hand

Table 7. Molecular weight and standard deviation (n=4) for HMW glutenin subunits of cv. Neepawa

MW($x10^{-3}$)	136.6	127.8	115.0	96.5	91.7
SD	1.4	0.9	0.4	0.3	0.5

Figure 15. SDS-PAGE patterns. Lanes 1 and 6 = Marquis, 2 = Neepawa, 3 = Holdfast, 4 = Halberd, 5 = Diplomat. MWs of Marquis subunits listed on left, other cultivar HMW glutenin subunits listed on right.


1
2
3
4
5
6
MWx10⁻³
MWx10⁻³
146.6
128.3
114.7
97.4
92.4
71.9
60.5
57.7
56.1
53.8
50.7
45.2
37.0
34.6
(Front)
147.0
137.4
129.1
115.1
101.1
96.6
91.8

side of the figure. Five of the HMW glutenin subunits of cv. Holdfast (Fig. 15, lane 3) are probably subunits 1, 5, 7, 8 and 11 according to Payne et al. (1980a). The subunits 1 and 8 have MWs of 145,000 and 106,000, respectively, determined by SDS-PAGE by Payne et al. (1980a). These values compare well with 147,000 and 101,100 obtained in the present study. The agreement between the two sets of MW values is considered to be quite good in view of the overall precision of the SDS-PAGE method (Bunce et al., 1985). It should be noted that MW values obtained in the present study apply only to the gel system used; other conditions would require a separate calibration.

F. Routine Method for Determination and Nomenclature of HMW Glutenin Subunits

The proposed routine method based on using glutenin subunits of Marquis as reference proteins for determination of MWs of glutenin subunits of other wheat cultivars by SDS-PAGE has several practical advantages. The reference proteins are similar to the unknown proteins in chemical and physical structure, the entire range of MWs is covered by a large number of reference proteins, and the source of reference proteins is inexpensive.

The subject of nomenclature of glutenin subunits deserves comment. Currently, most published reports use the nomenclature proposed by Payne et al. (1980a and 1981b) for HMW glutenin subunits in which the subunits are identified by numbers beginning with 1 for the largest subunit. However, it should be pointed out that some confusion may arise by using this numerical nomenclature. For example, Payne and co-workers, identified 12 bands (1 to 12) according to their relative

electrophoretic mobilities on SDS-PAGE (1980a) of seven wheat varieties. In the following year, they found a few more bands in patterns of different varieties (Payne et al., 1981b) and thus the order of these bands by decreasing MW became: 1, 2, 2*, 3, 4, 5, 6, 7, 13, 14, 15, 16, 17, 18, 19, 8, 9, 10, and 12. They deleted subunit 11 because of problems in discriminating subunits 10, 11, and 12. It appears that this nomenclature is not a system that can readily accommodate new subunits without confusion. Furthermore, a different numbering system was used by Moonen et al. (1983). Accordingly I propose that glutenin subunits be identified by their SDS-PAGE MW as shown in Figure 15. This nomenclature is preferred because it will obviate the difficulties encountered with the "closed" systems referred to above. Hereinafter, the subunits will be identified by their MW in kilodaltons.

Although it appears that SDS-PAGE overestimates the MW (Bunce et al., 1985), the system for estimating MWs of subunits proposed in this thesis is valid since the MWs are obtained relative to characterized reference proteins. All the MW values can be subsequently corrected when the exact values for some of the subunits are determined from complete amino acid sequencing.

G. HMW Glutenin Subunits of the 26 1983 UQN Varieties

1. Determination of Relative Molecular Weights

The MWs of the HMW glutenin subunits of the 26 varieties were determined by SDS-PAGE using the method described above. SDS-PAGE electrophoregrams of the 26 varieties are shown in Figs. 16 and 17. The MWs of the reference proteins (Marquis glutenin subunits) are

Figure 16. SDS-PAGE patterns of 1983 UQN varieties 1 to 13. M = cv. Marquis; lane no. corresponds to variety no. as listed in Table 1. MWs of Marquis subunits listed on left, HMW glutenin subunit region shown on right.

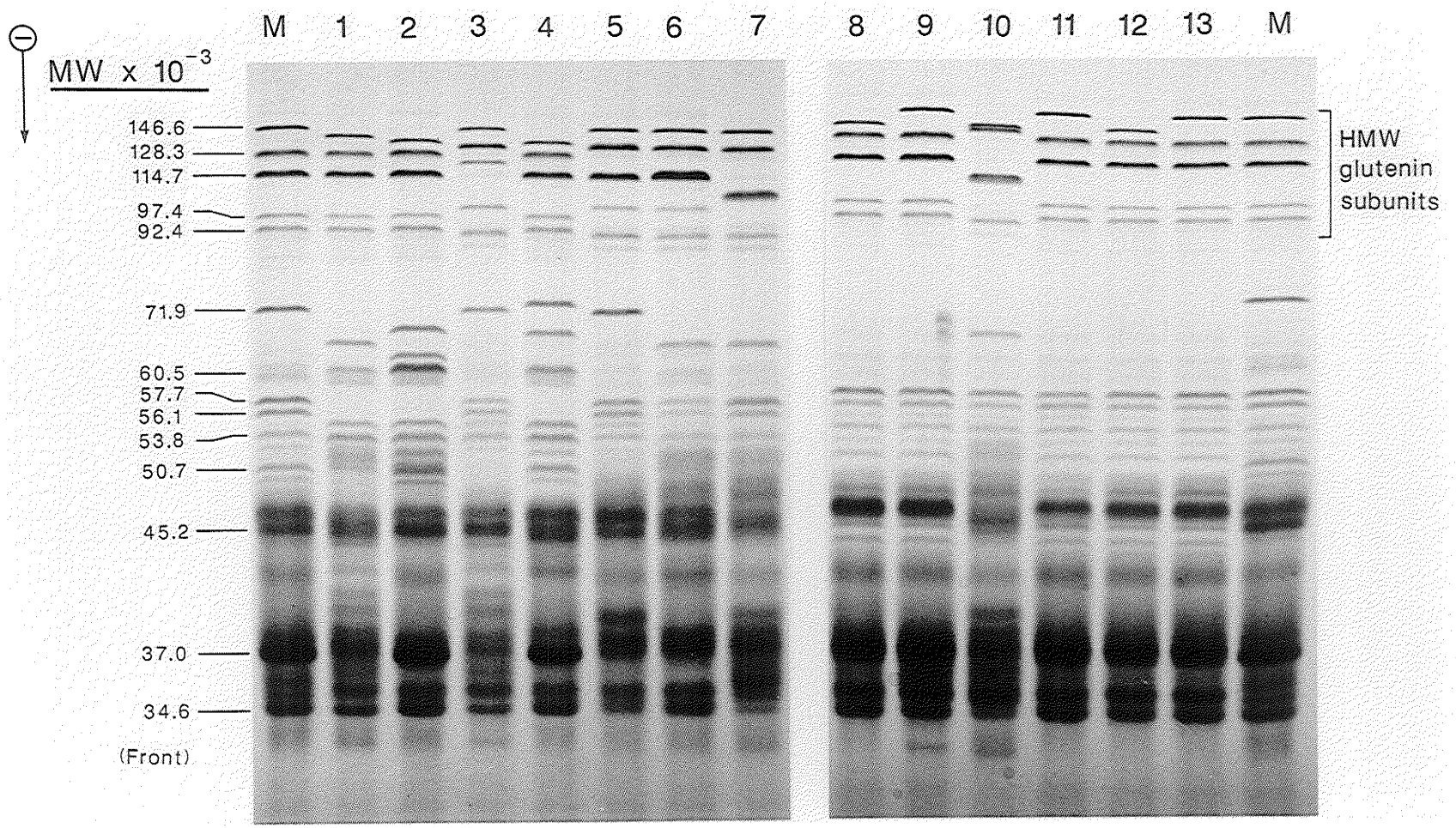
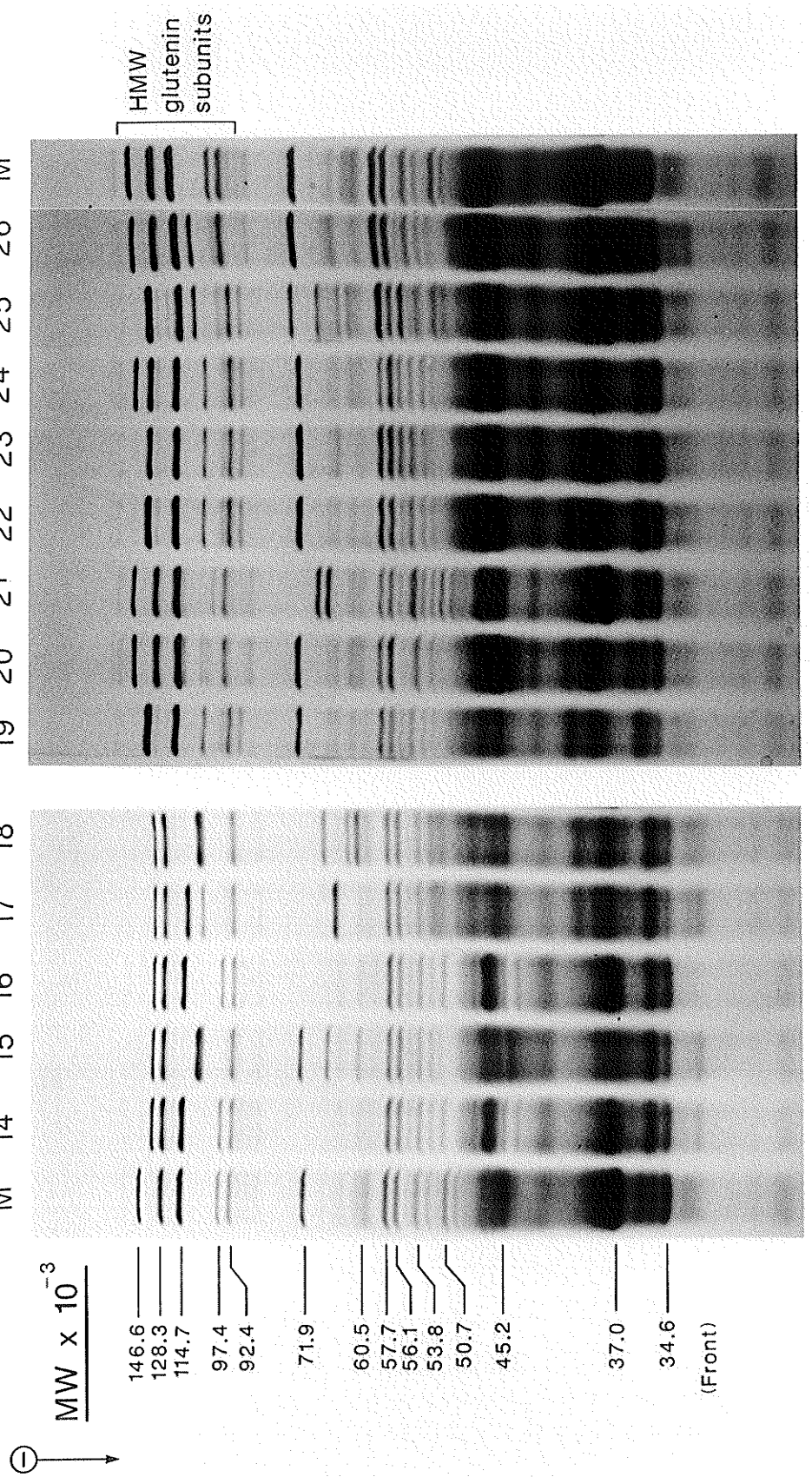

M 1 2 3 4 5 6 7 8 9 10 11 12 13 M
MW x 10^{-3}
146.6
128.3
114.7
97.4
92.4
71.9
60.5
57.7
56.1
53.8
50.7
45.2
37.0
34.6
(Front)
HMW glutenin subunits

Figure 17. SDS-PAGE patterns of 1983 UQN varieties 14 to 26. M = cv. Marquis; lane no. corresponds to variety no. as listed in Table 1. MWs of Marquis subunits listed on left, HMW glutenin subunit region shown on right.


HMW glutenin subunits
M 14 15 16 17 18 19 20 21 22 23 24 25 26 M
MW x 10^-3
146.6
128.3
114.7
97.4
92.4
71.9
60.5
57.7
56.1
53.8
50.7
45.2
37.0
34.6
(Front)

listed on the left hand side. The relative mobility (Rm) of each subunit for each of the 26 varieties was determined relative to the position of subunit 34.6 of Marquis in the same pattern. Then MWs of the subunits of the UQN varieties were determined using the regression equation for log MW vs. log Rm obtained for Marquis in each pattern (each analysis). SDS-PAGE data were replicated three times.

The pattern for each variety contained more than 15 bands of glutenin subunits. Accordingly, it was necessary to define the HMW glutenin subunit range for the purpose of the present study. According to Payne et al. (1980a), the HMW range was from subunit 1 to subunit 12, which have MWs of 145.0 and 95.0 kilodaltons (kD), respectively. It was impossible to use their nomenclature to label the subunits in the present study since it would be necessary to have the same set of varieties used by Payne et al. (1980a and 1981b). The MW range of 90.0 to 147.4 kD was adopted to delineate the HMW glutenin subunit region for the 26 varieties used in the present study (as indicated on the right hand side of Figs. 16 and 17). This range includes all the subunits which Payne et al. (1980a and 1981b) referred to as HMW glutenin subunits.

As discussed in section F of this chapter, the nomenclature proposed by Payne et al. (1980a and 1981b) presents problems in naming "new" subunits. In the present study, relative molecular weights will be used to identify subunits. To do this, it was first necessary to determine whether the difference in MW of some closely spaced bands was statistically significant. Using the range of MWs for the HMW glutenin subunit region indicated above, the 26 varieties contained a total of 13 different HMW glutenin subunits. Their average MWs are

tabulated in Table 8. The number of observations (N) of each subunit was the total number for the subunit in all 26 varieties, multiplied by three (no. of replicates). The standard deviation and t-test values are also included.

It is clear that all 13 subunits are statistically different from each other. On the basis of this evidence, it was concluded that the set of varieties investigated contained 13 different HMW glutenin subunits as determined by SDS-PAGE. Accordingly, it is justified to use these subunits for further studies.

2. Intervarietal Relationships Between HMW Glutenin Subunits

Table 9 shows the distribution of the HMW glutenin subunits from SDS-PAGE for the 26 varieties (presence is indicated by a dash). None of the varieties contained all 13 subunits. Most varieties contained 4 or 5 subunits. These observations are in general agreement with published data of Payne et al. (1981b). The present study showed that subunit 114.7 occurred in highest frequency (69.2%) while subunits 141.0 and 121.1 occurred only once each in the 26 varieties. For this reason these two subunits were not considered in further analyses because their recurrence was too low (3.8% frequency) to justify relating them to intravarietal variability of breadmaking quality.

It should be pointed out that certain absence/presence patterns of subunits occurred with respect to specific other subunits. For example, subunits 128.1 and 90.0 never occurred together in the same variety; the same was true for subunits 147.4 and 135.8, except for variety 24 which had both of the subunits 147.4 and 135.8. On the other hand, subunits 128.1 and 91.6 always appeared together in the

Table 8. Mean and standard deviation (SD) of molecular weights of HMW glutenin subunits of the 26 1983 UQN varieties

Mean[1]		SD	N	t
147.4	±	1.6	33	
				6.72***
141.0	±	1.6	3	
				6.51***
135.8	±	1.3	45	
				6.62***
133.1	±	1.5	15	
				12.26***
128.1	±	1.3	48	
				9.26***
121.1	±	0.6	3	
				11.13***
114.7	±	1.0	54	
				3.09**
113.6	±	1.1	9	
				17.37***
105.5	±	1.2	21	
				17.57***
100.2	±	0.7	21	
				17.66***
96.3	±	0.8	33	
				24.34***
91.6	±	0.9	48	
				9.14***
90.0	±	0.6	30	

[1]Mean molecular weight, $MWx10^{-3}$.
N, number of observations.
t, Student t-test between two means (Mendenhall, 1979).
,*, Significant at 1% and 0.1%, respectively.

Table 9. Patterns of HMW glutenin subunits for the 26 1983 UQN varieties[1]

Subunit[2]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	Sununit
147.4			–		–	–	–		–		–		–							–	–			–		–	147.4
141.0	–																										141.0
135.8		–		–				–		–		–		–	–	–	–	–	–			–	–	–	–		135.8
133.1			–		–	–	–			–																	133.1
128.1	–	–		–				–	–		–	–	–	–	–	–	–	–		–	–					–	128.1
121.1			–																								121.1
114.7	–	–		–	–	–		–	–		–	–	–	–		–			–	–	–	–	–	–			114.7
113.6																	–								–	–	113.6
105.5							–			–					–		–	–							–	–	105.5
100.2			–		–	–													–			–	–	–			100.2
96.3	–	–		–				–	–		–	–	–	–		–					–						96.3
91.6	–	–		–				–	–		–	–	–	–	–	–	–	–		–	–					–	91.6
90.0			–		–	–	–			–									–			–	–	–	–		90.0

[1]Variety no. corresponds to variety no. listed in Table 1.
[2]Subunit with MW x 10^{-3}

same variety. Perhaps this results from gene linkages as described by Payne et al. (1981b). Furthermore, varieties 9, 11 and 13 had identical HMW glutenin patterns, whereas varieties 8 and 12 had the same patterns but differed from the characteristic pattern of the other three varieties. These five varieties are all selections of the variety Veery (Table 1).

Some researchers have attempted to quantify the amount of protein present in each band. Payne et al. (1981b) used a spectrophotometric method to estimate the relative intensities of the subunit bands, i.e., the relative amount of dye bound by the subunits. This approach would be helpful for obtaining information on interactions between quantitative and qualitative aspects of certain subunits to breadmaking quality. Quantification, however, commands two provisos: (1) an accurate and sensitive instrument for measuring band intensity, and (2) an assumption that similar amount of protein enters the gel for each variety. The latter information is difficult to obtain with present technology.

Branlard and Dardevet (1985) used densitometric analysis to determine the protein content in a particular band. The quantity of proteins present in the band was obtained by multiplying the relative concentration by the glutenin content of the flour. The glutenin fraction was prepared by a micromethod according to Bourdet et al. (1972). Of course, this approach assumes that similar proportions of proteins of all wheat varieties examined entered the gel.

Glutenin subunit band intensity has also been determined by visual rating. DuCros (1987) rated the intensity of HMW glutenin bands on a scale of 1 to 4 where 1 indicated no band present, 2 was a faint

band, 3 was a medium-stained band, and 4 was a strongly-stained band. The author observed 16 HMW glutenin bands in the group of durum cultivars that she examined. However, only nine bands were used for analysis since the other seven bands were always rated 2 (faint). This introduced confusion as to whether the faint bands were due to low protein content of the cultivars that were examined or if the bands were indeed minor constituents of the HMW glutenin subunits.

In the present study, the relationship between HMW glutenin subunits and breadmaking quality was based on presence and/or absence of specific subunits that were detected by SDS-PAGE. Intensity of the band was not part of the analysis. Simple correlation was used first to determine inter-subunit relationships for the 26 varieties.

Table 10 gives the correlation coefficients between the 11 subunits in the MW range from 147.4 to 90.0. As mentioned above, subunits 128.1 and 91.6 always appeared together and, as was expected, the correlation coefficient of the subunits was perfect ($r = 1.000$). On the other hand, subunits 128.1 and 90.0 never appeared together ($r = -1.000$), and likewise for subunits 91.6 and 90.0 ($r = -1.000$). There were also some subunits positively or negatively correlated to each other at significant levels.

The number in brackets (see footnote 3) under Subunit in Table 10 is the identity of subunit according to the nomenclature of Payne et al. (1980a and 1981a, b). It was impossible in the present study to be sure that Payne's identity of the subunits and the identity of subunits in the present study, as presented in Table 10, were identical. As discussed before, it would be necessary to have the appropriate set of varieties used by Payne and coworkers in order to make

Table 10. Correlation[1] between high molecular weight glutenin subunits for the 26 1983 UQN varieties

Subunit[2]	147.4 (1)[3]	135.8 (2)	133.1 (2*)	128.1 (5)	114.7 (7)	113.6 (13-16)	105.5 (17-18)	100.2 (8)	96.3 (9)	91.6 (10)	90.0 (12)
147.4	1										
135.8	-842***	1									
133.1	372	-372	1								
128.1	-123	-037	-617***	1							
114.7	065	-065	-309	158	1						
113.6	-066	066	-176	038	542**	1					
105.5	-169	169	144	-055	-910***	595**	1				
100.2	182	-007	364	-768***	217	-219	-368	1			
96.3	-103	-055	-418*	677***	571**	-309	-520**	-520**	1		
91.6	-123	-037	-617***	1***	158	038	-055	-768***	677***	1	
90.0	123	037	617***	-1***	-158	-038	055	768***	-677***	-1***	1

[1]Decimals omitted, except for those with perfect correlations (i.e., r = -1 or 1).
[2]Subunit with MW x 10^{-3}
[3]Payne's nomenclature (1980a and 1981a,b; see text for details).
*, **, *** significantly correlated at 5%, 1%, 0.1%, respectively.

accurate cross-identification. The identity of subunits by Payne and co-workers were matched with the identity of subunits in the present study, as reported in Table 10, to the best of the author's ability, as gleaned from the literature and aided with the SDS-PAGE pattern of cv. Holdfast (Fig. 15) run along with cv. Marquis. Assuming that the match-up of the subunit numbers by Payne et al. (1980a and 1981a, b) and the identities of the subunits reported in the present study (Table 10) was correct, then subunits 135.8, 128.1, 91.6 and 90.0 would be the same subunits reported by Payne et al. (1980a, 1981a, b) as their numbers 2, 5, 10, and 12, respectively.

H. Statistical Relationships Between HMW Glutenin Subunits and Technological Characteristics of the 26 Varieties

The technological characteristics of the wheat varieties used in the present study were presented in Table 5. In section D of this chapter, these technological characteristics were discussed and their relationships to each other were reported. It was pointed out that not all of the characteristics that were significantly correlated with each other were relevant to the thesis project.

The relationships between the 11 HMW glutenin subunits of the 26 varieties and these technological characteristics are presented in Table 11. Again not all significant correlations are relevant to the main objective of this thesis project. For example, subunit 105.5 was significantly correlated with amylograph viscosity (AV). Amylograph viscosity is a measurement obtained from the amylograph which measures α-amylase activity through the viscosity of a flour slurry, and viscosity measurement from the amylograph most likely would not relate to

Table 11. Correlation[1] between technological data[2] and HMW glutenin subunits for the 1983 UQN varieties (n=26)

Subunit[3]	147.4	135.8	133.1	128.1	114.7	113.6	105.5	100.2	96.3	91.6	90.0
HW	-116	-075	262	141	098	-337	-105	-119	368	141	-141
GP	-341	157	-083	047	-228	374	224	-225	063	047	-047
FN	-352	386	203	-132	-134	-129	208	037	-165	-132	132
FY	166	-236	130	147	059	-223	005	046	023	147	-147
GT	049	-083	-004	-334	027	180	-082	275	-339	-334	334
FP	-297	114	-130	151	-075	328	178	-327	243	151	-151
ASH	215	-046	-373	088	074	178	-036	027	-124	088	-088
SV	024	-140	151	147	-259	105	399*	-211	-161	147	-147
AV	-269	314	221	-276	-399*	240	523**	039	-501**	-276	276
WG	-293	245	001	-294	-018	215	013	085	043	-294	294
FA	-160	297	-141	232	-074	-073	191	-247	257	232	-232
DT	-192	059	-149	579**	-056	-043	192	-511**	340	579**	-579**
MTI	051	-051	052	-565**	-023	123	-080	531**	-498**	-565**	565**
E	330	-264	288	-561**	-059	159	015	548**	-574**	-561**	561**
R	-079	-093	-053	526**	-151	001	288	-525**	249	526**	-526**
R/E	-205	057	-150	598**	-084	-067	205	-586**	393*	598**	-598**
A	140	-290	071	335	-181	094	316	-333	-025	335	-335
LV	-086	-012	-153	522**	023	-050	133	-418*	362	522**	-522**
BSI	008	-037	-124	516**	042	-170	104	-355	308	516**	-516**
ULV	001	-034	-130	520**	041	-162	104	-357	310	520**	-520**

[1]Decimals omitted.
[2]As defined in Table 5.
[3]Subunit with MW x 10^{-3}
*, **, *** significantly correlated at 5%, 1%, 0.1% respectively.

HMW glutenin subunit but rather to the starch component in the flour. Furthermore, it can be seen from Table 11 that of the technological tests for grains and flours, the HMW glutenin subunits were only related to the rheological (farinograph and extensograph) and remix baking tests. Thus, based on the varieties used in the present study, HMW glutenin subunits cannot be used to predict many of the technological tests used in this study; the exceptions are the farinograph, extensograph and baking tests.

The parameters derived from farinogram are farinograph absorption (FA), dough development time (DT), and mixing tolerance index (MTI), whereas those derived from extensogram are extensibility (E), maximum resistance (R), and area under the curve (A). All these parameters were discussed fully in section D of this chapter. Each parameter except for FA and A (Table 11) correlated significantly with at least four subunits; FA and A did not correlate with any of the subunits. Furthermore, not every subunit correlated with at least one parameter; only subunits 128.1, 100.2, 96.3, 91.6 and 90.0 did. Subunits 128.1 and 91.6 correlated to the strength of the flour, as based on relationships between DT and subunit 128.1 ($r = 0.579^{**}$), MTI and subunit 128.1 ($r = -0.565^{**}$), DT and subunit 91.6 ($r = 0.579^{**}$), and MTI and subunit 91.6 ($r = -0.565^{**}$). The coefficients between DT and subunit 128.1, and DT and subunit 91.6 were the same, as expected, since subunits 128.1 and 91.6 were perfectly correlated (Table 10, $r = 1.000$). If, as suspected, subunit 128.1 is indeed subunit 5, and subunit 91.6 is subunit 10 of Payne *et al.* (1981a, b), then the results of this study are in agreement with Branlard and Dardevet (1985). They reported that subunits 5 and 10 (equivalent to subu-

nits 128.1 and 91.6 in the present study) were significantly correlated with dough strength as measured by the Chopin Alveograph.

Payne et al. (1981a) reported that subunits 2 and 12 (subunits 135.8 and 90.0 in this study) were associated with poor breadmaking quality. The results obtained in the present study also indicated a similar trend. Subunit 90.0 correlated significantly with DT (negative correlation) and MTI (positive correlation), r = -0.579** and 0.565**, respectively. As discussed in section D, a weak dough gives a shorter DT and a higher MTI. But in the set of the varieties used in the present study, subunit 135.8 did not correlate with any of these parameters.

The results discussed above are supported by correlations between HMW subunit composition and extensograph parameters. Subunit 100.2 was significantly correlated positively to extensibility (E) and negatively to maximum resistance (R), r = 0.548** and -0.525**, respectively. Likewise, subunit 90.0 was also correlated to E and R, r = 0.561** and -0.526**, respectively. This is in general agreement with published data by Branlard and Dardevet (1985). They reported that their subunit 12 (subunit 90.0 in the present study) was significantly correlated to tenacity measured from the Chopin Alveograph (r = -0.338**). Tenacity measurement is related to the measurement of resistance of dough to deformation, which yields similar information to maximum resistance (R) of dough measured from extensograph. These results indicate that subunits 100.2 and 90.0 are related to poor rheological properties of extensograph parameters of dough, for the set of varieties used in the present study.

On the other hand, the correlation coefficients of subunits 128.1

and 91.6 to E and R showed that these two subunits were definitely associated with good rheological properties of dough as measured by extensograph parameters. These findings are also in general agreement with the published data of Branlard and Dardevet (1985).

As mentioned in section D of this chapter, the remix baking test is the all-inclusive breadmaking quality test for Canadian bread wheats. If any of the HMW glutenin subunits are related to breadmaking quality, those subunits should, by definition, correlate with loaf volume (LV). Indeed, the subunits significantly correlated to parameters from farinograph and extensograph were also significantly correlated with LV (Table 11). As mentioned before, LV is affected by protein content of the flour, therefore it would be more appropriate to use the baking strength index (BSI) or loaf volume per unit protein (ULV) for examining the relationship between HMW glutenin subunits and breadmaking quality in qualitative terms than to use LV. The subunits 128.1 and 91.6 showed strong positive correlations to these two criteria whereas subunit 90.0 was negatively correlated. The subunit 100.2 was neither correlated to BSI nor ULV ($r = -0.355$ and -0.357, respectively). These findings are in general agreement with published data by Payne *et al.* (1981a) and Branlard and Dardevet (1985). They reported that their subunits 5 and 10 (equivalent to subunits 128.1 and 96.6, respectively, in the present study) were contributors to good breadmaking quality, whereas their subunit 12 (subunit 90.0 in this study) contributed to poor breadmaking quality.

Based on the above findings, one can conclude that subunits 128.1, 91.6, and 90.0 are important to breadmaking quality; the first two contribute positively and the third, negatively.

I. Regression Equations for Predicting Breadmaking Quality on the Basis of HMW Glutenin Subunit Composition

The ultimate objective of a study of HMW glutenin subunits and breadmaking quality is to be able to predict the breadmaking quality from the composition of HMW glutenin. Thus, it was necessary to generate an equation to predict breadmaking quality from HMW glutenin subunit composition. The following section will deal with attempts to generate equations from HMW glutenin subunits to predict breadmaking criteria.

Stepwise multiple regression technique was used to generate equations for predicting certain breadmaking quality criteria based on HMW subunit composition. All 11 HMW subunits from the 26 varieties were used in stepwise multiple regression analysis as independent variables. DT, and MTI from farinograph, E, R and R/E from extensograph, and BSI and ULV from remix baking test were each used as dependent variables in the regression analysis.

In the previous section (H), discussion focused on relationships of individual subunits to breadmaking quality criteria. However, most likely some subunits have additive or antagonistic effects. If so, stepwise multiple regression technique should reveal the combined effects of subunits. Table 12 presents prediction equations from the 11 HMW glutenin subunits (the independent variables) for seven technological tests of breadmaking quality. The coefficient of determination (r^2) and the probability of F values are also included.

It can be seen that all r^2 values were significant below the 1% level (see last column, Prob > F). Furthermore, not all of the 11 subunits were selected for any one of the prediction equations. As

Table 12. Coefficients of HMW glutenin subunits, intercept, r^2, F, and probablity of F of the prediction equations for seven breadmaking quality criteria[1]

Subunit[2]	147.4	135.8	133.1	128.1	114.7	113.6	105.5	100.2	96.3	91.6	90.0	INTERCEPT	r^2	F	PROB > F
DT	-1.10		3.57	5.54	3.74		4.98	2.02				-1.99	0.565	4.111	0.0082
MTI	-15.45	-20.87	-29.35		-19.21	3.22	-43.01	-22.43	-26.17		32.57	84.66	0.738	4.995	0.0026
E		-17.08	-9.69		17.34	13.20			-26.67		17.27	185.10	0.576	4.304	0.0066
R			323.33	479.31	384.72		708.47	305.69	169.85			-329.03	0.576	4.295	0.0067
R/E	-0.60		1.66	2.57	1.44	-0.96	2.31					0.48	0.576	4.295	0.0067
BSI	3.98		11.78	34.47	27.85	-15.69	65.68	41.53	21.99			7.70	0.675	4.416	0.0049
ULV	2.47		7.79	22.87	18.10	-10.03	42.96	27.48	14.54			4.96	0.669	4.289	0.0056

[1]As defined in Table 5.
[2]Subunit with MW x 10^{-3}

mentioned before, some subunits were present or absent together with another subunit (Table 10, r with perfect correlation). These subunits would not both be selected into a prediction equation by the stepwise multiple regression analysis. For example, within each pair, subunits 128.1 and 91.6, subunits 128.1 and 90.0, subunits 91.6 and 90.0, only one subunit was selected for the prediction equation.

The range of r^2 values for the seven prediction equations was 0.565 for DT to 0.738 for MTI. This means that 56.5% of the variation for DT is explained by six subunits and 73.8% of the variation for MTI is explained by nine subunits. For the ULV, 66.9% of the variation is explained by eight subunits in the range of MWs from 96.3 to 147.4 kD. As an example, the prediction equation of ULV is as follows:

$$\begin{aligned} ULV = {} & 4.96 + 42.96\ (105.5) + 27.48\ (100.2) + 22.87\ (128.1) + \\ & 18.10\ (114.7) + 14.54\ (96.3) + 7.79\ (133.1) + \\ & 2.47\ (147.4) - 10.03\ (113.6) \end{aligned}$$

To use this equation, the MW shown in brackets is replaced by the number 1 (one) if the subunit is present and by 0 (zero) if the subunit is absent in the SDS-PAGE pattern for a specific variety.

The predictive power of the equations generated by stepwise multiple regression analyses was tested by comparing predicted and actual values for a separate set of eight different wheat varieties from 1985 Bread Wheat Cooperative Test. The SDS-PAGE patterns of these varieties are shown in Figure 18 with the HMW glutenin subunit region delineated. Their HMW glutenin composition with their relative molecular weights are presented in Table 13. It is important to point out that all eight cultivars were considered relatively good breadmaking varieties since they were selected into the final stage of the Cooper-

Figure 18. SDS-PAGE patterns of 1985 Bread Wheat Cooperative Test cultivars.

M = cv. Marquis; C1 = cv. Marquis, C2 = cv. Neepawa,

C3 = cv. Sinton, C4 = cv. Benito, C5 = cv. Columbus,

W1 = cv. Katepwa, W2 = cv. Leader, W3 = cv. Lancer;

C = Central region, W = Western region.

MWs of cv. Marquis subunits listed on left,

HMW glutenin subunit region listed on right.

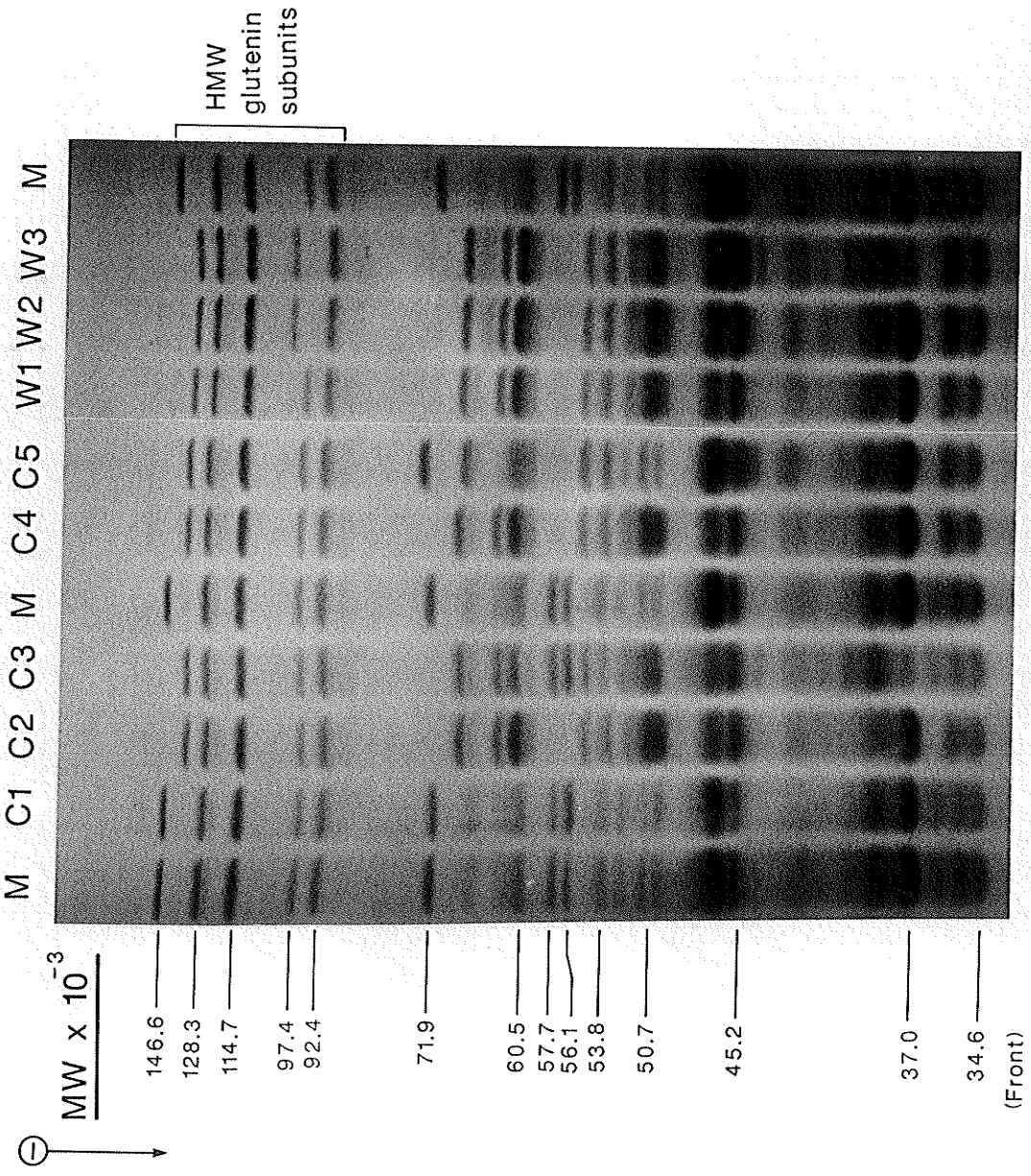

M C1 C2 C3 M C4 C5 W1 W2 W3 M
HMW glutenin subunits
MW x 10^-3
146.6
128.3
114.7
97.4
92.4
71.9
60.5
57.7
56.1
53.8
50.7
45.2
37.0
34.6
(Front)

Table 13. Pattern of HMW glutenin subunits from 1985 Bread Wheat Cooperative Test cultivars

SMW[1]	C1	C2	C3	C4	C5	W1	W2	W3[2]
147.4	-							
141.0								
135.8		-	-	-	-	-	-	-
133.1								
128.1	-	-	-	-	-	-	-	-
121.1								
114.7	-	-	-	-	-	-	-	-
113.6								
105.5								
100.2							-	-
96.3	-	-	-	-	-	-		
91.6	-	-	-	-	-	-	-	-
90.0								

[1]Subunit with MW x 10^{-3}.
[2]Variety no. corresponds to variety no. listed in Fig. 18.

ative Test for bread wheat varieties. Their technological data were obtained from the data published in the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986)

The first five parameters in the Table 12, DT, MTI, E, R, R/E, were tested against their respective predictive equations. The main thrust of the thesis project was to examine the relationship between HMW glutenin subunit composition and breadmaking quality. And, as indicated before, the all-inclusive test for breadmaking quality is the baking test (BSI and ULV). For this reason, the results for the five parameters, DT, MTI, E, R, and R/E, are presented in Appendix III.

The quality criteria DT and R were predicted within 95% confidence limits for the eight varieties. One of the eight varieties was predicted outside of the 95% confidence limit for MTI, two varieties were outside the 95% confidence limit for R/E, and all eight varieties were outside the limit for E (Appendix III, Table 18).

As mentioned in section D part 4 of this chapter, gliadin contributes to extensibility (E) and glutenin to elasticity. Thus, it was not surprising that the prediction of E from the glutenin subunit composition was outside the 95% confidence limit.

Actual BSI values were obtained from the data published in the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986) and ULV was calculated from the data published in the same minutes. The HMW glutenin subunit patterns of the eight cultivars were used to calculate the predicted values for BSI and ULV with the equations generated from the data for the 26 UQN varieties. The predicted and actual values are presented in Tables 14 (ULV) and

15 (BSI). All predicted values are within the 95% confidence limit. However, it should be emphasized that the results obtained here can not be generalized for all bread wheats since the prediction equations were generated from data for a limited number of varieties (26). By way of extension of this study, it would be useful to apply the prediction equations to a large group of varieties covering a much wider range of breadmaking quality.

Table 14. Predicted ULV of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	62.9	63.9	48.0	77.9
C2	60.5	65.0	46.0	75.0
C3	60.5	66.5	46.0	75.0
C4	60.5	64.1	46.0	75.0
C5	60.5	68.3	46.0	75.0
W1	60.5	67.2	46.0	75.0
W2	73.4	65.3	54.5	92.3
W3	73.4	72.8	54.5	92.3

[1]Values calculated from results obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).

Table 15. Predicted BSI of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	96.0	97.7	73.7	118.3
C2	92.0	99.1	70.3	113.7
C3	92.0	101.4	70.3	113.7
C4	92.0	97.5	70.3	113.7
C5	92.0	104.0	70.3	113.7
W1	92.0	102.3	70.3	113.7
W2	111.5	99.3	83.3	139.8
W3	111.5	110.7	83.3	139.8

[1]Values obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).

V. GENERAL DISCUSSION

Wheat flour proteins have long occupied the attention of cereal scientists. The ability of wheat flour to form a dough which can be baked into a tasty loaf of bread depends mostly on the proteins. Of course, other flour constituents, such as carbohydrates and lipids, and added ingredients, and the right processing all contribute to the quality of the final product, bread.

There are two aspects of flour proteins that are important to breadmaking characteristics of flour: protein content and protein quality. Protein content can be determined precisely by several different methods of analysis. Protein content of wheat mainly depends on agronomic and environmental factors, such as soil nitrogen, soil moisture, and temperature during the growing season (Bushuk, 1984; CIGI, 1983).

Protein quality is related to the type and number of protein molecules that constitute the total flour proteins. These are mainly inherited characteristics. However, they can also be affected by abnormal environmental conditions, such as disease, high temperature during maturation, wet harvest conditions, and improper postharvest storage (Bushuk, 1984). Protein quality generally refers to the combination of chemical and physical properties that collectively contribute to the desirable quality of the end product. In the case of bread, high quality generally means large loaf volume and good crumb texture.

Flour proteins are comprised of a group of many components with widely different amino acid compositions and sequences and hence different secondary, tertiary and quaternary structures. Wheat endosperm

contains a wide variety of non-storage proteins and over 100 storage-protein components (Payne et al., 1984). The storage proteins form the major portion of the protein of gluten---the viscoelastic substance responsible for the breadmaking ability of wheat. Storage proteins are generally classified into two groups of proteins, the gliadins and the glutenins. It has been shown that insoluble glutenin is directly proportional to the loaf volume (Orth and Bushuk, 1972). Furthermore, Payne et al. (1979) demonstrated that high molecular weight (HMW) subunits of glutenin correlate with breadmaking quality of English bread wheat varieties. Thus, information on the relationship between HMW glutenin subunits and breadmaking quality would be useful in developing an early-generation test for screening bread wheat varieties in breeding programs. With these considerations in mind, the present research was undertaken to explore further possible relationships between HMW glutenin subunits and breadmaking quality using 26 varieties of diverse breadmaking quality grown in four locations of Western Canada.

As the first step in the research, it was necessary to verify the identity of samples from each of the four locations. This variety identification was made by polyacrylamide gel electrophoresis (PAGE), an internationally accepted method for the purpose. All but one sample showed the expected intravarietal uniformity of electrophoregrams. Results for the Regina location sample of the variety Cook indicated that this sample contained an admixture of one or more unknown varieties. The admixed sample was discarded.

Large and obvious differences were observed among the gliadin electrophoregrams of the 26 UQN varieties, indicating the group of

varieties was highly suitable for the purpose of the present study.

The next step in this study was the development of the technique of polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate (SDS-PAGE) for routine analysis of a large number of wheat samples of different varieties. At the outset, it was necessary to determine what starting materials should be used as the source of HMW glutenin: namely, wholewheat meal, flour or extracted glutenin. All three were assayed for each of four varieties. It was found that there were no qualitative differences (there were some differences in intensity of some of the bands) in the SDS-PAGE patterns of the HMW glutenin region within any one variety from the three different starting materials. Thus, it was decided that flour would be used as starting materials for SDS-PAGE studies for the present study. This finding is of benefit to breeding programs because it showed that it is not necessary to prepare extracted glutenin as the first step in determining the HMW glutenin subunit composition.

Next, six of the 26 varieties of the 1983 UQN were selected, based on their broad spectrum of breadmaking quality, for the test set up to investigate possible environmental effects on the HMW glutenin patterns. No qualitative differences in the subunit patterns of the samples from the four locations for each variety were observed. These findings were consistent with published information by Orth and Bushuk (1973b) and confirmed the generally held view that the SDS-PAGE pattern, like the gliadin electrophoregram, is a fingerprint of the genotype (variety).

The findings from the above studies all led to the conclusion that the grain of each variety from the four locations could be compo-

sited to provide the required larger sample for further study.

Technological tests on the 26 varieties selected for this study showed they indeed exhibited a broad range of breadmaking quality. The results of the technological tests deserve some general comments. As other investigators had reported, so also the results of this study bore out that the harder the wheat, the higher the yield of flour upon milling, and the better the color. Interestingly, the present study showed that the sedimentation value (SV) was highly significantly correlated to loaf volume (LV) but not to protein content; similar results were published by Orth et al. (1972). The correlation between SV and protein content indicates that SV is related to the strength of the flour protein rather than protein quantity.

Results of rheological studies further suggested the notion that SV measures the strength of the flour. Both farinograph dough development time and extensograph maximum resistance were significantly correlated with SV.

To further perfect the SDS-PAGE method for the present study, it was necessary to have a set of standard proteins suitable for the study and readily available at nominal cost. It would be beneficial if the standard proteins had physical and chemical properties similar to those of the unknown proteins under investigation. In addition, the standard proteins should have a wide range of molecular weights (MWs), similar to that of glutenin subunits. Presently, it is not possible to obtain this type of standard reference proteins from commercial suppliers. Thus, the 14 glutenin subunits of the wheat cv. Marquis were adopted as the reference proteins, after their MWs were carefully determined by conventional SDS-PAGE analysis. The new procedure based

on the use of Marquis glutenin subunits gave highly reproducible results. MWs of two subunits of cv. Holdfast determined previously (Payne et al., 1980a) were confirmed by results of the present study.

Another point that required clarification was the nomenclature of glutenin subunits. Currently, most published reports use the nomenclature proposed by Payne et al. (1980a and 1981b) for HMW glutenin subunits. In this nomenclature, glutenin subunits are identified by number, initially in chronological order with No. 1 being assigned to the largest subunit. The problem with this "closed" system is that the chronological numbering was disrupted when new subunits were discovered with MWs between those of already numbered subunits. To minimize confusion as "new" glutenin subunits are discovered in new wheat varieties, it is proposed that the MW value in kilodaltons (kD) determined by SDS-PAGE be used as the identity of each subunit. This system has been used throughout this thesis. This is a completely "open" system and new subunits can be added as they are discovered without any difficulty. Although it has been shown that MWs estimated by SDS-PAGE appear to be overestimated (Bunce et al., 1985), the proposed nomenclature would still be meaningful since all MWs are obtained relative to the standard proteins. The MW values can be subsequently corrected when the exact values for some of the subunits are determined from complete amino acid sequences.

The methodology gave good results when applied for analysis of the selected varieties. From the 26 varieties, thirteen different HMW glutenin subunits were identified by SDS-PAGE and were within the MW range of 90.0 to 147.4 kD. The range of MWs agrees well with published data of Payne et al. (1980a). Only 11 of the HMW glutenin subunits

were subjected to further analyses since the other two subunits (141.0 kD and 121.1 kD) occurred only once in all the 26 varieties. It was also observed that certain pairs of subunits always occurred in tandem. This probably results from linked genes as described by Payne et al. (1981b). There were some subunits that were never found together.

To analyze the relationship between HMW glutenin subunits and breadmaking quality, the author used 1 to denote the presence and 0 to denote the absence of a subunit for statistical purposes. Differences in intensity of bands were not considered in this analysis.

First, simple correlations were carried out to determine the relationships between HMW glutenin subunits. It was found that subunits 128.1 and 91.6 were perfectly positively correlated. They always occurred together. On the other hand, subunits 128.1 and 90.0 were perfectly negatively correlated. These never occurred together. Subunits 128.1, 91.6, and 90.0 are probably the same as subunits 5, 10, and 12, respectively, of Payne et al. (1980a and 1981a,b).

The next step in the thesis project was to carry out statistical analyses of HMW glutenin subunits and technological characteristics of the wheats and their products. It was found that subunits 128.1 and 91.6 were each significantly correlated to farinograph MTI and DT. These findings were in general agreement with published data by Branlard and Dardevet (1985). Subunit 90.0 was found to be associated with poor breadmaking quality as measured by loaf volume (LV). Subunits 128.1 and 91.6 were positively significantly correlated to both ULV and BSI, whereas subunit 90.0 was negatively significantly correlated to both of these quality indices. These findings are in general

agreement with results published by Branlard and Dardevet (1985) and Payne et al. (1981a).

The main objective of the present study was to develop equations that would predict various breadmaking quality indices from the HMW glutenin subunit composition. Stepwise multiple regression technique was applied to generate such equations. All 11 HMW glutenin subunits of the 26 varieties were used in the analysis. First, it was found that subunits other than subunits 128.1, 91.6, and 90.0, were important to breadmaking quality. Seven equations with DT, MTI, E, R, R/E, BSI, and ULV as the dependent variables, were developed based on relevant subunits from the total of 11 subunits as independent variables. Each equation contained, at most, nine subunits.

The equations were then tested with a separate set of eight different cultivars (1985 Bread Wheat Cooperative Test cultivars). None of the predicted values for extensograph extensibility, E, fell within the 95% confidence limit even though the r^2 for this particular prediction equation was significant at the 1% level. It was not surprising to obtain these results since extensibility (E), as found by other investigators (Hoseney, 1986; Khan and Bushuk, 1978; Schofield and Booth, 1983), is strongly dependent on gliadin proteins and the present prediction equation was developed by using glutenin subunits only. One of the eight predicted values for MTI and two of the eight predicted values for R/E were outside the 95% confidence limit. Predicted values for DT, R, BSI, and ULV all agreed with actual values within the 95% confidence limit. It should be emphasized, however, that the results obtained here could not be generalized since the prediction equations were generated from a limited number of varieties

(26). It would be very useful to apply the prediction equations to a group of varieties covering a much wider range of breadmaking quality.

Although some progress has been made by the present study, there is still room for further studies in the understanding of the role of HMW glutenin subunits in breadmaking quality. The present study added some new information to this understanding. Moreover, the present study showed that HMW glutenin subunit composition has some potential for predicting the breadmaking quality of varieties in wheat breeding programs.

VI. CONTRIBUTIONS TO KNOWLEDGE

The major contributions to knowledge resulting from the present study are as follows:

(1) The SDS-PAGE procedure modified for the present study gave a high resolution of the high molecular weight (HMW) glutenin subunits. Modifications included: (a) higher concentration of acrylamide and bisacrylamide, (b) lower cross-linking of acrylamide, (c) lower constant current, and (d) longer running time.

(2) For the same variety, there were no differences in the SDS-PAGE patterns of the HMW glutenin subunit region from three different starting materials; wholewheat meal, flour, and extracted glutenin. Accordingly, flour can be used to obtain the SDS-PAGE patterns of the HMW subunits that are characteristic of the wheat variety.

(3) Fourteen subunits of the glutenin of the variety Marquis were proposed for use as the reference proteins for routine analysis of wheat glutenin by SDS-PAGE. The main advantages of this proposal are: (a) the reference proteins are processed in exactly the same way as the unknown wheat proteins, (b) the reference and unknown proteins are similar in physical and chemical properties, (c) the reference proteins cover the same molecular weight (MW) range as the unknown wheat proteins, and (d) the reference proteins are available at low cost.

(4) The methodology developed in this study gave excellent reproducibility of the MWs of glutenin subunits.

(5) On the basis of the results of this study, it was recommended that molecular mass in kilodaltons (kD) be used to identify the

HMW subunits for nomenclature and statistical analysis purposes. This nomenclature is open and will accommodate, without confusion, new subunits as they are discovered.

(6) Thirteen different HMW glutenin subunits (MWs ranging from 90.0 to 147.4 kD), were identified in the SDS-PAGE patterns of the 26 varieties of the 1983 Uniform Quality Nursery (UQN). Each variety contained four or five of the 13 HMW glutenin subunits.

(7) Correlation analysis showed that subunits 128.1 and 91.6 had the greatest positive influence on breadmaking quality, whereas subunit 90.0 had the greatest negative influence.

(8) Stepwise multiple regression technique revealed that other HMW glutenin subunits also contributed to breadmaking quality.

(9) Seven prediction equations, with r^2 values significant at the 1% level, were generated through the stepwise multiple regression technique using the HMW glutenin subunit compositions of the 26 varieties as the predictors for dough development time (DT), mixing tolerance index (MTI), extensibility (E), maximum resistance (R), ratio of R and E, baking strength index (BSI), and loaf volume per unit protein (ULV).

(10) The prediction equations were tested with data for a separate set of eight different cultivars from the 1985 Bread Wheat Cooperative Test. The prediction equations had high predictive power for all quality parameters except extensograph extensibility (E). Predicted ULV agreed with actual values within a 95% confidence limit. The prediction equation for E gave poor predictions indicating that factors other than glutenin contribute substantially to this characteristic.

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APPENDICES

APPENDIX I. SDS-PAGE Procedure for Routine Analysis of Wheat Protein on the LKB 2001 Vertical Electrophoresis Unit

1. Chemicals

Tris(hydroxymethyl)aminomethane	Tris
Sodium dodecyl sulfate	SDS
Acrylamide	
Bisacrylamide	
Mercapto-ethanol	ME
Ammonium persulfate	
Coomassie Brilliant Blue G-250	
N,N,N',N'tetramethyl ethylene diamine	TEMED
Pyronin Y	
Glycerol	
HCl	
H_2SO_4	
KOH	
Trichloroacetic acid (electrophoretic or analytical grade)	TCA

1.1 Stock solutions

A. Acrylamide 35% W/V (250 ml).
B. Bisacrylamide 2% W/V (100 ml).
C. 1 M Tris, pH 8.8 (adjust with HCl; 250 ml).
D. 1 M Tris, pH 6.8 (adjust with HCl; 250 ml).
E. Sample buffer stock soln: 20 ml glycerol, 12.5 ml soln D, 24.1 ml distilled water, 4 g SDS (solid) and 20 mg of Pyronin Y.
F. 10% W/V SDS (200 ml).
G. 1% W/V ammonium persulfate (10 ml).

Solns A to D should be stored in dark bottles at refrigerator temperature for no longer than 2 weeks. Solns E and F can be stored at room temperature for a couple of months. Soln G should be made fresh daily. All stock solutions except soln G were filtered through Whatman No. 1 filter paper before storage.

2. Procedure

2.1 Sample preparation

Extracting buffer: immediately before use, mix up stock soln E, ME and distilled water in the proportions below:

Distilled water	stock soln E	ME	no. of samples
6.0 ml	2.55 ml	0.45 ml	9
12.0 ml	5.10 ml	0.90 ml	18

Suspend 40 mg of flour in 1 ml extracting buffer. Each flour-buffer mixture is allowed to stand at room temperature for 2 hr with occasional shaking. They are then heated for 2.5 min in a boiling water bath and allowed to cool to room temperature. The flour suspension is allowed to settle and an aliquot of the

clear top layer is used as the experimental protein extract. 7.5 to 10.0 µl of protein extract solution is loaded into each slot in the gel.

2.2 Gel preparation (for two gels)

Separating-gel solution: Mix 49.3 ml soln A, 3.88 ml soln B, 37.6 ml soln C , and 5.67 ml distilled water together, then degas. After degassing, add 1.0 ml soln F, 2.5 ml soln G, and 50 µl TEMED, then immediately and gently pour half into the gel cassettes to a height of 14 cm each. Overlay the gel mixture with water and allow to polymerize 1.5 to 2 hr.

Stacking-gel solution: Mix 1.71 ml soln A, 0.433 ml soln B, 2.5 ml soln D, and 14.4 ml distilled water together, then degas. After degassing, add 0.2 ml soln F, 0.75 ml soln G, and 15 µl TEMED, then immediately and gently pour into the gel cassettes to a height of 1 cm each. Insert slot formers into the gel cassettes. Overlay the gel mixture with water and allow to polymerize 1 hr.

Note: Before pouring stacking-gel solution, drain off free water from the separating gel and rinse with some stacking-gel solution. Then pour the stacking-gel solution to about 1 cm above the separating gel.

2.3 Electrophoresis

Electrophoresis buffer: Add 12.114 g of Tris, 57.6538 g of glycine and 40 ml of soln F and make up 4 liters with distilled water; pH should be 8.3 (if necessary adjust with HCl).

Electrophorese for 2 hr at a constant current of 5 mA (per gel), followed by 18 hr at 10 mA and finally for 2 hr at 15 mA. The electrode buffers are circulated continuously during electrophoresis at 20°C.

Notes: If running two gels, the currents should be doubled. By the end of the running time, the tracking dye will have run off the gel.

2.4 Gel staining

Preparation of staining solution:

(1) Mix 1 g of Coomassie Brilliant Blue G-250 in 500 ml water, then add 500 ml 2 N H_2SO_4, mix well and allow to stand at least 4 hr;
(2) filter, and to 900 ml of the filtrate add 100 ml 10N KOH;
(3) then add 140 ml of 100% (W/V) TCA to the 1 liter of dye solution and again filter through Whatman No. 1 paper. Store in a dark bottle.

Gels are stained overnight in the staining solution, rinsed with distilled water, and then photographed (immediately

after rinsing) through an orange filter.

Note: To make 2 N H_2SO_4, dilute 54.99 ml conc. H_2SO_4 to 1 l with distilled water; to make 10 N KOH, dissolve 56.11 g of KOH and make up to 100 ml with distilled water.

APPENDIX II. Gliadin Electrophoregrams of 1983 UQN Varieties

Figure 19. Gliadin electrophoregrams for samples of varieties 1 to 4 and 7 to 10 of four locations and the Marquis reference samples.

Letters represent M, Marquis; L, Lethbridge location; R, Regina; S, Saskatoon; SC, Swift Current.

See Table 1 for identity of varieties.

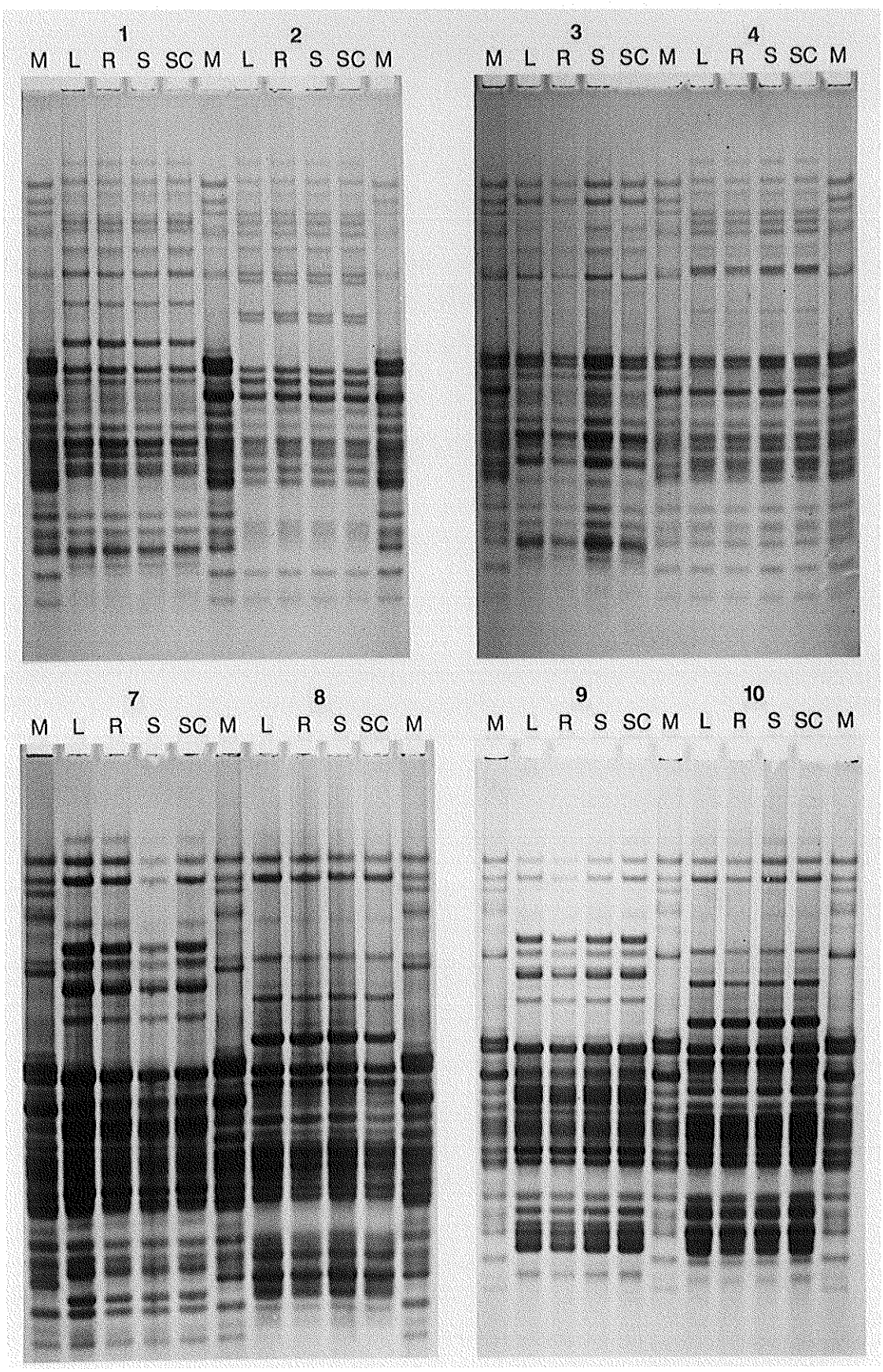

1
M L R S SC M L R S SC M
2
3
M L R S SC M L R S SC M
4
7
M L R S SC M L R S SC M
8
9
M L R S SC M L R S SC M
10

Figure 20. Gliadin electrophoregrams for samples of varieties 11 to 18 of four locations and the Marquis reference samples. Letters represent M, Marquis; L, Lethbridge location; R, Regina; S, Saskatoon; SC, Swift Current. See Table 1 for identity of varieties.

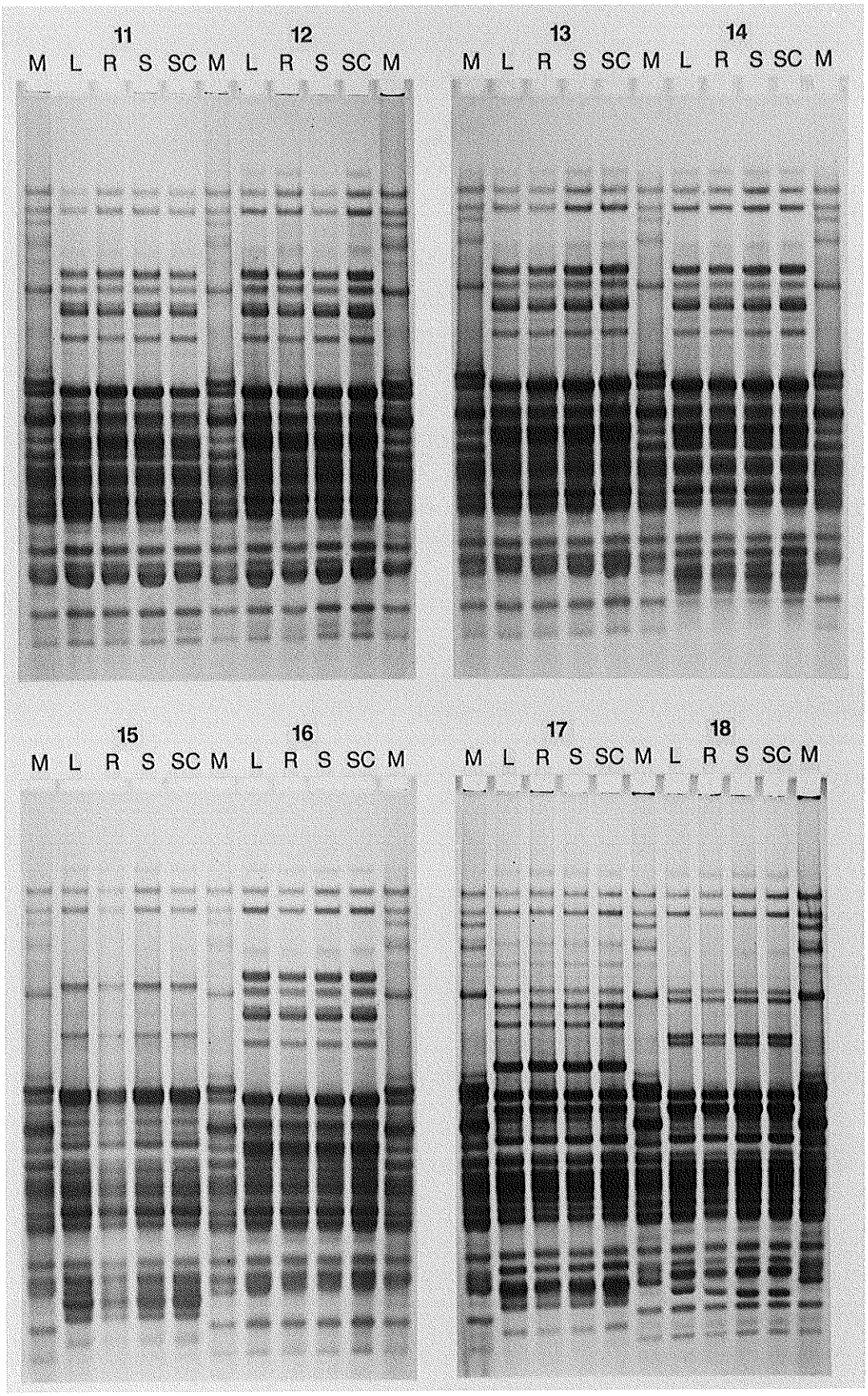
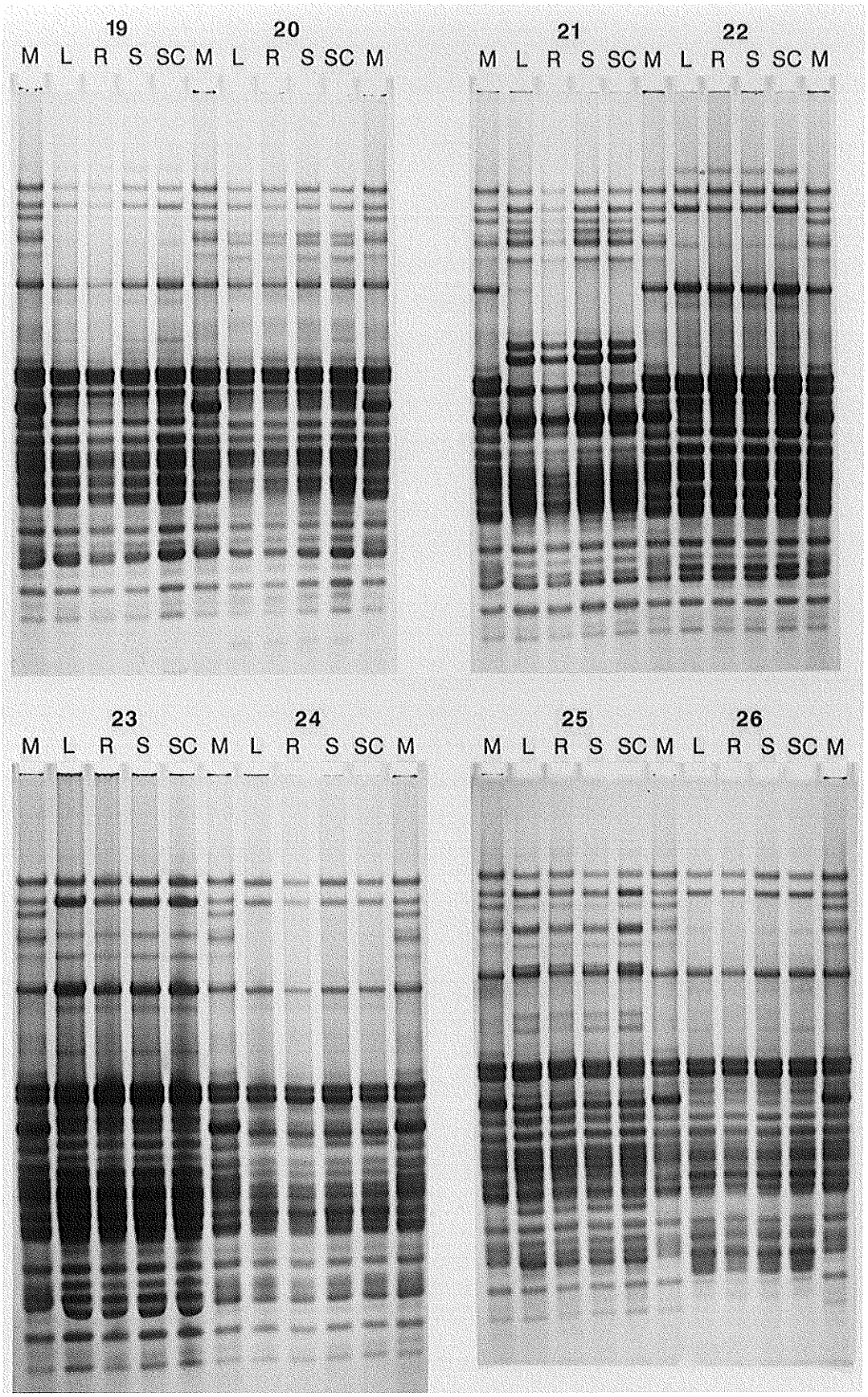

11
M L R S SC M L R S SC M
12
13
M L R S SC M L R S SC M
14
15
M L R S SC M L R S SC M
16
17
M L R S SC M L R S SC M
18

Figure 21. Gliadin electrophoregrams for samples of varieties 19 to 26 of four locations and the Marquis reference samples. Letters represent M, Marquis; L, Lethbridge location; R, Regina; S, Saskatoon; SC, Swift Current. See Table 1 for identity of varieties.


19
20
M L R S SC M L R S SC M
21
22
M L R S SC M L R S SC M
23
24
M L R S SC M L R S SC M
25
26
M L R S SC M L R S SC M

APPENDIX III. Additional Tables for Predicted Criteria of 1985 Bread Wheat Cooperative Test Cultivars from Stepwise Multiple Regression Equations

Table 16. Predicted DT of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	6.19	4.50	2.13	10.25
C2	7.29	5.25	3.29	11.29
C3	7.29	5.50	3.29	11.29
C4	7.29	4.75	3.29	11.29
C5	7.29	5.00	3.29	11.29
W1	7.29	5.75	3.29	11.29
W2	9.31	5.25	4.12	14.51
W3	9.31	5.75	4.12	14.51

[1]Values obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).

Table 17. Predicted MTI of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	23.8	50.0*	0.3	47.4
C2	18.4	40.0	-4.7	41.5
C3	18.4	25.0	-4.7	41.5
C4	18.4	30.0	-4.7	41.5
C5	18.4	35.0	-4.7	41.5
W1	18.4	25.0	-4.7	41.5
W2	22.1	30.0	-7.6	51.9
W3	22.1	30.0	-7.6	51.9

[1]Values obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).
*Outside of the 95% confidence interval.

Table 18. Predicted E of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	175.8	230.0	135.2	216.3
C2	158.7	230.0	118.5	198.9
C3	158.7	225.0	118.5	198.9
C4	158.7	235.0	118.5	198.9
C5	158.7	205.0	118.5	198.9
W1	158.7	230.0	118.5	198.9
W2	185.4	240.0	138.7	232.0
W3	185.4	240.0	138.7	232.0

[1]Values obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).

Table 19. Predicted R of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	704.5	355.0	330.6	1078.5
C2	704.5	425.0	330.6	1078.5
C3	704.5	430.0	330.6	1078.5
C4	704.5	405.0	330.6	1078.5
C5	704.5	355.0	330.6	1078.5
W1	704.5	480.0	330.6	1078.5
W2	840.7	455.0	359.6	1321.8
W3	840.7	515.0	359.6	1321.8

[1]Values obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).

Table 20. Predicted R/E of 1985 Bread Wheat Cooperative Test cultivars from the stepwise multiple regression equation

Variety no.	Predicted value	Actual value[1]	95% Confidence interval	
			Lower	Upper
C1	3.9	1.5	1.3	6.5
C2	4.5	1.9	1.9	7.1
C3	4.5	1.9	1.9	7.1
C4	4.5	1.7*	1.9	7.1
C5	4.5	1.7*	1.9	7.1
W1	4.5	2.1	1.9	7.1
W2	4.5	1.9	1.9	7.1
W3	4.5	2.2	1.9	7.1

[1]Values obtained from the minutes of the 1986 meeting of the Expert Committee in Grain Quality (Anonymous, 1986).
*Outside the 95% confidence interval.