# WHEAT FLOUR PROTEIN SOLUBILITY IN RELATION TO BREADMAKING QUALITY OF DIFFERENT GENOTYPES

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by

Abdul Kipruto Faraj

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BY

ABDUL KIPRUTO FARAJ

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

MASTER OF SCIENCE

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Dedicated to my mum for her encouragement and support and in memory of my dad

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## ABSTRACT

Protein solubility fractionation of sixteen samples of wheat (varieties and commercial classes) in relation to breadmaking quality was investigated. The effect of milling on two laboratory mills was also studied. Protein solubility in 2 % sodium dodecyl sulphate, 0.05 *M* acetic acid, 70 % ethanol, 2 % sodium chloride and distilled water was determined. The effects of temperature (4 °C vs 23 °C) and changes in flour:solvent ratio on protein extractability were also examined. Also investigated was the performance of the computerized micromixograph in terms of the precision and the discrimination ability of computed mixing parameters.

Significant differences were observed among the technological characteristics of flours milled on the Buhler experimental mill (Buhler) and Brabender Quadrumat Jr. (Quad) mill. Flour yield, starch damage and Zeleny sedimentation values were higher for the Buhler than Quad mill. Protein and ash contents were lower for the Buhler than for the Quad milled flour. However, rheological properties (farinograph, extensigraph and micromixograph) and baking quality characteristics, in terms of loaf volume and baking strength index, were not significantly different between the two mills. The Buhler mill had a higher milling efficiency than the Quad as determined by flour ash:wheat ash ratio.

Among the parameters generated by computerized mixograph, first minute slope (FMS), peak band width (PBW) and slope after peak (SAP) were found to be less reliable as compared with the other mixograph parameters. By determining the coefficient of variation ($CV_1$) for a particular mixograph parameter in each set of samples and the degree of variation ($CV_2$) within the samples, it was shown that the $CV_1$:$CV_2$ ratio gives an overall estimate of the analytical precision of the instrument to measure the particular parameter. FMS, PBW and

SAP had the lowest $CV_1:CV_2$ ratio for both Buhler and Quad flours.

Significant correlations were observed between the mixograph parameters and some of the other technological quality characteristics. For the Buhler milled flour, Peak height (PHG), peak band width (PBW) and total energy under the peak (TEG) were positively correlated ($P < 0.01$) to total flour protein (FP) content and parameters associated with FP content, viz; sedimentation value (SV), farinograph absorption (FAB), mixing tolerance index (MTI), extensibility (EXT) and loaf volume (LV). While energy to peak (ETP) and band width energy (BWE) were positively correlated ($P < 0.01$) with farinograph dough development time (DDT), remix time (RT) and mixograph development time (MDT). Similar correlations were observed for the Quad-milled flour but with slightly lower r-values.

More protein was extracted from the Buhler than Quad milled flours by the extracting solutions used. This was attributed to the flour particle size. The Buhler milled flour was finer than the Quad flour. Electrophoretic patterns of the proteins from the two mills were identical under reducing and non-reducing conditions. The relationship between the technological quality and protein extraction data was determined. For flours obtained from both mills the amount of acetic acid soluble protein was significantly correlated ($P < 0.01$) with MDT, ETP and BWE. The amount of acetic acid soluble protein was also positively correlated to MTI, RT and WO for the Buhler milled flour.

The amount of ethanol soluble proteins was positively correlated ($P<0.01$) with SAP and negatively correlated ($P<0.05$) with FP, FAB, PHG and MDT for the Quad milled flour only. The amount of water soluble protein was correlated ($P<0.05$) with MDT, ETP and BWE for both types of flour, and positively correlated with MTI for the Quad milled flour. For both types of flour, the amount of salt soluble protein was negatively correlated ($P<0.05$) with FP, DDT, WO, ETP and BWE but positively correlated with MTI for Buhler milled flour.

Stepwise multiple linear regression analysis was used to generate prediction equations for mixograph parameters based on the amount or proportion of extracted protein by various solvents for both types of flour. Most of the variation in the mixograph parameters could be explained by the same type of protein fractions in both types of flour. The best single variable models were: acetic acid soluble proteins for MDT ($R^2$ =0.70 and 0.59); sodium chloride residue proteins with PHG ($R^2$ =0.92 and 0.88) and TEG ($R^2$ =0.86 and 0.83). The $R^2$ values are for the Buhler and Quad milled flours, respectively. Two variable models gave significantly higher $R^2$ values.

The relationship between the protein fractions and mixograph parameters was also examined for a different set of 26 varieties milled on the Quad mill. When this set of samples was compared with the 16 Quad-milled samples in relation to mixograph parameters, some common and satisfactory regression models were found in terms of independent variables (protein fractions). For example, ETP and BWE were most closely associated with the amount of acetic acid residue protein ($R^2$ =0.52 and 0.49, respectively). For the two-variable models, the amount of acetic acid soluble and NaCl residue protein explained 54.0 % of the variation observed in MDT. The amount of water residue and acetic acid residue protein explained 74 % of the variation in ETP. Total flour protein and acetic acid soluble proteins explained 67.0 % of the variation in BWE.

These findings indicate that the type of experimental milling (Buhler vs. Quad mill) does not affect the breadmaking quality characteristics and the type of proteins extracted but affected the amount of protein extracted. Protein solubility fractionation may therefore be useful for screening early generation lines in a breadwheat breeding programme.

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# I. INTRODUCTION

Wheat which ranks first in production among the cereal grains grown in the world, contributes a third of the total annual cereal protein production (Lookhart, 1991) and provides almost 20 % of the total calories (Bushuk, 1982). It has been estimated that, of the 497 million tonnes (MT) of wheat consumed during 1984-1985, 323 MT was used for food, 104 MT for feed, 40 MT for seed and 30 MT for other uses (International Wheat Council, 1985). Wheat is clearly an important cereal grain as a source of calories and protein for human beings as well as livestock. Most of the wheat produced is used in making bread and other leavened products because of the unique rheological characteristics of hydrated meal or flour. It is the aim of most wheat breeding programs to maintain or improve the current yield and at the same time maintain or improve the end-use quality. Simple and reliable small-scale tests for screening varieties for good breadmaking potential would help plant breeders in carrying out their programs. These tests should be capable of predicting the rheological and breadmaking properties of dough to facilitate early elimination of unsuitable lines in a breeding program, thereby saving time and resources.

Determination of breadmaking quality in the early stages of a breeding programme is complicated by the large number of entries (lines), the small size of sample available, and the influence of environment. Variation in grain protein content and enzyme activity can be the result of pre-harvest sprouting. Simple and rapid screening tests measuring intrinsic wheat quality are of great value to the breeders. Protein fractionation based on solubility has been found to be one such test, with some utility for screening purposes. Some of the solubility-related tests developed for use in wheat breeding programmes include the Zeleny sedimentation test (Zeleny, 1947)

and the sodium dodecyl sulphate (SDS) sedimentation test (Axford *et al*, 1978). Other solubility tests that have been developed include the urea dispersible protein (UDP) test (Pomeranz, 1965) and the acetic acid insoluble (residue) protein test (Orth and O'Brien, 1976). Only the latter test utilizes principles of Osborne fractionation (Chen and Bushuk, 1970), which provides an effective separation of the various classes of wheat endosperm protein according to solubility. The residue protein test however, is tedious and time consuming to perform because it relies on a sequential fractionation strategy involving four solvents (water, 0.5 *M* sodium chloride, 70 % aqueous ethanol and 0.05 *M* acetic acid), as well as dialysis and freeze-drying steps. Accordingly, it was the goal of this study to evaluate the potential of a protein solubility test utilizing direct extraction of flour proteins with the Osborne solvents as a screening method for breadmaking quality.

The main objectives of this thesis project were: (i) to examine the effect of experimental milling on the technological quality parameters for breadmaking and on solubility fractionation of wheat proteins, (ii) to evaluate the quantitative relationship between the different protein fractions and the technological quality parameters, particularly the mixing characteristics as determined by a computerized mixograph and (iii) to examine whether the quantitative relationship could be used to generate prediction models with potential to screen early generation material in breadwheat breeding programmes.

# II. LITERATURE REVIEW

## A. INTRODUCTION

All the constituents of wheat flour are important in determining its overall functional properties. The unique viscoelastic properties of dough have been largely attributed to the protein fractions (Bloksma and Bushuk, 1988 and references cited therein) and the bread making quality of a flour is directly related to protein content and quality (Finney and Barmore, 1948; Bushuk *et al*, 1969; Khan *et al*, 1989; He and Hoseney, 1992). However, it has not been conclusively determined precisely which proteins govern wheat end-use quality.

The desire to understand the basis of breadmaking quality has led many researchers to examine correlations between the relative amounts of various flour constituents and baking quality. These investigations have mainly relied on protein solubility fractionation and reconstitution methods. The commonly used protein fractionation procedure is the classical Osborne fractionation method (Osborne, 1907) and the modification by Chen and Bushuk (1970). This procedure yields five fractions: (i) the water soluble proteins (albumins), (ii) salt soluble proteins (globulins), (iii) 70 % ethanol soluble proteins (gliadins) (iv) dilute acid or alkaline soluble proteins (glutenins) and (v) acetic acid insoluble proteins (residue proteins). The Osborne fractionation procedure has been the basis for providing considerable knowledge on the structure and function of cereal proteins (Bushuk, 1981). However, the Osborne procedure has some limitations. It is extremely tedious and time consuming, and there is also some degree of overlapping solubility of the different protein fractions.

This review will cover literature dealing with some of the extraction procedures, physicochemical properties of the fractions, and the relationship of the fractions to

technological parameters related to breadmaking quality. The literature on the effects of milling on breadmaking properties will also be reviewed.

## B. PROTEIN EXTRACTION AND FRACTIONATION

Following the classical solubility fractionation of proteins by Osborne (1907), a considerable number of protein extraction, fractionation and reconstitution procedures have been developed in trying to study the possible effects of the different wheat flour proteins on functionality. Most extraction methods are based on the original solvents of Osborne. However, the sequence in which such solvents are used, the vigour of extraction and the starting material all affect the yield and the composition of the various proteins due to their specific solubility, tendency to associate and possible chemical interactions (Bietz and Wall, 1975). While the fractions obtained by the modified Osborne fractionation procedure, are not homogeneous and absolutely distinct, the Osborne nomenclature is still commonly used and will be used in this review.

### 1. Differential extraction methods

In differential extraction procedures, preliminary fractionation is based on the solubility or molecular weight of the components. The components obtained may be examined further by other techniques with higher resolution. Pomeranz (1965) used urea to fractionate wheat flour proteins. He reported that the solubility of flour proteins in 3 *M* urea in 0.01 *M* pyrophosphate buffer pH 7.0 at 4 °C was much higher for flours of poor bread making quality. Lee and MacRitchie (1971) by extracting with progressively increasing concentration of urea (2-6*M*) followed by centrifugation,

managed to separate wheat gluten proteins into various fractions reportedly corresponding to gliadins, glutenins and residue proteins. MacRitchie (1987,1989) fractionated flour proteins from glutens of six varieties into nine or ten fractions by successive extraction with hydrochloric acid of different concentrations. Thus, it is evident that by varying the concentration of the extracting solutions different proteins are solubilized and therefore different fractions can be obtained.

**2. Total solubility methods**

Total solubility methods attempt to dissolve all the proteins and to subsequently resolve the complex mixture by differential precipitation, gel filtration or other separation techniques. Acetic acid has been widely used at various concentrations by many researchers (Bietz and Wall, 1975; Orth and Bushuk, 1972) to solubilize flour proteins. This has not been a very successful approach as a significant portion of the flour protein remains undissolved as a gel-like residue. Another widely used solvent for total protein extraction is a mixture of acetic acid-urea-cetyltrimethylammonium bromide (AUC) at concentrations of 0.1 *M*, 3.0 *M*, and 0.01 *M*, respectively (Meredith and Wren, 1966). According to these workers, AUC dissolves a higher proportion of the flour proteins than acetic acid alone. However, other researchers have reported that although AUC has provided valuable information it does have a number of disadvantages. Goforth *et al* (1977), reported that acetic acid at the concentration used (0.1 *M*) in AUC can cause acid catalysed peptide bond cleavage. The removal of the cetyltrimethyl ammonium bromide (CTAB) is also difficult and leads to problems in subsequent analyses such as Kjeldahl nitrogen determination (Bottomley *et al*, 1982).

Salts of long-chain fatty acids (soaps) have also been used to dissolve glutenin proteins into a visibly clear solution (Kobrehel and Bushuk, 1977). Sodium stearate and sodium palmitate were found to be very effective solvents. It was reported that sodium stearate, dissolved almost all the glutenin at the lowest concentration used (1 mg/10 mg glutenin in 2 ml water). Salts of fatty acids apparently do not disrupt disulphide bonds on protein solubilization (Wasik *et al*, 1979; Hamauzu *et al*, 1979).

Danno *et al* (1974) used 1 % sodium dodecyl sulphate (SDS) solution to dissolve flour proteins and extracted about 76 % of the total flour proteins from Canada Western No. 1 hard red spring wheat, experimentally milled to 60 % extraction. They found that a 1 % SDS solution in 0.05 *M* phosphate buffer at pH 6.8 was more effective in extracting wheat proteins than the other solvents used; acetic acid (0.05 *M*) extracted 62 %, aluminium lactate-lactic acid buffer at pH 3.1 - 66 %, AUC -72 %, 70 % ethanol - 62 % and 3,5- diiodosalicylic acid lithium salt solution - 66 %. Most of the SDS soluble proteins had molecular weights less than 75 kDa, suggesting single chain proteins; a small number of relatively high molecular weight (HMW) proteins were also detected in the SDS-polyacrylamide gel electrophoresis (PAGE) results. Virtually 100 % of the protein was extracted when 2-mecarptoethanol (1 m*M*) was included. Graveland *et al*, (1979,1982) developed a procedure in which a 1.5 % SDS solution was used to separate flour proteins into SDS-insoluble and SDS-soluble fractions. The solution dissolved 65-67 % of the total flour nitrogen for two Dutch wheat varieties. They further fractionated the soluble fraction by a combination of ethanol precipitation, ammonium sulphate precipitation and gel filtration chromatography into albumins, globulins, gliadins, glutenins II and III, and glutelins I, II, III and IV. The SDS-insoluble material was further separated by centrifugation into

glutenin I and glycoproteins.

Bottomley *et al* (1982) compared three solvents; AUC, SDS-Tris and urea-CTAB solution buffered to pH 5.5 with sodium citrate (CUC), in their abilities to solubilize wheat flour proteins and their suitability for chromatography. Their results indicated that SDS-based solvent extracted flour proteins more efficiently than either of the CTAB-based solvents. It was additionally shown that SDS-Tris buffer gave better resolution of flour proteins during gel filtration on columns of Sepharose CL-4B than the CUC buffer. According to their technique, the flour proteins were resolved into three major groups which were identified as glutenins, gliadins and albumins plus globulins.

Singh *et al* (1990) obtain almost complete extraction of proteins from a weak and strong wheat flour by sonication in 0.05 *M* sodium phosphate buffer, pH 6.90 containing 2 % SDS. These researchers speculated that the increased solubility of the flour proteins by sonication, compared with simple stirring, was similar to that achieved by mixing dough in a mixograph.

Recently, Gao and Bushuk (1992) dissolved glutenin, prepared from gluten, in 6 *M* urea/6 % (w/v) SDS solution at 50 °C for 24 h. They observed that at least 99 % of the glutenin protein dissolved under the extracting conditions used. The SDS-PAGE of three of four cultivars used showed that the HMW subunit composition of the solubilized reduced glutenin was the same as that of the respective flour. They did not observe any HMW subunits in the SDS-PAGE pattern of unreduced glutenin, an indication that the extracting conditions used did not break the interpolypeptide disulphide bonds.

### 3. Sequential extraction procedure

Wheat proteins have different solubilities in different solvents. Most sequential extraction processes currently used are based on a modified Osborne fractionation procedure (Chen and Bushuk, 1970). Large differences in the relative proportions of the proteins of the five major solubility fractions have been observed by many researchers (Orth and Bushuk, 1972; Tanaka and Bushuk, 1972; Bietz and Wall, 1975). Orth and Bushuk (1972) found intervarietal differences in the amount of soluble glutenin and insoluble glutenin. Substantial variations in the proportions of each protein fraction may also be attributed to the differences in the extraction methodology.

### 4. Effect of defatting

Defatting the flour material can affect subsequent extraction of the proteins. Maes (1966) reported that upon defatting of flour with water-saturated butanol, the total protein extractable by sequential fractionation with distilled water, 40 % isopropyl alcohol (v/v), 3.8 % lactic acid and 0.5 % potassium hydroxide did not change very much. But the fraction soluble in isopropyl alcohol decreased, on the average about 40 % for flour and 10 % for whole wheatmeal. Hoseney *et al* (1970) reported that petroleum ether also extracts purothionins. Chung and Pomeranz (1978) examined the effect of defatting (using acetone and isopropanol) on protein extractability in 0.05 *M* acetic acid. They observed that more protein was extracted from good and poor control flours than from corresponding defatted flours. They also reported that protein extractability of the defatted good flour was higher after acetone than isopropanol treatment.

Byers *et al* (1983) observed that partial denaturation of the cytoplasmic protein

may occur during defatting and as a consequence, result in a slight change in the proportion of the different types of proteins. Using several solvents (ethanol/diethylether/water; 2:2:1 (v/v/v), butan-1-ol and water saturated butan-1-ol), these workers observed that the extractability of alcohol soluble-proteins was not affected but the extractability of salt soluble proteins (albumins and globulins) decreased which they attributed to denaturation.

From the above discussion it can be seen that numerous extraction and fractionation procedures, using a wide range of solvents, have been employed in wheat protein research. Most of these studies have been aimed at understanding physico-chemical properties of the different fractions, their structure, rheological properties and their relationship to breadmaking quality particularly loaf volume.

## C. NON-STORAGE PROTEINS

The term "non-storage proteins" refers to those proteins of wheat kernel which are cytoplasmic in nature but not gliadins or glutenins (Wrigley and Bietz, 1988). Traditionally only gliadins and glutenins have been regarded as storage proteins while the albumins and globulins have been considered to be simple polypeptides having metabolic or structural functions (Shewry and Miflin, 1985). Recent data by Singh and Shepherd (1985, 1987) have indicated that certain globulins in wheat kernel, viz triticin (or triplet proteins) are also storage proteins and form disulphide linked aggregates. Gupta *et al* (1991) have also reported the presence of high molecular weight albumins in bread-wheat flour.

Generally 0.4-0.5 *M* NaCl is used to extract albumins and globulins from wheat flour. Subsequent dialysis of the extract against water can separate albumins from

globulins which precipitate. The soluble proteins typically amount to about 18-25 % of the total protein in the wheat kernel (Pence *et al*, 1954a; Bushuk and Wrigley, 1974; Wrigley and Bietz, 1988; Pogna *et al*, 1991). Of these albumin makes up about 15-20 % and globulin about 3-5 %. Direct extraction of flour with water solubilizes, in addition to albumins some globulin, some gliadins, pentosans and glycoproteins (Pence *et al*, 1950; Hoseney *et al*, 1969a). Pence *et al* (1954c) indicated that as many as 11 water soluble protein components could be detected by paper electrophoresis. While Nimmo *et al* (1963) reported that at least 15 protein components could be detected in the water soluble fraction. The soluble proteins have received less attention because they have been presumed to be less important in breadmaking quality than the gluten proteins. This may be partially attributed to the results that have been less than definitive perhaps because of the great heterogeneity of these fractions (Wrigley and Bietz, 1988).

**1. Albumins**

Albumins are the flour proteins that are soluble in water (Osborne, 1907). The amino acid composition of albumins differ greatly from those of other wheat flour proteins. Pence *et al* (1954a) using straight grade flour experimentally milled from pure varieties, reported that albumins have a high tryptophan and lysine contents and a lower amide contents than the other wheat proteins. Pence and Elder (1953) reported that the molecular weight of albumin was about 21 kDa by sedimentation equilibrium and 28 kDa by osmotic pressure measurements in 0.1 *M* sodium chloride. The role of albumins in breadmaking is not well understood.

Recently Gupta *et al* (1991) observed that some seed protein bands (M, 65, 63,

60 and 45 kDa) that were separated by 2-step SDS-PAGE were controlled by genes on the chromosome arms 4DL, 4AL, 5AL and 5DL, respectively. These workers reported that these proteins had extractability characteristics consistent with albumins. Analysis by size-exclusion high performance liquid chromatography (SE-HPLC) showed that these albumin bands were part of the polymeric protein fraction in bread wheat. By employing two-dimensional (2-D) diagonal electrophoresis (unreduced first dimension, reduced second) it was revealed that the HMW albumin bands occurred in both polymeric (disulphide linked aggregates) and monomeric forms in their native states. The effects of the disulphide linked HMW albumins (presumably ß-amylases based on antibodies reactions) on the functionality of the polymeric protein fraction in wheat is not yet known (Gupta *et al*, 1992).

**2. Globulins**

Globulins are the salt soluble proteins (Osborne, 1907). Compared with other wheat endosperm proteins, globulins are characterized by high arginine contents and low tryptophan and amide nitrogen contents (Pence and Elder, 1953). These workers, using mostly commercial unbleached flour milled from soft wheats, found three globulin components by ultracentrifugation. The molecular weight of wheat globulin determined from sedimentation constants ranged from 24 kDa to over 200 kDa (Meredith and Wren, 1966).

Singh *et al* (1991) prepared a globulin fraction highly enriched in triticin by exploiting the differential solubility of triticin in salt solutions of various concentration. Isoelectric focusing (IEF) revealed that triticin has large acidic and small basic polypeptides linked together by disulphide bonds similar to legumin-like storage

proteins of oats, rice and legume seeds. These workers also found that the amino acid compositions of purified triticin and the large and small basic polypeptides are similar to those of legumin-like proteins, but much different from those of gliadin and glutenin.

The role of globulin proteins in breadbaking is not well understood. Koenig *et al* (1964) observed that long-mixing flour had more globulin protein than short-mixing flour. However, Mullen and Smith (1965) found similar amounts of salt-soluble protein in short-mixing (weak) and long-mixing (strong) wheat flours. Pence and coworkers (Pence, 1962) found that removal of the salt soluble proteins from flour produced a smaller loaf volume response and tended to increase mixing time. However, they were not able to determine if this negative effect was due to the removal of globulins or due to the effect of added salt ions on the gluten proteins. MacRitchie (1987) also reported that more protein was extractable with 0.75 M NaCl from glutens of poor quality compared with better performing flours. It was observed that the relative proportion of these globulin-type proteins to glutenins appeared to be important in relation to baking quality.

Okada *et al* (1987) using heavily ground flour, fractionated globulins into HMW, medium molecular weight (MMW) and low molecular weight (LMW) globulins by gel filtration on Sephacryl S-300. These researchers found that MMW-globulin was especially reactive and polymerized into HMW-globulin by forming disulphide bonds during mixing. The flour from the over-ground mill stream (late-break flour) was more reactive than the other streams (early reduction flour). They suggested that globulins in wheat flour may play an important role as binders between proteins during dough mixing thus affecting the rheological properties of dough.

## 3. Electrophoretic patterns of the soluble proteins

Orth and Bushuk (1972) using 26 varieties of diverse baking characteristics observed that there were no qualitative varietal differences in polyacrylamide disk-gel electrophoretic patterns (pH 3.2) of water and salt-soluble proteins. Other research examining specific albumin and globulins has revealed probable relationship to quality and functionality. Elton and Ewart (1960) detected two globulin bands which migrated most rapidly in starch-gel electrophoresis of dialysed salt extract. Fisher *et al* (1968) identified the fast moving doublet as purothionin. Ohms (1980) examined reduced wheat albumin and globulin by PAGE. He observed two bands which occurred preferentially in cultivars having good baking quality. Pogna *et al* (1991) reported that numerous protein components were present in SDS-PAGE fractionation of albumins from bread wheat flour. Using their extraction procedure, the major proteins in this fraction occurred in two different size groups; HMW albumins (about 60 kDa) and LMW albumins (14-16 kDa).

## 4. The relationship between water soluble fractions and breadmaking properties of wheat flour

Pence *et al* (1954b, 1954c) examined flours differing in baking quality and found significant variations in the water soluble protein content. Absolute amounts of soluble proteins increased directly with the total protein but decreased when the total soluble protein was expressed as a percentage of the total flour protein. This observation was recently confirmed by Graybosch *et al* (1990). Pence and coworkers also observed that the amount of soluble protein and the ratio of soluble to gluten protein were not significantly correlated with breadmaking quality; but the ratio of albumin to globulin was significantly correlated with breadmaking quality. These

workers used the coefficient of regression of loaf volume on flour protein for each of the flours as an estimate of baking quality of the protein system for the flours. Maes (1966) reported that the baking quality of flour was negatively correlated with the percentage of water soluble proteins. Tanaka and Bushuk (1972) reported that soft wheat had higher contents of water soluble protein and lower contents of 70 % ethanol and acetic acid soluble proteins compared with hard wheat. They postulated that these quantitative differences could be the reason wheats differ in physical properties.

Mattern and Sandstedt (1957) studied the influence of water soluble constituents of wheat flour on mixing and baking characteristics. They reported that the principal factor responsible for determining mixing requirement of wheat flour was water soluble. These researchers found that the removal of water soluble constituents lengthened the mixing time of a flour while incorporation of the soluble components reversed this effect. They also reported that the factor responsible for shortening the mixing time was non - dialysable and heat stable. According to Mattern and Sandstedt (1957) the factor was precipitated by low concentration of ammonium sulphate and had a high nitrogen content indicating that it consists of gliadin. They observed that flour extracted with 5 % sodium chloride (dialysed and dried) retained its normal mixing time.

Mecham *et al* (1960) reported that after extraction with water, the gluten-starch residue differed markedly in mixing properties from the original flour or salt extracted residue. Similar findings have been observed by many other researchers (Mattern and Sandstedt, 1957; Shuey and Gilles, 1973; Sievert *et al*, 1991b), who have generally found that dough from water extracted flour does not breakdown during

extended mixing in the farinograph. Mecham *et al* (1963) also observed that the water-extracted residue remained sensitive to the addition of N-ethylmaleimide (NEMI), a sulfhydryl blocking agent. NEMI treatment of the water soluble constituents did not modify their effects on the mixing-curve characteristics. However, Yoshida and Danno (1989) reported that partial removal of gliadin by water extraction prevents the formation of a viscoelastic gluten mass and thereby precludes the development and breakdown of dough during mixing.

Hoseney *et al* (1969a), reported that albumins and globulins do not appear to influence breadmaking performance. These researchers observed that one non-dialysable fraction was involved in gluten modification. This fraction was assumed to be a glycoprotein since water extracts of flour contain glycoproteins responsible for gelation of aqueous extracts of wheat flour in the presence of oxidizing agents. The carbohydrate portion of these glycoproteins is necessary for normal baking characteristics. Hoseney *et al* (1969a) also reported that the water soluble fraction played no major part in mixing, and they proposed that polar lipids bind to gliadins by hydrophobic bonds and to glutenins by hydrophillic bonds, and in this way contribute to the gas retention complex in bread. D'Appolonia *et al* (1970) associated the increased loaf volume of the gluten-starch loaves with the addition of water soluble fraction to the residual protein in the pentosans extract, supporting the findings of Pence *et al* (1950). Hoseney (1984) however, reported that reduced loaf volume occurred only after the water soluble pentosans were removed and was not affected by the water soluble proteins, albumins and globulins.

Marais and D'Appolonia (1981a) examined the correlation of albumin and globulin with bromate requirement in breadmaking. They found that only 28 % of the

total variation in bromate requirement could be accounted for by albumin and globulin contents. In a subsequent study, Marais and D'Appolonia (1981b) found that the amount of water soluble material and albumins positively affected mixogram peak time and curve width. However, the albumin proteins sometime tended to have a negative effect on peak time, depending on the manner in which they interacted with entities in the gluten and tailing fractions.

Dreese and Hoseney (1990) suggested that water-soluble flour components are involved in the effects of iodate in dough. These workers washed commercial dry gluten with distilled water. Control (unwashed) and washed gluten performed equally in baking tests when they were added to the flour. However, when only 1.3 % of the gluten was removed, dynamic rheological tests showed that the washed gluten had higher storage modulus (G') and lower loss tangent (G''/G') values, i.e relatively more elastic and less viscous, therefore more resistant to deformation, than the control flour. They also observed that mixograph and dynamic rheological tests showed that the washed gluten gave no response to mixing with iodate, whereas the control gluten responded to iodate. Based on these observations, they suggested that the soluble material removed from washed gluten is involved in the effect of potassium iodate. The nature of this material is not known.

Most recently Sievert *et al* (1991b) found that during mixing, there was a decrease in staining intensity of triplet zone proteins (presumably triticin) of normally processed dough. However, mixing of doughs from water extracted flour gave no change in staining intensity of triplet zone proteins. On the basis of these findings they hypothesized that the events in the triplet band region during mixing were mediated through water soluble components of the flour.

It can be concluded from the above that the role water soluble proteins play in dough behaviour still remains ambiguous, and is dependant upon the wheat class variety of wheat, grade of flour and extraction technique employed.

## D. STORAGE PROTEINS

The term "storage or insoluble wheat proteins" is generally used to refer to the gluten proteins comprising mainly gliadins and glutenins. Gluten is the yellowish-grey elastic material remaining when a piece of dough has been kneaded in a stream of water to remove the starch and other water soluble components. The unique rheological properties of wheat flour have been attributed to the glutenin and gliadin protein fractions (Bloksma and Bushuk, 1988).

Finney (1943) was one of the first workers to recognize that differences in breadmaking quality of wheat were entirely accountable by differences in their gluten fractions. This observation was based on results obtained with flours reconstituted from gluten, starch and water solubles. Since then a number of researchers have confirmed these findings.

### 1. Gliadins

Gliadin represents the major wheat storage protein fraction in the starchy endosperm, and accounts for 30-60 % of the total grain nitrogen depending on the nutritional status and genotype of the plant (Bright and Shewry, 1983). It is generally accepted that wheat gliadins are: (i) proteins of wheat endosperm soluble in alcohol such as 70 % ethanol (v/v) at room temperature (ii) migrate in polyacrylamide and starch gels without reduction as reasonably discrete bands and (iii) are not excluded

during gel filtration on Sephadex G100 (Lasztity, 1984). Gliadin has also been defined as low molecular weight storage proteins and may be further classified into four main groups; $\alpha$-, ß-, $\gamma$- and $\omega$-gliadins based on their mobilities by starch gel electrophoresis at low pH (Woychik *et al*, 1961). Amino acid analysis showed that the gliadin proteins fall into two groups, with the $\omega$-gliadins in one group and the $\alpha$-, ß-, and $\gamma$-gliadins in the other (MacRitchie *et al*, 1990). The $\omega$-gliadins are characterized by high proportions of glutamine/glutamic acid, proline and phenylalanine and almost none of the sulfur containing amino acids, cysteine/cystine and methionine (MacRitchie *et al*, 1990). The $\alpha$-, ß-, $\gamma$-gliadins contain cysteine/cystine and methionine and lower contents of glutamine/glutamic acid. Gliadins, in general, are poor in basic amino acids especially lysine, arginine and histidine (Lasztity, 1984). They also have low amounts of aspartic acid and asparagine. In contrast with glutenins, gliadin are distinguished by being comprised of single-chain polypeptides, ranging in molecular weight from about 30 - 80 kDa (MacRitchie *et al*, 1990).

Differences in hydrophobic interactions in proteins between flours of good and poor breadbaking quality have been observed by a number of researchers (Chung and Pomeranz, 1979; Kobrehel, 1984; Popineau and Pineau, 1987). It has been reported that hydrophobicity of gliadins is fairly high and may play a role in the lipid-protein interaction (Lasztity, 1984). Kobrehel (1984) reported that good quality flours required a higher concentration of ionic detergent for an equivalent dissolution of proteins than poor quality flours. Based on this observation, he suggested that the hydrophobic interactions in proteins of good quality flours are stronger (or greater in number) than those of poor quality.

Chung and Pomeranz (1979) reported that the gliadin fraction from a poor

quality flour was more hydrophobic compared with the good quality flour. Nonchaotropic salts have been observed to increase dough strength of flours of different qualities (He *et al*, 1992). These workers observed that $Na_2SO_4$ had a more pronounced effect than did NaCl. Compared with NaCl, $Na_2SO_4$ greatly improved the rheological properties and gas retaining ability as well as the loaf volume and crumb grain of the poor-quality flour (presumably by increasing the hydrophobic interactions between gluten proteins).

The gliadin electrophoregram is not affected qualitatively and quantitatively by growing conditions, protein content, effects of sprouting, dusting of fumigants or by mild heat treatment. However, Schofield *et al* (1983) using SDS-PAGE showed that the gliadin patterns were essentially unaltered up to 75 °C but at 100 °C ω-gliadins dominate. Wrigley *et al* (1984) have also reported that significant changes occur in the relative intensities of the gliadin bands when sulphur is deficient during growth. There is also evidence that the sulfur nutrition, particularly when considered in relation to nitrogen supply can affect the baking properties (Timms *et al*, 1981). Huebner and Bietz (1988) showed that wheats grown under different sulfur fertilization levels exhibit major quantitative variation in gliadin components by reversed phase (RP)-HPLC and that significant quantitative variations also existed for gliadins extracted from wheat grown in different locations. Gliadin proteins resolved by PAGE are therefore best suited and most often used for variety identification (Zillman and Bushuk, 1979., Konarev *et al*, 1979).

## 2. Glutenins

There is no standard procedure for the extraction and preparation of glutenin

proteins, and accordingly an acceptable definition of glutenin according to solubility does not exist. However, it is generally agreed by many cereal chemists (Lasztity, 1984 and references cited therein) that glutenin has two main characteristics, (i) it is not soluble in dilute salt solution and (ii) it is a macromolecule composed of polypeptide chains bound by disulphide bonds. The term glutenin is now reserved for the native (unreduced) oligomeric molecules of this class of protein, while the term "subunit" refers to the single chain polypeptides obtained after reduction of disulphide bonds of glutenin (Bushuk, 1991).

Amino acid composition of glutenin is similar to that of gliadins, except that it has an increased amount of glycine and a slightly higher amount of basic amino acid and lower amounts of glutamic acid and proline (Bushuk and Wrigley, 1974). The average content of amino acids with hydrophobic side chains is lower (Lasztity, 1984). Chung and Pomeranz (1979) reported that the glutenin from a poor quality flour is less hydrophobic than that from a good quality flour.

Glutenin may be separated from other flour proteins and constituents by different techniques into a number of fractions. Separation based on differential solubility is one possibility. Acetic acid (e.g 0.05-0.1 *M*) has been used frequently to solubilize glutenin, however, the ratio of soluble glutenin:residue may vary widely (Orth and Bushuk, 1973a; Bietz and Wall, 1975). Chen and Bushuk (1970) reported that the acetic acid soluble fraction (glutenin) represented 10-30 % of the total flour protein and acetic acid insoluble protein (residue) represents about 35 % of the total protein. Subsequent fractionation of the extracted (soluble) proteins has been achieved by centrifugation, gel filtration, HPLC and other techniques. Techniques such as SDS-PAGE or SE-HPLC divide glutenin subunits into two relatively distinct groups - the

HMW and the LMW subunits (Bushuk, 1991). The LMW subunits of glutenin overlap some of the gliadins in SDS-PAGE.

Mecham *et al* (1962) found that exhaustive extraction of the flour with 0.01 *N* acetic acid resulted in a highly hydrated gelatinous residual material containing about 30 % of the total protein. This gel forming protein has been named "gel protein" (Graveland *et al*, 1979; Lasztity, 1984). Gel protein has also been prepared by other procedures (Bietz and Wall, 1975; Graveland *et al*, 1982; Moonen, et al, 1982). Moonen *et al* (1982) isolated gel protein from wheat flour by extraction with aqueous 1.5 % SDS solution. Remarkable intervarietal differences were observed both in the amount, viscosity and subunit compositions of these proteins. A high correlation (r=0.949) was observed between the amount of gel protein and SDS-sedimentation volume. Since the SDS-soluble proteins consists largely of glutenin proteins this leads to the conclusion that breadmaking quality is governed by both the amount and composition of glutenin proteins (Hamer *et al*, 1992).

Payne and Corfield (1979) reported that the apparent molecular weight of glutenin subunits determined by SDS-PAGE ranged from 12 - 134 kDa. Miflin *et al* (1983) reported that reduced glutenin fraction separated by SDS-PAGE contain bands of 100 kDa - 44 kDa, the latter comigrating with gliadin bands. Native glutenin has a molecular weight of over one million (Meredith and Wren, 1966). Studies using SDS-PAGE techniques have shown that reduced wheat glutenin consists of approximately 17-20 subunits (Lasztity, 1984). Treatment of native glutenin with reducing agents (such as 2-mercaptoethanol) greatly lowers the molecular weight with accompanying loss of elasticity and cohesiveness (MacRitchie *et al*, 1990), an indication that the native glutenin is stabilized by intermolecular disulphide bonds.

Using 164 samples, Hamer *et al* (1992) observed that the HMW glutenin-A subunits based models correlate positively but poorly ($R^2$=0.30-0.36) with loaf volume. Extending the models with other quality related parameters especially SDS-sedimentation volume led to an improved model ($R^2$=0.73). Hamer *et al* (1992) therefore concluded that breadmaking quality is not only governed by the quality of the HMW glutenin-A subunits but also by other factors, one of which is correlated with amount of glutenin proteins.

**3. The relationship between dough mixing and wheat gluten protein fractions**

Mixing of flour into dough is generally considered a critical step in breadmaking as it influences the overall bread quality in conventional breadmaking methods. At optimum mixing, the protein matrix is developed into a continuous network of sheets with embedded starch granules. In undermixed dough the swollen proteins forms compact masses in which some starch granules occur, while other starch granules lack a protein envelope (Bloksma and Bushuk, 1988). Mixing to below optimum consistency results in small loaf volume and poor crumb texture, while overmixing produces a wet sticky dough (Bloksma and Bushuk, 1988). Mixing properties are therefore important parameters in the quality assessment of flours. Their importance has become more significant with the introduction of new continuous breadmaking processes in which dough development is achieved by high-speed mixing. The baking industry in recent years has therefore placed greater emphasis on the mixing requirements of flour. Optimum control of this factor would be of great value to bakers, the milling industry and wheat breeders.

Mullen and Smith (1965) and Smith and Mullen (1965) showed that the main

difference between a short and long-mixing flours was that the short mixing had more of the ß-gluten components resolved by moving boundary electrophoresis and less acetic acid insoluble proteins. They also reported that the addition of protein-starch residues (glutenins) resulted in long mixing requirements whereas the addition of water solubles (gliadins) markedly shortened the mixing requirements. These workers concluded that mixing development time is determined by the type of protein-starch residues (i.e after extraction with distilled water and 0.1 *M* NaCl) and the presence or absence of the water solubles and not by the level of sulfhydryl groups in the system.

According to Huebner and Wall (1974, 1976) strong flours exhibiting long mixing times have high ratios of HMW to LMW glutenins resolved by gel filtration chromatography. These authors also reported that flours producing weak-doughs generally have lower amounts of HMW glutenins and unextracted protein. Hoseney *et al* (1969b) fractionated gluten into gliadin and glutenin using 70 % ethanol. The ratio of gliadin to glutenin was 53:47 for four wheat flours that varied in breadmaking quality. By reconstitution studies these researchers reported that gliadin controlled loaf volume while the glutenin fraction governed the mixing requirements. Since the ratio of gliadin and glutenin did not vary between short to long mixing flours, they postulated that the mixing requirement was governed by the differences in one of the fractions or by the type of interactions between the fractions.

Preston and Tipples (1980) observed dough weakening effects when particular gluten protein fractions were added to the base flours. Dough weakening was mainly due to the proteins in the acetic acid insoluble gluten fraction. Acetic acid soluble gluten proteins had no dough weakening effects. These workers reported that the addition of acetic acid soluble gluten increased loaf volume while the addition of acetic

acid insoluble gluten significantly reduced loaf volumes. Similar findings have been reported by MacRitchie (1987). By using HCl at various concentrations it was observed that the addition of early-extracted fractions (gliadins) to a base flour decreased mixing time and slightly depressed loaf volume. The intermediate fractions (glutenin) induced large increases in dough development time and loaf volume. While the last fraction (residue proteins) caused weakening of the dough and depression of loaf volume. Legouar *et al* (1979) reported that the mixing strength of wheat flours was due to the glutenins soluble in acetic acid and that the superior loaf volume depended on the glutenins insoluble in acetic acid. Their results supported the findings of Orth and Bushuk (1972) who were the first to show that the loaf volume per unit protein content (ULV) was positively correlated with acetic acid insoluble residue protein and negatively correlated to acetic acid soluble glutenins.

Booth and Melvin (1979), studied the effect of breadmaking quality by interchanging flour components of a Canadian hard red spring wheat and a European wheat variety. They observed that lactic acid-soluble and insoluble proteins were responsible for the poor baking quality of a high yielding European wheat variety.

Many researchers (Mecham *et al*, 1962; Tsen, 1967; Orth and Bushuk, 1972; Tanaka and Bushuk, 1973; Orth and O'Brien, 1976; Axford *et al*, 1978; and Sievert *et al*, 1991b) have observed a direct relationship between the amount of residue protein and functionality, particularly mixing strength of different genotypes. The underlying cause of the observed relationship has been attributed mainly to HMW glutenin. The latter can be released from the residue as polypeptide "subunits" in the presence of reducing agents. Orth and Bushuk (1972) and Orth *et al* (1972) developed prediction equations relating dough strength to the proportion of insoluble (residue)

protein since there was a highly significant correlation between the two factors. Orth and O'Brien (1976) described a fractionation procedure in which they showed that the proportion of residue protein, after direct extraction with 0.05 *M* acetic acid, as a percentage of total flour protein was significantly correlated with dough strength and represented a potential index of functional quality.

Fullington *et al* (1987) examined by SDS-PAGE, quality related endosperm proteins in sulfur deficient and normal wheat grain. They reported that the proportion of HMW components corresponding to HMW glutenin ($M_r$ > 80 kDa) in the residue was positively correlated with resistance to extension and negatively correlated with dough extensibility and breakdown. ω-gliadins ($M_r$ 51 - 80 kDa) showed the same degree of correlation as HMW glutenins whereas proteins of $M_r$ 38 - 50 kDa (*α*-, ß- and *γ*-gliadins) and 28 - 39 kDa (LMW glutenins) were positively correlated to dough extensibility. In earlier studies a number of researchers (Moss *et al*, 1983; Wrigley *et al*, 1984) observed that sulfur deficiency during grain development drastically altered dough properties. Wrigley *et al* (1984) reported that as a result of sulfur deficiency, protein synthesis was redirected in favour of the low sulfur-containing proteins particularly ω-gliadins and the HMW glutenin subunits. These workers reported that a loss of dough extensibility and an increase in resistance to extension accompanied these changes in protein composition.

Khan *et al* (1989) prepared glutenin using the procedure similar to the one described by Graveland *et al* (1982). They obtained water soluble, salt soluble proteins and gliadins by sequential extraction using water, 0.5 M NaCl, and 70 % ethanol. They varied the order of the extracting solutions, resulting in three different procedures of extraction. They reported that all the glutenin fractions gave a positive correlation with

mixing time whereas all gliadin fractions gave a negative correlation. Both gliadin and glutenin fractions were positively correlated with loaf volume.

Yoshida and Danno (1989) studied the effects of a water soluble protein fraction on the viscoelasticity of wheat gluten by means of a reconstitution study. They prepared the gluten and water soluble fractions from defatted flour by two methods: (i) water extraction of a flour/water slurry and (ii) dough mixing followed by water extraction and freeze-drying. These researchers observed that with the dough mixing method, the water soluble components especially gliadin, were incorporated into the gluten mass, while the gluten prepared by water extraction of flour was poor in gliadin. They reported that the reconstituted gluten with gluten obtained by dough mixing had the same rheological properties compared with gluten prepared from the flour. The same results were obtained when gliadin was used instead of the water soluble fraction obtained from flour/water slurry. On the other hand, the reconstituted gluten using gluten obtained from water extraction, was elastic and less extensible despite the same ratio of gliadin and glutenin. Based on these findings Yoshida and Danno (1989) suggested that for the formation of a viscoelastic mass, gliadin must be mixed with glutenin to form a homogeneous gluten network.

**4. Recording dough mixers**

Dough mixing characteristics of wheat flour have generally been determined using the farinograph and the mixograph. The first procedure requires large quantities of sample (e.g 50 - 300 g). It is therefore suitable where large quantities of samples are available. The mixograph has become popular especially for small quantities of samples, e.g in screening early generation material in wheat breeding programme. It

has been scaled down from the 35 g dough mixer, designed and built by Working in 1939 (Shogren, 1990) to one requiring only 10 g (Finney and Shogren, 1972) and 5 g (Finney, 1989). Recently Rath and coworkers (Rath *et al*, 1990) developed a mixer using the mixograph principle and requiring only 2 g of flour. This mixer combines the direct-drive design with electronic sensing instead of the traditional mechanical sensing, plus improved software facilitating automated interpretation, display, and recording of results. The 2-g mixer gave a good correlation to the standard 35 g mixograph with 34 samples (r=0.83, for time to peak and r=0.89 for peak dough resistance). Many traditional 35 g and 10 g mixographs have been modified to electronically record the measurements on the curve (Voisey *et al*, 1966a; Rubenthaler and King, 1986; Stearn and Barta, 1990; Navickis *et al*, 1990; Gras *et al*, 1990). Voisey *et al* (1966a) for example, developed a fixed-bowl recording mixer for 5- or 10-g flour samples that was similar in design to the 35-g mixograph. Dough resistance to mixing was measured by four strain gauges mounted on a beam (two each side) and connected to a Wheatstone bridge. This permitted an easier and faster analysis of the dough mixing characteristics and more objective interpretation of the measurements.

The electronic/computerized mixograph, however, has some limitations. One is that the instrument and procedure has not been standardized; different researchers have used different methods for data acquisition, interpretation and storage. Stearn and Barta (1990) described a procedure in which the bowl platform is immobilized and stabilized by a linear variable differential transformer (LVDT) type of transducer. Signals from the transducer travel via a signal conditioner to a computer containing a data acquisition board for analog to digital conversion, with programmes for data collection, storage and manipulation. Standardization for routine use has been difficult because

the cut-off voltage is by itself subject to visual determination. A similar procedure was described by Rubenthaler and King (1986).

Navickis *et al* (1990) evaluated mixograph parameters by electronic torque sensing. In their procedure, the mixograph parameters were computed from digitized data acquired from a 10 g mixograph via rotary potentiometer whose output was proportional to the bowl and pen positions and thus to the torque being encountered. However, the variation among replicates was higher for the objective computer-derived data compared with subjectively acquired data by a skilled operator. Shelton (1990) described a procedure in which the mixograph curve was acquired from the hardcopy result by computer using an optical scanner. The disadvantage of this procedure is that external noise and markings have to first be filtered out. However, the procedure has an advantage in that it is applicable to farinograph and extensigraph records.

Currently the disadvantages of the electronically recording mixograph are: (i) use of different electronic recording mechanism and computer software and (ii) the initial cost and the replacement of some vital components, e.g transducers. These disadvantages may be ascribed to the fact that most of the instruments have been custom made by each researcher. However, Gras and O'Brien (1992) recently reported on the application of the 2-g mixograph for early generation selection for dough strength. These researchers reported that a good range of variation in mixograph parameters combined with acceptable errors of determination was found for flours derived from the seeds of $F_2$ single-plant selections and their $F_3$ progeny. Their results agreed well between $F_2$ and $F_3$ generations for the mixograph mixing time and tolerance to mixing as evidenced by the medium-to-high offspring-parent heritability within and between crosses. The 2-g mixograph can also be used as a research tool.

Recently, Bekes and Gras (1992) demonstrated the usefulness of the direct-drive 2-g mixograph in their study of the effects of particular wheat proteins and their aggregates with lipids during dough mixing. This work further illustrates the versatility of the electronic mixograph in determining the mixing characteristics of wheat flour.

In Canada wheat quality screening of early generation materials typically involves determination of flour yield by the Quadrumat Jr. mill, determination of protein content by NIR and dough mixing strength using a mixograph. The prediction of baking quality has typically focused on the relationship of the various mixograph parameters to loaf volume. Lukow (1991) in carrying out regression analysis of thousands of samples, concluded that mixograph peak height may be more important in predicting baking quality than mixograph development time. Mixograph peak height has been shown to be positively correlated with loaf volume and is largely a function of protein content, water absorption and dough strength (Lukow, 1991).

## E. EFFECTS OF MILLING ON FLOUR PROTEIN EXTRACTION

Protein solubility studies on wheat flours of varying flour extraction rates have generally been carried out on straight run wheat flours obtained on various mills, e.g Buhler experimental mill, pilot-scale mills, Allis-Chalmers mill, Brabender Quadrumat mill and commercial mills. Different mills and extraction rates have an effect on the extractability of the flour proteins. Williams and McEwin (1967) used different grinding techniques to produce wheatmeal ranging in particle size from 0.5 to 2.0 mm. They showed that protein content, as determined by the Biuret test, of the coarser meals was significantly less than that of the fine meals. The standard error was higher for the coarser meals. They also observed that differences in extractability were significantly

correlated with kernel hardness but not with protein content.

Venkateswara *et al* (1986) fractionated proteins of flour of different extraction rates (70, 75, 80 and 85 %), milled from four wheat varieties of diverse mixing strength. They observed that as the flour extraction rate increased, the proportion of albumin, globulin and residue fractions as a percentage of total flour protein and the total flour protein of the samples increased but the increase in globulins was comparatively higher. Gliadin content decreased with increasing extraction rate, except for the weak wheat (Pissi) where gliadin content increased. Glutenin increased from 70 to 80 % extraction flour but decreased at 85 % extraction. They also observed that, as the extraction rate increased, the ratio of albumin and globulin to gliadins, glutenins and residue protein increased, while the gliadin to glutenin ratio was not affected.

Okada *et al* (1987) produced overground flour by passing it several times through the rolls. These researchers found that overgrinding caused little change in the gel filtration elution profiles of the extracts of flour or dough. However, the rate of aggregation (as shown by the rate of change of turbidity) of proteins stored at -20 $^{o}$C in 0.5 % SDS solution containing 0.0016 *M* NEMI increased with more passes through the rolls and with dough mixing. They suggested that the conformation of flour protein was changed by overgrinding and mixing so that denaturation of flour protein was more easily caused by frozen storage.

Kunerth and D'Appolonia (1985) showed that flours of different grades (patent, straight grade, first and second clears) had different farinograms. The lower quality grades showed weaker curves. Dexter *et al* (1990) milled representative samples of different wheat classes to extraction rates of 75, 79 and 83 %. At each extraction rate

they also removed up to 24 % (wheat basis) high quality middlings (farina). They reported that as the extraction rate increased, the straight run flour (no middlings removed) showed an increase in ash content and darkening of colour. At the same time the farinograph, extensigraph and alveograph curves weakened and bread quality deteriorated. But the removal of up to 24 % farina had only marginal effects on the rheological and baking properties of the residual flour. The type of milling therefore has an important influence on protein solubility and the technological quality and breadmaking characteristics of flour.

# III. MATERIALS AND METHODS

## A. MATERIALS

### 1. Wheat Samples.

Six pure wheat varieties with diverse quality characteristics (Table 29, Appendix I, page 171), were used in the preliminary studies. Five of the samples; Glenlea, Roblin, Neepawa, Augusta and Kenya were provided by Dr. P.K.W. Ng, Food Science Department, University of Manitoba. HY368 was provided by Dr. K. Preston of the Grain Research Laboratory (GRL), Canadian Grain Commission (CGC), Winnipeg.

A set of 16 samples was used for comparative study of experimental milling effects on technological quality and protein solubility fractionation. The samples are described by pedigree and/or wheat class in Table 1. The samples, 1 CWRW, 1 CWHRS, 1 CWSWS, 1 CEWW and Utility were composites of varieties of the same class grown in different sites. These were provided by Dr. K. Preston of the GRL, CGC, Winnipeg. Norwin and Norstar (1991 crop year) were provided by Dr. A. Brùle-Babel of Plant Science Department, University of Manitoba. Glenlea, Katepwa, Biggar, Oslo, Nordic, Guard, HY612 and Roblin were provided by Dr. R. McKenzie of Agriculture Canada, Winnipeg. These samples were grown in Lethbridge, Alberta in 1991 except Roblin which was grown in Indian Head, Saskatchewan in 1988.

A third set of wheat samples, comprising 26 varieties of the 1983 Uniform Quality Nursery (UQN), was used to develop and evaluate computer models which relate electronic recording mixograph parameters with protein solubility data. These samples were grown in four locations in western Canada at Lethbridge, Regina, Saskatoon and Swift Current Agriculture Canada Research Stations. The samples, provided by Dr. P.K.W. Ng, had been kept in cold storage (4 °C) in tightly closed

containers. Their pedigrees and origins are presented in Table 2; technological quality data is presented in Table 30, Appendix IV (page 191).

TABLE 1. The pedigree, class and origin of the samples used for comparative studies.

| No. | Sample | Pedigree, Class and Origin |
|---|---|---|
| S01 | 1 CWRW | composite, No. 1 Canada Western Red Winter |
| S02 | 1 CWHRS | composite, No. 1 Canada Western Hard Red Spring |
| S03 | 1 CWSWS | composite, No. 1 Canada Soft White Spring |
| S04 | 1 CEWW | composite, No. 1 Canada White Winter |
| S05 | Utility | composite, Utility, Canada |
| S06 | Norwin | Froid/Winoka//TX 65A1508/Westmount/Trader, hard red winter, USA |
| S07 | Norstar | ........., hard red winter, USA |
| S08 | Katepwa | Neepawa*6/Lee*2/Kenya Farmer/Neepawa*6//C.1 8154/2*Frocor, hard red spring, Canada |
| S09 | Augusta | Genesee/Redcoat, x A 2747//Yorkstar, soft white winter, USA |
| S10 | Glenlea | Pembina*2/Bage/3/Sonora 64/TZPP /Nairn 60, utility, Canada |
| S11 | Biggar | tested as HY 368, Canada Prairie Spring (CPS), Canada |
| S12 | Oslo | Sonora64/Yaqui50E/Guajolote/3/Inia/4/Crano/Elgan/ Sonora 64, dark northern spring,USA |
| S13 | Nordic | .........., dark northern spring, USA |
| S14 | Guard | .........., dark northern spring, USA |
| S15 | HY612 | .........., CPS, Canada |
| S16 | Roblin | Manitou x Tobari 66/ CT 934/ Neepawa//Era/Park/CT 615 Neepawa, Canada |

TABLE 2. 1983 Uniform Quality Nursery varieties[1]

| 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/R6 4137, Canada |
| 5 | Cook | WW31/Timgalen, Australia |
| 6 | Tobari 66/Romany | Tobari 66=Tezanos Pintos Precoz/Sonora 64A, Mexico<br>Romany=Colotana 261//51/Yaktana 54A, Kenya |
| 7 | Neelkant sib | HD1220/KAL*3//NAC, Mexico |
| 8 | Veery[2] #4 | CM33027-F-12M-1Y-10M-1Y-3M-1Y-0M, Mexico |
| 9 | Veery[2] #5 | CM33027-F15M-500Y-0M, Mexico |
| 10 | Nacozari 76 | TZPP/PL//7C, Mexico |
| 11 | Veery[2] #1 | CM33027-F-8M-1Y-8M-1Y-2M-0Y, Mexico |
| 12 | Veery[2] #2 | CM33027-F-12M-1Y-4M-2Y-2M-0Y, Mexico |
| 13 | Veery[2] #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[2] sib | Mexico |
| 17 | ------- | G11-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//Texaros 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 |

[1]Ng (1987).

[2]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 2. Cont.

| No. | Variety | Cross/Pedigree and Origin |
|---|---|---|
| 22 | Condor | Carstens VI/?/Dankowes/Carstens V/?/Wjatka, Australia |
| 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 |

### 2. Reagents and chemicals

Acrylamide, bisacrylamide (N, N'-methylene-bisacrylamide) and sodium dodecyl sulphate (SDS) were electrophoretic grade and were obtained from BioRad, Richmond, CA. Coomassie Brilliant Blue G-250 (CBB G-250), glycerol, glycine, potassium hydroxide and Tris (tris (hydroxylmethyl) amino methane) were purchased from Sigma Chemical Company, St. Louis, MO. All other chemicals and reagents were of analytical grade. Distilled and deionized (DD) water was used in all the experiments.

## B. PREPARATION OF COMPOSITE SAMPLES AND MILLING

The six samples used in the preliminary studies were milled on a Buhler laboratory mill after tempering to 15.5 % moisture basis (mb).

In the study of the technological and protein solubility distribution differences between Buhler laboratory and Quadrumat Jr. experimental mills (hereafter referred to as the Buhler and Quad mills, respectively), 2 kg of each of the 16 samples was used. One kg was tempered to 14.5 % mb, blended and left to stand overnight at 23 °C. Each of the samples was divided in four (@ 250 g) fractions and milled sequentially on a Quad mill. The resulting flour was composited and rebolted through a 50 GG (390 $\mu$m) sieve then blended. The other 1 kg was tempered to 15.5 % mb, blended and allowed to stand overnight at 23 °C before being milled on the Buhler mill. The flour was rebolted through a 70 GG (236 $\mu$m) sieve and blended. Both flours were kept in cold storage (4 °C) until required. The samples for Quad milling were tempered to only 14.5 % mb because the design of the mill and small size of the rolls results in less moisture loss during milling. The Quad samples were sieved using a wider mesh to give an equal amount of rebolt material as the Buhler flour. There was no milling replication

for individual samples because from experience and literature the two mills give very good reproducibility. For example, Bequette and Potts (1962) showed that the standard deviation of duplicate determinations on a Quad mill was 0.18, 0.02, and 0.21 % for flour moisture content, ash, and protein content, respectively. For the Central Breadwheat Coop. Test, duplicate milling on Buhler mill is routinely done for all the samples under review. Milling results indicate that there is good reproducibility (Rick Zillman, *personal communication*).

The 1983 UQN samples of each variety from the four locations were blended to form a single uniform wheat sample. Grain (100 g) from each location was used to give a 400 g composite. Each composite was tempered, blended, milled and rebolted as described for the 16 Quad samples. It was kept in cold storage (4 °C) until required.

## C. EXTRACTION AND FRACTIONATION OF FLOUR PROTEINS

### 1. Solubility studies

#### (i) Optimum protein extraction conditions

Optimum extracting conditions were determined for extraction time, temperature (4 °C vs 23 °C), solvent to sample ratio and mixing action (manual vs mechanical) using 0.05 *M* acetic acid (HAc), water and 2 % sodium dodecyl sulphate (SDS) solution.

#### (ii) Extracting solutions

Solutions of 2 % SDS, 2 % NaCl, 0.05 *M* HAc, 70 % ethanol (EtOH) and distilled water were used for direct extraction of flour proteins for the preliminary study. For the two other sample sets, 2 % SDS was omitted because this solvent

represents a "total" protein extracting solvent rather than specific groups of protein. It was the aim of the study to extract and quantify more discrete group of protein fractions.

## 2. Extraction

During each condition examined two extractions were performed on each sample and duplicate protein determination was done for each extraction. Preliminary studies showed that protein extraction using 1 g flour with 10 ml of solvent gave same amount of protein results with using 25 ml for all the solvents except water. Therefore subsequent extractions were done using 25 ml of solvent for all samples.

In the initial procedure, 1 g of flour was extracted with 25 ml solvent in 50 ml centrifuge tubes. The tubes were briefly agitated manually every 15 min for 2 h and centrifuged at 16000 *g* for 30 min at 20 °C in a Sorvall RC5C centrifuge (Dupont Co. Wilmington, Delaware). The supernatant was decanted and subsequently freeze-dried, along with the residues. The freeze-dried residues were weighed, finely ground by mortar and pestle, and stored in sealed containers at 4 °C until needed. The freeze-dried supernatants which were fluffy in appearance, were stored in sealed containers at 4 °C until needed.

In order to eliminate the freeze drying and manual shaking steps, the initial procedure was modified. The extraction procedure of Orth and O'Brien (1976) was used with minor modifications. Flour (1 g) was extracted in 25 ml of solvent in a 50 ml centrifuge tube. The tubes were mechanically shaken using a Ika-Vibrax-VXR shaker (Terochem laboratories Ltd., Edmonton, Alberta) at a speed setting of 1400 for 2 h. After centrifuging at 16000 *g* for 30 min at 20 °C, the supernatant was decanted into

a high precision (±0.1) measuring cylinder to determine total volume. Three ml of supernatant was used for protein determination using the micro-Kjeldahl procedure (AACC method 46-13, 1983).

## D. MIXING CHARACTERISTICS

The mixing characteristics of the samples were determine using both the farinograph and mixograph. The tests were performed according to the standard AACC methods 54-21 and 54-40A (1983), respectively. Flour samples of 50 and 35 g, were used for the farinograph and mixograph, respectively. Although the two methods measure mixing characteristics of the dough, both were used here to compare the mixing characteristics of the samples because of their different modes of mixing (Kunerth and D'Appolonia, 1985). It has also been reported by Mecham *et al* (1962), that a mixograph converts more protein to an extractable form more rapidly than the farinograph. Recently, Gupta *et al* (1992) reported that the correlations of protein extraction with mixograph and farinograph mixed doughs were different.

The mixing characteristics of the 26 UQN samples and the 16 samples for comparative milling studies were determined using a 10 g computerized electronic micromixograph at constant (62 %) water absorption. The procedure which was followed was that described by Voisey *et al* (1966b).

## E. SDS - PAGE ELECTROPHORESIS

### 1. Sample preparation

Samples for SDS-PAGE were prepared according to the procedure of Sievert *et*

*al* (1991a) with minor modifications. Protein solutions were prepared by dissolving (i) 40 mg of flour in 1 ml extracting buffer (see below) or (ii) an amount of freeze dried sample to give 6 mg protein in 1 ml of buffer. Ethanol extracted supernatants were dried using 5 ml test tubes in a rotary evaporator (Speedvac concentrator, Savant instruments Inc. NY) overnight at -92 °C. The dried samples were dissolved in extracting buffer (0.25 - 1.0 ml) to a protein concentration of 6 mg/ml. The buffer solution comprised of 0.063 M Tris/HCl at pH 6.8, 2 % (w/v) SDS, 10 % (v/v) glycerol, and 0.01 % (w/v) pyronin Y. Where reduction of disulphide bonds was required, 5 % (v/v) ß-mercaptoethanol (ME) was included in the buffer solution. Each supernatant mixture was allowed to stand at room temperature for 2 h with occasional vortexing on a Vortex-Genie mixer (Scientific Industries, Inc. Bohemia, NY) at a speed of 10 to disperse the solute. The samples were subsequently heated for 3 min in a boiling water bath and allowed to cool to room temperature. Suspensions were centrifuged in a table top centrifuge (Biofuge A, Heraus-christ GmbH, West Germany) for 10 min at 12,000 *g*. Supernatants (8-10 $\mu$ls) were used for loading into gel slots.

The same extraction procedure was followed for the residue fractions except they were let to stand overnight instead of two hours. For the NaCl, extractions samples were extracted at 30 °C for 2 h instead of room temperature. This improved the resolution of the protein bands by SDS-PAGE.

**2. Electrophoresis.**

SDS-PAGE was carried out in a LKB 2001 electrophoresis unit (Pharmacia LKB), according to the procedure described by Ng and Bushuk (1988). The acrylamide concentration in the stacking gel was 3.03 % and in the separating gel was 17.3 %.

Gel thickness was 1.5 mm. The two outer slots were always left blank to avoid curving of the bands. Electrophoresis was carried out at 35 mA for 45 minutes and at 10 mA until the tracking dye migrated to the end of the gel. Sievert *et al* (1991a) described this as the short run method which results in all the proteins being retained on the gel slab.

## F. TECHNOLOGICAL TESTS

### 1. Protein content

The total nitrogen content of flour and extracts was determined by the micro-Kjeldahl procedure (AACC method 46-13, 1983). Protein content was obtained by multiplying the Kjeldahl nitrogen by 5.7 (Tkachuk, 1969). The protein content determination was based on the amount of nitrogen in 3 ml aliquot of supernatants. Total protein content in the extract was then calculated by multiplying with the total supernatant volume.

### 2. Moisture content of the grain and flour

Moisture content of whole grain was determined according to the AACC standard procedure (AACC method 44-11,1983), using an electronic moisture meter (Canadian Aviation Electronics Ltd., model CAE 919). The moisture content of the flour was determined by near infra red (NIR) using the Dicky John NIR product analyzer (Dicky-John Corp., Auburn, IL) calibrated according to the standard AACC method (AACC method 44-15A, 1983), using a Brabender Rapid Moisture Meter (C.W. Brabender Instruments Inc. South Hackensack, NJ).

**3. Ash content of the grain and flour**

Ash content of meal and flour was determined according to the standard AACC method (AACC method 08-01, 1983). The material, 5 g (14 % mb) was incinerated overnight at 560 °C. Wheatmeal was prepared by milling 50 g of sample through a Udy cyclone mill (Udy Corporation, Fort Collins, CO).

**4. Starch damage value**

Starch damage was determined according to the method of Farrand (1964), using 5 g (14 % mb) of flour. Fresh bacterial $\alpha$-amylase from *Bacillus subtilis* (United States Biochemical Corp. Cleveland, Ohio) solution was prepared for each lot of determination.

**5. Falling number value**

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

**6. Particle size index (PSI)**

The PSI was determined on wholemeal using NIR according to the procedure of Williams (1979).

**7. Flour particle size (FPS)**

The FPS was determined according to the procedure of Williams (1979) with minor modifications. A sample (10 g) of well mixed flour was sieved for 5 min on a Rotap sieve shaker, using 200-mesh stainless-steel screens with an aperture of 75 $\mu$m.

The weights of throughs and overs on top of the sieve was determined. The proportion of throughs as a percentage of the total flour sieved was used as the means to compare the particle size between the two mills.

### 8. Extensigraph test

Extensigrams were obtained according to the procedure of Holas and Tipple (1978) using a Brabender extensigraph with minor modifications. The dough was mixed in a GRL mixer for 3.5 min at 130 R.P.M. Fermentation and resting period was done in a baking fermentation cabinet at 30 °C and 86 % relative humidity. Doughs pieces were stretched at 45 and 135 min. Data for the 135 min extensigrams are reported. The area under the curve was measured using a polar compensation planimeter (Sokkiska Ltd. Japan).

### 9. Sedimentation value

Zeleny sedimentation value (Zeleny, 1947) was determined according to the standard AACC procedure (AACC method 56-61A, 1983).

### 10. Baking test

A modified remix baking test (Irvine and McMullen, 1960) was used. Appropriate amount of water, as determined from the farinograph absorption less 2 %, was added to the flour (100g) in a GRL mixer. The dough was then mixed for 3.5 min. After fermentation, each dough was remixed to peak which varied depending on the dough strength. Loaf volume was determined by the rape seed displacement method. Baking strength index (BSI) was determined according to the procedure of Tipples and

Kilborn (1974). BSI reported as a percentage, is the remix loaf volume expressed as a percentage of the loaf volume normally expected for top quality Canadian red spring wheat of the same protein content and calculated as follows:

BSI = (loaf volume x 100)/((flour protein x 70) - 58)

where 70 and 58 are the slope and intercept, respectively as developed from the regression data of Tipples and Kilborn (1974), using 210 samples.

## G. STATISTICAL ANALYSIS

All Statistical analysis was performed on a HP 9000/380 minicomputer using SAS 6.0 statistical analysis software (SAS Institute, 1990). Prediction equations were generated for mixograph parameters (dependent variables) using protein extraction values (independent variables). Stepwise multiple linear regression analysis was used with the maximum $R^2$ improvement option to select the best prediction models. Only results for the best one and two-variable models are presented.

# IV. RESULTS AND DISCUSSION

The results of this study are presented and discussed in three sections. Section A deals with the results of the preliminary study carried out to determine optimum protein extraction conditions. Section B presents and discusses the technological quality characteristics and extracted protein distribution of flours milled on the Buhler and Quad mills. Milling, rheological and baking results for flour from both mills are discussed. The section also deals with the relationship between the technological quality of the flour and protein extraction data for water, 2 % NaCl, 70 % ethanol and 0.05 *M* acetic acid fractions. In the last section (Section C), the mixing characteristics and protein distribution of the 1983 UQN samples are presented and discussed.

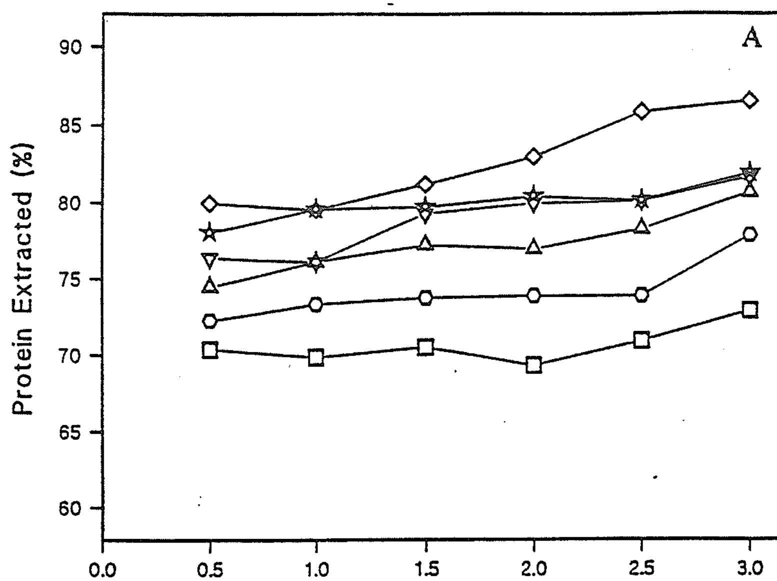
## A. PRELIMINARY STUDY OF PROTEIN EXTRACTION CONDITIONS

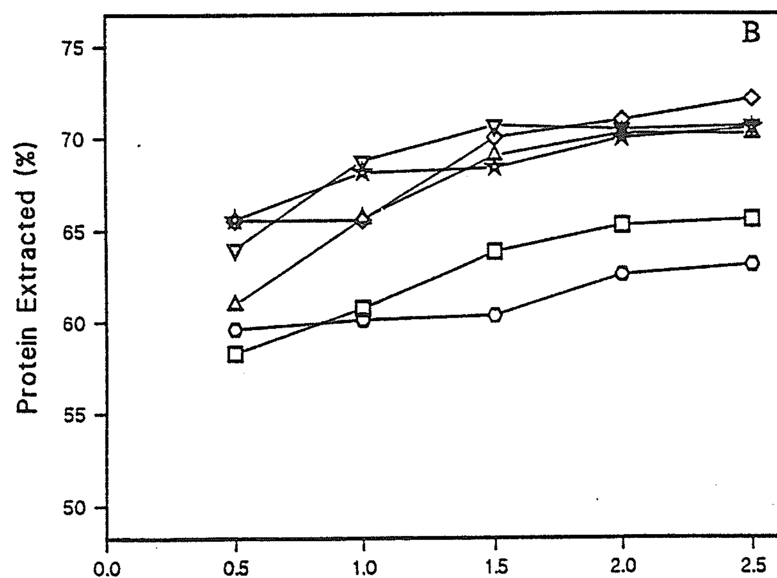
The technological quality data for the samples used in the preliminary study are reported in Table 29, Appendix I (page 169). Duplicate extraction on 1 g of flour was performed on all samples, under the conditions specified.

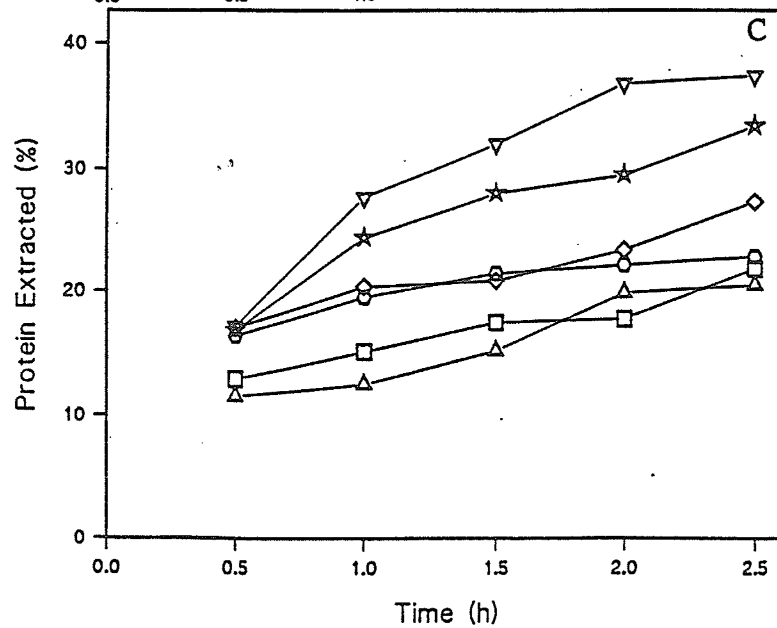
### 1. Rate and amount of protein extracted by the different solvents

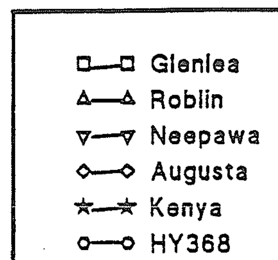
The technological quality data for the samples used in the preliminary study are reported in Table 29, Appendix I (page 171). The rate and amount of protein extracted by 2 % SDS solution, 0.05 *M* acetic acid and deionized distilled water at 0.5, 1.0, 1.5 2.0 and 2.5 and/or 3.0 h at room temperature were examined. Figure 1 illustrates that the amount and rate of extraction were different for the six varieties. The amount of protein extracted by 2 % SDS for each flour at each of the times tested (Fig. 1A), did not correlate (correlation matrix not shown) with flour protein content, but was

FIGURE 1. The rate and amount of protein extracted by 2 % SDS solution (**A**), 0.05 *M* acetic acid (**B**) and distilled deionized water (**C**). Based on duplicate determinations, the mean variation in protein content extracted were 1.4, 0.8 and 0.9 % for 2 % SDS solution, 0.05 *M* acetic acid and water, respectively.


A
Protein Extracted (%)
90
85
80
75
70
65
60
0.0
0.5
1.0
1.5
2.0
2.5
3.0


B
Protein Extracted (%)
75
70
65
60
55
50
0.0
0.5
1.0
1.5
2.0
2.5


C
Protein Extracted (%)
40
30
20
10
0
0.0
0.5
1.0
1.5
2.0
2.5
Time (h)


Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368

significantly correlated ($P < 0.1$) with the grain texture evaluated by the particle size index (PSI) with r-values of 0.80 and 0.73 at 30 min and 2 h extraction times, respectively. For the SDS extractions, the soft wheats (Augusta and Kenya) had more protein extracted than the harder wheats (Glenlea and Roblin); this is consistent with previous findings (He and Hoseney, 1991). It has been suggested (He *et al*, 1991), that the difference in protein solubility between strong and weak flours could be due to the difference in average molecular weight of the proteins. Based on the observed higher relative viscosity of protein solution from a strong flour than that from a weak flour, these workers ascribed the difference to molecular weight being slightly higher in strong flour. Protein solubility could also be affected by differences in protein aggregation. However, the very high correlation between PSI and the amount of protein extracted with SDS is hard to ignore as possibly the key factor in this result. HY368 with a PSI value of 65 should have had a higher amount of protein extracted but it did not. The reason for this discrepancy was not investigated.

The rate and amount of protein extracted by 0.05 *M* acetic acid was not related to the grain texture as shown in Fig. 1B. Both Glenlea, a hard wheat with a protein content of 11.8 % and HY368, a softer variety with a protein content of 12.3 % had low protein extraction compared with the other varieties. The amount of protein extracted by acetic acid at 30 min and 2 h was not correlated to the particle size index (PSI) or protein content.

The rate and amount of protein extracted with distilled water was not related to the kernel texture or the amount of protein in the grain (Fig. 1C). Roblin and Glenlea both hard wheats but of different protein content had the lowest amount of water extractable proteins while Neepawa had the highest amount. HY368 and Augusta had

an intermediate amount of protein extracted with water compared with the other varieties. This is in agreement with results and conclusions of other researchers. For example, Shuey and Gilles (1973) observed that the amount of protein extracted by water was higher for soft wheat compared with hard red spring wheat.

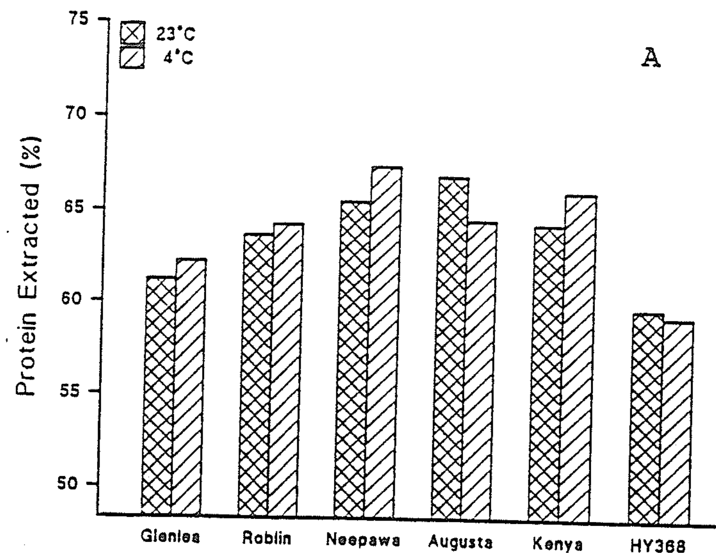
Protein solubility of the variety Glenlea was unique compared with the other five varieties. For all the extracting solutions used, this variety had the lowest amount of protein extracted indicating that the type, proportion or interaction among these proteins may be different compared with the other varieties. This may be a factor or a reflection of the unique mixing strength characteristics of Glenlea flour described in section B. The correlation between the amount of protein extracted at 30 min and 2 h was 0.93, 0.78 and 0.64 for SDS, acetic acid and water, respectively.

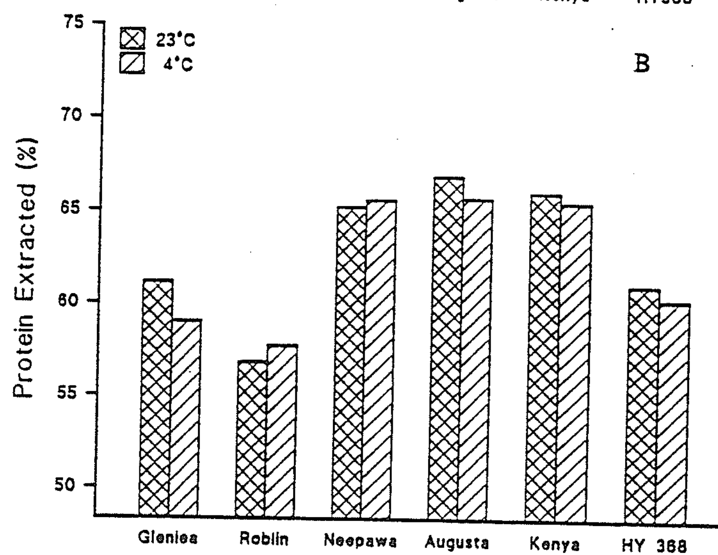
The total amount of water soluble proteins extracted was less than that extracted using either 2 % SDS or 0.05 *M* acetic acid, a reflection of the low relative proportion of albumins and globulins proteins. The results in this study further supports earlier work (Danno *et al*, 1974; He *et al*, 1992; Gao and Bushuk, 1992), as it was observed that the rate and the amount of protein extracted from a wheat flour was affected by the type of extracting solution, grain texture and total flour protein.

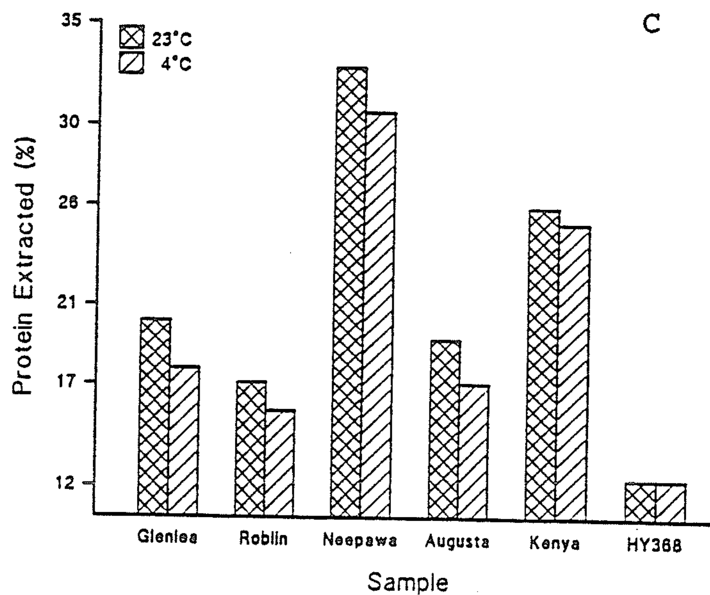
**2. Effect of temperature, flour:solvent ratio, and manual vs mechanical shaking**

Temperature (4 °C vs 23 °C) had a small effect, depending on the variety, on the amount of protein extracted using 2 % SDS, 0.05 *M* acetic acid and distilled water (Fig. 2). For 2 % SDS the difference in protein extractability ranged from 59.7 % (HY368) to 66.9 % (Augusta) at 23 °C and 59.3 % (HY368) to 67.4 % (Neepawa) at 4 °C. With acetic acid it ranged from 56.8 % (Roblin) to 66.9 % (Augusta) at 23 °C

FIGURE 1. The rate and amount of protein extracted by 2 % SDS solution (A), 0.05 *M* acetic acid (B) and distilled deionized water (C). Based on duplicate determinations, the mean variation in protein content extracted were 1.4, 0.8 and 0.9 % for 2 % SDS solution, 0.05 *M* acetic acid and water, respectively.


75
70
65
60
55
50
23°C
4°C
A
Protein Extracted (%)
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368


75
70
65
60
55
50
23°C
4°C
B
Protein Extracted (%)
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY 368

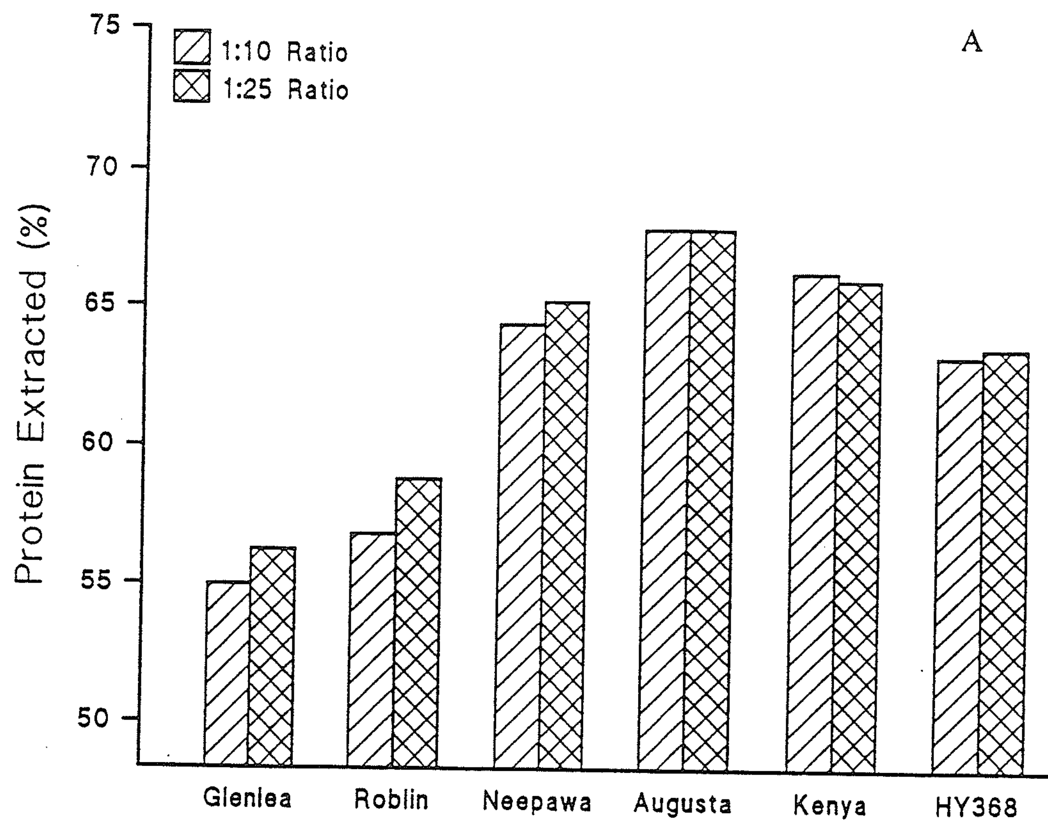

35
30
26
21
17
12
23°C
4°C
C
Protein Extracted (%)
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368
Sample

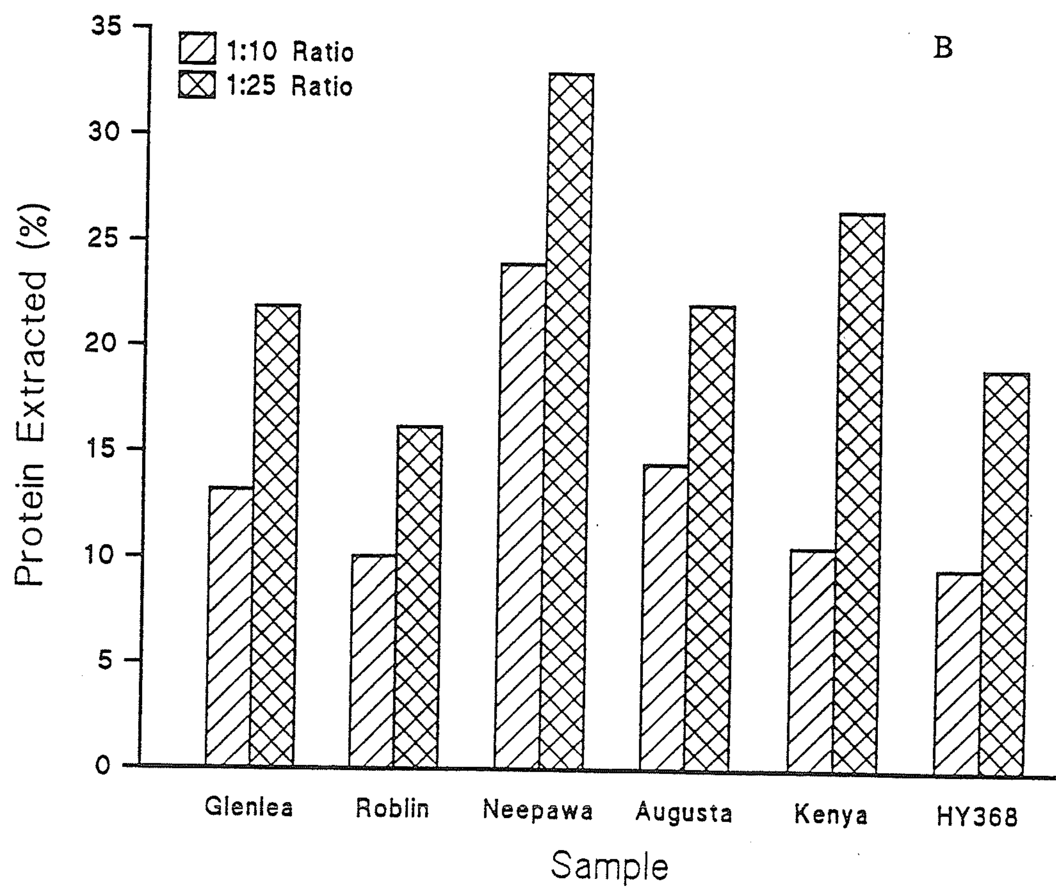
and 57.7 % (Roblin) to 65.7 % (Augusta) at 4 °C. Extractability in water ranged from 12.4 % (HY368) to 32.8 % (Neepawa) at 23 °C and 12.4 % (HY368) to 30.6 % (Neepawa) at 4 °C. In the modified Osborne fractionation procedure, Chen and Bushuk (1970) carried out extractions at 4 °C to minimize enzymic activity. In this study we were mainly concerned with quantitative rather than qualitative (specific protein groups) aspects, and our results indicate that about the same amount of protein was extracted at 4 °C and 23 °C (room temperature).

He *et al* (1991) reported that enzymatic activity did not significantly affect protein solubility particularly during the first 24 h of extraction based on results of SDS-PAGE and measurement of relative viscosity of unheated and heated protein. Because only 2 h was used in these extraction studies, and the grain appear visually sound, the effects of enzymic activity on protein solubility was not significant.

The amount of protein extracted with 0.05 *M* acetic acid and distilled water at flour:solvent ratios of 1:10 and 1:25 are shown in Fig. 3. The amount extracted using acetic acid (Fig. 3A) did not differ very much at the two flour:solvent ratios used. In contrast, the amount of protein extracted with distilled water (Fig. 3B) was very different at the two ratios. Far less protein was extracted at 1:10 than at 1:25 ratio. For example at 1:10 ratio the amount of protein extracted was 13.2, 24.0 and 14.5 % while at 1:25 it was 21.9, 33.0 and 22.1 % for Glenlea, Neepawa and Augusta, respectively. The increase in protein content of the water-soluble proteins with increased dilution could be due to protein partitioning due to varying intrinsic flour salt concentrations as suggested by Shuey and Gilles (1973). Similar observations have been reported by Mattern and Sandstedt (1957), Mullen and Smith (1965) and Hoseney *et al* (1969a). Hoseney *et al* (1969a) attributed the increase in protein

FIGURE 3. The effect of flour:extraction solution ratio (1:10 and 1:25) on protein extraction by 0.05 *M* acetic acid (**A**) and distilled deionized water (**B**). Based on duplicate determinations, mean variation in protein extracted at 1:10 and 1:25 ratio were 0.5 and 0.6, 0.9 and 1.2 % for acetic acid and water extraction, respectively.


1:10 Ratio
1:25 Ratio
A
75
70
65
60
55
50
Protein Extracted (%)
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368


1:10 Ratio
1:25 Ratio
B
35
30
25
20
15
10
5
0
Protein Extracted (%)
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368
Sample

extraction with decrease in flour:water ratio due to other types of extracted proteins, especially gliadin; an increase in relative quantity of solvent decreases flour salt concentration thereby increasing the amount of gliadin proteins extracted (Hoseney et al, 1969a). In a later study, Dreese and Hoseney (1990) observed that a 10:1 (water:flour) washing ratio caused a much larger rheological effect in gluten than did a 3:1 washing ratio. The amount of water soluble proteins removed was 2.9 % and 2.0 % of the total gluten proteins for the 10:1 and 3:1 washing ratios, respectively.

Initially, protein extraction was carried out by manually shaking the centrifuge tubes every 15 min for 2 h. A mechanical shaker was subsequently used. The amount of water and 0.05 *M* acetic acid protein extracted by manual and mechanical shaking is shown in Fig. 4. It can be observed that the amount of protein extracted using a mechanical shaker was slightly larger than by manual shaking but the difference was small, using 0.05 *M* acetic acid except for Glenlea and HY368 (Fig. 4A). Differences between the two procedures were observed in more samples with water extraction (Fig. 4B).

### 3. Protein recovery

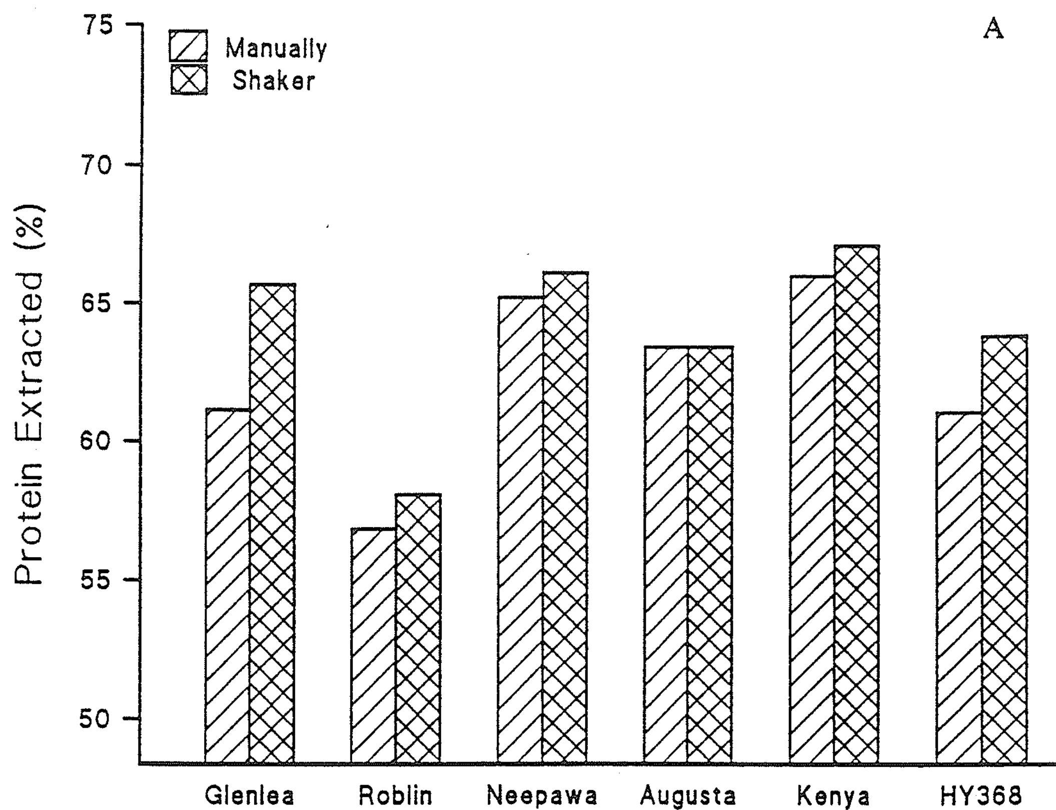
To determine the amount of protein extracted and subsequent percentage of protein recovered, the 26 varieties of 1983 UQN samples were used. If the recovery is precise then by determining the amount of protein in the flour and supernatant only, the amount of protein in the residue can be calculated by difference. This eliminates freeze-drying of the residue and saving of time in sample preparation. The 0.05 *M* acetic acid residue and supernatant extracted from 1 g of flour were freeze dried and the protein contents determined by micro-Kjeldahl procedure. The results are reported

in Table 3.

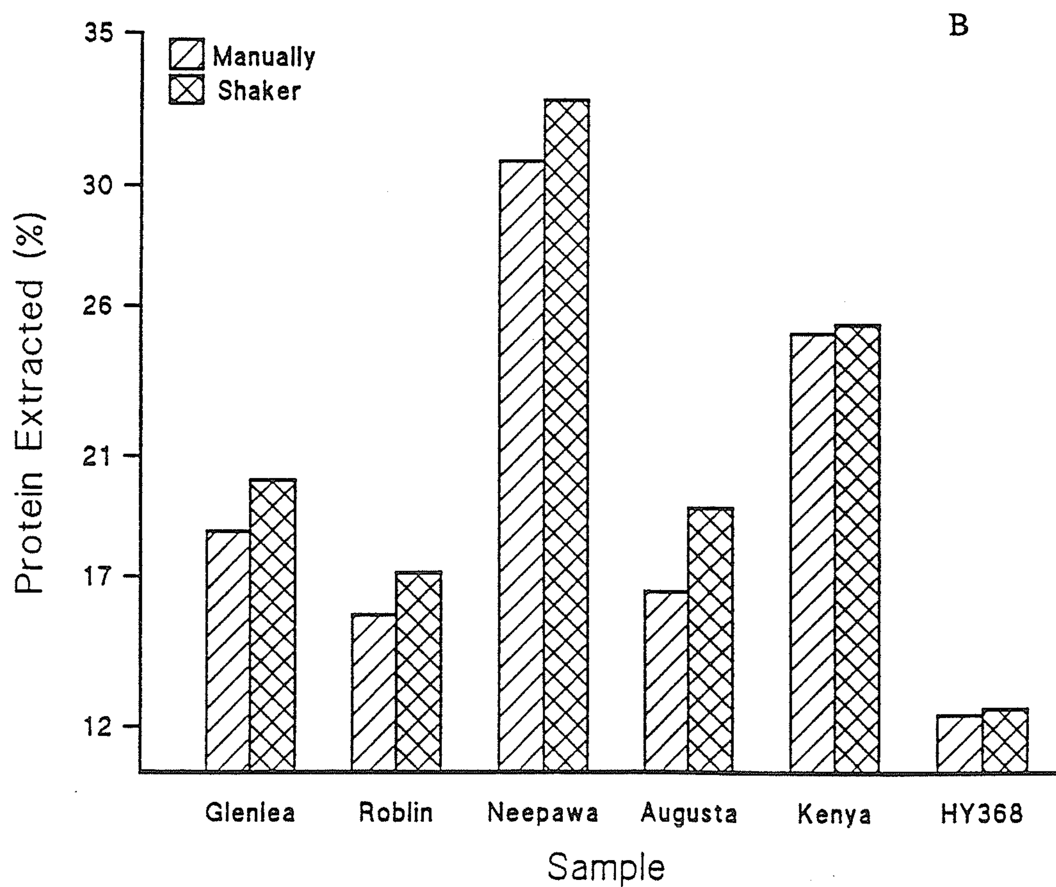
The mean coefficient of variation for the percent supernatant average over all 26 samples was 19.4 % an indication of the variability of the samples used. The recovery by this method ranged from 95.4-102.1 %, with a mean of 99.2 %. This represents very good recovery. The precision of the supernatant determination was excellent (CV < 2.0 %). Subsequent protein determination of the supernatants was done by direct wet determination of the aliquots and the residue determined by difference. The wet method is superior because no residue protein can be lost. In the modified Osborne fractionation procedure lower recoveries have been reported by many researchers. Orth and Bushuk (1972) attributed the low recovery to loss of low molecular weight proteins during dialysis and the cumulative error due to the numerous extraction steps involved in the modified Osborne fraction procedure. Less protein is lost by the direct extraction procedure.

It can be concluded from the preliminary studies that satisfactory and consistent amount of protein can be extracted from 1 g of flour using 25 ml of solvent after 2 h of mechanical shaking at room temperature. Subsequently, all the extractions were performed under these conditions.

FIGURE 4. The effect of manual vs mechanical shaking on the amount of protein extracted by 0.05 *M* acetic acid (A) and distilled deionized water (B). Based on duplicate determinations, the mean variation in amount of protein extracted manually and using a shaker were 0.8 and 0.6 %, 1.3 and 1.2 % for acetic acid and water extraction, respectively.



A
Manually
Shaker
Protein Extracted (%)
75
70
65
60
55
50
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368

B
Manually
Shaker
Protein Extracted (%)
35
30
26
21
17
12
Glenlea
Roblin
Neepawa
Augusta
Kenya
HY368
Sample

TABLE 3. The amount of protein extracted[1] and percentage recovery using 0.05 *M* acetic acid (n=26).

| Sample | Total protein (mg) | Super[2] (mg) | Super (%) | Residue (mg) | Residue (%) | Recovery (%) |
|---|---|---|---|---|---|---|
| UQN 99 | 139.2 | 92.1±0.1 | 66.2±0.1 | 44.5±0.8 | 32.0±0.5 | 97.9 |
| UQN 130 | 144.4 | 99.6±0.7 | 69.0±0.6 | 39.7±0.2 | 27.5±0.1 | 96.5 |
| UQN 176 | 125.6 | 90.1±0.5 | 71.8±0.4 | 36.9±0.9 | 29.4±0.7 | 100.9 |
| UQN 199 | 147.6 | 102.5±1.8 | 69.4±1.2 | 44.5±0.1 | 31.1±0.0 | 100.0 |
| UQN 200 | 137.5 | 94.8±0.9 | 69.0±0.7 | 41.8±0.2 | 30.4±0.1 | 99.4 |
| UQN 201 | 123.4 | 82.8±0.7 | 67.1±0.6 | 39.7±0.1 | 32.2±0.0 | 99.3 |
| UQN 203 | 133.0 | 88.1±1.1 | 66.2±0.8 | 41.9±0.1 | 31.3±0.4 | 97.7 |
| UQN 204 | 134.7 | 92.9±1.1 | 69.0±0.8 | 40.1±0.3 | 30.6±1.7 | 99.1 |
| UQN 205 | 126.3 | 86.3±1.2 | 68.4±1.0 | 39.9±1.0 | 31.0±1.6 | 99.9 |
| UQN 206 | 135.9 | 92.6±1.6 | 68.2±1.2 | 44.2±0.3 | 32.1±0.7 | 100.6 |
| UQN 207 | 139.0 | 91.9±1.5 | 66.2±1.1 | 45.2±0.4 | 33.3±0.8 | 99.2 |
| UQN 208 | 132.7 | 90.8±0.7 | 68.4±0.6 | 42.7±0.1 | 31.8±0.6 | 100.4 |
| UQN 209 | 136.3 | 91.7±1.2 | 67.3±0.9 | 42.8±0.7 | 32.3±0.7 | 99.3 |
| UQN 210 | 129.0 | 82.7±0.1 | 63.9±0.1 | 43.3±1.0 | 33.4±1.0 | 97.6 |
| UQN 211 | 130.4 | 79.9±1.4 | 61.3±1.1 | 44.4±1.4 | 33.6±1.7 | 95.5 |
| UQN 212 | 134.3 | 89.5±2.0 | 66.7±1.5 | 41.3±1.0 | 30.8±0.7 | 97.7 |
| UQN 213 | 124.9 | 83.3±1.2 | 66.7±1.0 | 41.3±0.3 | 31.7±1.2 | 99.2 |
| UQN 214 | 131.1 | 78.6±0.1 | 60.0±0.1 | 46.0±1.2 | 35.1±1.0 | 95.4 |
| UQN 215 | 128.0 | 84.1±0.8 | 65.7±0.6 | 42.7±1.7 | 33.4±1.4 | 99.6 |
| UQN 216 | 118.8 | 82.8±0.1 | 69.7±0.1 | 38.4±0.1 | 32.3±0.1 | 102.1 |
| UQN 217 | 123.8 | 90.3±2.4 | 72.9±1.9 | 32.2±0.4 | 26.0±0.3 | 99.1 |
| UQN 218 | 137.1 | 93.2±2.5 | 68.0±1.8 | 41.0±1.7 | 29.9±1.2 | 98.3 |
| UQN 219 | 124.1 | 96.7±0.6 | 78.0±0.5 | 29.5±0.6 | 23.8±0.5 | 101.9 |
| UQN 220 | 121.7 | 86.7±1.2 | 70.4±1.0 | 35.8±1.2 | 29.5±1.0 | 100.2 |
| UQN 221 | 138.4 | 99.2±0.3 | 71.8±0.2 | 41.3±0.8 | 39.9±0.6 | 101.9 |
| UQN 222 | 148.8 | 98.6±1.8 | 66.2±1.2 | 48.8±0.6 | 32.8±0.5 | 99.2 |
| mean | 132.5 | 86.6 | 68.0 | 41.2 | 31.4 | 99.2 |
| sd[3] | 7.9 | 16.8 | 3.6 | 4.2 | 3.0 | 1.7 |
| cv[4] | 5.7 | 19.4 | 5.2 | 10.1 | 9.4 | 1.7 |

[1]14.0 % moisture basis.
[2]Super = supernatant.
[3]Standard deviation, based on two separate extracts and duplicate Kjeldahl protein analysis on each sample.
[4]Coefficient of variation.

## B. TECHNOLOGICAL QUALITY CHARACTERISTICS AND PROTEIN SOLUBILITY DISTRIBUTION OF FLOUR MILLED ON BUHLER AND QUAD JR. MILLS

The effect of experimental milling (Buhler vs Quad) on the technological quality parameters for breadmaking and solubility fractionation of wheat proteins was examined. The samples were milled once on either mill. It has been shown from experience and literature (refer to section III B:) that there is good reproducibility of milling on both mills under study. The technological quality data for the Buhler laboratory and Quad Jr. mills are given in Tables 4 and 5, respectively. The Student's t-test was used to compare some of the quality parameters between Buhler and Quad milled flours and the results are shown in Table 6. The correlation coefficient between these technological data plus those of the mixograph are reported in Tables 10 and 11.

### 1. Milling and flour characteristics

#### (i) Flour yield (FN) and particle size index (PSI) values

A higher flour yield was observed for the Buhler than for the Quad mill. Flour extraction on the Buhler mill ranged from 68.2 to 75.2 % while for the Quad it ranged from 59.5 to 68.6 %. The milling action of the two mills is different and could account for the difference in flour yield. The results show a normal range of flour yield. The samples varied widely in hardness, from very soft (sample 09) to very hard (sample 10) as shown by their PSI values which ranged from 74 to 48 %. The correlation between FY and PSI was not significant ($r = 0.33$ and 0.44 for Buhler and Quad mills, respectively).

#### (ii) Flour protein and ash contents

Quad milled flour had higher protein and ash contents compared with the Buhler

flour (Tables 4 and 5). The average ash content was 0.39 % and 0.43 % for the Buhler and Quad mills, respectively. These results are consistent with the findings of Bequette *et al* (1960). Both protein and ash contents were significantly different between the two mills for most of the samples (Table 6). It is generally accepted that for a single mill the higher the flour extraction rate the higher the flour protein and ash contents and vice versa (Kent and Evers, 1969; Orth and Mander, 1975). However, the results comparing mills (Tables 4 and 5) show a different pattern of variation as the Quad mill had a lower extraction rate than the Buhler, but gave higher flour protein and ash contents. Interestingly McCluggage *et al* (1939) observed a similar trend, when they compared the Allis-Chalmers and Buhler mills. They reported that Buhler mill gave a lower flour yield but higher protein content and ash content compared with the Allis-Chalmers mill. The cause of this relationship was not investigated further. Hinton (1959) reported that aleurone layer and the bran in wheat had a higher ash content than the endosperm, hyaline layer, scutellum and embryo. This was further supported by Kent and Evers (1969). This suggests that the separation of the endosperm and bran by the two mills used in this study were different.

The results observed in this study may be attributed to the lower milling efficiency of the Quad as compared with that of the Buhler mill. This was investigated using a proposal put forward by Posner (1991) who suggested the use of the flour ash (FA) to wheat ash (WA) ratio as a better indicator of the separation (milling efficiency) between bran and endosperm compared with using percentage flour ash alone; better milling wheat should have FA/WA ratio as low as possible. Figure 5 illustrates the milling efficiency using this approach. The milling efficiency was very different for all the samples in either mill. The hard wheats had a lower efficiency especially with the

TABLE 4. Technological quality data[1] of flours milled on Buhler mill.

| SAMPLE #[2] | FY | GP | PSI | FPS | FP | ASH | FN | SD | SV | FAB | DDT | MTI | EXT | RMAX | R/E | AREA | RT | WO | LV | BSI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S01 | 73.7 | 11.6 | 59 | 40.4 | 10.8 | .38 | 405 | 23 | 51 | 56.8 | 5.5 | 35 | 148 | 440 | 2.97 | 88 | 2.1 | 10.0 | 705 | 101 |
| S02 | 70.3 | 13.7 | 55 | 36.7 | 13.0 | .43 | 509 | 27 | 56 | 63.1 | 6.0 | 20 | 153 | 560 | 3.66 | 118 | 2.6 | 12.5 | 822 | 96 |
| S03 | 68.2 | 11.1 | 74 | 68.1 | 9.9 | .42 | 402 | 14 | 23 | 55.1 | 2.0 | 110 | 143 | 180 | 1.25 | 36 | 0.8 | 4.2 | 477 | 76 |
| S04 | 71.0 | 10.5 | 71 | 67.0 | 9.5 | .44 | 363 | 12 | 25 | 50.8 | 1.5 | 100 | 116 | 220 | 1.90 | 38 | 0.8 | 3.7 | 450 | 74 |
| S05 | 71.2 | 12.9 | 48 | 35.8 | 12.2 | .46 | 443 | 32 | 51 | 60.5 | 27.0 | 0 | 176 | 800 | 4.54 | 133 | 4.0 | 19.0 | 795 | 99 |
| S06 | 75.2 | 11.9 | 57 | 42.3 | 10.6 | .38 | 456 | 30 | 52 | 58.6 | 3.0 | 5 | 150 | 490 | 3.27 | 103 | 2.4 | 11.4 | 720 | 105 |
| S07 | 72.4 | 12.8 | 65 | 47.7 | 11.7 | .35 | 364 | 23 | 70 | 57.5 | 6.0 | 20 | 193 | 470 | 2.41 | 110 | 2.4 | 12.0 | 757 | 99 |
| S08 | 72.3 | 14.5 | 48 | 37.7 | 13.8 | .36 | 599 | 25 | 54 | 64.1 | 6.0 | 20 | 145 | 790 | 5.44 | 170 | 2.7 | 12.2 | 840 | 92 |
| S09 | 70.6 | 9.8 | 71 | 66.7 | 8.8 | .42 | 356 | 9 | 25 | 51.8 | 1.5 | 90 | 102 | 330 | 3.23 | 52 | 0.7 | 2.7 | 430 | 77 |
| S10 | 72.4 | 12.8 | 52 | 37.1 | 11.7 | .41 | 488 | 33 | 58 | 59.7 | 23.0 | 0 | 165 | 1150 | 6.30 | 255 | 4.1 | 18.0 | 782 | 102 |
| S11 | 73.6 | 11.4 | 60 | 45.4 | 10.2 | .40 | 453 | 21 | 48 | 56.2 | 3.5 | 35 | 165 | 780 | 4.73 | 208 | 1.9 | 9.0 | 670 | 102 |
| S12 | 74.8 | 11.5 | 60 | 45.6 | 10.4 | .38 | 399 | 19 | 54 | 56.6 | 6.0 | 20 | 135 | 560 | 4.15 | 101 | 1.9 | 9.0 | 720 | 109 |
| S13 | 72.5 | 12.1 | 56 | 39.0 | 11.1 | .38 | 428 | 26 | 56 | 62.1 | 6.5 | 20 | 160 | 840 | 5.25 | 185 | 2.4 | 11.0 | 777 | 108 |
| S14 | 70.5 | 12.6 | 53 | 39.5 | 11.4 | .38 | 392 | 26 | 62 | 62.4 | 4.0 | 15 | 153 | 600 | 3.92 | 124 | nd[3] | nd | 780 | 105 |
| S15 | 71.8 | 10.3 | 64 | 56.6 | 9.7 | .36 | 642 | 18 | 31 | 58.6 | 3.5 | 30 | 114 | 340 | 2.98 | 51 | 1.4 | 6.0 | 532 | 86 |
| S16 | 71.7 | 16.0 | 56 | 38.2 | 15.5 | .34 | 642 | 16 | 67 | 67.6 | 10.0 | 0 | 180 | 780 | 4.37 | 190 | 2.6 | 13.5 | 947 | 92 |

[1]FY=Flour yield (%); GP=Grain protein (%); PSI=Particle size index (%); FPS=Flour particle size (% flour through 75 µm sieve); FP=Flour protein (%); FN=Falling number (sec); SD=Starch damage (FU); FAB=Farinograph absorption (%); DDT=Dough development time (min); MTI=Mixing tolerance index (BU); Ext=Extensibility (mm); RMAX=Maximum resistance (EU); R/E=Resistance/Extensibility; Area=Area under the curve ($cm^2$); WO=Work input (watt hr/kg); LV=Loaf volume (cc); BSI=Baking strength index (%); PHG=Peak height (Nm); MDT=Mixograph development time (min); ETP=Energy to peak (Nm); PBW=Peak band width (Nm); BWE=Band width energy (Nm). All calculations are based on 14 % m.b.

[2]Sample # corresponds to samples as defined in Table 1.

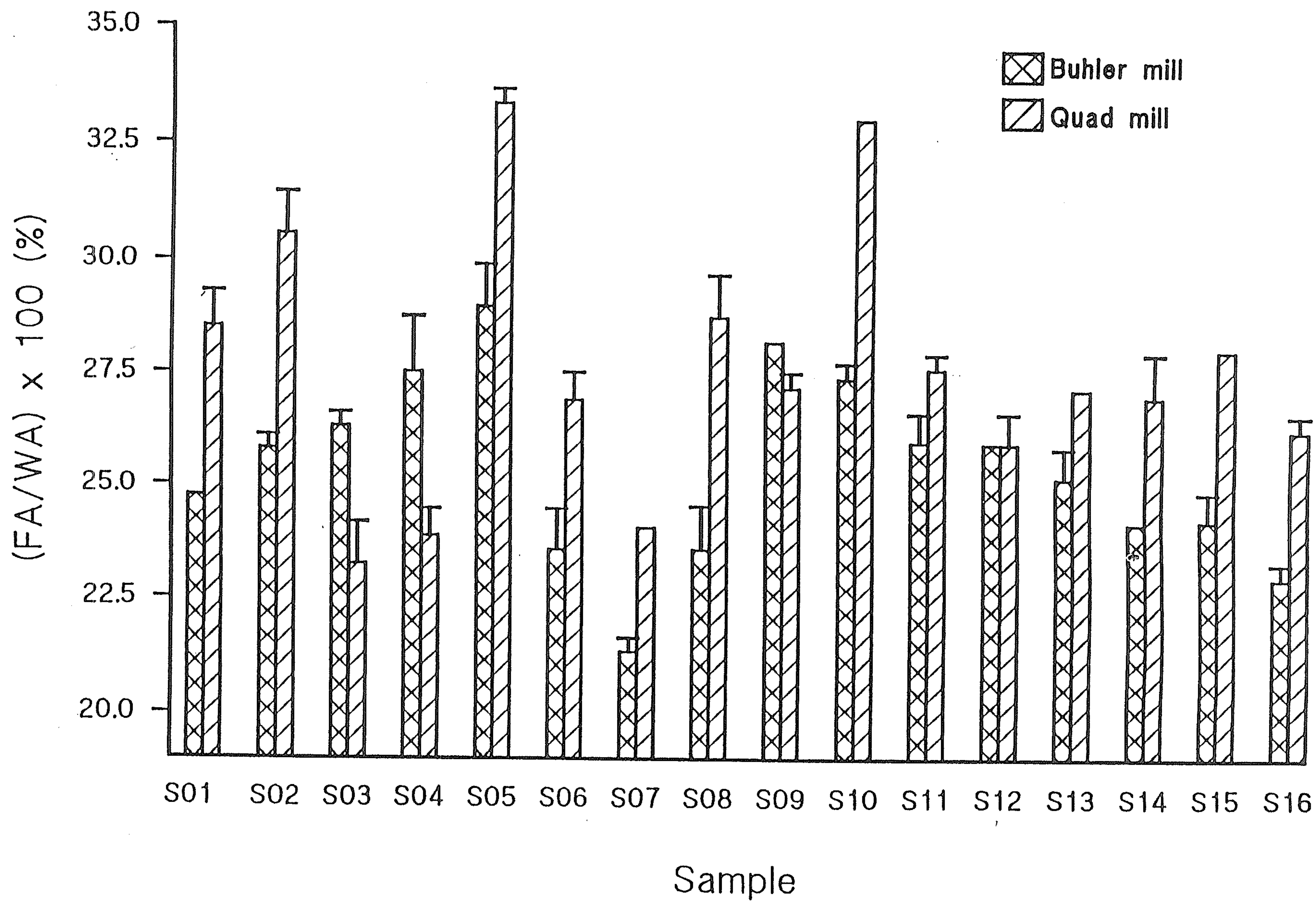
[3]nd Not determined, insufficient samples.

TABLE 5. Technological quality data[1] of flours milled on Quadrumat Jr. mill

| SAMPLE #[2] | FY | GP | PSI | FPS | FP | ASH | FN | SD | SV | FAB | DDT | MTI | EXT | RMAX | R/E | AREA | RT | WO | LV | BSI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S01 | 68.6 | 11.6 | 59 | 28.6 | 11.4 | .43 | 421 | 17 | 36 | 58.0 | 6.5 | 30 | 122 | 520 | 4.26 | 92 | 2.1 | 10.75 | 722 | 97.0 |
| S02 | 63.7 | 13.7 | 55 | 23.6 | 13.4 | .51 | 491 | 25 | 36 | 65.4 | 5.5 | 20 | 144 | 500 | 3.47 | 97 | 2.5 | 12.05 | 867 | 98.1 |
| S03 | 59.5 | 11.1 | 74 | 43.5 | 10.4 | .37 | 401 | 11 | 22 | 56.4 | 1.5 | 100 | 130 | 225 | 1.73 | 42 | 1.2 | 6.00 | 447 | 66.4 |
| S04 | 60.9 | 10.5 | 71 | 44.8 | 10.2 | .39 | 411 | 8 | 22 | 52.4 | 1.5 | 90 | 107 | 250 | 2.34 | 41 | 1.2 | 5.25 | 450 | 68.6 |
| S05 | 62.0 | 12.9 | 48 | 20.2 | 12.7 | .53 | 453 | 28 | 35 | 60.5 | 25.0 | 0 | 148 | 660 | 4.46 | 135 | 3.6 | 18.25 | 857 | 102.7 |
| S06 | 67.5 | 11.9 | 57 | 26.1 | 11.6 | .44 | 464 | 22 | 37 | 59.3 | 2.5 | 5 | 146 | 480 | 3.29 | 100 | 2.5 | 12.00 | 782 | 103.8 |
| S07 | 65.6 | 12.8 | 65 | 31.8 | 12.6 | .40 | 408 | 23 | 54 | 57.9 | 7.0 | 15 | 140 | 575 | 4.11 | 109 | 2.3 | 12.25 | 840 | 101.5 |
| S08 | 63.4 | 14.5 | 48 | 24.2 | 14.3 | .42 | 522 | 24 | 40 | 64.9 | 6.0 | 20 | 150 | 730 | 4.87 | 158 | 2.6 | 11.75 | 885 | 93.5 |
| S09 | 60.2 | 9.8 | 71 | 44.6 | 9.3 | .40 | 390 | 8 | 21 | 52.3 | 1.5 | 90 | 102 | 320 | 3.14 | 59 | 1.0 | 3.75 | 432 | 72.9 |
| S10 | 63.7 | 12.8 | 52 | 20.9 | 12.6 | .50 | 487 | 27 | 37 | 59.1 | 27.0 | 0 | 175 | 1200 | 6.86 | 312 | 4.0 | 21.45 | 817 | 98.8 |
| S11 | 63.6 | 11.4 | 60 | 31.6 | 11.0 | .42 | 385 | 16 | 40 | 56.8 | 4.0 | 35 | 165 | 930 | 5.64 | 210 | 1.8 | 9.00 | 680 | 95.0 |
| S12 | 64.5 | 11.5 | 60 | 30.5 | 11.4 | .38 | 415 | 17 | 43 | 56.9 | 7.5 | 20 | 137 | 580 | 4.23 | 114 | 2.1 | 11.50 | 705 | 95.3 |
| S13 | 64.5 | 12.1 | 56 | 26.6 | 11.8 | .41 | 482 | 20 | 46 | 62.8 | 6.0 | 10 | 163 | 860 | 5.28 | 192 | 2.3 | 11.75 | 817 | 105.9 |
| S14 | 63.9 | 12.6 | 53 | 22.2 | 12.4 | .44 | 405 | 23 | 45 | 62.9 | 6.0 | 20 | nd[3] | nd | nd | nd | 2.8 | nd | 875 | 107.5 |
| S15 | 63.0 | 10.3 | 64 | 36.1 | 10.1 | .39 | 422 | 14 | 25 | 56.9 | 3.5 | 35 | nd | nd | nd | nd | 1.9 | 7.25 | 470 | 72.4 |
| S16 | 64.4 | 16.0 | 56 | 26.8 | 15.9 | .41 | 602 | 16 | 50 | 69.0 | 10.0 | 0 | 220 | 840 | 3.82 | 264 | 2.5 | 13.75 | 790 | 74.8 |

[1]Abbreviations as defined in Table 10. All calculation are based on 14 % m.b.
GP and PSI were determined from wheatmeal.
[2]Sample # corresponds to sample as defined in Table 1.
[3]nd Not determined, insufficient samples.

FIGURE 5. The relationship between flour ash to wheat ash ratio and Buhler vs Quad milled flour.



35.0
32.5
30.0
27.5
25.0
22.5
20.0
(FA/WA) x 100 (%)
Buhler mill
Quad mill
S01
S02
S03
S04
S05
S06
S07
S08
S09
S10
S11
S12
S13
S14
S15
S16
Sample

TABLE 6. Comparison of some technological data[1] of flours from Buhler and Quad Jr. mills using Student's t-test.

| No.[2] | FP | FPS | ASH | SD | SV | LV | BSI | EXT | RMAX | R/E | AREA |
|---|---|---|---|---|---|---|---|---|---|---|---|
| S01 | * | *** | ns | ** | *** | ns | * | * | * | * | ns |
| S02 | ** | *** | ** | ns | *** | * | ns | ns | * | ns | * |
| S03 | ** | *** | * | * | ns | ns | * | * | * | * | ns |
| S04 | ** | *** | ** | * | ** | ns | ns | ns | ns | ns | ns |
| S05 | ns | *** | * | ** | *** | ** | ns | ** | * | ns | ns |
| S06 | ** | *** | * | ns | *** | ** | ns | ns | ns | ns | ns |
| S07 | ** | *** | ** | ns | *** | ** | ns | *** | ** | ** | ns |
| S08 | ** | *** | ns | ns | *** | ns | ns | ns | ns | * | ns |
| S09 | ns | *** | * | * | ** | ns | ns | ns | * | ns | ns |
| S10 | ** | *** | ** | * | *** | * | * | ns | ns | * | *** |
| S11 | ns | *** | ns | ns | * | ns | * | ns | * | * | ns |
| S12 | ** | *** | ns | ns | ** | ns | ** | ns | ns | ns | ns |
| S13 | ** | *** | * | ** | * | ns | ns | ns | ns | ns | ns |
| S14 | ** | *** | *** | *** | * | *** | nd[5] | nd | nd | nd | nd |
| S15 | ns | *** | ns | ns | ** | * | ** | ** | nd | nd | nd |
| S16 | ns | *** | * | *** | *** | *** | *** | *** | * | * | *** |

[1]FP=flour protein (%), FPS=Flour particle size (% flour through 75 μm sieve), ASH=flour ash (%), SD=starch damage (FU), SV=sedimentation value (cc), RT=remix time (min), LV=loaf volume (cc), BSI=baking strength index (%), EXT=extensibility (mm), RMAX=maximum resistance (EU), R/E=resistance/extensibility, AREA=area under the, extensigraph curve ($cm^2$).
[2]Sample No. corresponds to samples as defined in Table 1.
[3]*, **, *** Significantly different at P< 0.1, 0.05, and 0.01 level, respectively.
[4]ns-Not significant.
[5]nd-Not determined, insufficient samples.

Quad mill as indicated by the results for samples S01, S02, S05, S08 and S10. It was observed also that the Buhler mill had a better milling efficiency than Quad Jr. mill, except for the very soft wheats - S03, S04 and S09. The low milling efficiency for soft wheats milled on Buhler mill may be attributed to the fact that the mill was not optimized for soft wheat because it is routinely used for milling hard wheats. The results of the present study are consistent with Posner's (1991) suggestion, that FA/WA ratio gives a better indication of milling efficiency than FA alone. FA/WA ratio was significantly correlated with PSI and SD ($r = -0.73^{**}$ and $0.54^{*}$, respectively) for Quad milled flour but did not show any significant correlations with PSI and SD ($r = 0.13$ and $0.06$, respectively) for the Buhler milled flour (Tables 10 and 11).

**(iii) Falling number (FN) value**

Buhler milled flours generally had higher FN values than the Quad flour samples. The lower FN values for the Quad milled flour could be a reflection of poorer separation of the embryo and aleurone tissues from the endosperm compared with the Buhler milled flour. Alpha-amylase is usually abundant in the embryo and the aleurone layer close to the embryo (Liu *et al*, 1986). However, the FN values obtained for the flours from both mills showed normal variability and were within acceptable limits. Grain FN values ranged from 309-485 sec (data not shown) indicating that the grain samples were sound and had not suffered any significant pre-harvest sprouting.

**(iv) Starch damage (SD)**

A higher starch damage was observed for the Buhler flour than the Quad flour. The starch damage for the Buhler milled flour ranged from 9-33 Farrand Units (FU),

while for the Quad ranged from 7.5-27.0 FU. The difference in SD between the two mills was significant for a number of samples, especially the hard kernel varieties (Table 6). However, Dexter *et al* (1990) reported that starch damage was not significantly influenced by either extraction rate or the amount of farina removed. The difference observed here could be attributed to the type (size and shape of corrugations) and number of break and reduction rolls in the Buhler mill which is greater than in the Quad mill. Differential speed of the rolls could also contribute to the differences in SD. All the flours showed a high and significant correlation, between SD and kernel hardness (Table 10 and 11 for Buhler and Quad mills, respectively). PSI ranged from 48 % (S05 and S08) to 74 % (S03). In general the results indicate that the harder the wheat kernel the higher the SD. This is consistent with previous findings (Jones, 1940; Evers and Stevens, 1985; Bass, 1988). It has been suggested (Mok and Dick, 1990) that the stronger interaction between starch granules and endosperm protein in hard wheat may contribute to a higher degree of starch damage compared with soft wheats. The only unusual SD result was found for the Roblin (S16) sample. This cultivar is known to be a hard wheat; its kernel hardness was normal (PSI) However, both Buhler and Quad flours of this variety showed a much lower SD than previously reported (Anonymous, 1986). The reason for this inconsistency between SD and PSI is unknown. Other quality parameters for this sample were in the normal range.

Starch damage has been implicated as one of the factors that influences water absorption during dough mixing (Evers and Stevens, 1985). However, the difference between mills in terms of farinograph absorption cannot be accounted for by the differences in SD; Quad milled flours had lower SD values than the Buhler milled flours

but gave slightly higher water absorptions. The difference in farinograph absorption is probably due to the differences in flour protein content of flours from the two mills. De la Roche and Fowler (1975) showed that there was a significant effect of milling X cultivar interaction on flour yield, ash content, amylograph peak viscosity, farinograph departure time, farinograph absorption, valorimeter value and mixograph development time. It was suggested that increased SD along with an increase in protein content at the higher milling settings are probably the major factors responsible for the differences. Moreover, Evers and Stevens (1985) showed that the higher the SD the higher the water absorption. The difference in farinograph absorption observed in the present study was most likely due to the small but significant difference in protein content of the flours from the two mills as FAB was highly correlated with FP (Tables 10 and 11).

**(v) Sedimentation value**

Sedimentation value (SV), developed by Zeleny (1947), is widely used to estimate flour strength. The Buhler flours had a SV range of 23-70 $cm^3$, while Quad flour had SV's ranging from 21-54 $cm^3$. For all samples, Buhler milled flours had higher SV's than Quad milled flours. The difference in SV between the two mills was highly significant for almost all the samples (Table 6). Generally sedimentation values range 10-20 $cm^3$ for weak wheats and 70 $cm^3$ or more for very strong wheats (Ng, 1987).

The samples used in this study covered a wide range of SV an indication of diverse dough strength. The results showed that SV depends not only on protein content and protein quality but also on the type of mill used to grind the grain. Ng (1987) using Buhler milled 1983 UQN samples did not find any correlation between SV

and flour protein (FP) content supporting the findings of Orth *et al* (1972). While Fowler and De La Roche (1975) observed a significant correlation between SV and FP. For the samples used in this study there was a highly significant correlation ($P < 0.01$) between SV and FP for flours for each set of samples; $r = 0.71$ for Buhler flours and 0.70 for Quad flours. Correlation between SV and loaf volume (LV) was also highly significant; $r = 0.92$ and 0.81 for Buhler and Quad milled flours, respectively. Ng (1987) found a highly significant correlation ($P < 0.01$) between SV and LV ($r = 0.72$) using the remix baking procedure. However, recently Preston *et al* (1992) using a "world wheat collection" of 33 hard-textured cultivars demonstrated that SDS-sedimentation value was generally a poor predictor of dough strength and baking quality using the remix to peak procedure (among others).

Fullington *et al* (1987) observed that the electrophoretic patterns of the residue protein after SDS extract were identical to those obtained from SDS- and Zeleny sedimentation tests and suggested that these two sedimentation tests could be providing a measure of the proportion of HMW glutenin subunits present in the flour. From the results observed in this study and related work cited herein, it appears that the SV value is only good for predicting dough strength in those wheat varieties that have a wide range of protein content and dough strength. The type of mill used to grind the grain also has a large effect on the SV values. The correlation between SV values and LV may also be influenced by the baking procedure used.

**2. Rheological properties**

The farinograph and extensigraph were used to evaluate the rheological properties of the doughs obtained from the flours used in this study. Farinograms

representing the 16 samples used in this study are shown in Figs. 20-23 in Appendix II (pages 173-179). The samples had a broad range of dough mixing characteristics (see Tables 4 and 5).

Farinograph absorption (FAB) is the amount of water added to the flour to give a dough with a maximum consistency of 500 Brabender Units (BU). The samples showed a wide range of FAB. Buhler milled flours had slightly lower absorptions (51.8-67.6 %) than the Quad milled flours (52.4-69.0 %) for most of the samples. This difference can be attributed to the small differences in protein content between the two mills as discussed above. FAB was highly significantly correlated ($P<0.01$) with FP and LV ($r=0.89$ and 0.88, respectively) for Buhler milled flours (Table 10). Similar high correlations were obtained for the Quad milled flours (Table 11).

Dough development time (DDT) is the time from the addition of water until the dough piece reach maximum consistency. Generally the longer the DDT the stronger the dough. The samples showed a wide range of DDT, 1.5-27.0 min for both types of mills. The DDT values derived for Buhler milled flour were comparable to those of Quad milled flours. There was no significant differences between the DDT of the two types of flours. DDT was significantly correlated ($P<0.01$) with SD ($r=0.66$), RMAX ($r=0.70$), RT ($r=0.75$) and WO ($r=0.84$) for the Buhler milled samples (Table 10). Similar correlations between DDT and the above parameters were also observed for Quad milled samples.

Mixing tolerance index (MTI) is the difference in BU between the top of the curve at maximum consistency and the top of the curve 5 min after the peak. MTI indicates the ability of the dough to withstand overmixing. Flour samples with a high MTI must be mixed over a precise time, while those with a low MTI can withstand

some overmixing without much adverse effect on the dough. The samples used in this study showed a wide range of MTI. The values ranged from 0-110 BU for Buhler and 0-100 BU for Quad milled samples, indicating very strong to very weak doughs. There was a significant negative correlation ($P<0.01$) between MTI with most of the quality parameters for both types of mills (Tables 10 and 11).

The extensigraph test is used to measure the resistance to extension and the extensibility of a specially shaped dough piece. The maximum resistance (RMAX) in BU is the height of the curve at its highest point, while extensibility (EXT) is the length of the curve in millimetres 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 and is a measure of dough strength. The extensigrams for the samples used in this study are shown in Figs. 24-27, Appendix III (pages 182-188). Like the farinograms, the extensigrams reflect samples with a broad range of rheological properties. There were some minor differences in the extensigram parameters between the Buhler and Quad milled flours as summarized in Tables 4 and 5, however, these differences were not consistent (Table 6). Except for sample S07 that showed significant differences ($P<0.05$) between the two mills in all the three extensigraph parameters. While samples S05, S07, S15 and S16 were significantly different in EXT. The differences seem to be related to the inherent quality of the flours rather than the mills.

An optimal balance in the ratio of RMAX/EXT is considered important for proper dough development and good loaf volume. Doughs of strong wheat varieties have a high RMAX/EXT ratio and doughs of weak varieties have a low value (Shuey, 1975). The samples used in this study had a wide range of RMAX/EXT derived from both

mills, but the differences were not significant at the 5 % probability level except for S07. Results of both mills showed moderate correlation between RMAX/EXT ratio and mixing strength (TEG), tolerance (MTI) and LV.

### 3. Mixograph quality characteristics

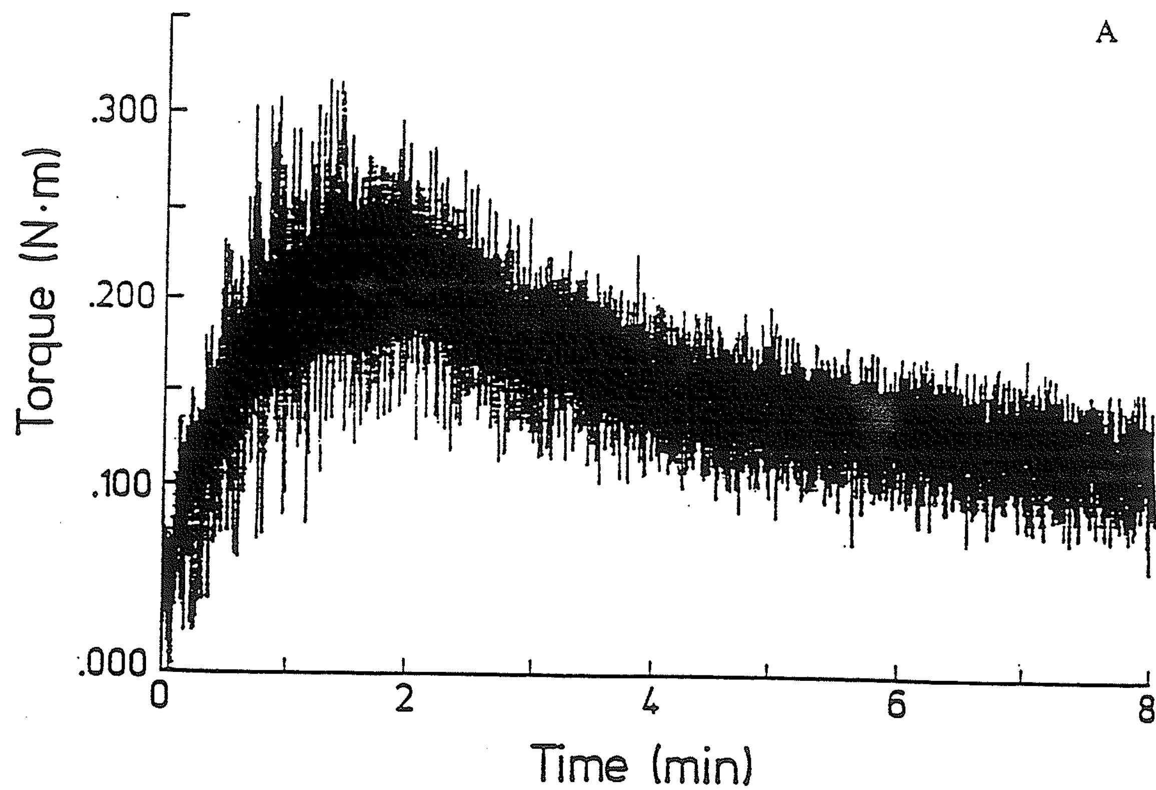
The rheological properties of the flour from the two mills were assessed using the mixograph (Tables 7 and 8). The mixograph is commonly used to evaluate mixing quality characteristic of flour especially in research and in screening of early generation material in wheat breeding programmes. Mixing quality parameters were electronically determined from the mixograph curve as illustrated in Fig. 6. The electronic computerized recording mixograph gives a more extensive evaluation of the flours than the farinograph or extensigraph because it required a smaller sample, shorter testing time and easier to operate. A large number of parameters were also quantified. The mixograph was modified to allow electronic acquisition, recording, analysis, and storage of data (Pon *et al*, 1989).

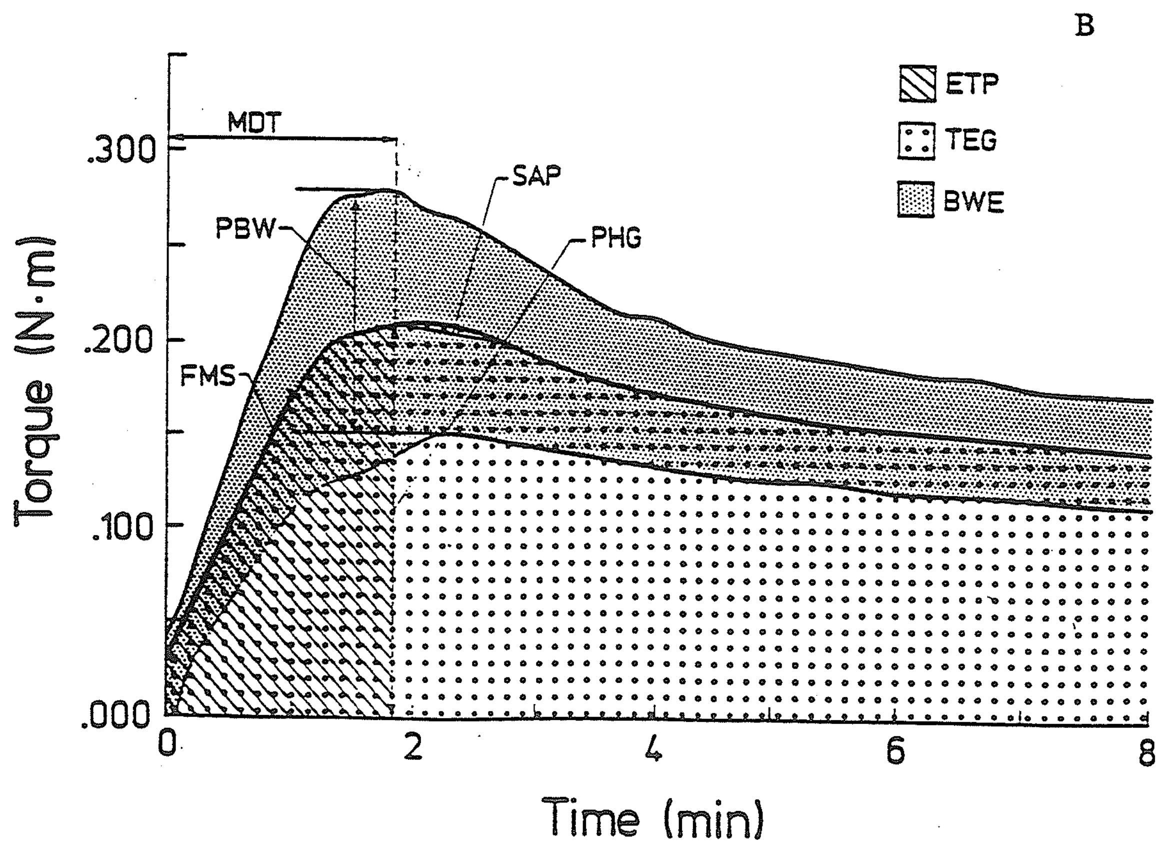
Mixograph peak height (PHG) represents the energy required at maximum resistance during dough mixing. Generally the stronger the dough the higher the PHG, although it was subsequently found to be highly correlated with flour protein (see below) along with TEG and PBW. Our samples had a PHG of 0.09-0.27 Nm for the Buhler milled flours and 0.11-0.28 Nm for the Quad milled flours. This is a further indication that samples possessed diverse mixing strength.

Mixograph development time (MDT) is the time required to mix the dough to optimum consistency or minimum mobility, the inference is that this is the point to which a dough should be mixed to produce a good loaf of bread (Hoseney, 1985).

FIGURE 6. An illustration of an electronically determined mixograph. **(a)** Typical mixing torque verses time curve **(b)** filtered maximum, mean and minimum curves estimated from the raw torque verses time curve.

PHG = Peak height of mean curve (Nm);
MDT = Mixing development time (min);
ETP = Energy to peak (Nm);
FMS = First minute slope (Nm/min);
PBW = Peak bandwidth (Nm);
SAP = Slope after peak (Nm/min);
TEG = Total energy under the mean curve (Nm);
BWE = Bandwidth energy (Nm).


A
Torque (N·m)
.300
.200
.100
.000
0
2
4
6
8
Time (min)


B
ETP
TEG
BWE
MDT
SAP
PBW
PHG
FMS
Torque (N·m)
.300
.200
.100
.000
0
2
4
6
8
Time (min)

While energy to peak (ETP) is the amount of energy required to reach this stage. Both parameters (MDT and ETP) are commonly used as indices of dough strength. Generally the stronger the dough the longer the MDT and the higher the ETP. Buhler milled flours had MDT values of 0.90-5.6 min and ETP values of 5.44-49.32 Nm while the Quad milled flours had MDT values of 1.1-5.2 min and ETP values of 7.22-46.11 Nm. The Quad flours had generally higher values than the Buhler flour for these two parameters. Finney and Shogren (1972) observed that the optimum mixing tolerance (i.e point of minimum mobility or maximum plasticity) is achieved when mixing requirements approaches four minutes. Therefore selecting new varieties with MDT greater than 4 min would give increased tolerance but in many instances increased bakery production costs. Most of the samples used in this study had MDT's less than 4 min except samples S05 and S10 both being the variety Glenlea. This variety is well known in Canada as an overly strong mixing variety (Bushuk, 1980).

The area under the curve provides an estimate of the maximum shear stress in the dough during mixing (Voisey *et al*, 1966c). This is represented as the total energy under the curve (TEG) in Tables 7 and 8. It can be observed that TEG varied widely among the samples used.

Band width energy (BWE) indicates the energy between the maximum and minimum along the curve. The width of the development curve at each stage of the mixing indicates the variation in shear stress in the dough during mixing. BWE could therefore be a major consideration than individual torque variations in assessing dough strength during mixing (Voisey *et al*, 1966a). There was a wide variation in the BWE for the samples used in this study (Tables 7 and 8).

**4. The interrelationship between the mixograph parameters**

The coefficient of variation (CV) expresses the standard deviation as a percent of the mean. In general, it allows direct comparisons of the variability of measurements that differ in scale or units measured e.g mixograph parameters. By calculating the CV of any parameter the degree of variation among all sample means was determined ($CV_1$). All the mixograph parameters (Tables 7 and 8) had high $CV_1$ values, i.e above 25 %. The high $CV_1$ is a further indication of the wide diversity of samples used in this study.

For each mixograph parameter, by calculating the overall mean and average standard deviation of the means, a different coefficient of variation ($CV_2$) was calculated, which is a relative measure of the analytical precision of the particular parameter; the higher the $CV_2$, the higher the standard deviation among duplicate or triplicate measurements of same sample and therefore the lower the reliability of the measurement of that particular parameter. The variation between and within the samples can be evaluated by calculating the ratio of $CV_1$:$CV_2$. This ratio is an overall indicator of the utility of the mixograph parameters as it incorporates both discrimination ability and precision. The parameters with the three lowest ratios for both mills were FMS, PBW and SAP, showing low reliability in the measurement of these three parameters.

These results are complementary to the findings of Pon *et al* (1989). By comparing the regression coefficient of manual vs computer generated parameters, these workers observed that the slopes of the calculated parameters were not significantly different from unity except for the computer generated PBW. They attributed the variation in PBW to the higher frequency response of the computer data

TABLE 7. Mixograph quality data[1] of Buhler milled flours.

| NO[2]. | PHG | MDT | ETP | FMS | PBW | SAP | TEG | BWE |
|---|---|---|---|---|---|---|---|---|
| S01 | 0.16±.01 | 2.9±.1 | 22.5±1.0 | 0.09±.01 | 0.09±.01 | 0.01±.005 | 61.3±3.2 | 16.1±0.9 |
| S02 | 0.20±.01 | 2.0±.2 | 17.2±1.0 | 0.13±.00 | 0.13±.01 | 0.01±.001 | 74.8±2.4 | 13.7±0.3 |
| S03 | 0.12±.00 | 0.9±.2 | 5.4±1.4 | 0.03±.04 | 0.09±.02 | 0.02±.003 | 40.0±2.6 | 4.6±0.9 |
| S04 | 0.09±.01 | 1.7±.2 | 8.3±2.1 | 0.04±.01 | 0.06±.00 | 0.01±.004 | 35.4±2.5 | 6.7±1.0 |
| S05 | 0.19±.00 | 5.2±.1 | 47.6±1.5 | 0.11±.00 | 0.12±.00 | 0.01±.003 | 77.9±1.3 | 38.3±0.7 |
| S06 | 0.14±.00 | 5.1±.7 | 36.7±7.1 | 0.07±.01 | 0.09±.00 | 0.00±.003 | 58.8±3.3 | 29.1±3.7 |
| S07 | 0.15±.00 | 3.7±.4 | 28.6±4.6 | 0.08±.01 | 0.09±.01 | 0.01±.003 | 63.9±2.5 | 20.6±2.5 |
| S08 | 0.20±.01 | 1.8±.2 | 15.6±2.1 | 0.14±.02 | 0.14±.01 | 0.01±.001 | 77.8±1.0 | 11.9±1.7 |
| S09 | 0.09±.00 | 2.0±.4 | 9.6±2.1 | 0.04±.01 | 0.06±.01 | 0.00±.001 | 36.8±0.1 | 7.6±0.7 |
| S10 | 0.18±.01 | 5.6±.4 | 49.3±1.8 | 0.08±.01 | 0.12±.00 | 0.01±.002 | 74.2±2.9 | 39.0±0.4 |
| S11 | 0.16±.01 | 2.1±.1 | 14.9±0.0 | 0.08±.01 | 0.09±.01 | 0.01±.004 | 59.2±2.0 | 10.9±0.5 |
| S12 | 0.15±.00 | 2.8±.5 | 20.9±3.9 | 0.05±.01 | 0.10±.01 | 0.01±.004 | 60.7±1.3 | 15.9±1.9 |
| S13 | 0.17±.00 | 2.8±.4 | 23.5±4.6 | 0.09±.01 | 0.11±.00 | 0.01±.001 | 69.5±2.0 | 18.2±3.2 |
| S14 | 0.16±.00 | 3.5±.2 | 27.8±2.2 | 0.11±.02 | 0.12±.01 | 0.01±.001 | 68.9±0.0 | 23.2±1.7 |
| S15 | 0.13±.01 | 2.3±.1 | 14.3±0.1 | 0.07±.01 | 0.08±.01 | 0.01±.001 | 48.6±.80 | 11.4±0.4 |
| S16 | 0.27±.01 | 2.1±.1 | 24.7±1.5 | 0.07±.01 | 0.16±.02 | 0.03±.003 | 95.9±6.2 | 18.6±0.8 |
| Mean | 0.16±.01 | 2.9±.3 | 22.9±2.3 | 0.08±.01 | 0.10±.01 | 0.01±.002 | 62.8±2.2 | 17.9±1.3 |
| SD[3] | 0.04±.00 | 1.4±.2 | 12.9±1.9 | 0.04±.01 | 0.03±.01 | .007±.001 | 16.5±1.5 | 10.3±1.0 |
| $CV_1$[4] | 25.00 | 47.42 | 56.11 | 44.66 | 30.00 | 70.00 | 26.29 | 57.53 |
| $CV_2$[5] | 3.12 | 8.93 | 10.05 | 12.50 | 9.90 | 20.00 | 3.49 | 7.40 |
| $CV_1/CV_2$ | 8.01 | 5.31 | 5.58 | 3.57 | 3.03 | 3.5 | 7.53 | 7.77 |

[1]PHG= Peak height (Nm), MDT= Mixograph development time (min), ETP= Energy to peak (Nm), FMS= First minute slope (Nm/min), PBW= Peak band width (Nm), SAP= Slope after peak (Nm/min), TEG= Total energy under the curve (Nm), BWE= Band width energy (Nm).
[2]NO. correspond to the sample # as defined in Table 1.
[3]SD=Standard deviation
[4]$CV_1$=(SD/mean) x 100; an index of discrimination ability (see text).
[5]$CV_2$=(SD of the mean/mean) x 100; an index of repeatability (see text).

TABLE 8. Mixograph quality data[1] of Quadrumat Jr. milled flours.

| SAMPLE #[2] | PHG | MDT | ETP | FMS | PBW | SAP | TEG | BWE |
|---|---|---|---|---|---|---|---|---|
| S01 | 0.16±.01 | 2.4±.3 | 17.1±1.3 | 0.10±.02 | 0.11±.01 | 0.01±.003 | 60.8±3.6 | 13.8±0.2 |
| S02 | 0.21±.01 | 2.1±.1 | 18.2±1.3 | 0.13±.00 | 0.14±.00 | 0.02±.003 | 78.0±3.3 | 14.4±0.8 |
| S03 | 0.14±.02 | 1.1±.1 | 7.2±0.1 | 0.06±.02 | 0.09±.03 | 0.02±.004 | 44.0±4.5 | 5.8±0.1 |
| S04 | 0.11±.01 | 2.2±.4 | 11.8±0.9 | 0.05±.00 | 0.07±.00 | 0.01±.001 | 41.5±3.1 | 8.9±1.3 |
| S05 | 0.19±.00 | 5.2±.0 | 46.1±0.1 | 0.10±.01 | 0.12±.00 | 0.01±.005 | 75.9±0.7 | 36.8±1.2 |
| S06 | 0.16±.01 | 4.9±.4 | 37.0±4.3 | 0.10±.01 | 0.10±.01 | 0.00±.002 | 61.6±2.4 | 27.8±1.8 |
| S07 | 0.16±.01 | 3.5±.2 | 26.9±2.8 | 0.09±.00 | 0.10±.00 | 0.01±.002 | 63.9±2.3 | 19.5±0.8 |
| S08 | 0.21±.00 | 1.8±.1 | 15.4±1.2 | 0.13±.00 | 0.13±.00 | 0.01±.001 | 78.2±0.3 | 11.4±1.0 |
| S09 | 0.10±.00 | 2.9±.1 | 14.5±0.6 | 0.04±.00 | 0.06±.00 | 0.01±.001 | 38.5±2.3 | 10.9±0.8 |
| S10 | 0.19±.00 | 4.8±.1 | 40.3±0.3 | 0.11±.00 | 0.13±.01 | 0.01±.001 | 74.6±0.1 | 31.9±0.5 |
| S11 | 0.17±.01 | 2.2±.0 | 16.7±1.0 | 0.09±.01 | 0.11±.01 | 0.01±.001 | 61.8±1.4 | 12.2±0.4 |
| S12 | 0.16±.00 | 3.1±.1 | 22.4±1.8 | 0.08±.00 | 0.10±.00 | 0.00±.001 | 63.7±1.1 | 16.0±0.9 |
| S13 | 0.20±.00 | 2.4±.1 | 22.2±0.2 | 0.13±.01 | 0.15±.01 | 0.01±.003 | 81.1±0.3 | 17.2±0.5 |
| S14 | 0.19±.01 | 3.6±.1 | 31.5±1.2 | 0.12±.01 | 0.13±.01 | 0.01±.001 | 75.8±1.8 | 24.4±2.4 |
| S15 | 0.12±.00 | 2.6±.5 | 16.7±2.6 | 0.07±.01 | 0.07±.01 | 0.01±.003 | 50.5±1.7 | 12.9±2.3 |
| S16 | 0.28±.00 | 2.2±.1 | 25.6±0.5 | 0.16±.00 | 0.16±.00 | 0.03±.001 | 99.5±1.2 | 18.2±0.3 |
| Mean | 0.17±.01 | 2.9±.2 | 23.1±1.2 | 0.10±.01 | 0.11±.01 | 0.01±.002 | 65.6±1.9 | 17.6±1.0 |
| SD[3] | 0.04±.01 | 1.2±.2 | 10.9±1.4 | 0.03±.01 | 0.03±.01 | .007±.001 | 16.5±1.3 | 8.6±0.7 |
| $CV_1$[3] | 23.53 | 39.80 | 46.95 | 30.00 | 27.27 | 70.00 | 25.08 | 48.61 |
| $CV_2$[3] | 2.94 | 5.17 | 5.36 | 7.00 | 4.55 | 20.00 | 2.87 | 5.39 |
| $CV_1/CV_2$ | 8.11 | 7.86 | 8.76 | 4.29 | 5.99 | 3.50 | 8.74 | 9.02 |

[1]Abbreviations as defined in Table 7.
[2]Sample # corresponds to samples as listed in Table 1.
[3] SD, $CV_1$ and $CV_2$ as defined in Table 7.

acquisition system. The values generated for the mixograph parameters in this study other than FMS, PBW and SAP are relatively more precise and can be used to discriminate flours according to their mixing properties. Baker and Campbell (1971) found that MDT was highly repeatable but had a moderately low heritability due to the occurrence of a significant cultivar by year interactions. However, De La Roche and Fowler (1975) found that MDT and LV had a relatively low repeatability and attributed it to large measurement errors. In this study the measurement of PHG, TEG and BWE on both types of flour were quite reliable as shown by their high $CV_1/CV_2$ ratio. This ratio was high for MDT and ETP for Quad flour but slightly lower for Buhler milled flour. These values were generated from 16 samples, more samples need to be analyzed to confirm the validity of these results.

Highly significant ($P < 0.01$) interrelationship among some of the mixograph parameters were observed. For example for the Buhler milled flour ETP with MDT ($r=0.96$); BWE with MDT and ETP ($r=0.95$ and 0.99, respectively). MDT, ETP, and BWE were not significantly correlated with protein content. While PBW was highly correlated with PHG ($r=0.96$); TEG with PHG and PBW ($r=0.097$ and 0.94, respectively). PHG, PBW, and TEG parameters were also significantly correlated with protein content. Therefore the relationships involving PHG, PBW, TEG and technological quality (next section) could be more apparent than real because of the protein influence. Similar inter-relationships were observed for the Quad milled flour.

Student's t-test was used to compare the mixograph parameters between the two mills. These comparisons are reported in Table 9. A few of the parameters showed significant differences between the two mills for some of the samples but not in others. For example, ETP was significantly different ($P<0.05$ level) between the two

TABLE 9. Comparison of the mixograph quality data[1] between flours from Buhler and Quad mills using Student's t-test.

| Sample #[2] | PHG | MDT | ETP | FMS | PBW | SAP | TEG | BWE |
|---|---|---|---|---|---|---|---|---|
| S01 | ns[3] | *[4] | ** | ns | ns | ns | ns | * |
| S02 | ns | ns | ns | ** | ns | ns | ns | ns |
| S03 | ns | ns | ns | ns | ns | ns | ns | ns |
| S04 | ns | ns | ns | ns | ns | ns | ns | ns |
| S05 | ns | ns | ns | ns | ns | ns | ns | ns |
| S06 | ns | ns | ns | * | ns | ns | ns | ns |
| S07 | ns | ns | ns | * | ns | ns | ns | ns |
| S08 | ns | ns | ns | ns | ns | ** | ns | ns |
| S09 | ns | ns | * | ns | ns | * | ns | ** |
| S10 | ns | * | ** | * | ns | ns | ns | *** |
| S11 | ns | * | ns | ns | ns | ns | ns | ns |
| S12 | * | ns | ns | * | ns | ns | ns | ns |
| S13 | *** | ns | ns | * | * | ns | * | ns |
| S14 | * | ns | ns | * | ns | ns | ns | ns |
| S15 | ns | ns | ns | ns | ns | ns | ns | ns |
| S16 | ns | ns | ns | ns | ns | ns | ns | ns |

[1]Abbreviation as defined in Table 7.
[2]Sample # corresponds to the samples as defined in Table 1.
[3]ns-Not significantly different.
[4]*, **, *** Significantly different at P< 0.1, 0.05, 0.01 level, respectively.

mills for the samples S01 and S10. While BWE was significantly different for samples S09 and S10. PHG, FMS and SAP were significantly different for S13, S02 and S08, respectively. There was no particular trend or relationships in these differences except that samples S10 and S13 were significantly different between the mills in more parameters than the other samples. These results are complementary with those reported by Bequette *et al* (1960). These workers reported that the protein content, farinograph peak, farinograph stability and valorimeter values of the Buhler and Quad milled flours were closely related. The variations in the mixogragh parameters between the two type of flours could therefore not be accounted for by the instrument or methodology but most likely by the differences in the inherent quality of the samples. The significant relationship in most of the mixograph parameters for the two types of flours is an indication that the mixograph can be used to determine the mixing characteristics of wheat varieties from either Buhler or Quad mill.

## 5. The relationship between the mixograph data and other technological quality parameters

Pearson correlation analysis was performed to determine the relationship between the mixograph data and the other breadmaking quality parameters. Only mixograph parameters with significant correlations are reported in Tables 10 and 11 for Buhler and Quad mills, respectively. For Buhler flour, PHG, PBW, and TEG were highly significantly correlated with flour protein ($P<0.01$), with r-values of 0.95, 0.91 and 0.91, respectively (Table 10). A similar level of correlation was found for the Quad milled flours (Table 11). The parameters PHG, PBW, and TEG are also highly intercorrelated. These parameters (PHG, PBW and TEG) were also highly correlated with farinograph absorption and loaf volume. For both mills MDT, ETP and BWE, were

TABLE 10. The correlation coefficients between the technological data for the Buhler milled flour.

| | FY | FP | F/W | PSI | FPS | SD | SV | FAB | DDT | MTI | EXT | RMAX | R/E | AREA | RT | WO | LV | BSI | PHG | MDT | ETP | PBW | TEG | BWE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FY | 1.0 | | | | | | | | | | | | | | | | | | | | | | | |
| FP | -01 | 1.0 | | | | | | | | | | | | | | | | | | | | | | |
| F/W | -26 | -38 | 1.0 | | | | | | | | | | | | | | | | | | | | | |
| PSI | -33 | -65** | 13 | 1.0 | | | | | | | | | | | | | | | | | | | | |
| FPS | 41 | -71** | 27 | 92** | 1.0 | | | | | | | | | | | | | | | | | | | |
| SD | 30 | 40* | 06 | -82** | -82** | 1.0 | | | | | | | | | | | | | | | | | | |
| SV | 43 | 71** | -52** | -69** | -86** | 63** | 1.0 | | | | | | | | | | | | | | | | | |
| FAB | 03 | 89** | -42 | -79** | -77** | 52* | 72** | 1.0 | | | | | | | | | | | | | | | | |
| DDT | 02 | 39 | 37 | -61* | -61* | 66** | 34 | 35 | 1.0 | | | | | | | | | | | | | | | |
| MTI | -53* | -63** | 38 | 91** | 87** | -75* | -85** | -76** | -55* | 1.0 | | | | | | | | | | | | | | |
| EXT | 15 | 64** | -33 | -67* | -49 | 58* | 80** | 57* | 51* | -63** | 1.0 | | | | | | | | | | | | | |
| RMAX | 31 | 55* | 08 | -79** | -79** | 74** | 67** | 62* | 70** | -74** | 59* | 1.0 | | | | | | | | | | | | |
| R/E | 36 | 45 | 13 | -73** | -79** | 65** | 55* | 57* | 57* | -69** | 34 | 95** | 1.0 | | | | | | | | | | | |
| AREA | 32 | 56* | -03 | -67* | -69** | 64** | 69** | 60* | 54* | -68** | 64** | 96** | 90** | 1.0 | | | | | | | | | | |
| RT | 16 | 61* | -05 | -89** | -87** | 86** | 74** | 67** | 75** | -83** | 66** | 77** | 67** | 67** | 1.0 | | | | | | | | | |
| WO | 23 | 68** | -07 | -87** | -87** | 86** | 77** | 71** | 84** | -85** | 76** | 79** | 65** | 70** | 99** | 1.0 | | | | | | | | |
| LV | 31 | 88** | -41 | -93** | -83** | 66** | 92** | 88** | 47 | -87** | 77** | 73** | 64** | 73** | 79** | 84** | 1.0 | | | | | | | |
| BSI | 66** | 30 | -29 | -82** | -67** | 71** | 78** | 49 | 33 | -83** | 61* | 64** | 59* | 62* | 67** | 66** | 71** | 1.0 | | | | | | |
| PHG | 10 | 95** | -29 | -79** | -72** | 47* | 74** | 90** | 52* | -73** | 70** | 73** | 64** | 73** | 68** | 74** | 91** | 43 | 1.0 | | | | | |
| MDT | 37 | 11 | 03 | -52* | -52* | 74** | 46 | 21 | 72** | -66** | 47 | 53* | 41 | 40 | 74** | 72* | 39 | 59** | 16 | 1.0 | | | | |
| ETP | 28 | 35 | 01 | -66* | -66** | 80** | 59** | 40 | 84** | -74** | 60* | 68** | 54* | 56* | 87** | 87** | 58** | 63** | 42 | 96** | 1.0 | | | |
| PBW | 12 | 91** | -23 | -78** | -74** | 54** | 69** | 90** | 52* | -75** | 63** | 76** | 70** | 73** | 70** | 73** | 87** | 40 | 96** | 17 | 41 | 1.0 | | |
| TEG | 22 | 91** | -31 | 89** | -83** | 61** | 84** | 90** | 58* | -85** | 72** | 80** | 72** | 77** | 79** | 84** | 97** | 59** | 97** | 34 | 57** | 94** | 1.0 | |
| BWE | 26 | 33 | 05 | 67 | -67** | 81** | 57** | 39 | 84** | -73** | 56* | 66** | 53* | 54* | 87** | 86** | 56** | 60** | 41 | 95** | 99** | 42* | 56** | 1.0 |

FY=Flour yield (%), FP=Flour protein (%), F/W= Flour ash/Wheat ash ratio, PSI=Particle size index (%), FPS=Flour particle size (% flour through 75 $\mu$m sieve %), SD=Starch damage (FU), SV=Sedimentation value (ml), FAB=Farinograph absorption (%), DDT=Dough development time (min), MTI=Mixing tolerance index (BU), Ext=Extensibility (mm), RMAX=Maximum resistance (EU), R/E=Resistance/Extensibility, Area= area under the curve ($cm^2$), RT=Remix time (min), WO=Work input (watt hr/kg), LV=Loaf volume (cc), BSI=Baking strength index (%), PHG=Peak height (Nm), MDT=Mixograph development time (min), ETP=Energy to peak (Nm), PBW=Peak band width (Nm), TEG=total energy under the curve (Nm), BWE=Band width energy (Nm).
*, ** Significant at $P < 0.05$ and 0.01 levels, respectively. The decimals are omitted except for the perfect correlations (1.0)

TABLE 11. The correlation coefficients between the technological data[1] for the Quad Jr. milled flour.

| | FY | FP | F/W | PSI | FPS | SD | SV | FAB | DDT | MTI | EXT | RMAX | R/E | AREA | RT | WO | LV | BSI | PHG | MDT | ETP | PBW | TEG | BWE |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| FY | 1.0 | | | | | | | | | | | | | | | | | | | | | | | |
| FP | 32 | 1.0 | | | | | | | | | | | | | | | | | | | | | | |
| F/W | 09 | 42 | 1.0 | | | | | | | | | | | | | | | | | | | | | |
| PSI | -44 | -69** | -73** | 1.0 | | | | | | | | | | | | | | | | | | | | |
| FPS | 54 | -71**[2] | -70** | 95** | 1.0 | | | | | | | | | | | | | | | | | | | |
| SD | 34 | 33 | 54* | -73** | -79** | 1.0 | | | | | | | | | | | | | | | | | | |
| SV | 58* | 67** | 16 | -54* | -66* | 40 | 1.0 | | | | | | | | | | | | | | | | | |
| FAB | 33 | 91** | 38 | -71** | -76** | 34 | 62* | 1.0 | | | | | | | | | | | | | | | | |
| DDT | 05 | 41 | 80** | -61* | -61** | 56* | 27 | 25 | 1.0 | | | | | | | | | | | | | | | |
| MTI | -67** | -69** | -54 | 86** | -92** | -66** | -76** | -69** | -57* | 1.0 | | | | | | | | | | | | | | |
| EXT | 26 | 78** | 24 | -55* | -63* | 15 | 69** | 77** | 42 | -68** | 1.0 | | | | | | | | | | | | | |
| RMAX | 31 | 53 | 47 | -69** | -71** | 46 | 65** | 47 | 64* | -72** | 76** | 1.0 | | | | | | | | | | | | |
| R/E | 36 | 34 | 54* | -69** | -68** | 57* | 56* | 28 | 62* | -67** | 50 | 94** | 1.0 | | | | | | | | | | | |
| AREA | 23 | 59* | 41 | -61* | -63* | 28 | 61* | 54* | 62* | -67** | 87** | 96** | 82** | 1.0 | | | | | | | | | | |
| RT | 33 | 62* | 78** | -86** | -90** | 77** | 49 | 52* | 89** | -80** | 52 | 69** | 67* | 64* | 1.0 | | | | | | | | | |
| WO | 35 | 64** | 74** | -80** | -88** | 77** | 56** | 51 | 91** | -79** | 57* | 71** | 67* | 68** | 98** | 1.0 | | | | | | | | |
| LV | 57* | 78** | 55** | -85** | -93** | 75** | 81** | 76** | 47 | -86** | 59* | 64* | 61* | 55* | 79** | 79** | 1.0 | | | | | | | |
| BSI | 64** | 34 | 47** | -69** | -80** | 82** | 66** | 39 | 37 | -74** | 23 | 52 | 65* | 33 | 67** | 66** | 85** | 1.0 | | | | | | |
| PHG | 37 | 93** | 46** | -70** | -74** | 29 | 70** | 96** | 41 | -76** | 87** | 64* | 45 | 70** | 60** | 63** | 76** | 38 | 1.0 | | | | | |
| MDT | 21 | 07 | 53** | -44 | -50* | 58** | 21 | -08 | 65** | -50* | 07 | 29 | 36 | 24 | 68** | 65** | 39 | 54** | 03 | 1.0 | | | | |
| ETP | 28 | 38 | 64** | -64** | -72** | 62** | 42 | 31 | 79** | -73** | 42 | 52 | 49 | 49 | 84** | 84** | 62** | 63** | 38 | 93** | 1.0 | | | |
| PBW | 47 | 85** | 51** | -77 | -81** | 45** | 69** | 90** | 50* | -83** | 81** | 73** | 60* | 74** | 67** | 68 | 82** | 53* | 95** | 08 | 42 | 1.0 | | |
| TEG | 45 | 91** | 52** | -79** | -83** | 41 | 77** | 93** | 46 | -84** | 85** | 70** | 54* | 73** | 70** | 72** | 84** | 51* | 97** | 16 | 49* | 96** | 1.0 | |
| BWE | 26 | 35 | 68** | -65** | -72** | 64** | 37 | 27 | 80** | -69** | 37 | 50 | 48 | 46 | 86** | 85** | 60** | 63** | 36 | 93** | 99** | 42 | 47** | 1.0 |

[1]FY=Flour yield (%), FP=Flour protein (%), F/W= Flour ash/Wheat ash ratio, PSI=Particle size index (%), FPS=Flour particle size (% flour through 75 μm sieve %), SD=Starch damage (FU), FAB=Farinograph absorption (%), DDT=Dough development time (min), MTI=Mixing tolerance index (BU), Ext= Extensibility (mm), RMAX=Maximum resistance (EU), R/E=Resistance/Extensibility, Area=Area under the curve ($cm^2$), RT= Remix time (min), WO=Work input (watt hr/kg), LV=Loaf volume (cc), BSI=Baking strength index (%), PHG=Peak height (Nm), MDT=Mixograph development time (min), ETP=Energy to peak (Nm), PBW=Peak band width (Nm), TEG=Total energy under the curve (Nm), BWE=Band width energy (Nm).

[2]*, ** Significant at $P< 0.05$ and 0.01 levels, respectively. The decimals are omitted except for the perfect correlations (1.0).

not correlated with FP but highly intercorrelated with each other. The results observed in this study are consistent with previous findings. Gupta *et al* (1992) did not find any correlations between MDT and FP (r=-0.012). Dong *et al* (1992) reported similar observations, i.e no association between FP with MDT and MTI. These results indicate that the type of mill used to grind the grain had no effect on the correlations between the breadmaking quality data. The high r-values observed in this study may be attributed to the broad and diverse mixing strength of the samples used.

Energy to peak (ETP) and band width energy (BWE) were highly and significantly correlated ($P<0.01$) with starch damage (SD), with r-values of 0.80 and 0.81, respectively for the Buhler milled flour. Similar correlations were also observed for the Quad milled flours. Starch damage has been reported to be highly correlated with grain hardness (Evers and Stevens, 1985). The highly significant correlation between ETP and BWE with SD therefore indicate that these two mixograph parameters may also be highly correlated with grain texture. That is, genotypes with harder kernels tend to produce flours with higher ETP and BWE. Additionally highly significant correlations ($P<0.01$) values were obtained between ETP and BWE with DDT, RT, WO and to a lesser degree LV, which have all been shown to be influenced by dough strength. Similar correlations were observed for the Quad milled flour. MDT did not show any correlation with LV or FP for flour from both mills.

Highly significant ($P < 0.01$) interrelationship among some of the mixograph parameters were observed as mentioned previously. The significant interrelationship between the mixograph parameters (PHG,PBW and TEG) and flour protein content emphasizes the substantial contribution of the flour protein in dough mixing characteristics (refer to section II). However, it would appear that MDT, ETP and BWE

are very useful parameters of mixing strength independent of flour protein.

## 6. Baking Test

The baking test is the final evaluation test in quality determination of bread wheat. It is the all-inclusive test of breadmaking quality, where the composite effects of many quality factors are expressed together. The remix baking test developed by Irvine and McMullen (1960) is commonly used to assess Canadian bread wheats. This test was originally designed to bring out the potential of strong flours which often do not achieve their full loaf volume potential under gentle mixing, such as in the AACC straight dough method (Tipples and Kilborn, 1974). A modified remix method of De la Roche and Fowler (1975) with some minor modifications was used in this study because the samples being examined were of very diverse mixing strength. To allow proper handling of the dough during the remix stage, the samples were remixed to optimum consistency rather than for a constant time (e.g 2.5 min after the method of Irvine and McMullen (1960)). In addition to loaf volume (LV) and baking strength index (BSI), the time required for the remix (RT) and the work input (WO) were recorded. Kilborn and Tipples (1972) reported that in the Chorleywood breadmaking process (CBP), to achieve proper dough development the mixing intensity (impeller speed) must be above a minimum critical level (varying with both flour and mixer). These researchers also observed that the work input imparted to the dough must be greater than a minimum critical amount depending on the flour used. The total energy used in developing the dough is generally accepted as a measure of dough strength (Voisey *et al*, 1966c).

The conditions to which each dough was subjected in the modified remix test used in this study were considered to be optimized for each sample. Bushuk *et al* (1969) showed that when strong wheats received their optimum remix requirements, the LV were similar to those of standard hard red spring wheats with the same protein content. LV in an optimized baking test is considered to be a measure of dough strength (MacRitchie, 1987). The larger the LV the stronger the dough. The results were shown in Tables 4 and 5 in the section dealing with description of test samples. The Buhler flours had RT values ranging from 0.70 min (very weak) to 4.10 min (very strong) with corresponding WO values of 2.7-18.0 watt hr/kg. For the Quad flours, the RT range was 1.0-4.0 min with corresponding WO of 3.8-21.5 watt hr/kg. The LV values ranged from 439-947 cc for the Buhler milled flours and 432-885 cc for the milled Quad flours. Dough handling properties after mixing and fermentation stages appeared to be normal for most of the samples except Roblin. This variety is known to be very strong which was also confirmed by its milling and rheological properties (Tables 4 and 5). However, during the final fermentation stage the dough collapsed and the resulting LV was lower than expected. SD of Roblin flour (Section IV:B (iv)) was considerably lower than expected for a hard wheat. But the low oven-spring and LV does not seem to be accounted for by the observed low SD. Both Buhler and Quad flour for this sample showed the same trend. The cause of this unique dough behaviour was not investigated further; it is speculated that the result is related to an insufficient amount of fermentable sugars available.

Statistical analysis results for Buhler milled flours, (Table 10), showed that LV was highly and significantly correlated ($P < 0.01$) with FP ($r = 0.88$), SV ($r = 0.92$), FAB ($r = 0.88$), MTI ($r = -0.87$), RT ($r = 0.79$) and WO ($r = 0.84$). Similar correlations were

obtained for the Quad milled flours (Table 11). These relationships are consistent with previous findings reported in literature (Section II). It is well established that LV is highly correlated to both protein content and protein quality. However, LV was not significantly correlated with MDT ($r=-0.39$), consistent with the findings of Dong *et al* (1992).

Remix time (RT) and WO were significantly correlated ($P < 0.01$) to SD, SV, DDT, RMAX, LV and the mixograph parameters. Fowler and De la Roche (1975) reported similar correlations between RT and MDT ($r= 0.84$). This findings indicates that RT is largely determined by the dough strength.

Generally, Quad milled flours gave larger LV's than Buhler milled counterparts. The baking-test results for the flours from the two mills were compared using paired Student's t-test (Table 6). For most samples, the difference in LV and BSI for the flours from one sample milled on the two mills was not consistent. A few samples showed some significant differences ($P<0.05$). For example, the LV for samples S05, S06, S07, S14 and S15 were significantly different between the two mills. The differences in BSI values between the two mills, like the LV data was not consistent. This is probably due to the small differences in protein content of flour obtained the flours from the two mills.

The results and discussion presented in this section indicate that the type of mill (Buhler vs Quad Jr.) used for grinding grain affected some milling and flour characteristics. This was shown by the significant differences in flour yield, protein and ash contents, starch damage and sedimentation value. However, the type of mill did not significantly affect the rheological properties (as determined by farinograph, extensigraph and mixograph) and baking quality properties. The analytical precision of

TABLE 12. The correlation coefficient between Buhler and Quad technological quality characteristics[1].

| Mixograph parameters | r-values |
|---|---|
| FY | **0.76** |
| FP | 0.99 |
| ASH | **0.47** |
| FN | **0.71** |
| SD | 0.93 |
| SV | 0.95 |
| FAB | 0.98 |
| DDT | 0.98 |
| MTI | 0.99 |
| EXT | **0.71** |
| RMAX | 0.96 |
| R/E | 0.89 |
| AREA | 0.96 |
| RT | 0.96 |
| WO | 0.97 |
| LV | 0.93 |
| BSI | 0.91 |
| ULV | 0.92 |
| PHG | 0.98 |
| MDT | 0.96 |
| ETP | 0.97 |
| FMS | 0.89 |
| PBW | 0.94 |
| SAP | 0.93 |
| TEG | 0.98 |
| BWE | 0.98 |

[1]Abbreviations as defined in Table 10.

the electronic computerized mixograph parameters PHG, TEG and BWE were more reliable than FMS, PBW and SAP. Peak height, PBW and TEG were highly correlated with flour protein. The correlation coefficient of the technological parameters between Buhler and Quad milled flour was high (r-value >0.90) for most of the parameters (Table 12). Except for FY (r=0.76), ASH (r=0.47), FN (r=0.71), and EXT (r=0.71). It can be concluded that despite the differences in some of the milling and flour quality characteristics e.g SD and FP either mill can be used to produce flour for determining the rheological and breadbaking properties of wheat varieties.

**7. Protein distribution among the extracting solutions**

Data for the total amount of protein in mg extracted from 1 g flour (termed "unnormalized") and amount of protein extracted reported as a percentage of flour protein (termed "normalized" ) are shown in Tables 13 and 14, and Figs. 7 and 8, respectively. The amount or range of protein extracted by the different solvents, distilled water ($H_2O$), 2 % sodium chloride (NaCl), 70 % ethanol (EtOH), and 0.05 *M* acetic acid (HAc), were characteristically different which reflects the different types of proteins extracted by each solvent.

Acetic acid (0.05 *M*) extracted the largest amount of protein (Fig. 7A) compared with other solvents (Figs. 7B, 8A and 8B) ranging from 62.4 % to 74.2 % (mean=66.8 %) for Buhler milled flours and 58.7 % to 69.2 % (mean=62.5 %) for the Quad milled flours. The amount of protein extracted was correlated ($P<0.05$) to some extent with the grain hardness for the Buhler milled flour (r=-0.52) and not for Quad milled flour for the normalized data as shown in Tables 16 and 17, respectively.

The amount of protein extracted from Buhler milled flour was generally greater

TABLE 13. The amount of protein extracted from Buhler milled flour by the different extracting solutions.

| SAMPLE[1] | FPRO[2] | Amounts of protein extracted (mg/g) | | | |
|---|---|---|---|---|---|
| | | $H_2O$ | 2 % NACL | 70% ETOH | 0.05 *M* HAc |
| 1 | 108.5±2.1 | 25.3±1.2 | 21.8±0.7 | 56.9±1.4 | 70.1±0.7 |
| 2 | 130.5±0.7 | 34.5±0.1 | 23.7±0.6 | 70.8±1.1 | 89.2±0.2 |
| 3 | 99.5±0.7 | 27.2±1.5 | 18.9±0.2 | 54.7±1.0 | 73.8±0.2 |
| 4 | 95.0±1.4 | 19.6±1.0 | 18.4±0.6 | 48.7±2.1 | 64.9±1.0 |
| 5 | 122.5±3.5 | 20.9±1.2 | 18.4±0.6 | 60.9±0.4 | 77.4±1.0 |
| 6 | 106.5±0.7 | 18.8±0.9 | 16.8±0.4 | 56.4±1.4 | 70.2±0.4 |
| 7 | 117.5±2.1 | 25.2±1.1 | 20.2±1.6 | 59.3±1.2 | 75.8±0.4 |
| 8 | 138.0±1.4 | 35.0±1.8 | 23.9±0.3 | 76.9±0.5 | 95.5±0.0 |
| 9 | 88.0±2.8 | 19.0±0.2 | 17.9±0.9 | 42.4±0.4 | 58.7±0.1 |
| 10 | 117.5±2.1 | 26.1±0.1 | 20.3±0.5 | 58.2±0.6 | 73.3±0.8 |
| 11 | 102.0±4.2 | 24.7±0.7 | 18.9±1.3 | 50.6±0.2 | 69.3±0.4 |
| 12 | 104.5±2.1 | 24.2±0.1 | 17.8±1.0 | 48.6±2.0 | 70.1±0.7 |
| 13 | 111.5±0.7 | 31.2±1.5 | 19.9±2.7 | 53.7±1.2 | 73.8±1.0 |
| 14 | 114.5±0.7 | 26.8±0.5 | 20.9±0.2 | 55.7±0.3 | 73.9±0.2 |
| 15 | 97.0±2.8 | 20.0±1.1 | 19.3±1.0 | 43.7±2.2 | 65.4±0.4 |
| 16 | 155.0±1.4 | 33.6±0.2 | 22.4±0.3 | 80.6±3.0 | 106.0±1.0 |
| Mean | 112.5 | 25.8 | 20.0 | 57.4 | 75.4 |
| SD | 17.0 | 5.4 | 2.2 | 10.7 | 11.8 |
| CV | 15.1 | 21.1 | 11.0 | 18.6 | 15.6 |

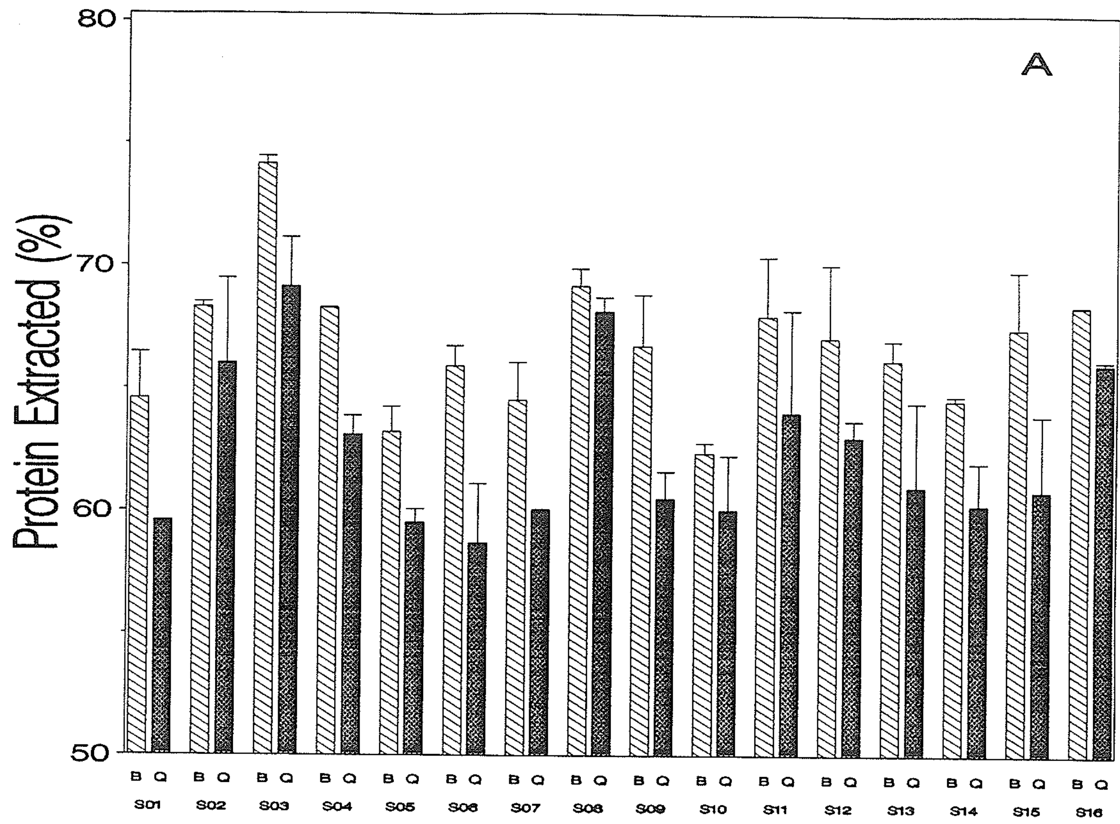
[1] Samples as defined in Table 1.
[2] FPRO=Flour protein (mg/g).

TABLE 14. The amount of protein (mg/g) extracted from Quad milled flour by the different extracting solutions.

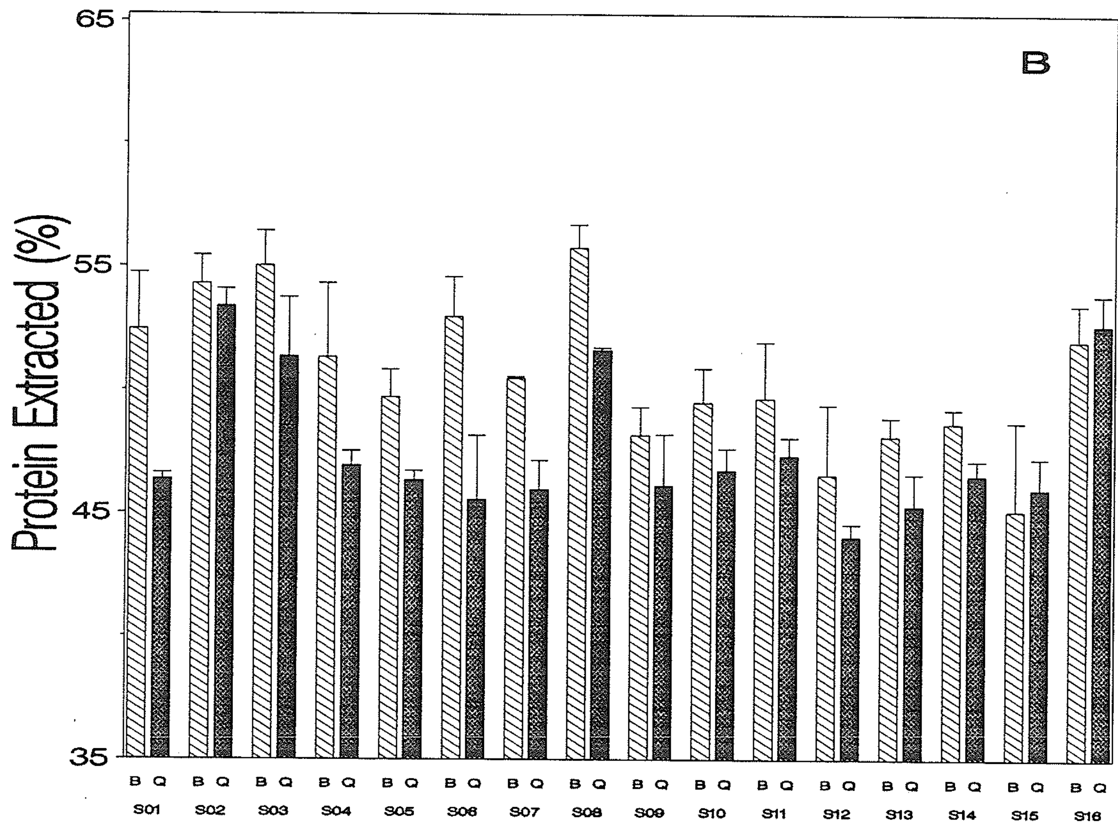
| SAMPLE | FPRO[1] | Amount of protein extracted (mg/g) | | | |
|---|---|---|---|---|---|
| | | $H_2O$ | 2 % NACL | 70 % ETOH | 0.05 *M* HAc |
| 1 | 114.5±0.7 | 21.3±0.1 | 20.7±0.5 | 53.1±0.1 | 68.2±0.4 |
| 2 | 132.5±3.5 | 31.0±0.9 | 23.8±0.6 | 70.7±1.0 | 87.4±2.2 |
| 3 | 104.5±0.7 | 28.5±0.2 | 18.8±0.3 | 53.7±0.9 | 72.3±2.6 |
| 4 | 102.5±0.7 | 23.1±0.1 | 15.6±0.3 | 48.1±0.9 | 64.7±1.2 |
| 5 | 127.5±0.7 | 22.1±0.7 | 16.9±0.4 | 59.1±0.2 | 75.9±0.3 |
| 6 | 116.0±1.4 | 19.0±0.1 | 17.2±0.1 | 52.8±0.7 | 68.1±3.6 |
| 7 | 126.5±0.7 | 23.7±0.1 | 20.2±0.0 | 58.2±0.8 | 76.0±0.5 |
| 8 | 143.5±0.7 | 29.8±0.4 | 23.9±0.3 | 74.1±0.2 | 97.8±0.3 |
| 9 | 93.0±1.4 | 19.5±0.8 | 17.9±0.4 | 42.9±0.3 | 56.3±1.9 |
| 10 | 126.5±0.7 | 24.0±0.2 | 19.8±0.1 | 59.1±0.8 | 75.9±2.4 |
| 11 | 110.5±2.1 | 23.3±0.1 | 19.9±1.0 | 52.3±0.2 | 70.7±3.3 |
| 12 | 114.0±1.4 | 24.9±0.1 | 18.2±0.6 | 50.2±0.1 | 71.8±0.1 |
| 13 | 118.5±2.1 | 30.1±0.8 | 19.4±0.3 | 53.7±0.5 | 72.2±2.8 |
| 14 | 124.5±2.1 | 23.8±0.8 | 20.6±0.2 | 57.9±0.2 | 75.0±0.9 |
| 15 | 101.0±1.4 | 20.3±0.8 | 19.0±0.7 | 46.4±0.6 | 61.4±2.3 |
| 16 | 159.0±1.4 | 28.1±0.1 | 22.8±0.8 | 83.7±0.6 | 105.0±1.1 |
| Mean | 119.7 | 24.3 | 19.6 | 57.2 | 74.8 |
| SD | 16.6 | 3.5 | 2.5 | 10.6 | 12.3 |
| CV | 13.9 | 14.6 | 12.6 | 18.5 | 16.5 |

[1] Samples as defined in Table 1.
[2] FPRO=Flour protein (mg/g).

FIGURE 7. The amount of protein extracted by 0.05 *M* acetic acid (Fig. 7A) and 70 % ethanol (Fig. 7B) from Buhler mill (**B**) and Quad mill (**Q**). The number of the samples corresponds to the samples as defined in Table 1.

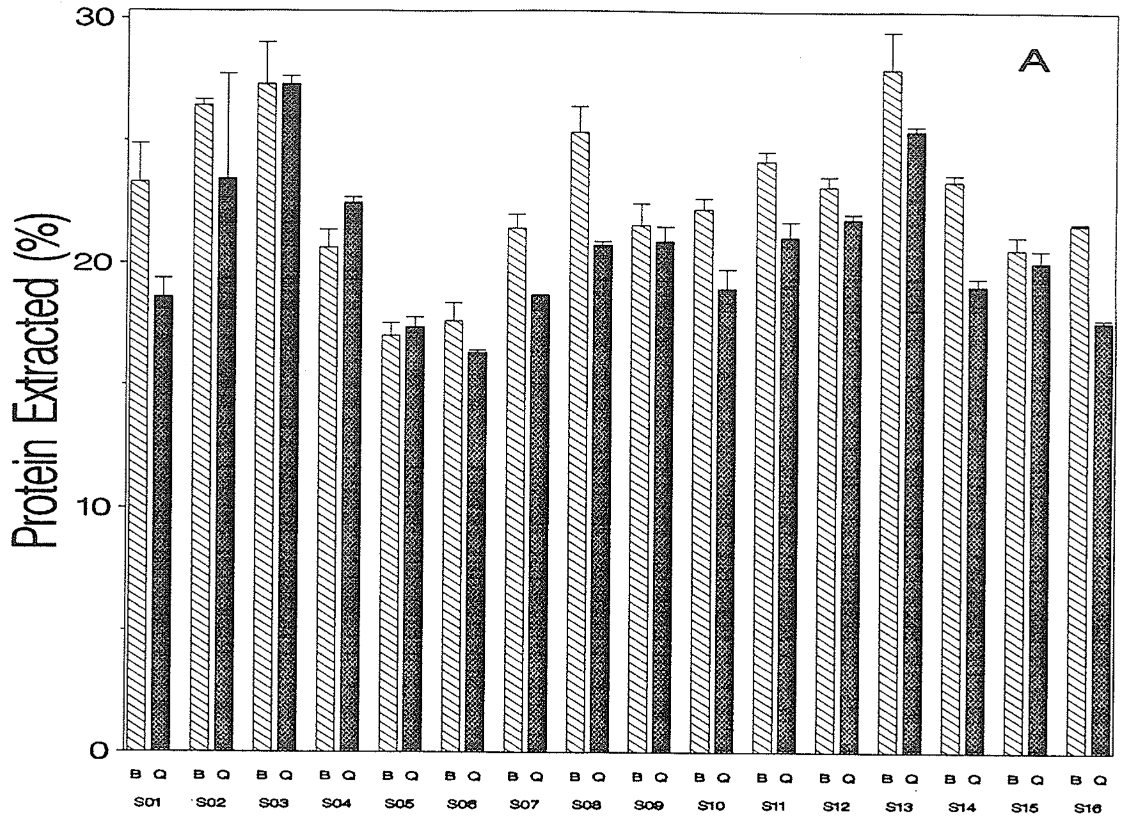


A
Protein Extracted (%)
80
70
60
50
B Q
S01
S02
S03
S04
S05
S06
S07
S08
S09
S10
S11
S12
S13
S14
S15
S16

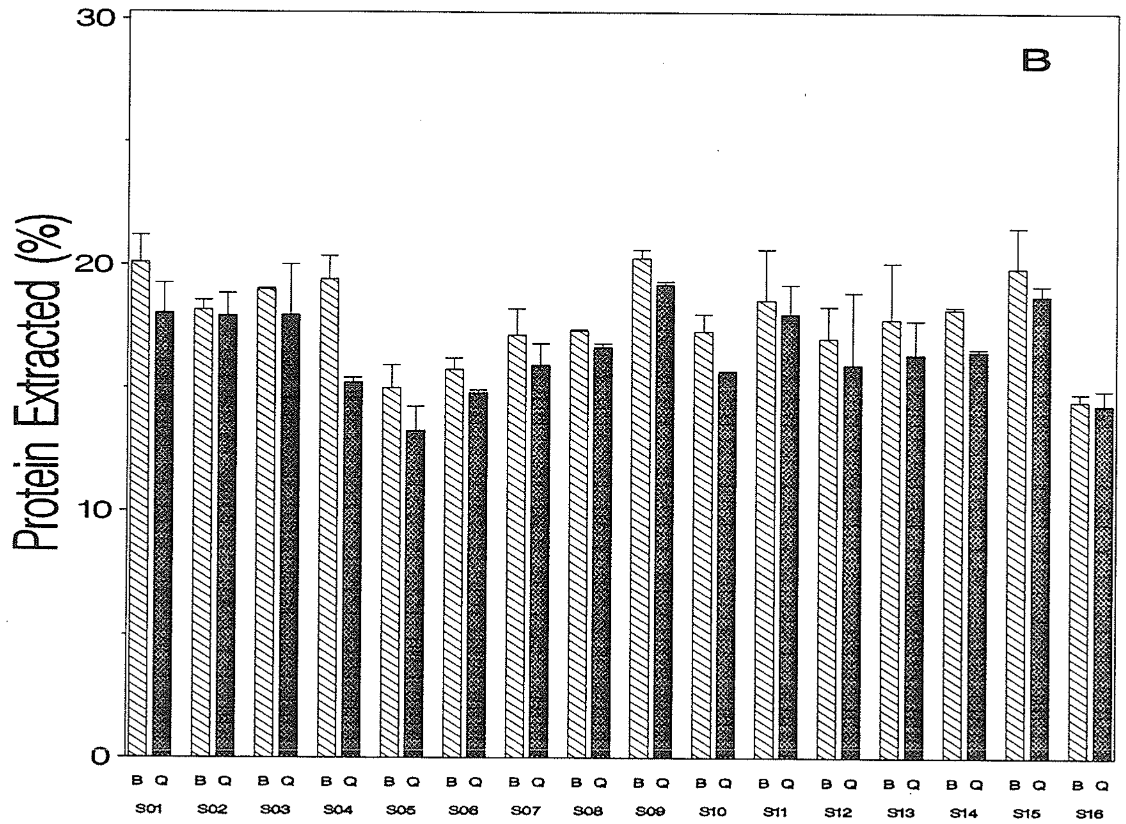



B
Protein Extracted (%)
65
55
45
35
B Q
S01
S02
S03
S04
S05
S06
S07
S08
S09
S10
S11
S12
S13
S14
S15
S16

FIGURE 8. The amount of protein extracted by distilled water (Fig. 8A) and 2 % NaCl solution (Fig. 8B) from Buhler mill (B) and Quad mill (Q). The number of the samples corresponds to the samples as defined in Table 1.



A
Protein Extracted (%)
30
20
10
0
B Q
S01
S02
S03
S04
S05
S06
S07
S08
S09
S10
S11
S12
S13
S14
S15
S16

B
Protein Extracted (%)
30
20
10
0
B Q
S01
S02
S03
S04
S05
S06
S07
S08
S09
S10
S11
S12
S13
S14
S15
S16

than for the Quad milled flour (Figs. 7 and 8). At first glance, this seems rather odd because Quad flour had higher protein than Buhler flour (Tables 4 and 5). However, the difference can be attributed to the particle size; the Buhler milled flours were finer than the Quad flours (see Tables 4 and 5). The average flour particle size (FPS) values as shown by the percentage of flour through a 75 $\mu$m sieve, were 46.5 and 30.1 % for Buhler and Quad milled flours, respectively.

The proportion of total flour protein extracted by EtOH (Fig. 6B) ranged from from 45.1 % to 55.7 % (mean=50.6 %) for Buhler milled flour and 44.0 % to 53.4 % (mean=47.6 %) for the Quad milled flour. Bushuk and Wrigley (1974) using the modified Osborne fractionation procedure reported that the amount of gliadins in wheat flour is about 33 % of the total wheat protein, but varies depending on the extraction procedure and flour sample. Some of the hard red spring varieties used in this study showed a high amount of ethanol extractable proteins, although over all the varieties, the amount of ethanol soluble protein was negatively correlated with hardness.

The texture of the residue obtained after EtOH extraction deserves mention. After removal of EtOH soluble proteins, the wet residue lacked extensibility by manual (subjective) stretching; on drying, it became hard and brittle. Both freeze-dried and air-dried EtOH residues were very hard and brittle, and difficult to grind to fine particle size compared with the HAc and NaCl residues which were softer and easier to grind into a fine powder. This could be attributed to the type of protein extracted by EtOH. It has been proposed that gliadin proteins are responsible for dough extensibility (Hoseney, 1986; MacRitchie *et al*, 1990). As gliadins are presumed to be removed by direct extraction with 70 % EtOH, the brittle nature of the EtOH residue provides further support of the effect of removing this fraction or may be the effect of EtOH on residue

proteins. However, a similar hard and brittle freeze-dried residue was observed after water extraction which was also found to remove substantial quantity of gliadin protein (Fig. 17) supporting the former hypothesis that the nature of the residue is due the removal of the EtOH soluble fraction.

Sodium chloride (2 %) extracted 14.5 % to 20.3 % (mean = 17.9 %) for Buhler flour and 14.3 % to 19.2 % (mean = 16.5 %) for Quad milled flour. Ewart (1968) with three consecutive extractions, using 0.04 *M* NaCl solution extracted about 18.2 % protein from Buhler milled flour. Compared with 14.5-20.3 % observed in this study, this shows that a single extraction with 2 % NaCl for 2 h is sufficient to extract most of the salt soluble protein fractions. The amount of protein extracted using distilled water or 2 % NaCl was less for the overly strong hard wheats, S10 (Glenlea) and S16 (Roblin) than for the other samples used (Figs. 8A and 8B). The coefficient of variation (CV) for the amount of 2 % NaCl soluble protein (11.0 and 12.6 % for Buhler and Quad milled flours, respectively) was the lowest compared to the other solvents used (Tables 13 and 14). An indication of the narrow range in the amount of this fraction for the samples used.

Distilled water extracted more protein than 2 % NaCl solution as shown in Fig. 8A and 8B, respectively. The amount of protein extracted by water ranged from 17.0 % to 28.0 % (mean = 22.8 %) for Buhler flours and for the Quad it was 16.4 % to 27.3 % (mean = 20.6 %). Mullen and Smith (1965) observed a marked difference in their water-soluble extracts, 49 % for the short mixing flour compared with 23 % for the long mixing flour.

The amount of protein extracted by the different solvents from the two mills were compared using the Student's t-test. The results (Table 15) show that the total

amount of protein (unnormalized) extracted by HAc and 2 % NaCl solution was not significantly different in the two mills most of samples. The only samples that were different ($P<0.10$) between the two mills were S01, S08, S09 and S14 for HAc soluble proteins and S04 for NaCl soluble proteins. More samples showed significant differences in protein extracted using $H_2O$ and EtOH. It has been reported in literature (e.g Minetti *et al*, 1975) that a single extraction of flour with water does not extract all of flour water soluble proteins. Direct extraction with EtOH is also known to extract water soluble proteins as shown by the electrophoregrams (compare Figs. 17 and 19). The significant differences between the mills in the amount of protein extracted by $H_2O$ and EtOH may therefore be attributed to the inconsistency in extraction by these solvents. Or may be the higher ash content of the Quad milled flour, suggesting contamination by bran could have affected significantly the protein extractability by $H_2O$ and EtOH. When the amounts of protein extracted were compared as a percentage of total flour protein (normalized) more samples showed significant differences for HAc and 2 % NaCl extracts. For EtOH and $H_2O$ extracts the number of samples significantly different was similar to the unnormalized data but different samples were involved. There was no any consistent differences in protein extracted with HAc and 2 % NaCl between the two mills. The differences observed may be attributed to the differences in flour protein content and the particle size in flours from the two mills.

The relationship between the Buhler milled flour protein solubility fractions and those of Quad mill was very good. The amount of protein extracted between the two mills was significantly correlated ($P< 0.01$) with r-values of 0.79, 0.85, 0.73 and 0.86 for $H_2O$, 2 % NaCl, 70 % EtOH and 0.05 *M* acetic acid, respectively. The degree of

TABLE 15. Comparison of protein extraction data of flours from Buhler and Quad mills using Student's t-test[1].

| Sample #[1] | Unnormalized | | | | | Normalized | | | |
|---|---|---|---|---|---|---|---|---|---|
| | FP | $H_2O$ | HAc | EtOH | NaCl | $H_2O$ | HAc | EtOH | NaCl |
| S01 | *[2] | * | * | * | ns | * | * | * | ns |
| S02 | ns[3] | * | ns | ns | ns | ns | ns | ns | ns |
| S03 | ** | ns | ns | ns | ns | ns | * | ns | ns |
| S04 | ** | * | ns | ns | * | * | ns | ns | * |
| S05 | ns | ns | ns | * | ns | ns | * | ns | * |
| S06 | ** | ns | ns | ns | ns | ns | * | * | ns |
| S07 | * | ns | ns | ns | ns | ns | ns | * | ns |
| S08 | * | * | * | * | ns | * | ns | * | * |
| S09 | ns | ns | * | ns | ns | ns | * | ns | * |
| S10 | * | ns | ns | ns | ns | * | ns | ns | ns |
| S11 | ns | ns | ns | * | ns | * | ns | ns | ns |
| S12 | * | * | ns | ns | ns | * | ns | ns | ns |
| S13 | * | ns | ns | ns | ns | ns | ns | ns | ns |
| S14 | * | * | ns | * | ns | *** | * | * | *** |
| S15 | ns | ns | ns | ns | ns | ns | ns | ns | ns |
| S16 | ns | *** | ns | ns | ns | *** | *** | ns | ns |

[1]Sample # corresponds to the samples as listed in Table 1.
[2]*, **, *** Significantly different at $P < 0.10$, 0.05 and 0.01 level, respectively.
[3]ns-Not significant.

correlation was low for EtOH and $H_2O$. This was not surprising because the two solvents had a lower extraction efficiency as shown by the high CV's (Tables 13 and 14). The overall protein content of the flours milled on the two mills was significantly correlated ($P < 0.01$) with an r-value of 0.99.

## 8. The relationship between technological quality and protein fraction data

The relationships between breadmaking quality data and the protein fractions are shown in Tables 16 and 17 for the Buhler and Quad mills, respectively. The correlations between the total amount of protein extracted-mg/g flour (termed "unnormalized") are included in Tables 16 and 17 for comparison with proportion of protein extracted expressed as a percentage of the total flour protein (termed "normalized"). It can be observed that quite different relationships can be derived between the technological parameters and the protein fractions quantitated as normalized or unnormalized terms. The most appropriate and commonly used quantization of the fractions is as a percentage of the total flour protein (i.e normalized). This is because the normalizing factors out the effect of differences in total flour protein. This is particularly important in relation to the EtOH and HAc soluble fractions which are very highly correlated with (FP). Flour protein content has great influence on the relationship between the unnormalized fraction values and the technological data for both type of flours. The technological parameters that are highly and significantly correlated ($P<0.01$) with FP content e.g FAB, LV, PHG and TEG, are also significantly correlated to all the four fractions of the unnormalized data. Most of the discussion below will be based on the normalized data.

The proportion of HAc soluble protein from the Buhler milled flour gave a

TABLE 16. Correlation[1] between technological and protein fraction data for Buhler milled flour.

| | Protein Solubility Classes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normalized[2] | | | | | Unnormalized[3] | | | |
| | FP | $H_2O$ | NaCl | EtOH | HAc | $H_2O$ | NaCl | EtOH | HAc |
| FP[4] | 1.0 | 08 | -71** | 44 | -07 | 76** | 73** | 96** | 96** |
| PSI | -66** | 09 | -56* | 05 | -52* | -42 | -46 | -57* | -52* |
| FPS | -71** | 02 | 60* | -10 | 53* | -52* | -50* | -61* | -56* |
| SD | 41 | -12 | -50* | 11 | -59* | 21 | 21 | 36 | 24 |
| SV | 72** | 01 | -64** | 05 | -51* | 51* | 46 | 60* | 57* |
| FAB | 89** | 17 | -63** | 21 | -12 | 74** | 67** | 80** | 84** |
| DDT | 39 | -35 | -56* | -09 | -57* | 04 | 06 | 30 | 24 |
| MTI | -63** | 24 | 68** | 14 | 62** | -30 | -30 | -48 | -46 |
| EXT | 64** | -00 | -69** | 23 | -32 | 43 | 31 | 59* | 54* |
| RMAX | 55* | 05 | 55* | -07 | 50* | 43 | 31 | 44 | 43 |
| R/E | 45 | 09 | -42 | -17 | -46 | 40 | 29 | 34 | 34 |
| AREA | 56* | 15 | -51* | 01 | -38 | 50* | 36 | 47 | 46 |
| RT | 61* | -18 | -61* | 01 | -67** | 30 | 33 | 48 | 40 |
| WO | 68** | -23 | -74** | 10 | -61* | 33 | 33 | 59* | 51* |
| LV | 88** | 06 | -74** | 21 | -36 | 66** | 59* | 79** | 77** |
| BSI | 30 | 02 | -46 | -21 | -59* | 22 | 09 | 17 | 14 |
| PHG | 95** | 11 | -71** | 28 | -14 | 75** | 67** | 88** | 91** |
| MDT | 11 | -60* | -51* | -23 | -83** | -32 | -27 | -00 | -12 |
| ETP | 35 | -50* | -65** | 14 | -79** | -08 | -07 | 23 | 13 |
| FMS | 91** | 07 | -54* | 26 | -20 | 70** | 77** | 84** | 85** |
| PBW | 91** | 16 | -69** | 31 | -11 | 77** | 68** | 87** | 89** |
| SAP | 58* | 29 | -32 | 39 | 53* | 57* | 44 | 60* | 71** |
| TEG | 91** | 03 | -74** | 18 | 33 | 67** | 61* | 81** | 82** |
| BWE | 33 | -50* | -61** | -14 | -78** | -10 | -08 | -21 | 12 |

[1]Decimals are omitted.
[2]Amount of protein extracted using specified solvent expressed as a proportion of flour protein.
[3]Amount of protein extracted per gram of flour.
[4]Abbreviations as defined in Tables 4 and 7.
[5]*, ** Significant at P< 0.05 and 0.01 level, respectively.

TABLE 16. Cont.

| | Protein Solubility Classes | | | |
|---|---|---|---|---|
| | Residue Protein[1] | | | |
| | $H_2O$ | NaCl | EtOH | HAc |
| FP | 97** | 99** | 91** | 88** |
| PSI | -68** | -67** | -71** | -81** |
| FPS | -69** | -71** | -75** | -85** |
| SD | 43 | 42 | 42 | 64** |
| SV | 71** | 72** | 79** | 85** |
| FAB | 83** | 88** | 89** | 81** |
| DDT | 50* | 44 | 51* | 64** |
| MTI | -69** | -65** | -77** | -83** |
| EXT | 66** | 67** | 65** | 72** |
| RMAX | 55* | 58* | 67** | 72** |
| R/E | 44 | 47 | 58* | 61* |
| AREA | 53* | 58* | 64** | 67** |
| RT | 64** | 61* | 68** | 84** |
| WO | 74** | 71** | 74** | 89** |
| LV | 86** | 89** | 89** | 92** |
| BSI | 29 | 31 | 44 | 53* |
| PHG | 92** | 96** | 94** | 88** |
| MDT | 26 | 15 | 24 | 50* |
| ETP | 48 | 39 | 48 | 69** |
| FMS | 88** | 90** | 89** | 86** |
| PBW | 89** | 94** | 91** | 85** |
| SAP | 52* | 58* | 48 | 25 |
| TEG | 90** | 93** | 95** | 94** |
| BWE | 46 | 38 | 46 | 67** |

[1]Amount of protein (mg) left in the residue per g of flour.

TABLE 17. Correlation[1] between technological and protein fraction data for Quad milled flour.

| | Protein Solubility Classes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normalized[2] | | | | | Unnormalized[3] | | | |
| | FP | $H_2O$ | NaCl | EtOH | HAc | $H_2O$ | NaCl | EtOH | HAc |
| FP[4] | 1.0 | -34 | -52* | 56* | 29 | 56* | 72** | 96** | 95** |
| PSI | -69** | 47 | 43 | -09 | 14 | -22 | -46 | -56* | -55* |
| FPS | -71** | 47 | 47 | -07 | 21 | -23 | -46 | -56* | -54* |
| SD | 33 | -26 | -25 | -11 | -29 | 11 | 24 | 19 | 17 |
| SV | 67** | -34 | -42 | -01 | -10 | 32 | 48 | 54* | 57* |
| FAB | 91** | -17 | -34 | 58* | 30 | 63** | 76** | 89** | 88** |
| DDT | 41 | -40 | -55* | -08 | -29 | 00 | 00 | 28 | 25 |
| MTI | -69** | 57* | 52* | 07 | 32 | -12 | -38 | -51* | -49 |
| EXT | 78** | -28 | -44 | 35 | 18 | 40 | 49 | 73** | 73** |
| RMAX | 53 | -30 | -27 | -05 | -15 | 19 | 37 | 38 | 40 |
| R/E | 34 | -30 | -13 | -25 | -31 | 05 | 28 | 16 | 18 |
| AREA | 59* | -31 | -32 | 09 | -05 | 23 | 39 | 49 | 50 |
| RT | 62* | -52* | -58* | -02 | -31 | 10 | 25 | 46 | 43 |
| WO | 64** | -47 | -62* | -00 | -26 | 15 | 24 | 48 | 46 |
| LV | 78** | -40 | -49 | 13 | -13 | 36 | 54* | 63* | 62** |
| BSI | 34 | -34 | -31 | -31 | -44 | 05 | 20 | 14 | 14 |
| PHG | 93** | -22 | -47 | 51* | 27 | 61* | 68** | 90** | 89** |
| MDT | 07 | -70** | -57* | -54* | -76** | -54* | -39 | -13 | -19 |
| ETP | 38 | -68** | -71** | -32 | -60* | -27 | -25 | 17 | 13 |
| FMS | 90** | -22 | -41 | 43 | 17 | 60** | 71** | 84** | 83** |
| PBW | 85** | -16 | -43 | 41 | 17 | 62** | 66** | 81** | 80** |
| SAP | 51* | 20 | -04 | 85** | 74** | 55* | 49 | 68** | 67** |
| TEG | 91** | -30 | -50* | 35 | 10 | 53* | 63** | 83** | 82** |
| BWE | 35 | -65** | -68** | -31 | -60* | -27 | -16 | 16 | 10 |

[1]Decimals are omitted.
[2]Amount of protein extracted using specified solvent expressed as a proportion of flour protein.
[3]Amount of protein extracted per gram of flour.
[4]Abbreviations as defined in Tables 4 and 7.
[5]*, ** Significant at P< 0.05 and 0.01 level, respectively.

TABLE 17. Cont.

| | Protein Solubility Classes | | | |
|---|---|---|---|---|
| | Residue Protein[1] | | | |
| | $H_2O$ | NaCl | EtOH | HAc |
| FP | 97** | 99** | 90** | 78** |
| PSI | -71** | -69** | -78** | -76** |
| FPS | -73** | -71** | -82** | -84** |
| SD | 34 | 32 | 47 | 53* |
| SV | 71** | 70** | 85** | 76** |
| FAB | 85** | 88** | 78** | 68** |
| DDT | 46 | 45 | 54* | 61* |
| MTI | -75** | -71** | -87** | -89** |
| EXT | 77** | 80** | 75** | 65* |
| RMAX | 53 | 52 | 65* | 61* |
| R/E | 36 | 32 | 54* | 53 |
| AREA | 61* | 60* | 67** | 61* |
| RT | 67** | 65** | 77** | 83** |
| WO | 68** | 67** | 79** | 83** |
| LV | 78** | 78** | 88** | 85** |
| BSI | 37 | 35 | 59* | 65** |
| PHG | 89** | 93** | 85** | 74** |
| MDT | 22 | 14 | 37 | 58* |
| ETP | 50* | 45 | 63** | 78** |
| FMS | 86** | 89** | 86** | 77** |
| PBW | 81** | 86** | 83** | 75** |
| SAP | 42 | 48 | 17 | 01 |
| TEG | 89** | 91** | 91** | 86** |
| BWE | 47 | 42 | 60* | 76** |

[1]Amount of protein (mg) left in the residue per g of flour.

significant (P<0.01) positive correlation with MTI (r=0.62) and negative correlation with RT (r=-0.67), MDT (r=-0.83), ETP (r=-0.79) and BWE (r=-0.78). For the Quad milled flours, the proportion of HAc soluble protein was positively correlated with SAP (r=0.74) and negatively correlated with MDT, ETP and BWE, but had lower r-values than those obtained for the Buhler milled flours. The highest correlation between any protein fraction variable and technological quality test was between HAc (normalized) and MDT (which is not correlated with FP), with r=-0.83 and 0.76 for Buhler and Quad milled flours, respectively. The proportion of HAc soluble protein was not correlated with LV for flours from both mills. The proportion of HAc soluble protein had a small but significant correlation (P<0.05) with PSI (r=-0.52), FPS (r=-0.53), SD (r=-0.59), SV (r=-0.51), DDT (r=0.57), WO (r=-0.61) and BSI (r=-0.59) for Buhler milled flours but not for the Quad milled flours.

These results are generally consistent with previous findings by others workers. MacRitchie (1979) observed that the amount protein extracted by single extraction with dilute HAc (0.002M) varied appreciably from one gluten sample to another and that the amount appeared to be correlated with baking performance of the parent flours as determined by the loaf volume index. Graybosch *et al* (1990) using samples of wheat lines with a wide range of breadmaking characteristics observed a small but significant positive correlation between potassium hydroxide soluble proteins - glutenins (after prior sequential extraction of samples with 0.04 *M* NaCl and 70 % EtOH) with MDT, MTI and LV with r-values of 0.39, 0.41 and 0.45, respectively. Using a sequential procedure, it was shown by Orth and Bushuk (1972) that the proportion of HAc soluble proteins (glutenin after NaCl and EtOH extraction) was negatively correlated with loaf volume per unit protein (ULV), LV, DDT and Zeleny

sedimentation value and positively correlated with MTI. They also observed that the proportion of residue protein (HAc insoluble after sequential extractions procedure) was positively correlated with ULV, LV, DDT and Zeleny sedimentation value.

The proportion of ethanol soluble protein was not significantly correlated with the breadmaking quality parameters for the Buhler milled flour, but significantly correlated ($P<0.05$) with FP ($r=0.56$), FAB ($r=0.58$), PHG ($r=0.51$), MDT ($r=-0.54$) and SAP ($r=0.85$) for the Quad milled flour. Graybosch *et al* (1990) observed that 70 % EtOH soluble protein was positively correlated with FP, FAB and DDT.

The proportion of sodium chloride extracted proteins was negatively correlated ($P< 0.01$) with FP, DDT, RT, WO, MDT, ETP and BWE for both types of flour. For the Buhler flour it was also negatively correlated ($P<0.01$) with PSI ($r=-0.56$); SV ($r=-$64); FAB ($r=-0.63$) and EXT ($r=-0.69$). Similar associations between salt-soluble proteins with breadmaking quality parameters were observed by Graybosch *et al* (1990). They observed that the proportion of salt-soluble protein was negatively correlated with FP ($r=-0.52$), FAB ($r=-0.58$), DDT ($r=-0.63$), LV ($r=-0.57$) and MDT ($r=0.32$). These workers did not observe any significant correlation between salt-soluble proteins and mixing or baking quality characteristics for the wheat lines that had undergone preliminary selection for mixing characteristics. It can be concluded from the observations in this study and those by Graybosch *et al* (1990) that an increase in the proportion of salt-soluble protein is associated with marked reductions in overall mixing and baking quality.

The proportion of water soluble proteins for flours from both mills were significantly correlated ($P<0.01$) with MDT, ETP and BWE. For the Quad milled flours, the water soluble proteins were also related to MTI ($r=0.57$) and RT ($r=-0.52$). In

general, the correlation coefficients were higher for the Quad milled flours. The data indicate that an increasing proportion of water soluble protein lowers the mixing characteristics of the flour.

Variation between the two mills in the level and significance of the correlation results may be attributed to the milling effects. The number of correlated relationships between technological quality and protein fractions was substantially higher for the Buhler milled flours ($n=37$) compared with Quad milled flours ($n=21$), particularly for HAc and NaCl fractions. As mentioned previously, the significant differences in terms of FP, FPS, SV and SD could have led to the differences observed in the correlations between the flours from the two mills. However, despite these differences there were some correlations that were similar to flours from both mills. Nevertheless the results indicate that accurate assessment of the technological quality of flours in terms of biochemical factors may be obscured for wheat experimentally milled on the Quad mill compared to the Buhler counterpart.

More technological parameters were significantly correlated ($P<0.01$) with the amount of residue protein than the normalized or unnormalized values for all the four solvents. The only parameters that did not show any significant correlation with $H_2O$, NaCl and EtOH residue proteins are SD, R/E, BSI, MDT and BWE. SAP was not related to any of the residue fractions for the Quad milled flour but showed significant correlation ($P<0.05$) with $H_2O$ and NaCl residue protein for the Buhler milled flour.

**9. Predicting mixograph parameters characteristics on the basis of protein fractions**

Stepwise multiple linear regression analysis was used to generate equations for predicting mixograph quality parameters from the protein solubility data. The amount

of supernatant and residue fractions, supernatant protein as a percentage of total flour protein and the total flour protein content were used as the independent variables while the MDT, ETP, PHG, TEG, and BWE plus the LV and BSI were used as dependent variables in the regression equations to generate one variable prediction models. FMS, PBW and SAP were omitted as dependent variables because of their high coefficients of variation ($CV_2$) and low $CV_1/CV_2$ values (see Tables 7 and 8) which indicated that they were less reliable as discussed above (Section B:3). A combination of the independent variables was also used to generate two variable prediction equations. The results are reported in Tables 18 and 19 for Buhler and Quad milled flours, respectively. The best three variables that showed $R^2$ values with significance below the 1 % probability level are reported. In all cases, model parameters are only listed if they contribute significantly ($P < 0.01$) to the model.

The $R^2$ values for Buhler flours ranged from 0.47 to 0.92, the corresponding range for the Quad flours was 0.42 to 0.88 for the single variable models. Some of the models showed very high predictive capacity as indicated by the high coefficients of determination using both mills. For example, for the dependent mixograph parameters PHG and TEG, single variable regression models using the predictor (independent variable) R-NaCl (the amount of NaCl residue protein) resulted in model $R^2$ values of 0.92 and 0.86 for Buhler milled flours and 0.83 and 0.86 for Quad milled flours. The percentage of acetic acid soluble protein also had a relatively high coefficient of determination with MDT for Buhler milled flour ($R^2 = 0.70$), but lower $R^2$-value (0.59) for the Quad milled flour. Using 250 lines (1974 crop season) and 328 lines (1975 crop season), Kosmolak and Baker (1975) found that a linear function of flour protein content and MDT was the most suitable for predicting remix loaf volume. While

consideration of the relative magnitudes of these two characteristics was best for predicting BSI. However, Lukow (1991) reported that through regression analysis of thousands of samples, it appears that parameters such as PHG may be more important in predicting baking quality than the MDT. It should be noted, however, that this study found PHG (among other mixograph parameters) to be highly correlated with flour protein content.

The relationship between the observed and predicted MDT in this study for Buhler milled flour using the prediction equation in Table 18 is illustrated in Fig. 9. A similar relationship for LV using acetic acid insoluble protein as the independent variable is illustrated in Fig. 10. Generally for one variable models the Buhler milled flour gave a higher $R^2$ values than the Quad flours. This may be attributed to the total amount of protein extracted from the two type of flours.

Combination of the variables increased the $R^2$ values as can be seen from the two variable models for both types of flour. The combination of acetic acid soluble protein and R-NaCl proteins gave high coefficient of determinations for both MDT and ETP for flours from both mills. The $R^2$-values were 0.85 and 0.86 for Buhler and 0.78 and 0.82 for Quad milled flours, respectively. The correlation between observed and predicted MDT for Buhler flour using the amount of HAc and NaCl insoluble protein as the independent variable is illustrated in Fig. 11. It can be observed that the predicted values are highly correlated with the observed MDT's. A similar relationship is illustrated for LV (Fig. 12) using the amount of water soluble protein and acetic acid insoluble protein as the independent variable.

A number of models (Table 20), were common for Buhler and Quad milled flours. Acetic acid soluble and residue and sodium chloride residue proteins can explain

TABLE 18. Linear least square regression models for mixograph parameters[1] and loaf volume as a function of protein fraction variables of Buhler milled flours.

| P[2] | Regression coefficient | b[3] | $R^2$ | Prob >F |
|---|---|---|---|---|
| **Single Variable Models** | | | | |
| MDT | (i) -41.10 (% HAc) | 30.37 | .70 | .0001 |
| | (ii) -26.72 (% $H_2O$) | 8.99 | .36 | .0144 |
| | (iii) -40.16 (% NaCl) | 10.08 | .26 | .0449 |
| ETP | (i) -363.86 (% HAc) | 265.98 | .63 | .0002 |
| | (ii) 1.37 (R[4]-HAc) | -28.58 | .48 | .0029 |
| | (iii) -481.39 (% NaCl) | 108.82 | .43 | .0062 |
| PHG | (i) .003 (R-NaCl) | -.09 | .92 | .0005 |
| | (ii) .002 (Fpro) | -.12 | .92 | .0001 |
| | (iii) .0056 (R-EtOH) | -.15 | .89 | .0001 |
| TEG | (i) 2.08 (R-EtOH) | -52.93 | .90 | .0001 |
| | (ii) 2.39 (R-HAc) | -26.90 | .88 | .0001 |
| | (iii) .97 (R-NaCl) | -27.41 | .86 | .0001 |
| BWE | (i) -286.04 (% HAc) | 208.93 | .61 | .0004 |
| | (ii) 1.07 (R-HAc) | -22.17 | .45 | .0042 |
| | (iii) -379.91 (%-NaCl) | 85.65 | .41 | .0071 |
| LV | (i) 21.41 (R-HAc) | -103.22 | .85 | .0001 |
| | (ii) 17.97 (R-EtOH) | -298.80 | .80 | .0001 |
| | (iii) 8.49 (R-NaCl) | -89.50 | .79 | .0001 |
| BSI | (i) -241.89 (% HAc) | 256.89 | .35 | .0159 |
| | (ii) 0.946 (R-HAc) | 59.81 | .29 | .0329 |
| | (iii) -299.94 (% NaCl) | 148.82 | .21 | .0764 |
| **Two Variable Models** | | | | |
| MDT | -0.36 (HAc)+0.27 (R-NaCl) | 4.49 | .85 | .0004 |
| | -37.35 (% HAc)-26.40 (% NaCl) | 32.57 | .80 | .0001 |
| | -10.66 (% $H_2O$)-35.21 (% HAc) | 28.86 | .74 | .0002 |
| ETP | -3.09 (HAc)+2.70 (R-NaCl) | 17.33 | .86 | .0001 |
| | -311.77 (% HAc)-366.54 (%NaCl) | 296.60 | .86 | .0001 |
| | -4.26 (NaCl)+2.17 (R-HAc) | 26.41 | .80 | .0001 |
| PHG | 0.369 (% EtOH)+0.006 (R-EtOH) | -.329 | .95 | .0001 |
| | 0.002 (Fpro)+.002 (R-EtOH) | -.143 | .94 | .0001 |
| | 0.004 (Fpro)-0.002 (EtOH) | -.143 | .94 | .0001 |
| TEG | 1.16 (R-HAc)+1.17 (R-EtOH) | -45.72 | .93 | .0001 |
| | 0.76 ($H_2O$)+2.06 (R-HAc) | -34.13 | .93 | .0001 |
| | 1.40 (R-HAc)+0.46 (R-NaCl) | -32.66 | .93 | .0001 |
| BWE | -2.45 (HAc)+2.03 (R-NaCl) | 14.17 | .84 | .0001 |
| | -244.87 (% HAc)-289.71 (%NaCl) | 233.12 | .84 | .0001 |
| | -3.43 (NaCl)+1.71 (R-HAc) | 22.47 | .74 | .0002 |
| LV | 7.12 ($H_2O$)+18.33 (R-HAc) | -171.14 | .90 | .0001 |
| | 1080.70 (% $H_2O$)+22.30 (R-HAc) | -382.54 | .89 | .0001 |
| | 11.05 (HAc)-2891.52 (% HAc) | 1798.16 | .87 | .0001 |
| BSI | -0.96 (R-$H_2O$)+2.77 (R-HAc) | 75.11 | .51 | .0100 |
| | 0.86 ($H_2O$)-288.04 (% HAc) | 265.46 | .51 | .0102 |
| | 156.09 (% $H_2O$)-328.25 (% HAc) | 279.05 | .48 | .0140 |

[1]Abbreviation as defined in Table 7.
[2]P=Quality parameters.
[3]b=intercept.
[4]R=Amount of protein left in the residue.

TABLE 19. Linear least square regression models for mixograph parameters[1] and loaf volume as a function of protein fraction variables of Quad milled flours.

| P[2] | Regression coefficient | b[3] | $R^2$ | Prob >F |
|---|---|---|---|---|
| | **Single Variable Models** | | | |
| MDT | (i) -27.27 (% HAc) | 19.98 | .59 | .0006 |
| | (ii) -27.62 (% $H_2O$) | 8.64 | .49 | .0027 |
| | (iii) 0.11 (R-HAc) | -2.04 | .34 | .0006 |
| ETP | (i) 1.38 (R[4]-HAc) | -38.73 | .61 | .0003 |
| | (ii) -455.57 (% NaCl) | 98.47 | .50 | .0022 |
| | (iii) -249.80 (% $H_2O$) | 74.66 | .46 | .0037 |
| PHG | (i) 0.0025 (Fpro) | -.132 | .88 | .0001 |
| | (ii) 0.0027 (R4-NaCl) | -.106 | .86 | .0001 |
| | (iii) 0.0001 (EtOH) | -.046 | .81 | .0001 |
| TEG | (i) .9008 (Fpro) | -42.21 | .84 | .0001 |
| | (ii) .990 (R-NaCl) | -33.42 | .83 | .0001 |
| | (iii) 1.270 (EtOH) | -7.12 | .68 | .0001 |
| BWE | (i) 1.059 (R-NaCl) | -29.75 | .58 | .0006 |
| | (ii) -346.42 (%NaCl) | -74.94 | .47 | .0036 |
| | (ii) -188.97 (% $H_2O$) | 56.63 | .43 | .0061 |
| LV | (i) 20.71 (R-EtOH) | -577.74 | .77 | .0001 |
| | (ii) 23.45 (R-HAc) | -333.96 | .73 | .0001 |
| | (iii) 7.88 (Fpro) | -227.68 | .61 | .0001 |
| BSI | (i) 1.53 (R-HAc) | 22.41 | .42 | .0064 |
| | (ii) 1.83 (R-EtOH) | 17.07 | .34 | .0173 |
| | (iii) -200.97 (% HAc) | 216.48 | .21 | .0749 |
| | **Two Variable Models** | | | |
| MDT | -0.224 (HAc)+0.183 (R-NaCl) | 1.39 | .78 | .0001 |
| | -24.36 (%HAc)-30.81 (% NaCl) | 23.26 | .77 | .0001 |
| | -0.335 (NaCl)+0.16 (R-NaCl) | 2.54 | .74 | .0001 |
| ETP | -1.86 (HAc)+1.75 (R-NaCl) | -12.73 | .82 | .0001 |
| | -2.21 (NaCl)+1.68 (R-HAc) | -8.57 | .82 | .0001 |
| | -0.46 (EtOH)+1.09 (R-HAc) | -33.94 | .74 | .0001 |
| PHG | 0.0026 (Fpro)+0.18 (% $H_2O$) | -0.183 | .89 | .0001 |
| | 0.0023 (Fpro)+0.001 ($H_2O$) | -0.146 | .89 | .0001 |
| | 0.0039 (Fpro)-0.002 (R-$H_2O$) | -0.146 | .89 | .0001 |
| TEG | 2.022 ($H_2O$)+2.12 (HAc) | -78.90 | .91 | .0001 |
| | 1.151 ($H_2O$)+1.89 (R-EtOH) | -80.68 | .89 | .0001 |
| | 1.788 (NaCL)+1.81 (R-EtOH) | -82.27 | .88 | .0001 |
| BWE | -1.46 (HAc)+1.36 (R-NaCl) | -8.93 | .79 | .0001 |
| | -1.75 (NaCl)+1.29 (R-HAc) | -5.86 | .78 | .0001 |
| | 1.41 (Fpro)- 1.72 (HAc) | -22.12 | .72 | .0002 |
| LV | 13.08 ($H_2O$)+22.86 (R-HAc) | -628.59 | .82 | .0001 |
| | 2079.86 (% $H_2O$)+30.36 (R-HAc) | -1072.36 | .80 | .0001 |
| | 19.49 (NaCl)+20.83 (R-HAc) | -599.93 | .79 | .0001 |
| BSI | 3.67 (R-EtOH)-1.27 (R-NaCl) | -11.21 | .60 | .0026 |
| | -1.25 (R-$H_2O$)+3.62 (R-EtOH) | -15.93 | .57 | .0042 |
| | -0.81 (HAc)+2.25 (R-EtoH) | 10.76 | .55 | .0053 |

[1]Abbreviation as defined in Table 7.
[2]P=Quality parameters.
[3]b=Intercept.
[4]R=Amount of protein left in the residue.

TABLE 20. Linear least square regression models for mixograph parameters[1] and loaf volume as a function of protein fraction variables common to both Buhler and Quad milled flours.

| P[2] | Common Independent Variable | Buhler ($R^2$) | Quad ($R^2$) |
|---|---|---|---|
| | **Single Variable Models** | | |
| MDT | (i) % HAc | .70 | .59 |
| | (ii) % $H_2O$ | .36 | .49 |
| ETP | (i) $R^3$-HAc | .48 | .50 |
| | (ii) % NaCl | .43 | .46 |
| PHG | (i) R-NaCl | .92 | .88 |
| | (ii) Fpro | .92 | .86 |
| TEG | (i) R-NaCl | .86 | .83 |
| BWE | (ii) R-HAc | .45 | .58 |
| | (iii) %-NaCl | .41 | .47 |
| LV | (i) R-HAc | .78 | .42 |
| | (ii) R-EtOH | .47 | .39 |
| BSI | (i) % HAc | .35 | .51 |
| | (ii) R-HAc | .29 | .51 |
| | (iii) % NaCl | .21 | .48 |
| | **Two Variable Models** | | |
| MDT | (i) HAc and R-NaCl | .85 | .78 |
| | (ii) % HAc and % NaCl | .80 | .77 |
| ETP | (i) HAc and R-NaCl | .86 | .82 |
| | (ii) % HAc and % NaCl | .80 | .74 |
| PHG | none | --- | --- |
| TEG | none | --- | --- |
| BWE | (i) HAc and R-NaCl | .84 | .79 |
| | (ii) NaCl and R-HAc | .74 | .78 |
| LV | (i) $H_2O$ and R-HAc | .90 | .82 |
| | (ii) % $H_2O$ and R-HAc | .89 | .80 |
| BSI | none | --- | --- |

[1]Abbreviation as defined in Table 7.
[2]P=Quality parameters.
[3]R=Amount of protein left in the residue.

FIGURE 9. The relationship between the predicted and observed mixograph development time (MDT) for Buhler flour using the amount of acetic acid soluble proteins as the independent variable.

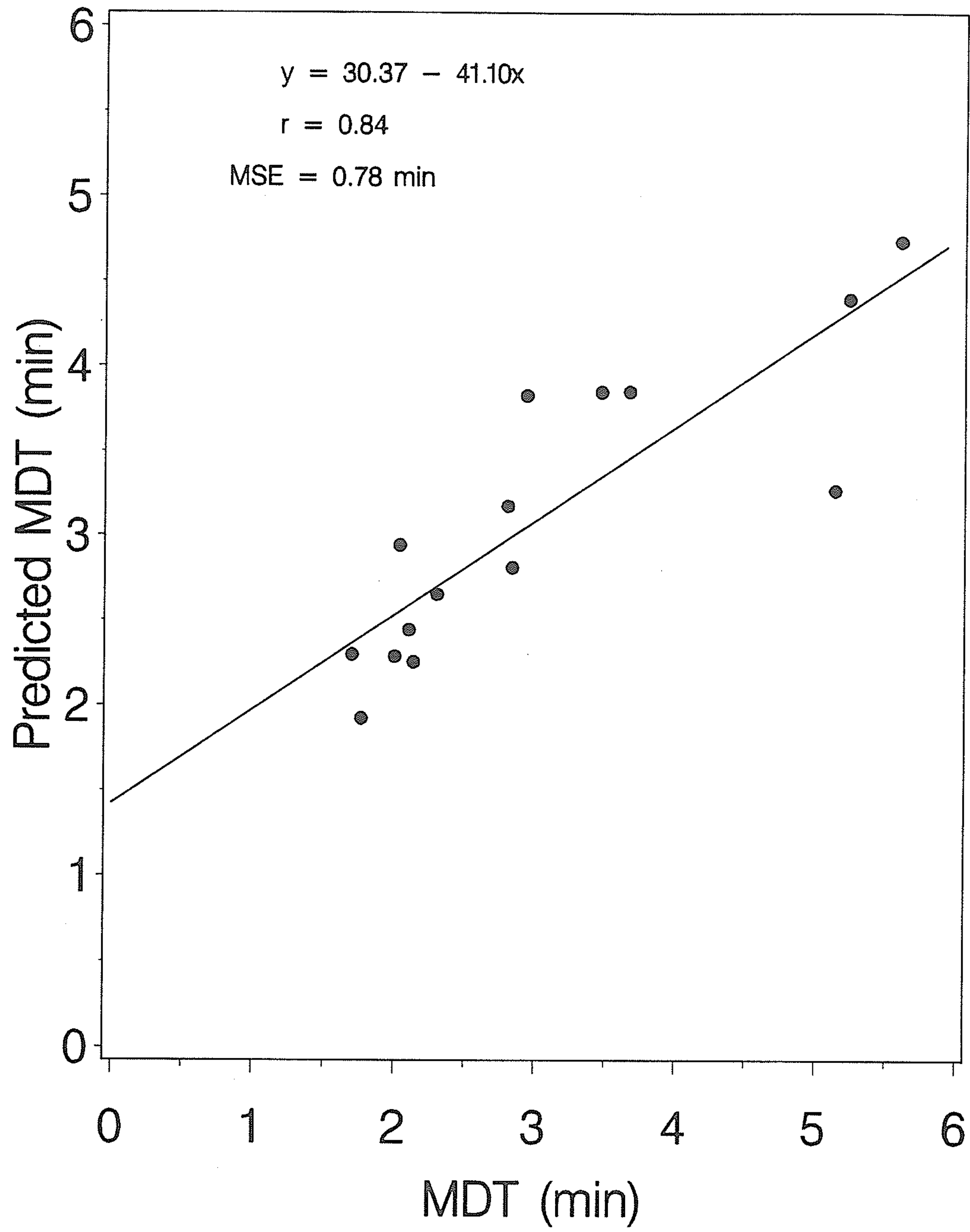
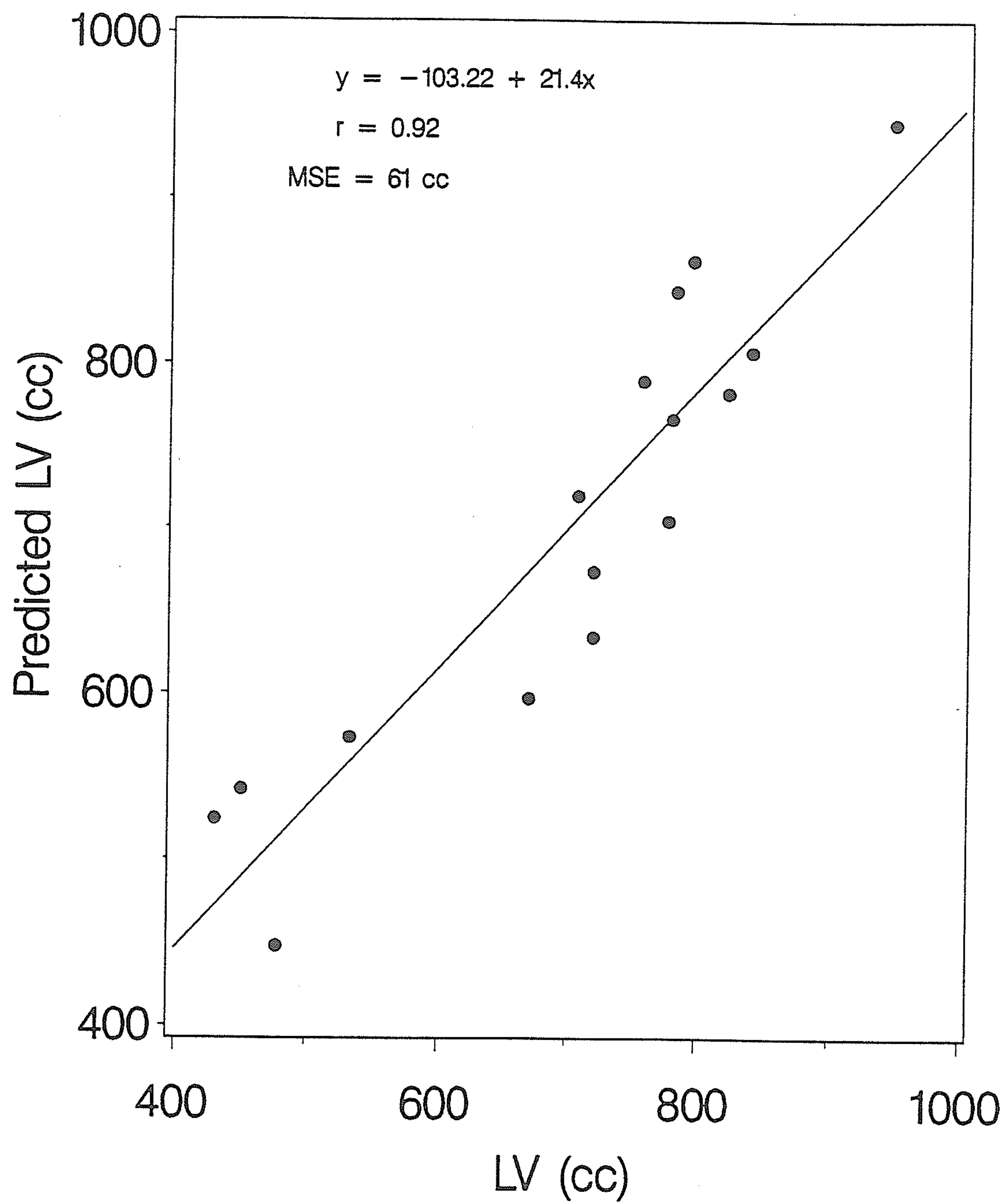
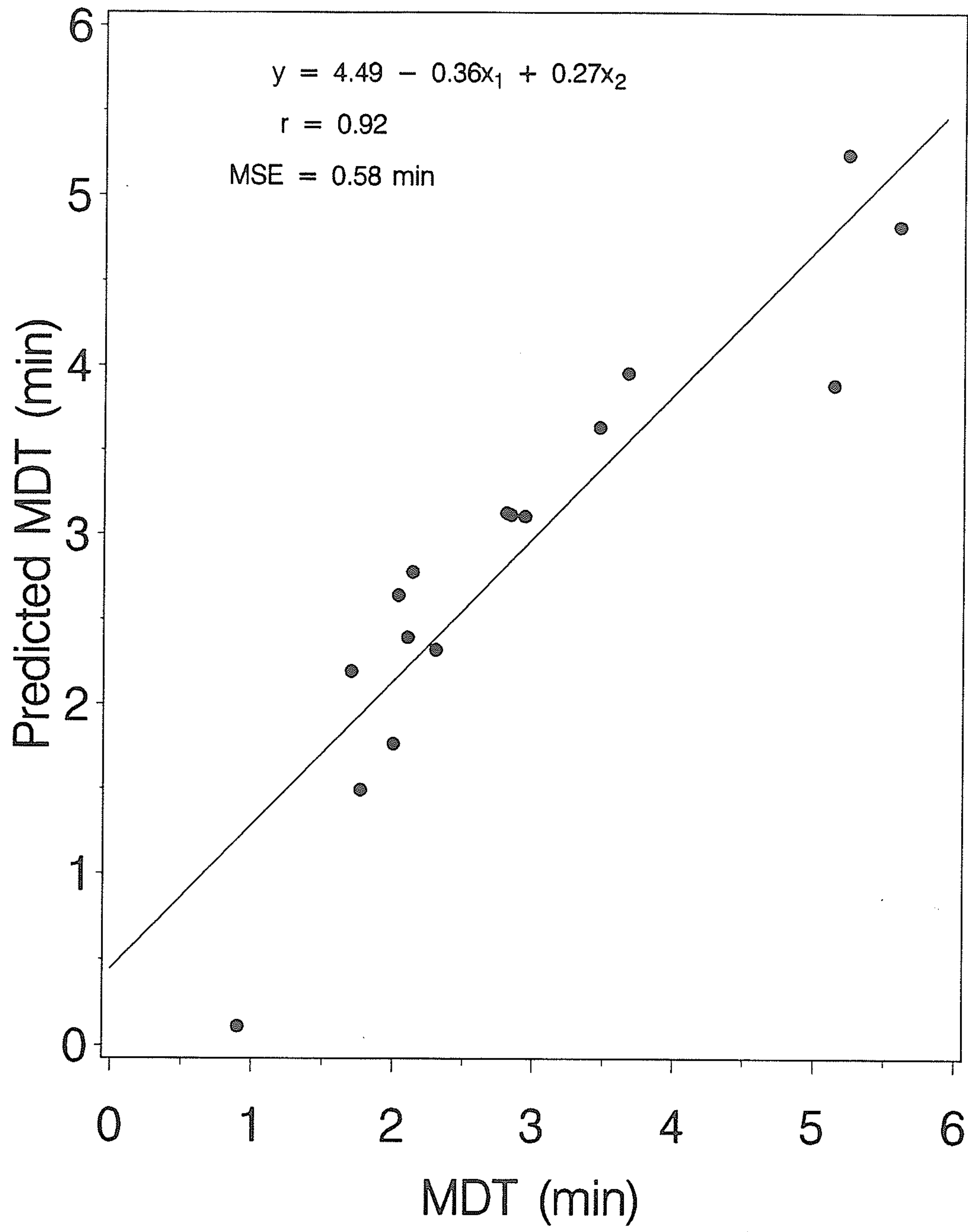

y = 30.37 − 41.10x
r = 0.84
MSE = 0.78 min
Predicted MDT (min)
MDT (min)
0
1
2
3
4
5
6

FIGURE 10. The relationship between the predicted and observed loaf volume (LV) for Buhler milled flour using the amount of acetic acid insoluble (residue) protein as the independent variable.



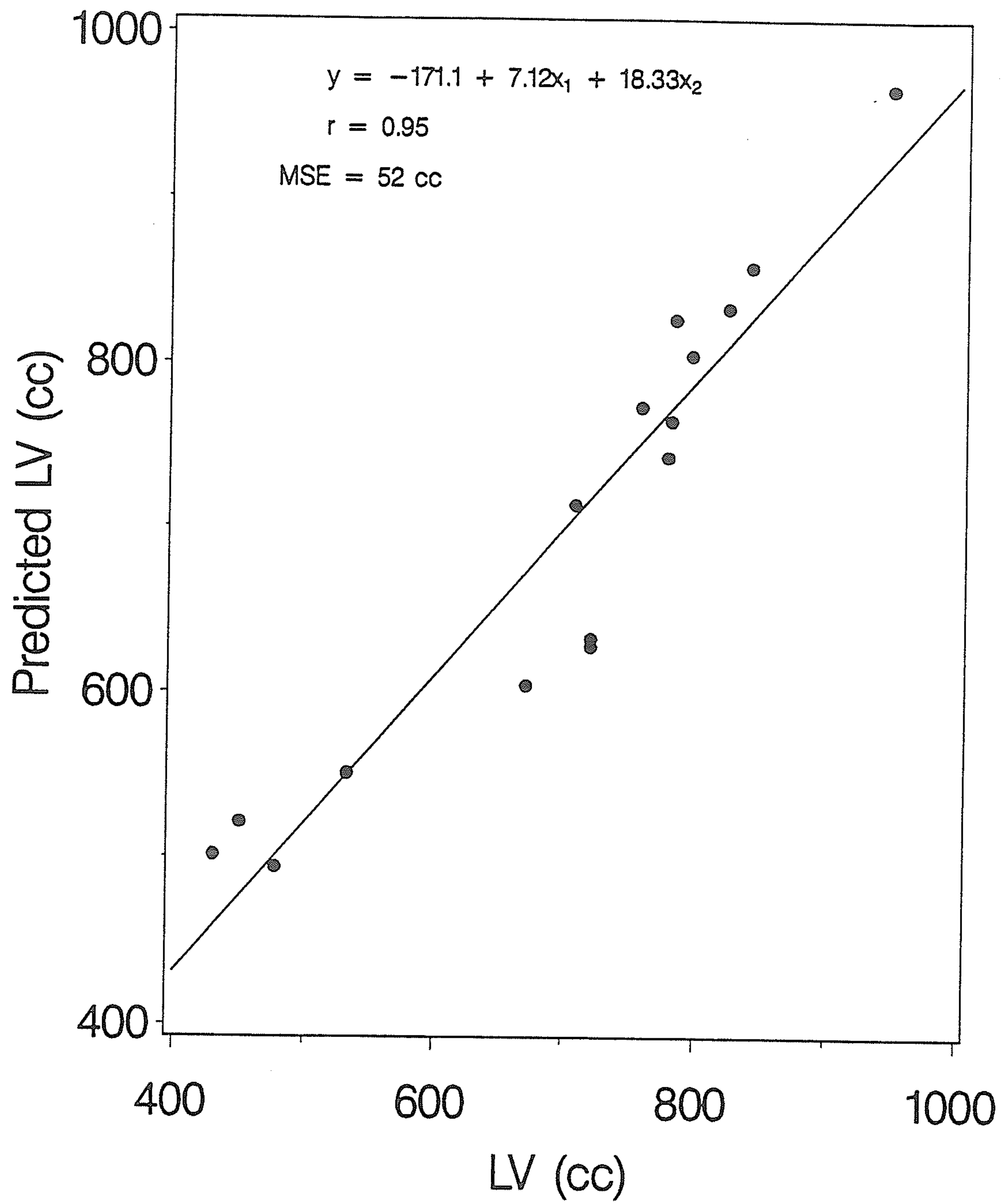
y = −103.22 + 21.4x
r = 0.92
MSE = 61 cc
Predicted LV (cc)
LV (cc)
400
600
800
1000

FIGURE 11. The relationship between the predicted and observed mixograph development time (MDT) for Buhler flour using the amount of acetic acid soluble and sodium chloride residue protein as the independent variables.



y = 4.49 − 0.36x1 + 0.27x2
r = 0.92
MSE = 0.58 min
Predicted MDT (min)
MDT (min)
0
1
2
3
4
5
6

FIGURE 12. The relationship between the predicted and observed loaf volume (LV) for Buhler flour using the amount of water soluble and acetic acid insoluble (residue) protein as the independent variables.



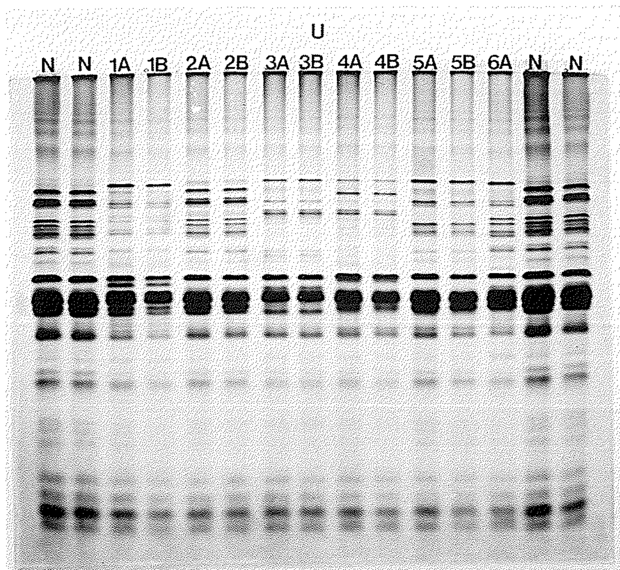
$y = -171.1 + 7.12x_1 + 18.33x_2$
$r = 0.95$
MSE = 52 cc
1000
800
600
400
Predicted LV (cc)
400
600
800
1000
LV (cc)

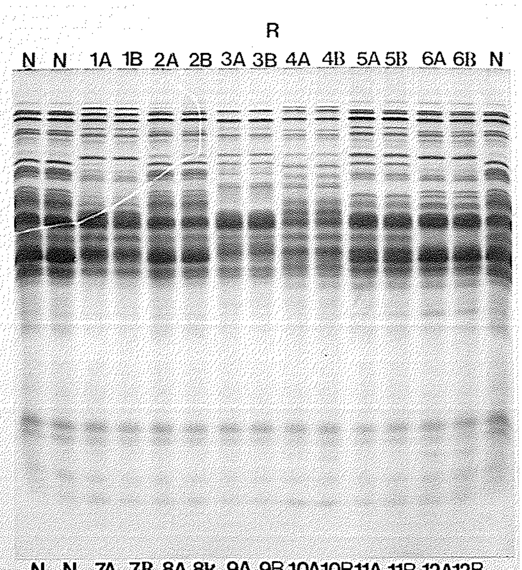
most of the variations observed in both mills. For example 70 % and about 60 % (for Buhler and Quad mills, respectively) of the variations in MDT could be explained by the amount of HAc soluble protein. While NaCl residue proteins could explain over 80 % of the variations observed in ETP and PHG for both mills. Loaf volume was highly correlated with acetic acid residue proteins with $R^2$ values of 0.85 and 0.73 for Buhler and Quad milled flours, respectively. For the two variable models HAc and NaCl residue proteins could explain over 78 % of the variations in MDT, ETP and BWE. Water soluble and HAc residue proteins could explain 90 % and 82 % of the variations in loaf volume for Buhler and Quad milled flours, respectively.

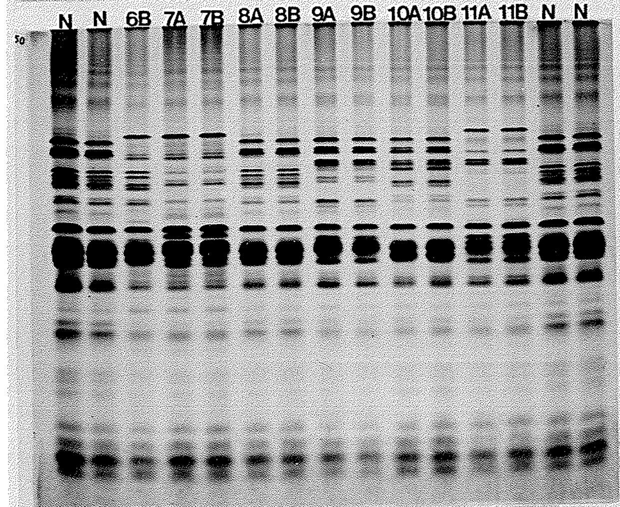
### 10. Electrophoretic patterns of the Buhler and Quad flours

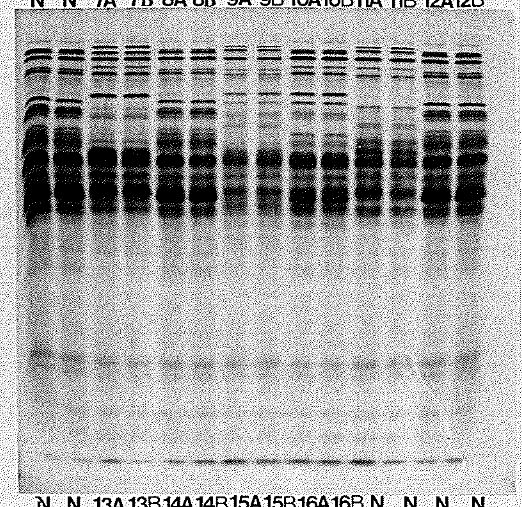
As discussed in Section IV:B, there were some differences between Buhler and Quad milled flours on a sample by sample basis. For example, more protein was extracted from the Buhler milled flours than from the Quad milled flours (Figs. 7 and 8). SDS-PAGE was performed to determine whether there were any differences in the protein composition of flours from these two mills. Results (Fig. 13) showed that the electrophoretic patterns of the proteins from the two mills were identical under both reduced and non-reducing conditions. The results show that the protein composition was not affected by milling. Differences in electrophoretic patterns between the samples represent normal genotypic differences (Ng *et al*, 1988). Other researchers (refer to Section II) have reported that differences in flour extraction rates (flour yield) affect the type and proportion of protein fractions that are extracted from the flour. Apparently the different extraction rates, averaging 72.0 % for the Buhler and 63.7 % for the Quad mills, did not affect the SDS-PAGE patterns of SDS extracted fractions.

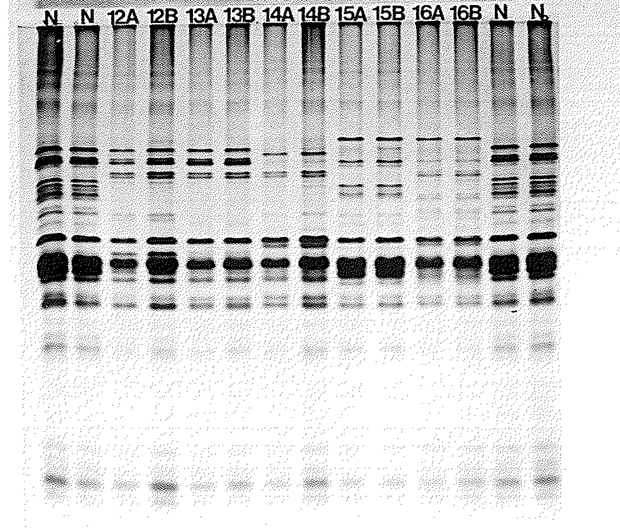
FIGURE 13. The SDS-PAGE patterns of SDS extract from (**A**) Buhler and (**B**) Quad milled flours. The number of the samples corresponds to the samples as defined in Table 1. N=control flour (Neepawa). **U**=Unreduced and **R**=Reduced (by mercaptoethanol) extracts.

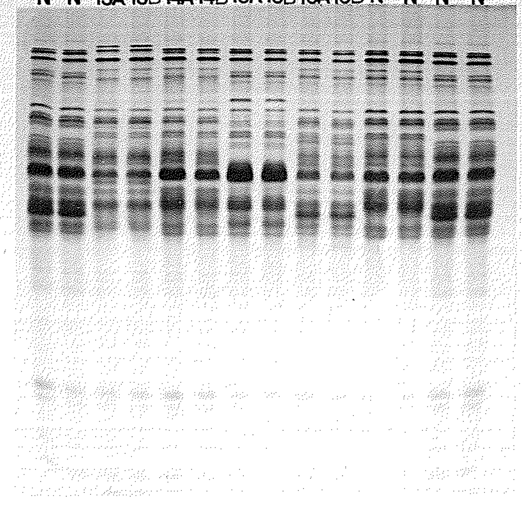

U
N N 1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A N N


R
N N 1A 1B 2A 2B 3A 3B 4A 4B 5A 5B 6A 6B N


N N 6B 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B N N


N N 7A 7B 8A 8B 9A 9B 10A 10B 11A 11B 12A 12B


N N 12A 12B 13A 13B 14A 14B 15A 15B 16A 16B N N


N N 13A 13B 14A 14B 15A 15B 16A 16B N N N N

## C. MIXING CHARACTERISTICS AND PROTEIN SOLUBILITY DISTRIBUTION OF THE 1983 UQN SAMPLES

The 1983 UQN samples (n = 26) represented wheat varieties with a very narrow range of protein content. The grain protein content range from 13.2-15.5 % as shown in Table 21. Accordingly, these samples are representative of selected late generation lines in a hard red spring wheat breeding programme. However, the breadmaking quality characteristics of the samples were very diverse, from very poor to very good as shown by the technological data in Table 30, Appendix VI (page 191). The purpose of carrying out this part of the study was to (i) compare the mixing behaviour and protein solubility between the samples used in the first part (samples of broad range of protein content) with the 1983 UQN samples (of narrow range of protein content) and (ii) examine whether the prediction equations developed in part B of the study could be used to predict the mixing characteristics of the UQN samples. The UQN samples were milled on the Quad mill. Accordingly, the discussion and prediction equations for the 16 Quad milled flours described previously will be used for comparison.

### 1. Dough mixing characteristics

The mixing characteristics of the UQN samples were examined using the micromixograph; other technological and baking quality tests could not be performed due to the limited amount of sample available. Inspite of the narrow range of protein content (Table 21), the mixing properties were very diverse (Table 22). For example, MDT ranged from 1.5 to 5.7 min and PHG was 0.07-0.11 Nm compared with the 16 Quad samples where the MDT was 1.1-5.2 min and PHG was 0.10-0.28 Nm. All the other mixograph parameters had lower values compared with the samples used in

TABLE 21. Some milling properties[1] of the 1983 UQN Quad milled samples.

| Sample | GP (%) | FY (%) | FP (%) | ASH (%) |
|---|---|---|---|---|
| UQN 099 | 14.7 | 65.5 | 13.9 | 0.45 |
| UQN 130 | 15.0 | 64.5 | 14.4 | 0.47 |
| UQN 176 | 14.2 | 61.0 | 12.6 | 0.60 |
| UQN 199 | 15.5 | 66.5 | 14.8 | 0.49 |
| UQN 200 | 14.2 | 67.0 | 13.8 | 0.47 |
| UQN 201 | 13.2 | 62.5 | 12.4 | 0.43 |
| UQN 203 | 14.0 | 65.0 | 13.3 | 0.48 |
| UQN 204 | 14.2 | 63.5 | 13.5 | 0.49 |
| UQN 205 | 13.2 | 62.5 | 12.6 | 0.53 |
| UQN 206 | 14.4 | 64.5 | 13.6 | 0.49 |
| UQN 207 | 14.0 | 64.0 | 13.9 | 0.55 |
| UQN 208 | 14.0 | 64.5 | 13.3 | 0.53 |
| UQN 209 | 14.1 | 66.0 | 13.6 | 0.53 |
| UQN 210 | 13.8 | 62.0 | 12.9 | 0.47 |
| UQN 211 | 13.9 | 67.0 | 13.0 | 0.51 |
| UQN 212 | 13.7 | 63.0 | 13.4 | 0.49 |
| UQN 213 | 13.9 | 64.5 | 12.5 | 0.53 |
| UQN 214 | 13.6 | 64.0 | 13.1 | 0.51 |
| UQN 215 | 13.9 | 66.0 | 12.8 | 0.54 |
| UQN 216 | 13.9 | 60.0 | 11.9 | 0.44 |
| UQN 217 | 13.3 | 66.5 | 12.4 | 0.52 |
| UQN 218 | 14.7 | 66.5 | 13.7 | 0.49 |
| UQN 219 | 14.0 | 59.0 | 12.4 | 0.41 |
| UQN 220 | 13.2 | 66.0 | 12.2 | 0.52 |
| UQN 221 | 15.2 | 52.0 | 13.8 | 0.42 |
| UQN 222 | 15.4 | 68.0 | 14.9 | 0.53 |

[1]GP=Grain protein content (%); FY=Flour yield (%); FP=Flour protein content (%); ASH=Flour ash content (%). All values are based on 14 % mb. The average variation between duplicate determinations of GP, FP and ASH were 0.16 %, 0.25 % and 0.10 %, respectively.

TABLE 22. Mixograph quality parameters[1] of the 1983 UQN samples.

| SAMPLE | PHG | MDT | ETP | FMS | PBW | SAP | TEG | BWE |
|---|---|---|---|---|---|---|---|---|
| UQN 099 | 0.10±.01 | 3.4±0.2 | 14.7±1.0 | 0.05±.00 | 0.06±.01 | 0.005±.001 | 48.8±1.9 | 10.5±1.0 |
| UQN 130 | 0.09±.01 | 2.2±0.2 | 9.2±1.1 | 0.05±.01 | 0.06±.00 | 0.005±.002 | 45.9±1.6 | 6.5±0.8 |
| UQN 176 | 0.08±.00 | 2.1±0.2 | 7.6±0.2 | 0.04±.01 | 0.05±.01 | 0.005±.001 | 37.9±1.7 | 5.5±0.2 |
| UQN 199 | 0.10±.01 | 2.4±0.1 | 10.4±1.5 | 0.05±.01 | 0.07±.01 | 0.006±.002 | 47.8±2.8 | 8.2±0.5 |
| UQN 200 | 0.10±.01 | 2.9±0.1 | 13.0±0.8 | 0.04±.01 | 0.07±.00 | 0.009±.001 | 48.1±3.0 | 10.2±0.5 |
| UQN 201 | 0.09±.01 | 3.7±0.5 | 14.6±2.2 | 0.03±.01 | 0.06±.01 | 0.004±.003 | 43.4±3.4 | 11.5±0.4 |
| UQN 203 | 0.10±.01 | 2.7±0.5 | 11.7±2.2 | 0.05±.00 | 0.07±.02 | 0.006±.002 | 48.7±2.2 | 9.4±1.1 |
| UQN 204 | 0.08±.00 | 3.4±0.2 | 12.5±1.2 | 0.03±.00 | 0.05±.01 | 0.003±.002 | 40.1±1.5 | 9.4±1.1 |
| UQN 205 | 0.08±.00 | 3.5±0.4 | 13.2±1.8 | 0.04±.00 | 0.05±.01 | 0.004±.001 | 41.0±1.8 | 10.0±0.3 |
| UQN 206 | 0.11±.01 | 2.5±0.2 | 11.8±1.4 | 0.05±.01 | 0.08±.01 | 0.007±.002 | 50.6±2.7 | 9.9±1.2 |
| UQN 207 | 0.09±.01 | 4.0±0.5 | 16.0±1.4 | 0.04±.01 | 0.05±.01 | 0.005±.002 | 43.7±3.3 | 11.3±1.2 |
| UQN 208 | 0.08±.01 | 2.9±0.3 | 11.0±0.7 | 0.04±.01 | 0.05±.00 | 0.003±.001 | 42.5±2.2 | 7.7±0.8 |
| UQN 209 | 0.08±.01 | 3.3±0.1 | 12.3±0.5 | 0.03±.00 | 0.05±.01 | 0.004±.001 | 41.4±2.6 | 9.2±1.3 |
| UQN 210 | 0.08±.01 | 5.6±0.6 | 20.4±1.1 | 0.03±.01 | 0.05±.01 | 0.002±.001 | 39.7±2.9 | 15.8±1.6 |
| UQN 211 | 0.09±.01 | 4.4±0.2 | 19.2±1.7 | 0.04±.01 | 0.07±.01 | 0.005±.002 | 47.8±3.3 | 16.2±2.4 |
| UQN 212 | 0.08±.01 | 3.5±0.4 | 12.7±1.5 | 0.04±.01 | 0.05±.01 | 0.003±.001 | 39.8±3.3 | 9.6±0.9 |
| UQN 213 | 0.08±.01 | 3.0±0.3 | 10.7±0.8 | 0.03±.01 | 0.06±.02 | 0.003±.001 | 41.4±3.6 | 8.7±1.5 |
| UQN 214 | 0.09±.01 | 3.6±0.1 | 14.9±1.0 | 0.04±.00 | 0.07±.02 | 0.003±.002 | 46.4±3.9 | 12.9±3.1 |
| UQN 215 | 0.09±.01 | 2.8±0.1 | 10.6±0.6 | 0.04±.01 | 0.07±.02 | 0.009±.002 | 42.0±4.4 | 8.8±2.1 |
| UQN 216 | 0.07±.01 | 3.7±0.6 | 12.3±0.7 | 0.03±.01 | 0.05±.00 | 0.004±.001 | 36.7±4.6 | 9.3±0.7 |
| UQN 217 | 0.07±.00 | 2.4±0.2 | 7.5±1.1 | 0.03±.00 | 0.05±.01 | 0.005±.001 | 35.1±3.2 | 6.0±1.8 |
| UQN 218 | 0.11±.01 | 2.0±0.1 | 9.2±0.9 | 0.06±.01 | 0.08±.02 | 0.007±.002 | 50.8±3.7 | 7.6±1.0 |
| UQN 219 | 0.07±.00 | 1.5±0.3 | 5.2±1.3 | 0.04±.00 | 0.06±.01 | 0.006±.003 | 35.1±0.9 | 4.6±0.9 |
| UQN 220 | 0.10±.00 | 2.3±0.2 | 9.9±0.4 | 0.05±.01 | 0.07±.02 | 0.005±.001 | 51.0±6.1 | 8.2±1.6 |
| UQN 221 | 0.09±.00 | 2.1±0.2 | 10.0±1.0 | 0.06±.00 | 0.07±.02 | 0.007±.001 | 46.5±2.2 | 9.1±2.8 |
| UQN 222 | 0.11±.00 | 3.2±0.2 | 15.2±0.8 | 0.05±.00 | 0.07±.01 | 0.006±.002 | 52.6±0.8 | 11.1±1.1 |
| Mean[2] | .09±.01 | 3.0±0.3 | 11.7±1.1 | 0.04±.01 | 0.60±.01 | 0.005±.001 | 44.0±2.8 | 9.5±1.2 |
| SD | .01±.00 | 0.9±0.2 | 4.0±.51 | 0.01±.00 | 0.01±.00 | 0.002±.001 | 5.1±1.1 | 2.7±.73 |
| $CV_1$ | 13.5 | 28.5 | 34.3 | 20.0 | 16.5 | 40.0 | 11.6 | 28.3 |
| $CV_2$ | 6.7 | 8.9 | 9.5 | 12.5 | 18.3 | 20.0 | 6.4 | 12.8 |
| $CV_1/CV_2$ | 2.0 | 3.2 | 3.6 | 1.6 | 1.0 | 2.0 | 1.9 | 2.2 |

[1]Abbreviations as defined in Table 7.
[2]Mean of triplicate determinations.

the first part of the study. As was found previously (see Table 8), the coefficient of variation for mixograph parameters ($CV_1$) was quite variable. However, the $CV_1$ range was 25-70 for the Quad samples and 11.57-40 for the UQN samples an indication of the range in mixograph characteristics between these two set of samples. But most mixograph parameters had higher $CV_2$ for the UQN samples than for the 16 Quad samples. FMS, PBW, SAP and BWE had $CV_2$ values greater than 10.0 and a low $CV_1/CV_2$ ratio. As discussed earlier (see section IV:B:4) the higher the $CV_2$ (and the lower the $CV_1/CV_2$ ratio) the lower the precision of the instrument in measuring that particular parameter. However, separate but similar micromixographs were used, unintentionally, to determine the mixing characteristics of the UQN and the samples used in the first part of this study. Based on data for set of standard samples, the two machines did not give similar absolute mixing quality values (Table 23). This is in agreement with the results of Pon *et al* (1989). The difference in mixograph values obtained may be attributed to the transducers used in the individual mixographs. The correlation between the mixograph parameters of the equipments varied widely from a high of 0.97 for TEG to a low of 0.36 for FMS as shown in Table 24. The two set of mixograph data could therefore not be compared statistically.

TABLE 23. Mixograph parameters[1] data comparing two different mixographs

| Sample | PHG | MDT | ETP | FMS | PBW | SAP | TEG | BWE |
|---|---|---|---|---|---|---|---|---|
| **MIXOGRAPH # 1 PARAMETERS** | | | | | | | | |
| UQN130 | .10±.01 | 2.2±.2 | 9.2±1.1 | .05±.01 | .06±.00 | .005±.002 | 45.9±1.6 | 6.5±0.8 |
| UQN209 | .08±.00 | 2.1±.2 | 7.6±0.2 | .04±.01 | .05±.01 | .005±.001 | 37.9±1.7 | 5.5±0.2 |
| UQN212 | .08±.01 | 3.5±.4 | 12.7±1.5 | .04±.01 | .05±.01 | .003±.001 | 39.8±3.3 | 9.4±0.9 |
| UQN217 | .07±.00 | 2.4±.2 | 7.5±1.1 | .03±.00 | .05±.01 | .005±.001 | 35.1±3.2 | 6.0±1.8 |
| UQN219 | .08±.00 | 1.5±.3 | 5.2±1.3 | .04±.00 | .06±.01 | .006±.003 | 35.1±0.9 | 4.6±0.9 |
| **MIXOGRAPH # 2 PARAMETERS** | | | | | | | | |
| UQN130 | .18 | 1.6 | 11.7 | .12 | .12 | .010 | 65.9 | 8.6 |
| UQN209 | .15 | 2.7 | 17.6 | .08 | .84 | .010 | 56.6 | 11.5 |
| UQN212 | .15 | 2.6 | 17.4 | .07 | .09 | .008 | 57.2 | 11.8 |
| UQN217 | .15 | 1.9 | 12.0 | .07 | .07 | .024 | 50.4 | 7.2 |
| UQN219 | .14 | 0.9 | 5.8 | .01 | .09 | .019 | 47.5 | 4.3 |

[1] Abbreviation as defined in Table 7.
[2] Mixograph # 1 and mixograph # 2 were used in the 2nd and 1st part of the study, respectively.

TABLE 24. The correlation coefficient between mixograph #1 and mixograph #2 parameters[1].

| Mixograph parameters | r-values |
|---|---|
| PHG | 0.93 |
| MDT | 0.68 |
| ETP | 0.70 |
| FMS | 0.36 |
| PBW | -0.50 |
| SAP | 0.56 |
| TEG | 0.97 |
| BWE | 0.68 |

[1]Abbreviations as defined in Table 10.

**2. Protein distribution among the extracting solutions**

The amount of protein extracted by the different extracting solutions varied. The proportion of the proteins extracted as a percentage of the total flour protein is shown in Figs. 14 and 15, while the total amount (mg/g flour) extracted is shown in Table 25.

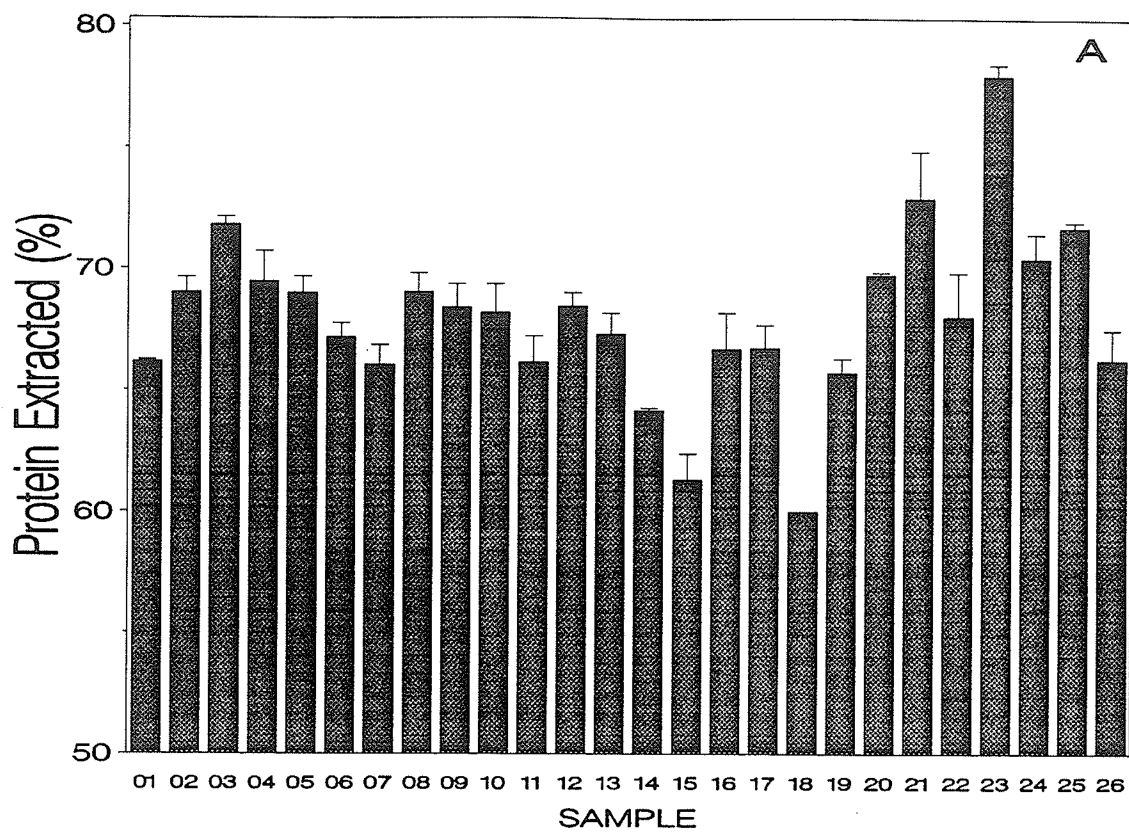
The largest amount of protein was extracted using 0.05 *M* acetic acid with a range 60.0-78.0 % while NaCl extracted the least amount of protein ranging from 14.6-24.9 %. A similar trend was found with the 16 Quad samples (section IV:B:7) except that the UQN samples had a wider range of protein extracted. The range of protein extracted using water (16.9-27.4 %) and 70 % ethanol (48.4-56.8 %) was similar in both sets of samples.

TABLE 25. The amount of protein extracted (mg/g flour) from the 1983 UQN samples using different extracting solutions[1].

| Sample | Fpro[2] | $H_2O$ | 0.05M HAc | 70 % EtOH[3] | 2 % NaCl[3] |
|---|---|---|---|---|---|
| UQN 099 | 139.2 | 28.5±2.0 | 92.1±0.1 | 73.8 | 22.5 |
| UQN 130 | 144.4 | 24.6±0.1 | 99.6±0.9 | 80.0 | 21.6 |
| UQN 176 | 125.6 | 26.4±0.1 | 90.2±0.5 | 71.3 | 20.2 |
| UQN 199 | 147.6 | 26.7±0.3 | 102.5±1.9 | 79.9 | 21.5 |
| UQN 200 | 137.5 | 26.7±0.3 | 94.8±0.9 | 70.7 | 21.1 |
| UQN 201 | 123.4 | 28.0±0.9 | 82.9±0.7 | 61.5 | 19.4 |
| UQN 203 | 133.0 | 22.9±0.7 | 87.8±1.1 | 67.3 | 20.9 |
| UQN 204 | 134.7 | 32.8±1.4 | 93.0±1.1 | 73.3 | 22.0 |
| UQN 205 | 126.3 | 29.8±0.9 | 86.4±1.2 | 67.9 | 31.4 |
| UQN 206 | 135.9 | 25.1±0.3 | 92.7±1.6 | 73.7 | 31.2 |
| UQN 207 | 139.0 | 34.6±0.4 | 91.9±1.5 | 74.6 | 21.1 |
| UQN 208 | 132.7 | 32.3±0.4 | 90.8±0.7 | 69.6 | 30.1 |
| UQN 209 | 136.3 | 33.1±1.3 | 91.7±1.2 | 70.7 | 30.4 |
| UQN 210 | 129.0 | 29.5±0.8 | 82.7±0.1 | 65.7 | 28.8 |
| UQN 211 | 130.4 | 24.3±0.5 | 79.9±1.4 | 63.2 | 20.6 |
| UQN 212 | 134.3 | 34.1±0.2 | 89.6±2.0 | 69.6 | 24.7 |
| UQN 213 | 124.9 | 23.9±1.1 | 83.3±1.2 | 62.4 | 23.5 |
| UQN 214 | 131.1 | 23.3±1.2 | 78.6±0.0 | 66.8 | 23.2 |
| UQN 215 | 128.0 | 25.7±1.7 | 84.1±0.8 | 62.0 | 23.2 |
| UQN 216 | 118.8 | 29.7±1.1 | 82.8±0.1 | 60.2 | 21.3 |
| UQN 217 | 123.8 | 24.0±1.9 | 90.3±2.4 | 62.4 | 21.7 |
| UQN 218 | 137.1 | 23.1±3.3 | 93.2±2.5 | 69.8 | 23.4 |
| UQN 219 | 124.1 | 34.1±0.1 | 96.8±0.6 | 70.5 | 22.5 |
| UQN 220 | 121.7 | 23.2±2.2 | 85.7±1.2 | 62.4 | 24.0 |
| UQN 221 | 138.4 | 33.9±2.1 | 99.2±0.3 | 71.9 | 23.6 |
| UQN 222 | 148.8 | 27.0±2.9 | 98.6±1.9 | 81.8 | 21.7 |

[1]Extracting solutions as defined in Table 22.
[2]Fpro = Flour protein.
[3]The average variation between duplicate determinations of 70 % EtOH and 2 % NaCl was 0.4 mg/g and 0.5 mg/g, respectively.

FIGURE 14. The amount of protein extracted by 0.05 *M* acetic acid (A) and 70 % ethanol (B) from the 1983 UQN samples.


A
Protein Extracted (%)
80
70
60
50
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
SAMPLE

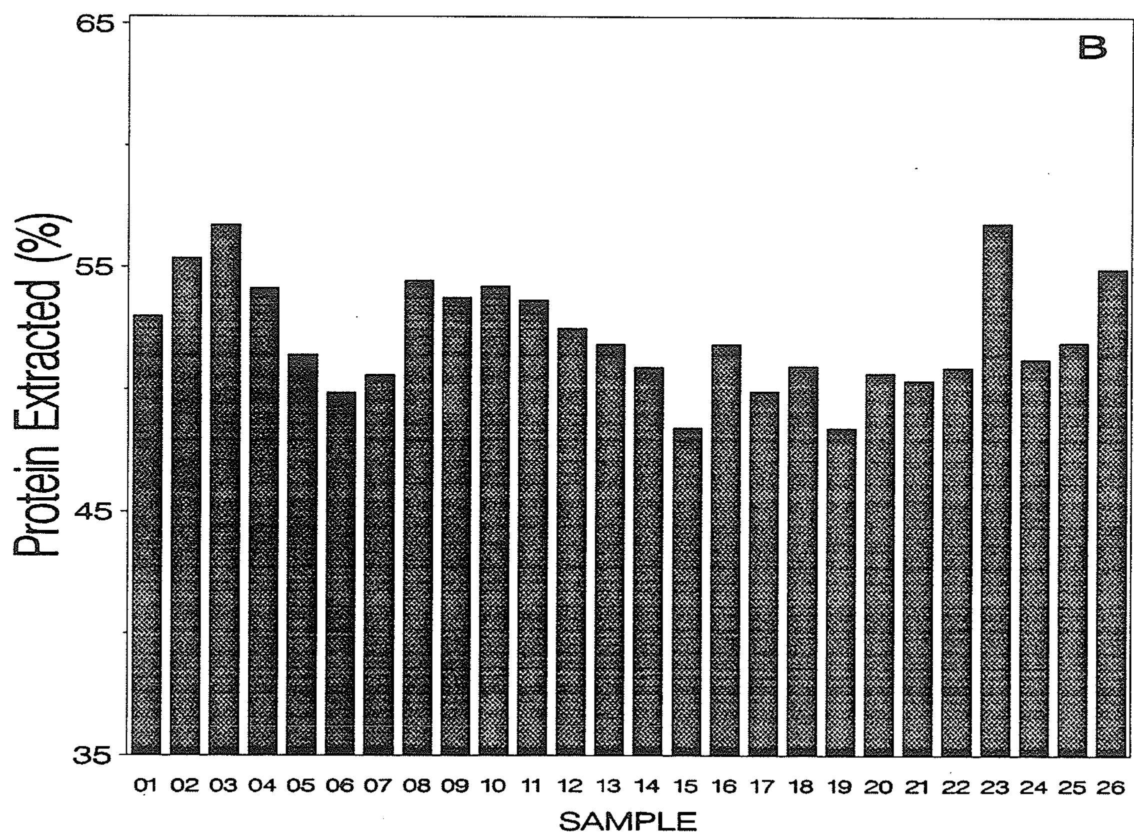
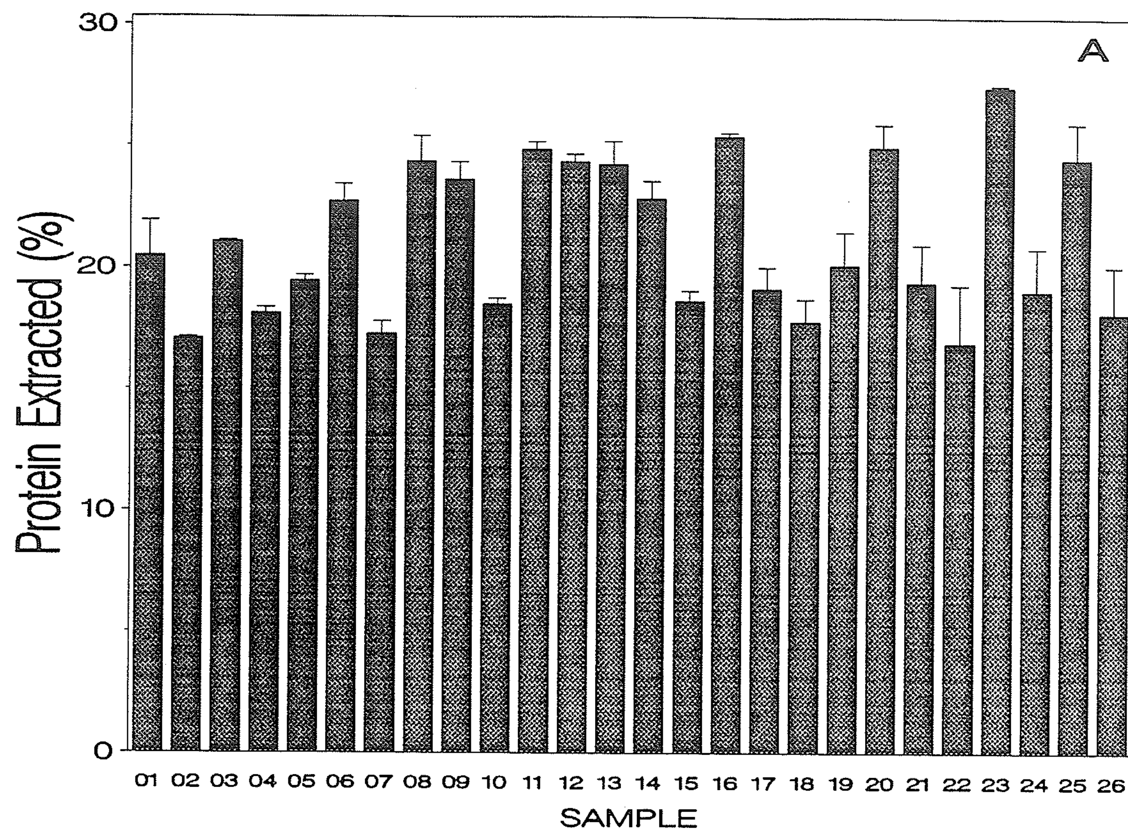
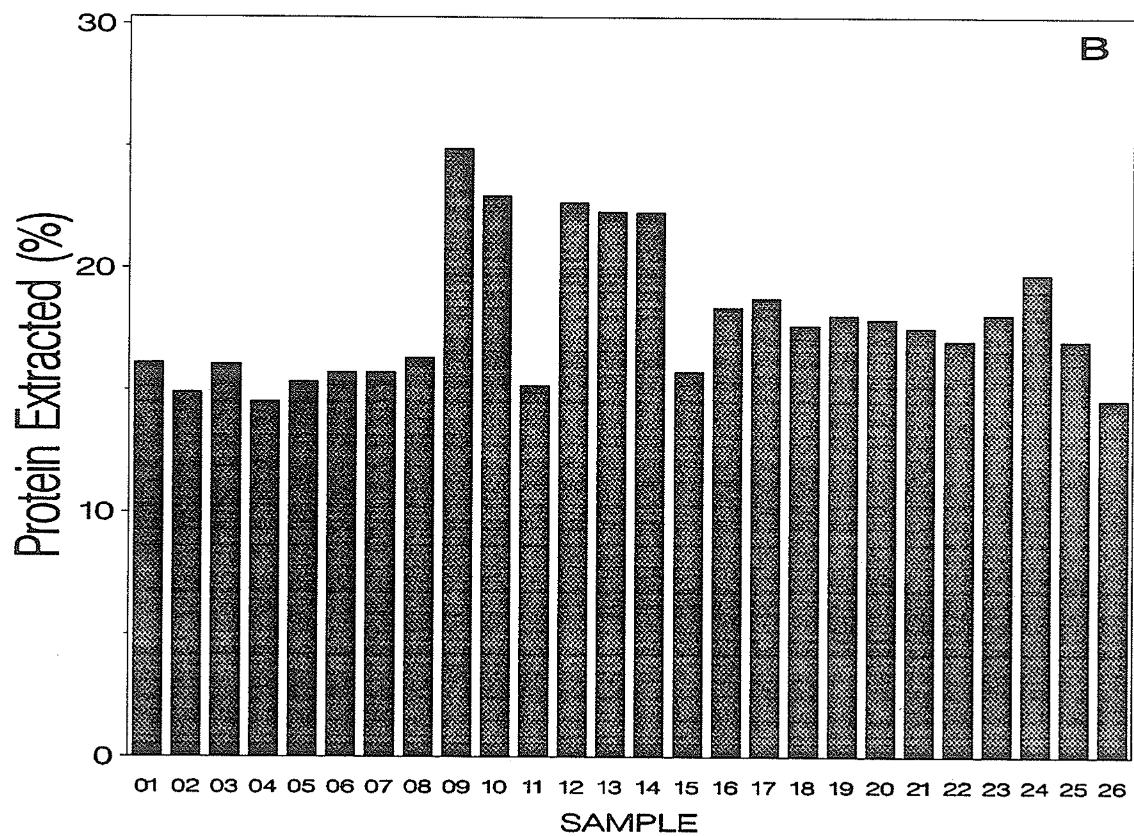

B
Protein Extracted (%)
65
55
45
35
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
SAMPLE

FIGURE 15. The amount of protein extracted by distilled deionized water (A) and 2 % NaCl (B) solution from the 1983 UQN samples.


A
Protein Extracted (%)
30
20
10
0
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
SAMPLE


B
Protein Extracted (%)
30
20
10
0
01 02 03 04 05 06 07 08 09 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26
SAMPLE

**3. The relationship between protein fractions and the mixing quality characteristics for the UQN samples**

Pearson correlation analysis was used to examine the relationship between the mixograph parameters and the amount of extracted protein by the different solvents. Flour protein had a strong influence on some dough mixing properties as the results (Table 26) show that flour protein was significantly correlated ($P < 0.01$) with PHG, FMS and TEG; r-values were 0.60, 0.53 and 0.59, respectively. A similar effect of protein content with the above parameters but with higher r-values was found with the 16 Quad and Buhler samples. Likewise, MDT did not show any correlation with flour protein content. This is in agreement with the findings of Khan *et al* (1989) using 44 hard red spring cultivars. Dong *et al* (1992) also did not find any association between total protein content and mixing time and tolerance using 135 hard red winter wheat genotypes from randomly mated population. This indicates that MDT is not affected by protein quantity but by protein quality while PHG, FMS and TEG are influenced more by protein quantity.

The total amount of acetic acid extracted protein was significantly correlated ($P < 0.01$) with MDT ($r = -0.56$), ETP ($r = -0.44$), FMS ($r = 0.46$), SAP ($r = -0.41$) and BWE ($r = -0.51$). When the amount was examined as a percentage of total protein (normalized), higher levels of correlation ($P < 0.01$) were obtained for the parameters MDT, ETP and BWE with r-values of -0.70, -0.80 and -0.82, respectively. Khan *et al* (1989) using an elaborate sequential extraction procedure, reported that the amount of glutenin and gliadin had a positive and negative effect, respectively, on MDT. The effect of acetic acid soluble protein reported here, though containing gliadins and glutenins, is similar to their findings.

Comparing the UQN results with the results for the 16 Quad samples (section

IV:B), ETP and BWE showed different levels of correlations (r = -0.60 and -0.60 for 16 Quad and -0.80 and -0.82 for UQN samples, respectively) with the percentage of acetic acid soluble proteins.

The amount of 70 % ethanol soluble protein (normalized) was significantly correlated with only a single mixograph parameter, BWE (r = -0.47**), and slightly correlated with MDT (r = -0.38*) and ETP (r = -0.36*). Neither the amount nor the percent total protein extracted by 2 % NaCl solution was related to any of the mixograph parameters. These results are not consistent with results obtained using the 16 Quad samples. The amount of NaCl extracted proteins for the 16 Quad samples had a highly significant correlation ($P < 0.01$) with ETP, and BWE (r-values = -0.71 and -0.68, respectively). Graybosch *et al* (1990) likewise did not find any correlation between flour mixing characteristics and 2 % NaCl soluble proteins. The inconsistency between the results for the two sets of samples used in this study may be attributed to the broader range of protein content and mixing characteristics in the 16 Quad samples compared with the UQN samples. For example, the range of flour protein was 118.8-148.8 mg/g for the UQN while the 16 Quad samples had a range of 93.0-159.0 mg/g, thus affecting the amount of protein extracted by the different solvents.

The total amount of water soluble fractions was negatively correlated ($P < 0.01$) with PHG (r = -0.49), PBW (r = -0.59) and TEG (r = -0.45). When the amount of water soluble protein was analyzed as a percentage of total flour protein, the magnitude of the correlations were enhanced to r-values of -0.70, -0.71 and -0.66 for PHG, PBW and TEG, respectively. Unlike the 16 Quad samples where the amount of water soluble proteins (normalized) showed significant correlations ($P < 0.01$) with MDT, ETP and BWE with r-values of -0.70, -0.68 and -0.65, respectively.

TABLE 26. Correlation coefficient between mixograph[1] data and protein fractions of the 1983 UQN samples.

| | Protein Solubility Classes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Normalized[2] | | | | | Unnormalized | | | |
| P[3] | Fpro | $H_2O$ | HAc | NaCl | EtOH | $H_2O$ | HAC | NaCL | EtOH |
| FPRO | 1.0 | -.30 | .19 | -.36* | -.36* | .09 | .71*** | -.02 | .88*** |
| MDT | .07 | .19 | -.70*** | .18 | -.38* | .17 | -.56*** | .16 | .24 |
| ETP | .16 | -.02 | -.80** | .05 | -.36* | .06 | -.44** | .10 | -.07 |
| PHG | .60*** | -.70*** | -.29 | -.29 | -.04 | -.49** | .30 | -.09 | .40** |
| FMS | .53*** | -.50*** | .01 | -.21 | .17 | -.31 | .46** | -.03 | .45** |
| PBW | .28 | -.71*** | -.26 | -.23 | -.20 | -.59*** | .13 | -.11 | .16 |
| SAP | .28 | -.35* | .23 | .31 | .02 | -.25 | .41** | -.23 | .20 |
| TEG | .59*** | -.66*** | -.40 | -.24 | -.08 | -.45** | .21 | -.04 | .38* |
| BWE | .09 | -.08 | -.82*** | .05 | -.47** | -0.37 | -.51*** | .09 | -.17 |

[1]Abbreviation as defined in Tables 4 and 7.
[2]Refers to values expressed as a percentage of flour protein.
[3]P=mixograph parameters.
[4]*, ** and *** Significant at P< 0.10, 0.05 and 0.01 level, respectively.

sets of samples; r-values of -0.65 and -0.70 for the 16 Quad and UQN samples, respectively.

**4. Predicting mixograph quality characteristics on the basis of protein fractions.**

The correlation data reported in Table 26 suggested that quantitative protein solubility data can be used to explain some of the variation observed in some mixograph parameters. These relationships were further examined using stepwise multiple regression analysis (Table 27) to select single or two variable computer generated models for each mixograph quality parameter; only significant correlations ($P < 0.05$) are reported. The results indicate that protein solubility data have varying influence on the mixograph parameter values, as found previously for the 16 Quad samples.

The amount of HAc soluble protein could explain a considerable proportion of the variation in MDT ($R^2 = 0.48$), ETP ($R^2 = 0.64$) and BWE ($R^2 = 0.67$) while the amount of water residue (R-$H_2O$) proteins could explain some of the variation observed in PHG ($R^2 = 0.61$) and TEG ($R^2 = 58$). Combinations of variables increased the $R^2$ values e.g the amount of HAc and water soluble protein explained over 70 % of the variation observed in MDT, ETP and BWE.

To evaluate the robustness of the prediction equations for mixograph parameters as a function of protein solubility, UQN and Quad milled wheat samples were compared on the basis of computed models with common protein fractions variables. Potentially robust models would be those having comparable and high $R^2$ values. For example, for MDT, the amount of acetic acid soluble protein (variable % HAc) as a single independent variable resulted in computed models with $R^2$ of 0.58

and 0.48 for the Quad and UQN samples, respectively. The Buhler samples (counterpart of Quad samples) had a $R^2$ value of 0.70 for the same parameter. The difference in the $R^2$ values may be attributed to the differences in amount of protein extracted from the samples in each of the three sets, which is speculated to be due to both the flour particle size and the range of protein content among the samples examined.

Other single variable models which appear to be potentially useful for mixograph parameter prediction include HAc residue protein with dependent variables ETP and BWE, and $H_2O$ residue protein with dependent variables PHG and TEG. In the case of two variable predictor models, Table 28 shows that there were potentially more satisfactory models for each of the five dependent mixograph parameters listed. Unfortunately, it was not possible to further evaluate the relative value of any of the two variable models.

As mentioned previously (section IV:C1), different mixograph were used to determine the mixing characteristics of these two sets of samples. The main objective of analysing the mixograph parameters and protein solubility fractions of the UQN samples was to verify whether the models generated for the 16 samples when applied to a different set of genotypes. Because of the differences in mixograph values obtained, the objective of this part of the study was not fulfilled.

One of the stated objectives and advantages of the electronic recording mixograph is to facilitate intra as well as inter-laboratory comparisons. But as demonstrated above this can only be realized if the mixographs can be fully standardized, including the software used for data acquisition and processing. Otherwise intra as well as inter-laboratory comparisons cannot be effectively achieved.

TABLE 27. Linear least square regression models for mixograph parameters as a function of protein fractions variables for 1983 UQN samples.

| P[2] | Regression coefficient | b[3] | $R^2$ | Prob> F |
|---|---|---|---|---|
| | **Single Variable Models** | | | |
| MDT | (i) -17.049 (% HAc) | 14.63 | .48 | .0001 |
| | (ii) -0.0765 (HAc) | 9.93 | .32 | .0028 |
| | (iii) -0.083 (R[4]-HAc) | -.50 | .29 | .0047 |
| ETP | (i) -77.235 (% HAc) | 64.67 | .64 | .0001 |
| | (ii) 0.443 (R-HAc) | -6.66 | .52 | .0001 |
| | (iii) -0.237 (HAc) | 33.53 | .20 | .0235 |
| PHG | (i) 0.001 (R-$H_2O$) | -.017 | .61 | .0001 |
| | (ii) -0.248 (% $H_2O$) | .142 | .49 | .0001 |
| | (iii) 0.002 (R-EtOH) | -.022 | .36 | .0001 |
| TEG | (i) 0.45 (R-$H_2O$) | -3.04 | .58 | .0001 |
| | (ii) -106.620 (% $H_2O$) | 66.60 | .44 | .0002 |
| | (iii) 0.84 (R-EtOH) | -9.06 | .40 | .0006 |
| BWE | (i) -61.919 (% HAc) | 51.59 | .67 | .0001 |
| | (ii) 0.338 (R-HAc) | -4.88 | .49 | .0001 |
| | (ii) -0.216 (HAc) | 28.94 | .26 | .0075 |
| | **Two Variable Models** | | | |
| MDT | 14.094 (% $H_2O$)-21.680 (% HAc) | 14.80 | .71 | .0001 |
| | 0.087 (R-$H_2O$)+0.169 (R-HAc) | 4.93 | .71 | .0001 |
| | 0.088 ($H_2O$)-19.936 (% HAc) | 21.12 | .64 | .0001 |
| ETP | -.242 (R-$H_2O$)+0.681 (R-HAc) | 8.49 | .74 | .0001 |
| | 0.270 ($H_2O$)-86.062 (% HAc) | 63.10 | .73 | .0001 |
| | 35.201 (% $H_2O$)-88.818 (% HAc) | 65.08 | .73 | .0001 |
| PHG | -.001 (EtOH)+0.001 (R-$H_2O$) | -.01 | .66 | .0001 |
| | 0.003 ($H_2O$)-0.588 (% $H_2O$) | 0.13 | .66 | .0001 |
| | 0.001 (Fpro)-0.002 (% $H_2O$) | 0.01 | .66 | .0001 |
| TEG | -.275 (EtOH)+0.587 (R-$H_2O$) | 1.77 | .62 | .0001 |
| | -.202(HAc)-267.86 (H2O) | 6.84 | .61 | .0001 |
| | 1.378 ($H_2O$)-267.864 (% $H_2O$) | 62.16 | .61 | .0001 |
| BWE | 21.763 (% $H_2O$)-69.060 (% HAc) | 51.84 | .72 | .0001 |
| | -0.194 (R-$H_2O$)+0.529 (R-HAc) | 7.28 | .72 | .0001 |
| | 0.1674 ($H_2O$)-26.479 (% $H_2O$) | 50.74 | .71 | .0831 |

[1]Abbreviation as defined in Table 7.
[2]P=Quality parameters.
[3]b=Intercept.
[4]R=Amount of protein left in the residue.

TABLE 28. Linear least square regression models for mixograph parameters as a function of protein fraction variables common to both the 16 Quad and 1983 UQN samples.

| P[2] | Common Independent Variable(s) | QUAD ($R^2$) | UQN ($R^2$) |
|---|---|---|---|
| | **Single Variable Models** | | |
| MDT | (i) % HAc | .58 | .48 |
| | (ii) % $H_2O$ | .49 | .29 |
| | (iii) % EtOH | .29 | .15 |
| ETP | (i) R[4]-HAc | .61 | .52 |
| | (ii) R-EtOH | .40 | .18 |
| | (iii) % HAc | .36 | .64 |
| PHG | (i) Fpro | .88 | .35 |
| | (ii) R-$H_2O$ | .80 | .61 |
| | (iii) R-EtOH | .73 | .36 |
| TEG | (i) Fpro | .84 | .34 |
| | (ii) R-EtOH | .83 | .40 |
| | (iii) R-$H_2O$ | .79 | .57 |
| BWE | (i) R-HAc | .58 | .49 |
| | (ii) % HAc | .36 | .67 |
| | (iii) R-EtOH | .36 | .20 |
| | **Two Variable Modes** | | |
| MDT | (i) % HAc and R-NaCl | .70 | .54 |
| | (ii) % HAc and R-$H_2O$ | .69 | .64 |
| | (iii) % $H_2O$ and % HAc | .69 | .71 |
| ETP | (i) R-$H_2O$ and R-HAc | .72 | .74 |
| | (ii) % HAc and R-$H_2O$ | .70 | .66 |
| | (iii) HAc and R-HAc | .74 | .64 |
| PHG | (i) Fpro and % $H_2O$ | .89 | .66 |
| | (ii) % $H_2O$ and R-$H_2O$ | .89 | .66 |
| | (iii) $H_2O$ and % $H_2O$ | .88 | .65 |
| TEG | (i) $H_2O$ and % $H_2O$ | .86 | .61 |
| BWE | (i) Fpro and HAc | .72 | .67 |
| | (ii) Fpro and R-HAc | .72 | .67 |
| | (iii) R-$H_2O$ and R-HAc | .70 | .72 |

[1]Abbreviations as defined in Table 7.
[2]P=Quality parameters.
[3]b=Intercept.
[4]R=Amount of protein left in the residue.

### 5. Electrophoretic patterns of the UQN protein fractions

The SDS-PAGE results reported in literature (refer to Section II) contains mainly specific proteins. The 26 UQN set of samples were pure varieties of narrow protein content range but of diverse baking quality characteristics (Ng, 1987). It was therefore a good set of samples to show the type of proteins solubilized by direct extraction. The composition of the supernatants from the different extractions were analyzed by SDS-PAGE. Subsequent extraction of the remaining residue in the presence of mercaptoethanol was performed to provide information complementary to that obtained for the extracted protein.

The electrophoretic results are shown in Figs. 16-19. By the direct extraction method used in this study 0.05 *M* acetic acid extracted some HMW, MMW and LMW proteins (Fig. 16). The absence of bands in the LMW region of the residue (Fig. 16, (R)), indicates that 0.05 *M* acetic acid extracted almost all of LMW (presumably albumin and globulin) proteins. Interestingly, compared with the control flour extract using 2 % SDS, acetic acid extracted a much higher proportion of MMW (gliadins) and LMW glutenins proteins relative to the HMW glutenin subunits (Fig. 16).

The dense bands in the MMW region in Fig. 17 (S) indicated that 70 % ethanol extracted most of the gliadins, with some HMW and LMW proteins. It can be observed from Fig. 17 (R) that most of the HMW glutenins were not extracted by ethanol. By comparison, no HMW glutenin were extracted by 2 % NaCl solution (Fig. 18S), apparently some gliadins and most of the LMW proteins were extracted by this solution as observed from the dense bands in Figure 18S. The arrow indicates a prominent globulin protein that was absent or very faint in the other supernatant fractions.

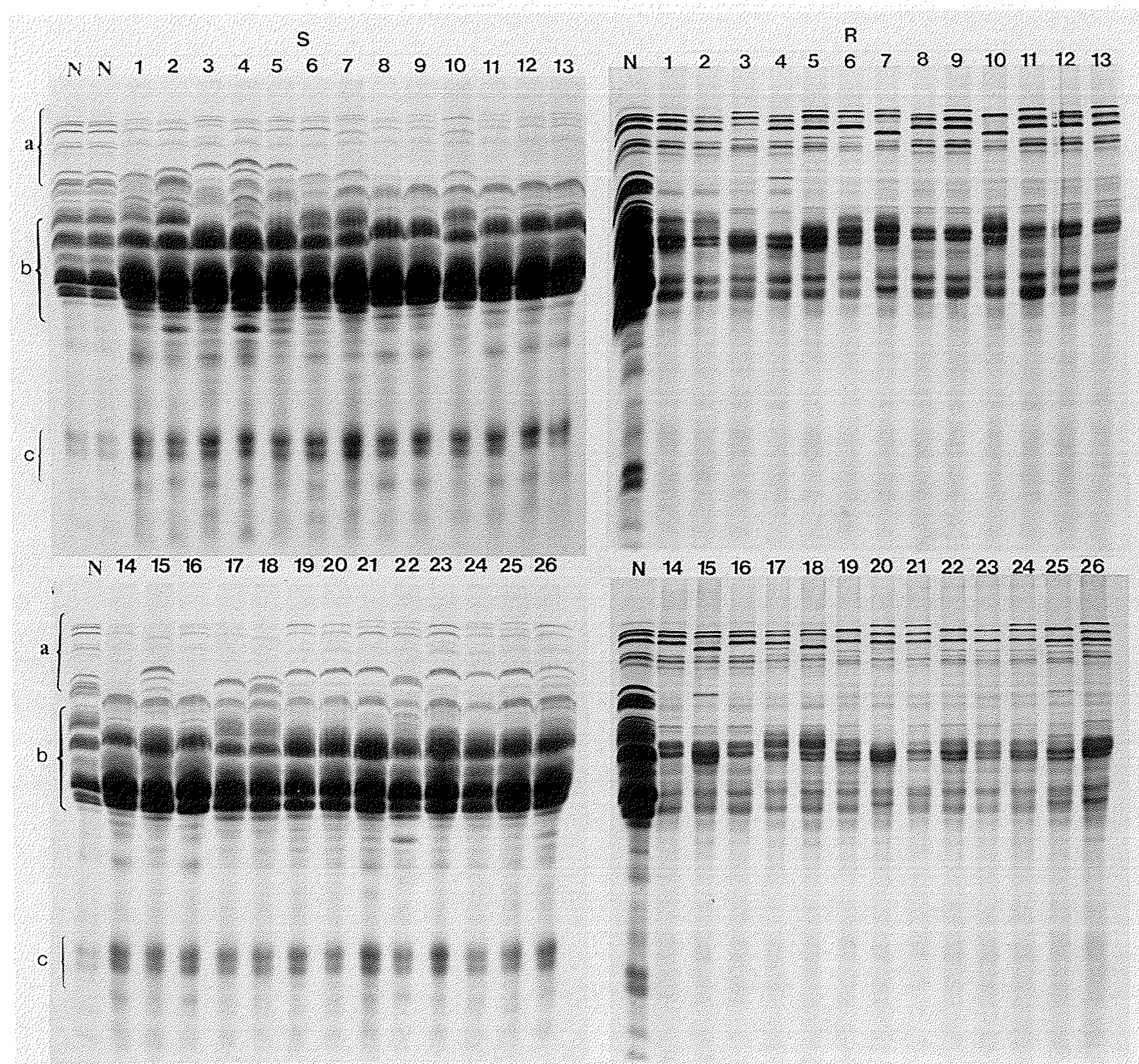
Like 2 % NaCl, distilled water extracted no HMW glutenins but most of the MMW (gliadins) and LMW (albumins and globulins) proteins (Fig. 19 (S)). It can be observed from the 2 % NaCl residue result (Fig. 19 (R)) that not all of the LMW proteins were extracted by distilled water. This may indicate either that single extraction results in insufficient extraction of the water soluble proteins or more likely that water did not extract some of the globulins particularly the LMW doublet which is most prominent in these residue patterns (Fig. 19 (R)). The electrophoretic patterns observed in the supernatant and residue for all the extractions are consistent with those reported in literature (Section II). The relatively high proportion of HMW glutenin in the residue of the water and salt extraction is largely attributed to their total insolubility in these solvents.

Equal quantities of protein for each extraction solvent were applied into each slot. Subjective evaluation shows that apart from qualitative differences there were also quantitative differences among the samples in all the gels as indicated by the band intensities. It has been suggested by Lawrence *et al* (1988) that quantitative differences in the HMW subunit compositions may also explain some of the breadmaking property differences among cultivars. This aspect was not examined in this study. The HMW protein groups have been associated with good breadmaking characteristics (refer to section II). This may explain the high $R^2$ values observed between HAc, R-HAc and R-NaCl with some of the mixograph parameters. Estimation of 0.05 *M* acetic acid or 2 % sodium chloride residue proteins may therefore be a useful screening tool for mixing characteristics in early generation lines.

Finally, for the acetic acid extractions (Fig. 16) it was observed that there was a lack of correspondence in the HMW glutenin subunit composition between the supernatant and residue fractions. Specifically for samples 20 and 26, some of the HMW subunits were missing in the supernatant but present in the residue. This would appear to suggest that these additional HMW proteins observed in the residue were not extractable by 0.05 *M* acetic acid solution. In the present work, chemical reduction of samples for SDS-PAGE was carried out on the freeze-dried protein fraction extracted with 0.05 *M* acetic acid under non-reducing conditions. Extraction with 2 % SDS solution under reducing conditions using mercaptoethanol (Ng, 1987) resulted in similar HMW protein composition compared with the acetic acid residue of these two samples, supporting the above hypothesis. On the other hand, for samples 22 and 25 some HMW glutenin subunits were visible in the acetic acid extract but not in the residue; there is no obvious explanation for this result. As above, the results of Ng (1987) showed that, extraction of samples UQN 22 and 25 with SDS solution under reducing conditions gave HMW glutenins compositions similar to the residue fraction results obtained here. Further investigation should be carried out to look into the nature of these discrepancies.

FIGURE 16. The SDS-PAGE patterns of 0.05 *M* acetic acid extracted flours. **S** = supernatant and **R** = residue proteins. The sample # corresponds to the varieties as defined in Table 2. **N** = control flour (Neepawa).

**a** = High molecular weight region (mainly glutenin)
**b** = Medium molecular weight region (mainly gliadin)
**c** = Low molecular weight region (mainly albumin and globulin).



S
R
N N 1 2 3 4 5 6 7 8 9 10 11 12 13
N 1 2 3 4 5 6 7 8 9 10 11 12 13
a
b
c
N 14 15 16 17 18 19 20 21 22 23 24 25 26
N 14 15 16 17 18 19 20 21 22 23 24 25 26
a
b
c

FIGURE 17. The SDS-PAGE patterns of 70 % ethanol acid extracted flours. **S** = supernatant and **R**-residue proteins. The sample # corresponds to the varieties as defined in Table 2. **N** = control flour (Neepawa).

**a** = High molecular weight region (mainly glutenin)
**b** = Medium molecular weight region (mainly gliadin)
**c** = Low molecular weight region (mainly albumin and globulin).

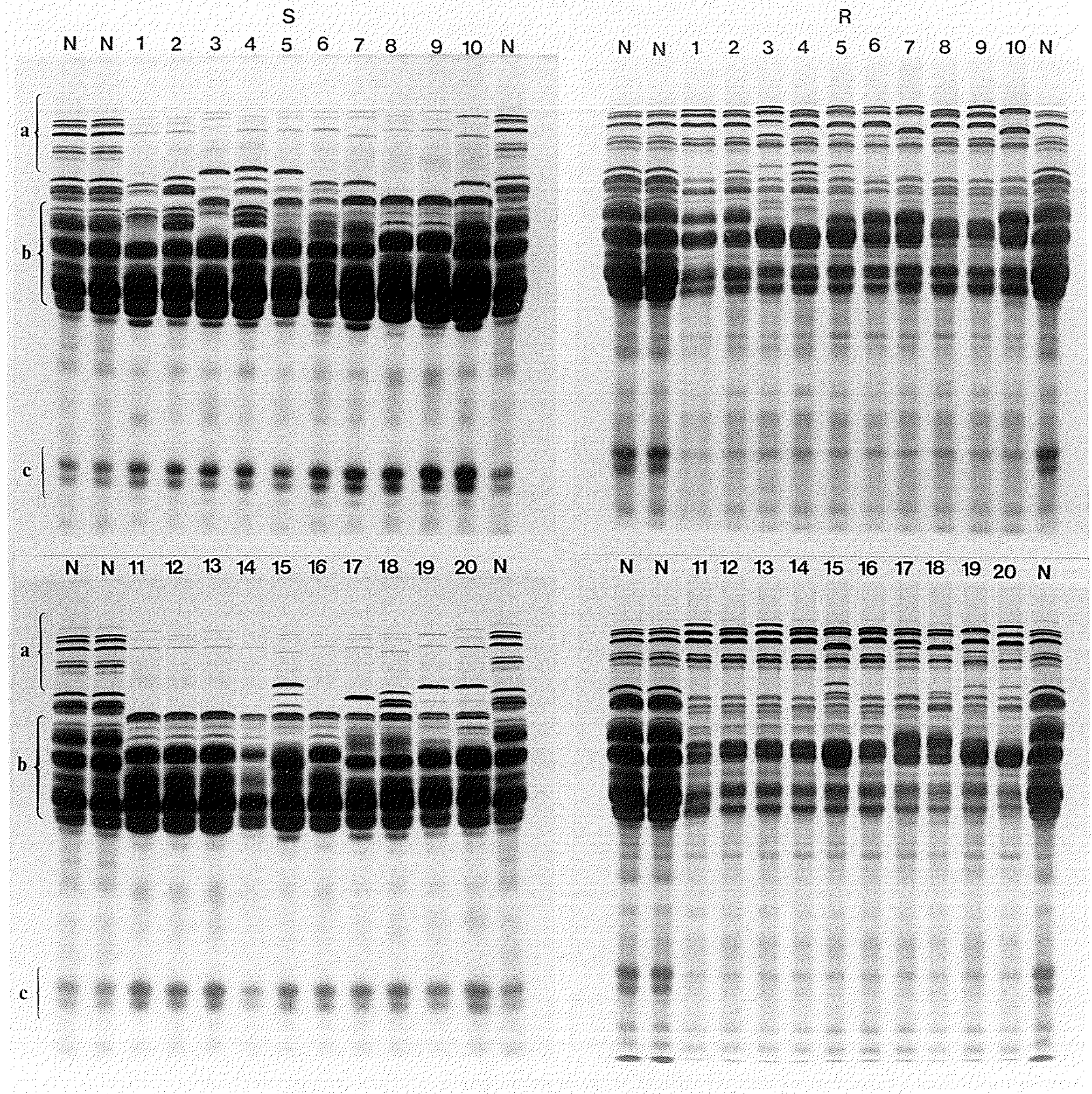

S
N N 1 2 3 4 5 6 7 8 9 10 N
a
b
c
R
N N 1 2 3 4 5 6 7 8 9 10 N
N N 11 12 13 14 15 16 17 18 19 20 N
N N 11 12 13 14 15 16 17 18 19 20 N

FIGURE 18. The SDS-PAGE patterns of 2 % sodium chloride extracted flours. **S** = supernatant and **R** = residue proteins. The sample # corresponds to the varieties as defined in Table 2. **N** = control flour (Neepawa).

**a** = High molecular weight region (mainly glutenin)
**b** = Medium molecular weight region (mainly gliadin)
**c** = Low molecular weight region (mainly albumin and globulin).

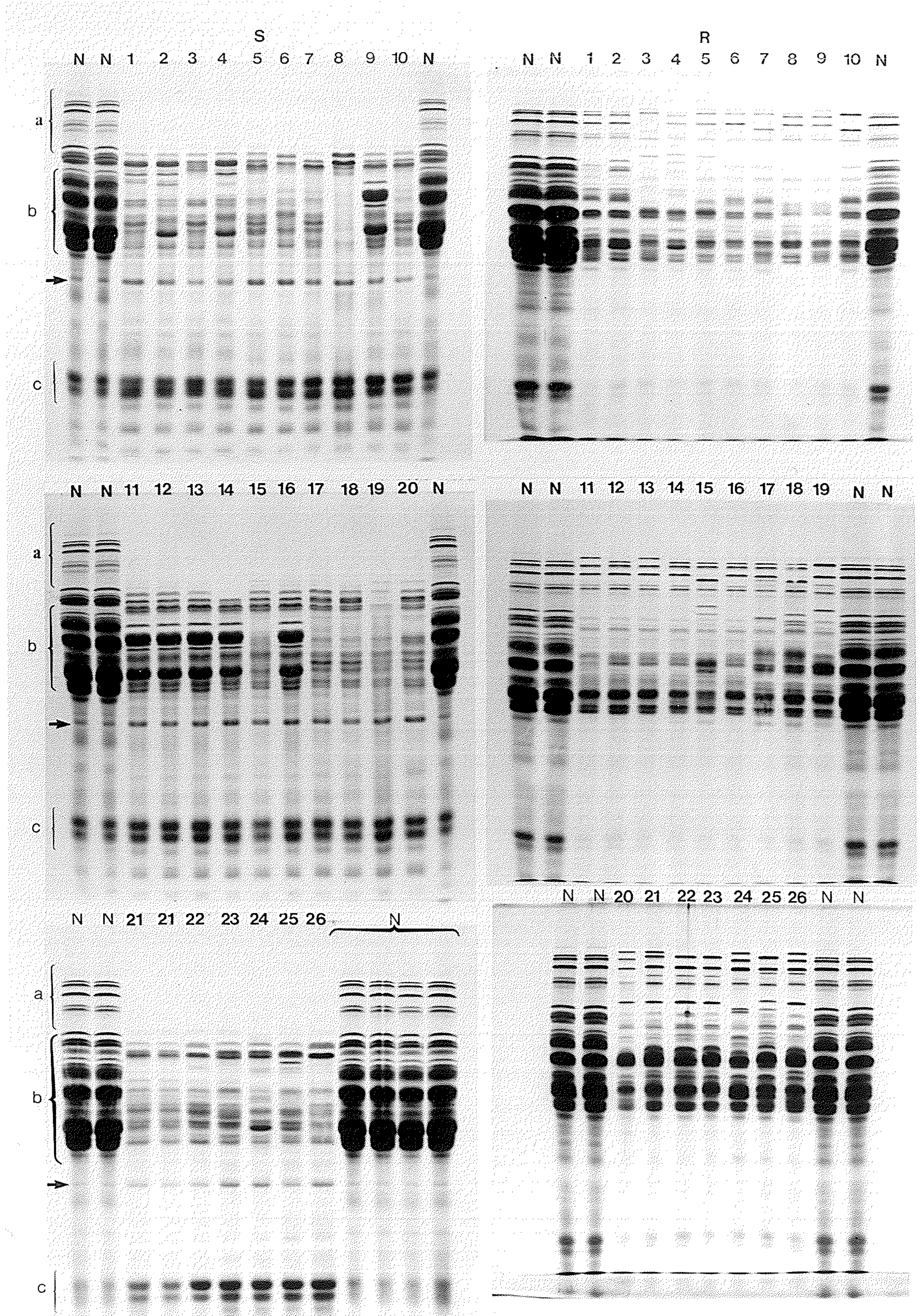
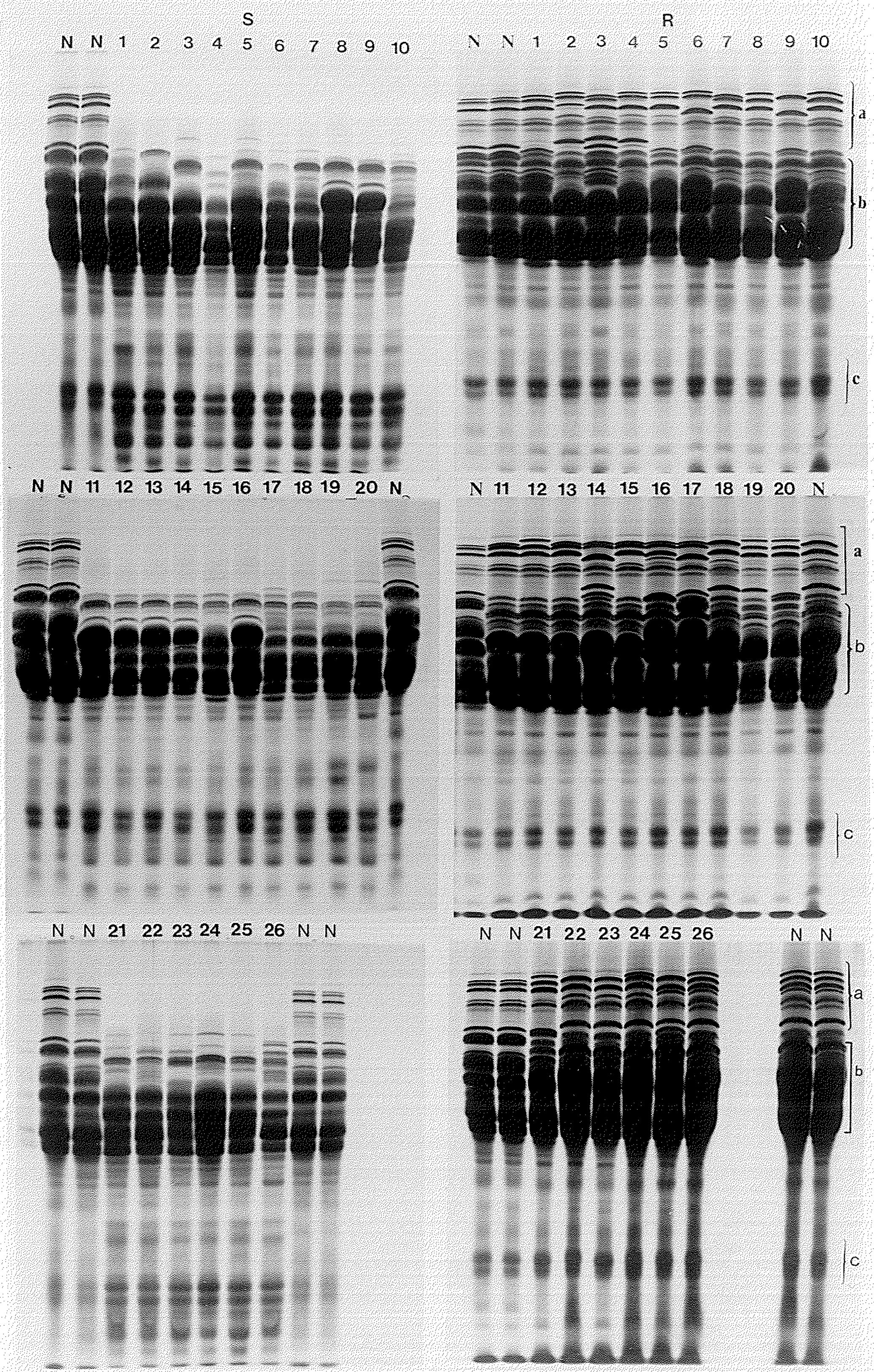

S
N N 1 2 3 4 5 6 7 8 9 10 N
a
b
c
R
N N 1 2 3 4 5 6 7 8 9 10 N
N N 11 12 13 14 15 16 17 18 19 20 N
N N 11 12 13 14 15 16 17 18 19 N N
N N 21 21 22 23 24 25 26 N
N N 20 21 22 23 24 25 26 N N

FIGURE 19. The SDS-PAGE patterns of distilled water extracted flours. **S** = supernatant and **R** = residue proteins. The sample # corresponds to the varieties as defined in Table 2. **N** = control flour (Neepawa).

**a** = High molecular weight region (mainly glutenin)
**b** = Medium molecular weight region (mainly gliadin)
**c** = Low molecular weight region (mainly albumin and globulin).


S
N N 1 2 3 4 5 6 7 8 9 10
R
N N 1 2 3 4 5 6 7 8 9 10
a
b
c
N N 11 12 13 14 15 16 17 18 19 20 N
N 11 12 13 14 15 16 17 18 19 20 N
a
b
c
N N 21 22 23 24 25 26 N N
N N 21 22 23 24 25 26 N N
a
b
c

# V. SUMMARY AND CONCLUSIONS

1. In the first part of this study, the extractability of proteins of flours milled on two different experimental mills (Buhler and Quadrumat Jr.), using 16 different wheat samples of diverse breadmaking quality, was determined using four solvents, i.e. distilled water, 2 % NaCl solution, 70 % aqueous ethanol and 0.05 *M* acetic acid solution.

2. Protein recoveries based on freeze-drying of supernatants and insoluble residues ranged from 95.4 % to 102.1 % depending on the solvent. Small losses were incurred in the free :e-drying stage.

3. The extraction temperature (4 °C vs 23 °C) had no significant affect on the amount of protein extracted. Accordingly, extraction at room temperature (ca. 23 °C) was used for subsequent experiments.

4. Significant differences were found in the properties of flour obtained by the two mills. Higher flour yields were obtained using the Buhler mill. These flours however, were lower in protein and ash content indicating that the Buhler mill gave a more efficient separation of the endosperm and bran during milling. Additionally, the Buhler-milled flours had higher starch damage but lower farinograph absorption, higher Zeleny sedimentation values and finer particle size. The two flours had similar rheological properties (farinograph, extensigraph and mixograph) and bread of similar loaf volume. It can be concluded, that despite some differences in milling and flour characteristics, the two mills can be used to produce flour for determining the rheological and breadbaking properties of wheat varieties; although given its greater efficiency, the Buhler mill is apparently more satisfactory in this regard.

5. Determining the flour ash:wheat ash ratio gave a better indication of the milling efficiency than flour ash alone.
6. Among the computer generated mixograph data, peak height (PHG), mixograph development time (MDT), energy to peak (ETP), total energy under the curve (TEG) and band width energy (BWE) were found to be relatively more reliable and useful parameters in characterizing the mixing properties of wheat flour than first minute slope (FMS), peak band width (PHG) and slope after peak (SAP).
7. There appears to be two sets of interrelated (highly correlated) mixograph parameters; (i) MDT/ETP/BWE and were insensitive to flour protein content, and (ii) PHG/TEG/PBW, which were also highly correlated with flour protein content.
8. For all solvents, more protein was extracted from the Buhler milled flours than Quad flours. It is postulated that the finer flour particle size of Buhler flours resulted in higher protein extractions. The amount of protein extracted with each solvent as well as the amount of residue protein calculated by difference, was statistically evaluated by correlation and multiple regression analysis with a variety of technological quality parameters for breadmaking.
9. Significant and high correlations ($P<0.01$) for both types of milled flours were observed between percentage of acetic acid soluble proteins with ETP, MDT, and BWE. For Buhler flours, the amount of acetic acid soluble protein was also correlated with mixing tolerance index (MTI), remix time (RT), and work-input (WO) with r-values of 0.62, -0.67 and -0.61, respectively. The amount of ethanol soluble protein was significantly correlated ($P<0.05$) with farinograph absorption (FAB), PHG, MDT, and SAP for the Quad milled flour only. The amount of salt soluble protein was negatively correlated with flour protein (FP), FAB, DDT, WO, LV, MDT

and BWE for both types of flour. Water soluble protein was significantly correlated ($P < 0.01$) with ETP, PHG and BWE for both types of flours.

10. The highest correlations with technological quality were obtained with the residue protein variables for all the solvents. Compared with Quad milled flours, protein fraction data from Buhler milled flours gave higher and a greater number of (ca. 75 %) significant correlations with technological quality parameters. Presumably the higher levels of protein extracted with Buhler flour was a contributing factor.
11. Stepwise multiple linear regression analysis was used to generate equations for predicting loaf volume and mixograph quality parameters from protein solubility fraction data. The results indicated that quantitative protein solubility data has considerable value in predicting or explaining the variation in LV and flour mixing properties. For example the amount of HAc residue protein could explain 85 % of the variation in LV for Buhler milled flour, while the amount of HAc soluble protein could explain 70 % of the variation observed in MDT. The $R^2$ was generally higher for Buhler flour compared with the Quad milled flour.
12. Two variable models gave higher $R^2$ values than single variable models. The two independent variables in the models, e.g HAc (the amount of acetic acid soluble protein) and R-NaCl (the amount of sodium chloride residue protein) for MDT of the Buhler milled flour, each did not affect the dependent variable to the same degree and were therefore not additive in their effect in predicting the MDT.
13. Flour protein content did not influence the prediction equations except for PHG and TEG for the Quad milled flour.
14. In the second part of the study, protein extraction results for UQN genotype flours milled on the Quad mill were used in an attempt to verify or validate the prediction

equations obtained in the first part of the study.

15. Mixograph values for the UQN samples and the 16 Quad samples were different. The values could not be compared statistically because they were obtained from two different computerized mixographs electronic transducers which behaved quite differently, as assessed using a set of common flour samples.

16. The amount of protein extracted by the four extracting solutions for the UQN samples showed similar trends as the Quad samples. Similar correlations with a slightly lower r-values for the UQN samples were observed for the mixograph quality parameters and 0.05 M acetic acid and water soluble proteins. Different correlations between the two sets of samples were observed for the 70 % ethanol and 2 % NaCl soluble proteins.

17. SDS-PAGE patterns of the proteins from the two mills were identical under both reducing and non-reducing conditions.

18. It can be concluded that the amount of acetic acid soluble proteins and sodium chloride residue proteins can be used to predict the mixograph development time. The amount of acetic acid residue protein could be used to predict loaf volume.

19. It can be concluded from the correlation analysis study that direct protein extraction data can be used at least as effectively as sequential extraction data for predicting breadmaking quality parameters on the basis of the high $R^2$ values for some relationships.

20. Electronic recording mixographs need to be standardized including the software used for data acquisition and processing, to ensure that satisfactory and effective intra- and inter-laboratory comparisons can be achieved.

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# APPENDIX I

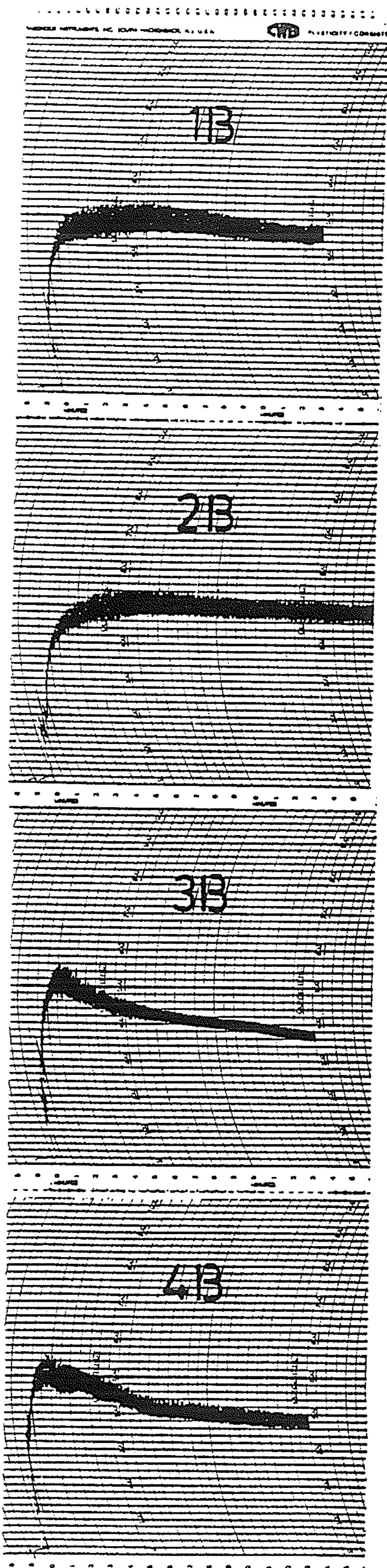
TABLE 29. Quality parameter[1] data for the samples used in preliminary study.

| SAMPLE | FP | FARINOGRAPH | | | MIXOGRAPH | | PSI |
|---|---|---|---|---|---|---|---|
| | (%) | FAB (%) | DDT (min) | MTI (B.U[2]) | PKT (min) | PKH (M.U[3]) | (%) |
| Glenlea | 11.8 | 61.8 | 3.0 | 15 | 8.5 | 500 | 50 |
| Roblin | 15.4 | 65.8 | 6.0 | 15 | 2.0 | 660 | 47 |
| Neepawa | 15.4 | 65.2 | 5.5 | 20 | 2.0 | 560 | 48 |
| Augusta | 7.9 | 50.6 | 1.0 | 100 | 4.0 | 270 | 71 |
| Kenya | 12.6 | 58.0 | 4.0 | 40 | 2.5 | 430 | 62 |
| HY368 | 12.3 | 59.6 | 5.8 | 25 | 3.0 | 530 | 65 |

[1]Abbreviations as defined in Table 10.
[2]B.U=Brabender units.
[3]M.U=Mixograph units.

# APPENDIX II

FIGURE 20. Comparison of the farinograms of Buhler and Quad milled flour samples no. 1-4. The numbers corresponds to the varieties as listed in Table 1.
B = Buhler milled flour
Q = Quad milled flour.


1B
2B
3B
4B

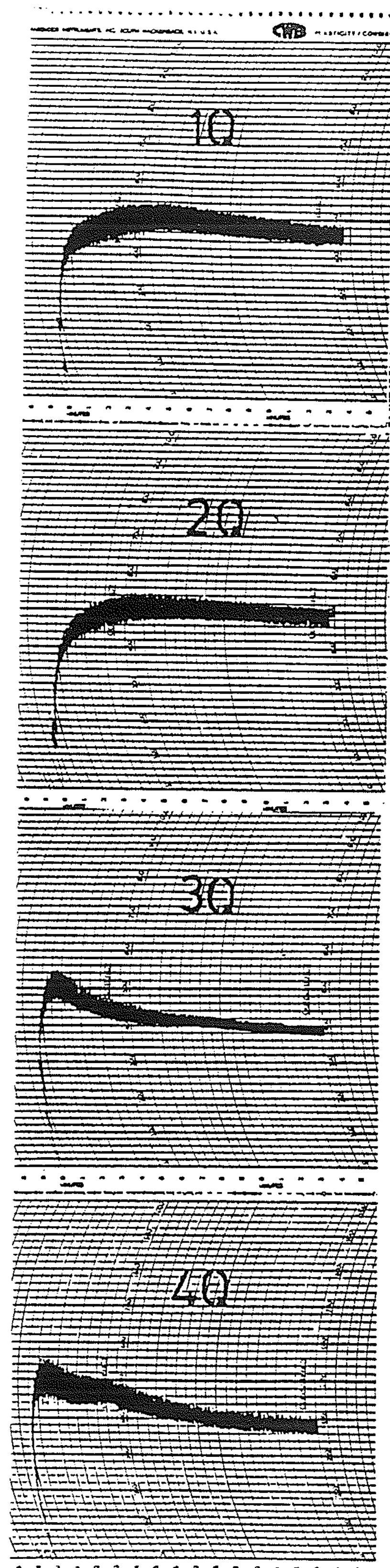
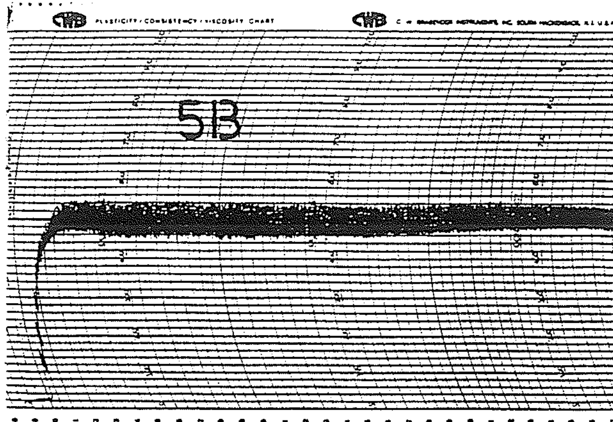
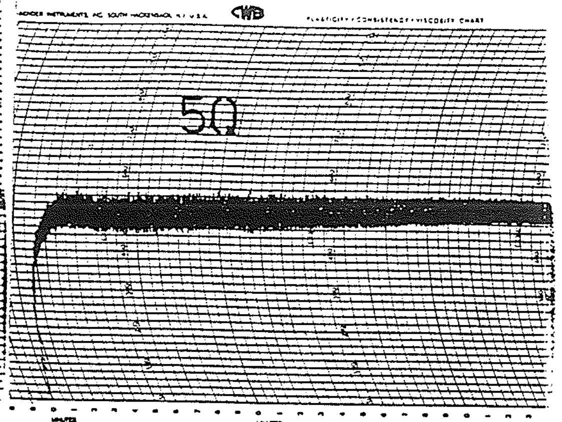
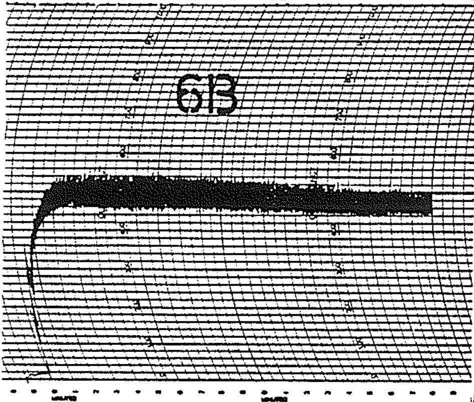
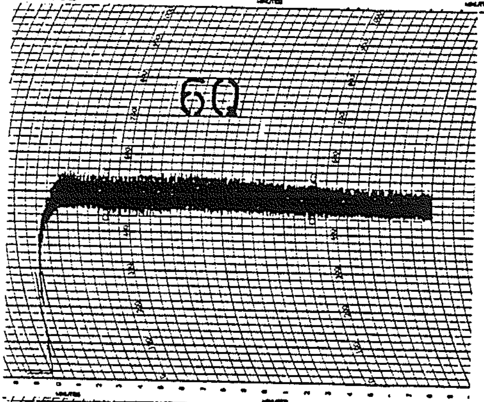

1Q
2Q
3Q
4Q

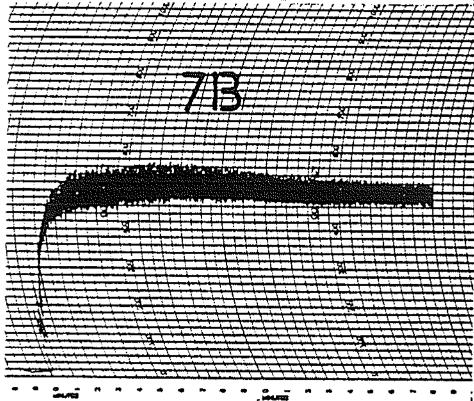
FIGURE 21. Comparison of the farinograms of Buhler and Quad milled flour samples no. 5-8. The numbers corresponds to the varieties as listed in Table 1.
B = Buhler milled flour
Q = Quad milled flour.

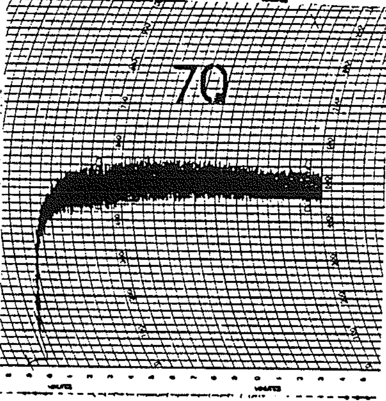

5B

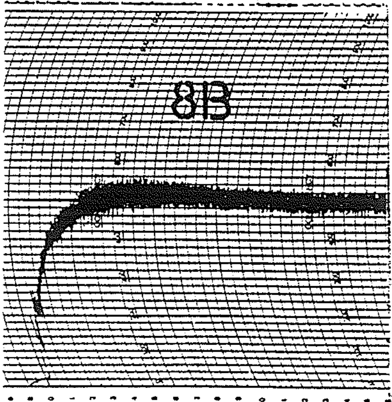

5Q


6B


6Q


7B


7Q


8B

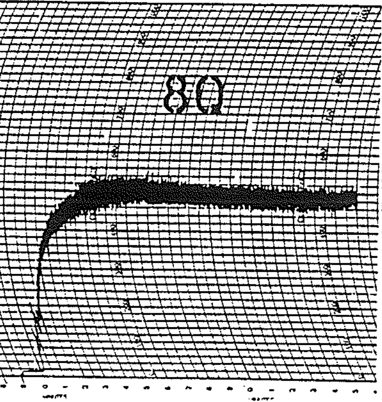
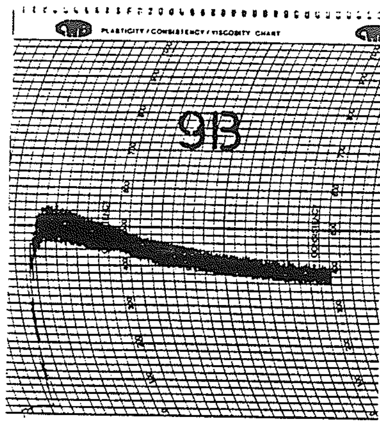
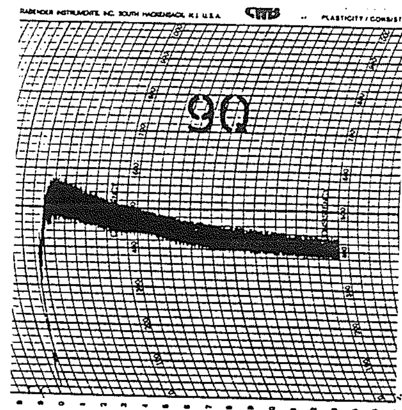
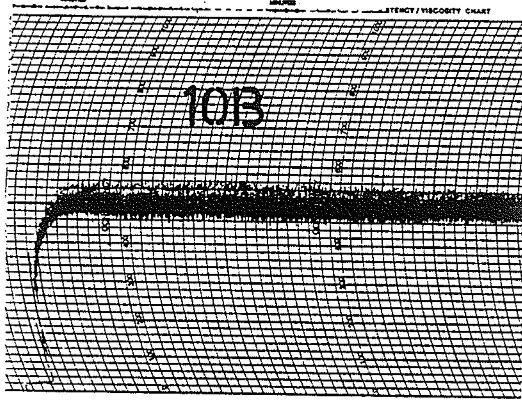
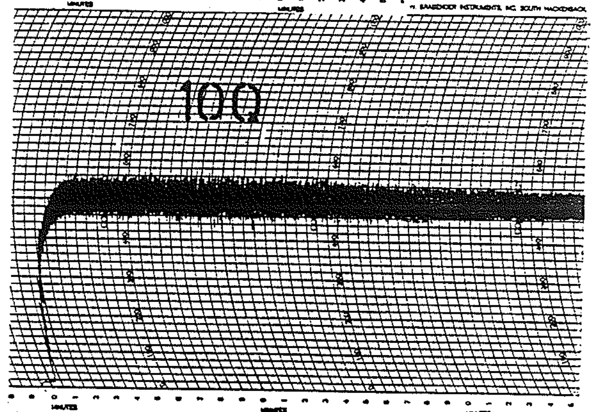

8Q

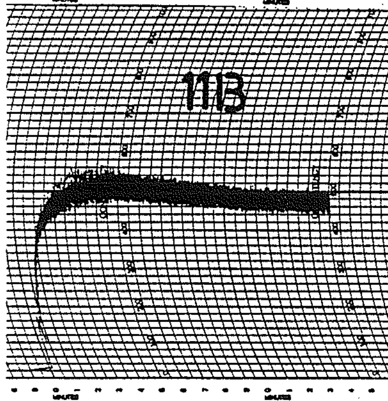
FIGURE 22. Comparison of the farinograms of Buhler and Quad milled flour samples no. 9-12. The numbers corresponds to the varieties as listed in Table 1.
B = Buhler milled flour
Q = Quad milled flour.

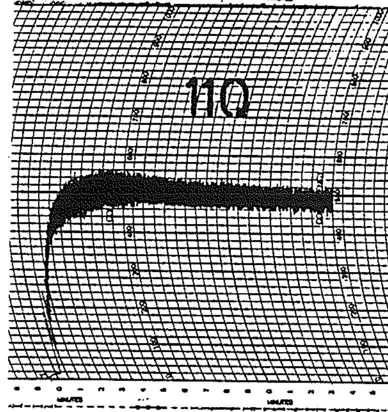

9B

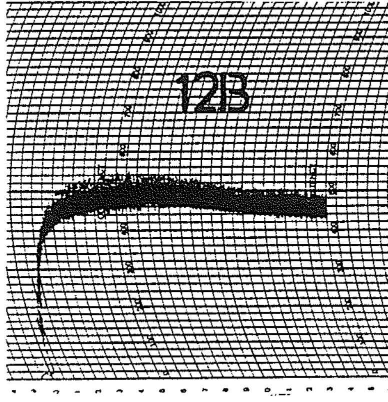

9Q


10B


10Q


11B


11Q


12B

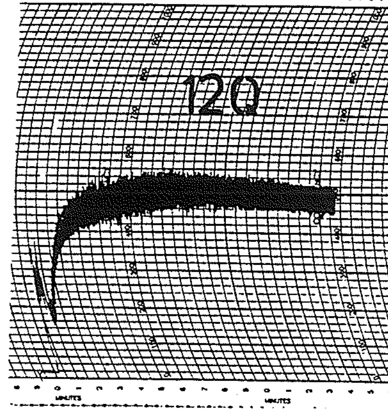
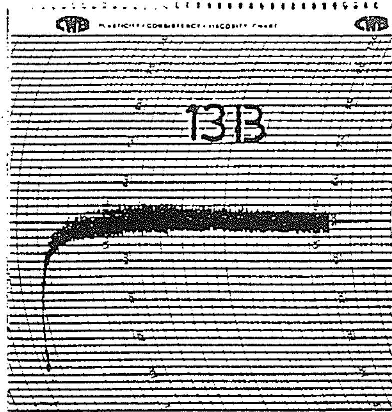
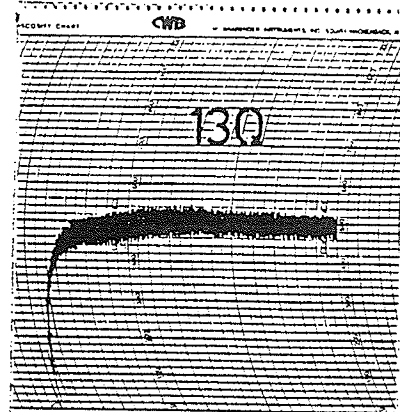

12Q

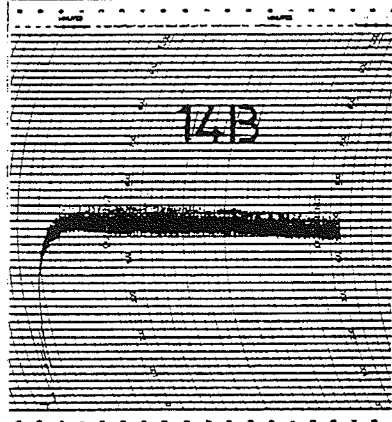
FIGURE 23. Comparison of the farinograms of Buhler and Quad milled flour samples no. 13-16. The numbers corresponds to the varieties as listed in Table 1.

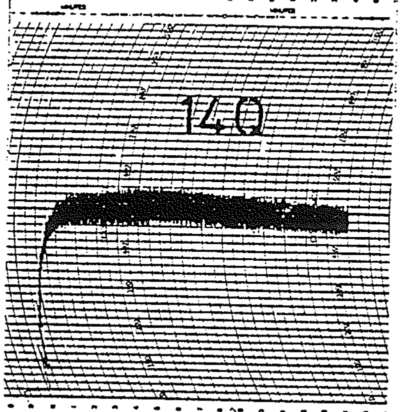
B = Buhler milled flour

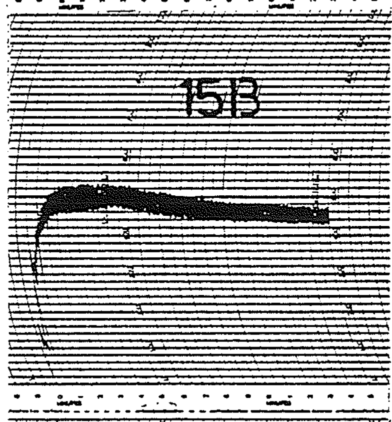
Q = Quad milled flour.

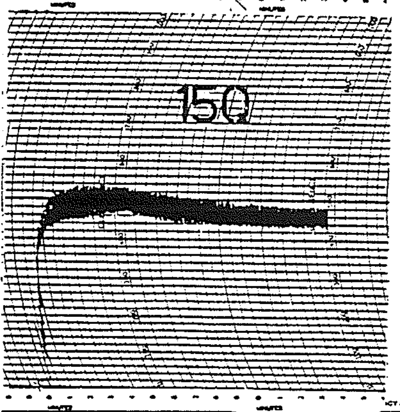

13B

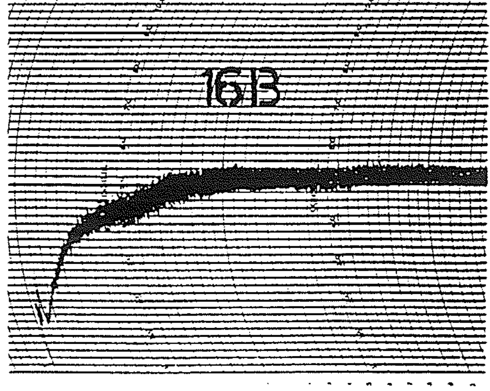

13Q

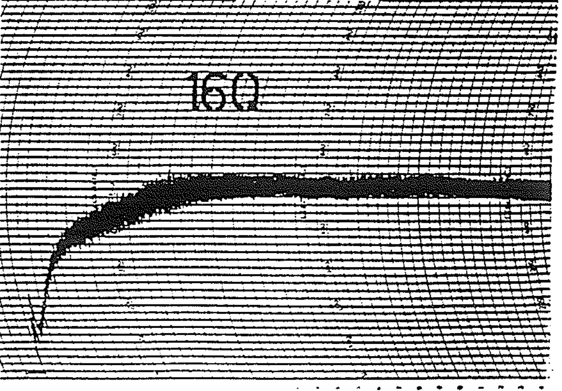

14B


14Q


15B

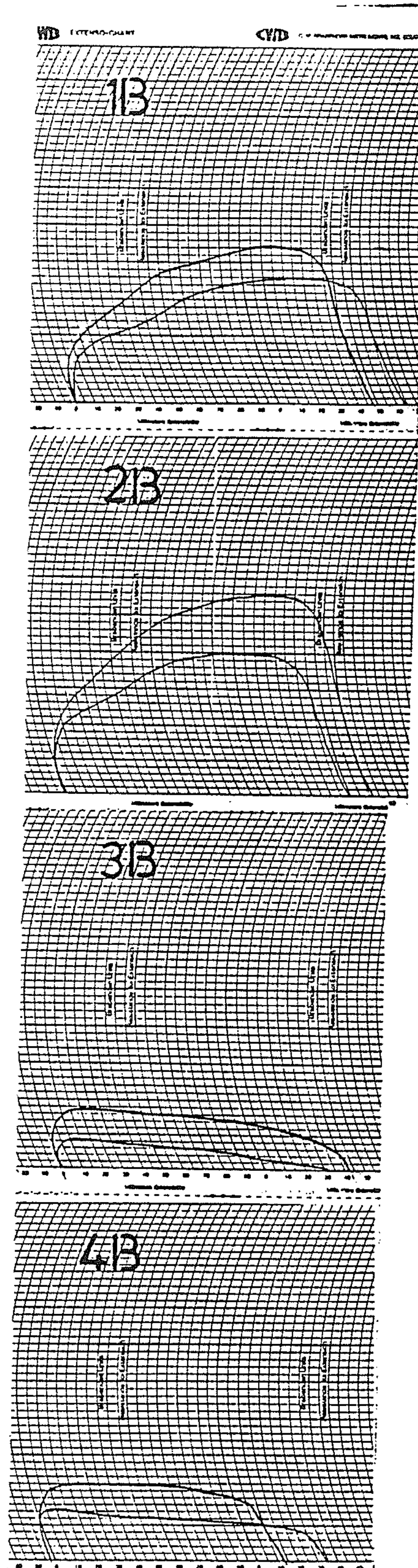

15Q


16B


16Q

# APPENDIX III

FIGURE 24. Comparison of the extensigrams of Buhler and Quad milled flours samples no. 1-4. The numbers corresponds to the samples as listed in Table 1.
B = Buhler milled flour
Q = Quad milled flour.


1B
2B
3B
4B

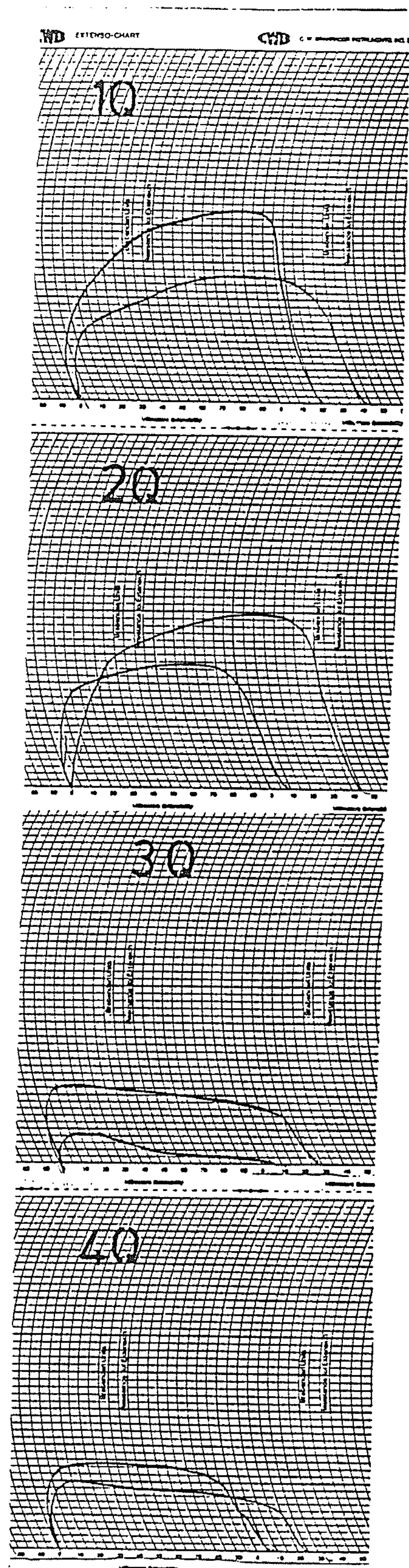
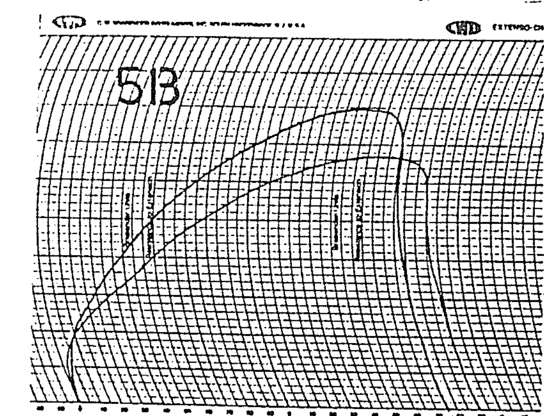
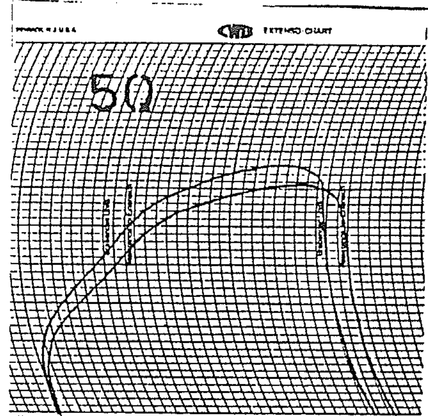
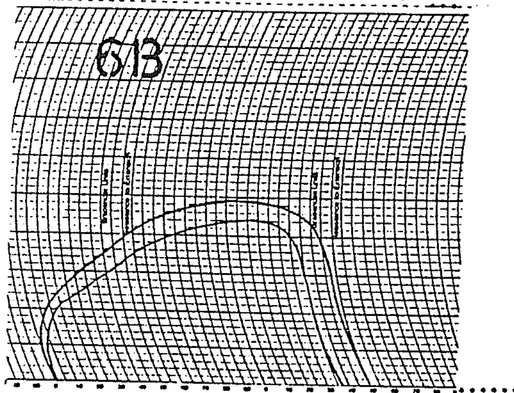
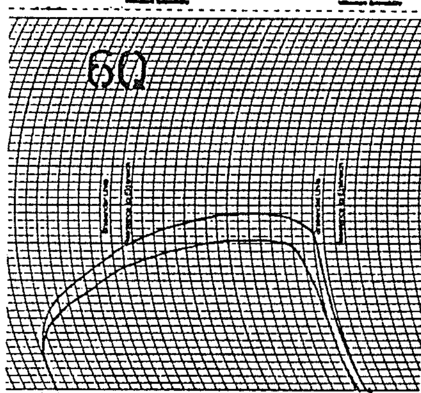

1Q
2Q
3Q
4Q

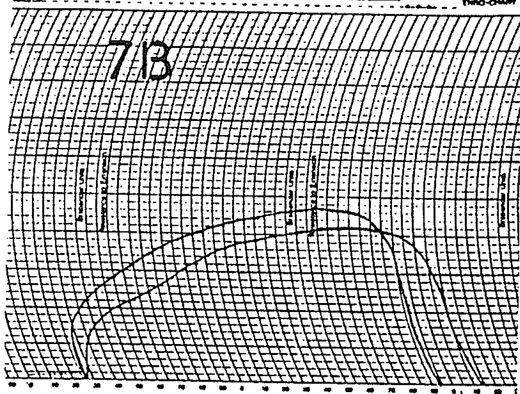
FIGURE 25. Comparison of the extensigrams of Buhler and Quad milled flours samples no. 5-8. The numbers corresponds to the samples as listed in Table 1.
B = Buhler milled flour
Q = Quad milled flour.

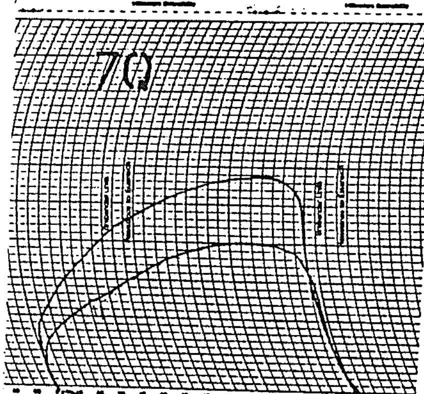

5B

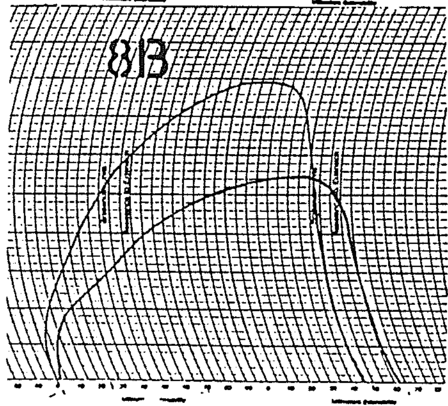

5Q


6B


6Q


7B


7Q


8B

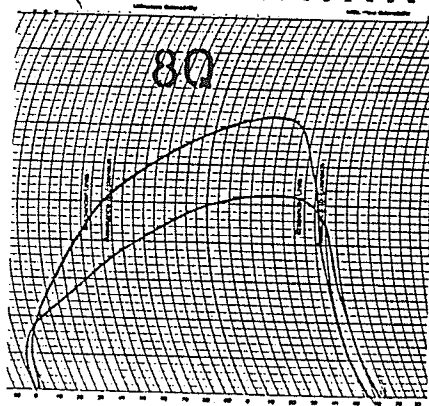
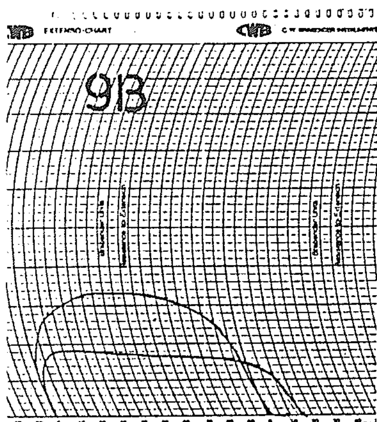
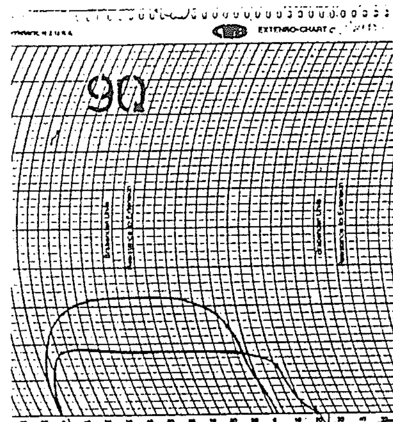
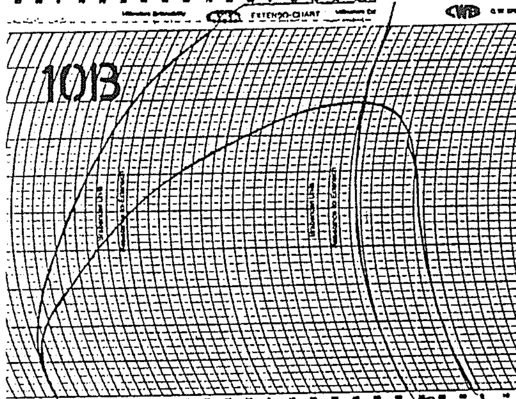
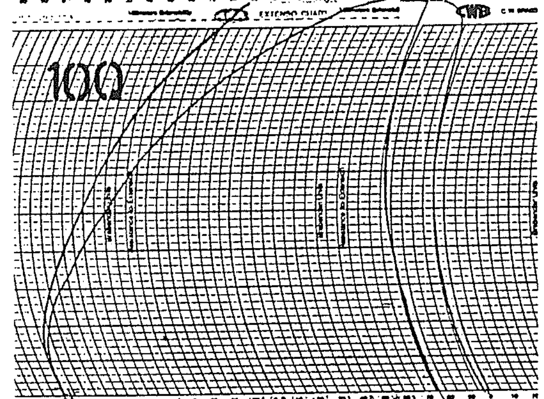

8Q

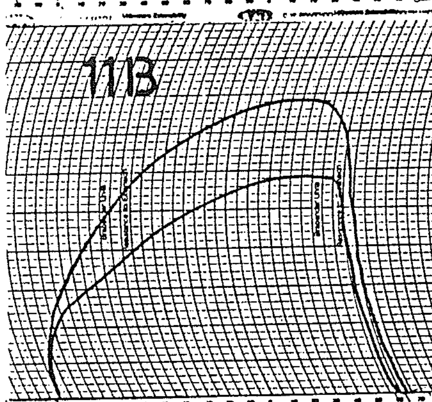
FIGURE 26. Comparison of the extensigrams of Buhler and Quad milled flours samples no. 9-12. The numbers corresponds to the samples as listed in Table 1.
B = Buhler milled flour
Q = Quad milled flour.

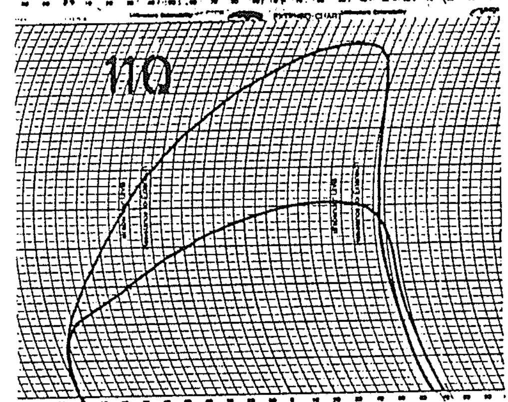

9B
EXTENSO-CHART

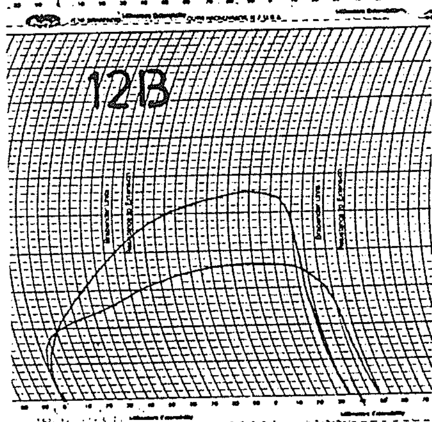

9Q
EXTENSO-CHART


10B
EXTENSO-CHART


10Q


11B


11Q


12B

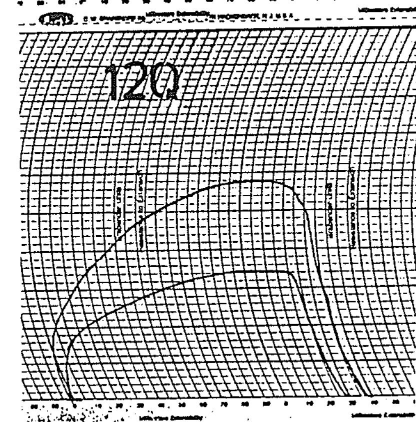
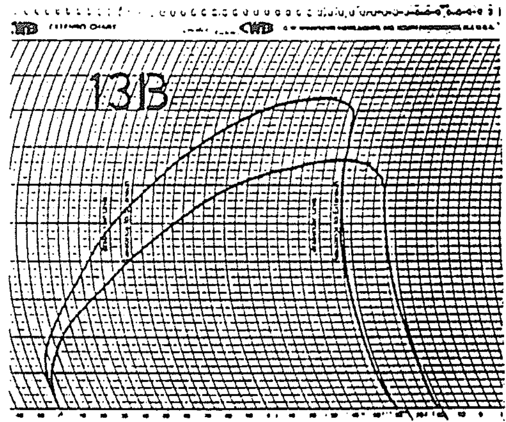
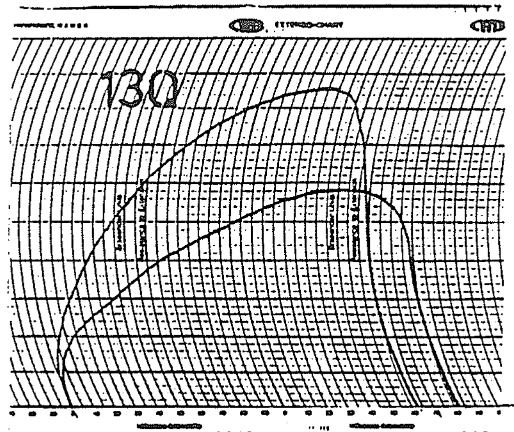

12Q

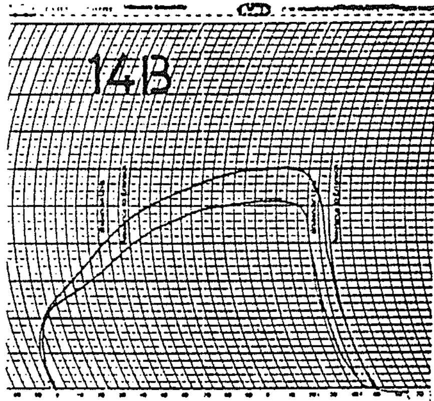
FIGURE 27. Comparison of the extensigrams of Buhler and Quad milled flours samples no. 13-16. The numbers corresponds to the samples as listed in Table 1. 14Q and 15Q are missing due to insufficient samples.

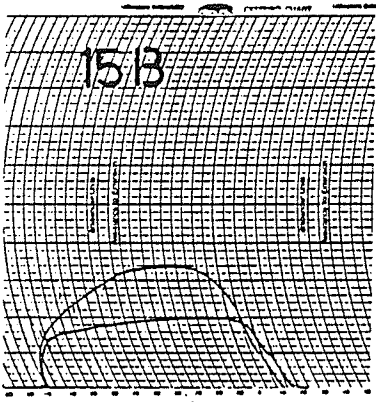
B = Buhler milled flour

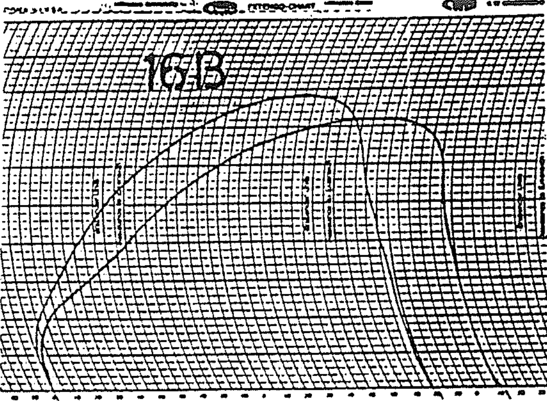
Q = Quad milled flour.

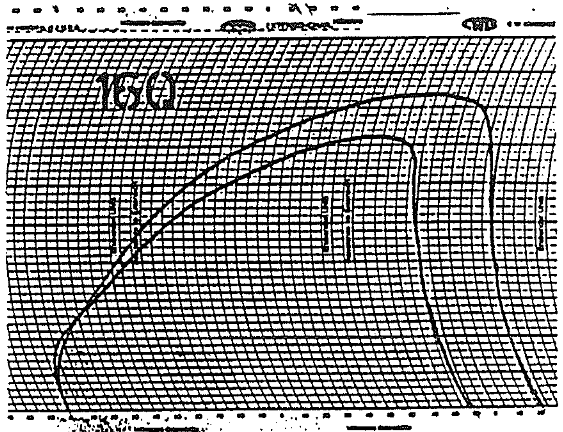

13B


13Q


14B


15B


16B


16Q

# APPENDIX IV

TABLE 30. Technological quality data[1] parameters of the 1983 UQN varieties[2]

| VARIETY[3] | HW | GP | FN | FY | GT | FP | ASH | SV | AV | WG | FA | DT | MTI | E | R | R/E | A | LV | BSI | ULV |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| UQN 99 | 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 |
| UQN 130 | 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 |
| UQN 176 | 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 |
| UQN 199 | 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 |
| UQN 200 | 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 |
| UQN 201 | 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 |
| UQN 203 | 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 | 64.6 |
| UQN 204 | 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 |
| UQN 205 | 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 |
| UQN 206 | 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 |
| UQN 207 | 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 |
| UQN 208 | 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 |
| UQN 209 | 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 |
| UQN 210 | 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 |
| UQN 211 | 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 |
| UQN 212 | 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 |
| UQN 213 | 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 |
| UQN 214 | 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 |
| UQN 215 | 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 |
| UQN 216 | 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 |
| UQN 217 | 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 |
| UQN 218 | 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 |
| UQN 219 | 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 |
| UQN 220 | 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 |
| UQN 221 | 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 |
| UQN 222 | 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=Hectolitre weight (kg/hl); GP=Grain protein (14 % m.b.; Nx5.7); FN=Falling number (sec; 14 % m.b.) FY=Flour yield (%); GT=Grinding time (sec.; "as is"); FP=Flour protein (14 % m.b.; Nx5.7); ASH=Flour 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.); Ext=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] Ng (1987).

[3] Variety no. corresponds to variety no. listed in Table 2.