THE INFLUENCE OF GLUTEN PROTEINS ON THE MIXING AND BAKING PROPERTIES OF FOUR SECONDARY HEXAPLOID TRITICALES

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Roberto Javier Peña

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ABSTRACT

Peña, Roberto J., Ph.D., The University of Manitoba, October 1984. The Influence of Gluten Proteins on the Mixing and Baking Porperties of Four Secondary Hexaploid Triticales. Major Professor: Dr. G.M. Ballance.

A baking test which combines dough development by sheeting rolls and a no-time baking regime was evaluated for its suitability to bake triticale and wheat flours. Extensigrams showed that sheeting rolls developed doughs to a similar extent to that produced by a pin mixer.

Triticale and wheat flours were baked with three baking procedures; the AACC-straight dough method, the no-time/mixing, and the no-time/sheeting methods. All flours performed better under the no-time baking procedures than with the AACC method. No-time/sheeting produced similar loaf volume but better crumb characteristics than the no-time/mixing procedure. The conditions of the no-time/sheeting procedure were adequate to detect differences in baking quality among triticales having similar protein contents.

The influence of gluten proteins on the dough strength (mixing and baking) characteristics of 4 secondary hexaploid triticales (4T, 11T, Impala and Carman) was examined. Two

wheats representing strong (cv. Marquis) and weak (cv. Fredrick) character were included for comparison.

The proportions of gluten in flour and gluten protein in flour protein varied widely among triticales. Gluten washing and Osborne-type solubility fractionation showed that the triticales tested had lower gluten contents than bread wheats, and less gluten protein as a proportion of flour protein than typical soft- or bread wheats.

A gluten stretching test showed that gluten strength varied widely among triticale flours. The results on mixing and baking properties of reconstituted flours prepared on an equal gluten protein basis showed that the triticale Impala had gluten quality similar to that found in strong bread wheats. Interchange of gluten between the wheat Marquis and the triticale Carman resulted in an interchange of mixing and baking properties present in the original flours. This indicated that the extent of the improvement of the dough strength of triticale, due to increase in gluten protein quantity, depends greatly on the quality of that gluten.

Triticale and wheat glutens were fractionated into acid insoluble (residue) and acid soluble (pH 5.8 insoluble and pH 5.8 soluble) fractions. The residue contained mainly highly aggregated glutenin-like protein, the pH 5.8-insoluble fraction combined both glutenin-like and gliadin-like protein, and the pH 5.8-soluble fraction was rich in low molecular weight gliadin-like protein. The pH 5.8-insoluble fraction was further fractionated into glutenin-

like and gliadin-like proteins. An apparent positive association between either the proportion of residue or glutenin-like protein, and mixing strength was observed. Mixographic results of a base flour with added gluten protein fractions and from synthetic flours having most of the gluten protein replaced by the pH 5.8-insoluble protein suggested that mixing strength in the triticales and wheats of this study was mainly controlled by the proportion of glutenin-like protein. The proportion of any of the solubility gluten protein fractions was not apparently related to loaf volume potential of the flours. Addition of either residue or pH 5.8-soluble fraction, from either triticale or wheat, decreased the loaf volume of the base flour, whereas addition of pH 5.8-insoluble fraction resulted in loaf volume improvement. The pH 5.8-insoluble fraction was more effective in increasing the loaf volume of the base flour than either reconstituted gluten or a combination of pH 5.8-insoluble + residue fractions. The loaf volumes of synthetic flours with pH 5.8-insoluble protein replacing most of the gluten proteins indicated that the control of the baking potential of the triticale and wheat flours resided in the combined effect of glutenin-like and gliadin-like proteins.

The differences in the magnitude of the glutenin-like/gliadin-like ratio shown by the triticale and wheat samples could not explain the differences in loaf volume observed among the synthetic flours. Thus, the contribution

by the pH 5.8-insoluble fraction to the baking potential of the synthetic flours seemed to reside in the protein composition of its glutenin-like and gliadin-like components. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis analysis showed that the pH 5.8-soluble protein fraction from the triticale samples had some nonaggregated protein bands in the molecular weight regions of approximately 44,500, 52,000 and 76,000 which were absent in the protein from the wheat samples. Some qualitative and quantitative differences in the high molecular weight protein subunit composition were observed among triticales and between triticales and wheats. However, no obvious relationship between these subunits and the mixing and baking characteristics of the triticale and wheat flours was observed.

I. INTRODUCTION

Cereal grains contribute more than 60% of both calories and protein in the human diet. In these times of large rates in population growth, the increase in food production becomes a major priority. To assist in this regard plant breeders have used hybridization techniques to introduce advantageous genes into cereals that already serve as human food sources. However, interspecific and intergeneric hybridization have also been used to produce new crops to supplement existing food and feed resources. Triticale (X Triticosecale Wittmack) is an example of an intergeneric hybridization.

Intensive work has been done during the last 20 years on improving the agronomic performance of triticale. As a result, many of the early problems such as disease susceptibility, lodging, day-length sensitivity, and seed shrivelling, among others, have been largely overcome (Bushuk and Larter 1980). Among the different wheat-rye genotypes that can be synthesized, the secondary hexaploid triticale (2n=42 chromosomes) is the most promising type. This type is obtained by crossing octoploid with hexaploid triticales or hexaploid triticales with hexaploid wheats, to produce secondary hexaploid triticales having the A-, B-, and R-genome chromosomes (in most cases, 1 or 2 rye

chromosomes are substituted most likely by their homoeologous D-genome chromosomes). Today, most of the experimental materials of the international triticale programs, and all the triticales developed commercially, are of the secondary hexaploid type (Bushuk and Larter 1980). In the present work, secondary hexaploid triticales were used. They will be, for convenience, simply referred as triticales.

In 1980, world cultivation of triticale was in excess of 1 million hectares (CIMMYT 1981). This, indicates that triticale is close to becoming an important commercial crop. However, the commercial success of triticale depends not only on its agronomic performance but also on the post-harvest quality of its grain.

Triticale is generally known to have inferior bread making quality and short mixing requirements relative to wheat (Haber et al. 1976, Lorenz et al. 1972, Tsen et al. 1973, Unrau and Jenkins 1964). This inferior quality along with inconsistency in quality characteristics of triticale cultivars from year to year, have limited its acceptability at the industrial level. If triticale is to succeed as a commercial crop, its industrial quality must be systematically improved.

Tsen et al. (1973) suggested that triticale doughs are too weak to withstand the stress of mixing, fermentation, and oven spring. In accordance with this, Lorenz et al. (1972) and Tsen et al. (1973), respectively, had recommended

the use of mild bread making conditions and a considerable reduction of the fermentation time when baking triticale doughs. For this reason, the first objective of this study was to examine the suitability of dough development by sheeting combined with a no-time baking procedure to test the baking quality of triticale flours.

Dough is a viscoelastic body that responds to an applied force in a way which varies with the flour used to prepare the dough. In this thesis "dough strength" will be used as a general term to refer to the way a dough responds to the forces applied during mixing and baking.

In terms of inherent factors in the flour, it is well established that the main factor controlling dough strength in wheat is the gluten protein (Finney 1943). Since triticale also forms gluten, it is very likely that dough strength in this cereal is also controlled primarily by its gluten protein. In this respect, Tsen et al. (1973) have suggested that the poor baking quality of triticale could be due to low gluten protein content and inferior gluten protein quality. This suggestion has, so far, not been experimentally substantiated. Therefore, this study had as a second (major) objective to examine the influence of gluten protein quantity and quality on the mixing and baking properties of triticale flours. For this, fractionation of flour into major components was extremely useful since it allowed the alteration of proportions and interchange of the gluten protein component in reconstituted flours in which

the effect of both, gluten protein quantity and quality could be expressed in terms of baking characteristics and mixing properties. Extraction of gluten protein by dilute acid was also used to render functional gluten protein fractions that could be tested for their contribution to the baking and mixing properties of a base flour. These experimental approaches allowed the study of the direct role of gluten proteins on dough strength. Finally, the use of electrophoretic techniques permitted the examination and comparison of protein subunit composition, as related to the variability in dough strength between the materials used.

To relate quantitative and qualitative differences in the analytical results to dough strength, secondary hexaploid triticales representing a range in dough strength were included. A good and a poor quality wheat flours were studied in parallel with the triticales to examine differences in terms of factors contributing to dough strength, between triticale and hexaploid wheat, an aspect that has not been reported in the literature.

It was expected that this research project would provide information about factors affecting dough strength in triticale. The understanding of what is deficient could help to define what is to be corrected. Thus, a better criteria could be used for the systematic improvement of the post-harvest quality of the triticale population.

II. LITERATURE REVIEW

A. Introduction

Triticale has been shown to be a highly productive crop but its processing quality needs to be improved. Improvement of the inherent quality of the grain along with modification of the standard technology should help this new cereal to find its place as an important food supplier for the world population.

There are various quality factors influencing the utilization of triticale. In this thesis, the attention is focused on the study of the baking potential of triticale and on the influence of the gluten proteins on both the baking quality and mixing properties of this cereal. This chapter reviews studies on the baking, rheology and biochemistry of triticale and wheat, pertinent to the work reported in this thesis.

B. Baking Technology

The attainment of good quality bread depends on both the inherent quality of the flour and the baking process. The baking methods currently in use vary, from one country to another, depending on flour types available, as well as on economical and cultural factors.

Baking procedures can be grouped into two general categories; bulk-fermentation methods, and no-time methods.

The main difference between methods in these two categories is the way the dough is developed to manifest its maximum baking potential.

1. Bulk-Fermentation Methods

This category includes the straight dough and the sponge-dough procedures. These procedures have a bulk-fermentation stage during which gluten undergoes chemical and physical changes which contribute to the "ripening", i.e. acquirement of appropriate viscoelastic properties, of the dough (Kent 1975). The main difference between the straight dough and the sponge-dough procedures is that in the first all ingredients are mixed to form the dough in a single step, followed by a bulk-fermentation stage of usually 3h, while in the second method only part of the flour is mixed with all the yeast and part of the water, to form a "sponge" which is allowed to ferment for 2 to 4h. The "sponge" is then combined with the rest of the flour, water, and salt, and mixed into a dough which is given a short bulk-fermentation time (Jackel 1978, Kent 1975). The fermented doughs are molded, proofed, and baked similarily in both procedures (Jackel 1978).

In the U.S.A., the sponge-dough method has been preferred, since its introduction in 1920, over the straight dough method because the latter presents problems such as non-uniform scaling and proofing (Jackel 1978). The sponge-dough method has the disadvantage of being wasteful of time, space, and labor.

2. No-Time Methods

Highly automated baking technology originated in the U.S.A. with the application of the method of Baker (1954), which included the concept of mechanical dough development of Swanson and Working (1926). These authors found that controlled application of intensive mixing action to the ingredients of a dough formula containing fair amounts of an oxidizing agent, can bring about the desirable viscoelastic dough obtained with the bulk-fermentation methods.

The American "Do-Maker" and "Amflow" continuous dough processes follow the principle described above. Their main features are the premixing of a pre-ferment and other ingredients followed by an intensive mixing (development) of the dough in a closed chamber, the elimination of the bulk-fermentation stage, and the extrusion of the dough directly into the baking pan to be then proofed and baked (Kent 1975). These highly automated methods have the advantage, over the bulk-fermentation methods, of reducing processing time, space, and labor.

Another major no-time method is the British Chorleywood Bread Process (CBP). The CBP can be used either on a batch or continuous fashion. In this method, the bulk-fermentation stage is eliminated. Instead, the dough is developed in a single step by vigorous mixing until the energy expended in the dough is 40 kJ per Kg of dough over a period of 5 min (Kent 1975). Other features of the method are the use of ascorbic acid (oxidizing agent) at a high

level (75 ppm) and the use of yeast at a higher level (1.5 to 2.0 times more) than in bulk-fermentation processes (Kent 1975). It is claimed that this process produces bread of high quality, results in an increase of 4% in yield of bread, and saves time and space (Kent 1975).

The no-time/chemically assisted methods are widely used in Canada (Jackel 1978). In these methods, the dough is developed in a single step using conventional moderate-speed mixing. The development process is assisted by the presence in the formula of a fast-acting oxidizing agent (potassium bromate or a mixture of potassium bromate and ascorbic acid) (Tsen 1973). The presence of cysteine reduces mixing time and accelerates the ripening of the dough.

Dough strength (mixing time and stability, and baking potential) is a critical factor to be considered before a baking procedure is to be adopted in the production of a particular type of bread. The strength of the flour is critical at all stages of a baking process because the dough structure is continuously subjected to physical strain. The amount of strain exerted upon the dough structure varies with baking conditions such as mixing intensity, presence of reducing and/or oxidizing agents, and presence and extent of a bulk-fermentation stage. Therefore, dough strength requirements differ from one process to another, or, in another view, baking requirements differ from one flour type to another. Fortunately there is enough processing and dough strength variability that adjustments can be made in

both factors to obtain the desirable product.

Economically it is more desirable that the wheat varieties destined to bread production have enough flour strength (not too much, not too little) to produce a good product over a wide range of baking conditions (Tipples et al. 1982). In cases where it is difficult to breed for wheat varieties with good dough strength, as in Europe, then a combination of mild mixing and baking conditions, such as those of the Chorleywood baking process, help to partially alleviate the problem. This process allows flours with limited strength to produce bread of better quality than if these flours were baked under any of the other conventional processes described above (Axford et al. 1963).

As suggested by Bushuk and Larter (1980) and evidenced by Lorenz et al. (1972) and Tsen et al. (1973), triticale flours have somewhat different rheological properties and inferior strength to those of bread wheat flours. Therefore even after modifications in standard baking processes, such as the use of mild baking conditions and considerable reduction of fermentation times, triticale flours have produced bread with quality generally inferior to that of bread wheats (Lorenz et al. 1972). However, it is possible that baking conditions similar to those of the Chorleywood baking process, particularly the elimination of the bulk fermentation time, and its baking formula, could be favourable to the baking requirements of triticale flours.

C. Dough Development

The process of dough development is essential to produce bread of satisfactory quality. Well developed doughs can be obtained by a combination of conventional moderate-speed mixing followed by fermentation and punching with the bulk-fermentation methods. The contribution of the bulk-fermentation stage to the development of the dough is made by providing an environment in which the gluten proteins undergo changes in their colloidal state due to chemical reactions, mainly those that acidify the dough (Pyler 1973), and to the repeated stretching and contraction of the protein network resulting from the aggregation of small gas cells into large ones, and from the dividing of the large cells again into small ones during the punching steps. Thus the bulk-fermentation stage finishes the ripening of the bread dough. However, as mentioned earlier, the application of intense mixing to the dough or the use of a combination of reducing and/or oxidizing agents and moderate-speed mixing result in an equally well-developed dough, and makes the bulk-fermentation stage unnecessary.

During mixing, the action of pins or blades accelerates the rate of hydration of the solid particles. As water penetrates the particles, the mixing action results in the removal of hydrated layers until all particles become hydrated and the free water of the aqueous mass drops to a level that permits the dough to oppose a defined resistance pattern to the mixing action (Hoseney and Finney 1974). At

this stage, mixing results in disaggregation of native protein aggregates which then reaggregate into an entanglement network. As mixing continues, the entanglements begin to slip loose as the molecules are aligned more in the direction of the shear (Moss 1974, Tsen 1973). At a certain stage, the strength of the network reaches a maximum, corresponding to the peak development of the dough. At this point the gas retention capacity of the dough is optimal (MacRitchie 1980b).

If mixing is terminated before the protein is properly altered, the dough is less able to retain gas (under-mixed dough). On the other hand, if mixing is continued beyond the maximum resistance point, the dough becomes wet, sticky, and its resistance decreases indicating that the dough structure has broken down (over-mixed dough). According to MacRitchie (1980b), over-mixing decreases entanglement but this can be recovered after a certain time in which the dough is allowed to relax. Over-mixing has been suggested to also produce an irreversible change namely mechanical scission of covalent bonds in the gluten proteins still entangled (MacRitchie 1980b, Tanaka and Bushuk 1973).

Large protein aggregates can be split by mixing and by chemical action. During mixing, shearing and tearing forces can break linkages -- mainly hydrophobic interactions between protein aggregates (Tsen 1973). This is based on the observation that more protein can be extracted, by dilute acids, from doughs at progressive stages of mixing

(Mecham et al. 1962). According to Tsen (1969), the increase in extractable protein indicated a decrease in the size of the protein aggregates.

Acceleration of dough development by the addition of cysteine was first observed by Henika and Rodgers (1965). Cysteine exerts its action by reducing disulfide bonds between protein aggregates (Tsen 1969). Thus cysteine facilitates the disaggregation action of mixing, promotes disulfide interchange, and therefore contributes to attaining dough development in a shorter time with less mixing intensity (Tsen 1969). A dough thus developed is matured (network-setting) by added oxidizing agents (e.g., ascorbic acid, potassium bromate) which cross-link some sulfhydryl groups into new disulfide bonds (Tsen 1969).

The practice of using sheeting rolls for dough development is common in large areas of the world including South America, Southeast Asia, and Africa (Bushuk and Hulse 1974, Stenvert et al. 1979). Kilborn and Tipples (1974) and Kilborn et al. (1981) studied factors affecting dough development by sheeting rolls (dough break). Kilborn and Tipples (1974) found that optimum dough development could be achieved by passing the dough several times through sheeting rolls, with the advantage of expending only 10-15% of the energy required to develop the dough with a high-speed mixer. The dough, thus developed, included ingredients common to the no-time baking procedures, and was baked as in the Chorleywood Bread Process, to produce bread with

satisfactory quality.

The combination of dough development by sheeting rolls and baking under the conditions of the no-time methods seems to provide mild conditions for the production of bread from a range of flour types (Stenvert et al. 1979), and from composite flours (Bushuk and Hulse 1974).

Therefore, dough-sheeting and no-time baking conditions seem to be a promising combination for improving the baking performance of triticale flours.

D. Mixing and Baking

Properties of Triticale

Wheat is the most widely used cereal in the world. The unique viscoelastic properties of its flour dough make it very suitable for the production of a number of baked products of which bread is the most valuable.

Many investigators have studied the mixing and baking properties of triticale flours. Their results consistently showed that, in general, triticale flours had lower absorption and considerably shorter mixing times and mixing tolerances than bread wheat flours, as well as a rather inferior baking quality as compared to that of wheat flours (Ahmed and McDonald 1974, Haber et al. 1976, Kaltsikes and Larter 1970, Lorenz et al. 1972, Peña 1979, Rooney et al. 1969, Tsen et al. 1973, Unrau and Jenkins 1964).

In some studies it was found that mixing and baking properties varied among the triticales tested (Lorenz et al.

1972, Peña 1979, Singh and Katragadda 1980). Examination of their results indicates that differences in mixing and baking properties among triticales can not be related to differences in flour protein content. However, the results of Lorenz and Welsh (1977) with one triticale cultivar at various flour protein levels, could indicate that within a triticale strain, as the flour protein increases its mixing requirement and bread loaf volume increases also.

Satisfactory breads have been obtained from some triticale flours when modifications in the standard baking procedures such as the use of mild mixing conditions (Lorenz et al. 1972), reduction or elimination of the bulk-fermentation stage (Lorenz et al. 1972, Lorenz and Welsh 1977, Peña 1979), and addition of additives such as sodium stearoyl-2-lactylate (Tsen et al. 1973) were made. The improved baking performance of some triticales has been explained as due to a combination of better baking practices for triticale, along with the actual improvement of the rheological characteristics of certain triticale strains; mainly those selected from crosses between triticales and bread wheats (CIMMYT 1977).

The inferior baking quality of triticale is in part contributed by high amylolytic (Klassen and Hill 1971, Peña and Bates 1982, Welsh and Lorenz 1974) and proteolytic (Madl and Tsen 1973, Singh and Katragadda 1980) activities. However, these detrimental enzymatic effects could be substantially overcome by reducing or eliminating the bulk-

fermentation stage during breadmaking (Tsen et al. 1973). Therefore, it appears that the inferior baking quality of triticale could be mainly due to low gluten protein content, as indicated by the low insoluble flour protein values obtained for triticales by Chen and Bushuk (1970a) and Ahmed and McDonald (1974). Additionally, it is likely that triticale has gluten with a quality inferior to that in bread wheats. This, could result from the partial or total absence of D-genome chromosomes in the background of hexaploid triticales. With wheat it has been demonstrated (Kerber and Tipples 1969, among others) that D-genome chromosomes make an important contribution to the baking and mixing properties of flours. Tetraploid wheats (durum), that lack D-genome chromosomes, have poor baking quality.

E. Relationship of Gluten Proteins to Dough Strength

Baking quality and mixing requirements vary widely among hexaploid wheats. It is well accepted that factors associated with mixing requirements are interrelated with factors associated with baking quality (Finney et al. 1982, Tipples et al. 1982) and therefore, the term dough strength is conventionally used to associate baking quality with mixing requirements. Thus, strong flours are generally considered to have longer mixing requirements than weak flours.

Flours with very long mixing requirements may produce very tenacious doughs and may perform poorly under conditions of slow speed- or minimal mixing and no-time

baking procedures. In contrast, flours with short mixing requirements generally do not tolerate over-mixing and the conditions of bulk-fermentation baking procedures. According to Tipples et al. (1982), a wheat flour performing well over a wide range of processing conditions normally should have mixing requirments that are neither unduly short nor unduly long.

The contribution of flour components to the variation in dough strength has been extensively studied. The first indication that the gluten protein controls dough strength in wheat flours was obtained by Aitken and Geddes (1938) who found that addition of dry gluten to a weak flour, imparted stronger character to such flour. The increase in flour strength varied with the source of the gluten added, indicating the influence of a gluten quality factor on the control of flour quality character (Aitken and Geddes 1938). The latter was confirmed by the fractionation-reconstitution studies of Booth and Melvin (1979), Finney (1943), and MacRitchie (1978). These authors found that differences in baking quality between flours were accounted for by differences in their gluten character. Butaki and Dronzek (1979a) found that addition of glutens from different flour sources increased differentially the mixing strength of a weak flour.

Gluten is the rubbery mass left after a dough is washed with sodium chloride solutions to remove starch and soluble materials. It comprises 78-85% of the total flour protein

in bread wheats (Pence et al. 1954) and is composed chiefly of protein (approximately 85%) (Kasarda et al. 1976). Gliadin (70% ethanol-soluble) and glutenin (70% ethanol-insoluble) are the main protein fractions of gluten. The glutenin fraction has been further fractionated into dilute acid soluble and insoluble (residue) fractions.

Several factors have made it difficult to find a relationship between specific gluten components and dough strength. These include interactions between gluten and other flour components as well as variability in the composition of isolated gluten protein fractions due to different extraction procedures which have been used. Also because of overlapping physical and chemical properties between gliadins and glutenins there is almost invariably a certain degree of cross-contamination of gluten fractions regardless of the extraction procedure used. In spite of these difficulties, important knowledge about gluten protein composition and its relation to dough strength has been obtained.

Use of dilute acid solution to disperse gluten is an effective procedure to fractionate gluten into functional protein fractions (Booth and Melvin 1979, Finney et al. 1982, Harris and Frokjer 1952, Hoseney et al. 1969, MacRitchie 1978, 1980a, Preston and Tipples 1980, Shogren et al. 1969). Fractionation of gluten with 2M urea also seems to yield functional protein fractions (MacRitchie 1972, 1973). In contrast, Booth and Melvin (1979) and Hoseney et

al. (1969) found that the use of 70% ethanol to fractionate gluten, yielded fractions with altered functionality.

Results of studies involving the addition of gluten protein fractions to a base flour (Harris and Frokjer 1952, MacRitchie 1973, Preston and Tipples 1980) and fractionation-reconstitution of flour components (Finney et al. 1982, Hoseney et al. 1969, MacRitchie 1980a, Shogren et al. 1969), indicate that the high molecular weight, residue protein (acid- and urea-insoluble gluten protein), is not the main factor controlling the baking potential (loaf volume) among flours. When this fraction is present in excess, it tends to decrease the loaf volume potential of a flour (Harris and Frokjer 1952, MacRitchie 1973, 1980a, Preston and Tipples 1980, Shogren et al. 1969). However, it is well recognized that this fraction contributes importantly to the mixing properties of wheat flours (Hoseney et al. 1969, MacRitchie 1972, 1973, Preston and Tipples 1980, Shogren et al. 1969). When added in excess, the residue protein tends to increase the elasticity and decrease the extensibility of a flour dough (MacRitchie 1972, 1973, Preston and Tipples 1980).

On the other hand, gluten protein fractions that combine glutenin and gliadin (i.e. acid soluble gluten proteins) have been found to house the main factors responsible for variation in dough strength. When added to a flour, they tend to increase mixing time, mixing tolerance, and loaf volume potential of a flour (Harris and

Frokjer 1952, Preston and Tipples 1980). When this fraction is used to replace whole gluten in reconstituted flours, all or most of the dough strength potential is maintained (Goforth et al. 1977, MacRitchie 1980a, Shogren et al. 1969). Also, when this gluten protein fraction is interchanged in reconstituted flours, the dough strength character is also interchanged (Booth and Melvin 1979, Finney et al. 1982, MacRitchie 1978).

Low molecular weight, gliadin-rich protein (gluten protein soluble above pH 6.0), when used to replace the other gluten proteins in a reconstituted flour (Shogren et al. 1969) or when added in excess to a base flour (Harris and Frokjer 1952), decreased mixing time, mixing tolerance, and loaf volume. MacRitchie (1980a) found that interchange of a gliadin-rich fraction between two flours of different baking quality, resulted in no change in the baking potential of the flours used.

Other approaches commonly used to study the functionality of gluten proteins include the statistical and nonstatistical comparisons between the proportions of gluten proteins and flour quality parameters. The amount of residue flour protein insoluble in 3M urea (Pomeranz 1965), in 0.05M acetic acid (Axford et al. 1978, Hamada et al. 1982, Orth and Bushuk 1972), and in AUC (0.1M acetic acid, 3M urea, 0.01M cetyltrimethyl-ammonium bromide) (Huebner and Wall 1976) has been found to be larger in the stronger wheats. The amount of glutenin was found not to correlate

(Hamada et al. 1982) or to correlate negatively (Marais and D'Appolonia 1981a, Orth and Bushuk 1972) with dough strength. Other studies have found no association between the amount of gliadin in a flour and that flour's dough strength (Huebner 1970, Huebner and Rothfus 1968, Huebner and Wall 1976, Orth and Bushuk 1972).

The protein solubility distributions of some triticale flours have been examined. Chen and Bushuk (1970b) and Wall et al. (1972) found that triticale flours had considerably less residue protein than had wheats. This deficiency in the amount of residue protein was suggested by both groups of investigators to be a major factor influencing the inferior quality of triticale flours.

Fractionation and reconstitution studies and those involving addition of gluten protein fractions to a base flour, favour the suggestion that gluten protein fractions combining both glutenin and gliadin are responsible for most of the variability in dough strength in wheat. Apparently these gluten protein fractions include glutenin and part of the gliadins found as high molecular weight aggregates (Shogren et al. 1969, Finney et al. 1982), which have been designated (Beckwith et al. 1966, Bietz and Wall 1980) high molecular weight gliadins. On the other hand, those researchers who have examined the association between protein solubility distribution and dough strength, favour the idea that the flour residue protein is the main factor controlling dough strength. This discrepancy could be due

to differences in composition between residue protein isolated from gluten and residue protein isolated from flour. When the source of gluten protein fractions is flour, more protein remains insoluble (see Miflin et al. 1983 for review), than when the source is gluten. The formation of a dough by mixing followed by the kneading action to wash gluten out, results in a certain degree of disaggregation of complex protein aggregates (Mecham et al. 1962, Tsen 1969, 1973). As a consequence of this partial disaggregation effect, the gluten proteins become more extractable than when they were in their native state in the flour. Thus, as suggested by Preston and Tipples (1980), part of the gluten protein (influencing dough strength variability) present in the residue protein becomes soluble protein when extracted from gluten.

The importance of glutenin in influencing dough strength is now well recognized. Therefore, great attention has been given to the study of its physical and chemical properties. Studies involving sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) of reduced glutenin have shown that glutenin is a rather heterogeneous protein complex of subunits ranging in molecular weight between 12,000 and 135,000 (Huebner and Wall 1976, Khan and Bushuk 1978).

Gel chromatography has been used to separate high molecular weight (HMW) glutenin from glutenin of lower molecular weight. Hamada et al. (1982), Huebner and Wall

(1976), and Field et al. (1983), found that the amount of HMW glutenin (Field et al. (1983) call this fraction "HMW prolamin") correlated positively with dough strength. Huebner and Wall (1976) found by SDS-PAGE that the protein subunit composition of reduced HMW glutenin was essentially the same as that of the lower molecular weight fraction indicating that the main difference between those two fractions was the extent of aggregation of their protein components. Field et al. (1983) using SDS-PAGE of reduced protein extracts found no difference in the amount of high molecular weight protein subunits (>90,000) between a strong and a weak wheat flour.

Some investigators have examined, by SDS-PAGE, the possible association of specific protein subunits with variation in dough strength. Orth and Bushuk (1973a) found that differences in number and molecular weight of the subunits of wheat cultivars were not related to the variability in dough strength. The same authors, as well as Wrigley et al. (1982), found no association between the presence of specific HMW glutenin subunits and dough strength. Both groups of investigators examined different large populations of wheats from different countries. In contrast, Bournof and Bouriquet (1980) found two high molecular weight subunits present in good quality flours and absent in those with unsuitable dough strength. In the same line, Payne et al. (1979, 1981) found that the presence of some HMW glutenin subunits was positively correlated with

dough strength. However, the cultivars used in each of the studies of these two groups of investigators, were genetically related. Payne et al. (1979) found that several unrelated cultivars of good bread making quality, did not possess the subunit that correlated with dough strength. Association between certain gliadin subunits and the dough strength of cultivars from several countries has also been reported (Wrigley et al. 1981, 1982). With respect to the association of gluten protein subunit composition and dough strength, Miflin et al. (1983) emphasized that this type of analysis does not explain cause and effect, and that it could only indicate linkage between "true quality genes" and structural genes for certain proteins.

From this review one can see that the baking technology can be modified to suit conditions required to obtain bread of appropriate quality from a variety of flour types. With respect to the flour, it appears that a combination of gliadin (mainly high molecular weight gliadin) and glutenin in suitable proportions are required to produce a dough with appropriate elasticity to tolerate mechanical work and maintain a stable structure at the different stages of bread making, and appropriate extensibility to allow for dough expansion which results in bread of good loaf volume and good crumb characteristics. Much is still to be understood about the chemistry and rheology of wheat flours, but even more has to be learned about the composition of triticale and the role of the endosperm components on its

inferior dough strength. Accordingly, this work was designed to study the influence of technological modifications and of the gluten proteins on the dough strength of secondary hexaploid triticales.

III. MATERIALS AND METHODS

A. Grain and Flour Samples

Four triticales -- three grown in Sonora, Mexico, during the 1980-1981 season as part of the International Maize and Wheat Improvement Center (CIMMYT) yield nurseries, and the fourth (cv. Carman) grown at the University of Manitoba, Winnipeg, Canada, during the summer of 1980; one hard red spring wheat (cv. Marquis), and flour from a soft white winter wheat (cv. Fredrick) and from a strong hard red spring wheat (c.v. Neepawa) were used. Identity and cultivar name for the triticale samples are listed in Table 1.

All triticales were identified as being of the secondary hexaploid type, having 2n=42 chromosomes (Gustafson 1982). The triticales 4T, 11T and Impala had chromosome 2R substituted and the triticale Carman had all rye chromosomes present (Gustafson 1982).

The wheat and triticale grain samples were tempered (to 15.5% and 14.5% moisture, respectively) overnight and milled on a Buhler Experimental Mill. The wheat flour streams were blended and rebolted through a 70-gg (236μ) sieve, while for the triticale flours, the last flour stream from the reduction system was first sifted through a 11-mesh (125μ) sieve to reduce ash content of the flour, prior to the blending and rebolting as with the wheat flour.

TABLE 1. Identity of the Triticale Cultivars

Identity	Name
W74.103-ADDAX/BGL "s"-M_2AxIRA[a] x-33470-c-1Y-3M-2Y-2M-0Y	4T
TRR"R"-MPE/PND"s"xM_2A-IRA[a] x-47220-A-2M-1Y-1Y-0H	11T
UMS/TCL BULK x1530A	Carman
BGC-BULK E_2[a] x-11066-A-6M-100Y-100B-101Y-0Y	Impala

[a] Pedigree identification system of CIMMYT.

The triticale samples used in this study were chosen because they are of the same type (secondary hexaploid triticale) and because they represented a range of dough strength. Additionally, the triticale Carman was included because it has all the rye chromosomes while the others lack one (2R). The chromosome substituting chromosome 2R in the samples was not identified.

The wheat samples were included for comparison purposes. The specific varieties included were chosen because they represent good-(cv. Marquis) and poor-(cv. Fredrick) dough strength.

B. Flour Analysis

Ash, moisture, protein (Nx5.7), alpha-amylase activity (Falling Number (FN) value), and the Zeleny sedimentation value, all were determined and replicated using AACC methods (1976) (methods 08-01, 44-15A, 46-12(13), 56-81b, 56-60, respectively).

C. Mixograph Characteristics

1. Swanson and Working-Mixer Mixographs

Mixograms of all original flours were determined with the Swanson and Working mixograph using 35g of flour (14% mb) at 60% absorption for the wheat Marquis, and at 59% absorption for the soft wheat and the triticales. The spring was set in position 8.

2. Electronic Recording-Mixer Mixographs

Mixograph characteristics of original, fractionated-reconstituted, and altered flours were evaluated with the electronic Recording Dough Mixer (Voisey et al. 1966) equipped with a Dynamaster recorder (The Bristal Company of Canada, Toronto). Sample size was 5g (14% mb) and the mixer speed was 95 rpm. Water absorption was used as noted later. Mixing time, peak height, and peak area (the area under the curve between time zero and the peak time) were measured in 5-min mixograms.

D. Extensigraph Characteristics

Extensigrams were obtained with the Brabender Extensigraph by using the AACC method 54-10 (1976) with the exception that doughs were developed either with the GRL-mixer described by Hlynka and Anderson (1955), or with sheeting rolls (National Mfg. Co. Lincoln, Nebraska) as described in the "Baking Procedures" section of this chapter. Two extensigrams were obtained from each dough; one immediately after dough preparation, and the other after a rest period of 45 min.

E. Gluten Stretching Test

Glutens were obtained from 15g of wheat flour and from 25g of triticale flour using the Theby gluten-washing apparatus according to the AACC method 38-11 (1976). Gluten strength was determined by measuring the force required to stretch and break a strand of fresh wet gluten using the

apparatus and technique of Matsuo (1978).

F. Baking Procedures

1. No-Time/Sheeting Procedure

A number of bread making experiments were performed. The procedure that gave satisfactory results and which was used to generate most of the baking data reported here is referred as no-time/sheeting procedure. The baking formula included 25g of flour (14% mb), 0.625g of sugar, 0.25g of sodium chloride, 0.75g of shortening, 1.0g of fresh yeast, 0.025g of ammonium phosphate, 70ppm of ascorbic acid, 30ppm of potassium bromate, and water to desired absorption. The procedure was performed as follows: dough ingredients were premixed for 1 min in a National-mixer (National Mfg. Co.) (in a GRL-mixer for 100g-flour formula). The dough was then developed by passing it 15 times through sheeting rolls (National Mfg. Co.) at a 5/64' gap for 25g-flour formula or at 5/32' gap for a 100g-flour formula. After each pass, the dough was shaped into a cylinder and rotated 90° for the next pass. The dough was then kept in a fermentation cabinet (32.2°C and 90-95% RH) for an intermediate proofing period of 20 min. The dough was next sheeted 3 times, at successively smaller gap distances and without folding between passes, passing it through the rolls at gap distances of 7/32', 5/32', and 5/64' for 25g-flour formula or at 5/16', 7/32', and 1/8' for 100g-flour formula. The sheeted dough was then rolled into a cylinder, panned, and

proofed for 60 min. After this, the dough was baked for 25 min at 216°C. Loaf volume was measured by rapeseed displacement.

The baking formula and other conditions were established after considering several baking formulas used by different investigators (Kilborn and Tipples 1974, Stenvert et al. 1979, Moss 1980, Kilborn et al. 1981), for the no-time baking system. Gap distance between rolls was chosen to be the minimum without tearing the dough. Intermediate proofing time of 20 min was adopted following recommendations of Kilborn and Tipples (1979) who found that intermediate proofing period is necessary for adequate gas production and to obtain satisfactory crumb characteristics.

2. No-Time/Mixing Procedure

A no-time/mixing procedure following the same formula and baking conditions as the no-time/sheeting procedure was also used when indicated. The only difference between the no-time/mixing and the no-time/sheeting procedures was that in the former dough development was achieved, in a single operation, by mixing (in a National-mixer) instead of by sheeting.

3. AACC Straight Dough Procedure

The AACC straight dough baking procedure and formula were used according to the AACC method 10-10 (1976) for a 25g-flour formula, when indicated.

G. Fractionation of Flour Into Starch-Water Solubles and Gluten for Fractionation-Reconstitution Studies

1. Extraction of Free Lipids

Prior to fractionation the flours were partially defatted, to prevent binding of free lipids to the gluten proteins, as follows: flour (200g) was extracted over night (16h) with n-hexane (1L) in a shaking-water bath at 22-26°C. The suspension was filtered on a Buchner funnel. The flour was then re-extracted with 0.5L of solvent for an additional 1h, filtered, and the flour washed with a small portion of solvent. The extracts were combined and the solvent evaporated on a rotary-evaporator. Recovered lipids were stored at -18°C and later used for reconstitution of flours. The flour was air-dried at room temperature until solvent odor was no longer detected.

2. Fractionation of Partially Defatted Flour

The partially defatted flours were fractionated as follows: flour (50g) was extracted by stirring with 0.001M NaCl solution (100ml) for 5 min and then centrifuged for 10 min at 15000xg at 4°C. The supernatant was kept at 0-4°C. The objective of this step was to separate most of the amylolytic activity from the starch extracted from the dough so that amylolysis could be prevented while washing the gluten out. The flour residue was made into a dough, placed in a metallic container and kneaded by hand in the presence of a small portion of the salt solution for 1 to 2 min to

gradually remove starch and remaining soluble material. The suspension obtained while kneading the dough was percolated through a 11-mesh (125μ) sieve. The kneading operation was repeated until the gluten mass was formed and the washing solution was relatively clear and not milky. The gluten was allowed to relax in distilled deionized water for 30 min before it was frozen and freeze-dried. The starch-water solubles suspension and the supernatant obtained at the beginning of the operation were combined, frozen, and freeze-dried. The freeze-dried gluten and starch-water solubles fractions were ground to pass a 9-mesh (156μ) sieve, and their moisture and protein content determined. These fractions were stored at 0-4°C until used for reconstitution studies. Results (not shown) from preliminary baking experiments with the wheat flour, Neepawa, indicated that the defatting step did not alter the functionality of the flour components.

H. Flour Reconstitution

Ground gluten and starch-water solubles (s-ws) were blended in the desired proportions before the lipid material was added. The desired amount of lipid was weighed as small droplets on the surface of a metallic tray. To this, small portions of the gluten + s-ws blend were added and the whole rubbed with a spoon to gradually incorporate lipids into the blend, to form a reconstituted flour. Reconstituted flours were rehydrated by keeping them in a fermentation cabinet for 32h at room temperature. The humidity control (giving

90-95% RH) was turned on for 15 min, 3 times during the rehydration time period. The samples reached a moisture content between 11 and 13%.

All original and reconstituted flours were tested in duplicate for the Zeleny sedimentation value, mixographic characteristics, and for baking quality using the no-time/sheeting procedure for 25g of flour. Three reconstitution experiments were conducted.

1. Reconstitution I

Flour fractions were reconstituted into flours having the same constituent levels as the original flours.

2. Reconstitution II

A base starch-water solubles (s-ws) component was obtained by blending s-ws fractions from all flours (20% from each of Impala, Carman, Marquis, and Fredrick; 10% from each of 4T and 11T). Similarly, a base lipid fraction was prepared by blending free lipids of all samples at equal proportions. The base s-ws component was blended with gluten isolated from each of the triticale and wheat samples. To this, the base lipid was incorporated at a 0.85% (db) level to produce reconstituted flours having the same s-ws and lipid composition and the same level of gluten protein (11.5% db, typical of strong wheat flours), but different gluten type.

3. Reconstitution III

The components fractionated from Marquis and Carman were reconstituted to the levels of their corresponding original flours, but interchanging their gluten component.

I. Fractionation of Flour Proteins by Solubility

The flour protein solubility distribution of all the samples was estimated by the fractionation procedure of Osborne as modified by Chen and Bushuk (1970a). Ten grams of flour were extracted sequentially with 0.5M sodium chloride, 70% ethanol, and 0.05M acetic acid. The salt-solution extracts combined both albumins and globulins. This was treated as a single solubility fraction. The solvent of the ethanol-extracts was evaporated on a rotary-evaporator, and the concentrated protein dispersed in 0.05M acetic acid. All fractions were frozen, freeze-dried, weighed, ground, and their moisture and protein contents determined. The fractionation procedure was performed on three replicates of each flour.

J. Preparation of Gluten for the Study of Gluten Protein Components

Untreated flour (200g) and a 0.001M NaCl solution (120ml) were mixed into a dough for 1 min in a GRL-mixer. The dough was placed in a metallic container and kneaded by hand in the presence of a small portion of the salt solution for 1 to 2 min to gradually remove starch and water solubles. The suspension obtained while kneading the dough

was percolated through a 11-mesh (125μ) sieve to recover small gluten particles in the suspension. The kneading operation was repeated until the gluten mass was formed and the washing solution was relatively clear and not milky. The gluten was then washed out with 3-50ml aliquots of distilled deionized water to remove remaining salt. The gluten was allowed to relax in distilled deionized water for 30 min before it was frozen and freeze-dried. The gluten was weighed, ground to pass a 9-mesh (156μ) sieve, and its moisture and protein contents determined. The ground gluten was stored at 0-4°C.

K. Fractionation of Gluten Proteins by Solubility and pH Precipitation

Gluten (6g protein, db) was extracted with 0.05M acetic acid (120ml) by shaking the suspension 2h at 0-4°C, followed by centrifugation (30 min at 15,300xg, at 4°C). The supernatant was decanted and the residue re-extracted as before with 2x80ml portions of the acid solution. The residue was then washed by gentle stirring for 5 min in the presence of 2x40ml portions of the acid solution followed by centrifugation. All supernatants were combined and centrifuged to remove contaminant insoluble material.

The pH (from 3.8 to 4.0) of the supernatant was adjusted to 5.8 by the addition of 1.0M sodium bicarbonate. Precipitation was allowed to take place overnight at 0-4°C. The supernatant (pH 5.8-soluble protein) was separated from the precipitate (pH 5.8-insoluble protein) by centrifugation

(15,300xg for 30 min, at 4°C). The pH 5.8-soluble protein solution was dialyzed for 48h at 0-4°C against 0.01M acetic acid to remove salt from the protein solution.

All three, residue, pH 5.8-insoluble and pH 5.8-soluble protein fractions were frozen, freeze-dried, weighed, and ground. Their moisture and protein contents were determined, and their proportions in their original glutens calculated. The fractionation was performed in at least 3 replicates to generate enough material for further studies.

L. Fractionation of the pH 5.8-Insoluble Gluten Component by Acid Solubilization-pH 5.8 Precipitation in the Presence of 70% Ethanol

This fractionation procedure is based on the method of Bietz et al. (1975) for the preparation of glutenin from gluten and was performed as follows: freeze dried sample (0.5g protein, db) from the pH 5.8-insoluble gluten component was shaken for 2h at 0-4°C in the presence of 30ml of a 0.05M acetic acid-70% ethanol solution. The pH (from 5.0 to 5.2) of the dispersion was adjusted to 5.8 by the addition of 1N sodium hydroxide. Precipitation was allowed to take place overnight at 0-4°C. The supernatant (70% ethanol-soluble protein) was separated from the precipitate (70% ethanol-insoluble protein) by centrifugation (20,000xg for 30 min, at 4°C). The supernatant was dialyzed for 48h against 0.01M acetic acid to remove ethanol from the protein solution.

Both, 70% ethanol-soluble and -insoluble protein fractions were frozen, freeze-dried, weighed, and ground. Their moisture and protein contents were determined, and their proportions in the pH 5.8-insoluble fraction calculated. The fractionation was performed in duplicate.

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

The SDS-PAGE method for determination of molecular weights of proteins of Bietz et al. (1975) was used but was modified as follows: samples (15mg protein db) were suspended in 1.0ml of a 0.125M Tris-borate buffer, pH 8.8, containing 2.0% (w/v) SDS, 5% (w/v) 2-mercaptoethanol (only when protein reduction was desired), 10% (v/v) glycerol, and 0.01% (w/v) bromophenol blue. After allowing the samples to stand overnight at room temperature with occasional shaking, they were placed in a boiling water bath for 3 min, then cooled, and centrifuged to separate supernatant (used for SDS-PAGE) from sediment (present in both unreduced and reduced extracts of the residue protein, and in the unreduced extracts of the pH 5.8-insoluble and 70% ethanol-insoluble fractions).

Electrophoretic work was conducted with a "Bio-Rad" model 221 vertical electrophoresis apparatus using 1.5mm thick gel slabs. The standard gel systems consisted of a 1.0-1.5 cm stacking gel (T=5.0%, C=2.4%) and a 28.5-29.0 cm running gel (T=7.5 or 10%, C=2.4%). The running gel was formed as follows: acrylamide, bisacrylamide, and sodium

sulfite (5.88g, 0.146g, and 0.014g, respectively for a 7.5% T gel or 7.84g, 0.195g, and 0.014g, respectively for a 10.0T T gel) were dissolved in 75ml of 0.125M Tris-borate buffer, pH 8.8, containing 0.1% (w/v) SDS. Three-hundred microliters of 3-dimethylamino-propionitrile were added to the gel solution. The whole was filtered and deaerated. Two milliliters of 2.0% ammonium persulfate were added to the gel solution which was mixed and poured into the electrophoresis apparatus. The stacking gel was formed as follows: acrylamide, bisacrylamide, and sodium sulfite (0.784g, 0.019g, and 0.004g, respectively) were dissolved in 15ml of 0.125M Tris-borate buffer, pH 8.8, containing 0.1% (w/v) SDS. Sixty microliters of 3-dimethylamino-propionitrile were added to the gel solution. The whole was filtered. Two hundred and fifty microliters of 2.0% ammonium persulfate were added to the gel solution which after mixing was poured onto the top of the running gel. Ten slots were formed.

For the electrophoresis run, the 0.125M Tris-borate buffer, pH 8.8, was used in both electrode chambers. Electrophoresis runs were conducted for 3 to 3.5h at constant current (18mA per gel) and at 15°C. Protein molecular weight markers were included in all electrophoretic runs.

On termination of electrophoresis, the length of the tracking dye migration and the length of the gel were measured. The gels were soaked for 5 min in a 10%

trichloroacetic acid solution before they were stained for 24h in a dye solution containing Coomassie Brilliant Blue R250 (0.2%, w/v) in water-methanol-acetic acid (53:40:7, v/v) (Payne and Corfield 1979). The gels were destained in several changes of a solution containing water-methanol-acetic acid (68:25:7, v/v) (Payne and Corfield 1979). The length of the gels, before staining and after destaining, was measured. Destained gels were photographed using "Technical Pan" film, ASA 100.

N. Molecular Weight Determination by SDS-PAGE

The Weber and Osborn (1969) equation was used to calculate the molecular weight of the electrophoretic protein bands.

$$\text{Relative mobility} = \frac{\text{Protein migration (cm)}}{\text{Dye migration (cm)}} \times \frac{\text{length of gel before staining (cm)}}{\text{length of gel after destaining (cm)}}$$

The following standard proteins were used to calibrate the gels:

Protein[a]	Molecular Weight (daltons)	Supplier
Lysozyme	14,300	Sigma
β-lactoglobulin	18,400	Sigma
Trypsinogen	24,000	Sigma
Pepsin	34,700	Sigma
Albumin (egg)	45,000	Sigma
Albumin (bovine plasma)	66,000	Sigma
β-galactosidase	116,000	Sigma
γ-globulin (bovine, Cohn fraction II, 99% γ-)	160,000	Sigma

[a] Electorphoretic analyses of unreduced and reduced samples was accompanied by unreduced and reduced, respectively, standard proteins.

IV. RESULTS AND DISCUSSION

A. Characteristics of Triticale and Wheat Flours

Table 2 shows that all triticale flours had from slightly low (11T) to very low (4T) falling number values. The wheats had high (Marquis) and slightly low (Fredrick) falling number values. The low protein contents, low sedimentation values, and short mixing times shown by the triticale flours are typical of weak flour types. Both the sedimentation values and mixing times indicated that a range in dough strength, although narrow, can be found among secondary hexaploid triticales of similar protein content. The wheats had protein contents close to those of the triticale flours (Table 2). Both sedimentation value and mixing time showed that Marquis is a strong wheat flour. In contrast, Fredrick had low sedimentation, corresponding to its type (weak), but its mixing time of 3.2 min was not as short as could be expected for such a flour.

B. The Suitability of the No-Time/Sheeting Procedure to Bake Triticale Flours

1. Effect of Method of Dough Development on Extensigrams

Mechanical dough development allows for the expression of resistance to extension and extensibility characteristics of a flour dough. These characteristics can be manifested

TABLE 2. Analysis of Triticale and Wheat Flours

Quality Characteristics	Triticale				Wheat	
	4T	11T	Carman	Impala	Marquis	Fredrick
Protein (%)[a]	11.3	11.9	11.8	12.4	12.6	10.0
Falling number value (sec)	66	221	129	169	404	274
Ash (%)[a]	0.56	0.44	0.45	0.46	0.46	0.52
Zeleny sedimentation (cc)	23	20	26	32	46	12
Mixing time (min)[b]	2.0	1.4	1.7	2.7	4.0	3.2

[a] Dry weight basis.
[b] Swanson and Working mixograph.

in an extensigram when the developed dough is tested in the Brabender extensigraph.

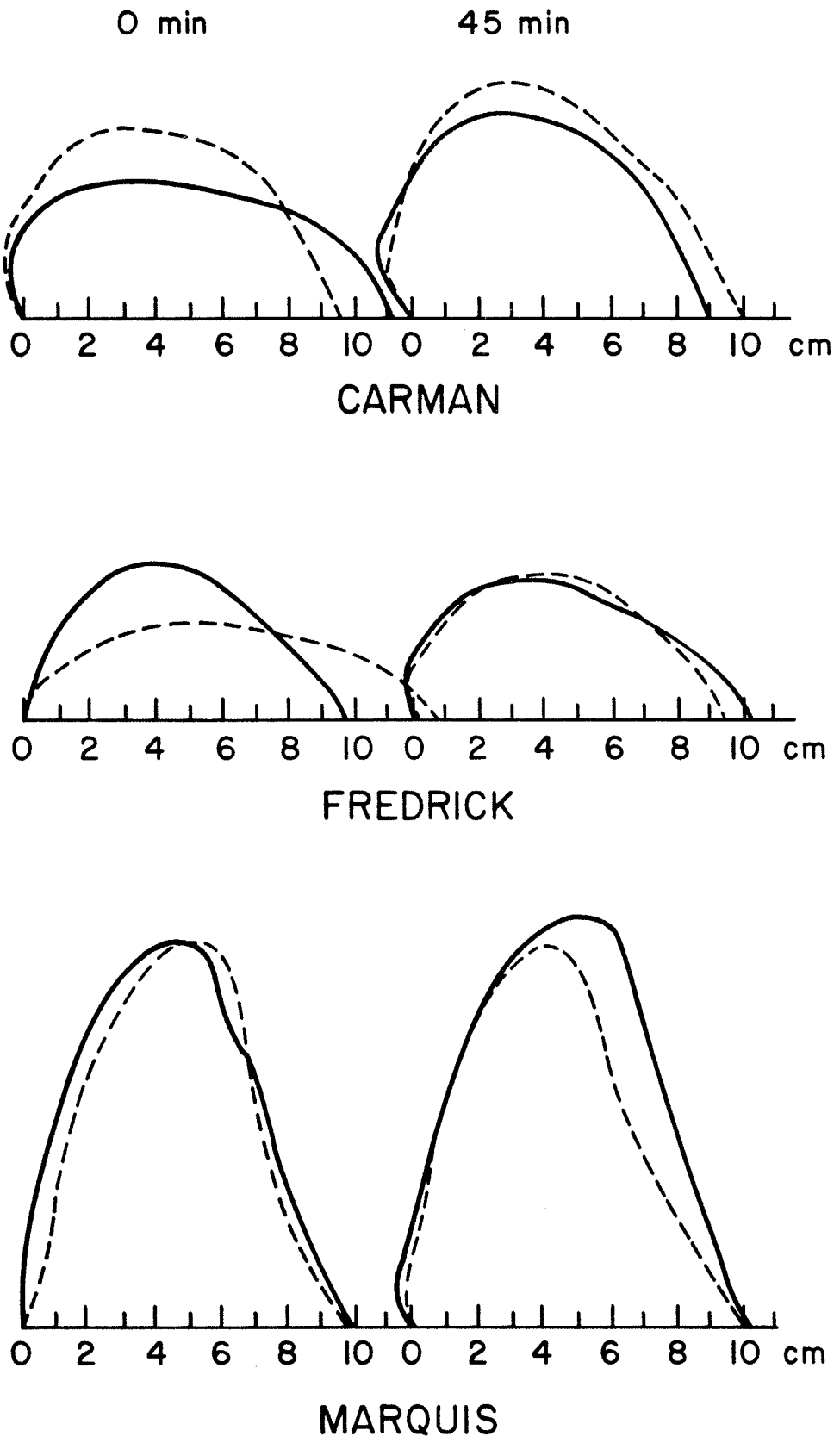
Extensigrams of the triticale Carman and of the two wheat doughs, developed both by mixing and by sheeting, were obtained to examine the effectiveness of sheeting rolls to develop doughs of different strength character, as compared to that of a conventional pin mixer. Carman was the only triticale used in the extensigraphic test as well as in the following (baking) experiment because this was the only triticale, among those used in this study, in enough quantity as to do so. Extensigrams are shown in Figure 1.

At the no rest stage, when the dough structure was in a stressed state, doughs developed by both procedures were similar for the strong wheat Marquis. For both Fredrick and Carman, the extensigrams of their sheeted doughs were different to those of their corresponding mixed doughs. The extensigrams of the doughs after a 45 min relaxation period showed that they were developed in a similar manner when mixed or sheeted. This confirmed the finding of Kilborn and Tipples (1974) that sheeting is an effective way to develop doughs. Additionally, these results also showed that sheeting can be used to develop doughs from a range of flour types.

2. Influence of Baking Procedure on Baking Performance

The baking performance of three wheat flours (representing: very strong, Neepawa at 13.8% protein; strong, Marquis; and weak, Fredrick, flour types) and of one

Figure 1. Extensigrams of triticale (Carman) and Wheat (Fredrick and Marquis) doughs developed by mixing and by sheeting. The extinsigrams of the doughs for Marquis as prepared here, were different from the typical extensigram obtained from doughs prepared according to the standard procedure (AACC method 54-10, 1976).


SHEETING
MIXING
0 min
45 min
0 2 4 6 8 10 0 2 4 6 8 10 cm
CARMAN
0 2 4 6 8 10 0 2 4 6 8 10 cm
FREDRICK
0 2 4 6 8 10 0 2 4 6 8 10 cm
MARQUIS

triticale flour (Carman) was tested under the conditions of each of three bread making procedures; the AACC straight dough method, the no-time/mixing, and the no-time/sheeting procedures. Bread making results are presented in Table 3 and illustrated in Figure 2.

Flour baked by the two no-time procedures produced similar-type breads with larger loaf volumes and better crumb characteristics than the corresponding breads obtained with the AACC baking method.

Marquis and Neepawa performed well under the conditions of the AACC straight dough procedure producing breads with large loaf volume and satisfactory crumb characteristics. In contrast, Fredrick and Carman produced breads with low loaf volume and unsatisfactory crumb characteristics, thus showing that both flours were too weak to withstand long fermentation times. Additionally, Carman produced bread with a soggy crumb. This was probably due to excessive amylolytic activity, which was favoured by the long-fermentation conditions of the straight dough method. The latter was suggested by the absence of sogginess when Carman was baked with the no-time baking procedures. Tsen et al. (1973) also found that both loaf volume and crumb characteristics of triticale flours improved substantially when the bulk-fermentation stage was eliminated.

The crumb characteristics of both Fredrick and Carman were unsatisfactory even under the conditions of the no-time procedures (Table 3, Figure 2). However, Figure 2 shows

TABLE 3. Influence of Baking Procedure on the Baking Performance of Wheat and Triticale Flours

Flour	AACC Straight Dough	No-Time/ Mixing	No-Time/ Sheeting
Fredrick			
Water absorption (%)	57.0	57.0	57.0
Mixing time (min)	3.2	3.2	--
Loaf volume (cc)	153	182	176
Crumb appearance[a]	U	U	U
Crumb structure[a]	U,O	U,SO	U,SO
Marquis			
Water absorption (%)	63.0	63.0	63.0
Mixing time (min)	4.8	4.8	--
Loaf volume (cc)	200	222	228
Crumb appearance	S	S	S
Crumb structure	S,SO	S	S,F
Neepawa			
Water absorption (%)	63.0	63.0	63.0
Mixing time (min)	4.8	4.8	--
Loaf volume (cc)	212	235	231
Crumb appearance	S	S	S
Crumb structure	S	S	S,F
Carman			
Water absorption (%)	57.0	57.0	57.0
Mixing time (min)	1.8	1.8	--
Loaf volume (cc)	154	182	183
Crumb appearance	U	U	Q-U
Crumb structure	U,O,SY	U,O	Q,SO

[a] S = satisfactory, Q = questionable, U = unsatisfactory, F = fine, O = open, SO = slightly open, SY = soggy.

Figure 2. Breads of flours baked under the conditions of three breadmaking procedures.

Baking procedures:
A = AACC straight dough
B = No-time/mixing
C = No-time/sheeting

Flours:

1	= Fredrick	(wheat)
2	= Marquis	(wheat)
3	= Neepawa	(wheat)
4	= Carman	(triticale)

NOTICE/AVIS

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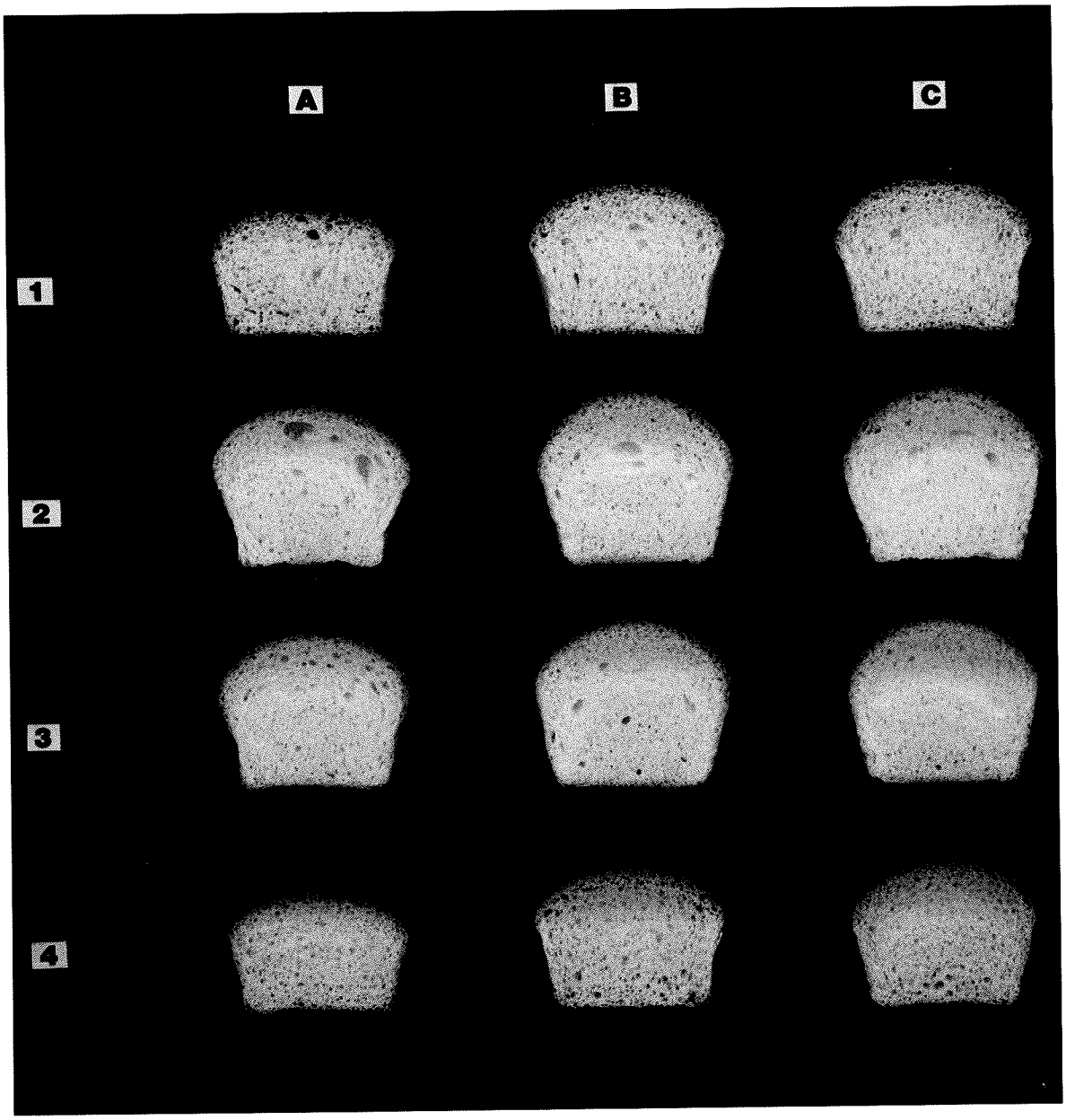
A
B
C
1
2
3
4

that doughs developed by sheeting produced breads with apparently less open more desirable crumb structure than those produced from no-time doughs developed by mixing. Kilborn and Tipples (1974), Kilborn et al. (1981), and Stenvert et al. (1979) have also found that sheeted doughs produce breads with better crumb characteristics than those of breads from mixed doughs.

3. Baking Potential of the Triticale Flours

The four triticale flours were baked with the no-time/sheeting procedure to observe if this procedure could show differences in dough strength among these samples. The results are shown in Table 4 and Figure 3.

The triticale 4T performed extremely poorly. Its dough became progressively more sticky and more difficult to handle as the number of passes through the rolls increased during the dough-development stage. At the end of the proofing stage, the dough was open on the surface indicating that it could not retain the gas produced. The resultant bread had a thick and non-uniform crust, compact crumb, and small loaf volume. The dark crust and soggy crumb of bread from 4T indicated that the detrimental effect of its very high alpha-amylase activity could not be overcome by eliminating the bulk-fermentation stage. Although the handling properties and overall baking performance of 11T and Carman were much better than those of 4T, these samples produced poor quality breads. The loaf volume of Carman was better than that of 11T. In contrast, Impala showed good

TABLE 4. Baking Potential of Triticale Flours[a]

Quality characteristic	Triticale Cultivar			
	4T	11T	Carman	Impala
Loaf volume (cc)	122	154	175	205
Crumb appearance[b]	U	U	U	S
Crumb structure[b]	U,C,SY	U,O	U,O	S

[a] All baked at 58% water absorption.

[b] S = satisfactory, U = unsatisfactory, O = open, SO = slightly open, SY = soggy, C = compact.

Figure 3. Breads from triticale flours.
1 = 4T
2 = 11T
3 = Carman
4 = Impala

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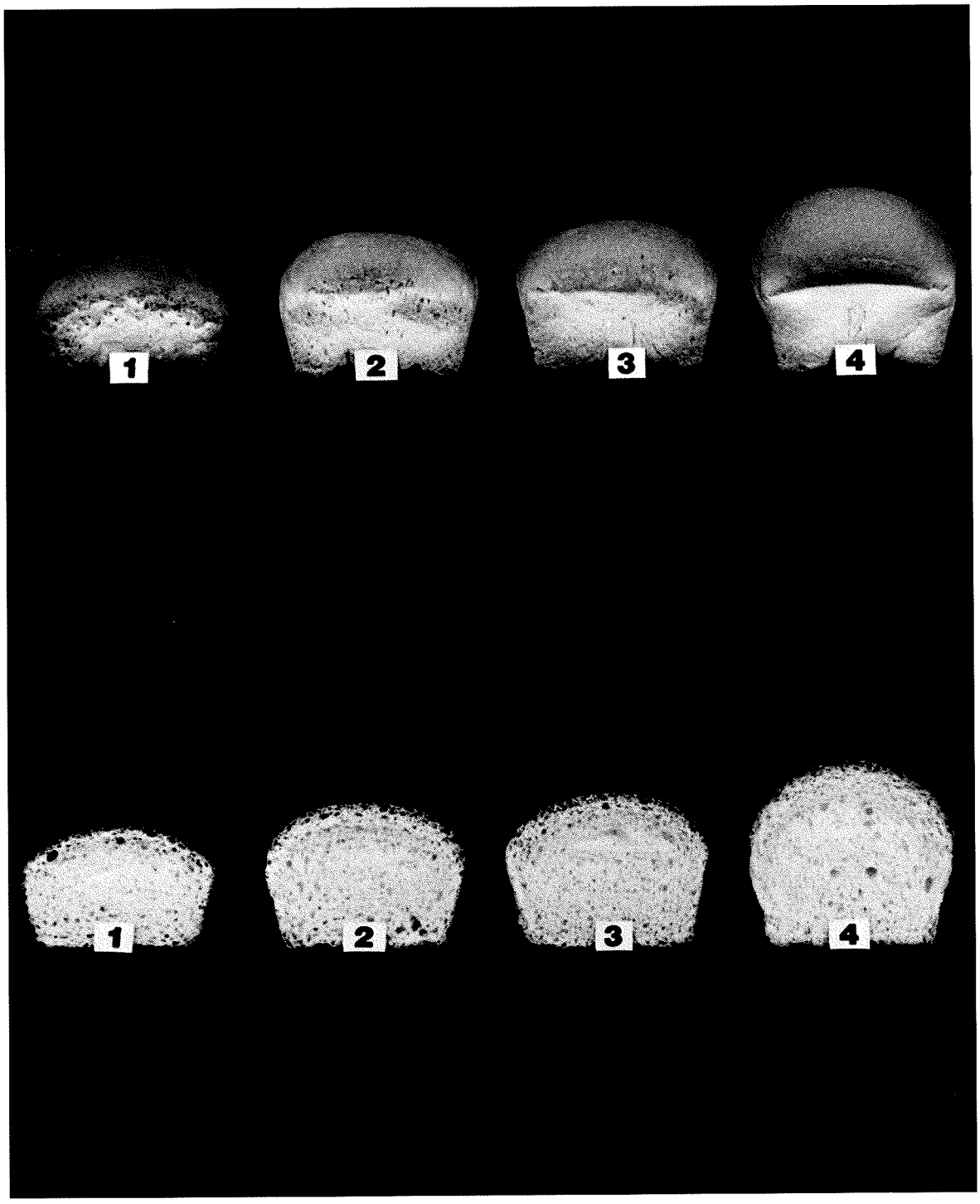
1
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4
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handling properties and resulted in bread with satisfactory crumb characteristics and very acceptable loaf volume.

Therefore, it appeared that the dough sheeting and other conditions of the no-time/sheeting procedure were adequate to show that there were marked differences in baking potential among triticales having similar protein content and only small qualitative dissimilarities (Zeleny sedimentation value and mixing time) (Table 2).

C. Influence of Gluten Quantity and Quality on the Dough Strength of Triticale and Wheat Flours

Gluten quantity and quality have been recognized to be the main factors controlling dough strength in wheat (Aitken and Geddes 1938, Finney 1943). Since triticale flours also form gluten, it is most likely that dough strength in this cereal is also controlled by its gluten properties. Therefore, the proportions of gluten in triticale and wheat flours and its qualitative characteristics as related to dough strength were examined.

1. Gluten and Gluten Protein Content

Table 5 shows gluten recovery and gluten protein content for partially defatted triticale and wheat flours. The quantity of gluten varied widely among triticale flours, with 4T containing the least and Impala the most gluten. Marquis had the highest gluten content while Fredrick had the third lowest of all the flours analysed. However, when gluten was expressed in terms of gluten protein as a percent

TABLE 5. Quantitative Gluten Characteristics of Partially Defatted Triticale and Wheat Flours[a]

Characteristic	Triticale				Wheat	
	4T	11T	Carman	Impala	Marquis	Fredrick
Flour Protein (%)	11.8	12.0	12.4	12.9	13.3	10.1
Gluten content (% of flour)	6.3	8.6	8.5	10.0	12.7	8.6
Gluten protein (% of gluten)	93.5	86.5	92.6	89.3	89.9	91.7
Gluten protein in flour protein (%)	49.9	62.0	63.5	69.2	85.8	78.1

[a] Percentage at dry basis.

of flour protein, the level of Fredrick was higher than the highest level in the triticales. Thus, the lower gluten content of Fredrick was due mainly to its lower flour protein content (Table 5). Therefore, it appeard that the generally inferior dough strength of most triticales (inferior to that found in bread wheats as judged by their baking quality, Tables 3 and 4) was due not only to their low flour protein content but also, and more critically, to their considerably lower gluten protein as percent of flour protein. This evidence supports the suggestion of Tsen et al. (1973) in that the poor baking quality of triticale could be due in part to low gluten content.

These results (Table 5) also suggested that, as in the case of wheat flours (Aitken and Geddes 1938, Finney 1943), gluten protein is a major factor controlling dough strength in triticales. For example, Impala, with the highest value for gluten protein as a percent of flour protein, showed the best baking quality while 4T, with the lowest value for the same parameter, showed the poorest baking quality.

The results of gluten protein as a percent of flour protein also suggested that besides the gluten protein quantity factor, the gluten protein quality could be another important factor controlling dough strength in triticale. The gluten protein as a percent of flour protein of 11T was close to that of Carman (62.0 and 63.5%, respectively) but the loaf volume of 11T was considerably inferior to that of Carman (154 and 175 cc, respectively, Table 4). Therefore,

the gluten strength of the triticale and wheat flours was examined.

2. Gluten Stretching Test

The force required to stretch and break a strand of gluten was determined as an indicator of gluten strength. The results (Table 6) showed that gluten strength varied considerably among the triticale flours. Flour 11T had the weakest gluten while 4T and Carman and the wheat Fredrick showed similar gluten strength. Impala had the gluten with the highest gluten strength, slightly higher than that of the wheat Marquis.

There was no apparent trend between gluten strength and loaf volume for the four triticales, although this may not be expected, considering the different levels of gluten protein and of other factors, such as alpha-amylase activity of the materials in this study. However, the fact that the best bread making triticale, Impala, had the highest gluten strength suggested that, as was shown with wheat (Finney 1943), gluten quality is a major factor controlling dough strength in triticale.

3. Fractionation-Reconstitution Studies

Because of the differences among the triticales for several parameters being measured it was difficult to evaluate the significance of any single parameter on dough strength. For this reason fractionation and reconstitution studies were undertaken in an attempt to normalize certain

TABLE 6. Gluten Stretching Values of Triticale and Wheat Flours

Sample	Gluten Strength (dynes x 10^{-4})[a]
Triticale	
4T	11.3 ± 0.4
11T	6.8 ± 0.5
Carman	11.8 ± 1.3
Impala	15.5 ± 0.1
Wheat	
Marquis	13.8 ± 0.7
Fredrick	10.3 ± 0.5

[a] Mean and mean difference for duplicates.

of these parameters and thus, to examine the influence of gluten quality on dough strength of triticale. The recoveries of gluten and starch-water solubles (s-ws) are given in Table 7.

a. The Zeleny Sedimentation Value of Reconstituted Flours

The Zeleny sedimentation value was determined in original and all reconstituted flours. The results are shown in Table 8. For reconstituted flours the standard sedimentation test with sediment volume determined after 5 min was not suitable because a discernible boundary did not exist after 5 min. For this reason a 10 min sedimentation time was employed. Although a boundary was apparent after this period, the phase above the boundary was hazy and shown to contain considerably more suspended material (5 times more starch, approximately 5% more protein) than the same phase from corresponding unfractionated flour (data not included). This suggested that the native close association between the flour components disrupted by fractionation was not regenerated upon reconstitution. It was observed that some of the relative differences in sedimentation value among unfractionated triticale flours could not be found for their corresponding reconstituted ones (Table 8). Therefore, it was concluded that the Zeleny sedimentation test is not suitable to evaluate quality differences between fractionated-reconstituted flours.

TABLE 7. Fractionation Data on Partially Defatted Triticale and Wheat Flours

	Recovery of Dry Matter (%)			Recovery of Protein (%)[a]		
		Starch-water			Starch-water	
Flour	Gluten	Solubles	Yield	Gluten	Solubles	Yield
Triticale						
4T	6.3	91.2	97.5	49.9	45.6	95.5
11T	8.6	87.2	95.8	62.0	35.6	97.6
Carman	8.5	88.7	97.2	63.5	31.6	95.1
Impala	10.0	88.8	98.7	69.2	28.2	97.4
Wheat						
Marquis	12.7	84.9	97.6	85.8	12.8	98.6
Fredrick	8.6	90.4	99.0	78.1	23.3	101.4

[a] Percent of total flour protein (db)

TABLE 8. Zeleny Sedimentation Values of Original and Reconstituted Triticale and Wheat Flours

Flour	Control A[a]	Control B[b]	Reconstitution I[b]	Reconstitution II[b]	Reconstitution III[b,c]
4T	23	22	16	22	--
11T	20	18	17	27	--
Carman	28	26	26	31	31
Impala	32	27	24	33	--
Marquis	44	38	36	36	32
Fredrick	13	12	14	24	--

[a] A: values (cc) taken after the standard 5 min-rest period.
[b] B: values (cc) taken after a 10-min rest period.
[c] Carman flour was reconstituted with starch-water solubles and free lipids of Carman plus gluten from Marquis; Marquis flour was reconstituted with starch-water solubles and free lipids of Marquis plus gluten from Carman. Largest difference between duplicates = 2cc

b. Reconstitution to Original Composition

(Reconstitutuion I)

Reconstitution I was designed to evaluate the effect of fractionation-reconstitution of the original flours to determine if this procedure had altered flour functionality (bread making and mixing properties). The results are presented in Table 9.

The falling number values of the reconstituted flours were essentially the same as those of their corresponding original flours. Therefore, the alpha-amylase activity was not altered by the fractionation-reconstitution procedure.

Although the fractionation-reconstitution procedure did not alter the mixographic patterns from those found in unfractionated flours (Figure 4), small variations in all mixing characteristics measured were observed (Table 9). These variations could have resulted from differences in rate of hydration, during mixing, between the original and the reconstituted flours.

Table 9 shows that fractionation-reconstitution resulted in approximately a 10% reduction in loaf volume potential, however, the order in loaf volume and the relative volume differences among unfractionated flours were maintained in the reconstituted ones. A small reduction in loaf volume due to fractionation-reconstitution is usually found in this type of study (Marais and D'Appolonia 1981a, Finney et al. 1982). The crumb characteristics of reconstituted flours were essentially the same as those of

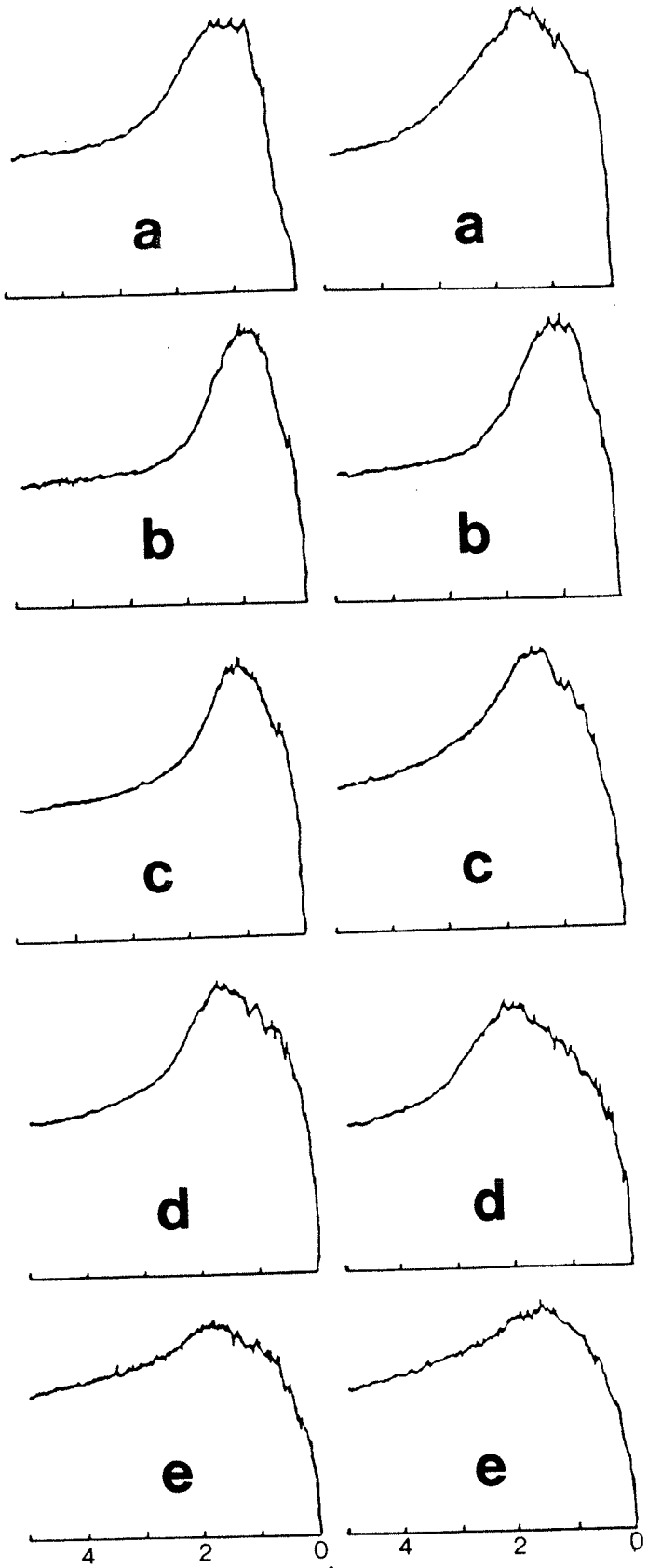
TABLE 9. Effect of Fractionation-Reconstitution (Reconstitution I) on Functional Properties of Triticale and Wheat Flours

Qualtiy	Triticale								Wheat		
	4T		11T		Carman		Impala		Fredrick		LSD[f]
Characeristic	O[a]	R[a]	O	R	O	R	O	R	O	R	(P=0.05)
Flour protein (%)[b]	11.3	11.6	11.9	12.2	11.8	12.1	12.4	12.5	10.0	10.3	
Falling number (sec)	66	65	221	203	129	129	169	180	274	280	
Mixograph[c]											
Mixing time (min)	1.3	1.4	1.0	1.0	1.1	1.4	1.6	2.0	1.8	1.6	0.2
Peak height (cm)	12.6	12.1	12.0	12.0	12.8	12.0	12.8	11.0	9.3	9.8	0.2
Peak area (cm^2)	25.0	36.1	22.2	22.7	28.4	32.2	43.0	41.6	32.6	29.2	3.2
Bread making[c]											
Loaf volume (cc)	134	118	160	155	174	159	203	-[e]	176	156	4.0
Crumb appearance[d]	U	U	U	U	U	U	S	-[e]	U	U	
Crumb structure[d]	U,C,SY	U,C,SY	U,O	U,O	U,O	U,O	S	-[e]	U,SO	U,O	

[a] O = original, R = reconstituted.
[b] Dry weight basis.
[c] All samples tested at 58% water absorption.
[d] S = satisfactory, U = unsatisfactory, O = open, SO = slightly open, SY = soggy, C = compact.
[e] These values were not obtained due to equipment failure and restricted amount of sample.
[f] LSD = Least Significant Difference.

Figure 4. Mixograms of original and reconstituted flours (reconstituted to original composition).

a = 4T
b = 11T
c = Carman
d = Impala
e = Fredrick


a
a
b
b
c
c
d
d
e
e
4
2
0
4
2
0
min
Original
Reconstituted

the corresponding unfractionated flours (Table 9).

c. Reconstitution to Constant Gluten Protein Content (Reconstitution II)

Reconstitution II was designed to evaluate the quality of glutens from the six different sources in the absence of quantitative differences which were present in the original flours. This was achieved using the same base starch-water solubles (s-ws) and free lipids, and adding equal amounts of gluten protein from each of the six sources. The results are presented in Table 10. All reconstituted flours had similar falling number values (range of 157 to 164 sec with an average of 161 sec).

Mixing characteristics were different from one flour to another (Table 10) indicating that, as did the gluten stretching test (Table 6), there was gluten quality variability among triticale flours. More importantly, the results suggest that gluten quality was a major factor controlling flour mixing properties. This latter suggestion is in full agreement with Marais and D'Appolonia (1981b) who found that gluten and water solubles had a major effect on the mixing properties of wheat flour doughs. Peak height could not be related to differences in gluten quality among the flours. Mixing time and peak area, a measurement that combines both mixing time and peak height, separated the flours in terms of gluten strength. The flours bearing glutens from the strong wheat, Marquis, and the strong triticale, Impala, had longer mixing times and larger peak

TABLE 10. Influence of Gluten Quality on Functional Properties of Reconstitution II Flours Having the Same Gluten Protein Content (11.5% db)

Quality Characteristics	Triticale				Wheat		LSD[d] (P=0.05)
	4T	11T	Carman	Impala	Marquis	Fredrick	
Flour protein (%)[a]	14.5	14.3	14.3	14.3	14.5	14.6	
Mixograph[b]							
Mixing time (min)	1.8	1.4	1.5	2.4	2.3	1.9	0.2
Peak height (cm)	10.0	10.4	13.2	11.1	9.5	8.5	0.8
Peak area (cm^2)	34.6	27.4	35.7	50.2	42.2	30.4	5.5
Bread making[b]							
Loaf volume (cc)	169	193	198	225	217	204	10.0
Crumb appearence[c]	U	Q	U	S	S	Q	
Crumb structure[c]	U,O	Q,SO	U,O	S	S	Q,SO	

[a] Dry weight basis.
[b] All samples tested at 60% water absorption for mixograms and at 61% for baking.
[c] S = satisfactory, Q = questionable, U = unsatisfactory, O = open, SO = slightly open.
[d] LSD = Least Significant Difference.

areas than the flours carrying gluten from the weaker type wheat and triticale flours (Table 10). The peak area value of 4T indicated, as did the gluten stretching test, that the gluten of this triticale had more strength than that of 11T and was similar to that of Carman.

The bread making dough is the system in which the gluten interact with other functional components to fully express its quality in terms of loaf volume and crumb characteristics. The results in Table 10 show that baking quality varied widely among the reconstituted flours tested. This variation evidenced that there is a wide gluten quality variability among the secondary hexaploid triticale group, and that, as observed with the gluten stretching test (Table 6), a triticale flour (Impala) can have gluten as strong as that of a strong wheat flour (Marquis).

The results of the gluten stretching test and of the mixographic peak time and peak area of Reconstitution II flours (Table 10), all indicated that the gluten of 4T is stronger than that of 11T. In contrast, the loaf volume values (Table 10) indicated the opposite. These results are not as contradictory as they appear. The gluten strength indicators may simply be showing that the gluten of 4T is less extensible than that of 11T. If this is the case then one might expect that the gluten of 4T would require more work input to break a gluten strand in the stretching test and a longer mixing time to develop its dough than for the gluten of 11T. The inferior loaf volume of 4T relative to

that of 11T may again reflect the greater resistance to extension which may in turn prevent proper expansion of the dough during the fermentation and oven spring stages. This situation is not unexpected and it is possible that the gluten of 4T behaves more as that of very strong wheats in that some wheat glutens can be very strong, elastic but not very extensible.

d. Reconstitution Involving Interchange of Gluten (Reconstitution III)

This experiment was designed to evaluate the effect of interchanging gluten between wheat and triticale flours. Evaluation was made by comparison of mixing and baking properties of the control flours with those having interchanged glutens. Results are presented in Table 11.

The differences in gluten quality between the wheat Marquis and the triticale Carman were manifested in the mixographic characteristics of the reconstituted flours tested. In examining the effect of foreign gluten on the mixographic characteristics of the non-interchanged flours, it was observed that the gluten of Carman decreased mixing time and peak area and increased peak height of the parent flour Marquis. The opposite occurred when the gluten of Marquis was placed in the background of Carman (Table 11).

It was observed that the general mixographic pattern, characteristic of each parent flour, was interchanged with the gluten interchange (Figure 5). This indicated that the mixographic pattern of a flour is mostly controlled by the

TABLE 11. Functional Properties of Reconstitution III Flours Involving Interchange of Gluten Between Wheat (Marquis) and Triticale (Carman)

Quality Characteristics	Flour			
	Marquis Control[a]	Marquis S-WS+ Carman Gluten[b]	Carman Control[a]	Carman S-WS+ Marquis Gluten[b]
Flour protein (%)[c]	13.3	13.2	12.1	12.0
Gluten protein in flour (%)[c]	11.5	11.5	8.3	8.3
Mixograph[d]				
Mixing time (min)	2.0	1.5	1.4	2.0
Peak height (cm)	12.8	14.6	12.0	9.9
Peak area (cm^2)	52.3	40.2	32.2	39.6
Bread making[e]				
Loaf volume (cc)	212	190	159	186
Crumb appearance[f]	S	Q	U	S
Crumb structure[f]	S	Q,SO	U,O	S,SO

[a] Reconstituted flours were used as controls.
[b] S-WS = starch-water solubles.
[c] Dry weight basis.
[d] Water absorption used was 58% for Carman control, and 60% for all others.
[e] Water absorption used was 63% for Marquis control and Marquis s-ws+ Carman gluten, and 58% for Carman control and Carman s-ws+ Marquis gluten.
[f] S = satisfactory, Q = questionable, U = unsatisfactory, O = open, SO = slightly open.

quality of the gluten it bears. A similar finding was obtained by Marais and D'Appolonia (1981b) who interchanged gluten between two wheat flours with different mixographic characteristics.

The ultimate test, bread making, also showed that Marquis gluten was superior to that of Carman. While the gluten from Carman negatively affected the loaf volume and crumb characteristics found in the parent flour Marquis, that from Marquis improved the baking performance of the parent flour Carman (Table 11). These results confirmed the finding of the Reconstitution II experiment (Table 10) in that, as in wheat, gluten protein quality influenced largely the baking potential of the triticale flours. This indicates the reason why the triticale Impala, having low gluten protein content (Table 5) but good gluten quality (Table 6, 10), produced bread with satisfactory loaf volume and crumb characteristics (Table 4, Figure 3).

D. Protein Solubility Distribution of Triticale and Wheat Flours

The proportions of various protein fractions within the triticale and wheat flours were examined by the modified Osborne technique of Chen and Bushuk (1970a). The purpose of this investigation was to determine if a relationship exists between the relative levels of one or more of the various protein fractions and the dough strength of the source materials. Results on protein solubility

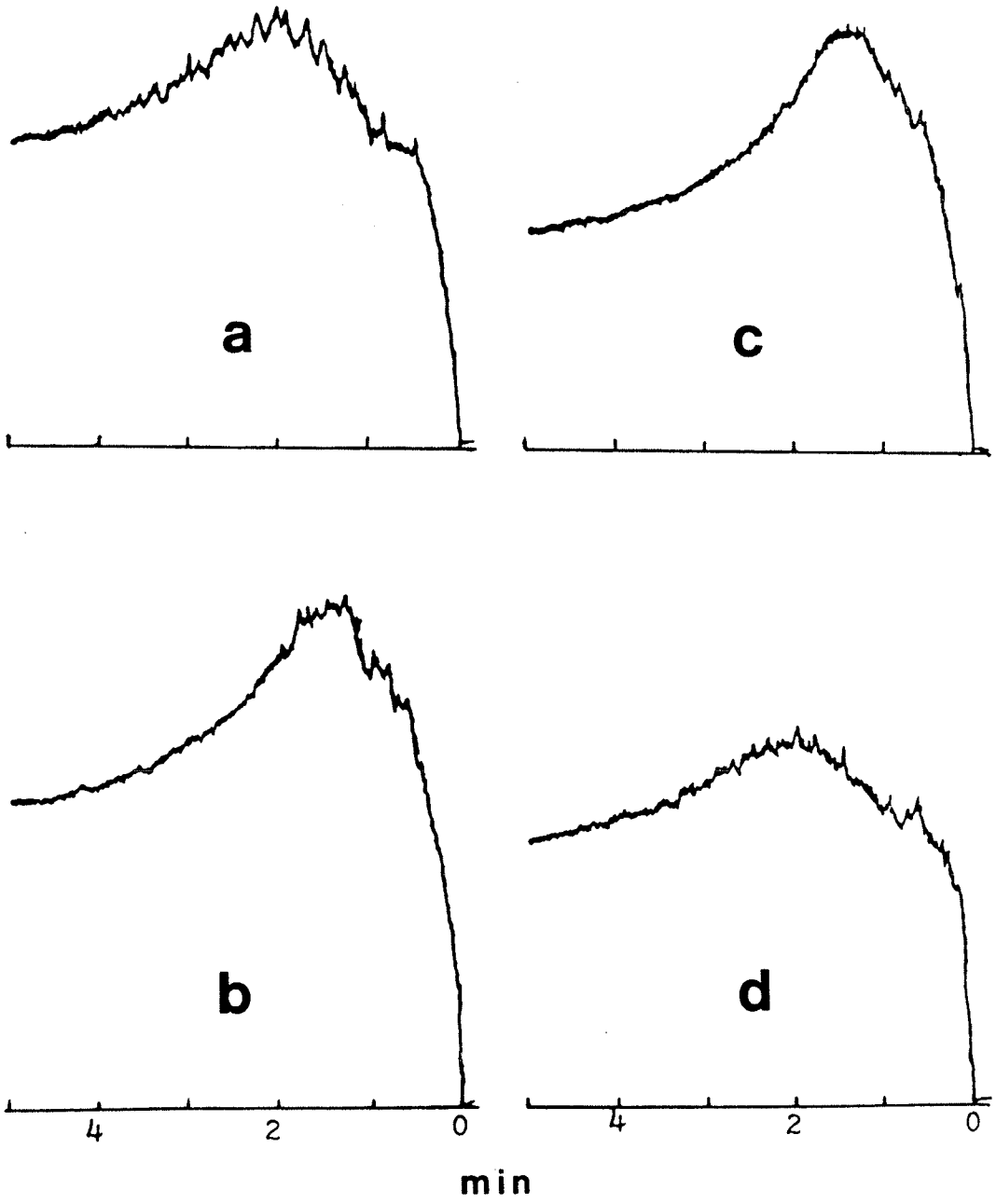
Figure 5. Mixograms of flours reconstituted with original components and of flours reconstituted with interchanged glutens.

a = Marquis with original components

b = starch-water solubles and free lipids from Marquis plus gluten from Carman

c = Carman with original components

d = starch-water solubles and free lipids from Carman plus gluten from Marquis


a
c
b
d
4
2
0
4
2
0
min

distribution are shown in Table 12.

The protein solubility distribution varied among triticales and between triticales and wheats. The amount of salt-soluble protein (includes albumins and globulins) was considerably higher in all the triticales than in the wheats. This indicated, as did the gluten content values (Table 5), that the amount of gluten-like protein was deficient in triticale. Among the triticales, the ethanol-soluble fractions were similar in level and relative variation to those of the salt-soluble fractions. The proportion of this fraction for the wheat flours fell within the range found for the triticales. The proportion of acid-soluble protein among triticales was lowest for 11T (7.1%) and highest for Carman (14.6%). Fredrick had a value similar to that of Carman while Marquis was slightly higher (16.1%). For the residue protein fraction, 4T and Impala had similar higher proportions of this fraction than 11T and Carman. Fredrick had a residue protein content similar to that of Impala while Marquis had the highest value. Chen and Bushuk (1970a) and Ahmed and McDonald (1974) found slightly higher values for the albumins + globulins and the acid-soluble fractions, and slightly lower values for the 70% ethanol and residue fractions than those found for the triticales of this study. However, their results and those reported in this work (Table 12) agree in that triticale flours have in general higher albumins + globulins and lower residue protein than bread wheat flours.

TABLE 12. Distribution (%)[a] of Flour Proteins Upon Osborne-Type Solubility Fractionation

Fraction	Triticale				Wheat		Standard Deviation[b]
	4T	11T	Carman	Impala	Marquis	Fredrick	
Salt-soluble	30.8	32.2	26.8	27.9	19.0	21.6	0.6
Alcohol-soluble	27.2	33.3	32.5	30.3	29.0	29.3	1.4
Acetic acid-soluble	10.0	7.1	14.6	9.4	16.1	14.8	0.8
Residue	28.4	23.9	24.9	30.4	35.8	30.0	0.6
Protein recovery	96.4	96.5	98.8	98.0	99.9	95.7	

[a] Percent of total flour protein (db).
[b] Largest standard deviation among 3 replicates.

With respect to the possible relationship between protein solubility distribution and dough strength, it was observed that, among triticales and between wheats, the flours with longer mixing times (Table 10) had the larger residue protein contents (Table 12). Thus, these results agree with those of Orth and Bushuk (1972) in that there appears to be a relationship between amount of residue protein and mixing requirements of a flour.

On the other hand in terms of baking quality, with the exception of Impala, which had good baking quality and high residue protein, no relationship was apparent between these parameters for the other triticales. Similarly, no relationship between the proportions of the other solubility fractions and dough strength in triticale could be seen.

It is noteworthy to indicate that 2 of the triticales tested in this study (4T and Impala) had more residue protein than the early triticales tested by Chen and Bushuk (1970a), Ahmed and McDonald (1974) and Wall et al. (1972). This indicates that the composition of some new secondary hexaploid triticales is changing favourably (improved strength) with respect to earlier materials.

E. Quantitative and Qualitative Characteristics of Gluten Protein Fractions from Triticale and Wheat Flours

1. Distribution of Gluten Protein Fractions Obtained by Acid Solubilization - pH 5.8 Precipitation

Based on the observed variation in gluten quality among

the triticale flours of this study, an examination of the gluten protein components and their association with dough strength was undertaken. For this, the gluten protein of the triticale and wheat flours was fractionated with 0.05M acetic acid into acid-soluble and residue (acid-insoluble) fractions. The acid-soluble component was further fractionated by adjusting the pH of the extract to 5.8 thus pH 5.8-soluble (acid soluble - pH 5.8 soluble) and pH 5.8 - insoluble (acid soluble - pH 5.8 insoluble) fractions were obtained. The pH value of 5.8 was chosen because Shogren et al. (1969) who used 0.005N lactic acid, found that gluten protein soluble at this pH value (and above) was rich in gliadin protein. Furthermore, Harris and Frokjer (1952) showed that at pH 5.8 (and above), gluten protein had similar solubility in lactic and acetic acid solutions. The distribution of gluten protein fractions is shown in Table 13.

Only a small proportion of the gluten protein was soluble at pH 5.8 (<16%). The largest amount of pH 5.8 - soluble protein corresponded to 4T (15.2%) and the lowest to Carman (8.7%). The proportions of this protein fraction for the wheats Marquis and Fredrick fell within the range found among the triticales. In contrast to the pH 5.8 - soluble fraction, the pH 5.8 - insoluble fraction contained the majority of the gluten protein and its proportion varied widely among triticales and between wheats. In the case of the residue protein fraction, the proportions also varied

TABLE 13. Distribution (%)[a] of Gluten Proteins Upon 0.05M Acetic Acid Solubilization - pH 5.8 Precipitation

Sample	Acid-Soluble		Acid-Insoluble (Residue)	Protein Recovery
	pH 5.8-Soluble	pH 5.8-Insoluble		
Triticale				
4T	15.2	65.3	16.5	97.0
11T	11.7	80.8	9.1	101.6
Carman	8.7	86.2	6.0	100.9
Impala	10.3	66.8	22.0	99.1
Wheat				
Marquis	9.3	69.9	13.8	93.0
Fredrick	9.0	77.4	6.3	92.7
Standard deviation[b]	1.1	1.1	0.9	

[a] Percent of total gluten protein (db).
[b] Largest standard deviation among 4 replicates.

widely among triticales and between wheats.

The relative proportions of protein found in the different fractions for the glutens of the two wheats of this study, are in general agreement with those found by Harris and Frokjer (1952) for wheat gluten protein fractions similar to those of this study. Shogren et al. (1969) found larger amounts (~30%) of gluten protein soluble at pH 5.8 than in the present study (~9%). This difference could be due to differences in the cultivars used, the acid used, and to differences in the extraction conditions such as temperature, time allowed for protein precipitation at a given pH value, and centrifugation speed.

When examining the possible relationship between the gluten protein solubility distribution and dough strength in the triticale and wheat flours, it was found that the proportion of pH 5.8 - soluble protein did not follow a trend that could be related to dough strength (baking and mixing properties). The proportions of both pH 5.8 - insoluble and residue protein were apparently associated with mixing strength. The triticales 4T and Impala, having longer mixing times than 11T and Carman (Table 2, 10), had considerably less pH 5.8 - insoluble and more residue protein than 11T and Carman. A parallel relationship between the proportions of these two protein fractions and mixing time was also seen when comparing the two wheat samples. The apparent positive association between gluten residue protein and mixing time agrees with similar finding

by Butaki and Dronzek (1979b) who compared the proportions of gluten residue protein with mixing characteristics of various wheats. In contrast, the proportions of both pH 5.8 - insoluble and residue protein did not show a trend that could be associated with the loaf volume potential shown by their respective flours (Table 4, 10).

2. Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) of Gluten Protein Fractions

The protein composition of the three gluten protein fractions (residue, pH 5.8-insoluble and pH 5.8-soluble) from the triticale and wheat samples was examined by SDS-PAGE. Major differences within fractions, due to the sample source, as well as among fractions were observed. Electrophoretic patterns of unreduced and reduced protein fractions are shown in Figures 6-8.

a. Residue Gluten Protein

The solubility, under nonreducing conditions, of the residue protein in the SDS-PAGE solvent was quite limited. A hydrated gel which absorbed most of the solvent was formed. Only a small portion of supernatant was separated upon centrifugation. Patterns for unreduced residue protein extracts are shown in Figure 6 (patterns 1a-6a). Some of the protein remained at the origin and some entered the gel unresolved. The presence of some bands in the patterns for unreduced residue protein suggested that this fraction was also composed of low molecular weight components probably

Figure 6. SDS-PAGE in 7.5% gels of unreduced gluten protein fractions.

Sample size: 20 µl (300 µg protein)

Pattern	Sample
1a,b,c	4T
2a,b,c	11T
3a,b,c	Carman
4a,b,c	Impala
5a,b,c	Marquis
6a,b,c	Fredrick

Fraction
a = residue
b = pH 5.8-insoluble
c = pH 5.8-soluble

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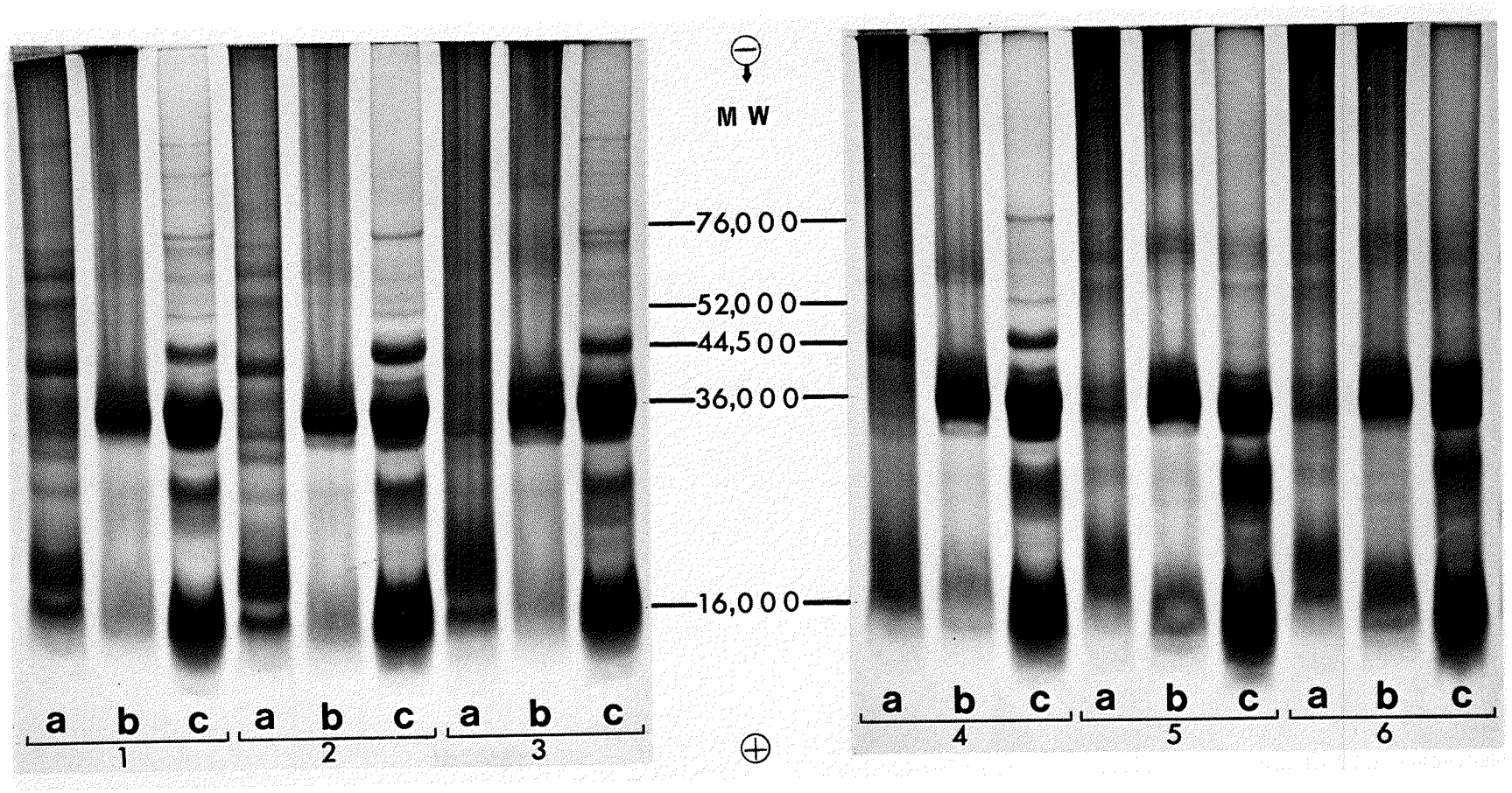
MW
76,000
52,000
44,500
36,000
16,000
a b c a b c a b c
1 2 3
a b c a b c a b c
4 5 6

held, by secondary forces, to the very high molecular weight protein complex. It could also indicate that some acid-soluble protein remained as contaminant in the residue protein fraction. Both explanations are possible since first, cross-contamination among the fractions could not be avoided under the fractionation conditions of this study. Secondly, even "highly purified" unreduced glutenin preparations have been found to contain low molecular weight proteins which were suggested to be part of the glutenin protein complex (Bietz and Wall 1975, Khan and Bushuk 1977). Bietz and Wall (1975) also found, by SDS-PAGE, that unreduced wheat residue gluten protein contained low molecular weight components.

The proteins of the residue fraction resolved into various subunits upon reduction of disulfide bonds (Figure 7, patterns 1a-6a). Some differences in number and location of subunits in the three main molecular weight regions (30,000-50,000, 60,000-70,000, and >80,000) were observed among triticales and between triticales and wheats. Only two bands, one at about 92,000 and the other at about 60,000 daltons (indicated by arrows in Figure 7, pattern 1a), were apparently common to all samples. The most prominent difference between triticale and wheat was that the number of high molecular weight (HMW) subunits (>80,000) was larger in wheat than in triticale. This, could be, as discussed later, a cereal-specific characteristic.

Figure 7. SDS-PAGE in 10% gels of reduced residue and pH-insoluble gluten protein fractions.

Sample size: 15 µl (225 µg protein)

Pattern	Sample	Fraction
1a,b	4T	a = residue
2a,b	11T	b = pH 5.8-insoluble
3a,b	Carman	
4a,b	Impala	
5a,b	Marquis	
6a,b	Fredrick	

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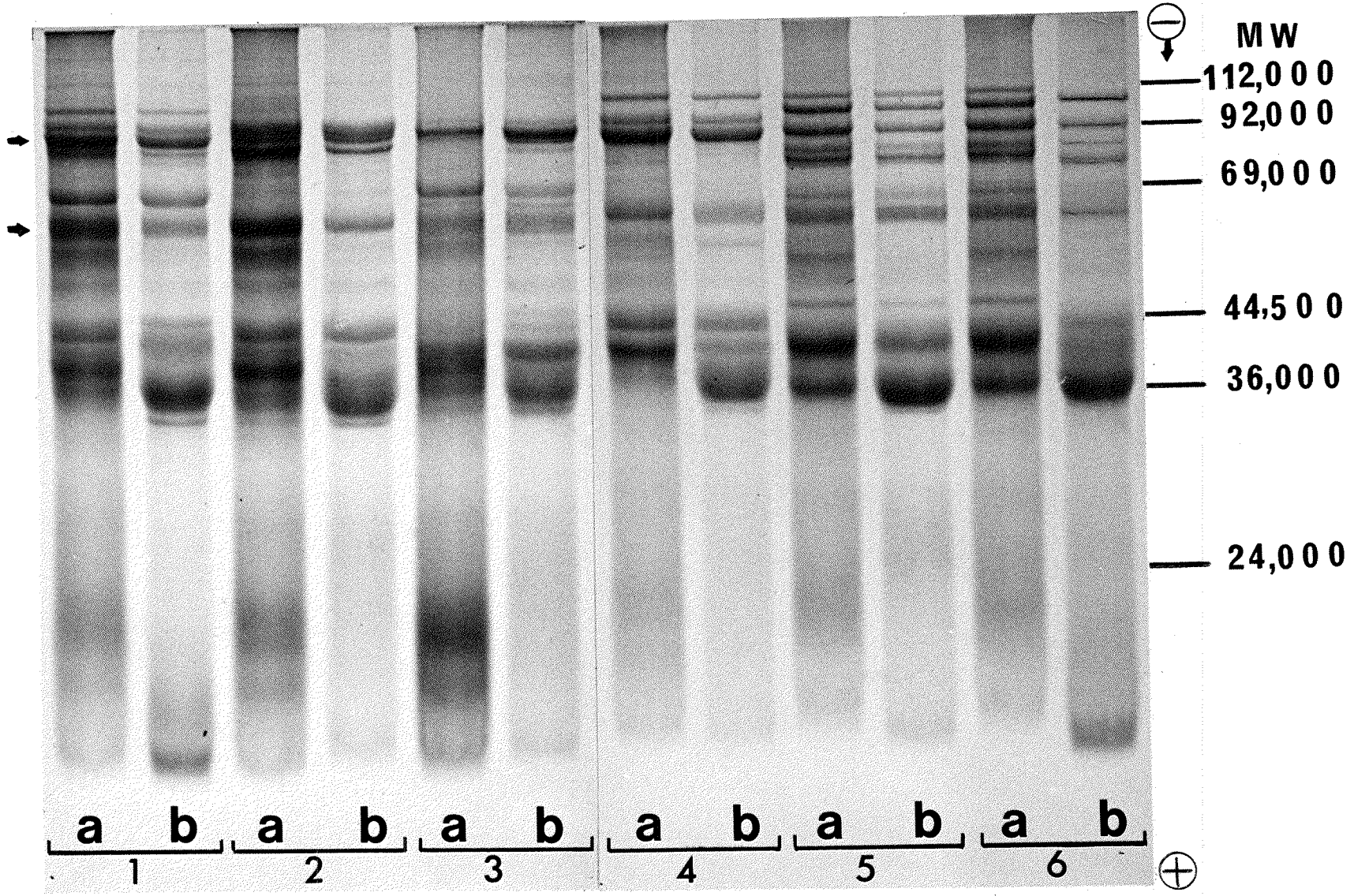
MW
112,000
92,000
69,000
44,500
36,000
24,000
a b a b a b a b a b a b
1 2 3 4 5 6

b. pH 5.8-Insoluble Gluten Protein

Patterns for the unreduced pH 5.8-insoluble fraction of all samples are shown in Figure 6 (patterns 1b-6b). Like the residue protein, this fraction was also composed of protein which remained at the origin, and of protein that entered the gel unresolved. Additionally, it contained some proteins that migrated to the molecular weight region of about 36,000. These latter protein components correspond in mobility to the low molecular weight gliadins of wheat (Bietz and Wall 1972). The unreduced pH 5.8-insoluble fraction also contained small amounts of fast moving proteins (~16,000 daltons).

The patterns for reduced pH 5.8-insoluble protein extracts are shown, in parallel with those for their corresponding residue protein counterparts, in Figure 7. The pH 5.8-insoluble fraction had protein components in the 36,000 molecular weight region in larger concentration than did the residue fraction. This quantitative difference could be due to absence or very low presence in the residue fraction of the nonaggregated low molecular weight gliadin-like protein found in the pH 5.8-insoluble fraction (Figure 6, patterns 1a,b-6a,b). It was also observed that there were many similarities in subunit composition between the two fractions within each of the samples. The similarities suggested that some proteins, probably glutenin-like in nature, were common to both fractions, but in a more complex state in the residue than in the pH 5.8-insoluble fraction.

This suggestion agrees with the observation of Bietz and Wall (1975) in that residue and glutenin resembled each other in subunit composition.

c. pH 5.8-Soluble Gluten Protein

Electrophoretic patterns for unreduced pH 5.8-soluble protein are shown in Figure 6 (patterns 1c-6c). In contrast to the complex protein composition of both residue and pH 5.8-insoluble unreduced fractions, the unreduced pH 5.8-soluble fraction was composed of proteins which resolved into mainly low molecular weight components. The majority of the proteins of this fraction had mobilites in the molecular weight neighborhoods of 36,000 and of 16,000. This indicated that the pH 5.8-soluble fraction was rich in gliadin-like protein, as well as being rich in the low molecular weight albumin-globulin protein that is usually found as contaminant in crude gluten preparations (Bietz and Wall 1972, Wall 1979). In addition, some protein remained at the origin, suggesting that small amounts of protein in this fraction had large molecular weight (>150,000), probably as an aggregated component, that could not enter the gel.

Major differences in band pattern composition were observed to occur between triticale and wheat samples. Some protein bands with mobilities of approximately 76,000, 52,000 and 44,500 daltons were present in the patterns of all triticales but absent in those of the wheats (Figure 6, patterns 1c-6c).

Electrophoretic patterns for reduced pH 5.8-soluble protein are shown in Figure 8. Reduction resulted in the appearence of at least one more band in all samples in the molecular weight region of 61,500. Furthermore, in the patterns of the two wheats an additional band in the 44,000 molecular weight region was also observed. This evidenced that the pH 5.8-soluble fraction contained, in the unreduced state, some high molecular weight aggregated protein. In the molecular weight region between 44,000 and 76,000 fewer differences in subunit composition were observed among the triticales than between the triticales and wheat samples. The bands between approximately 70,000 and 76,000 (two in 4T and Carman, one in 11T and Impala), and that at 52,000 daltons, which were present in the triticale samples, were absent in the two wheat patterns. The absence of bands above 76,000 daltons (Figure 8, patterns 1-6) evidenced that the pH 5.8-soluble fraction did not contain high molecular weight (HMW) subunits (>80,000). It appeared to be composed of mainly gliadin-like protein, and some albumin-globulin contaminant protein.

Therefore, the SDS-PAGE analysis showed that the three gluten protein fractions examined represent three distinct protein entities. The residue protein represented the most complex highly aggregated gluten protein. Its solubility characteristics and electrophoretic behaviour were similar to those of residue gluten proteins reported in the literature (Wall 1979). The presence of both glutenin-like

Figure 8. SDS-PAGE in a 7.5% gel of reduced pH 5.8-soluble gluten protein fraction.

Sample size: 20µl (300 µg protein)

Pattern	Sample
1	4T
2	11T
3	Carman
4	Impala
5	Marquis
6	Fredrick

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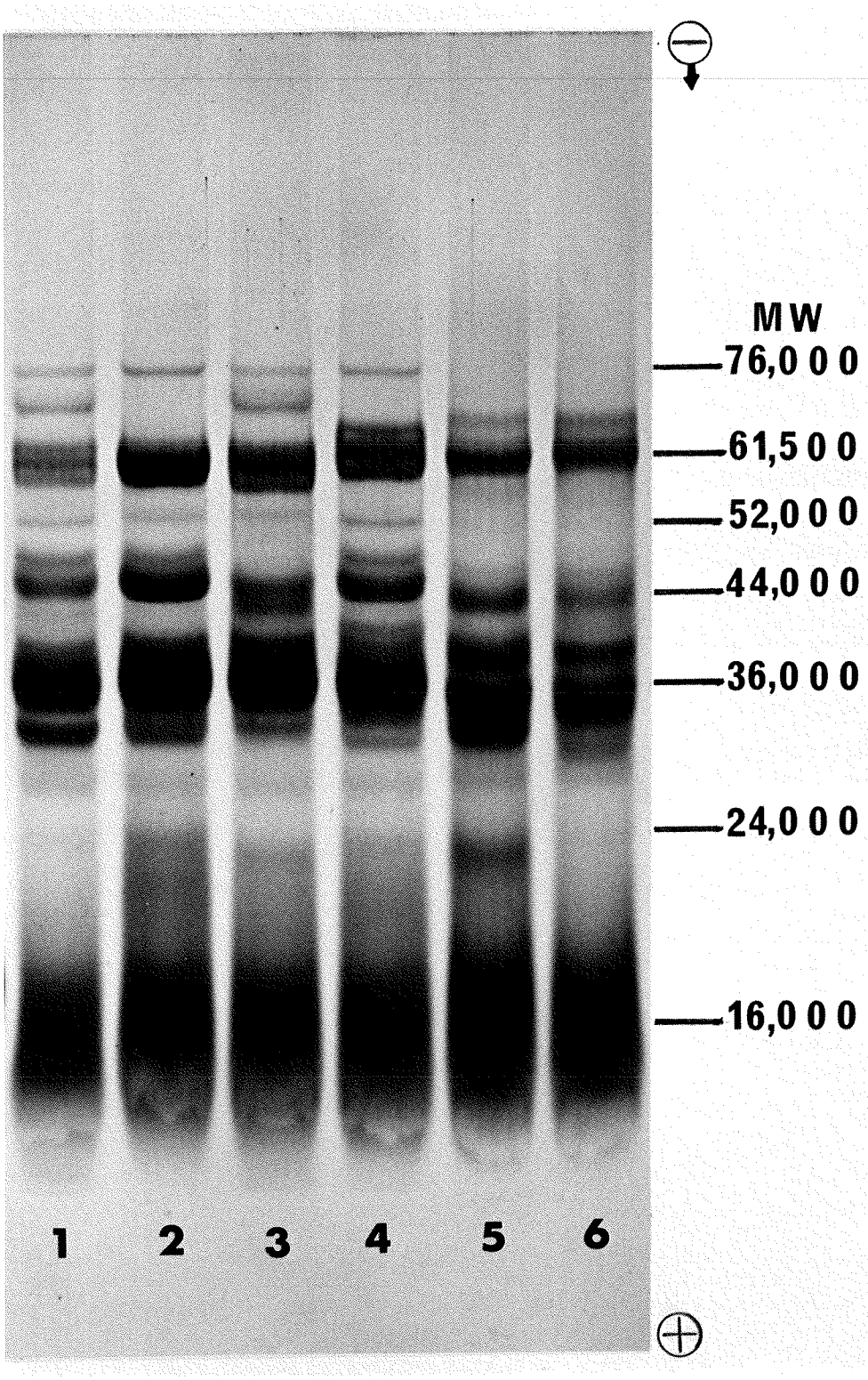

MW
76,000
61,500
52,000
44,000
36,000
24,000
16,000
1
2
3
4
5
6

and gliadin-like protein in the pH 5.8-insoluble fraction, and the predominance of gliadin-like protein in the pH 5.8-soluble fraction, have been reported for similar wheat gluten protein fractions by Shogren et al. (1969).

3. Effect of Gluten Protein Fractions on the Mixing Characteristics of a Base Flour

All gluten protein fractions (residue, pH 5.8-insoluble, and pH 5.8-soluble) were individually added (2.3% protein dry weight, flour basis) to the flour Fredrick and their effect on mixing characteristics tested with the electronic recording dough mixer. The soft wheat flour Fredrick, was chosen as a base flour that could potentially be improved and thus allowed examination of potential improving aspects of the various gluten protein fractions. Mixographic results are shown in Table 14 and Figure 9.

a. Residue Gluten Protein

Addition of residue protein did not affect peak height but resulted in a small effect on mixing time and peak area which varied (positively and negatively) with the sample source (Table 14). Apparently, this fraction improved the mixing stability of the base flour as could be judged from the more gradual falling of the mixograms for flours having residue protein as compared to that of the base flour (Figure 9).

The small effect on mixing characteristics due to addition of residue protein to the base flour observed in

TABLE 14. Effect of Residue, pH 5.8- Insoluble, and pH 5.8-Soluble Gluten Protein on the Mixing Characteristics of the Flour Fredrick

Flour[a]	Mixing Time (min)	Peak Height (cm)	Peak Area (cm^2)
Control	1.4	6.5	16.8
Control + residue[b]			
from: 4T	1.2	6.7	14.5
11T	1.5	6.5	19.8
Carman	1.7	6.1	16.0
Impala	1.5	6.3	19.4
Marquis	1.5	6.5	19.9
Fredrick	1.2	6.3	15.2
LSD (p=0.05)[c]	0.1	0.4	1.1
Control + pH 5.8-insoluble[b]			
from: 4T	1.6	7.4	22.8
11T	1.8	7.1	23.0
Carman	1.8	7.2	27.0
Impala	1.7	7.2	23.4
Marquis	1.6	7.4	23.3
Fredrick	1.4	7.6	21.1
LSD (p=0.05)[c]	0.2	0.1	1.4
Control + pH 5.8-soluble[b]			
from: 4T	1.4	7.2	20.3
11T	1.4	6.6	16.8
Carman	1.3	7.2	18.2
Impala	1.2	7.5	18.6
Marquis	1.8	8.0	27.4
Fredrick	1.8	8.0	27.2
LSD (p=0.05)[c]	0.2	0.3	2.5

[a] Water absorption used: 58% for control and 62% for all other flours.
[b] Level of addition: 2.3% protein, dry wt., flour basis.
[c] LSD = Least Significant Difference.

Figure 9. Effect of the addition (2.3% dry weight, flour basis) of pH 5.8-soluble (A), pH 5.8-insoluble (B), and residue (C) gluten protein on the mixographic patterns of the flour Fredrick (Control).

Source of fraction: 1 - 4T
2 - 11T
3 - Carman

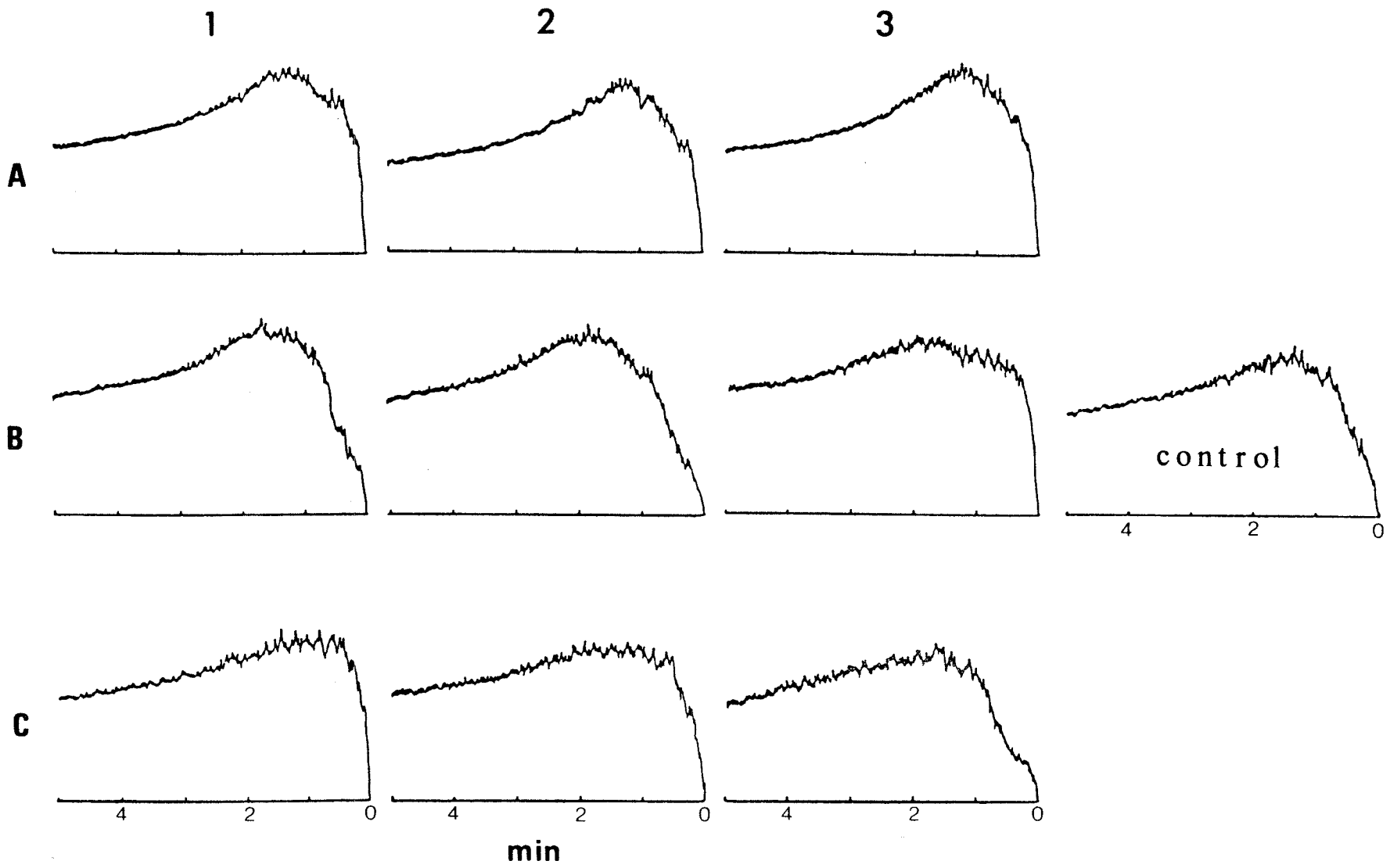
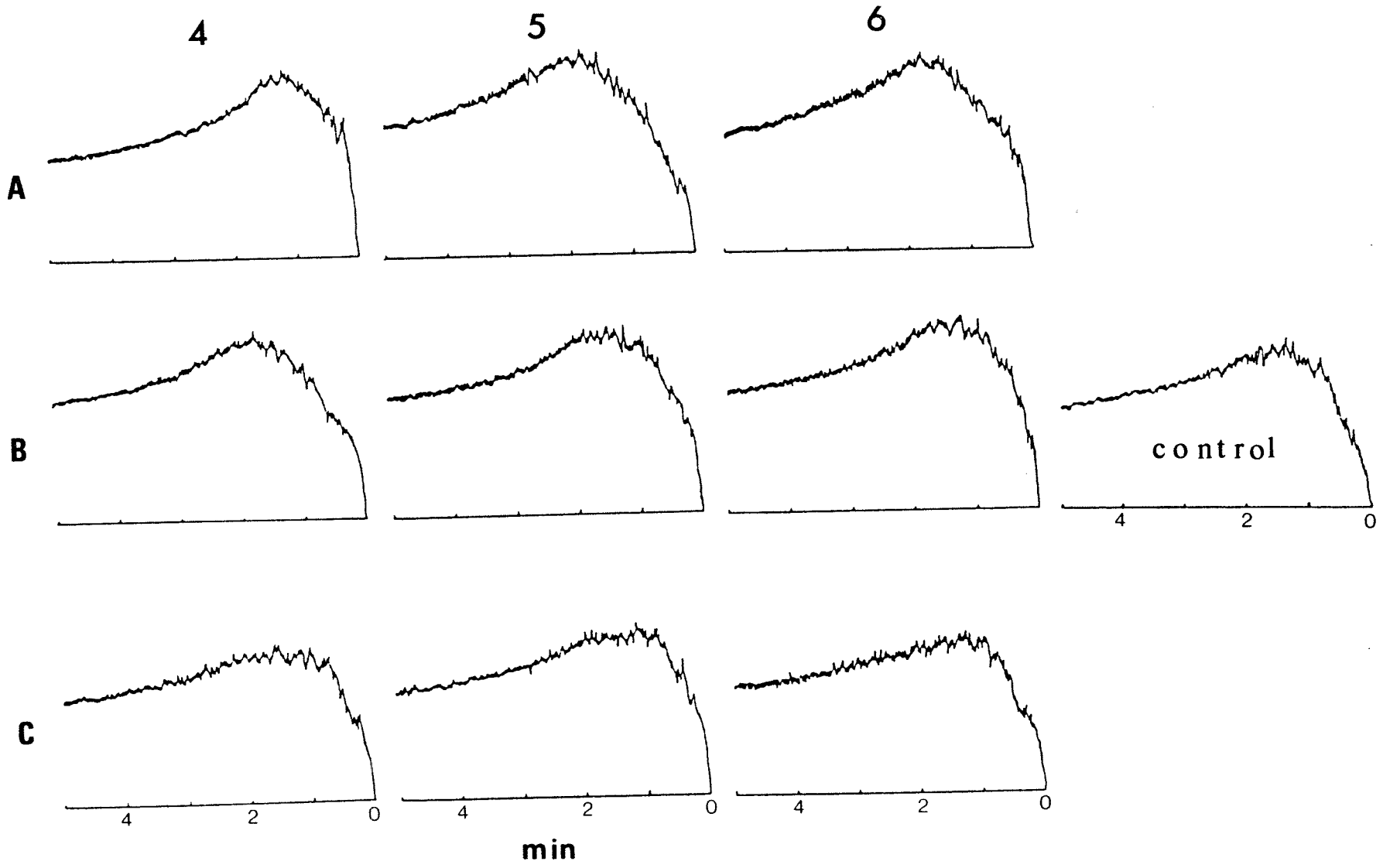

1
2
3
A
B
C
control
4
2
0
min

Figure 9. Continued

Source of fraction: 4 - Impala
5 - Marquis
6 - Fredrick


4
5
6
A
B
control
C
4
2
0
min

this experiment, agrees with similar findings by Harris and Frokjer (1952) and Preston and Tipples (1980) who worked with wheat gluten fractions. Improvement in mixing stability due to increase in residue protein was also observed by MacRitchie (1972) and Preston and Tipples (1980) with wheat gluten proteins.

b. pH 5.8-Soluble Gluten Protein

The pH 5.8-soluble protein affected the mixing characteristics of the base flour in a way different to that of the residue protein from corresponding sources (Table 14, Figure 9). pH 5.8-soluble protein from the triticales had no effect, or decreased slightly (Impala) the mixing time, had no effect, or increased slightly (4T) the peak area, and had no effect (11T) or increased the peak height of the base flour. In contrast, pH 5.8-soluble protein from both wheats considerably increased most of the mixing characteristics relative to those of the base flour. However, one effect that was common for the addition of the pH 5.8-soluble protein, regardless of its source, was the reduction of mixing stability as could be judged from their mixographic patterns (Figure 9). The results of this experiment were similar to those of Harris and Frokjer (1952) who found that addition of wheat gluten protein soluble at pH 5.5, increased the peak height and decreased the mixing stability of the base flour.

The marked differences between wheat and triticale in the effect of added pH 5.8-soluble protein on the overall

mixing characteristics could have resulted from the differences in protein composition observed by SDS-PAGE for unreduced pH 5.8-soluble protein. This fraction from the triticales had proteins that, in the unreduced state, resolved into bands which were not present in the same fraction from the wheats (Figure 6, patterns 1c-6c).

c. pH 5.8-Insoluble Gluten Protein

Addition of pH 5.8-insoluble protein increased the values of all mixing characteristics of the base flour (Table 14). One exception was the pH 5.8-insoluble protein from Fredrick which did not affect mixing time. The greatest increase in peak area corresponded to the protein from Carman. Judging from its mixographic pattern (Figure 9), the large peak area was due to a faster rate of hydration relative to that of the rest of the samples. The mixing stability of the flours with added pH 5.8-insoluble protein was similar to that of the base flour, and intermediate between that of flours with added residue protein and flours with added pH 5.8-soluble protein (Figure 9). The overall improvement in mixing strength contributed by the pH 5.8-insoluble protein agrees with findings by Harris and Frokjer (1952) for a similar wheat gluten protein fraction.

Synthetic flours having pH 5.8-insoluble protein as the major component of their gluten protein were prepared to test to what extent the pH 5.8-insoluble protein influenced

the mixing characteristics shown by the reconstituted flours bearing corresponding gluten protein (Table 10). The synthetic flours were prepared by adding, individually, pH 5.8-insoluble protein fraction to a base flour component consisting of a blend of equal amounts of Fredrick flour and wheat starch. The flours thus formed had 14.2% (db) gluten protein of which 77% was pH 5.8-insoluble protein and 23% was gluten protein contributed by the flour Fredrick. The results are summarized in Table 15 and illustrated in Figure 10.

Differences in all mixing characteristics were observed among some of the synthetic flours. The relative order in mixing time and peak area among the synthetic flours with constant pH 5.8-insoluble protein content were considerably different to those of corresponding reconstituted flours with constant gluten protein content (Table 10). This different effect on mixing strength of the pH 5.8-insoluble fraction relative to that of whole gluten, along with the differences in the effect on mixing strength and mixing stability of the base flour among the added protein fractions (Table 14, Figure 9), suggested that the factors in the gluten involved in the definition of the mixing properties of a flour were distributed among the three protein fractions. However, the pH 5.8-insoluble fraction contributed the most to mixing strength.

TABLE 15. Mixing Characteristics of Synthetic Flours[a] Containing a High Level (10.9% db) of pH 5.8-Insoluble Gluten Protein

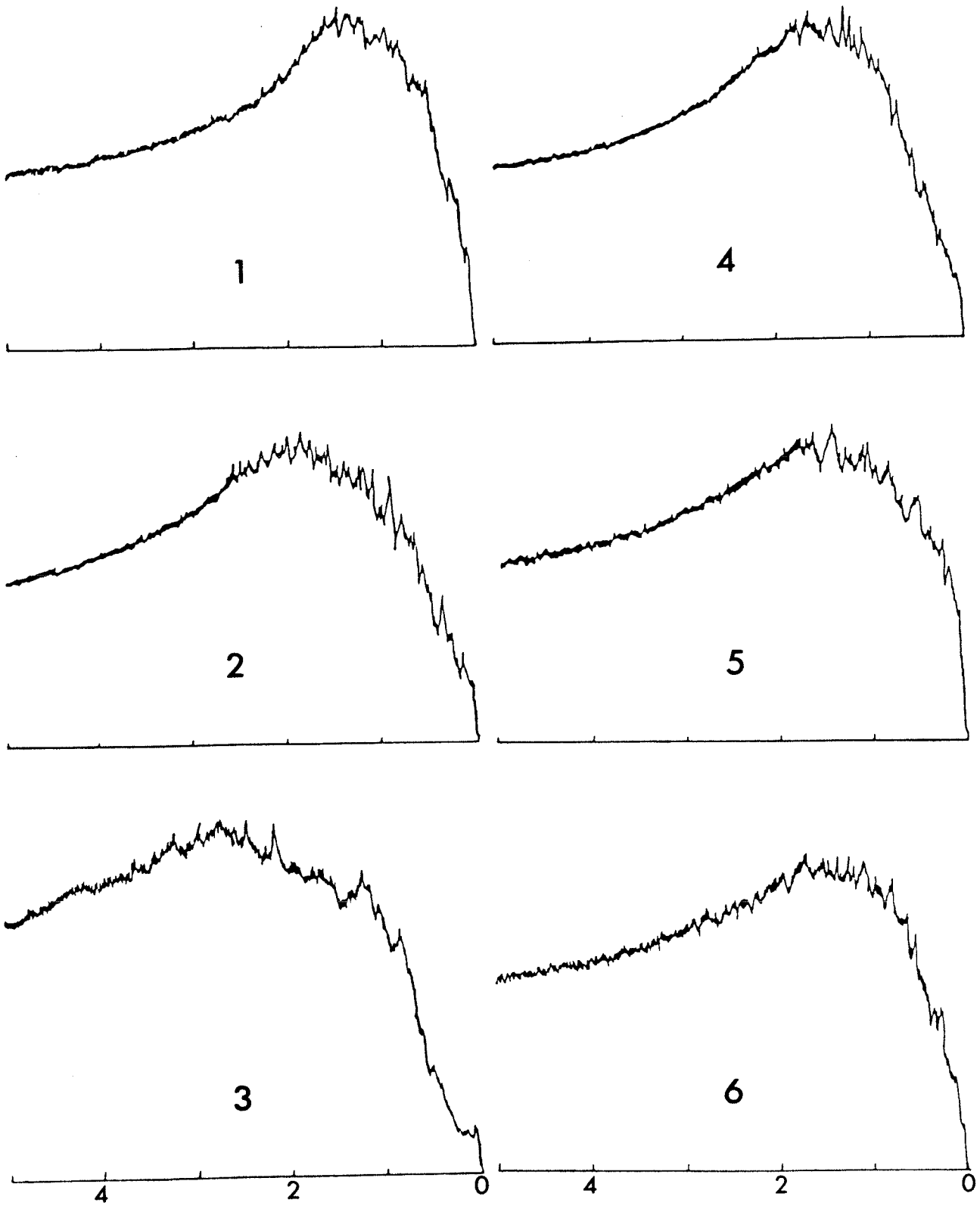
Quality Characteristics	Source of Protein Fraction						
	Triticale				Wheat		LSD[b] (P=0.05)
	4T	11T	Carman	Impala	Marquis	Fredrick	
Mixing time (min)	1.3	2.0	2.8	1.7	1.7	1.6	0.2
Peak height (cm)	8.6	8.3	9.5	8.5	8.0	8.2	0.6
Peak area (cm^2)	21.2	28.8	44.9	25.0	26.0	24.6	1.3

[a] A blend, 1 to 1, of Fredrick flour and wheat starch was used as a base component to which the pH 5.8-insoluble protein fraction was added to form the synthetic flours. The flours had 14.2% (db) gluten protein of which 77% was pH 5.8-insoluble protein and 23% was gluten protein contributed by the Fredrick flour. All flours were tested at 66% absorption.

[b] LSD = Least Significant Difference.

Figure 10. Mixograms of synthetic flours having a high level (10.9% db) of pH 5.8-insoluble gluten protein. Preparation of the flours is indicated in Table 15.

Source of protein:
1 - 4T
2 - 11T
3 - Carman
4 - Impala
5 - Marquis
6 - Fredrick


1
4
2
5
3
6
4
2
0
4
2
0
min

4. Effect of Gluten Protein Fractions on the Baking Potential of a Base Flour

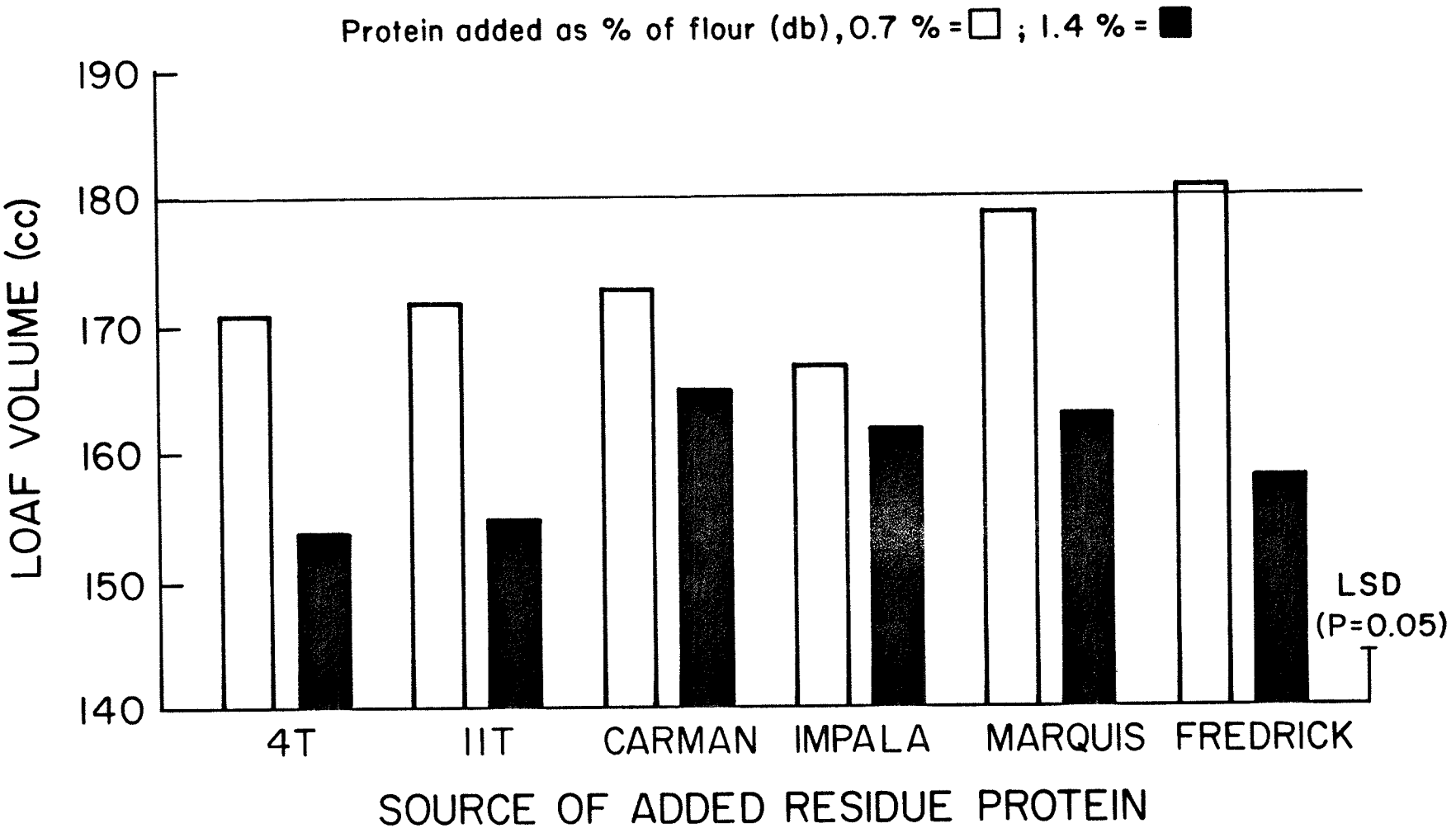
The contribution to baking potential of the three gluten protein fractions was studied by adding, individually, these fractions to the base flour Fredrick which was then baked according to the no-time/sheeting procedure for the 25g flour formula.

a. Residue Gluten Protein

The amount of residue protein added to the base flour was chosen to, besides examining the effect on loaf volume of adding residue protein to the base flour, test if the difference in baking quality between Fredrick and Marquis (Table 10) was due to their difference in residue protein content (Table 13). For this, residue protein from all triticale and wheat flours was individually added at two levels (dry weight, flour basis), 0.7% to match the level in Marquis, and 1.4% to have residue protein at an excessive level. The results are shown in Figure 11.

Increasing the level of residue gluten protein of the flour did not improve the baking quality of the sample. Rather, as the amount of residue protein, regardless of source, was increased, the loaf volume of the flour decreased. At the 0.7% level of addition, the decrease in loaf volume was significant only when the source of residue protein was triticale. Addition of 1.4% residue protein from any of the sources reduced the loaf volume of the flour even more. The extra residue protein could have restricted

Figure 11. Effect of the addition of residue gluten protein on the loaf volume of the base (control) flour Fredrick. Loaf volume of the control is indicated by the horizontal line. Water absorption was 58% for the control, 61% for the flours with 0.7% added protein, and 62% for flours with 1.4% added protein.
LSD:Least Significant Difference.


Protein added as % of flour (db), 0.7 % = □ ; 1.4 % = ■
LOAF VOLUME (cc)
190
180
170
160
150
140
LSD
(P=0.05)
4T
IIT
CARMAN
IMPALA
MARQUIS
FREDRICK
SOURCE OF ADDED RESIDUE PROTEIN

the extensibility and consequently, the expansion of the dough during baking. The observed reduction in loaf volume upon addition of residue gluten protein to the base flour, agreed with similar findings by Harris and Frokjer (1952) and Preston and Tipples (1980), although their residue protein fractions may not necessarily be the same as the residue fraction of this study.

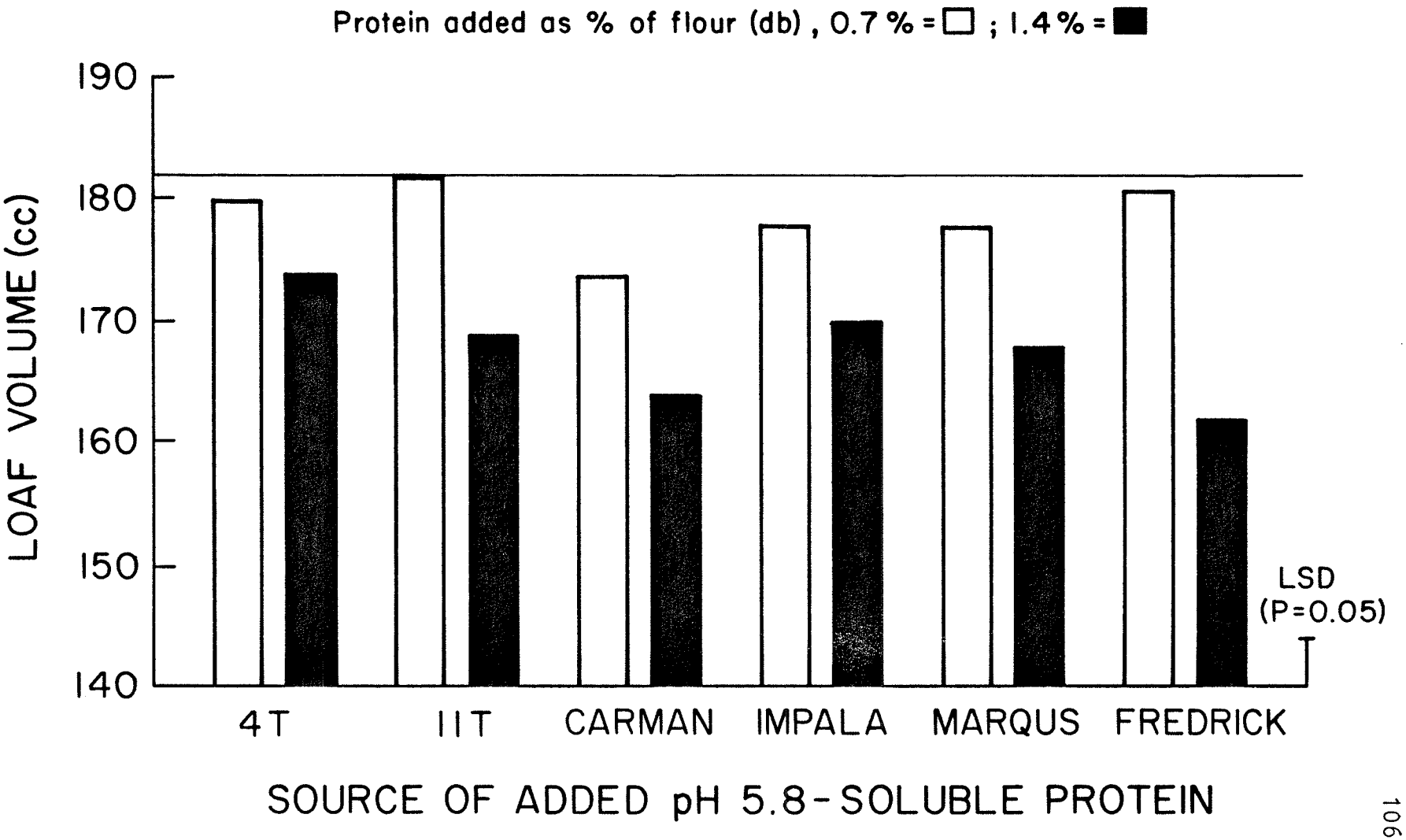
b. pH 5.8-Soluble Gluten Protein

The pH 5.8-soluble fraction was added to the base flour at 0.7 and 1.4% protein (dry weight, flour basis) levels. This allowed one to compare the effect on loaf volume of this fraction with that of the residue fraction at constant protein content. The results are shown in Figure 12. As in the case of residue protein, an increase in the proportion of pH 5.8-soluble protein of the base flour resulted in a decrease in loaf volume. At the 0.7% level of addition the decrease in loaf volume was significant only when the source of the protein added was Carman, Impala, or Marquis. At 1.4%, the pH 5.8-soluble protein from all samples significantly reduced the loaf volume of the flours.

This effect was expected since it is well known (Wall 1979) that the gliadin-rich portion of gluten is more viscous and much less elastic than the rest of the gluten protein (mainly glutenin). Since the pH 5.8-soluble protein fraction was rich in low molecular weight gliadin-like protein, its addition to the flour most likely decreased the elasticity and, consequently, the already deficient baking

Figure 12. Effect of the addition of pH 5.8-soluble gluten protein on the loaf volume of the base (control) flour Fredrick. Loaf volume of the control is indicated by the horizontal line. Water absorption was 58% for the control, 59% for the flours with 0.7% added protein, and 60% for flours with 1.4% added protein.

LSD: Least Significant Difference


Protein added as % of flour (db), 0.7 % = □ ; 1.4 % = ■
190
180
170
160
150
140
LOAF VOLUME (cc)
LSD
(P=0.05)
4T
11T
CARMAN
IMPALA
MARQUS
FREDRICK
SOURCE OF ADDED pH 5.8-SOLUBLE PROTEIN

strength of Fredrick. Reduction in loaf volume of a flour due to addition of wheat gluten protein soluble at pH 5.5 and above has been reported in the literature (Harris and Frokjer 1952, Shogren et al. 1969, respectively).

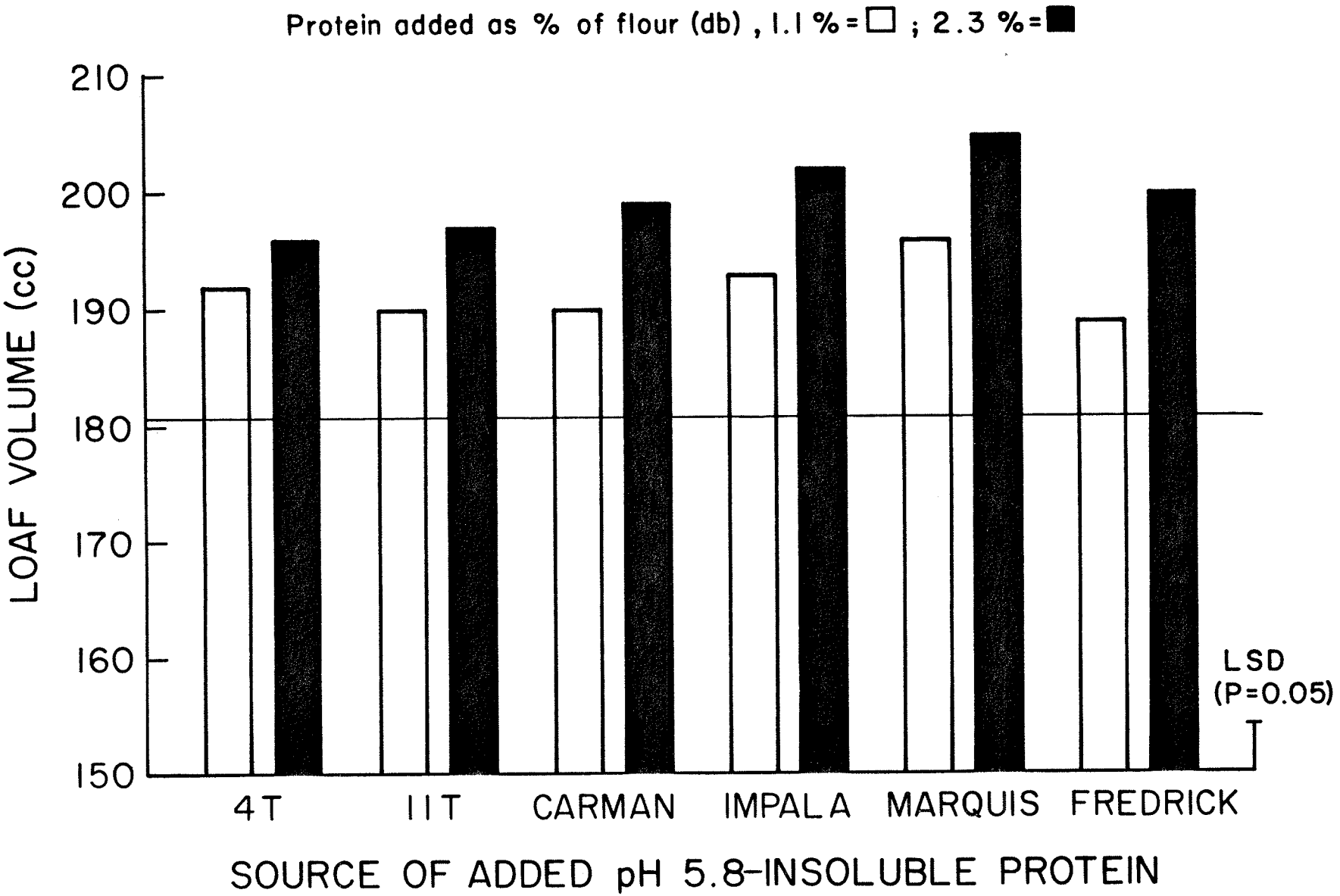
c. pH 5.8-Insoluble Gluten Protein

Since neither the residue protein nor the pH 5.8-soluble protein improved the loaf volume of the base flour, it was expected that addition of pH 5.8-insoluble protein will result in loaf volume increase. Therefore, the latter was tested at two levels (dry weight, flour basis), one (1.2% protein) similar to the highest used for the two previously tested fractions, and a higher (2.3% protein) level to further enhance any effect of the pH 5.8-insoluble protein on loaf volume. The results are shown in Figure 13. As expected, the pH 5.8-insoluble fraction significantly increased the loaf volume of Fredrick. Larger loaf volumes were obtained as the level of addition of this fraction increased. The largest increase, at both levels of addition, was due to the pH 5.8-insoluble protein from the wheat Marquis followed by that from the triticale Impala. The differences in loaf volume due to source of the fraction added were not significant.

To further test the extent of the positive contribution of the pH 5.8-insoluble protein on loaf volume, a comparison was made of the effect of adding (on an equal protein basis) a) a complete reconstituted gluten, b) a gluten component consisting of pH 5.8-insoluble and residue protein, or c)

Figure 13. Effect of the addition of pH 5.8-insoluble gluten protein on the loaf volume of the base (control) flour Fredrick. Loaf volume of the control is indicated by the horizontal line. Water absorption was 58% for the control, 61% for the flours with 1.2% added protein, and 63% for flours with 2.3% added protein.

LSD: Least Significant Difference


Protein added as % of flour (db), 1.1 % = □ ; 2.3 % = ■
LOAF VOLUME (cc)
210
200
190
180
170
160
150
LSD
(P=0.05)
4T
11T
CARMAN
IMPALA
MARQUIS
FREDRICK
SOURCE OF ADDED pH 5.8-INSOLUBLE PROTEIN

just pH 5.8-insoluble protein. The complete reconstituted glutens were based on the proportions fractionated from the donor sources (Table 13). The pH 5.8-insoluble + residue components were formed by combining pH 5.8-insoluble with residue protein in the proportions corresponding to their fractionation yields (Table 13) but assuming % pH 5.8-insoluble + % residue protein as 100%. Proportions of protein fractions used in reconstituted gluten and the pH 5.8-insoluble + residue (pHI-R) protein component are presented in Table 16. Reconstituted gluten, the pHI-R protein component, and the pH 5.8-insoluble protein were added at a 2.3% (dry weight, flour basis) protein level. Their effect on the loaf volume of the base flour is illustrated in Figure 14.

The three types of gluten protein all increased the loaf volume of the base flour. However, the general trend was that as the proportion of pH 5.8-insoluble protein in the three added materials increased, the respective loaf volume increased accordingly. This trend occurred for all gluten components from all triticale and wheat sources that were tested. Therefore, these results provided further evidence that the pH 5.8-insoluble fraction combined proteins which contributed to the viscoelastic properties involved in the control of baking potential of a flour.

The improving effect of the pH 5.8-insoluble protein on the loaf volume of the base flour also suggested that the decrease in loaf volume upon addition of residue or of pH

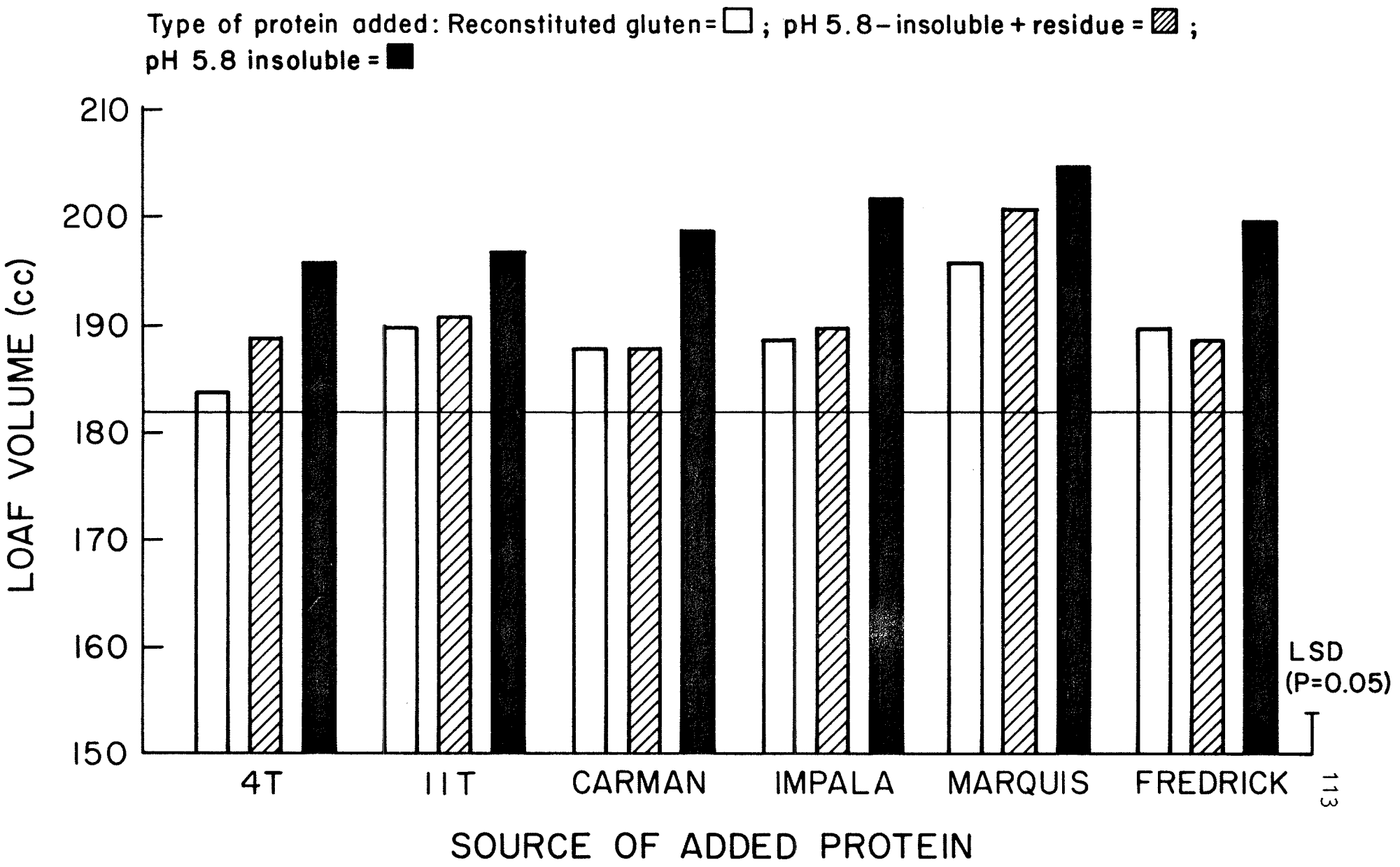
TABLE 16. Proportions of Gluten Protein Fractions Present in Reconstituted Gluten and in the pH 5.8-Insoluble + Residue Component Used in Breadmaking Studies

Component and Source	pH 5.8-soluble (%)[a]	pH 5.8-insoluble (%)[a]	Residue (%)[a]
Reconstituted gluten:			
4T	15.7	67.3	17.0
11T	11.5	79.5	9.0
Carman	8.6	85.5	5.9
Impala	10.4	67.6	22.0
Marquis	10.0	75.2	14.8
Fredrick	9.7	83.5	6.8
pH-insoluble + residue:			
4T	--	79.8	20.2
11T	--	89.8	10.2
Carman	--	93.5	6.5
Impala	--	75.4	24.6
Marquis	--	83.5	16.5
Fredrick	--	92.5	7.5

[a] Dry weight basis.

Figure 14. Effect of the addition (2.3% dry weight, flour basis) of reconstituted gluten, pH 5.8-insoluble + residue, and pH 5.8-insoluble protein on the loaf volume of the base (control) flour Fredrick. Loaf volume of the control is indicated by the horizontal line. Water absorption used was 58% for the control and 63% for all other flours.

LSD: Least Significant Difference


Type of protein added: Reconstituted gluten = □ ; pH 5.8 – insoluble + residue = ▨ ;
pH 5.8 insoluble = ■
LOAF VOLUME (cc)
210
200
190
180
170
160
150
LSD
(P=0.05)
4T
11T
CARMAN
IMPALA
MARQUIS
FREDRICK
SOURCE OF ADDED PROTEIN

5.8-soluble protein was in part due to a decrease in pH 5.8-insoluble protein. The proportion of the latter protein in the base flour was shifted to a lower level when the residue and the pH 5.8-soluble protein were added to the flour.

The synthetic flours prepared for the mixographic experiment discussed earlier (Table 15), were used to evaluate if the pH 5.8-insoluble protein could reflect the differences in baking potential shown by reconstituted flours bearing gluten from the various triticale and wheat flours (Table 10). The results are summarized in Table 17.

The loaf volume potential of the synthetic flours varied with the source of pH 5.8-insoluble protein. The flour with protein from Marquis had, as expected, the largest loaf volume followed by that with protein from Impala. Flours with protein from Carman and Fredrick had lower loaf volume than that with 4T protein. This was not expected since Carman and Fredrick flours had better loaf volume than 4T, at constant gluten protein content (Table 10). This change in order of loaf volume potential could be due to differences in protein composition between the whole gluten and the pH 5.8-insoluble fraction. In any case, these results indicated that the factors responsible for the differences in baking potential of the flours of this study were mainly housed in the pH 5.8-insoluble fraction. These results agree with those obtained by Harris and Frokjer (1952) and Shogren et al. (1969) in similar studies with wheat gluten protein fractions.

TABLE 17. Baking Quality of Synthetic Flours[a] Containing a High Level (10.9% db) of pH 5.8-Insoluble Gluten Protein

Quality Characteristics	Source of Protein Fraction					
	Triticale				Wheat	
	4T	11T	Carman	Impala	Marquis	Fredrick
Loaf volume (cc)[b]	188	200	177	210	232	163
Crumb appearence[c]	Q	Q S	U	S	S	U
Crumb structure[c]	Q,SO	Q S,SO	U,O	S,SO	S,F	U,O

[a] Synthetic flours were prepared as indicated in Table 15. All flours were baked at 66% absorption.
[b] Least Significant Difference (p=0.05) = 3.0 cc.
[c] S = satisfactory, Q = questionable, U = unsatisfactory, O = open, SO = slightly open, F = fine.

5. Subfraction Composition of the pH 5.8-Insoluble Gluten Protein

The pH 5.8-insoluble fraction was found to contribute importantly to flour mixing strength as well as to be the main gluten protein factor controlling the baking potential of a flour. The differences in mixing characteristics and loaf volume potential shown by the synthetic flours (Tables 15, 17) could be due to differences in the proportion of protein subfractions (specifically gliadin-like and glutenin-like proteins) among the several pH 5.8-insoluble protein fractions evaluated and/or to their subunit composition. To evaluate these possibilities, both the proportion of protein subfractions from the pH 5.8-insoluble gluten protein, as well as their composition were examined.

a. Fractionation of pH 5.8-Insoluble Gluten Protein into Gliadin-like and Glutenin-like Components

The pH 5.8-insoluble protein was resuspended in and extracted with 0.05M acetic acid solution containing 70% ethanol. It was assumed that the gliadin-like protein would be solubilized in this solvent. Subsequent adjustment of the pH to 5.8 would precipitate any co-solubilized glutenin-like proteins. This pH value was chosen because SDS-PAGE (Figure 8) had shown that high molecular weight subunits (>80,000), i.e. glutenin-like protein, were not present in the protein soluble at pH 5.8. Results on the proportions of pH 5.8-70% ethanol soluble (gliadin-like) and insoluble (glutenin-like) proteins are shown in Table 18.

TABLE 18. Distribution(%)[a] of pH5.8-Insoluble Gluten Proteins Upon pH 5.8 Precipitation in the Presence of 70% Ethanol

Source of Fraction	Ethanol-soluble[b]	Ethanol-Insoluble[b]	Recovery	Ratio Insoluble/Soluble
Triticale				
4T	53.2[c]±0.2	41.2[c]±0.2	94.4	0.77
11T	50.8±1.6	43.5±0.4	94.3	0.86
Carman	48.9±0.2	50.7±0.5	99.6	1.04
Impala	55.1±0.3	44.2±0.1	99.3	0.80
Wheat				
Marquis	56.4±0.1	38.5±0.8	94.9	0.68
Fredrick	55.4±1.0	40.2±1.8	95.6	0.73

[a] Percent of total pH 5.8-Insoluble gluten protein (db).
[b] Ethanol-soluble and ethanol-insoluble are referred in the text as gliadin-like and glutenin-like, respectively.
[c] Mean and mean difference for duplicates.

The relative proportions of gliadin-like and glutenin-like protein varied among all samples. Carman had the largest glutenin-like/gliadin-like ratio followed by 11T, while Marquis had the smallest one followed by Fredrick. The variation in the magnitude of this ratio among the samples corresponded positively to the trend in mixing time and peak area followed by the synthetic flours (Table 15). This relationship was clear among triticale sources and between wheats and less apparent when all samples were considered in the comparison. This finding further supports the idea of the importance of the proportion of glutenin-like protein in determining mixing strength, and agrees with results by MacRitchie (1972, 1973, 1980a) in that as the amount of glutenin-like protein in a flour increases, its mixing strength increases.

In contrast to the positive relationship between the mixing strength of the synthetic flours and the ratio of glutenin-like to gliadin-like protein, the ratios showed no trend which corresponded to the loaf volume obtained with the synthetic flours (Table 17). This finding is in general accord with those of Hoseney et al. (1969) and MacRitchie (1980a) who found that the differences in baking quality between wheat flours were not due to differences in the ratio of glutenin to gliadin. It was therefore apparent that the composition of the pH 5.8-insoluble proteins was more important than their proportions in contributing to the baking quality of the flours.

b. SDS-PAGE of Gliadin-like and Glutenin-like Proteins

Electrophoretic patterns for unreduced and reduced gliadin-like and glutenin-like proteins from the pH 5.8-insoluble fraction are shown in Figures 15 and 16. The patterns for unreduced gliadin-like proteins (Figure 15, patterns 1a-6a) showed that this component was composed of high molecular weight protein that remained at the origin or entered the gel, unresolved, producing smearing just below the origin. It also contained protein that resolved into bands with from fast to slow mobilities. At least one more major band was seen to be present (at about 40,000 to 45,000 daltons) in the patterns for the triticale proteins, which was absent in those for the wheat samples. A similar compositional difference was previously observed to occur in the pH 5.8-soluble fraction (Figure 6, patterns 1c-6c).

The patterns for unreduced glutenin-like protein (Figure 15, patterns 1b-6b) showed that the majority of the protein remained at the origin or just entered the gel suggesting that this fraction was composed of mainly high molecular weight protein. A band, at about 44,500 daltons, present in the patterns for the glutenin-like protein from the triticale samples was absent in the patterns for the same protein from the wheat samples (Figure 15, patterns 1b-6b).

The patterns for reduced gliadin-like protein (Figure 16, patterns 1a-6a) showed that 70% ethanol extracted small amounts of protein containing HMW subunits (>80,000

Figure 15. SDS-PAGE in 10% gels of unreduced gliadin-like and glutenin-like proteins from the pH 5.8-insoluble gluten protein fractions.

Sample size: 20 µl (300 µg protein)

Gliadin-like	Glutenin-like
1a = 4T	1b = 4T
2a = 11T	2b = 11T
3a = Carman	3b = Carman
4a = Impala	4b = Impala
5a = Marquis	5b = Marquis
6a = Fredrick	6b = Fredrick

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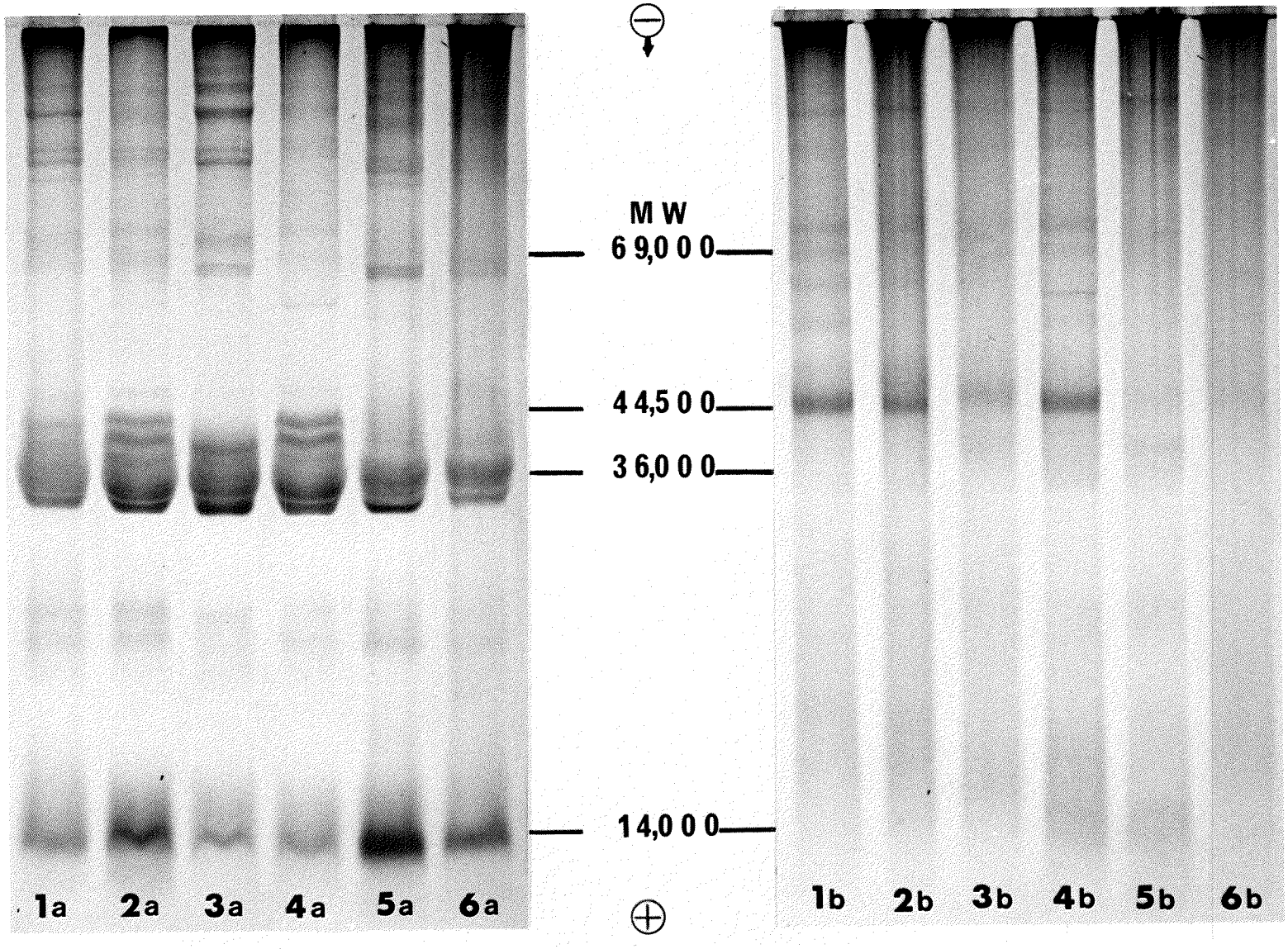
⊖
MW
69,000
44,500
36,000
14,000
⊕
1a
2a
3a
4a
5a
6a
1b
2b
3b
4b
5b
6b

Figure 16. SDS-PAGE in 10% gels of reduced gliadin-like and glutenin-like proteins from the pH 5.8-insoluble gluten protein fractions.

Sample size: 20 µl (300 µg protein)

Gliadin-like	Glutenin-like
1a = 4T	1b = 4T
2a = 11T	2b = 11T
3a = Carman	3b = Carman
4a = Impala	4b = Impala
5a = Marquis	5b = Marquis
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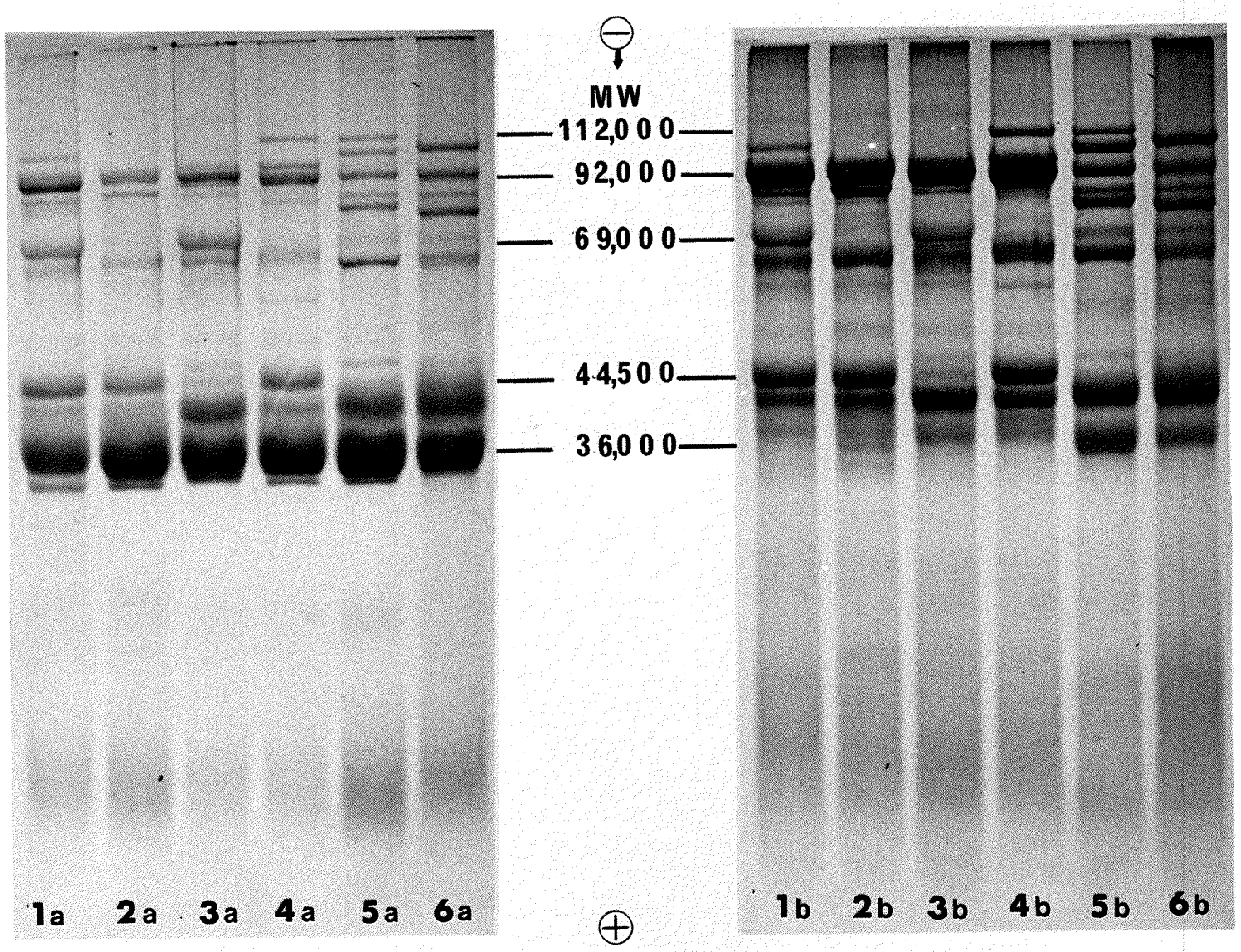
MW
112,000
92,000
69,000
44,500
36,000
1a
2a
3a
4a
5a
6a
1b
2b
3b
4b
5b
6b

daltons). These are generally classified as a unique characteristic of glutenin-like (70% ethanol-insoluble) protein (Pyler 1983, Wall 1979) but have been reported previously as part of gliadin preparations (Lukow 1983, McMaster 1982, Paredes-Lopez 1980, Preston et al. 1975). In addition, to the HMW subunits, the gliadin-like protein contained a small amount of protein that resolved into subunits in the neighborhood of 69,000 daltons, as well as by subunits in the range of molecular weight between 50,000 and 30,000.

The reduced glutenin-like protein had a subunit composition similar to that of its corresponding gliadin-like counterpart (Figure 16). The reduced gliadin-like and glutenin-like proteins differed primarily in that the majority of the gliadin-like protein was low molecular weight (30,000-50,000) while the glutenin-like component had more protein concentrated in the HMW subunits. Thus, the differences between the two protein components were mainly quantitative. This was not unexpected since it has been shown that, under reducing conditions, the HMW gliadin proteins are the same as the LMW glutenin proteins (Bietz and Wall 1980, Wall 1979). These are the proteins that in SDS-PAGE concentrate in the region between 35,000 and 45,000 (Bietz and Wall 1973, Payne and Corfield 1979). Gliadin and glutenin proteins both have subunits with mobilites in the neighborhood of 69,000 daltons (Bietz and Wall 1972).

The banding patterns for both gliadin-like and

glutenin-like reduced proteins, showed differences in the number and molecular weight of the bands in the molecular weight region between 36,000 and 69,000 among triticales and between triticales and wheats. However, these differences could not be associated to the variation in mixing and baking properties shown by reconstituted (Table 10) or synthetic flours (Table 15, 17).

Since the HMW protein subunits are in general recognized as characteristic of glutenin-like protein, it was convenient to discuss them with the patterns for the glutenin-like protein (Figure 16, patterns 1b-6b). Some major differences in HMW subunit composition were found among triticale samples and between triticale and wheat samples. Samples from 4T and Impala, which had the better mixing time and peak area values among the triticales (Table 10), had one band each of approximately 105,000 and 112,000 daltons, respectively. These bands were absent in the patterns for the 11T and Carman proteins. The protein from the triticale samples had at least one less HMW subunit than that from the wheats. Additionally, most of the HMW subunit protein in the triticale samples concentrated close to the molecular weight region of 92,000, while that from the wheats spreaded out more, into bands within the region of approximately 80,000 to 112,000 daltons. It has been found that bread wheats have more HMW subunits than durum wheat or wheats lacking D-genome chromosomes (Wall 1979 for review). Considering this, it is possible that the differences in

number and, perhaps, in molecular weight of HMW subunits observed among triticales and between triticale and wheat samples, could be related to the variation in flour strength observed among the samples of this study. This possibility deserves further study with more triticale samples.

V. GENERAL DISCUSSION

During the early 1970's, it was well accepted that triticale had little chance to become a commercial crop due to its poor grain quality characteristics. However, new crosses between hexaploid triticales and hexaploid wheats have resulted in the improvement of both agronomic and grain quality characteristics in triticale.

Most emphasis has been placed on the improvement of grain quality characteristics that affect yields and little has been done to raise the industrial quality of the average population of high-yielding triticales. The reason for this is that on one hand, the standard dough-testing and bread making practices established for bread wheats, but commonly used to test triticale are not completely suitable for the evaluation of triticale quality. Consequently, the quality of the materials tested could be misjudged. On the other hand, little attention has been given to the study of compositional grain factors that could be responsible for the differences in flour strength observed among triticale flours. Thus, the factors to be improved are not well defined.

Given the potential of triticale has as an important source of nutrients for humans, this study was undertaken, 1) to investigate the suitability of the relatively new

technological practice of dough development by sheeting for the evaluation of the baking performance of triticale flours and, most importantly, 2) to study the influence of gluten protein quantity and quality on the dough strength variability observed among triticales. The results of this work provide new information that could perhaps be of use in improving the dough strength of secondary hexaploid triticales.

Sheeting rolls were found to be effective in developing bread doughs from diverse types of triticale and wheat flours. This practice seems to present three advantages over the use of dough mixers that could be rather critical when dealing with weak type flours, particularly those from triticale. First, it requires considerably lower work input, relative to that required by a pin mixer (Kilborn and Tipples 1974), to develop a dough. Second, the elimination of the dough tearing, that occurs in a dough mixer, could reduce considerably the mechanical, irreversible, scission of covalent bonds in the gluten proteins that has been suggested to occur during dough mixing (MacRitchie 1975, 1980a, Tanaka and Bushuk 1973). This could reduce the risk of overdeveloping the dough, something that could easily happen if a dough of poor stability is mixed just past its optimum. Third, the dough-folding action between the sheeting steps results in a more finely interconnected dough structure than in the case of dough mixing (Stenvert et al. 1979). This could be reflected in an improved crumb

structure.

Elimination of the bulk-fermentation stage, addition of oxidizing agents, and use of a higher yeast level were contrasting conditions of the no-time baking procedures that contributed to the improvement in the baking performance of wheat and triticale flours as compared to their performances under the conditions of the AACC method. The major advantage of eliminating the bulk-fermentation stage seems to be the reduction in the detrimental effect of high alpha-amylase activity to the structure of the bread dough. This is a desirable baking feature when dealing with triticale flours which in general have undesirably high alpha-amylase activity levels.

The no-time/sheeting baking procedure proved to be a valuable test, a) to detect differences in baking quality among triticales having similar flour protein content and small differences in their mixing properties and sedimentation values, b) to evaluate the baking potential of flours from fractionation-reconstitution studies, and c) to estimate the influence of the addition of gluten proteins or the baking potential of a base flour.

Mixing time and the Zeleny sedimentation value have been found to be relatively good predictors of baking quality of bread wheats (Orth et al. 1972, Pinckney et al. 1957). In this study, both parameters classified the triticale flour as weak but indicated that Impala had better dough strength than the other triticales. These results

agreed with those of the no-time/sheeting procedure with respect to the general dough strength of the triticales tested and therefore it is suggested that both mixing time and the sedimentation value could be used, along with the baking test, to screen for dough strength in triticale.

The generally inferior dough strength shown by all the triticales, relative to that of the bread wheat Marquis, was in part due to the considerably lower gluten protein contents of the triticales. Additionally, the fractionation-reconstitution studies showed that gluten quality was also a factor contributing to the dough strength of the triticale flours. These findings suggest that if the dough strength of triticale is to be improved, it will not be enough to just increase the content of gluten protein of this cereal because the magnitude of the effect of gluten content on dough strength depends greatly on the quality of that gluten. For example, 4T, 11T, and Carman, at high gluten protein content still resulted in poor baking quality and poor mixing characteristics. When the triticales and wheats were tested at equal gluten protein contents, only Impala was shown to have dough strength as good as that of the bread wheat Marquis.

Due to the important contribution of gluten protein quality on dough strength, the gluten complex was fractionated in an attempt to determine if a specific protein fraction or subfraction was largely responsible for the dough strength. With respect to mixing characteristics,

it was found that the proportion of the protein fraction with the largest content of high molecular weight protein (i.e. the residue from the Osborne-type fractionation and from the acid solubilization - pH 5.8 precipitation, as well as the glutenin-like protein from the pH 5.8 precipitation in the presence of 70% ethanol) was positively associated with the mixing time of the triticales and wheats studied. The apparent relationship between the proportion of acid-soluble high molecular weight protein and mixing strength was confirmed by mixographic studies involving the effects of gluten protein fractions on the mixing characteristics of a base flour. In contrast, the mixographic results showed that the acid-insoluble gluten residue protein affected only slightly the mixing characteristics of the base flour. According to Preston and Tipples (1980) the slight effect of residue protein on mixing strength could result from restricted interaction of the highly aggregated residue proteins with other flour components. The results of this study are in general agreement with the concept that mixing strength of a flour depends mainly on its proportion of glutenin-like protein (Miflin et al. 1983 for review).

The control of baking quality of a flour appears to be more complex than that of mixing strength which seems to reside mainly in the proportion of glutenin-like protein in the flour. Recent studies involving fractionation and reconstitution (Finney et al. 1982, MacRitchie 1980a) and studies of the effect on baking quality of adding gluten

protein fractions to a base flour (Preston and Tipples 1980) seem to favour the idea that the majority of the control of baking potential resides in the acid-soluble gluten protein. This fraction contains both glutenin and gliadin proteins. Similarly, it was found in this study that the main factor(s) responsible for loaf volume potential was housed in the acid-soluble fraction (pH 5.8-insoluble) which combined both glutenin-like and gliadin-like proteins. It is possible that the combination of these two protein types is a required condition to provide the bread dough with the appropriate viscoelasticity that results in the expression of the maximum loaf volume potential of a flour. Too elastic or too extensible flours do not generally produce bread of acceptable loaf volume. However, an appropriate balance in viscoelasticity seems not to depend on fixed ratios of glutenin to gliadin. This was indicated by the finding that the variation in the magnitude of the glutenin-like/gliadin-like ratio observed among the various triticale and wheat sources did not correspond to the variation in loaf volume of the synthetic flours bearing corresponding pH 5.8-insoluble protein. Consequently, an appropriate viscoelastic balance which allows for the gas retention and expansion of the bread dough seems to depend on the composition of the acid-soluble, glutenin-like and gliadin-like proteins and the way they interact with each other and with the other flour components.

The analysis by SDS-PAGE of unreduced and reduced

gluten protein fractions was performed with the aim of examining if there were major qualitative differences among triticales and between triticales and wheats, that could be associated with the observed variability in dough strength of the samples of this study. The unreduced pH 5.8-soluble protein from the triticale samples had subunits in the molecular weight regions of 44,500, 52,000, and 76,000, which were absent in the same fraction from the wheat samples. Reduction of disulfide bonds neither changed the mobilities of these protein bands nor made them appear in the patterns for the wheat proteins. Therefore, it appeared that these bands are nonaggregated proteins which could be cereal specific, inherited by triticale from its rye parent. This latter suggestion is based on the resemblance in electrophoretic mobility between these bands and the 40,000-dalton-γ-secalins the 52,500-dalton-ω-secalins, and the 75,000-dalton-γ-secalins, present in the unreduced 60% ethanol extracts from the rye cultivars studied by Shewry et al. (1983). Similarly, both the gliadin-like and glutenin-like unreduced protein from the triticale samples had a protein band with mobility of approximately 44,500 daltons which was absent in the same proteins from the wheat samples.

The presence of the extra bands in unreduced triticale gluten protein fractions, which are not changed in mobility upon reduction of disulfide bonds, could indicate that the gluten from the triticales contains a larger number of

nonaggregated proteins than the gluten from the wheats. It might be suggested that not only the number of nonaggregated proteins but also the amount of such protein could be responsible for some of the observed differences in dough strength. This possibility is highly speculative but if true, this could be one of the qualitative factors affecting the dough strength of triticale. This speculation is based on the work by Field et al. (1983) who found a positive correlation between the variations in the ratio of aggregated to nonaggregated protein and the baking potential of various wheat flours, as well as on that by Wrigley et al. (1980) who found that the dough strength of several wheat varieties decreased when the amount of cysteine decreased due to sulfur-restricted growing conditions. Reduction of cysteine content would have resulted in a decrease or the proportion of aggregated protein.

Another major finding of the SDS-PAGE analysis concerns the high molecular weight (HMW) protein subunits of the glutenin-like protein. It appears now to be well accepted that the HMW subunits contributed by the chromosome 1D of the D-genome play an important role in the viscoelastic properties that characterize bread wheats. This suggestion comes from the results of Bietz and Wall (1972) who found that some of the HMW subunits present in bread wheats were absent in the durum wheats. This, was later substantiated by the results of Bietz et al. (1975) and Orth and Bushuk (1973b) who found that elimination of the D-genome

chromosomes from bread wheats resulted in the deletion of certain HMW subunits originally present in the parent wheats. Further studies (Bietz et al. 1975, Orth and Bushuk 1974) showed that the synthesis of some HMW subunits was controlled by chromosome 1D. Chromosomes 1A and 1B control the synthesis of the rest of the HMW subunits (Bietz et al. 1975, Payne et al. 1980).

Considering the above findings, it is very likely that the lower number of HMW subunits in the protein from the triticale samples relative to that in the protein from the wheats, reflected the absence of the majority of the D-genome chromosomes (all absent in Carman), particularly of chromosome 1D, in the background of the triticales. This could be implicated in the definition of dough strength in triticale.

Another interesting observation concerns the differences in the HMW subunit composition among the triticale samples. The presence of one more HMW subunit in both 4T and Impala than in either 11T or Carman could have contributed to the better mixing strength of the former relative to that of the latter pair. Moreover, the extra HMW subunit of Impala was the largest among those of the triticales and the soft wheat Fredrick. Its mobility was similar to that of the largest HMW subunit of Marquis. Both, Impala and Marquis showed the best dough strength among all the samples studied. Considering that the presence or absence of certain HMW glutenin subunits appear

to be related to differences in baking potential among several wheat cultivars (Bournof and Bouriquet 1980, Payne et al. 1979, 1981), it is possible that the presence of the largest HMW subunit in both Impala and Marquis could be related to their superior gluten quality.

At this point the possibilities noted in the above paragraph concerning the probable influence on dough strength of the extra HMW subunits of 4T and Impala can be taken only as a speculation that warrant the further study of this aspect. If this speculation was true, then the extra HMW subunit in 4T and Impala could have been coded for by genes of any of the 1A, 1B, or 1D chromosomes that could have been involved in translocations with the 1R rye chromosome. May and Apples (1980) found translocations between wheat (B-genome) and rye chromosomes in wheat x rye crosses, and Lukaszewski and Gustafson (1983) found translocations between wheat (including A-, B-, and D-genomes) and rye homoeologous and non-homoeologous chromosmes in triticale x wheat crosses.

VI. CONTRIBUTIONS TO KNOWLEDGE

The results of this study contributed to knowledge as follows:

1. Sheeting rolls were found to be effective in developing bread doughs from triticale and wheat flours of diverse strength.
2. The combination of dough development by sheeting rolls and a no-time baking formula and regime resulted in the no-time/sheeting procedure. This proved to be an effective tool in investigating the contribution of gluten proteins to the baking potential of triticale and wheat flours.
3. Gluten protein as percent of flour protein varied widely (from 50-70%) among the triticales of this study. The triticales had in general lower gluten protein in flour protein than the wheat flours examined.
4. Gluten quality, as judged by its influence in the mixing and baking characteristics of reconstituted flours, also varied widely among triticales. The gluten quality among triticales went from inferior to similar to that found in good quality bread wheats.
5. The well accepted relationship between mixing strength and the proportion of glutenin-like protein in wheat flours was found to exist in triticale flours.

6. Addition of highly insoluble (residue) and low molecular weight, gliadin-rich (pH 5.8-soluble fraction), gluten fractions from any of the triticale and wheat samples decreased the loaf volume of the base flour. In contrast, addition of the gluten fraction which included both glutenin-like and gliadin-like protein (pH 5.8-insoluble fraction) increased the loaf volume of the base flour.
7. The pH 5.8-insoluble protein was more effective in increasing the loaf volume of the base flour than either whole reconstituted gluten or a combination of pH 5.8-insoluble + residue protein. This along with the large contribution of the pH 5.8-insoluble protein to the baking performance of synthetic flours, indicated that the control of the baking potential of the triticale flours resided, as has been found previously for wheat flours, in the combined effect of glutenin-like and gliadin-like proteins.
8. The contribution of the pH 5.8-insoluble protein to the definition of the baking potential of the triticale and wheat flours did not depend on an optimum glutenin-like/gliadin-like ratio. Therefore, the factors responsible for the differences in baking quality among the triticales tested seemed to reside in the composition of both the glutenin-like and gliadin-like proteins. These factors could not be identified from the SDS-PAGE analysis of these proteins.

9. Electrophoretic analysis (SDS-PAGE) showed that pH 5.8-soluble fraction from the triticales had some nonaggregated protein components with molecular weights of approximately 44,500, 52,000 and 76,000, which were absent in the pH 5.8-soluble protein from the wheats. These three protein components were apparently cereal specific and could have been inherited by the triticales from their rye parents.
10. The high molecular weight (HMW) subunit composition of the glutenin-like protein varied among triticales and between wheats. The smaller number of HMW subunits in the triticales relative to that of the wheats could be due to the absence of the majority of the D-genome chromosomes (all absent in Carman), particularly of chromosome 1D, which is known to contribute with at least 2 HMW protein subunits in bread wheats.

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