EFFECTS OF DOUGH MIXING AND RELAXATION ON THE PROTEIN SOLUBILITY AND COMPOSITION OF TWO DIVERSE BREAD WHEATS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

M. Theresa Almonte

In Partial Fulfilment of the

Requirements for the Degree

of

Master of Science

Department of Food Science

May 1998

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ACKNOWLEDGEMENTS

I would like to express my thanks and appreciation to Dr. H. D. Sapirstein, for his scientific guidance and patience during the course of this research and for his constructive criticism in the preparation of this thesis.To Dr. R. Holley for his support and encouragement, to Dr. W. Bushuk, for being such an inspiration to many young cereal chemist, and to Dr. Watts for being in my committee.

Thanks are extended to Randy Roller, Wayne Johnson and Heather Delorme, for their technical assistance and for their friendship. I also would like to thank Rick Zillman of the baking and milling laboratory, Plant Science Department, for his technical assistance earlier on in the project.

For their support, appreciation goes out to my friends, colleagues and the staff of Food Science and FDC. To the "core" (Lisa, Andrea, Dale, Rick, and Uzor), thank you for the laughter, the memories, and most of all, for your friendship. Lisa and Jim, thank you for knowing when to avoid mentioning "it". Kenneth, thank you for all the "fun" times. To Cathe and Gerry, thank you for believing in me. I am grateful to Suroj for speaking her mind at the most appropiate times. Wiolette, the striking concert of thunder and lightning to the tune of Bethoveen will always bring a smile to my face. Ian, than you for your eternal optimism and encouraging words. Thank you all for seeing me through this phase of my life and keeping me reasonably sane. Finally, to my family (Ma, Pa, Nick, Mike, Antonio, Cathleen, Michelle, and Khayla), thank you for your silent faith in me.

ABSTRACT

The nature of the changes in composition and distribution of wheat flour proteins during dough mixing and dough relaxation was investigated. Two hard red spring wheats with contrasting dough mixing strengths were used, viz. Glenlea and Katepwa. Glenlea and Katepwa are cultivars of the Canada Western Extra Strong Red Spring and Canada Western Red Spring wheat classes, respectively. Doughs were mixed for various times (1 min, peak dough development time, 15 min and 30 min) in a GRL-200 mixer, and were subsequently divided into two equal portions. One piece was relaxed for 45 min and the other represented the control for a particular mixing stage and no relaxation time. Proteins were fractionated according to the Orth and O'Brien (1976) method of direct extraction of flour with 0.05 M acetic acid, and the modified Osborne procedure (Chen and Bushuk 1970). Quantitative protein distributions among the resulting fractions were determined. The protein compositions of the different fractions were analyzed by sodium dodecyl sulphate polyacrylamide gel electrophoresis (pH 8.3) (SDS-PAGE) under reducing and non-reducing conditions, two-step one dimensional SDS-PAGE (pH 8.3), and polyacrylamide gel electrophoresis (pH 3.1) (A-PAGE).

Direct extraction of flour with acetic acid showed that Glenlea contained less acetic acid-soluble (AAS) protein than Katepwa. For both cultivars, AAS protein increased progressively with increasing mixing time. Mixing Katepwa dough beyond 15 min did not extract more AAS protein, whereas significantly more AAS proteins was extracted for Glenlea. Glenlea and Katepwa were comparable in the effects of mixing on the acetic acid-insoluble (AAI) protein fraction; AAI protein content decreased with increasing mixing time.

Dough mixing had no significant effect on the amounts of the Osborne salt- and water-soluble protein fractions of Katepwa. For Glenlea, the amount of salt-soluble protein decreased

significantly (from 3.4% to 1.9% of flour protein) only upon initial dough mixing to 1 min. As was found for Katepwa, dough mixing had no significant effect on the water-soluble protein of Glenlea. Intercultivar differences in the amount of salt-soluble protein were not found for flour or any mixing treatment. However, Glenlea flour contained significantly less Osborne water-soluble protein compared to that in Katepwa flour. This intercultivar difference persisted in the undermixed, peak dough development (PDD) and overmixed samples. It was noteworthy that the water-soluble protein fractions of both cultivars contained significant quantities of gliadin proteins. This indicates that the modified Osborne procedure does not prevent the solubilization of gliadin.

In contrast to similarity of dough mixing effects on the Osborne salt- and water-soluble protein fractions of Glenlea and Katepwa flour, there were substantial and significant intercultivar differences, caused by dough mixing, in the pattern and amount of Osborne ethanol-soluble (ES) and Osborne AAS protein fractions.

Mixing of Katepwa dough significantly affected the proportion of Osborne ES protein in an unexpected way; the amount of ES protein decreased (from 36% to 25%) when the Katepwa dough was mixed for 1 min and significantly increased (back to 36%) with further mixing up to 15 min. Mixing beyond this time did not result in the extraction of more ES protein. This overall trend was not observed for Glenlea. It was noteworthy that there was essentially no change in Glenlea ES protein from flour through to doughs mixed for 30 min. Because of the complex effect of dough mixing on Katepwa ES protein, compared to Glenlea, Katepwa had a significantly lower amount of ES protein at 1 min mixing, but a significantly higher amount of ES protein at 15 and 30 min of mixing.

In contrast to the pattern of variation that was found for the Katepwa Osborne ES protein fraction with mixing, a significant increase in Katepwa AAS protein was observed upon 1 min of mixing, with no significant change thereafter. The effect of dough mixing on Glenlea Osborne AAS

protein was also different to that obtained for Glenlea ES protein, as well as being different from results obtained for Katepwa AAS protein. While there was no significant difference between Glenlea flour and dough mixed for 1 min, mixing Glenlea dough up to 30 min caused a steady and significant increase in the amount of Osborne AAS proteins. Katepwa had significantly more Osborne AAS protein than Glenlea in flour and undermixed samples. This difference was reduced to non-significance at 15 min of mixing.

The Osborne AAI protein fractions of both Glenlea and Katepwa showed similar patterns of variation with increasing mixing time. The amounts of AAI protein increased after 1 min of mixing and decreased with further mixing up to 15 min. Although the 3.6% increase (cf. flour) in Glenlea AAI protein at 1 min of mixing was not significant ($p < 0.05$), the increase is most likely real as the difference was significant at $p < 0.10$ compared to AAI protein of dough mixed to 15 and 30 min. Like the Osborne AAS protein fractions, cultivar differences were found in the AAI fractions of the flour and undermixed samples. The stronger Glenlea flour contained more AAI protein in the flour. However, after 1 min of dough mixing, Katepwa had more AAI protein than Glenlea.

The quantitative protein fractionation results for Katepwa flour and dough samples indicate that a significant effect on gliadin proteins (and/or possibly ethanol soluble glutenin) occurs upon the initial transformation of flour into dough at 1 min of mixing; gliadin proteins (nominally defined as the ES fraction) and/or glutenin (soluble in 70% ethanol) appear to become partially insoluble or unextractable. This would account for the decrease in ES protein and the concomitant increase in AAS and AAI protein at 1 min of mixing. Because the latter two fractions are mainly composed of glutenin protein, it is plausible that aggregation of gliadins with glutenin is the underlying reason for the decrease in extractability of gliadins upon initial dough mixing. In this scenario, further mixing would be expected to cause a disaggregation of gliadins and glutenin, resulting in an increase in ES protein solubility, as was observed for Katepwa. The absence of a similar pattern

of variation with Glenlea flour and mixed dough, especially the absence of any effects on the ES protein fraction, suggests that no similar aggregation of gliadin and glutenin proteins occurs. For Glenlea, dough mixing causes a gradual disaggregation of polymeric glutenin evidenced by the significant decrease and increase in Glenlea Osborne AAI and AAS protein, respectively. Electrophoresis results, abstracted below, contained evidence supporting this gliadin/glutenin interaction hypothesis.

SDS-PAGE of direct extracts of flour and dough with 0.05 M acetic acid showed dough mixing related effects only for Glenlea AAS protein and for AAI protein of both cultivar samples. SDS-PAGE of unreduced AAS protein extracts of Glenlea samples showed an increase in band staining and streaking in the high molecular weight (HMW) region of the electrophoresis gels. This streaking is caused by the presence of polymeric protein which cannot be resolved into discrete bands under non-reducing conditions. SDS-PAGE of reduced AAS protein of Glenlea showed an increase in HMW glutenin subunits (HMW-GS) from 1 min of mixing to PDD. SDS-PAGE of unreduced AAI protein samples of Glenlea showed a clear decrease in band staining and streaking in the HMW gel region in fractions isolated from dough mixed for up to 30 min of mixing. For Katepwa AAI protein samples, similar SDS-PAGE results were observed for doughs mixed to 15 min. For both cultivars, these results are consistent with an increase in the solubility of glutenin which presumably arises due to de-polymerization and/or disaggregation of polymeric glutenin during dough mixing.

Similar to the quantitative protein fractionation results, electrophoresis of the Osborne water-extractable protein fraction of both Glenlea and Katepwa showed no significant effects due to dough mixing. Interestingly, A-PAGE results of this protein fraction showed the presence of considerable amounts of gliadin proteins in flour and mixed doughs. This result conflicts with the widely held belief that the modified Osborne fractionation procedure, due to the use of 0.5 M NaCl

solution for the initial extraction step, prevents the subsequent extraction of gliadin proteins in the water-extractable fraction.

Electrophoresis of Osborne salt soluble protein showed some effects related to dough mixing. The unreduced salt-soluble protein fractions of both cultivars, and especially Glenlea, showed triplet band (triticin) proteins and substantial streaking in the HMW region of the gel which decreased in staining intensities as mixing time increased. It was noteworthy that SDS-PAGE of reduced salt-soluble showed the presence of a few faint bands in the HMW-GS region of the electrophoregram. These bands appeared to be x-type HMW-GS.

Electrophoresis of Osborne ES protein did not show any clear effects related to dough mixing. This was to be expected as the protein concentrations of the ES fraction did not vary significantly with mixing. An interesting finding was that SDS-PAGE of reduced ES slot protein (polymeric ES protein) of Katepwa flour and doughs contained considerably more HMW-GS and LMW-GS protein than Glenlea. In general, slot protein of the various Osborne fractions of Katepwa flour and doughs contained considerably more HMW-GS and LMW-GS protein than Glenlea.

Some dough mixing related effects were observed in electrophoretic analysis of Osborne AAS protein. In agreement with the quantitative AAS protein fraction results for Glenlea dough samples, SDS-PAGE of unreduced AAS protein showed a progressive increase in streaking in the HMW gel region as mixing time increased. This indicates an increase in the solubility of Glenlea glutenin with increasing dough mixing, which is likely due to polymer disaggregation. As well, a small but noticeable increase in the staining intensity of some predominant gliadin bands was also observed for doughs mixed from 1 to 30 min. Similar results were not obtained for Katepwa unreduced AAS protein analyzed by SDS-PAGE; the staining intensity of gliadin bands appeared to peak at 1 to 3.5 min of mixing, after which a substantial decrease in gliadin band intensity was observed.

SDS-PAGE of unreduced Osborne AAI protein provided results that were consistent with the

quantitative results obtained for this protein fraction, as well as gliadin/glutenin interaction. For Katepwa dough mixed to 1 min, compared to corresponding results for Katepwa flour, electrophoregrams showed a substantial increase in the concentration of all gliadin bands, as well as triticin protein in the HMW region of the gel. The staining intensity of all of these protein components decreased progressively with increasing mixing up to 15 min. A-PAGE of gliadins in the AAI protein fraction of Katepwa also showed a similar trend. Compared to the Katepwa results, SDS-PAGE of unreduced AAI protein of Glenlea, showed a similar, but less pronounced, increase in gliadin band concentration upon flour mixing to 1 min protein, and a comparable decrease in triticin protein with increasing dough mixing. For Glenlea AAI protein, SDS-PAGE under reducing conditions showed a noticeable decrease in the concentration of HMW-GS with increasing dough mixing time. No similar result was obtained for Katepwa reduced AAI protein analyzed by SDS-PAGE.

All of the above results taken together indicate that there are considerable differences between Katepwa and Glenlea in the response of their respective endosperm proteins to dough mixing. Differences which occur when flour is initially hydrated (1 min of mixing) appear to be critical to the resulting pattern of variation in protein composition caused by dough mixing. For Katepwa, a moderate mixing strength flour, a significant proportion of gliadin proteins became unextractable when flour was hydrated. With further dough mixing, gliadin extractability increased. No similar result was found in the case of the extra-strong Glenlea flour. For Katepwa, aggregation of gliadin and glutenin during flour hydration is a plausible explanation for this result. Such an aggregation event, which would tend to dilute or plasticize the polymeric glutenin protein, would also explain the lower dough mixing requirements of Katepwa flour compared to that of Glenlea. According to this hypothesis, dough mixing requirements are inversely related to the quantity of gliadin proteins aggregating with glutenin during the earliest stages of dough development. The degree of

aggregation may, in turn, be inversely related to the molecular size of glutenin. This would also explain why Glenlea, which presumably has glutenin of very large average molecular size and relatively low specific surface area, showed no similar gliadin aggregation/disaggregation effects with dough mixing.

In contrast to the many significant effects of dough mixing, dough relaxation up to 45 min showed no clear systematic effects, for either Glenlea and Katepwa, on the solubility of the different protein fractions that were studied or their electrophoretic compositions.

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LIST OF ABBREVIATIONS

Abbreviation	Definition
AAI	Acetic acid insoluble
AAS	Acetic acid soluble
A-PAGE	acidic polyacrylamide gel electrophoresis
AUC	Acetic acid (0.1M)-urea-(3M)-cetyltrimethyl ammonium bromide (0.01M)
BU	Brabender units
CV	coefficient of variation
CWES	Canada Western Extra Strong
CWRS	Canada Western Red Spring
DDT	dough development time
ES	Ethanol-soluble
Gl	Glenlea
G1	Glenlea, undermixed -1 min mixing
G2	Glenlea, peak dough development - 15 min mixing
G4	Glenlea, overmixed, 30 min mixing
GRL	Grain Research Laboratory
HRS	hard red spring
HMW-GS	high molecular weight glutenin subunit
HPLC	high performance liquid chromatography
kD	kiloDalton
Kp	Katepwa
K1	Katepwa, undermixed - 1 min mixing
K2	Katepwa, peak dough development - 3 min mixing
K3	Katepwa, 15 min mixing
K4	Katepwa, overmixed, 30 min mixing
LMW-GS	low molecular weight glutenin subunit
MTI	mixing tolerance index
Mr	relative molecular mass
MU	mixograph units
NIR	near infrared reflectance
NEMI	N-ethylmaleimide
PAGE	polyacrylamide gel electrophoresis
PDD	peak dough development
PSI	particle size index
RH	relative humidity
SDS-PAGE	sodium dodecyl sulfate-PAGE

I. INTRODUCTION

Dough mixing is the initial stage in the breadmaking process, and is widely considered to be the most critical step in the transformation of flour into bread. At this stage, the dough is developed into a continuous structure which has the proper characteristics of plasticity, elasticity, and viscosity for optimum quality of the final product (Hoseney 1985).

Information on the behaviour of dough during mixing is fundamental to the understanding of the dough development process and the breadmaking potential of wheat flour. Studies of the factors responsible for varietal differences in the physical properties of wheat flours and doughs have focused on the protein component. Protein quantity and quality provide measures of the potential of flour in relation to its end use. Protein quantity is related to the amount of total nitrogen in the flour and can be easily determined and precisely defined. It is widely believed that protein quality is related to the concentration, composition, and molecular size distribution of the two major wheat protein fractions, gliadin and glutenin; it is by nature, a more subtle and complex attribute and is accordingly less well-defined. Protein quality is related to the physicochemical characteristics of the protein, more specifically, the gluten-forming component (Bushuk 1982). Finney and Barmore (1948) convincingly showed that wheat gluten proteins are primarily responsible for the differences in loaf volume between wheat flours.

Flour proteins can be routinely differentiated into more or less distinct classes based on differences in solubility. Flour proteins can be classified as water/salt-soluble proteins (mainly albumins and globulins) and water/salt-insoluble proteins (gliadin and glutenin) or gluten proteins (MacRitchie 1984). One of the most widely used classification systems for wheat proteins is the modified Osborne procedure (Chen and Bushuk 1970) which involves sequential fractionation of proteins into five classes: (1) water-soluble (albumins); (2) salt-soluble (globulins); (3) alcohol-

soluble (gliadins); (4) acetic acid-soluble (glutenins); and (5) insoluble residue (high molecular weight (HMW) glutenins).

In dough mixing and baking, proteins, particularly the gluten proteins (gliadins and glutenins) are likely functional as a mixture, reflecting in part, their distinct functionality, as well as their interaction properties. To explain functionality for breadmaking on a molecular basis, comprehensive qualitative and quantitative information on the protein composition is required. Few studies on protein fractions have focused on all five classical Osborne protein fractions and the effects of dough mixing and relaxation.

The hypothesis that was tested in this thesis was the following: intercultivar differences exist in the response of the different protein constituents in endosperm to the effects of dough mixing and relaxation. The response of the different flour proteins to mixing was investigated in two ways: 1) quantitatively, by direct extraction of protein with dilute acetic acid, and by determining the distribution of protein among the five Osborne classes, and 2) qualitatively by electrophoretic analysis of the various protein fractions. The experimental plan that was adopted was to use two cultivars of hard red spring wheat of contrasting dough mixing strength, Glenlea and Katepwa. Glenlea is a cultivar of the Canada Western Extra Strong (CWES) red spring wheat class, and is known to possess very strong dough mixing properties for breadmaking. Katepwa is a cultivar of the Canada Western Red Spring (CWRS) wheat class and possesses moderately strong dough characteristics and optimum quality for a wide range of breadmaking processes.

Although much research has been carried out on wheat proteins and their relationships to dough mixing and baking, a considerable amount of information on the molecular and functional properties of the proteins remains elusive. The search by cereal scientists for the fundamental reasons for the intercultivar variation in wheat functional properties continues. To this end, it was a goal of this thesis research to obtain additional new information on the nature of the solubility

and compositional changes that occur in wheat flour proteins during dough mixing.

II. LITERATURE REVIEW

A. Introduction

There is ample evidence that physicochemical changes occur in the protein component of dough during mixing. Some of the technological parameters that are related to dough mixing characteristics include dough extensibility, development time, and mixing tolerance (Mecham 1964, Tsen 1967, Tanaka and Bushuk 1973a,b,c, Hoseney 1985, Paredes-Lopez and Bushuk 1983, Wrigley 1991, Sievert et al 1991a,b). Taken together, these parameters contribute to characterizing the overall dough strength of a flour.

Wheat flour dough possesses unique rheological properties that reflect the viscosity and elasticity of the gluten protein phase. The functional properties of dough during mixing are a reflection of the properties of the two main gluten protein constituents, the gliadins and glutenins, each contributing to the viscous and elastic properties of dough, respectively. Dough functionality depends on the combination of a number of factors: protein content, protein composition, the molecular properties of the protein (average molecular size and size distribution), and interactions with other proteins and non-protein constituents (Bushuk and MacRitchie 1989). Protein fractionation has contributed to a better understanding of the relations between protein composition and functional properties of flour for breadmaking, but much remains to be learned.

The focus of this thesis is on the effects of dough mixing and relaxation on the protein solubility and composition of bread wheat. Accordingly, the following literature review deals with (i) the relationships between wheat proteins and dough mixing, (ii) the extraction and fractionation of wheat endosperm proteins, and (iii) the effects of structural relaxation on protein composition and distribution.

B. The Relationship Between Wheat Proteins and Dough Mixing

The collective properties of the constituents of wheat flour, i.e.protein, starch, non-starch polysaccharides, lipids, and enzymes dictate its suitability for breadmaking. Of the major constituents present in flour, the proteins contribute only about 15% of the dry matter of flour, the rest being starch, lipids, and minor constituents (Bushuk 1982).

Numerous excellent reviews on flour proteins and their relation to dough mixing behaviour and breadmaking quality have been published (Simmonds and Orth 1973, Kasarda et al 1976, Shewry and Miflin 1985, Pomeranz 1987, Wrigley and Bietz 1988, Bloksma and Bushuk 1988, MacRitchie 1992). This section will focus on the non-storage proteins (albumins and globulins), storage proteins (gliadins and glutenins), dough mixing effects, disaggregation of glutenin during mixing, and lipid-protein interactions.

1. Non-storage Protein

Non-storage proteins are those proteins of the wheat kernel that are often called soluble, cytoplasmic or non-gluten proteins. They can be found in the embryo, aleurone layers and in the endosperm and constitute approximately 15-20% of the total proteins in the caryopsis (Wrigley and Bietz 1988, Bushuk 1993).

Non-storage proteins are mainly monomeric, i.e. single chain polypeptides, of relatively low molecular weight (<30 kDa) with specific metabolic, regulatory and/or protective functions (Porceddu et al 1983, Shewry and Miflin 1985, Bushuk 1993). Albumins and globulins are classified as non-storage proteins or low molecular weight (LMW) proteins based on their solubility properties. However, there have been reports of high molecular weight (HMW) albumins (70 kDa) (Gupta et al 1991) and globulins which have the ability to form disulphide-linked

aggregates (Singh and Shepherd 1985). This relatively new-found class of aggregating proteins occurs widely among hexaploid wheat cultivars. They were originally named triplet band proteins and more recently "triticins" by Singh and Shepherd (1984 and 1985), and appear to be affected by dough mixing (Sievert et al 1991b). Based on the nomenclature of Osborne (1907), albumins are water-soluble (Ewart 1969), while globulins are soluble in dilute salt solutions (e.g. 0.5 M NaCl), but are insoluble in water and in high salt concentrations. Albumins typically make up about 10% of total wheat protein and are usually present in greater amounts in wheat than globulins (Chen and Bushuk 1970, Wrigley and Bietz 1988).

Mullen and Smith (1965) and Smith and Mullen (1965a,b) described the effects of mixing on flour proteins using short and long-mixing flours optimally mixed in a farinograph. They found that the amounts of salt-soluble proteins (albumins and globulins) extracted from the two flours were similar and had little relationship to mixing characteristics. The salt-insoluble fraction contained about 90% of the total protein in the flours and was the fraction that was suggested to contain the factor responsible for major differences in mixing properties of the flours. However, even when the salt-soluble proteins were extracted, the remaining fractions had the same mixing properties as the original flours. Orth and Bushuk (1972) later confirmed this when they reported that quantitative and qualitative variations in the Osborne water and salt soluble proteins of 26 wheat varieties was not related to baking quality. Tanaka and Bushuk (1973a) also found that the amounts of Osborne water and salt soluble proteins were not affected by dough mixing.

2. Storage Protein

Storage proteins are those proteins of the wheat kernel that are present in protein bodies deposited in the starchy endosperm, and function in the seed primarily as a nitrogen store (Shewry et al 1986, Miflin et al 1983). Storage proteins comprise most of the protein in wheat endosperm

(over 80% of the flour protein) and are made up of gliadins and glutenins (Bushuk 1993). Gliadins are classified by the Osborne (1907) system as alcohol-soluble (e.g. 70% ethanol), whereas glutenins are defined by their solubility in dilute acids (e.g. 0.05 M acetic acid) or dilute basic solutions. Storage proteins of wheat, when mixed with water, form a cohesive mass called gluten. Gluten is fundamental to the baking quality of a flour because of its viscoelastic properties. Gluten was first described by Beccari in 1745 (Bailey 1941) as the visco-elastic mass that remains after thoroughly washing out the starch from a dough.

The gliadins form a heterogeneous group of polypeptides (about 40% of the flour protein) with molecular weights ranging from approximately 30 kDa to 75 kDa (Shewry and Tatham 1997) and an average molecular weight of 35 kDa (Graveland et al 1982). Gliadins are relatively small with compact structures and are considered to be the proteins that confer viscous flow and contribute to the extensibility and stickiness of doughs (MacRitchie 1984, Bushuk and MacRitchie 1989). The gliadins are single-chain polypeptides (monomers) that associate by hydrogen bonding and hydrophobic interactions (Woychick et al 1961, Nielsen et al 1968, Wall and Beckwith 1969, Graveland et al 1982). Based on their mobilities at pH 3 in starch gel electrophoresis, Woychick et al (1961) classified gliadins into four groups, α-, β-, γ- and ω-gliadins based on decreasing order of mobility and increasing molecular size. Bushuk and Sapirstein (1991) classified these groups by A-PAGE. Nielson et al (1968) found that the intrinsic viscosity of gliadin proteins was essentially unaffected by disulphide bond cleaving agents. This suggested that most, if not all, of the cysteine residues of gliadins, are involved in intramolecular disulphide bonds. This was later confirmed by Graveland et al (1985) using SDS-PAGE electrophoresis (without reduction). The polymeric proteins referred to as HMW-gliadins (Bietz and Wall 1980), or aggregated gliadins (Shewry et al 1983) containing inter-molecular disulphide bonds have been shown to be LMW-glutenin (Adrian et al 1987).

Mattern and Sandstedt (1957) studied the influence of the water-soluble constituents of wheat flour on its mixing and baking characteristics. Direct water extraction of the flour revealed that the water-soluble fraction was the principal factor responsible for determining the mixing requirement of wheat flour. When this fraction was extracted, the mixing time of the flour increased dramatically (from 1.5 min for the control flour to 18 min for the extracted flour), and more tolerance to overmixing was exhibited. A similar effect was shown by Shuey and Gilles (1973). Mattern and Sandstedt (1957) also showed that reconstitution of the water-soluble fraction to the same flour reversed the tolerance to overmixing (i.e. mixing time was shortened to 4 min). Examination of the extract revealed that it had a high amide-N content, which suggested that it consisted mainly of gliadin proteins. In addition, Mattern and Sandstedt (1957) noted a fundamental difference between water extracts of short and long mixing flours. They showed that extracts from the short-mixing flour had more soluble proteins and a higher amide-N value than extracts from long-mixing flours. Using a different experimental approach, Sullivan et al (1963) and Tkachuk and Hlynka (1963) further emphasized the importance of the water-soluble proteins in mixing. They both showed differences in the reactivity of the -SH groups in flour and found that about one half of the -SH groups were quite labile and more readily oxidized than the other half. The most labile -SH groups appeared to be water and salt-soluble and reacted readily with N-ethylmeleimide (NEMI), a sulphydryl-blocking agent. Due to the large effect of NEMI upon mixing, it was suggested that the soluble protein is important in determining dough mixing properties in the farinograph.

Mullen and Smith (1965a) and Smith and Mullen (1965b,c) found that added water-soluble gliadins markedly decreased the mixing time requirements of flour in a farinograph. Perhaps the water-soluble proteins referred to by Mattern and Sandstedt (1957), Sullivan et al (1963), and Tkachuk and Hlynka (1963), as non-storage protein, were in fact, gliadins soluble in water. Finally,

Yoshida and Danno (1989) studied the effects of the water-soluble fraction and the dough mixing procedure on the viscoelasticity of wheat gluten. They showed that water-soluble components, particularly gliadin, were incorporated into the gluten mass during dough mixing and these proteins led to the formation of a viscoelastic mass. Conversely, the gluten prepared by water extraction was low in gliadin content, and this resulted in the formation of an elastic mass with low extensibility. Moreover, the reconstituted gluten, with gluten obtained by water extraction, was elastic and less extensible than the reconstituted gluten obtained by dough mixing. This was observed despite using the same ratio of gliadin and glutenin during the reconstitution process. From these results, Yoshida and Danno (1989) suggested that gliadin must be well mixed with glutenin to form a homogeneous gluten network for the formation of a viscoelastic mass. They also proposed that gliadin could play an important role in the breakdown of dough during mixing.

It is worth noting however, that although major variation exists in the gliadin composition among different wheat cultivars, these differences have not been strongly related to intercultivar differences in breadmaking quality. In contrast, the relationships between glutenin proteins and breadmaking quality have been emphasized in many reviews (MacRitchie 1992, Bushuk 1993, Schofield 1994, and references cited within). Glutenin constitutes about 40% of the total flour protein and is made up of at least 22 polypeptide subunits covalently linked by disulphide bonds (Schofield 1994). Glutenin protein has very high average molecular weight (at least 1- 10 million kDa) and is highly polydisperse (Wrigley and Bietz 1988, Ng et al 1989). Based on SDS-PAGE, glutenin subunits (GS) have been divided into two groups: HMW-GS (90-140 kDa) and LMW-GS (35-60 kDa) (Ng and Bushuk 1987). The LMW-GS overlap with gliadins on the basis of their relative mass by SDS-PAGE. A bread wheat variety may contain three to five HMW-GS and 15 or more LMW-GS components (Bushuk 1993).

Mullen and Smith (1965) and Smith and Mullen (1965a,b) found that a short-mixing flour

contained less acid-insoluble proteins and had more water-soluble proteins, when the salt-insoluble fraction was further fractionated to yield a water-soluble fraction (gliadins) and a protein-starch residue (primarily glutenins). Compared to the long-mixing flour, more than twice as much of the protein of the short-mixing flour, was soluble in water under the conditions used. They postulated that the observed differences were related to the protein-starch residue (primarily glutenins) and the amount and molecular weight distribution of the water-soluble fraction. The long-mixing flour contained more acid-insoluble proteins with possibly higher molecular weights than the short-mixing flour. On the basis of these observations, the acid-insoluble protein was likely glutenin.

It has been shown (Khan and Bushuk 1979, Graveland et al 1982) that the glutenins constitute the most important protein fraction for creating a continuous protein network in dough. This network may result from the H-bonding potential arising from the unusually large numbers of glutamine side chains and/or the potential for hydrophobic interactions between the many non-polar amino acids present in glutenin (Bushuk 1985 and references cited therein). It is these aggregates that appear to be responsible for the unique strength exhibited by wheat flour doughs. Areca and Yonezawa (1975) found that gluten from flours of better baking quality, aggregates better than flours of poorer baking quality. They concluded that the aggregation behaviour was mainly determined by the nature of glutenin.

Mecham and coworkers (1962, 1964, 1965) showed a significant change in protein solubility during mixing. There was a gradual increase in the amount of protein extracted by dilute acetic acid with mixing time. The rate of increase in extraction of these proteins was higher for flours with poor mixing stability and shorter mixing times than for flours with good mixing stability and longer mixing times. Also, dilute acetic acid extracted a higher percentage of the original protein from the weak flours than from strong flours (Mecham et al 1962, Mecham 1964, Smith and Mullen 1965a,b, Mullen and Smith 1965, Tsen 1967, Orth and Bushuk 1972, Paredes-Lopez and

Bushuk 1983). Tsen (1967) further established that both the percentage of total extractable protein (0.05 M acetic acid) and the protein content of the extract increased with dough mixing.

3. Dough Mixing and Protein Solubility

The mixing time of wheat flour is affected by the environmental conditions (soil, temperature, availability of nitrogen, etc.) that the wheat is grown in (Hoseney 1985, Preston et al 1992) and protein content (Finney and Barmore 1948) of the flour. However, the mixing phenomenon of wheat dough is very complex and is still not completely understood.

At the macroscopic level, the processes of dough mixing can include three stages: flour hydration, dough development, and overmixing (Hoseney 1985, MacRitchie 1986). In hydration, water is absorbed by the flour and cohesive dough mass begins to be formed. In this stage, there is excess amount of water in the system and the dough is quite fluid, showing relatively little resistance to extension. Upon more work input, the dough forms a cohesive mass with minimum mobility and maximum consistency. At this point, the dough has well-defined viscoelastic properties and has attained its optimal or peak development. Upon further mixing, dough consistency decreases and it becomes wet and sticky. In this overmixed condition, a dough usually produces a deleterious effect on the ultimate quality of the bread (Hoseney 1985, MacRitchie 1986).

At the molecular level, the time required to mix a dough to peak development (at a given mixing intensity), is widely held to be related to the properties of the polymeric protein and in particular the insoluble or the largest glutenin molecules present (Ewart 1968, Graveland et al 1985).

Ewart (1968, 1972, 1977) proposed the "Linear Glutenin Hypothesis" which attempts to explain what occurs when a wheat flour is mixed into a dough. The hypothesis takes into account

the effect of mixing on the ordering of glutenin molecules. It describes the glutenin molecules as polypeptide chains joined by disulphide (SS) bonds into linear concatenations of subunits that are linked in an ordered manner and with only a small amount of branching. Ewart's argument against the highly branched model of glutenin (Graveland et al 1985, 1994, 1996) was that a branched glutenin would not need development by mixing since it could not be oriented and, therefore, would only need thorough wetting or hydration to reach its maximum development. Graveland proposes a highly branched model in which HMW-GS are linked together by disulphide bonds in a head-to-tail fashion to form a linear backbone in which clusters of the LMW-GS are bound, also by disulphide bonds.

In contrast, the "Linear Glutenin Hypothesis" proposes that in an undisturbed flour system, the polypeptide chains are in their compact, folded, native conformation, and are randomly orientated. When the flour is mixed, the shearing force breaks the weak, non-covalent forces and the polymers begin to orientate themselves parallel to the shearing force. The molecules align so that there is substantial overlap along their lengths. Once alignment of the glutenin subunits occurs, the non-covalent forces act co-operatively to increase the strength and extensibility of the dough. Stress relaxation or viscous flow may depend primarily on slippage of the linear chains of glutenins, and partly on mechanical scission and S-S interchange. The S-S interchange may in fact control the average length of concatenations. Thus, in a resting dough, the S-S interchange plays a greater contribution to stress relaxation.

4. Increase of Solubility of Polymeric Proteins by Dough Mixing

The increase in solubility of glutenin with dough mixing is believed to be caused by a decrease in protein aggregate size. Aggregated proteins are defined as polypeptides physically associated by strong non-covalent interactions. A decrease in protein aggregate size can be caused by physical

breakdown of flour particles, disruption of hydrogen and hydrophobic bond interactions, salt linkages, and depolymerization of large glutenin molecules as a result of cleavage of disulphide bonds (Mecham et al 1962, Mecham 1964, Tsen 1967, Tanaka and Bushuk 1973a,b,c). These studies show that the increase in solubility of protein during dough mixing was due to the breakdown of the HMW polymeric glutenin proteins.

Mecham et al (1965) postulated that the increase in protein extractability by dilute acetic acid during dough mixing could be attributed mainly to the disaggregation of large-sized protein aggregates originally present in the flour. They proposed that mixing decreased the size of the aggregates, rendering the proteins more extractable and soluble in acetic acid. Their results imply that the disaggregation step could influence the rate of development of doughs because disaggregation leads to a decrease in the mixing stability of the dough. Later work of Tsen (1967) and Tanaka and Bushuk (1973b,c) supported this hypothesis.

Tsen (1967), using flour samples ranging in dough strength, studied the molecular size distribution of protein components previously solubilized by mixing. He specifically investigated the molecular weight distribution of glutenin and gliadin fractions by gel filtration chromatography. The study showed that as the dough was mixed, component I (MW > 150 kDa excluded from the gel) increased, whereas, the amounts of the other components in the extract (gliadins, albumins, globulins, and soluble non-protein fraction) did not change significantly with mixing time. The results suggested that the insoluble residue protein was converted to a smaller soluble protein aggregate size. The very weak flour (soft wheat) contained a higher proportion of component I than the strong flour (hard wheat). The increase in the amount of component I in weak flours, was greatest during the first few minutes of mixing. These differences suggest that the molecular structure of protein aggregates in weak flours is not as stable as that in strong flours, which may explain the effect of mixing. Also, the results suggest that the protein of a weak flour may have a

smaller overall aggregate size than the protein from a strong flour, which may also explain the difference in protein extractability. Tsen (1967) concluded that mixing produces some disaggregation based on the observation that molecular weight distribution of the other soluble components or gliadins were not affected. Tsen therefore postulated that the increase in protein extractability during mixing must result from disaggregation of large protein aggregates present in the nondispersable fraction.

Tanaka and Bushuk (1973a,b,c) investigated the mechanisms of disaggregation and/or depolymerization during dough mixing using a farinograph. They found that although there was an increase in protein extractability when flour was mixed, the elution profiles (Sephadex G-150) of the AUC (0.1 M acetic acid, 3 M urea, 0.01 M cetyltrimethylammonium bromide) of extracts from the control and overmixed doughs were not significantly different. Their results also suggested that the main effect of mixing was disaggregation of flour particles and insoluble gluten complex. When the dough was mixed extensively in air or in the presence of sulphydryl-blocking reagents such as iodate and NEMI, more glutenin was solubilized from the residue protein. These results suggested that depolymerization of rheologically important gluten proteins also occurred when doughs were overmixed. The similar effects of sulphydryl-blocking agents and cysteine, strongly suggested that disulphide bonds were involved in depolymerization. However, SDS-PAGE showed that dough mixing had no significant effects on the protein composition of reduced Osborne AAS and AAI proteins. They suggested that the difference in solubility between the Osborne AAS and AAI proteins, was due to a difference in molecular weight (i.e. the number of subunits held together by disulphide bonds).

Paredes-Lopez (1980) found that under non-reducing conditions, Osborne glutenin fraction extracted from doughs subjected to various mixing treatments, showed qualitative and quantitative changes by SDS-PAGE. The SDS-PAGE of the unreduced glutenins from undermixed samples

of Glenlea, showed the presence of protein bands (58 kDa and lower MW) that were not present in the fractions from the peak or overmixed dough samples. The reason for this was attributed to the fact that these protein components formed HMW aggregates with further dough development, and thus were unable to enter the gel under non-reducing conditions.

Sievert et al (1991a,b) optimized fractionation and electrophoretic conditions to investigate the acetic acid-insoluble proteins of wheat flour and dough under non-reducing and reducing conditions. Under non-reducing conditions, the SDS-PAGE patterns of the acetic acid residue fractions showed a tight cluster of relatively high molecular weight proteins, which the authors referred to as "triplet band" proteins or triticins (Singh and Shepherd 1984). These triplet proteins and neighbouring bands, collectively called triplet zone proteins, decreased in staining intensity as mixing time increased. The undermixed dough showed the most intense triplet zone proteins and level of background streaking. As mixing continued, the bands became fainter, especially after 15 and 30 minutes of dough mixing. Sievert et al (1991b) concluded that depolymerization and/or association of triplet zone proteins with other dough constituents was responsible for the observed decrease in band intensity. Based on related effects of iodate treatment of dough, they also proposed that triplet zone proteins may be involved in the disulphide/sulphydryl interchange reactions during dough mixing, The triplet band proteins may also be involved in the formation of larger protein aggregates. Singh and Shepherd (1985) showed that the subunits of the triplet band interact with the subunits of glutenin forming a large number of higher order protein aggregates. These results suggest that triplet proteins might also influence the functional properties of dough, although no direct evidence has yet been reported. Also, the band intensity of the HMW- and LMW-GS of the AAI protein decreased with mixing time. These observations are consistent with the explanation that the increased solubilization of proteins during mixing results from an increased extractability of glutenin (Tsen 1967, Tanaka and Bushuk 1973a,b, Danno and Hoseney 1982a,

Sievert et al 1991a,b).

Danno and Hoseney (1982a,b) used viscometry to validate the Tanaka and Bushuk (1973a,b,c) hypothesis that both disaggregation and depolymerization occur during dough mixing, and that depolymerization is more evident under conditions of extensive dough breakdown. Using 1% SDS as the solvent, they found that as protein concentration increased with mixing (up to 15 min), the relative viscosity of the extracted proteins also increased. About 72% of the total flour nitrogen was extracted from flour by extracting with 1% SDS for 2 hr at room temperature with gentle stirring. Nitrogen extraction increased to 95% when the same flour was overmixed in a mixograph.The proteins solubilized as a result of mixing had higher relative viscosities than proteins extracted from flours. However, there was a decrease in the relative viscosities of SDS extracts from overmixed doughs. When the extracts were treated with a reducing agent, the relative viscosities of the proteins extracted from the flour and dough decreased but were not significantly different. Danno and Hoseney (1982a) postulated that the extracted protein components contained intermolecular disulphide bonds which indicated that HMW glutenin was cleaved during dough mixing. They maintained (Danno and Hoseney 1982b) that the increase in extractability of protein during mixing was not related to a decrease in protein size (i.e. disaggregation) because solubility apparently increased before the size of proteins (glutenins) was reduced. Their results suggested that glutenin of HMW can be extracted after a short period of mixing. This was supported by gel filtration and SDS-PAGE studies on the molecular weight distribution of the SDS-soluble fraction (Danno and Hoseney, 1982b).

Paredes-Lopez and Bushuk (1983) found that relatively high energy was required to mix a Glenlea dough to its peak development in comparison with strong and medium strength flours. They showed that the main characteristic of mixing was that optimally developed doughs from strong flours contained more of the Osborne AAS glutenin proteins than their corresponding flours.

The amount of glutenin proteins also increased with flour strength. A dramatic increase in the amount of AAS glutenin proteins was observed when the Glenlea dough was overmixed. For the weak flour, the amount of AAS glutenin proteins remained relatively constant as the dough was mixed beyond its peak. Dough development produced only minor qualitative and quantitative changes in the SDS-PAGE patterns of non-reduced and reduced albumins, globulins and gliadins. The observed changes seemed to depend on the mixing strength of the flour, where the influence of mixing was greater for the stronger flour than for the weaker flour.

Weegels et al (1995, 1997) studied the changes in the composition of the glutenin subunits in glutenin macropolymer (GMP) of flours and doughs from cultivars of different mixing strength. They found that after mixing in a mixograph, the amount of GMP, and the amount of HMW- and LMW-GS in the GMP, decreased, although to different extents. They concluded that changes in protein composition during dough mixing indicate that specific reactions involving glutenin subunits occur during depolymerization of GMP and that the most readily extractable glutenin polymers from flour are rich in LMW-GS.

5. Lipid-Protein Interaction

The effects of dough mixing on protein solubility and composition may be in part, related to associated effects on the lipid component of flour. The addition of water to flour induces gluten proteins to bind certain flour lipids (Olcott and Mecham 1947, Bekes et al 1983a, Zawistowska et al 1984). Dough mixing modifies lipid binding, depending on the mixing action and the mixing environment. Lipids normally extractable from flour with nonpolar solvents become bound and lipid binding increases as dough development increases (Olcott and Mecham 1947, Chung et al 1978, Frazier 1983, Frazier and Daniels 1986).

Dough mixing affects lipid binding and distribution of the flour lipids in the protein fractions

(Chung and Tsen 1975, Frazier et al 1981, Frazier and Daniels 1986, Bushuk 1986), which may affect protein solubility and composition. Chung and Tsen (1975) found that more than half of flour free lipids became bound when dough was optimally mixed to its peak in a farinograph, and that mixing greatly increased the amount of lipids associated with the glutenin fraction. Beyond peak dough development, little effect on lipid binding and extractability was observed. They also reported that the nonpolar bound lipids were mainly found in the AAS fractions, and highly bound polar lipids were found in the acid insoluble starch-lipid-protein fraction. It has been reported that glutenins bind fifteen times as much lipid as the gliadins upon dough mixing (Frazier and Daniels 1986).

Zawistowska et al (1984) fractionated the proteins of five hard red spring (HRS) wheat cultivars, according to the Osborne procedure and found the fractions contained the following amounts of lipids: albumin/globulin, 8.01-9.14% ; gliadin, 11.5-13.5% ; and ASS glutenin, 7.25-8.49%. The A-PAGE of the gliadin fractions extracted from flour by 70% ethanol showed that the same HMW gliadin components (>68 kDa) and small amounts of LMW components, were involved in binding the lipids for the five cultivars. The intensity of some of the bands in the electrophoregram depended on whether lipids were present in the starting flour material. This latter observation was also reported by Bekes et al (1983a,b). Zawistowska et al (1984) also found that the lipid content of the Osborne AAI fraction was quite low with Glenlea having the highest amount of lipid content (0.47%).

Because the binding of lipid by glutenin depends on the physical action of mixing, it has been postulated that formation of glutenin-lipid complexes is directly involved in mechanical dough development (Bushuk 1986). Lipids (extracted along with the gliadin proteins by 70% aqueous ethanol solution) that can mediate the association of some gliadins to form aggregates of HMW, have been reported by Bekes et al (1983). Differences in A-PAGE and SDS-PAGE patterns of

gliadins with and without lipids indicate that in the presence of lipids, mainly galactolipids, gliadins, as well as trace amounts of albumin and globulin proteins, can form aggregates of higher apparent molecular weight.

Although many studies on protein-lipid interaction in dough and its effects on protein solubility and composition are highly variable and full of contradictions, most results suggest that there is an affinity between specific gluten proteins and specific lipids. It can be concluded from the literature that protein-lipid interaction can affect protein solubility and its composition during dough mixing.

C. Extraction and Fractionation of Proteins

The protein composition of wheat flour is very complex and contains many molecular species with different composition, structure, size, and size distribution (Bietz and Wall 1980, Graveland et al 1982, Ng and Bushuk 1989).

1. Modified Osborne Fractionation

The classical separation of proteins by Osborne (1907,1924) was based on sequential solubility of the proteins in different solvents. This solubility fractionation procedure remains a widely used classification system for wheat proteins. The sequential extraction of wheat flour and dough with 0.5 M NaCl, water, 70% ethanol, and 0.05 M acetic acid, fractionated wheat proteins into five solubility classes: (i) salt-soluble proteins (globulins); (ii) water-soluble proteins (albumins); (iii) ethanol-soluble proteins (gliadins); (iv) acetic acid-soluble proteins (glutenins); and (v) acetic acid-insoluble (or residue) proteins (HMW glutenins).

Chen and Bushuk (1970) modified the Osborne fractionation procedure by initially extracting

the flour with 0.5 M NaCl, to prevent gliadins from becoming soluble and thus contaminating the water and salt soluble protein extracts. Also, mild stirring with a magnetic stirrer at 4°C was adopted to avoid artifacts that might be produced by high shear stirring and any proteolytic enzyme degradation that might occur at ambient temperatures.

The modified Osborne procedure was employed by Orth and Bushuk (1972) to rank 26 wheat varieties according to their breadmaking potential. It was shown that loaf volume, for a group of flours varying widely in mixing strength, was inversely related to the amount of protein (6 to 27.4% of total flour protein) that was soluble in 0.05 M acetic acid solution. They showed a direct correlation between the amount of insoluble (15 to 36.5% of total flour protein) protein in 0.05M acetic acid solution and loaf volume. Orth and Bushuk (1972) also reported that the amounts of albumin, globulin, and gliadin proteins, were relatively constant in the set of wheat genotypes that were studied.

This sequential approach to extracting and fractionating wheat flour proteins into different protein classes is tedious and time consuming. Over the years, as protein fractionation methods have become more refined, it has become evident that each of the five solubility fractions are heterogeneous and that considerable amount of overlap exists between the classes, especially between the two major storage protein fractions, the gliadin and glutenin (Bushuk and Wrigley 1974, Fu and Sapirstein 1996a, Dupuis et al 1996). This potentially limits interpretation of relationships between particular protein fraction(s) to dough and baking properties. Despite these limitations, however, fractionation of the wheat proteins on the basis of solubility has retained its importance.

2. Direct Extraction Procedures

To avoid the formation of gluten prior to extraction, a more direct approach to isolate

polymeric glutenin is to first quantitatively extract the monomeric proteins of flour with non-reducing solvents such as dilute acetic acid (Jankiewicz and Pomeranz 1965, Tsen 1967, Orth and O'Brien 1976), dimethyl sulfoxide (Burnouf and Bietz 1989, Gupta and MacRitchie 1991), or 50% 1-propanol (Byers et al 1986, Kruger et al 1988, Singh et al 1991, Fu and Sapirstein 1996a, Sapirstein and Johnson 1996). This direct approach leaves a starchy residue containing the polymeric glutenin which can then be extracted under reduced condition.

a. Direct Extraction with Acetic Acid. Acetic acid has been used by many researchers to solubilize flour and dough proteins (Jankiewicz and Pomeranz 1965, Tsen 1967, Orth and O'Brien 1976, Paredes-Lopez and Bushuk 1983). Dilute acetic acid (0.01-0.05 M) is a milder and more useful solvent compared to alkaline and strongly acidic solvents which can cleave some covalent bonds, particularly disulphide bonds (Meredith and Wren 1966). Like dilute hydrochloric acid (0.01N) (Macritchie 1987), dilute acetic acid can also extract glutenin without significantly reducing its native functional properties. However, one major disadvantage is that these milder solvents fail to dissolve all of the residue or glutenin proteins (Mecham 1964).

Numerous papers have established the importance of the relationship between residue proteins that remain after extraction of wheat flour with non-reducing solvents, and functionality, particularly the mixing strength of different genotypes. Previous studies found an inverse relationship between the protein content of the acetic acid-insoluble residue and time of mixing (Mecham et al 1962, Mecham 1964, Smith and Mullen 1965a,b, Mullen and Smith 1965, Orth and Bushuk 1972, Tanaka and Bushuk 1973a,b, Orth and O'Brien 1976, Sievert et al, 1991a,b).

The incomplete solubility of wheat flour proteins in acetic acid was exploited by Orth and O'Brien (1976) who developed a test for dough strength in wheat. They found that the proportion of residue protein (percentage of total flour protein) remaining after direct extraction of flour with

dilute acetic acid was significantly correlated with dough strength. Paredes-Lopez and Bushuk (1983) also showed that the amount of Osborne AAI protein was inversely related to the energy input during dough mixing. They reported a decrease in the amount of residue protein with an increase in flour strength, at equivalent mixing treatment.

b. Direct Extraction with Propanol. Although propanol was not utilized in the present study, it is a very effective solvent to solubilize gluten proteins. Fu and Sapirstein (1996a,b) recently reported this protein isolation procedure using the differential solubility of proteins in different aqueous solutions of 1-propanol to fractionate monomeric and polymeric (native unreduced) glutenin proteins of wheat flour. This fractionation scheme produced four relatively distinct fractions: monomeric proteins (albumins, globulins and gliadins), soluble glutenin, insoluble glutenin, and residue protein. Using this fractionation procedure, Fu and Sapirstein (1996b) and Sapirstein and Johnson (1996) showed a very strong relationship between the amount of 50%- propanol insoluble glutenin and dough mixing requirements. Fu and Sapirstein (1996b) reported intercultivar variability in the soluble glutenin (9.6 - 19.4% of flour protein) and insoluble glutenin (12-28% of flour protein) proteins. The latter fraction consisted of essentially pure HMW- and LMW-GS. Their results showed that a relatively constant amount of protein (14-18%) remained in the final residue and that 57-70% of flour protein was comprised essentially of monomeric proteins and a substantial amount of polymeric glutenins (Fu and Sapirstein 1996b).

Fu and Sapirstein (1996a,b) also found that the insoluble glutenin was the only fraction that was positively correlated with breadmaking quality (dough mixing requirement and loaf volume). They found that Glenlea, the sample among the cultivars studied with the strongest dough mixing characteristics, had the highest ratio of insoluble to soluble glutenin, and the lowest concentration of monomeric and residue proteins. This same procedure was used by Dupuis et al (1996) to better

interpret and characterize the AAS and AII fractions of bread wheat and their relations to breadmaking quality.

The procedure of extracting flour with 50% 1-propanol, followed by solubilizing the unextractable glutenin in 50% propanol plus 1% dithiothreitol (DDT), was recently exploited by Sapirstein and Johnson (1996), who developed a relatively simple spectrophotometric procedure to measure the concentration of insoluble glutenin in flour. They found a very strong relationship between spectrophotometrically determined insoluble glutenin content and dough mixing requirements, for samples of Canadian wheats of diverse breadmaking potential.

D. Effects of Structural Relaxation of Mechanically Developed Doughs on Protein Compositions and Distributions

Most studies on the effect of mixing on dough protein solubility and composition have been based on doughs without any rest or relaxation treatment. That is, the test doughs are immediately frozen after mixing, lyophilized, and subsequently analyzed. Frazier et al (1975 and 1985) and Daniels and Frazier (1978) found that analyses of rested doughs could lead to very different conclusions regarding optimum development prediction.

The physical and/or textural properties of a freshly mixed flour dough are different from those of a rested dough. A freshly made dough is often sticky, relatively inelastic and more resistant to extension or deformation and has been said to be in an activated state (Hlynka 1955a,b). In a relaxed dough, resistance to deformation is known to increase. This could be due to better alignment of glutenin molecules which would permit more physical cross-links between them. However, since a dough is a dynamic system, subtle changes continue to take place for some time

after a dough has been worked.

Extensigraph studies have been done on the relaxation of internal stresses in flour doughs (Munz and Brabender 1940, Dempster et al 1947, Hlyanka 1955a,b). When a dough is left to rest for increasing times after mixing, it exhibits a decrease in resistance to extension and an increase in extensibility . The dough seems to slacken or relax. This is called structural relaxation and is a rheologically time-dependent property of wheat flour dough which has been documented for conventional, low work-input doughs only.

Using an extensigraph, Hlyanka (1955a,b) defined structural relaxation of dough as the stabilization of the extensigram dimensions of the dough properties with time. Structural relaxation occurs when the dough spontaneously returns to an equilibrium structure with fewer physical cross-links between glutenin molecules. Dempster et al (1952) suggested that the rate of relaxation is indicative of the rate at which the network structure returns from the partly oriented, highly cross-linked arrangement caused by working the dough, to an equilibrium arrangement. Thus, they postulated that the relaxation of internal stresses is determined by the extent of cross linking of the network structure.

Dempster et al (1952) studied the rates of relaxation of internal stresses in doughs using a Brabender Extensigraph. They found that the behaviour of flour dough is consistent with an internal network structure of flexible, long-chain protein molecules with some cross links between polar groups of adjacent molecules. The structure is dynamic so that change within the network readily occurs. Therefore, during resting after the shaping stage, the kinetic motions of the molecular segments will cause the network to return to the equilibrium state. It has been suggested that relaxation is analogous to the unwinding of the dough from its strained structure to a more stable structure (Hlynka, 1955a).

Launay and Bure (1974) studied the stress relaxation of doughs following shear (cone and

plate rheometer) deformation. They investigated the effects of such parameters as flour type, dough-water content, mixing time, presence of sulphite or NEMI, and dough temperature. They reported an increase in relaxation time with flour strength. An increase in water content caused an increase in the stress relaxation rates or a decrease in relaxation times. Mixing time in a farinograph (3 to 60 min) for different flours had very little influence on the relaxation curves. Doughs formulated with sulphite or NEMI showed a decrease in relaxation times. The authors speculated that the sulphydryl groups were not directly involved in the stress-relaxation phenomena. Finally, they showed that an increase in dough temperature produced an increase in the relaxation rate.

Recently, Weegels and coworkers (1994, 1996, 1997) studied the glutenin subunit composition of GMP of doughs during mixing and resting. GMP was defined as the glutenin that was unextractable in 1.5% SDS. They found that during mixing, depolymerisation of the GMP occurred and became partly extractable in SDS. This resulted in changes in subunit composition of the remaining GMP. During resting, repolymerisation or reassembly of the glutenin subunits took place. The HMW- and LMW-GS were incorporated to different levels and at different rates. Finally, the incorporation rates appeared to be dependent on the total amounts of glutenins and the molecular masses of the subunits. The GMP from dough of weak flours breaks down fast and reassembly was slow whereas it was the opposite for dough from strong flours (Graveland et al 1996).

E. SUMMARY

A review of the pertinent literature leaves the impression that the phenomenon of dough mixing has been extensively studied. However, a clear picture of mixing effects on the total complement of proteins in the endosperm is not yet known. Perhaps the only concensus, which

exists in the literature, on the effects of dough mixing on wheat flour proteins relates to glutenin.

Good evidence exists that glutenin's quantity and quality is mainly responsible for intrinsic differences in dough mixing requirements among different wheats. Good evidence also exists that insoluble or large polymeric glutenin disaggregates and/or depolymerizes during dough mixing; accordingly, glutenin becomes smaller on average, and therefore more soluble with dough mixing. Some workers have provided evidence that dough mixing can also affect, or be influenced by, water extractable proteins, gliadins, and triplet band (triticins) proteins. Since 1980 (Paredes-Lopez 1980), there have been no reported studies on the effects of dough mixing on 4 to 5 different protein fractions that exist in flour. As well, an interesting hypothesis regarding the interaction of gliadins and glutenins and dough mixing has been recently proposed (Fu et al 1996, Dupuis et al 1996). In this hypothesis, the extent of the protein-protein interaction is inversely proportional to dough mixing requirements. In this study, by quantitatively and qualitatively examining the protein composition of the various Osborne protein fractions in flour and dough of two diverse HRS wheats, answers were sought for some of the many outstanding questions related to the response of different protein in flour to the effects of dough mixing.

III. MATERIALS AND METHODS

A. Materials

1. Wheat Cultivars

Wheat cultivars selected in this study were two hard red spring wheats of contrasting mixing strength, viz. Glenlea and Katepwa (1991 crop). Glenlea is a cultivar of the CWES class and is known to have exceptionally strong dough properties for breadmaking under Canadian baking conditions (Bushuk 1980). Katepwa, is a cultivar of the CWRS wheat class and possesses medium-strong dough characteristics and good breadmaking quality for a range of baking procedures. The wheat samples were tempered to 15.5% moisture content and milled into straight-grade flours on a Buhler Automatic laboratory mill. The flours were kept in cold storage (4°C) in tightly closed plastic containers. Flour extraction was similar for both wheats with flour yields of approximately 72%. Some pertinent technological results are presented in Table 1.

2. Reagents and Chemicals

SDS was from Bio-Rad (Richmond, CA). Acrylamide and bisacrylamide (N, N'-methylene-bisacrylamide) were obtained from Pharmacia LKB, Biotechnology AB (Uppsala, Sweden). Ascorbic and lactic acids were obtained from Mallinckrodt (Mississauga, ON). Aluminum lactate was obtained from Fluka Chemicals (Hauppauge, NY). Coomassie Brilliant Blue G-250, Coomassie Brilliant Blue R-250, glycerol, glycine, potassium hydroxide, Tris, and ferrous sulphate were from Sigma Chemical Company (St. Louis, MO). All chemicals and reagents were of analytical grade or better. Hydrogen peroxide (3% practical grade) was purchased from a local pharmacy. Distilled deionized water was used in all the experiments.

B. Preparation of Dough Samples

Doughs (200 g flour at 14% m.b. plus 1% salt) were mixed in air in a Grain Research Laboratory (GRL)-200 mixer equipped with a GRL energy-input meter (Kilborn 1979). The meter measured the energy used by the motor and recorded the energy input during mixing in watt-hours per kilogram (Whr/kg) of dough. The pin speed was set at 140 rpm and a thermostatically controlled bath held the temperature of the water-jacketed mixing bowl at 30 °C. Water absorptions (Table 1) were adjusted to give a dough consistency of 500 B.U. when mixed in a Brabender farinograph. The dough mixing treatments that were adopted for this study are listed in Table 2. A single mix of each cultivar was performed.

Upon completion of a mixing treatment, the dough sample was removed from the mixing bowl as cleanly as possible by rubbing some shortening on both palms. The dough was gently moulded by hand into a cylindrical shape, and cut into equal portions. One portion was placed in a humidity cabinet (30 °C and 95-100% R.H.) to relax for 45 min. The other half was cut into smaller pieces, immediately frozen in liquid nitrogen, freeze-dried and subsequently ground in a coffee grinder (Moulinex Coffee and Spice Mill). The latter represented the control sample at a particular mixing stage and zero relaxation. Relaxed dough pieces were subsequently treated like the control sample. The ground samples were kept in tightly sealed containers at 4 °C and were removed as required for analyses.

Table 1. Pertinent technological data of Glenlea and Katepwa.

Wheat	Glenlea	Katepwa
Moisture, %	9.3	9.2
Protein, % (13.5% m.b.)	13.1	14.1
Hardness (PSI)	46.6	49.3
Falling number, sec	545	492
Flour yield, %	72.4	72.3
Flour		
Moisture, %	14.0	13.9
Protein, % (14.0% m.b.)	12.4	13.6
Ash, % (14.0% m.b.)	0.42	0.34
Amylograph visc., BU	800	710
Sedimentation value, cc	53	55
Starch damage, Farrand Unit	26	26
Wet gluten content, %	27.1	41.4
Dough Farinograph		
Absorption, %	60.4	64.9
D.D.T., min	27.0	6.5
M.T.I., BU	5.0	15.0
Extensigraph		
Extensibility (E), mm	203	182
Max. resistance (R), EU	970	660
R / E	4.78	3.63
Area, cm^2	249	158
Mixograph		
Peak time, min	8.5	2.3
Peak height, MU	540	650

Table 2. Mixing and relaxation treatments of dough samples[a] studied.

Sample No.	Mixing Treatment[b] (min)	Relaxation Time[c] (min)
F	flour	-
1	undermixed (1 min)	0
2	undermixed (1 min)	45
3	peak development[d]	0
4	peak development	45
5[e]	15 min	0
6	15 min	45
7	30 min	0
8	30 min	45

[a] 200 g flour (14% m.b.) + water + 1% salt
[b] GRL-200 mixer with a GRL energy input meter operated at 140 rpm and 30 °C
[c] Humidity cabinet kept at 30 °C and 95-100% relative humidity
[d] Katepwa = 3.5 min
Glenlea = 15 min
[e] Katepwa only
See Appendix (Fig. 34 and 35) for the mixing curves.

C. Extraction and Fractionation of Flour and Dough Proteins

1. Direct Extraction of Protein with 0.05M Acetic Acid

AAS and AAI fractions of the flours and freeze-dried doughs, were prepared by extracting a 1 g sample (d.b.) with 25 ml of 0.05 M acetic acid in a 50 ml centrifuge tube as described by Orth and O'Brien (1976), with minor modifications. Immediately after the addition of the solvent, all tubes were vortexed at full speed for 15 sec on a Vortex-Genie mixer (Scientific Industries, Inc., Bohemia, NY). They were then placed in a mechanical shaker (Ika-Vibrax-VXR, Terochem Laboratories Ltd., Edmonton, Alberta), at a speed setting of 1400. After 2 hr of shaking, the tubes were centrifuged at 20,190 g for 30 min at 20 °C in a Sorvall RC-5C Superspeed Centrifuge (Dupont Co., Wilmington, DE).

The supernatants were decanted and volumes measured in a high precision (±0.1) graduated cylinder. Three ml of supernatant were used for protein determination by the micro-Kjeldahl method (AACC Method 46-13 1983). Total protein content in the extract was calculated based on the total supernatant volume. The remaining supernatants were freeze-dried. The freeze-dried supernatants, which were fluffy in appearance, were placed in a container and dispersed with a glass stirring rod. The residues were freeze-dried, weighed, and finely ground with a mortar and pestle and their protein content determined by the micro-Kjeldahl method. The freeze-dried samples were stored at 4 °C until required for analyses. Protein determination of all fractions was done in duplicate. Mean data are reported in this study.

2. Modified Osborne Procedure

The proteins of the flour and dough samples were extracted and fractionated by the modified Osborne method as described by Chen and Bushuk (1970), with some modifications.

Samples (10 g d.b.) were initially extracted with 0.5 M NaCl (40 ml) by stirring with a magnetic stirrer in a centrifuge bottle (250 ml) for 2 hr. Subsequently, each suspension was centrifuged at 1860 g for 30 min at 4 °C in a Sorvall RC-5C Superspeed centrifuge (Dupont Co., Wilmington, Delaware), and the supernatant decanted. To facilitate a more efficient extraction in the following fractionation stage, the precipitate was removed from the centrifuge bottle and cut into small pieces with a scissor. The dispersed precipitate was extracted again with 40 ml of 0.05 M NaCl for 1 hr, centrifuged, and the supernatant was decanted. The residue was then washed once with 40 ml of distilled and deionized water for 30 min, centrifuged, and the supernatant was decanted. The three supernatants were then combined and poured into a 40 cm dialysis tubing (Spectra/Por^{R}6 Membrane, Spectrum Medical Industries, Inc., Houston, TX, with a molecular weight cut-off of 1000 daltons. The combined extract was dialysed against cold distilled deionized water for 72 hr; the water was changed three times daily. After dialysis, the retentate was centrifuged producing two protein fractions: supernatant - salt-soluble proteins (mainly globulins) (fraction 1) and, residue - water-soluble proteins (mainly albumins) (fraction 2).

The 0.05 M NaCl insoluble residues in the centrifuge bottles were kept at 4 °C until the following day when the fractionation was resumed. Residues were extracted twice with 40 ml of 70% ethanol, centrifuged, and the supernatants decanted. The ethanol-extracts were combined and reduced almost to dryness using a rotary vacuum evaporator (Buchi 461 Water Bath, Buchi, Switzerland). A sufficient amount of 0.05 M acetic acid (5 ml) was used to quantitatively transfer the ethanol-extract from the glass container and subsequently freeze-dried. This step in the procedure produced aqueous ES proteins (mainly gliadins) (fraction 3).

The resulting residue was twice extracted with 0.05 M acetic acid (40 ml), centrifuged, and the supernatants decanted, combined, and freeze-dried. This last step in the procedure yielded two fractions: AAS proteins (mainly glutenins) (fraction 4) and, AAI proteins (mainly HMW glutenins)

(fraction 5).

Osborne extractions were performed at 4 °C and were done in duplicate. After freeze-drying, the four supernatant fractions were weighed and pulverized into homogeneous powder by a mortar and pestle. The residues were freeze-dried, weighed and subsequently ground in a coffee grinder. The micro-Kjeldahl method was used to determine nitrogen contents of the extracts and the residues. All freeze-dried protein fractions were kept in sealed containers at 4 °C and used as required for analyses.

D. Polyacrylamide Gel Electrophoresis

1. Sample Preparation

Samples for PAGE analysis were prepared according to the procedure of Sapirstein and Bushuk (1985), with a few modifications. Protein solutions (controls) were prepared from flour and freeze-dried doughs by extracting samples with 70% ethanol. The protein fractions obtained from direct acetic acid and modified Osborne procedures were solubilized in two solvents for PAGE analysis: (1) 70% ethanol and (2) the original solvent used to extract the protein fraction (i.e. NaCl, water or acetic acid). The amount of freeze-dried sample and solvent used, as well as the amount of sample applied onto the gel slots, was adjusted according to the detection of protein bands of reasonable intensity and resolution after staining. For each protein fraction, the amount of sample solubilized and applied was the same. This permitted any quantitative effects of mixing to be revealed. The detection of the bands were based on subjective judgement.

2. Electrophoresis

A-PAGE (6.3% gels) was performed at pH 3.1 using a custom-built electrophoretic apparatus described by Bushuk and Zillman (1978) and Sapirstein and Bushuk (1985). Electrophoresis was carried out at a constant current of 50 mA producing a voltage of 300-400 V. The duration of the electrophoresis was approximately 2 hr or when the tracking dye (Coomassie Brilliant Blue R) band with a faster mobility (blue-green in colour) migrated to the end of the gel. This short-run method retained proteins on the gel slab (albumins and globulins) which possess higher mobilities than the gliadins.

E. Sodium Dodecyl Sulphate - Polyacrylamide Gel Electrophoresis

1. Sample Preparation

Flours and freeze-dried doughs (controls) for SDS-PAGE analysis were extracted with 1 ml of 2% (w/v) SDS-extracting buffer solution at pH 6.8 as described by Ng and Bushuk (1988). The extraction solution was composed of glycerol, stacking-gel buffer solution (1.0 M Tris with HCl), water, SDS, and Pyronin Y dye. For SDS-PAGE of protein fractions isolated by direct acetic acid or modified Osborne procedures, protein solutions were prepared by solubilizing a given amount of freeze-dried sample in SDS-extracting buffer solution. The amounts of the freeze-dried sample, buffer solution, and sample applied onto the gel slots, were adjusted to yield optimally stained electrophoregrams. It is noted, however, that equal amounts of samples were solubilized and applied so that quantitative differences due to the mixing treatments could be observed in the resulting electrophoregrams. Analyses of the fractions were carried out under non-reducing and reducing conditions using 5% (v/v) 2-mercaptoethanol as the reducing agent.

2. Electrophoresis

SDS-PAGE, of non-reduced and reduced proteins, were performed in an electrode buffer solution (Tris, glycine, and SDS) of pH 8.3 according to Ng and Bushuk (1988). The separating gel (pH 8.8, 17.33% acrylamide concentration and 0.45% cross-linkage) was poured to a height of 14 cm into a vertical LKB 2001 electrophoresis unit (Pharmacia LKB). The stacking gel solution was composed of 3.03% acrylamide, with a pH 6.8, and 1.43% cross-linkage. Each gel was 1.5 mm in thickness and accommodated 15 samples.

Electrophoresis was carried out according to Sievert et al (1991a), with some modifications. Proteins were electrophoresed using constant current (35 mA) until the tracking dye reached the separating gel. The current was then lowered (usually 8-10 mA) until the tracking dye migrated to the end of the gel (usually overnight); this method resulted in all the proteins being retained on the gel slab. Two gels were electrophoresed simultaneously.

Two-step one-dimensional SDS-PAGE was performed as described by Singh and Shepherd (1985, 1988) and Sievert et al (1991b), with some modifications. In the first step, non-reduced protein extracts were loaded onto a 1.0 mm thick separating gel and electrophoresed overnight. As soon as the gels were removed from the plates, the top section of the gel (0.5 cm from the origin) was cut using a sharp scalpel and a straight edge ruler as a guide. The strip of gel containing unresolved proteins was reduced by immersing it in a glass beaker (400 ml) containing a solution of 5% (v/v) 2-mercaptoethanol (ca. 25 ml) for one and a half hour at 37^{0}C.

For the second step, a 1.5 mm thick gel composed of a separating (17.33% acrylamide solution) and a stacking (3.03% acrylamide solution) gel was prepared. This gel contained two slots, one normal size and one wide. The cut out gel was loaded onto the thicker gel by laying it flat on a ruler and carefully sliding it into the wider slot, resting flush against the stacking gel. A reduced total protein extract (8 µl) of Katepwa or Glenlea flour was applied onto the normal size

slot as a secondary standard (Glenlea - GL2 or Katepwa - KP2). The secondary standard also served as a marker for keeping track of the migration of the proteins. When the tracking dye of the secondary standard reached the end of the gel, the second electrophoretic separation was stopped.

F. Technological Tests

1. Moisture content

The moisture content of the whole grain was determined using an electric moisture meter (Canadian Aviation Electronics Ltd., model CAE 919/3.5) according to the AACC standard procedure (AACC Method 44-11 1983). The moisture content of the flour was determined by near-infrared reflectance (NIR) using the Dickey John Instalab 800 NIR instrument (Dickey-John Corp., Auburn, IL), calibrated using a Brabender Rapid Moisture Meter (C.W. Brabender Instruments Inc. South Hackensack, NJ), according to the standard AACC method (AACC Method 44-15A 1983).

2. Protein content

The NIR instrument was used to determine protein content of the whole grain. The micro-Kjeldahl method (AACC Method 46-13 1983) employing a Kjeltec System 1002 Distilling Unit from Tecator (Sweden) was used to determine nitrogen content of the flour extracts and residues. Total protein content was calculated by multiplying the nitrogen content by the conversion factor for wheat of 5.7 (Tkachuk 1969).

3. Ash content

The ash content of the grain (ground meal) and flour (5 g of sample at 14% m.b.) was determined according to the standard AACC method (AACC Method 08-01 1983). The meal was prepared by grinding 50 g of grain through a Udy Cyclone Grinder with a sieve size of 0.5 mm (Udy Corporation, Fort Collins, CO).

4. Hardness (PSI)

The hardness as measured on the wholemeal (Udy Cyclone ground) was determined by NIR spectroscopy according to Williams (1979).

5. Falling number test

The falling number value was determined, using a single sample apparatus, according to the standard AACC method (AACC Method 56-81B 1983).

6. Amylograph test

The amylograph viscosity or malt index of the flour was determined according to the standard AACC method (AACC Method 22-10 1983) using 65 g of flour (14% m.b.) and 460 ml of water.

7. Sedimentation test

Zeleny sedimentation value (Zeleny 1947) was determined following the standard AACC procedure (AACC Method 56-61A 1983).

8. Starch damage test

Starch damage was determined according to the Farrand Method (Farrand 1964) using 5 g

(14% m.b.) of flour. This was a chemical test that used bacterial α-amylase from *Bacillus subtilis* (United States Biochemical Corp., Cleveland, OH) to estimate the starch damage in Farrand Units.

9. Wet gluten content

The wet gluten content of the flour was determined according to the AACC standard procedure (AACC Method 38-11 1983).

10. Farinograph test

The dough mixing characteristics of the flours (farinogram) were determined according to the AACC standard method (AACC Method 54-21 1983). A small mixing bowl and a constant weight procedure was implemented (50 g of flour at 14% m.b.).

11. Extensigraph test

For the extensigraph measurement, a dough was prepared by mixing 100 g flour (14% m.b) for 3.5 min in a GRL-200 mixer set at 131 rpm. The amount of water added was adjusted to give a dough consistency of 500 B.U. when mixed in a farinograph. The dough was removed from the mixer, moulded by hand into a cylindrical shape, clamped into a modified extensigraph holder, and rested for 45 min in a fermentation cabinet at 30 °C and 95% R.H. After the rest period, the dough was stretched in the extensigraph until it separated. It was then reshaped manually and allowed to rest in the cabinet for another 45 min. At the end of the second rest period, the dough was reshaped by hand without stretching and was further rested for 45 min before it was stretched for the second time. Hence, the dough was stretched after 45 and 135 min of rest; measurements from the second stretch are reported. The area under the curve was measured by a polar compensation planimeter (Sokkiska Ltd., Tokyo).

12. Mixograph test

Mixograms were obtained according to the AACC standard Mixograph Method (AACC Method 54-40A 1983). The amount of flour used was 35 g (14% m.b.) and 63 % water absorption.

The above technological tests were carried out at least in duplicate.

G. STATISTICAL ANALYSIS

All statistical analyses were performed on a HP 9000/380 minicomputer using SAS 6.07 statistical analysis software (SAS Institute, 1990). Analysis of variance and least significant difference test were performed to determine significant differences between cultivars and protein fractions.

IV. RESULTS AND DISCUSSION

The results of this study are presented and discussed in five sections. Section A: examines the dough mixing results - the energy required to mix flour to dough; Section B: mixing and its effects on protein solubility; Section C: electrophoretic composition of the wheat flour proteins at the different mixing stages; Section D: dough relaxation.

A. Dough-Mixing Results

The optimum mixing time for a dough is mainly a varietal (genetic) characteristic and is relatively independent of protein content, i.e. environmental effects. As discussed in the literature review, the differences in dough mixing requirements among different wheats are largely determined by differences in protein quality or composition.

Glenlea and Katepwa substantially differ in mixing strength as reflected by their dough mixing curves (Fig. 1) using a GRL-200 mixer. Figure 2 presents the corresponding dough mixing curve using a 35g mixograph. The mixing curves of both cultivars reflect the expected mixing characteristics of each wheat (Marchylo et al 1992, Dupuis et al 1996). The observed difference in the dough mixing records of the GRL-200 and 35g mixograph mixers reflects the difference in mixing intensities of the two instruments. Kilborn and Tipples (1972) have shown that in order to achieve proper dough development, the mixing intensity (impeller speed) and the work imparted to the dough must be above a minimum critical level. This requirement varies with both flour and mixer, with the latter being dependent on the flour used. As the dough strength of a flour increases, the critical value to also increases. Clearly, the GRL-200 mixer operating at 140 rpm, has a substantially less intense mixing action than the mixographoperating at 88 rpm. The mixograph

Figure 1. Mixing curves of Glenlea and Katepwa doughs (1% salt, water absorption at 60.4 and 64.9%, respectively).

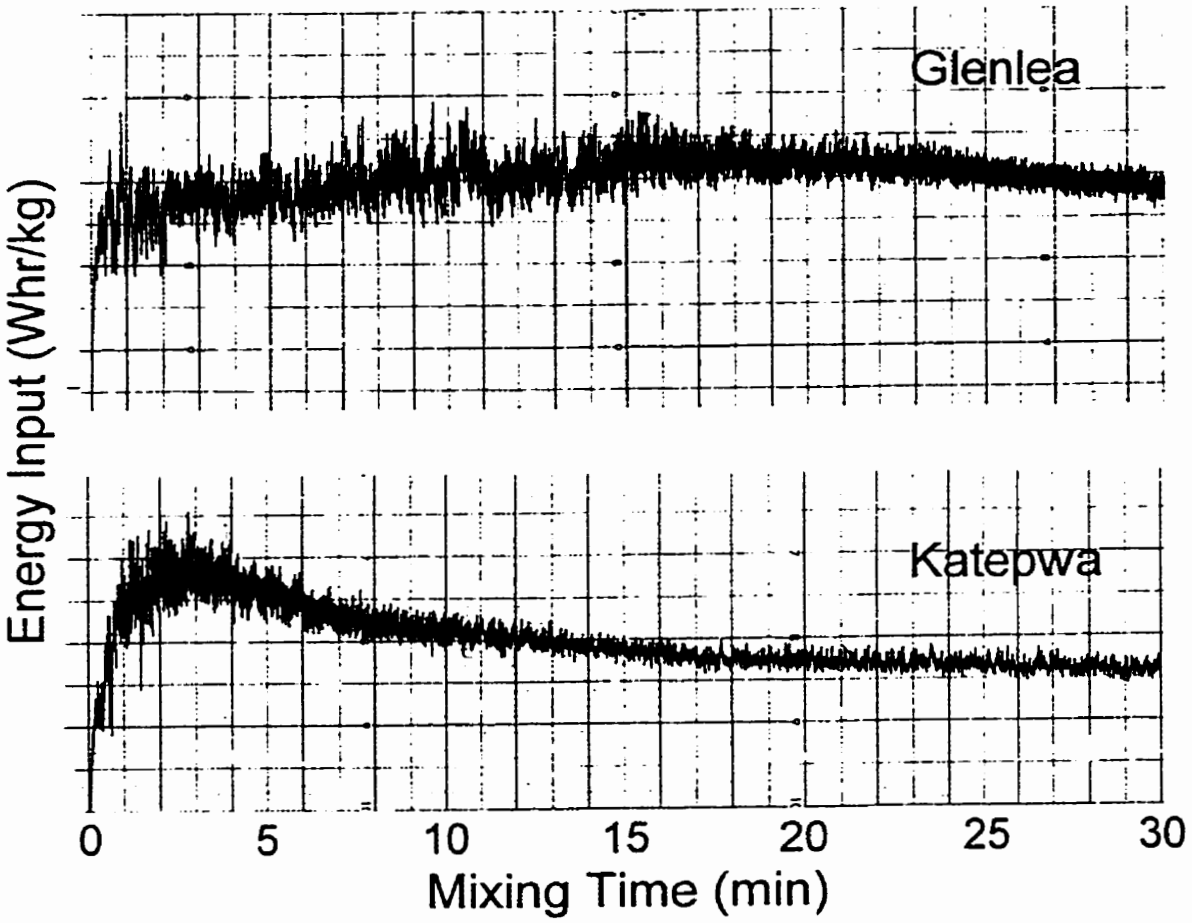
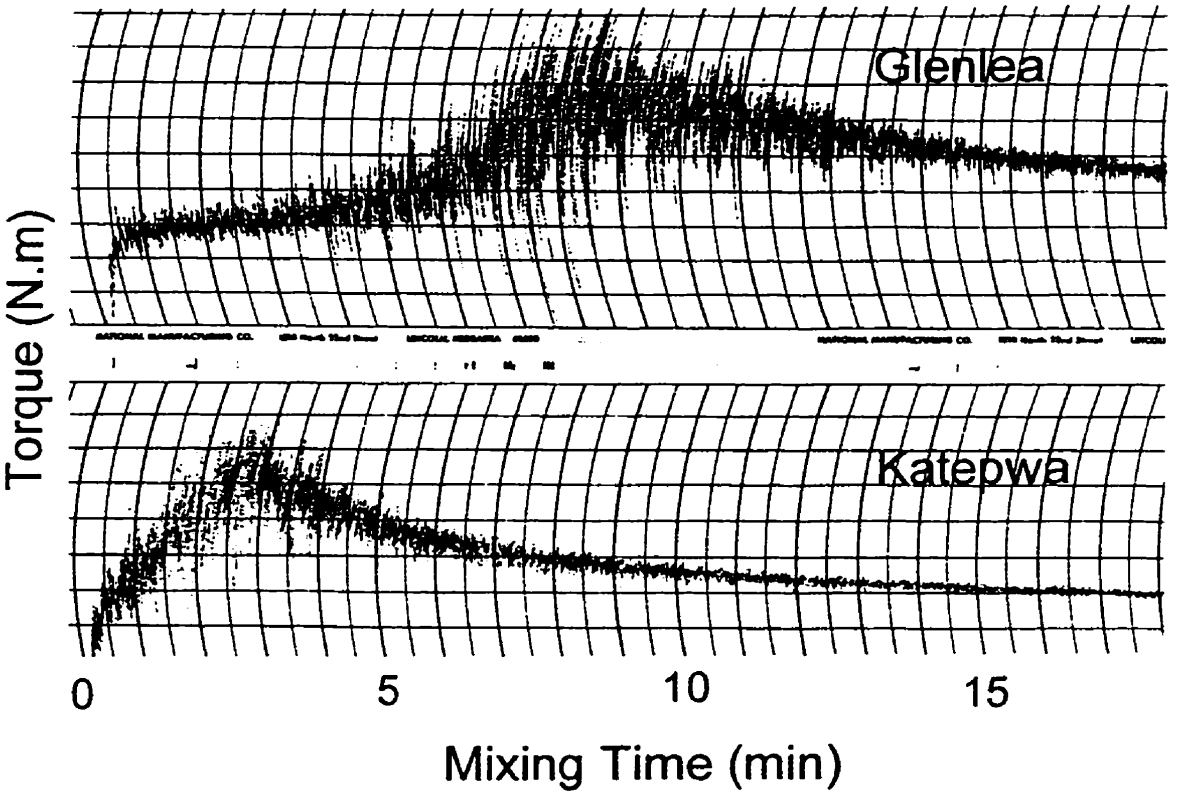

Glenlea
Katepwa
Energy Input (Whr/kg)
0
5
10
15
20
25
30
Mixing Time (min)

Figure 2. Mixograms of Glenlea and Katepwa doughs (1% salt, water absorption at 60.4 and 64.9%, respectively).

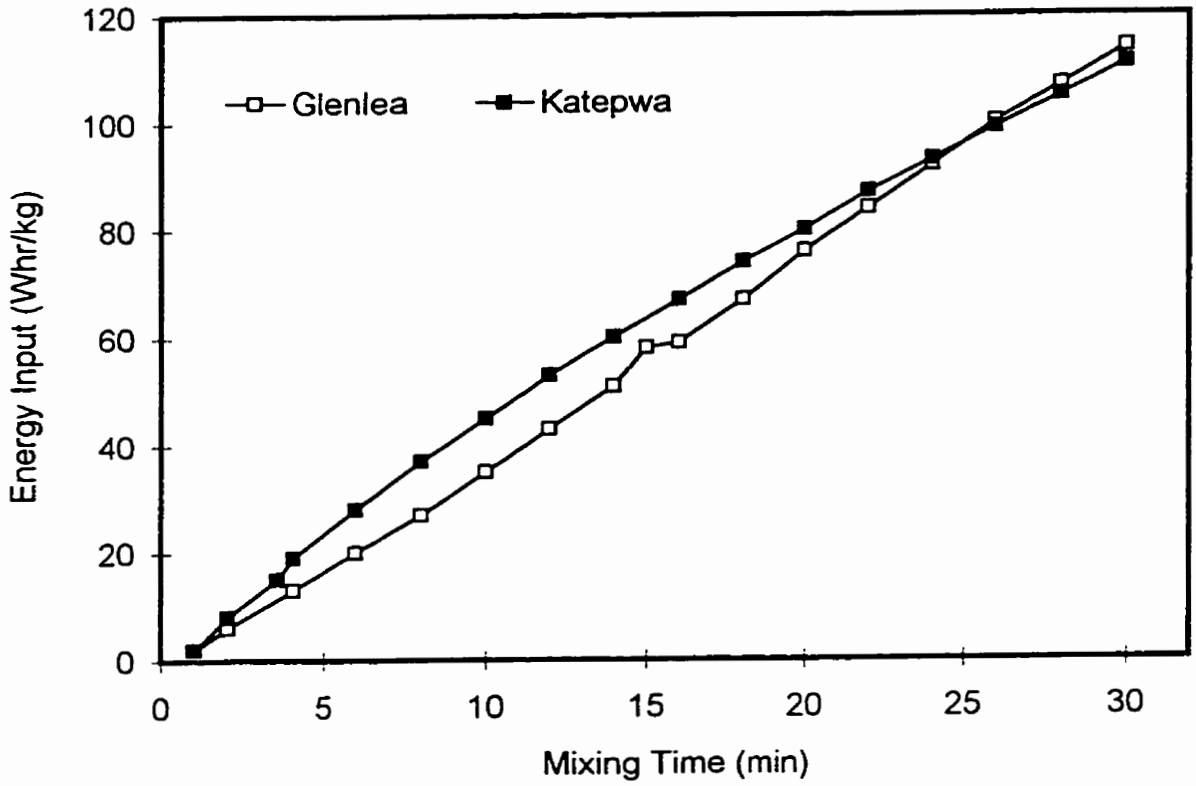

Glenlea
Katepwa
Torque (N.m)
0
5
10
15
Mixing Time (min)

with its six pin configuration and planetary mixing action is a much more efficient mixer particularly for strong flours like Glenlea for which a clear mixing peak was obtained (Fig. 2) in contrast to the relatively indistinct mixing peak produced by the GRL-200 mixing at 140 rpm. For the remaining Results and Discussion section, only the doughs mixed in the GRL-200 mixer are reported and discussed.

The term PDD is generally used to describe optimization of physical properties of dough by mixing (Bushuk et al 1968). PDD is defined physically by Hoseney (1985) as the stage in dough mixing when the dough achieves minimum mobility, becomes cohesive, partially elastic, and resistant to extension. To attain PDD, Glenlea flour required 15 min of mixing with a work input of 58 Whr/kg, whereas, Katepwa flour required only 3.5 min of mixing with a work input of 15 Whr/kg (Fig. 3). The PDD times for both cultivars are in general agreement with those obtained by Kilborn and Tipples (1973a) and Paredes-Lopez (1980). Due to the different dough formulation used such as the amount of salt added to the flours, differences in water absorptions, and as in the case of Paredes-Lopez (1980) using a different mixer, the energy requirements in this study are not exactly similar to that used by the above researchers.

The net energy utilized for mixing the flours to 30 min was 114 and 111 Whr/kg for Glenlea and Katepwa, respectively. Once peak dough development was attained, Katepwa quickly showed evidence of dough breakdown as the resistance to extension rapidly decreased. Katepwa's resistance to extension reached a plateau after about 15 min of mixing. For Glenlea, only a small decrease in resistance to extension was found even after 30 min of mixing.This tolerance of Glenlea doughs to overmixing has been observed previously. Paredes-Lopez (1980) found that Glenlea had an atypical farinogram at the normal farinograph speed of 60 rpm. At a higher speed (90 rpm), Glenlea's farinogram showed a second broad peak at about 16-17 min. The initial peak (90 rpm mixing) is therefore likely related to hydration of flour constituents only.

Figure 3. Effect of mixing times (min) and energy inputs (Whr/kg) on Glenlea and Katepwa doughs mixed in a GRL-200 mixer.


120
100
80
60
40
20
0
Energy Input (Whr/kg)
Glenlea
Katepwa
0
5
10
15
20
25
30
Mixing Time (min)

Paredes-Lopez (1980) found that Glenlea required extremely high energy input to reach its overmixed stage in comparison with both the strong and medium-mixing strength flours when mixed in a farinograph. This study reported very high energy requirements for dough mixing because unlike the GRL-200, the farinograph in comparison is a much less efficient mixer.

B. Influence of Dough Mixing on Wheat Protein Solubility

In this section, the effect on protein solubility when flour is mixed to its optimum time will be discussed. PDD times were 15 and 3.5 min for Glenlea and Katepwa, respectively. The changes in flour proteins during dough mixing from 1 min (undermixed) up to 30 min (overmixed) will also be presented in this section.

1. Dough Mixing and Direct 0.05M Acetic Acid Extracts

The amounts of AAS protein, as a percentage of total protein in flour were 60.2 and 69.9% for Glenlea and Katepwa, respectively (Table 3). The higher amount of protein extracted from the relatively weaker flour (Katepwa) is consistent with previous results (Orth and Bushuk 1972, Orth and O'Brien 1976,Paredes-Lopez 1980, Dupuis et al 1996). Paredes-Lopez (1980) observed that for an equivalent energy input, there was an inverse relationship between dough strength and protein extractability. He also found that Glenlea required an extremely high level of farinograph work input to solubilize a given amount of protein as compared to Katepwa.

For both Glenlea and Katepwa, the amount of AAS protein increased with mixing to 1 min and continued to increase with dough development (Fig. 4). For Glenlea, the proportion of AAS flour protein increased significantly from 60.2% (flour) to 80.7% (overmixed). For Katepwa,

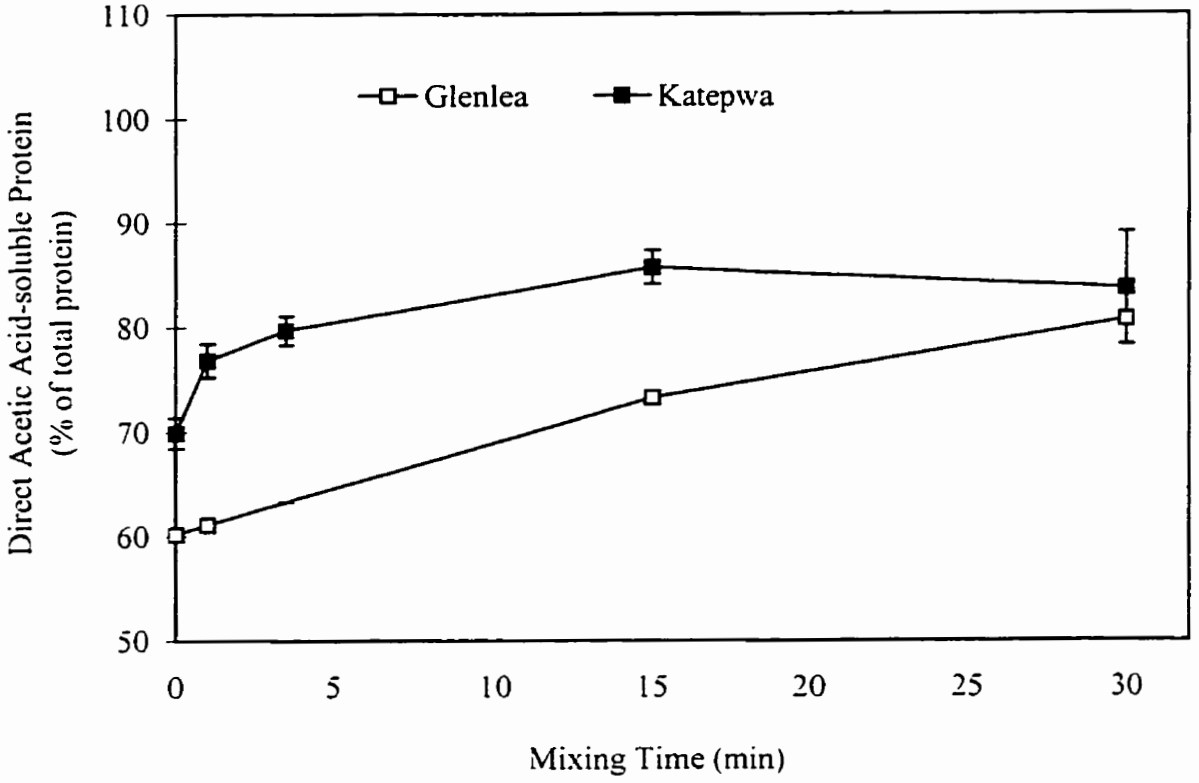
Table 3. Effect of Glenlea and Katepwa dough mixing on the distribution of protein in direct 0.05 AAS and AAI as a percentage of total protein.

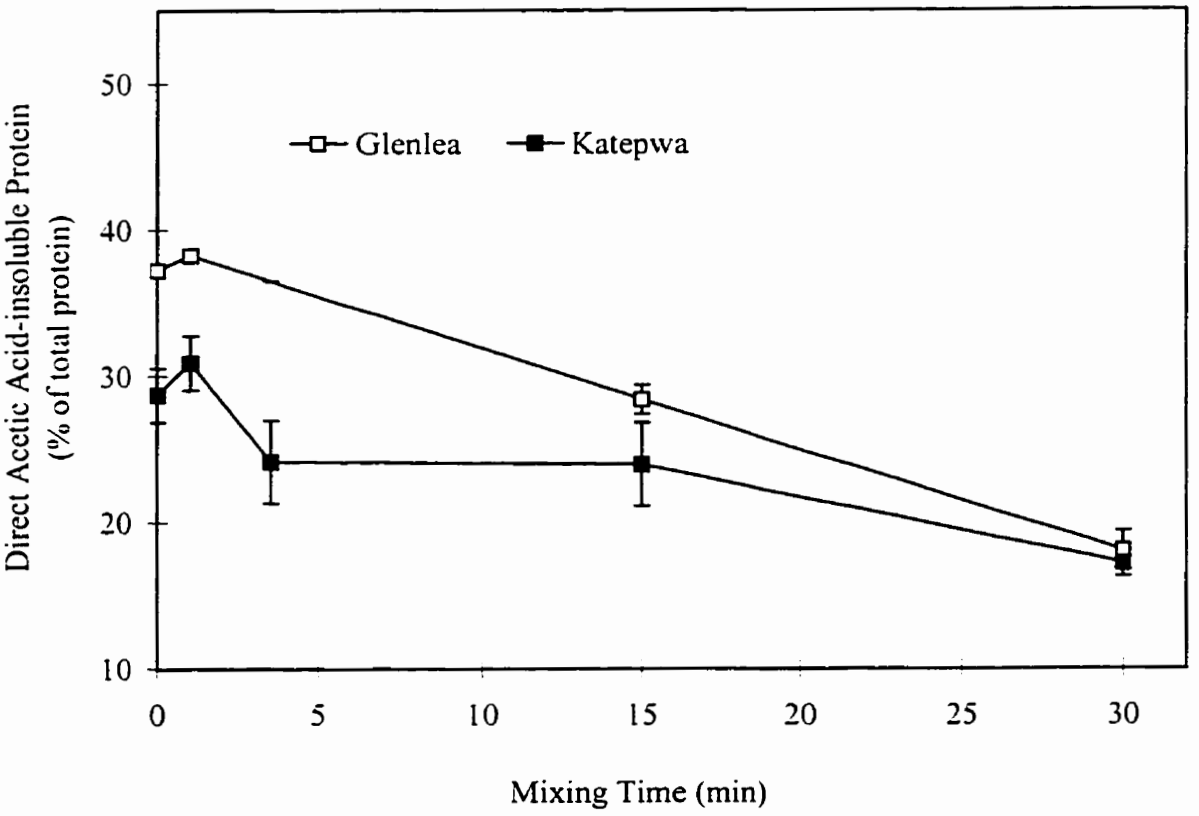
Glenlea				
Fraction	0 min (flour)	1 min	15 min (peak)	30 min
AAS	60.2±0.1c	61.1±0.1c	73.2±0.6b	80.7±0.2a
AAI	37.3±0.4a	38.2±0.1a	28.4±1.0b	18.0±1.3c
Total Recovery	97.5	99.3	101.6	98.7

Katepwa					
Fraction	0 min (flour)	1 min	3.5 min (peak)	15 min	30 min
AAS	69.9±1.5c	76.8±1.6b	79.7±1.4b	85.7±1.6a	83.7±5.4a
AAI	28.7±1.9ab	30.9±1.9a	24.1±2.8b	24.0±2.8b	17.2±0.9c
Total Recovery	98.6	107.7	103.8	109.7	100.9

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

Figure 4. Effect of mixing time on direct 0.05 M AAS (top) and AAI (bottom) protein of cvs. Glenlea and Katepwa.


Glenlea
Katepwa
Direct Acetic Acid-soluble Protein (% of total protein)
110
100
90
80
70
60
50
0
5
10
15
20
25
30
Mixing Time (min)


Glenlea
Katepwa
Direct Acetic Acid-insoluble Protein (% of total protein)
50
40
30
20
10
0
5
10
15
20
25
30
Mixing Time (min)

doughs, AAS protein extractability reached a plateau at 15 min of mixing, while for Glenlea mixing beyond 15 min resulted in significantly more extracted protein. For Glenlea and Katepwa, the amount of protein solubilized at 30 min of mixing were 20.5 and 15.8%, respectively.

Figure 4 also shows that the rate of increase in the amounts of AAS was greatest during the first few minutes of mixing for the relatively weaker flour Katepwa when compared to Glenlea. However, when mixed to PDD, the rate of increase for Glenlea surpassed that of Katepwa, whereby the total amounts of AAS protein to PDD were 13 and 9.8%, respectively. Similar findings were reported previously by Tsen (1967) who suggested that protein aggregates in weak flours are not as stable and may have a smaller overall aggregate size than the protein from a strong flour.

It should be mentioned that the high total protein recoveries for Katepwa doughs mixed for 1 and 15 min makes interpretation of precise trends in these results subject to some uncertainty. However, the trend of increasing and decreasing AAS and AAI protein with mixing is clear for both cultivar samples.

Previous work has established a relationship between the amount of AAS protein and mixing strength of wheat flour doughs. Acetic acid extracts a higher percentage protein from weaker flours than from stronger flours (Mecham et al 1962, Mecham 1964, Mullen and Smith 1965, Tsen, 1967, Orth and Bushuk 1972, Tanaka and Bushuk 1973a,b,c, Butaki and Dronzek 1979, Paredes-Lopez and Bushuk 1983). These references dealt with flour extracts, and not the breakdown of insoluble glutenin with mixing. When mixed to PDD, more AAS protein is extracted from Glenlea dough than from Katepwa. As Glenlea flour contains significantly more AAI protein than Katepwa, this result seems plausible.

The amount of AAI flour protein of both cultivars was similar to results of other studies on

high quality Canadian bread flours [(Paredes-Lopez 1980, Preston et al 1992, Dupuis et al 1996)] Tsen (1967), Orth and Bushuk (1972) and Butaki and Dronzek (1979) also observed that stronger wheat flours have more acid-insoluble gluten than weak flours. Butaki and Dronzek (1979) reported that acetic-acid soluble proteins constituted about 90% of total protein from weak gluten and about 70% from strong gluten. They also found that Glenlea's insoluble fraction contained the highest percentage of protein.

As expected from previous studies (Tsen 1967, Tanaka and Bushuk 1973a,b,c, Sievert et al 1991a,b), the proportion of AAI proteins declined with mixing for both Glenlea and Katepwa (Table 3, Fig. 4). The proportion of AAI fraction in the total flour protein decreased with dough development from 37.3% (flour) to 18.0% (overmixed) for Glenlea, and from 28.7% (flour) to 17.2% (overmixed) for Katepwa. Also, dough mixing to the overmixed stage resulted in nearly the equivalent proportions of AAI proteins for both cultivars, whereas flours differed by over 8% in the proportion of AAI proteins. The difference between undermixing and overmixing stages in the proportion of AAI proteins was 19.3% for Glenlea and 11.5% for Katepwa. These differences were produced by comparable amount of energy inputs of 114 Whr/kg for Glenlea and 111 Whr/kg for Katepwa. Dough mixing to 30 min solubilized significantly more AAI proteins for Glenlea than for Katepwa. These data indicate that when the flours are mixed beyond optimum development, intrinsic flour strength cannot be differentiated by the amount of remaining AAI proteins. This result shows that while Glenlea had a much higher concentration of AAI protein and Osborne residue (discussed below) protein in flour, this protein (mainly glutenin) is comparable to that of Katepwa in its susceptibility to depolymerization by mixing.

For Glenlea, the freeze-dried direct AAI fraction at different mixing times had protein concentrations ranging from 6.4% (undermixed) to 3.1% (overmixed) (Table 4). For Katepwa,

Table 4. Effect of dough mixing on the protein concentration of directly extracted 0.05 M AAS and AAI residue fraction[a].

Glenlea					
Fraction	0 min (flour)	1 min	15 min (peak)	30 min	
AAS	3.3±0.0	3.6±0.0	4.3±0.0	4.8±0.0	
AAI	6.2±0.0	6.4±0.0	4.7±0.2	3.1±0.2	
Katepwa					
Fraction	0 min (flour)	1 min	3.5 min (peak)	15 min	30 min
AAS	4.1±0.0	4.8±0.1	4.9±0.1	5.1±0.1	5.3±0.1
AAI	5.4±0.3	5.5±0.4	4.3±0.4	4.1±0.5	3.3±0.5

[a]The protein concentration of the AAS fraction is measured in mg/ml 0.05M acetic acid. The protein concemtration of the freeze dried AAI residue is measured in percent.

the protein concentration of the AAI residues varied from 5.5% (undermixed) to 3.3% (overmixed). Accordingly, the protein contents of the AAI residue fractions decreased when dough was mixed to peak, which is consistent with previously published results (Mecham et al 1962, Tsen 1967, Tanaka and Bushuk 1973a,b, Sievert et al 1991a,b). The relative decrease was greater for Glenlea (24%) compared to Katepwa (20%). The decline in AAI protein concentration with mixing is likely due to increased protein solubility caused by mixing which decreases the size of large-sized protein aggregates (Mecham et al 1965). For the direct AAS fraction, the protein concentration increased when dough was mixed to 30 min.from 3.3% (flour) to 4.8% (overmixed) and 4.1% (flour) to 5.3% (overmixed) for Glenlea and Katepwa, respectively (Table 4).

2. Dough Mixing and the Osborne Protein Fractions

a. Salt-Soluble (0.5 M NaCl) Protein. For flour of Glenlea and Katepwa, salt-soluble protein contents were 3.4 and 3.0% of total flour protein, respectively (Tables 5 and 6). Varietal differences were not observed in the amounts of salt-soluble fractions of the flour samples. Recently, however, Dupuis et al (1996) found that comparison of the protein solubility distribution between Glenlea and Katepwa showed a significant varietal difference in the Osborne salt-soluble protein fraction with Katepwa containing more of this protein than Glenlea. This difference in findings may have resulted because Dupuis et al (1996) did not further fractionate the salt-soluble protein fraction into albumins (water-soluble) and globulins (salt-soluble). Other studies indicate that the amount of salt-soluble protein extracted from weak and strong flours are similar and have little effect on mixing characterisitics and breadmaking quality (Mullen and Smith 1965, Smith and Mullen 1965a,b, Orth and Bushuk 1972, Tanaka and Bushuk 1973a).

Table 5. Effect of Glenlea dough mixing on the distribution of proteins in Osborne solubility fractions as a percentage of total protein.

Osborne Solubility Fraction	Dough Mix Time			
	0 min (flour)	1 min	15 min (peak)	30 min
Salt	3.4±0.3a	2.4±0.2b	1.9±0.2b	2.1±0.0bc
Water	9.7±0.5a	9.6±1.2a	8.9±0.9a	8.1±0.4a
Alcohol	32.8±2.8a	32.7±3.1a	32.8±2.7a	32.1±1.5a
Acetic-acid	12.1±1.2c	12.5±0.2c	16.7±1.9b	20.3±0.2a
Residue	33.9±2.6ab	37.5±0.1a	32.7±1.9ab	28.1±0.6b
N recovery	91.7	94.7	93.0	90.7

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

Table 6. Effect of Katepwa dough mixing on the distribution of proteins in Osborne solubility fractions as a percentage of total protein.

Osborne Solubility Fraction	Dough Mix Time				
	0 min (flour)	1 min	3.5 min (peak)	15 min	30 min
Salt	3.0±0.2a	2.2±0.1a	2.1±0.2a	1.9±0.1a	2.7±1.2a
Water	10.8±0.9b	11.4±0.3ab	11.5±0.7a	11.5±0.9a	11.1±0.9ab
Alcohol	35.9±1.3a	25.2±0.5c	29.4±0.8b	36.7±0.6a	36.2±1.5a
Acetic-acid	16.0±0.9b	19.8±1.2a	19.9±1.4a	19.8±1.2a	20.2±0.5 a
Residue	28.8±0.3d	41.2±1.1a	34.8±1.2b	31.5±0.1 c	30.1±0.9cd
N recovery	94.5	99.8	97.7	101.3	99.4

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

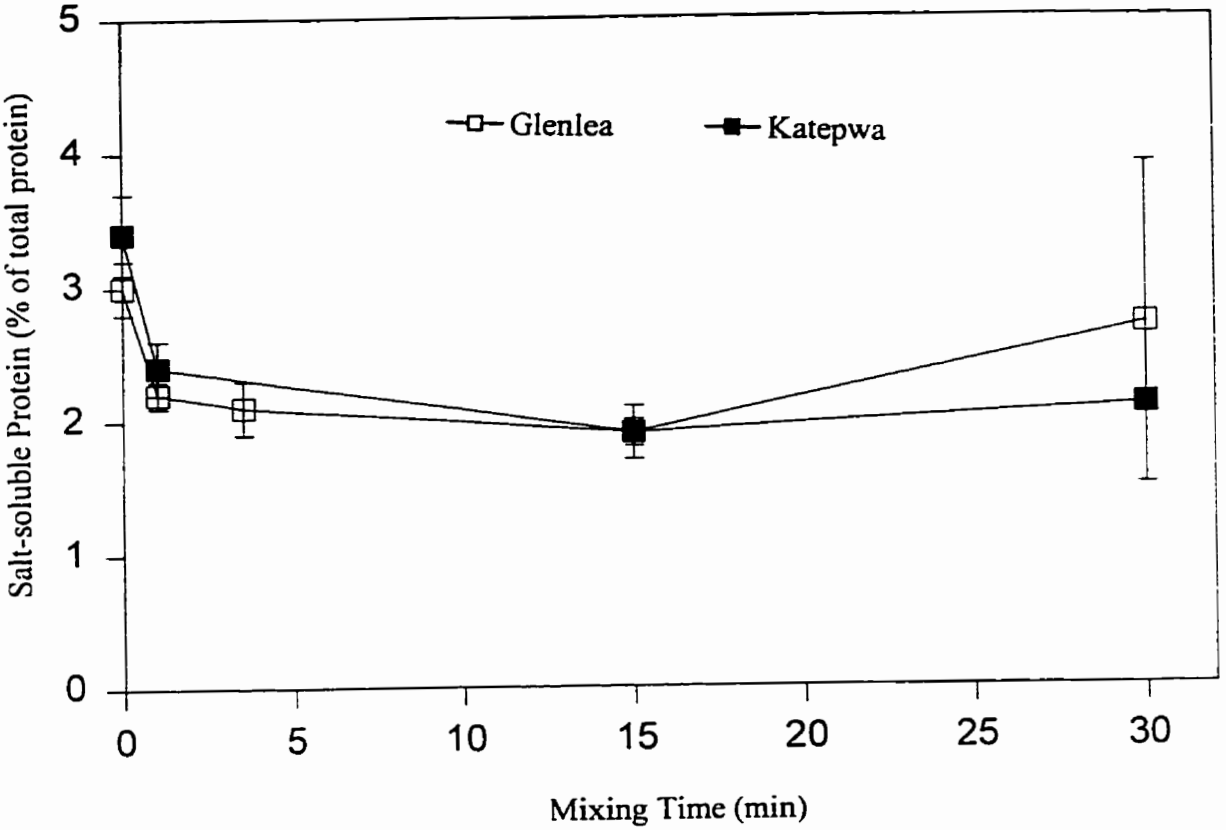
For Glenlea, the amount of salt-soluble protein decreased significantly with mixing from 3.4% (flour) to 1.9% (15 min mixing) (Fig. 5). Further mixing produced no change in this fraction. For Katepwa, comparable values were obtained, 3.9% (flour) to 1.9% (15 min mixing).

Paredes-Lopez (1980) reported a decreasing trend of approximately 1-1.5% of flour protein (not statistically evaluated) in the amount of salt soluble proteins with mixing in three cultivars of diverse mixing strength. In contrast, Tanaka and Bushuk (1973a) reported that the amounts of salt soluble protein were not affected by dough mixing in a farinograph. Koenig et al (1964) found that good quality flours, which also had longer mixing times, contained more salt-soluble proteins than poor quality flours. In contrast, Mullen and Smith (1965) and Smith and Mullen (1965a and 1965b) extracted similar amounts of salt-soluble proteins from short and long mixing flours.

In terms of protein concentration, for both Glenlea and Katepwa, flour had the highest protein concentration of 55.8 and 60.5%, respectively (Tables 7 and 8). Orth (1971) reported salt-soluble protein concentrations for 26 wheat samples, ranging from 43.0 to 88.5%. Chen and Bushuk (1970) found the Osborne salt-soluble protein concentration for a hard red spring wheat flour to be 76.2%. With mixing, the protein concentration decreased considerably compared to flour. Glenlea and Katepwa were comparable in their salt-soluble concentations at all the mixing. For Glenlea and Katepwa, the decreases in protein concentration from flour to 30 min of mixing were approximately 10 to 17%, respectively.

While it is generally accepted that salt-soluble proteins do not play a major functional role in breadmaking, in this study, some mixing related effects were observed on this protein fraction as indicated above and by electrophoresis (see below).

Figure 5. Effect of mixing time on Osborne salt (top) and water (bottom) soluble protein of cvs. Glenlea and Katepwa.


Glenlea
Katepwa
Salt-soluble Protein (% of total protein)
0
1
2
3
4
5
0
5
10
15
20
25
30
Mixing Time (min)

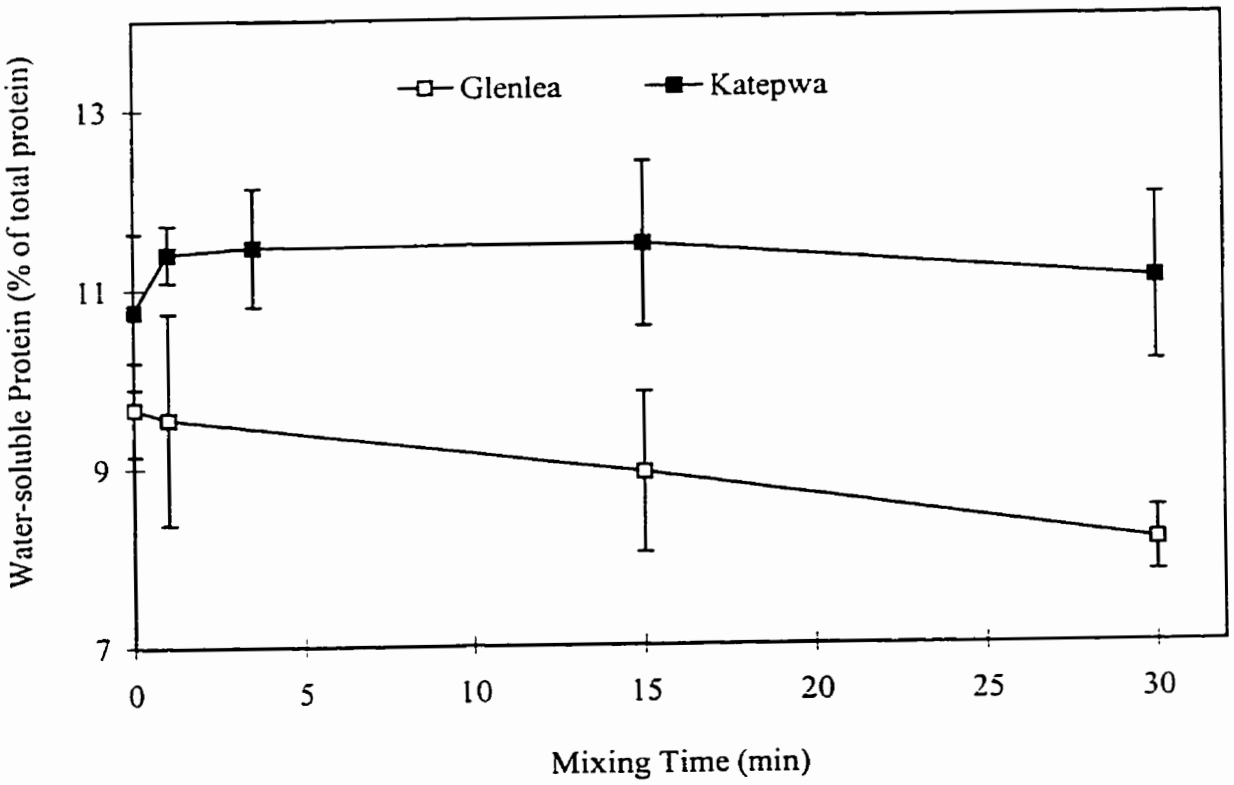

Glenlea
Katepwa
Water-soluble Protein (% of total protein)
7
9
11
13
0
5
10
15
20
25
30
Mixing Time (min)

Table 7. Effect of Glenlea dough mixing on the protein concentration of Osborne solubility fraction.

Osborne Solubility Fraction	Dough Mix Time			
	0 min (flour)	1 min	3.5 min (peak)	30 min
Salt	55.8±3.1a	50.4±0.8ab	47.2±0.2b	45.7±2.2b
Water	47.3±1.1a	47.1±2.9a	44.7±0.5a	44.9±0.4a
Alcohol	67.4±0.0b	71.5±2.1ab	71.3±0.7ab	72.5±1.8a
Acetic-acid	62.7±2.6d	68.4±3.3c	73.1±2.7b	75.8±1.6a
Residue	5.8±0.3b	6.5±0.1a	5.8±0.0b	4.9±0.0c

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

Table 8. Effect of Katepwa dough mixing on the protein concentration of Osborne solubility fraction.

Osborne Solubility Fraction	Dough Mix Time				
	0 min (flour)	1 min	3.5 min (peak)	15 min	30 min
Salt	60.4±1.6a	52.4±2.5ab	48.5±1.1b	47.8±2.3b	43.2±1.4b
Water	52.2±0.6a	48.4±0.0b	48.3±0.4b	47.1±0.0b	48.9±0.0b
Alcohol	72.7±0.8a	70.9±0.4a	73.3±1.6a	70.9±4.2a	73.4±3.2a
Acetic-acid	74.2±3.7a	76.3±4.3a	79.8±1.8a	71.2±0.4a	74.2±0.6a
Residue	5.5±0.1c	7.6±0.1a	6.5±0.3b	5.6±0.1c	5.6±0.2c

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

b. Water-Soluble Protein. The amount of water extractable protein did not change significantly during mixing for both Glenlea (8.1 to 9.7% total protein) and Katepwa (10.8 to 11.5% total protein) (Tables 5 and 6) . However, there seemed to be a slight trend of decreasing extractable protein, particularly at 30 min of mixing (Fig. 5). Paredes-Lopez (1980) found the water-soluble proteins of Glenlea flour (protein content of 12.6%) remained constant throughout mixing; although overmixing produced the greatest amount of proteins. For Neepawa (protein content of 14.1%), the undermixed sample showed the lowest amount of water-soluble protein which eventually increased with more mixing (Paredes-Lopez, 1980). Tanaka and Bushuk (1973a) also showed that mixing had no effect on the amount of water-soluble proteins.

Throughout the mixing stages, the amount of water extractable protein was significantly less for Glenlea than Katepwa (Fig. 5). This is in agreement with results from other researchers. Clusky et al (1961) showed hard and soft wheat flours contained the same amount of water-soluble protein, although total protein content was generally higher in hard wheats. In contrast, Orth (1971) found intervarietal differences in the proportion of water-soluble protein of 26 wheat varieties. Orth (1971) extracted water-soluble proteins in the range of 6.3 to 10.6%, Krull and Wall (1969) reported a range of 7 to 10%, while Wrigley and Bietz (1988) reported a higher amount of 15%. Shuey and Gilles (1973) also found that the amount of water-soluble protein was higher for soft wheat than for hard red spring wheat. Nemeth (1993) reported an average of 14.8% water extractable proteins from 11 soft wheat varieties. Finally, using a direct water extraction procedure, Faraj (1993) found that Glenlea contained the lowest amount of water-soluble protein while Neepawa contained the highest, among the varieties he studied.

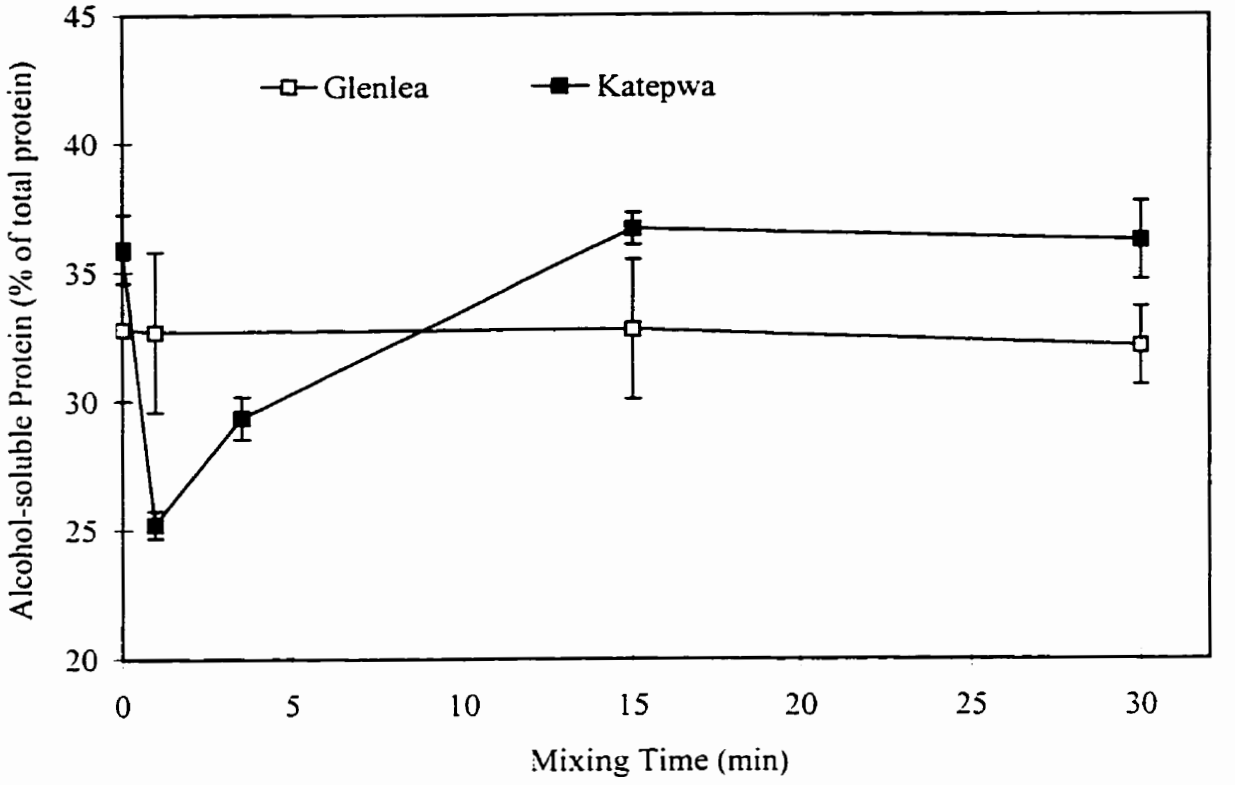
The low protein concentration (about 45%) of the water-soluble fraction shows the variability and the amount of non-protein material extracted by water at different mixing times

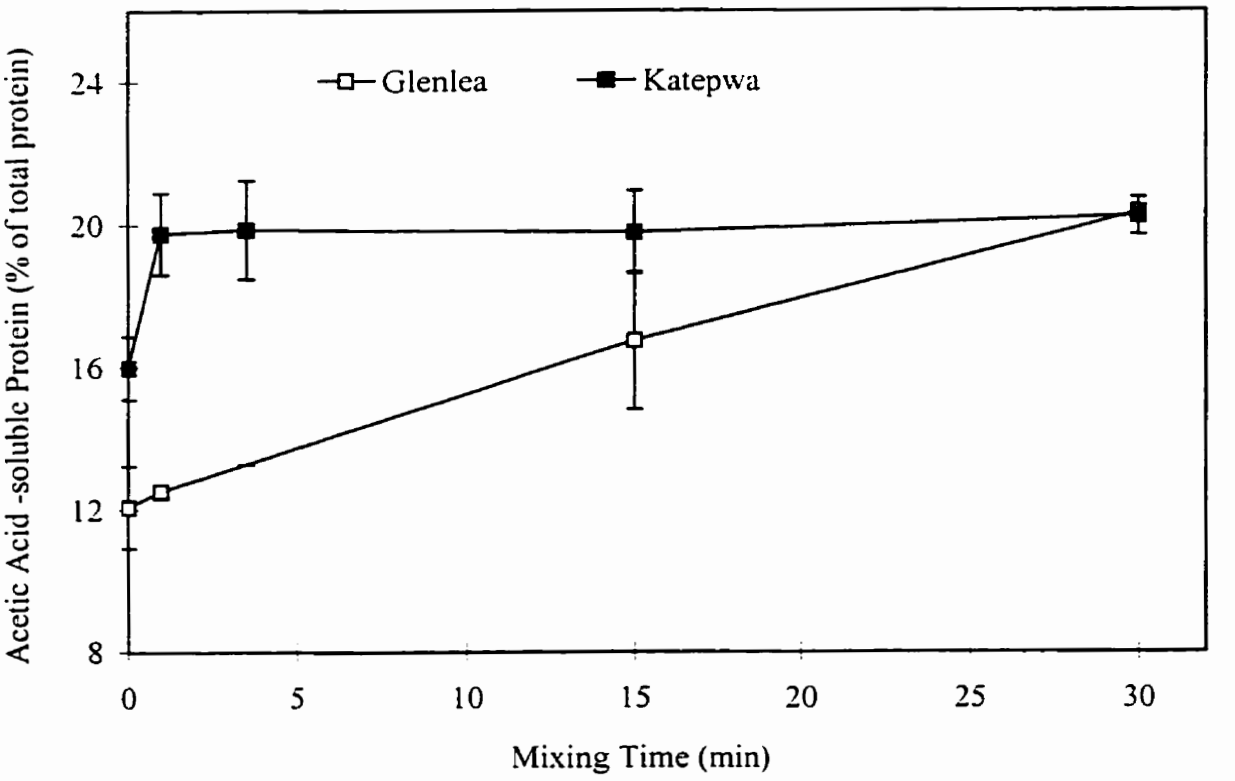
(Tables 7 and 8). For both wheat varieties, the flour samples contained the highest protein content. For Katepwa, a significant decrease in protein concentration (approximately 6%) was observed at 1 min of mixing. For 26 wheat varieties, Orth (1971) found the water-soluble protein fraction had protein concentration levels which ranged from 58.1 to 69.5%. Also, a hard red spring wheat with a flour protein content of 13.6%, had a water-soluble fraction with a protein concentration of 53.3% (Chen and Bushuk 1970).

c. Alcohol-Soluble (70% ethanol) Protein. Ethanol soluble (ES) protein fractions represented 32.8 and 35.9% of total flour protein for Glenlea and Katepwa, respectively (Tables 5 and 6). No significant varietal difference was observed with the flour samples. This is consistent with findings of Dupuis et al (1996) who also found no significant varietal differences between alcohol soluble proteins extracted from the flours of Glenlea and Katepwa. In contrast, Orth and Bushuk (1972) reported significant intercultivar differences in this fraction among flours of diverse breadmaking quality. Researchers using a modified Osborne procedure have reported varying values of ES proteins in flours. Ewart (1968) reported a value of 16.0% total protein content. Chen and Bushuk (1970) found a red spring wheat variety to contain 28.5%. Orth (1971) obtained a range from 29.1 to 41.6%. Tanaka and Bushuk (1973a) reported values of 27.6 to 31.6%. Wrigley and Bietz (1988) reported a value of 33.0% of total protein content. Lastly, Nemeth (1993) obtained a mean value of 35.4% for 11 soft wheat varieties ranging from 31.9 to 40.0%.

In contrast to the Osborne salt and water-soluble protein fractions, the Katepwa ES protein fraction was very susceptible to dough mixing effects. It is noteworthy that mixing had no significant effect on the amount of ES protein from Glenlea (32.8% of total protein) (Fig. 6). For

Figure 6. Effect of mixing time on Osborne alcohol (top) and AAS (bottom) soluble protein of cvs. Glenlea and Katepwa.


Glenlea
Katepwa
Alcohol-soluble Protein (% of total protein)
45
40
35
30
25
20
0
5
10
15
20
25
30
Mixing Time (min)


Glenlea
Katepwa
Acetic Acid -soluble Protein (% of total protein)
24
20
16
12
8
0
5
10
15
20
25
30
Mixing Time (min)

Katepwa, however, the amount of ES protein significantly decreased upon initial flour mixing (1 min) (35.9 to 25.2% total protein) and subsequently increased significantly to a maximum of 36.7% at 15 min of mixing, where it remained constant for the additional 15 min of mixing to 30 min (Table 6). As will be discussed later, the significant increase in the amount of ES protein with continued mixing was parallell by a concomitant decrease in AAI; electrophoresis results showed that the labile protein which is solubilized upon mixing of Katepwa dough, is gliadin which has apparently aggregated with glutenin upon initial mixing of the flour with water.

Tanaka and Bushuk (1973a) reported a gradual increase in the amount of ES protein when doughs were mixed in air up to 15 min; dough sampling was first performed at 5 min of mixing. Also, Paredes-Lopez (1980) found that overmixing produced a slight increase in the solubility of this fraction in Glenlea doughs, although statistical analysis was not performed. Paredes-Lopez (1980) did not observe a drop in ES protein from flour to 1 min of mixing for any of the three cultivar samples. In that study, the initial dough mixing was done at a much lowere mixer speed (37 rpm) compared to 135 rpm used in this study. Presumably, an adequate level of mixing intensity is required in order to produce the effects found in this study on the ES protein fraction.

Of the five Osborne solubility fractions, the ES fraction had one of the highest protein concentrations (about 70% for both cultivars) (Table 7 and 8). During mixing, Glenlea and Katepwa doughs did not vary in protein concentration. The ES protein concentrations are consistent with analogous results of other researchers: 75% to 92% for 26 wheat varieties (Orth 1971), and 90% for a hard red spring wheat variety (Chen and Bushuk 1970).

d. Acid-Soluble (0.05 M acetic acid) Protein. Acetic acid extracted 12.1 and 16.0% total protein from Glenlea and Katepwa flour, respectively; significant varietal differences were

observed (Tables 5 and 6). Mixing Glenlea dough for 1 min showed no significant change in the amount of protein solubilized in comparison to the amount from flour (Table 5). With further mixing to 30 min, the amount of AAS protein of Glenlea dough increased significantly and progressively (12.5 to 20.3% total protein) in a linear fashion (Fig.6)

For Katepwa however, a distinctly different solubility profile was obtained; the amount of AAS protein increased with initial mixing and remained stable for the duration of the mixing treatment (16.0 to 20.2% total protein). Katepwa flour and the undermixed treatment (1 min) sample contained significantly more AAS proteins compared to analogous samples of Glenlea. With further mixing to 30 min, the two varieties attained identical AAS protein levels. Katepwa showed a higher rate of increase in AAS protein solubility (about 4% more) in the initial mixing stage. This is in agreement with results of Tsen (1967) who found that the rate of increase of protein in the initial mixing stage, was higher for weak flours than for strong flours. Like other researchers (Mecham 1962, 1964, 1965), he also found that dilute acetic acid extracts a higher percentage of protein from weak flours than from strong flours. Orth and Bushuk (1972) suggested that a low proportion of acetic acid-soluble protein is a characteristic of a good quality wheat flour (see Literature Review). Paredes-Lopez (1980) found the amount of AAS protein increased substantially with optimum mixing for the very strong flour (Glenlea) whereas lower increases were observed for the two weaker flours in that study.

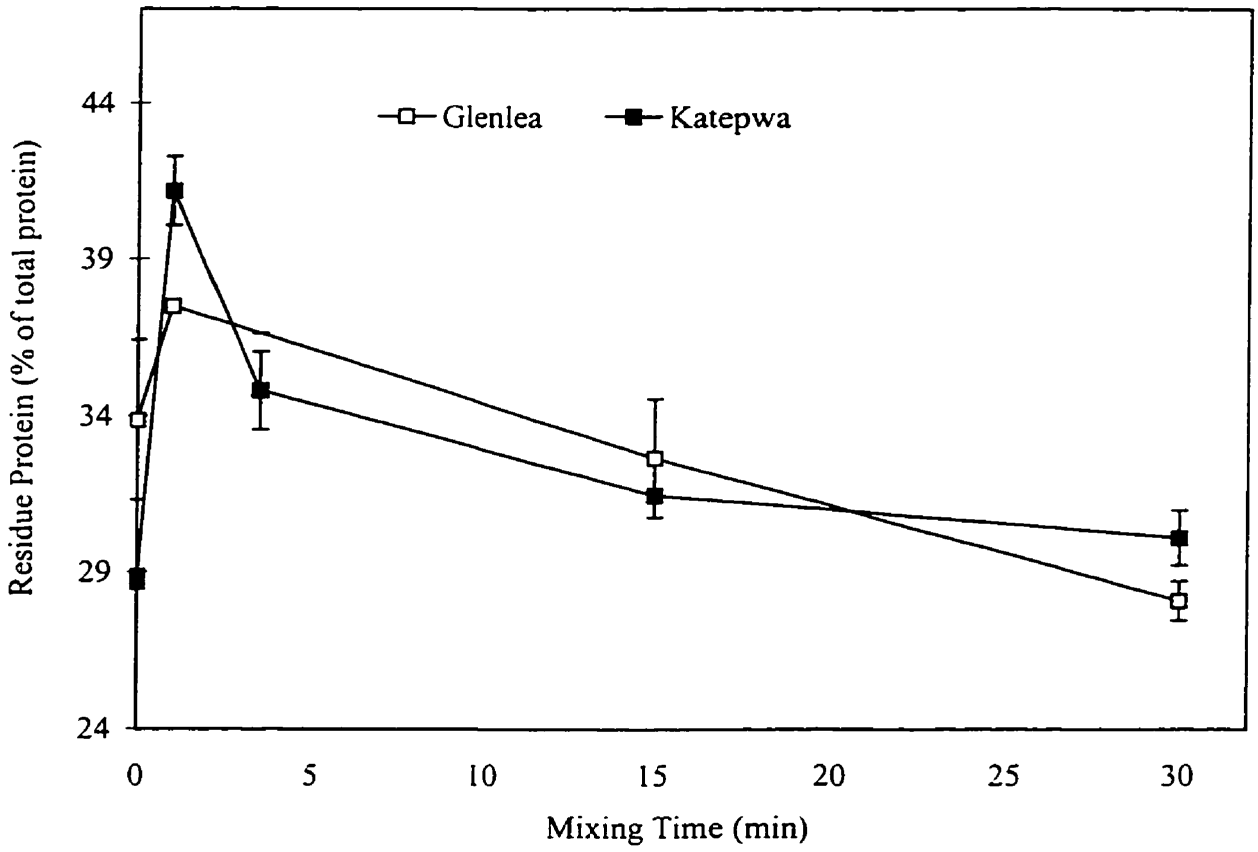
The amount of AAS protein of Glenlea and Katepwa was similar to values reported by other researchers. Paredes-Lopez (1980) obtained AAS protein content of 9.4% for flour and 10.8% for undermixed dough and 18.5% for dough for a Glenlea sample with a flour protein content of 12.6%. Wrigley and Bietz (1988) reported a value of 16% protein content for AAS soluble protein. Khan et al (1990) reported values expressed as percent of total gluten protein for

cultivars containing 12.4 and 13.2% flour protein; the former contained 14% AAS proteins and the latter to contain 16%. Finally, Nemeth (1993) obtained a mean value of 15.9% for 11 soft wheat varieties ranging from 11.8 to 22.7%

Like the ES fraction, this fraction also had a high protein concentration (Tables 7 and 8). The protein concentration of the AAS fraction varied from 62.7% (flour) to 75.8% (30 min mix) for Glenlea, and 71.3% (15 min mix) to 76.3% (1 min mix) for Katepwa. Thus, the protein concentration of the fractions of the Katepwa flour and undermixed samples was higher than that of Glenlea but equalized with further mixing. Chen and Bushuk (1970) reported a value of 70.2% for the AAS protein concentration of a hard red spring wheat with a flour protein content of 13.6%. These values are similar to those determined by Orth (1971) who obtained values with range 66.9 to 88.5% for 26 varieties.

e. Residue (0.05M acetic acid-insoluble) Protein. Compared to other Osborne fractions, the effect of dough mixing on residue protein solubility produced very distinct and similar results for Glenlea and Katepwa samples (Tables 5 and 6, Fig. 7); there was an initial increase in residue protein upon flour hydration (1 min mix), followed by a progressive decline in the amount of residue protein to 30 min of mixing. For Glenlea, the total amount of protein in the AAI fraction increased with initial mixing to 37.5% from 33.9% for flour (Table 5); mixing to 30 min reduced the amount of AAI protein to 28.1% (Fig. 7). Although the 3.6% increase (cf. Flour) in Glelea AAI protein at 1 min of mixing was not significant ($p < 0.05$), the increase is most likely real as the difference was significant at $p < 0.10$ compared to AAI protein of dough mixed to 15 and 30 min. Similarly, Katepwa AAI protein increased (cf. flour) significnatly by more than 12% (to 41.2% total protein) with 1 min of mixing and subsequently decreased

Figure 7. Effect of mixing on Osborne residue protein of Glenlea and Katepwa flours.


Glenlea
Katepwa
Residue Protein (% of total protein)
44
39
34
29
24
0
5
10
15
20
25
30
Mixing Time (min)

significantly to 31.5% at 15 min of mixing and then remained constant (Table 6, Fig. 7). Consistent with the differences in dough mixing requirements of Glenlea and Katepwa, the residue protein content of both cultivars were significantly different at the flour and premixed stages. Glenlea contained significantly more AAI protein than Katepwa (Fig. 7).

Paredes-Lopez (1980) showed that the amount of AAI protein in dough decreased with increasing work input for flours varying in mixing strength. He also found that the decrease was greatest for the strongest flour, Glenlea. Paredes-Lopez (1980) suggested that the stronger the flour, the greater the effect of development on glutenin solubilization. In contrast, Tanaka and Bushuk (1973a) found that the amount of AAI residue protein decreased with increased mixing time, with no relationship to intrinsic mixing strength of the flours examined.

Orth (1971) reported AAI protein contents ranging from 15 to 36.5% (mean of 26.9%) for 26 wheat varieties. Nemeth (1993) obtained a mean value of 29.3% for the Osborne residue protein fraction of 11 soft wheat varieties ranging from 21.0 to 36.0%.

Of the five fractions analyzed, the AAI fraction had the lowest protein concentration (Tables 7 and 8); i.e. 6% averaged over all mixing treatments. Orth (1971) found the protein concentration in 26 insoluble Osborne residues varied from 2.8 to 8.1%. The low protein concentration of the AAI residue was due to the predominance of starch in the residue fraction. The protein concentration of the AAI fraction of Katepwa increased significantly from 5.5% to 7.6% after 1 min of mixing. This significant increase in residue protein concentration upon flour hydration, was also observed in Glenlea but to a lesser degree, i.e. from 5.8% to 6.5%. With further dough development, both wheat cultivars showed decreasing trend in the AAI residue protein concentration, although after 15 min of mixing, the Katepwa fraction remained constant. The protein concentration of Katepwa was higher than Glenlea at the undermixed, PDD and

overmixed stages. At 15 min of mixing, however, the protein concentration of Glenlea and Katepwa residue fractions were equivalent.

D. Influence of Mixing on the Electrophoretic Compositions of the Protein Fractions

The possibility of qualitative and quantitative effects due to mixing on the protein composition was investigated using SDS-PAGE and A-PAGE. Gel electrophoresis can resolve a complex mixture of proteins based on their molecular size and/or charges. SDS-PAGE separates proteins according to their molecular size and A-PAGE separates proteins according to their molecular size and net positive charge. SDS-PAGE was chosen for this study because it can be applied to all the fractions of freeze-dried proteins both before and after reduction of disulphide bonds. Also, the effect of mixing on "slot" proteins were investigated with a two-step one-dimensional electrophoretic procedure. Slot protein refers to protein too large to enter the polyacrylamide gel by SDS-PAGE under non-reducing conditions. Accordingly, this protein remains in the sample application slot during the electrophoresis run.

In discussing an electrophoretic pattern result (electrophoregram), the term "qualitative" change is used to indicate the presence or absence of a band. The term "quantitative" change is used to indicate relative change in the staining intensity of a band as determined by visual inspection. Some of the electrophoregram results that are discussed may not be readily apparent from the figures due to loss of detail in the photographic results.

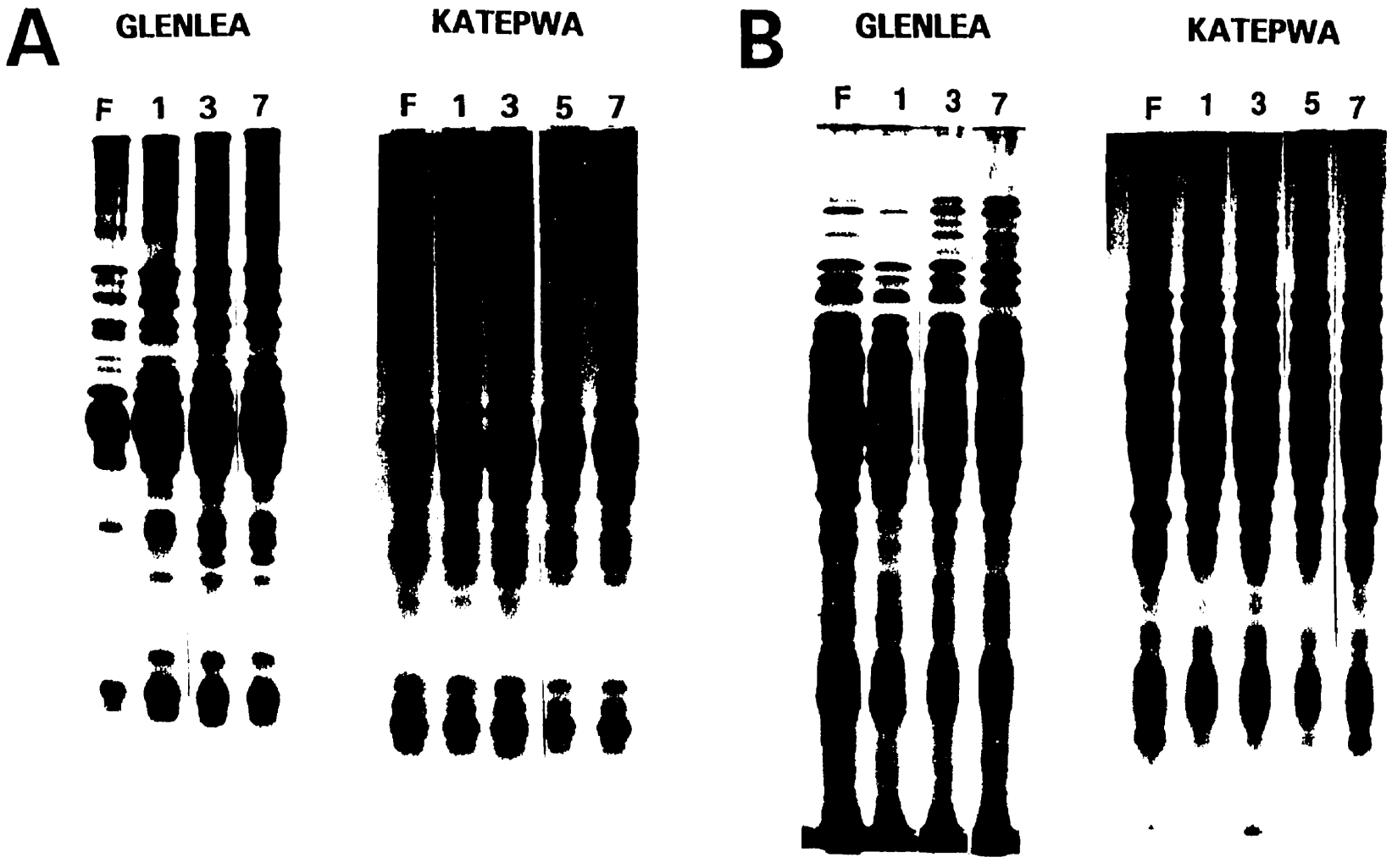
1. Electrophoretic Compositions of the Direct 0.05 M Acetic Acid Fractions

SDS-PAGE patterns of the AAS fraction (obtained by direct extraction) of Glenlea under non-reducing conditions (Fig. 8A) showed an increase in staining intensity with increasing dough mixing. Also, the background streaking became more intense as mixing time increased. This indicates that more of the glutenin was being rendered soluble. This electrophoregram is consistent with the increased solubilization of proteins during mixing (Fig. 4) which was an expected result based on previous work (see Literature Review). This solubilized polymeric proteins as glutenin is still much too large to resolve as discrete bands and is thus visualized under non-reducing conditions as background streaking. SDS-PAGE of direct extracts of AAS protein under reducing conditions (Fig. 8A) showed an increase in the band intensity of HMW-GS with increasing dough mixing.

There are many theories on what causes background streaking in electrophoresis results. Nakamura and Handu (1984) speculated that streaking is probably caused by the presence of lipids since Coomassie Brilliant Blue is known to be a dye for lipids. Others have suggested that the streaking is probably caused by other subunits of disulphide-linked poteins of the flour or dough that does not enter the gel. The streaking may also represent HMW proteins that have a strong tendency to aggregate (Singh and Shepherd 1985, Sievert et al 1991a). Singh and Shepherd (1985) have shown the disulphide-linked proteins to be involved in formation of the dark background streaking that is observed in electrophoregrams under non-reducing conditions. The identity of these streaking components remains to be elucidated. However, in this study, it appears to be due to unresolved polymeric protein.

The SDS-PAGE patterns of the AAS fraction of Katepwa under non-reducing and reducing conditions (Fig. 8) indicated no effect of mixing. Katepwa had more intensely stained stained

Figure 8. SDS-PAGE of unreduced (A) and reduced (B) direct 0.05 M acetic acid-soluble protein of flour and dough subjected to various mixing. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.


A
GLENLEA
F 1 3 7
KATEPWA
F 1 3 5 7
B
GLENLEA
F 1 3 7
KATEPWA
F 1 3 5 7

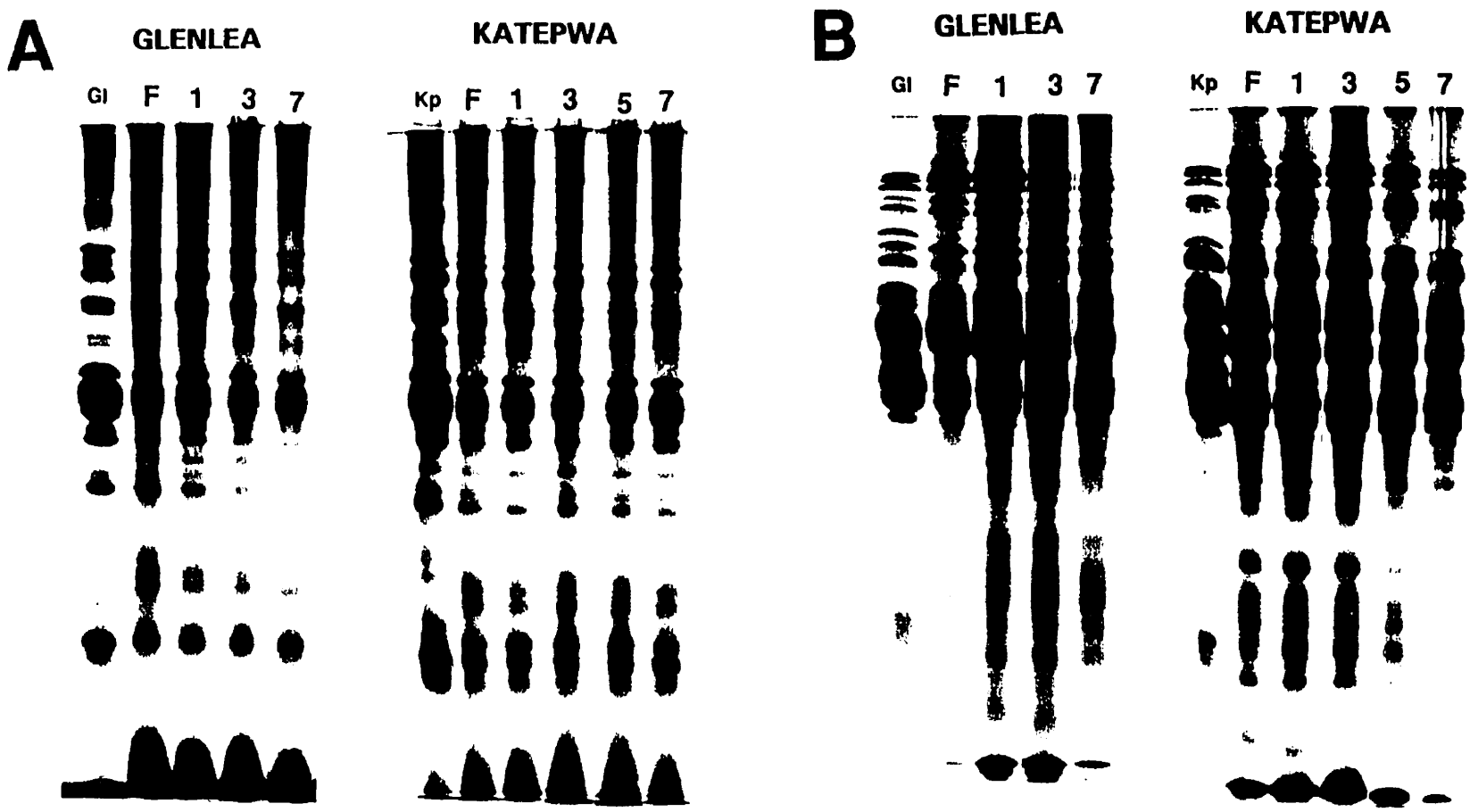
electrophoregrams, which is consistent with a higher extraction level by acetic acid (Table 3). The higher amount of extracted protein and therefore darker bands, made it much more difficult to show effects of mixing, which were significant only for the 1 min mixing treatment.

Electrophoregrams of the direct AAI protein fraction under non-reducing and reducing conditions are shown in Fig. 9. For the unreduced extract of Glenlea, there was a decrease in staining intensity of a tight cluster of relatively HMW diffused bands of low mobility, as mixing time increased. Concurrently, the staining intensity of the other bands and level of background streaking decreased with mixing time. SDS-PAGE patterns of direct extracts from AAI residues prepared under reducing conditions, showed a pronounced increase in background dtreaking for dough samples staining at (Fig. 9B, Gl- lane 3 and Kp- lane 5).

For Glenlea, the HMW cluster of bands in reduced AAI protein show the characteristic pattern of the triplet band (triticin) proteins described by Singh and Shepherd (1985) and Sievert et al (1991a,b). Under reduced conditions, these same protein bands disappeared from the electrophoretic patterns, thus, confirming that triplet band proteins are disulphide-linked and can be reduced to lower molecular weight components. This is consistent with observations by Sievert et al (1991a).

Compared to Glenlea, SDS-PAGE direct extracts of unreduced and reduced AAI proteins from flour and doughs of Katepwa (Fig.9A) did not show as noticeable effects. When the Katepwa samples were reduced (Fig. 9B) the background streaking appeared tp increase from flour to the PDD sample and declined with further mixing.

Figure 9. SDS-PAGE of unreduced (A) and reduced (B) direct 0.05 M acetic acid-insoluble protein of flour and dough subjected to various mixing treatments. As defined in Table 2. F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.


A
GLENLEA
KATEPWA
Gl F 1 3 7
Kp F 1 3 5 7
B
GLENLEA
KATEPWA
Gl F 1 3 7
Kp F 1 3 5 7

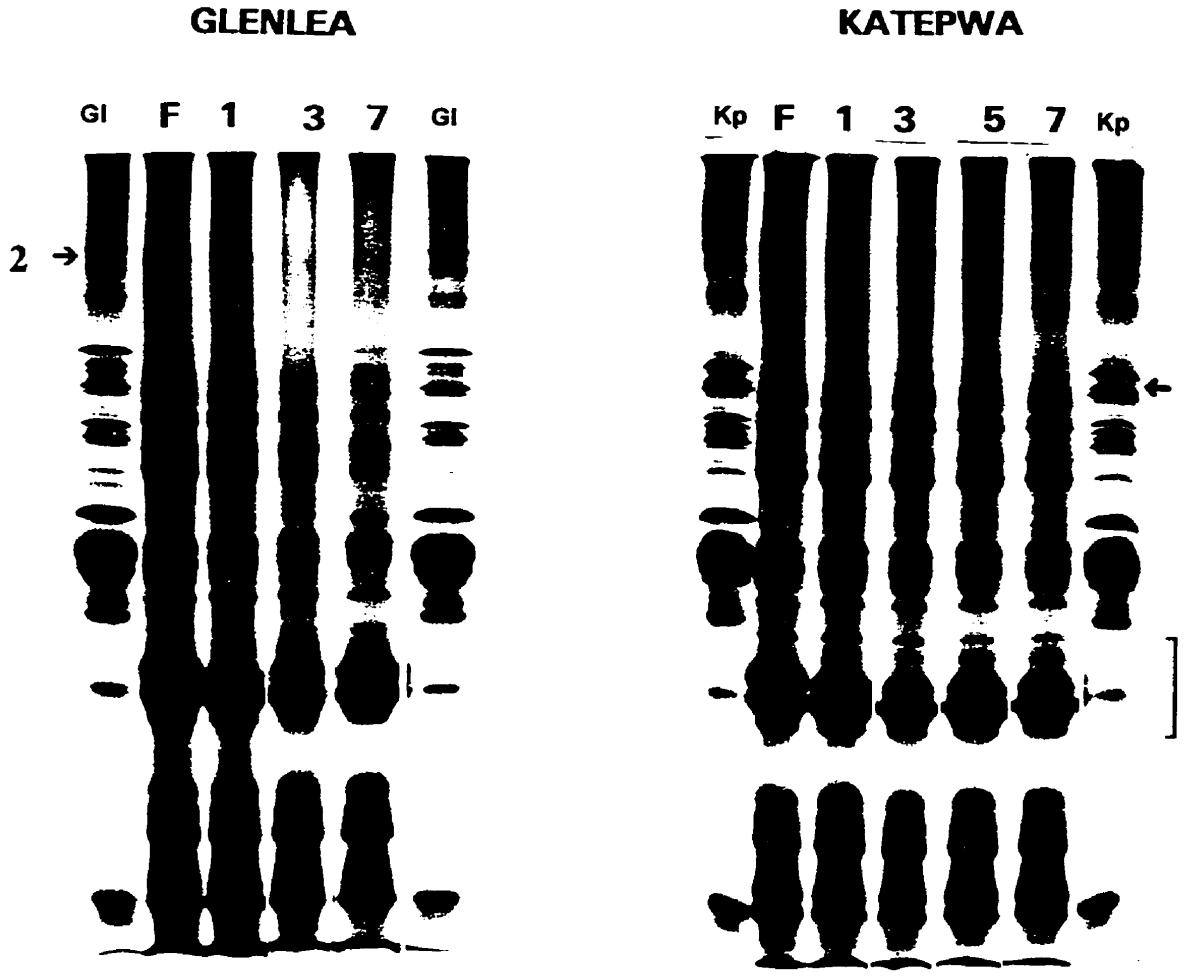
2. Electrophoretic Composition of the Osborne Protein Fractions

a. Salt-Soluble (0.5 M NaCl) Protein. Results of the SDS-PAGE under non-reducing conditions of the salt-soluble protein fractions are shown in Fig. 10. No clear trend in changing protein composition with mixing was evident except for Glenlea doughs mixed to PDD where a noticeable reduction in band intensities were observed. Theses results are on the whole consistent with the quantitative results (Table 5 and 6) showing a decline in proteincontent and concentration of the Osborne salt-soluble fraction with mixing. For Katepwa, no noticeable effects of dough mixing was observed for SDS-PAGE of unreduced Osborne salt-soluble protein. Prominent globulin proteins (brackets) can be seen in the electrophoregrams of both cultivars. The globulin proteins were faint in the water-soluble fractions and were very faint or absent in the other Osborne fractions.

The Glenlea flour and undermixed (1 min) samples (Fig. 10, lanes F and 1), under non-reducing conditions, stained the darkest and showed large amounts of background streaking. Triplet band proteins were also observed (Fig. 10, arrow 2). Compared to the flour and undermixed samples (lanes F and 1), the PDD (lane 3), and overmixed (lane 7) samples stained much less in the upper half of the gel slab, and were devoid of the triplet bands. Also, the PDD and overmixed samples showed only slight background streaking when compared to the other mixing treatments.

Like Glenlea, for Katepwa's salt-soluble protein result, the triplet zone bands are particularly evident in the non-reduced flour, undermixed, and PDD samples (Fig. 10, lanes F,1 and 3, respectively). The intensity of bands in this region of the gel decreased with increasing mixing. One band in particular was present only in the PDD sample (Fig. 10, arrow, lane 3). Like the Glenlea result (Fig 10), background streaking for Katepwa mixing treatments decreased as mixing

Figure 10. SDS-PAGE of unreduced Osborne salt-soluble protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.


GLENLEA
KATEPWA
Gl F 1 3 7 Gl
2
Kp F 1 3 5 7 Kp

time increased.

The unreduced salt-soluble protein fraction was characterized by a substantial amount of protein that did not enter the gel and remained in the slot. Two-step electrophoresis was performed to determine the nature of this HMW protein material. Figure 11 shows that Katepwa had much more of this protein than Glenlea. For Katepwa, Figure 11 shows background streaking of the samples increased with dough development. Interestingly, one Katepwa band (Fig. 11, arrow 1) decreased significantly after peak development (Fig. 11, lanes 3-7). Dough mixing had no effect on the reduced slot proteins of Glenlea. This result does not correspond with results shown in Fig. 10 indicating intense streaking and slot proteins for flour and 1 min mixed dough. Lastly, the background streaking in both Katepwa and Glenlea reduced slot protein samples were observed only in the Osborne salt-soluble protein fractions but not in slot protein of total protein extracts (Fig. 11, lanes Gl and Kp).

Upon reduction of the salt-soluble protein fractions (Fig. 12), flour and dough samples of both cultivars contained a few faint bands in the HMW-GS region of the electrophoregram. These HMW bands were most prominent in the undermixed (1 min) sample of Glenlea (lane 1) and especially the PDD sample of Katepwa (lane 3), and became fainter with increased mixing. These bands appear to be x-type HMW-GS. This was surprising as glutenin is generally believed to be insoluble in salt solutions. Another darkly stained band in the HMW region (arrow 1) was observed in the Katepwa results; the intensity of this band clearly decreased as mixing time increased. The considerable amount of background streaking and slot protein in the unreduced Osborne salt-soluble protein results has been noted in the discussion of Fig. 11.

The A-PAGE of the salt-soluble protein fraction (Fig. 13) showed the presence of intensely stained albumin and globulin proteins. There was also a general absence of bands co-migrating

Figure 11. Two-step one dimensional SDS-PAGE of unreducedOsborne salt-soluble slot protein of flour and dough subjected to various mixing treatments. As defined in Table 2. F = flour, 1 = premix, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, slot protein of total protein extracts. Gl2 = Glenlea and Kp2 = Katepwa, total protein extracts.

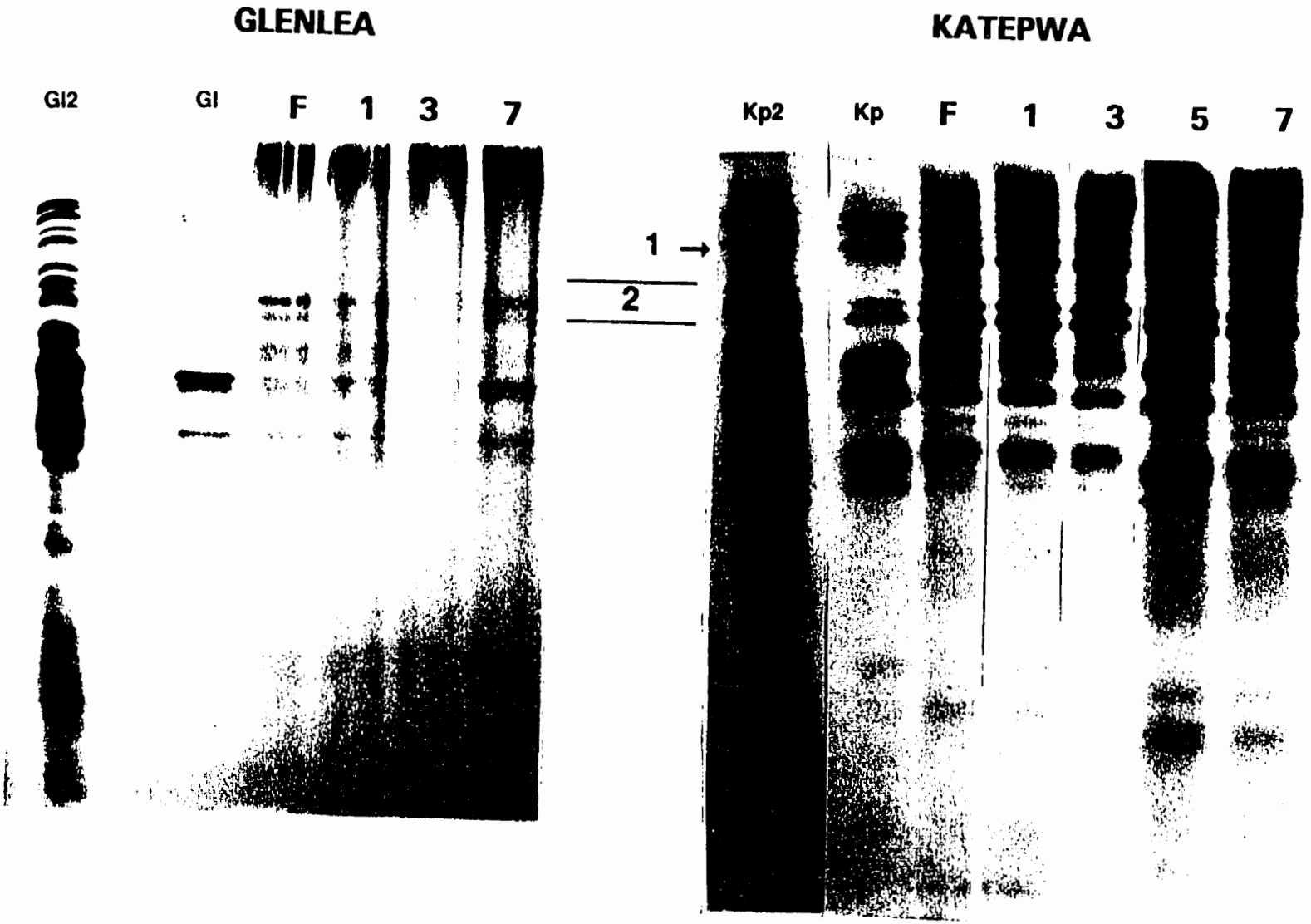

GLENLEA
KATEPWA
Gl2
Gl
F
1
3
7
Kp2
Kp
F
1
3
5
7
1
2

Figure 12. SDS-PAGE of reduced Osborne salt-soluble protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

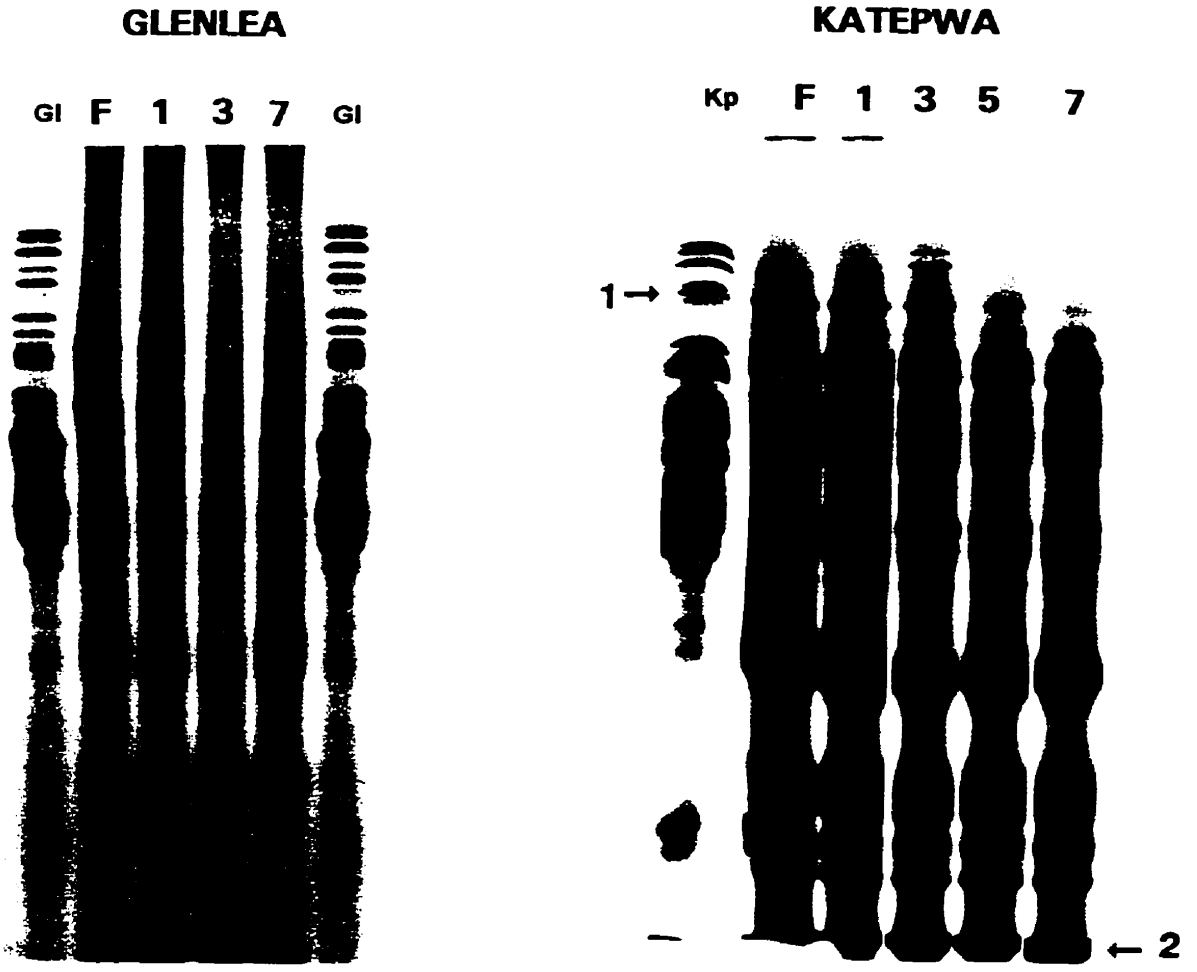
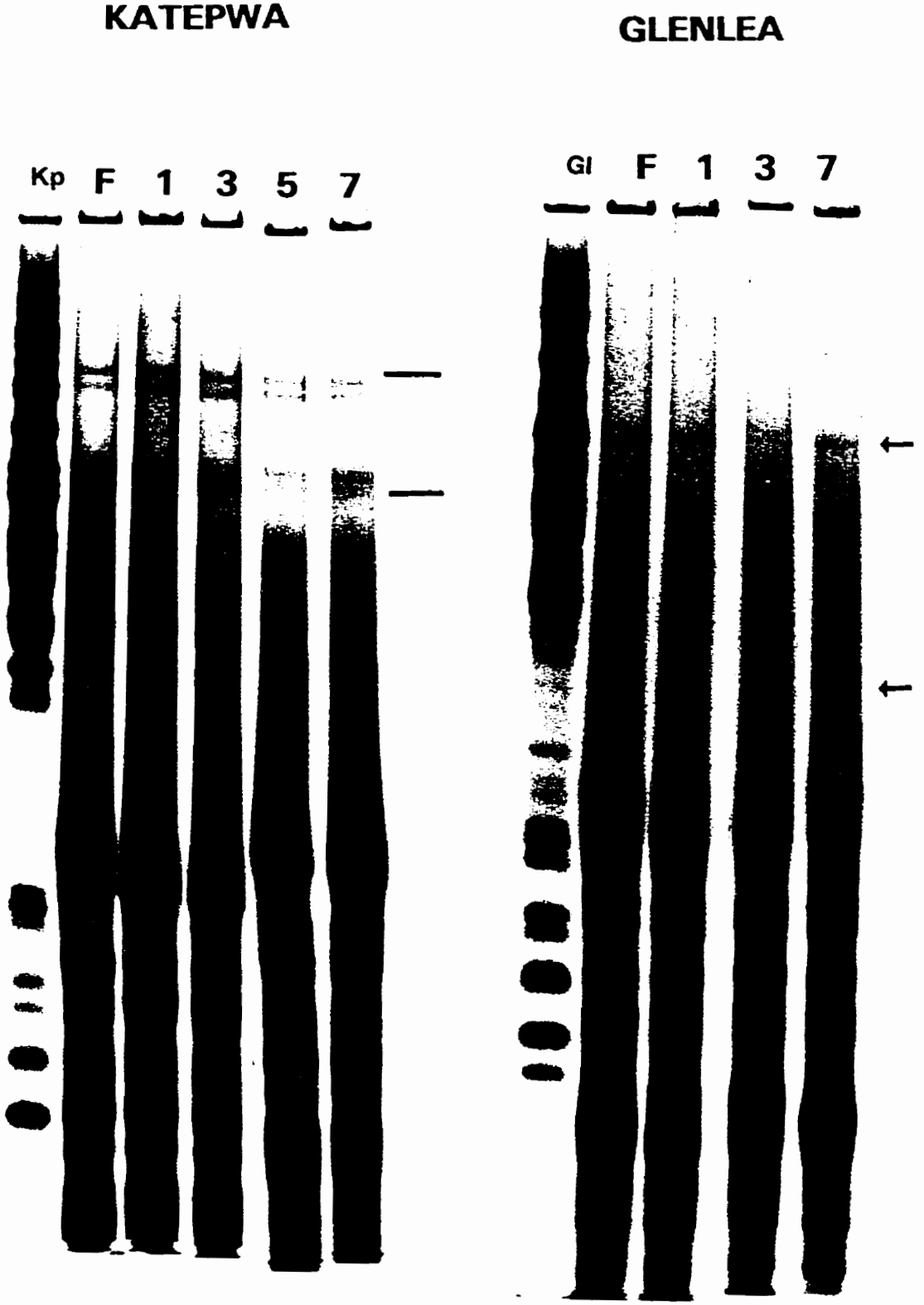

GLENLEA
Gl F 1 3 7 Gl
KATEPWA
Kp F 1 3 5 7
1→
← 2

Figure 13. A-PAGE of Osborne salt-soluble proteins of flours and dough subjected to various mixing treatments. The freeze-dried samples were resolubilized in 0.5 M NaCl solution.As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.


KATEPWA
GLENLEA
Kp
F
1
3
5
7
Gl
F
1
3
7

in the gliadin mobility range. Katepwa's electrophoregram did not reveal a significant dough mixing effect. Compared to Katepwa, Glenlea's A-PAGE results was similar, except for the absence of two pairs of relatively low mobility bands (bracketed) in the electrophoretic pattern of Katepwa.

b. Water-Soluble. SDS-PAGE of unreduced water-extractable protein fractions are shown in Fig. 14. For both Glenlea and Katepwa, this fraction contained substantial amounts of gliadins in addition to albumin proteins. The fact that a considerable quantity of gliadins were extracted suggest the initial fractionation step using 0.5 M NaCl does not prevent the solubilization of some gliadins. Chen and Bushuk (1970) modified the Osborne fractionation procedure (Osborne 1907) by initially extracting with 0.5 M NaCl ostensibly to prevent the gliadins from being extracted. However, the modified Osborne procedure is apparently not successful in this regard (Figs. 14, 15, and 16). Chen and Bushuk (1970) did not report electrophoresis results in their study.

Under non-reducing conditions (Fig. 14), some qualitative changes were observed in the pattern of water-soluble Glenlea extracts as mixing time increased. One band (arrow 1), which was quite prominent in the control total flour protein extract (Gl), was very faint in the Osborne water-extractable fraction of Glenlea flour (F). This band appeared to increase in staining intensity as mixing time increased. For Katepwa, a band with similar electrophoretic mobility showed an increase in staining intensity with overmixing (Katepwa, lane 7). Another Glenlea protein band (arrow 2) appeared to decrease in staining intensity as mixing time increased. As well, for Katepwa, there was a noticeable reduction in the intensity of the slot proteins with mixing. Two-step electrophoresis of the slot protein (results not shown) indicated that the reduction in staining of Katepwa slot protein was not due to glutenin protein, ie. no bands were present in the

Figure 14. SDS-PAGE of unreduced Osborne water-extractable protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed. 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

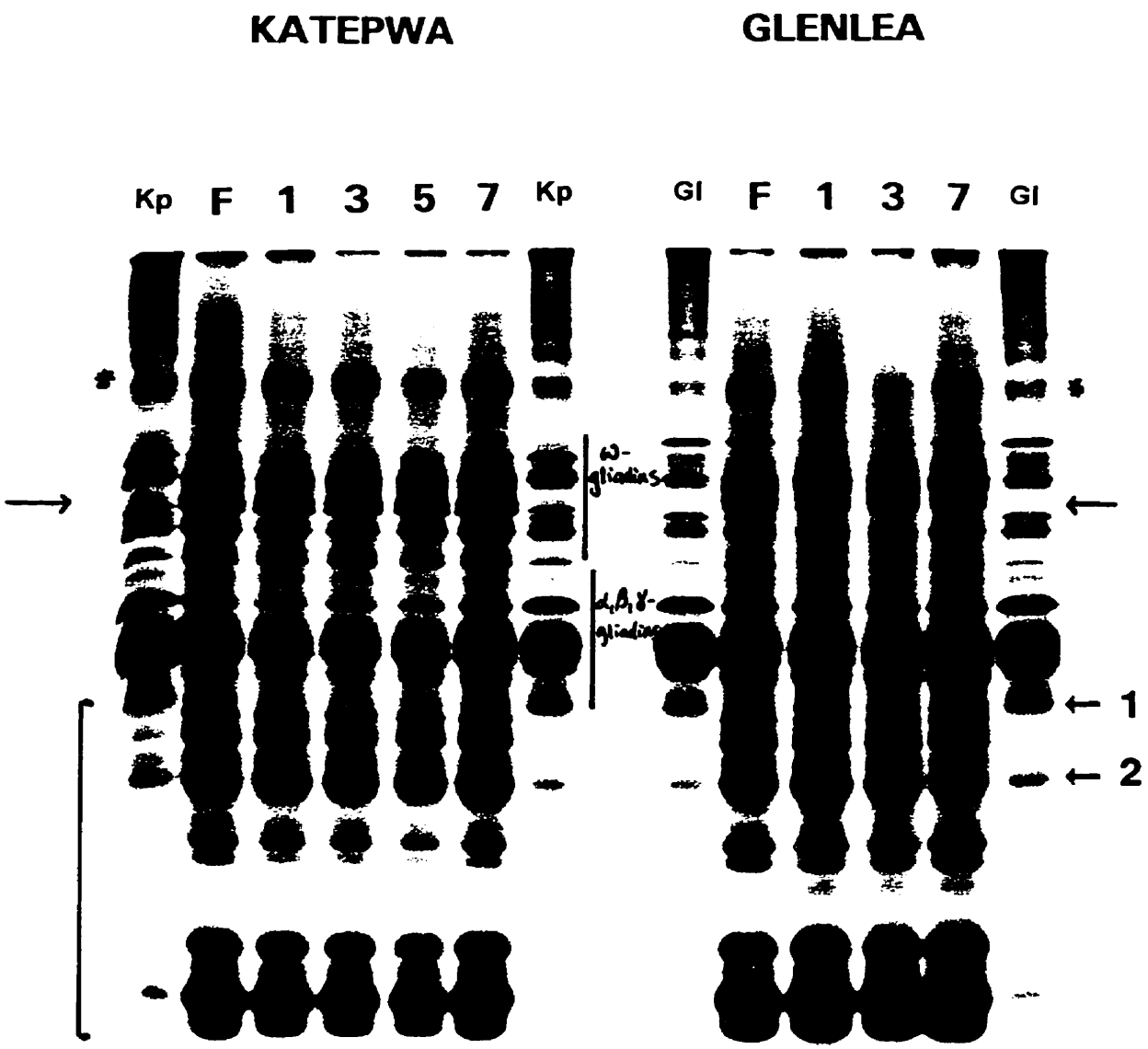
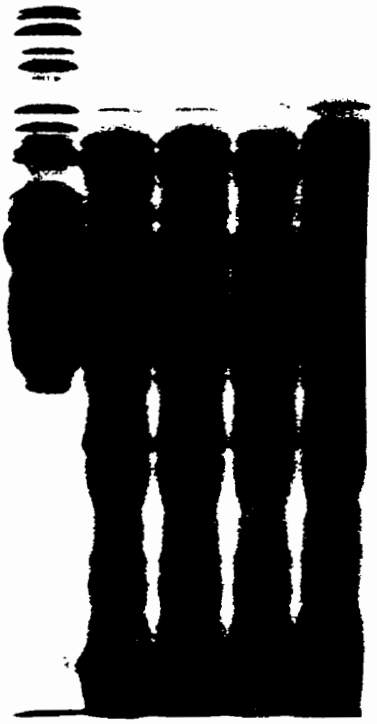

KATEPWA
GLENLEA
Kp
F
1
3
5
7
Kp
Gl
F
1
3
7
Gl
ω-gliadins
α,β,γ-gliadins
1
2

Figure 15. SDS-PAGE of reduced Osborne water-extractable protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.

GLENLEA

Gl F 1 3 7



KATEPWA

Kp F 1 3 5 7

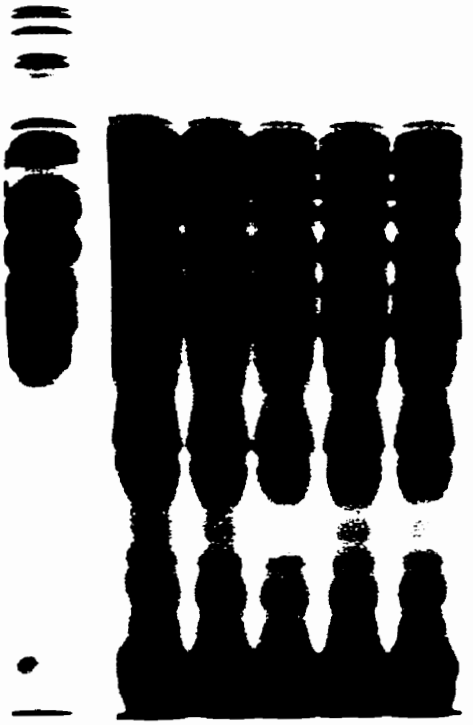
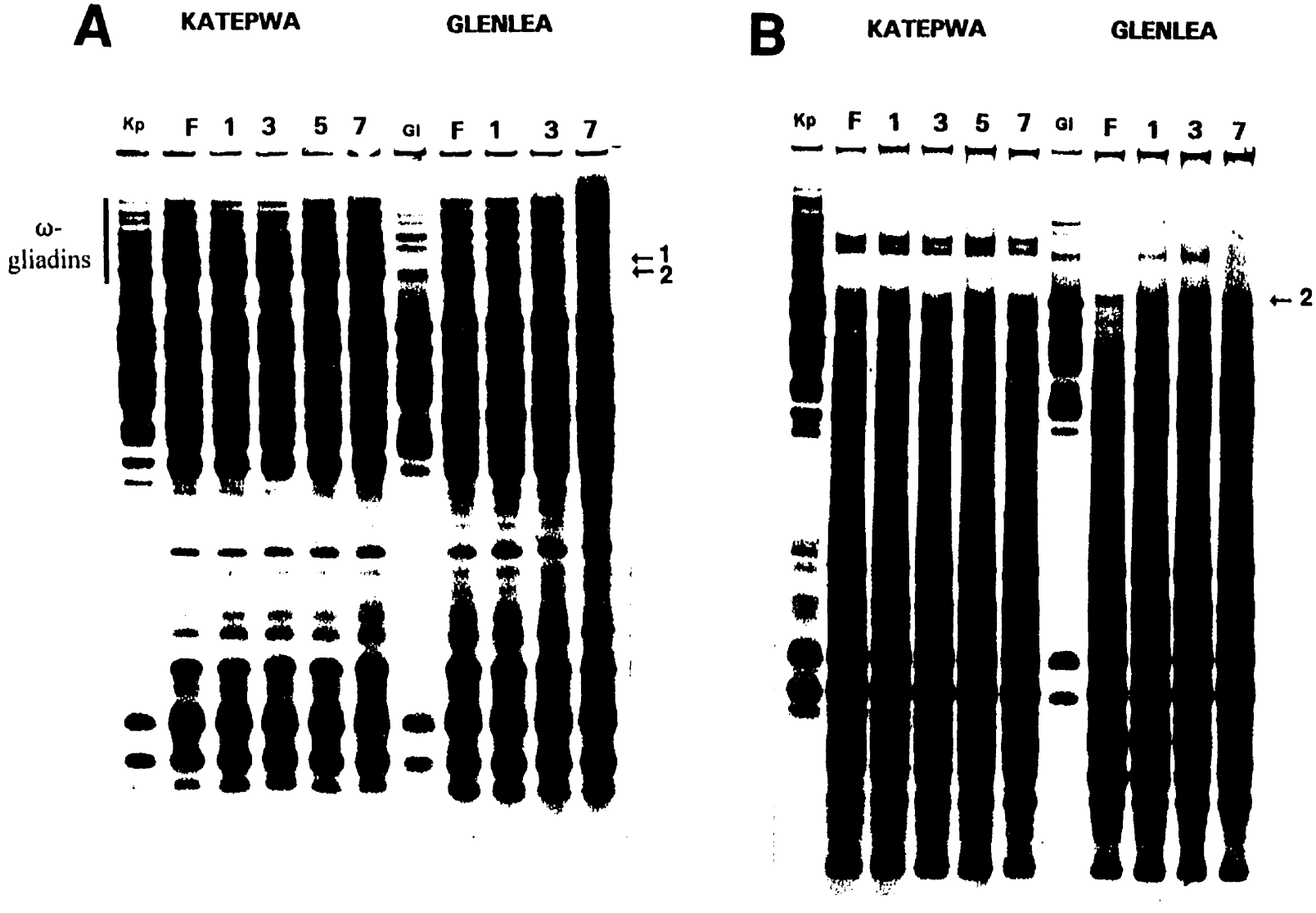


Figure 16. A-PAGE of Osborne water-extractable protein of flour and dough subjected to various mixing treatments. The freeze-dried samples were resolubilized for electrophoresis in 70% ethanol (A) and water (B). As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.


A
KATEPWA
GLENLEA
Kp F 1 3 5 7 Gl F 1 3 7
ω-
gliadins
1
2
B
KATEPWA
GLENLEA
Kp F 1 3 5 7 Gl F 1 3 7
2

electrophoregram of the two-step procedure.

SDS-PAGE of reduced water-soluble proteins (Fig. 15) was noteworthy for the complete absence of HMW-GS and likely LMW-GS as well. With one exception, no effect of mixing was found. The electrophoretic pattern of Glenlea at PDD (lane 3) was noticeably less intense than the other samples. The electrophoretic results of Osborne water-soluble protein corresponds with Fig. 5 and Tables 5 and 6 which showed no significant mixing effects.

For A-PAGE (Fig. 16) two different solvents were used to solubilize different groups of proteins. Fig. 16 showed that more of the gliadins and less of the albumin and globulin proteins were solubilized by ethanol. Conversely, less gliadin protein and more albumin and globulin proteins were solubilized by water. Compared to Glenlea, the electrophoretic patterns of the extracts of the water-soluble proteins of Katepwa resolubilized in 70% ethanol showed considerably more ω-gliadins (Fig. 16). This distinction was enhanced for water-soluble proteins resolubilized in water; Glenlea hardly showed any ω-gliadins. A-PAGE of the water-soluble protein fraction of Katepwa was not qualitatively affected by mixing. In contrast, some minor effects of mixing were observed in Glenlea (see bands denoted by arrows 1 and 2).

c. Alcohol-Soluble (70% ethanol) Protein. SDS-PAGE of unreduced ES protein (Fig. 17) showed, for both Glenlea and Katepwa, the presence of gliadins, some oligomeric glutenin (ladder-like bands near the slots), considerable amount of unresolved polymeric glutenin (as slot protein and background streaking), and some non-gluten proteins evidenced as relatively faint bands with high relative mobility. The ladder-like pattern of glutenin oligomers was much more pronounced for Katepwa samples than for Glenlea counterparts.

Two-step SDS-PAGE of the slot proteins (Fig. 18) confirmed that the Osborne alcohol-

Figure 17. SDS-PAGE of unreduced Osborne alcohol-soluble protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

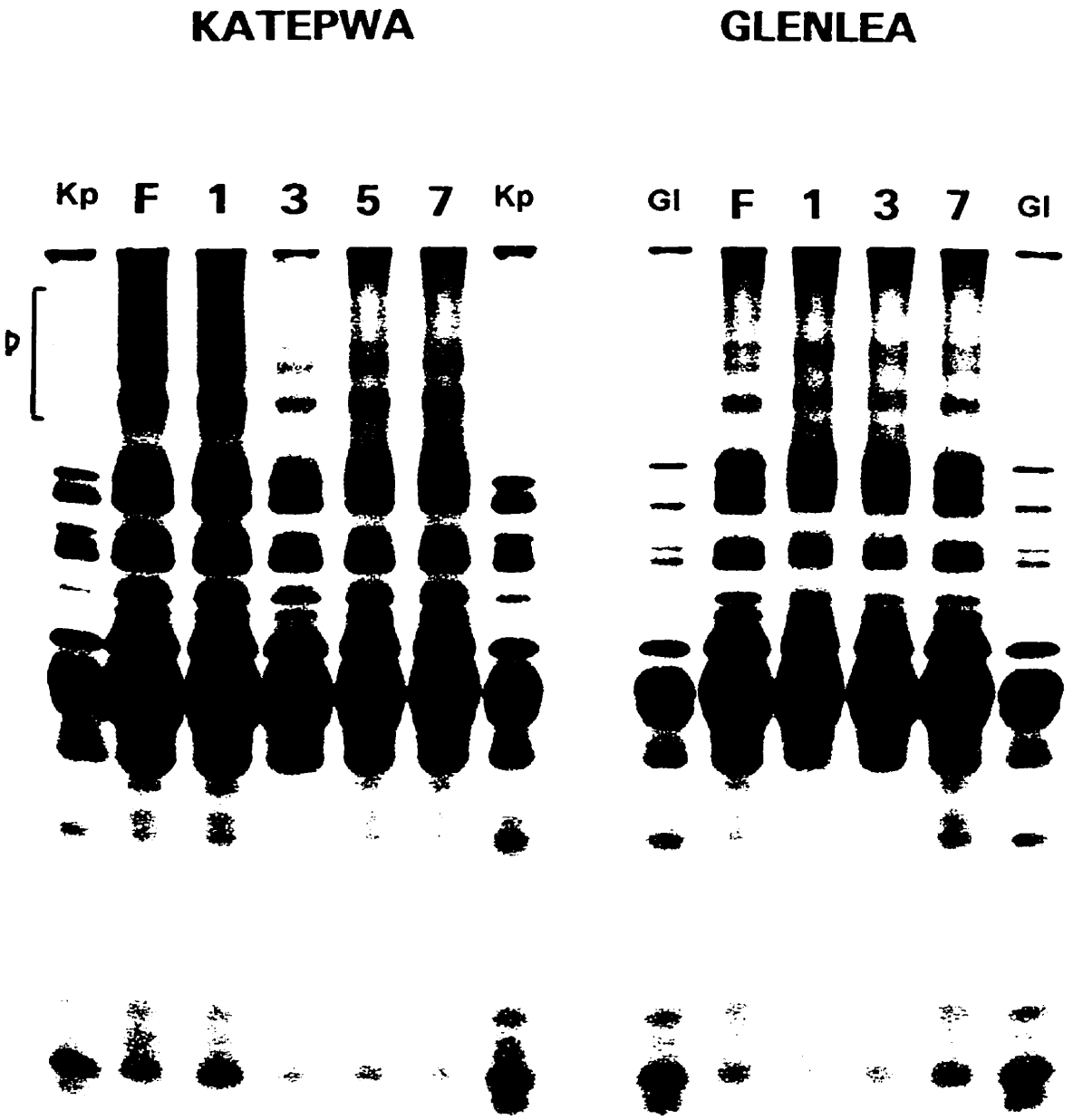
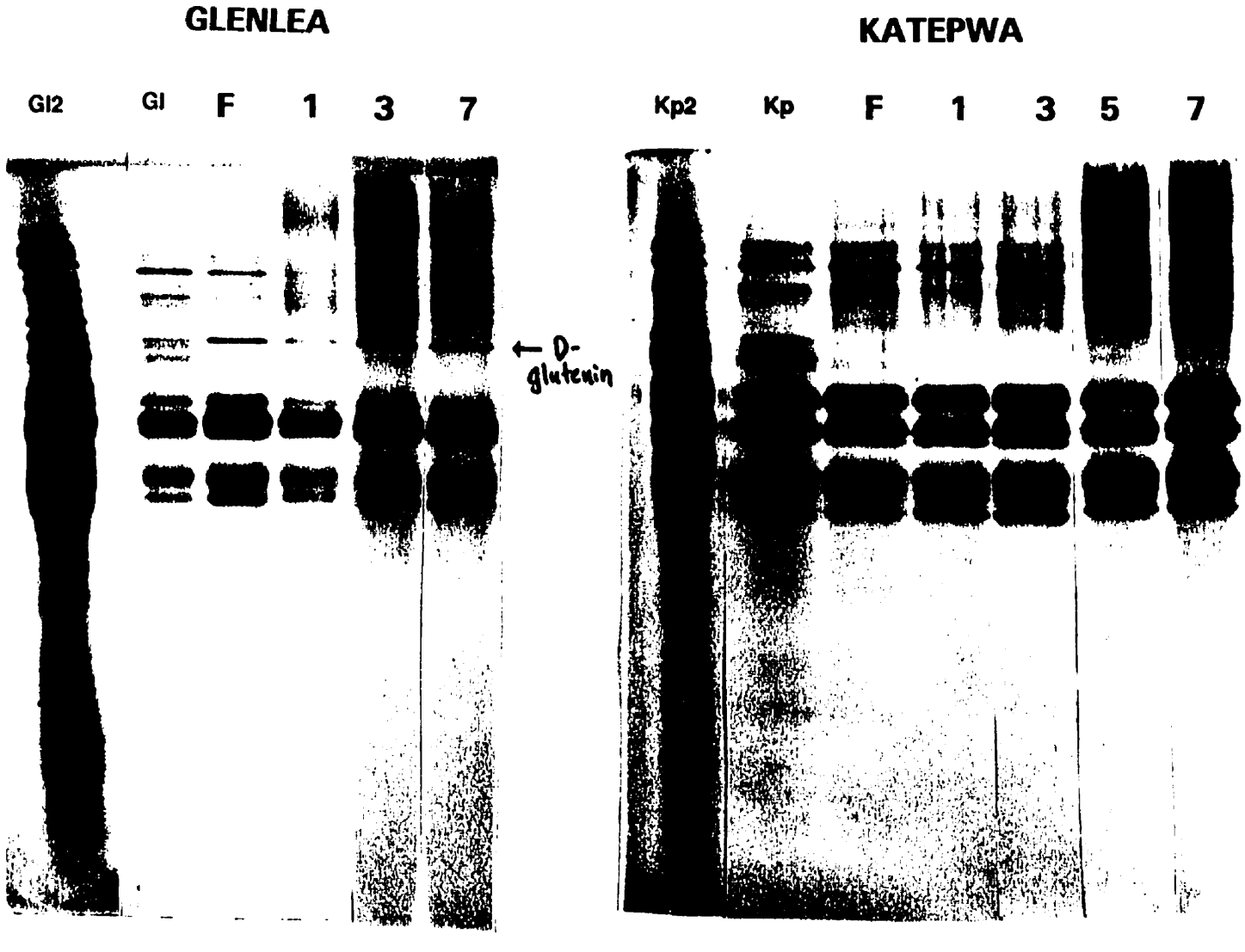

KATEPWA
GLENLEA
Kp
F
1
3
5
7
Kp
Gl
F
1
3
7
Gl
p

Figure 18. Two-step one dimensional SDS-PAGE of unreduced Osborne alcohol-soluble slot protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = premix, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, slot protein of total protein extracts. Gl2 = Glenlea and Kp2 = Katepwa, total protein extracts.


GLENLEA
Gl2
Gl
F
1
3
7
← D-glutenin
KATEPWA
Kp2
Kp
F
1
3
5
7

soluble protein fractions contained glutenin (predominantly LMW-GS), and that the Katepwa flour and mixing treatment samples contained more of the polymeric protein, than did the Glenlea extracts. It is interesting that the Katepwa slot protein contained both LMW-GS and all HMW-GS (in lesser amounts), whereas for Glenlea, relatively few HMW-GS bands were resolved in the slot protein of flour and dough. SDS-PAGE of the reduced alcohol-soluble protein (Fig. 19) showed the presence of large quantities of gliadins and presumably LMW-GS (which co-migrate with the gliadins). By contrast, very little HMW-GS polypeptides were present as indicated by the very faint bands in the HMW region of the reduced SDS-PAGE result. A-PAGE results (Fig. 20), were unremarkable as characterisitic patterns of gliadins were obtained for Katepwa and Glenlea flour and dough samples, and very little albumin/globulin proteins were present.

In regard to dough mixing, some evidence of an effect on alcohol-soluble proteins can be seen in the unreduced SDS-PAGE result (Fig. 17) and SDS-PAGE of reduced slot proteins (Fig. 19). For Katepwa, the SDS-PAGE of unreduced protein (Fig. 17) showed a noticeable reduction in the overall intensity of the PDD sample (Fig. 17, lane 3) followed by an increase in band intensities at 15 min mixing (Fig. 17, lane 5) and 30 min of mixing (Fig. 17, lane 7); not only were the gliadin bands affected in this way but also oligomeric and slot protein. For the Glenlea flour and dough samples (Fig. 17), no similar trend was observed, as the protein band intensities were largely constant with increasing mixing. For reduced slot protein, the electrophoresis results (Fig. 20) show some evidence of a mixing effect, particularly at 15 and 30 min mixing for both cultivars. However, it should be noted that in quantitative terms, slot protein (Osborne alcohol-soluble glutenin too large to enter the polyacrylamide gel) likely accounts for only a very small proportion of total Osborne alcohol-extractable protein. Accordingly, the electrophoresis results generally correspond to the pattern of Osborne alcohol-extractable protein (Fig. 6). However, for Katepwa, the substantial effect that initial dough mixing (1 min) had on ES protein was not

Figure 19. SDS-PAGE of reduced Osborne alcohol-soluble protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

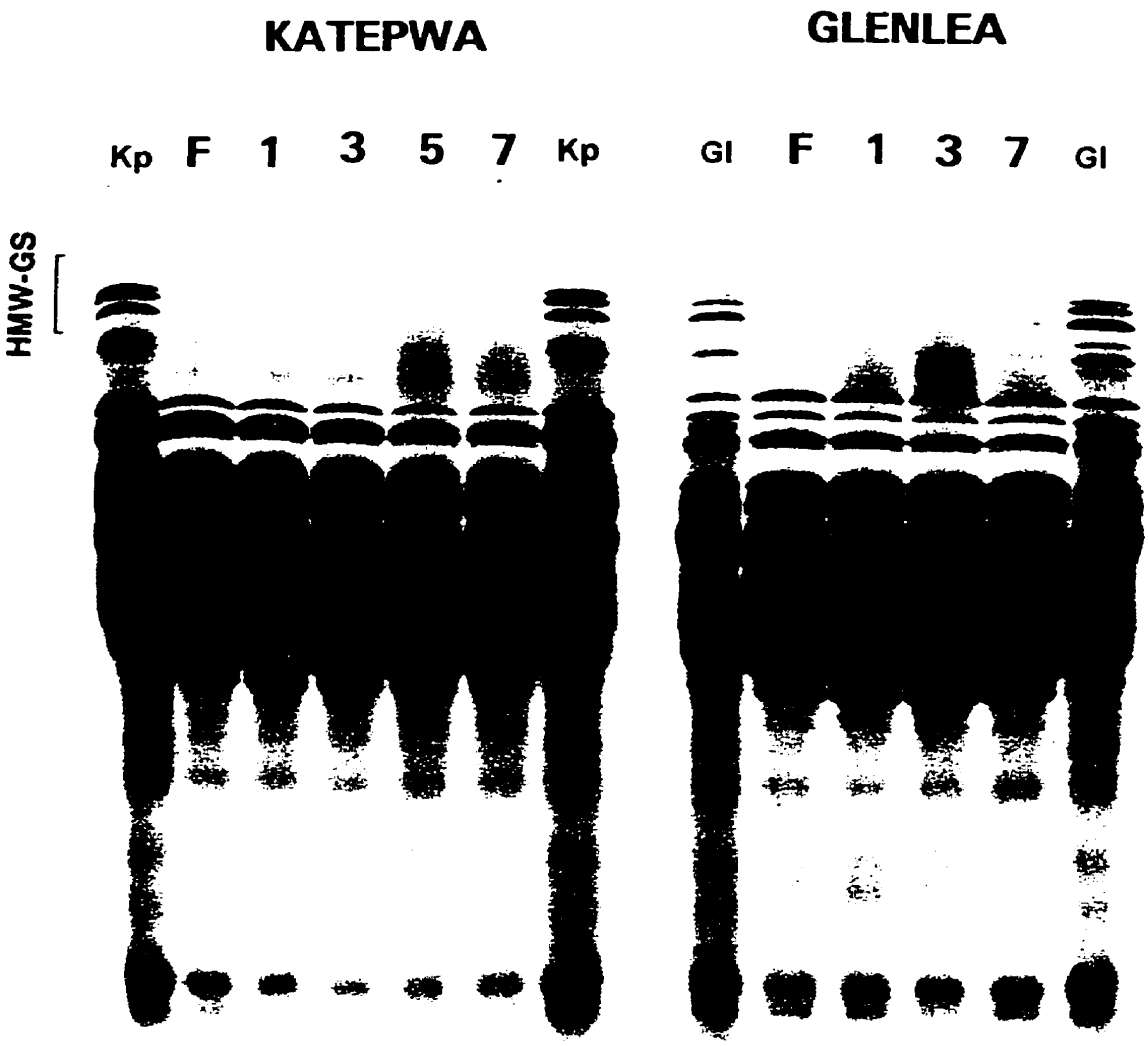
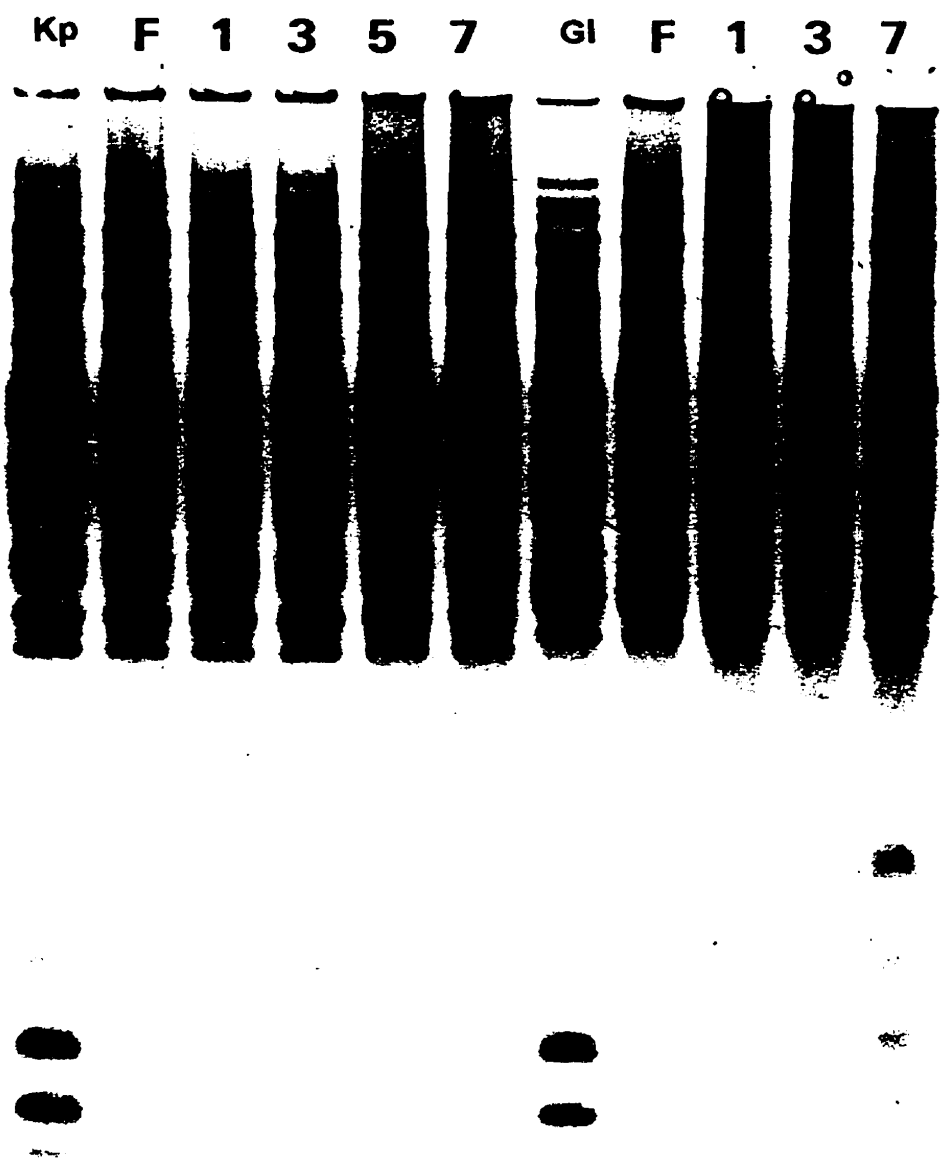

KATEPWA
GLENLEA
Kp F 1 3 5 7 Kp
Gl F 1 3 7 Gl
HMW-GS

Figure 20. A-PAGE of Osborne alcohol-soluble protein of flour and dough subjected to various mixing treatments. The freeze-dried samples were resolubilized in 70 % ethanol solution.As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.


KATEPWA
GLENLEA
Kp
F
1
3
5
7
Gl
F
1
3
7

observed in the electrophoresis results of this fraction. The relatively high amount of ES protein that was analyzed by electrophoresis may have been a factor in this result.

d. Acid-Soluble (0.05M acetic acid) Protein. Electrophoresis results of the Osborne AAS protein fraction are shown in Figs. 21- 24. SDS-PAGE of unreduced extracts (Fig. 21) indicates that the Osborne AAS fraction contains substantial amounts of gliadins, as well as smaller amounts of albumin and globulin proteins. Similar results were observed recently by Fu and Sapirstein (1996a) and Dupuis et al (1996). Dupuis et al (1996) found that the Osborne AAS protein fractions of Glenlea and Katepwa flours contained 2.0% and 4.6% (total flour protein basis) gliadin proteins, respectively. These quantities of gliadins represented about 36 % and 53% of the AAS fractions, respectively. Contamination of the Osborne AAS fraction with non-glutenin protein has been reported by other researchers (Orth and Bushuk 1973, Bietz and Wall 1975) but not quantified. Fu and Sapirstein (1996a) recently found that the Osborne fractionation scheme was problematic in the purity and identity of AAS protein. They reported that if water or salt solution is the first solvent used in flour protein extraction the subsequent glutenin fraction can be substantially contaminated with monomeric proteins, i.e. gliadins. Fu and Sapirstein (1996a) proposed that the apparent aggregation of gliadins and glutenins was promoted by salt. It is therefore not surprising that the AAS protein fraction obtained using the modified Osborne procedure which employs 0.5 M salt as the first solvent in the protein fractionation, contained gliadin proteins.

In addition to the presence of gliadin proteins, SDS-PAGE of the unreduced AAS protein fraction (Fig. 21) was noteworthy for the intense background streaking in the HMW region of the electrophoregram and oligomeric protein bands as observed earlier for the 70% ES fraction. Also,

Figure 21. SDS-PAGE of unreduced Osborne acetic acid-soluble proteins of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

GLENLEA

KATEPWA

Figure 22. Two-step one dimensional SDS-PAGE of unreduced Osborne acetic acid-soluble slot protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = premix, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, slot protein of total protein extracts. Gl2 = Glenlea and Kp2 = Katepwa, total protein extracts.

KATEPWA

GLENLEA

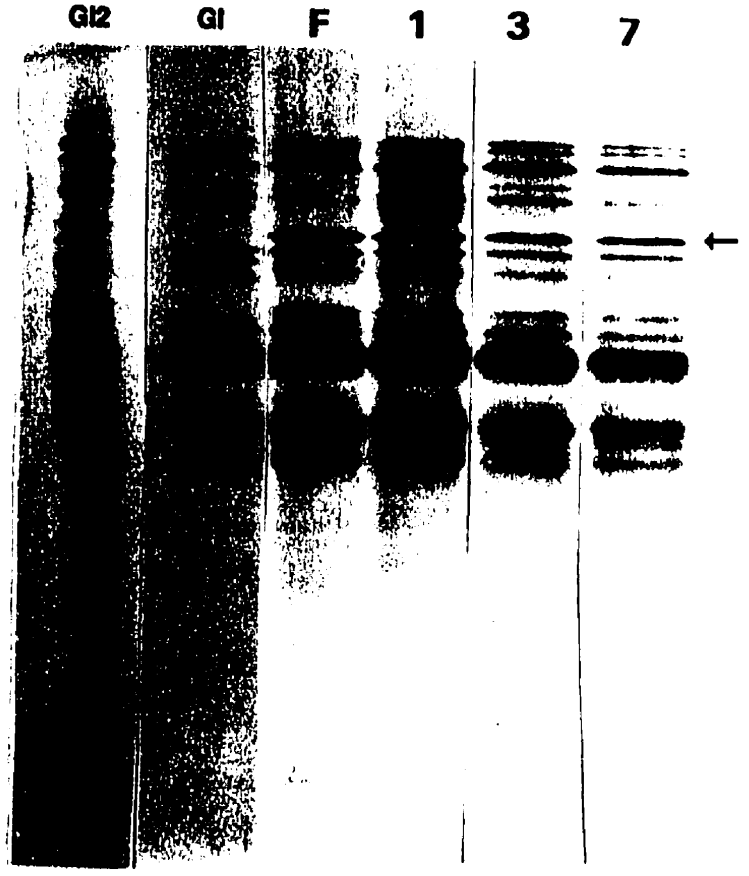


Figure 23. SDS-PAGE of reduced Osborne acetic acid-soluble protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

GLENLEA

KATEPWA

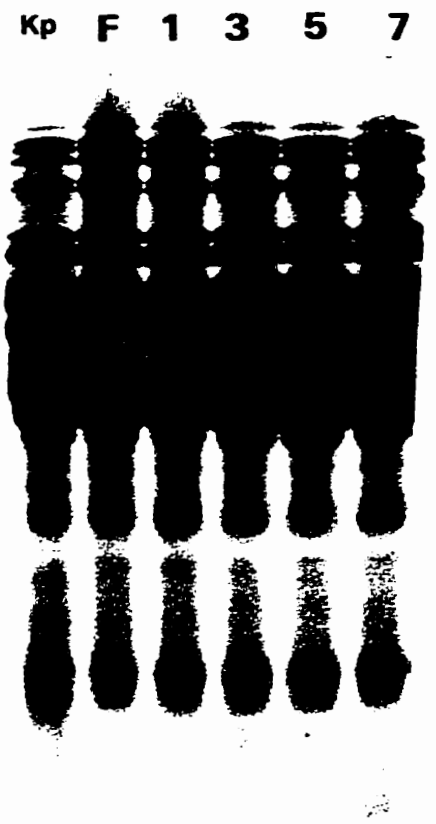
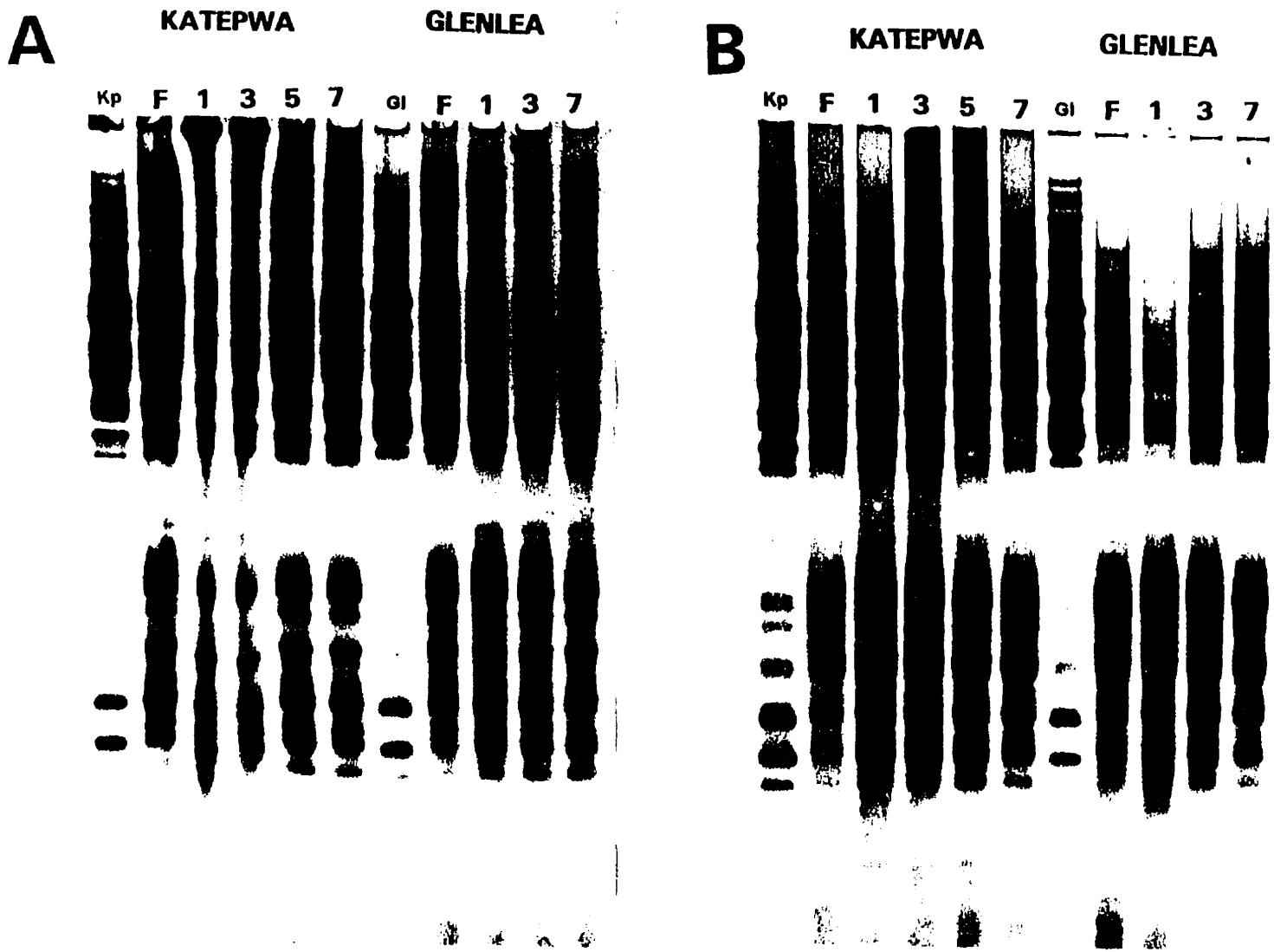


Figure 24. A-PAGE of Osborne acetic acid-soluble protein of flour and dough subjected to various mixing treatments. The freeze-dried samples were resolubilized for electrophoresis in 70% ethanol (A) and 0.05 M acetic acid (B) solutions. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.


A
KATEPWA
GLENLEA
Kp F 1 3 5 7 Gl F 1 3 7
B
KATEPWA
GLENLEA
Kp F 1 3 5 7 Gl F 1 3 7

excessive amounts of slot proteins were observed indicating the presence of polymeric glutenin in the AAS samples. This was confirmed by two-step SDS-PAGE results (Fig. 22) which showed that the AAS fraction contained relatively comparable amounts of LMW-GS and HMW-GS. This was in contrast to the corresponding ES protein fraction result (Fig. 18) which showed much greater quantity of LMW-GS compared to HMW-GS in ES slot protein.. Glenlea's AAS slot protein result also showed the presence of a strongly stained protein band (Fig. 22, arrow), which was absent in Katepwa. this polypeptide was identified (Fu and Sapirstein 1996) as a D-glutenin subunit.

SDS-PAGE of the reduced AAS protein (Fig. 23) showed the presence of large quantities of HMW-GS and LMW-GS and co-migrating gliadins. The A-PAGE results (Fig. 24) produced poorly resolved electrophoregrams, which was more evident for Katepwa than for Glenlea, especially for undermixed and PDD samples (Fig. 24, lanes 1 and 3). The streaking appears to be typical for these samples since it was observed in replicated gels. Dupuis (1995) also observed poor A-PAGE resolution of the AAS fractions from Glenlea and Katepwa using 70% ethanol as a reconstituting solvent. Using 0.05M acetic acid as a reconstituting solvent resolved fewer proteins (Fig. 24B) but produced better electrophoregrams.

In regard to dough mixing effects, some evidence of an increase in protein with mixing can be seen in the unreduced (Fig. 21) and reduced (Fig. 23) SDS-PAGE results for Glenlea. Theses electrophoretic results are consistent with the quantitative AAS protein fraction results for Glenlea as previously shown (Fig. 6). SDS-PAGE of unreduced AAS protein of Glenlea showed a progressive increase in streaking in the HMW region with mixing. This result is consistent with glutenin being disaggregated by mixing action causing the residue glutenin protein to be solubilized. SDS-PAGE of reduced AAS protein of Glenlea (Fig. 23) showed a noticeable

increase in the level of background streaking and staining of HMW-GS for the 15 min and 30 min dough samples. Similar results were not obtained for Katepwa flour and dough samples. SDS-PAGE of unreduced Katepwa AAS protein (Fig. 21) showed that the staining intensity of gliadin bands appeared to peak at 1 to 3.5 min of mixing (lanes 1 and 3), after which a substantial decrease in gliadin band intensity was observed. As for the reduced slot protein(Fig. 22) and A-PAGE (Fig. 24), the electrophoresis results did not show evidence of any mixing effects.

e. Residue (0.05M acetic acid-insoluble) Protein. SDS-PAGE of the Osborne residues under non-reducing conditions (Fig. 25), showed that the residue protein fractions contained significant amounts of gliadins. As well, there were traces of albumin and globulin, triticins, and considerable amounts of slot proteins, background streaking, the latter being consistent with the extraction of glutenin. In regard to triplet band (triticin) protein differences were noted between Katepwa and Glenlea whereby Katepwa showed the characteristic pattern of a distinct doublet, whereas, Glenlea showed a more diffused pattern. This observation was also noted by Sievert et al (1991a) for the same wheat cultivars. Two-step SDS-PAGE of the slot protein (Fig. 26) showed he presence of HMW and LMW-GS for both cultivars; Katepwa appeared to contain a greater amount of this polymeric protein as evidenced by more intense bands compared to Glenlea.

Under reduced conditions (Fig. 27), the SDS-PAGE of the residue fraction showed the presence of large quantities of HMW- and LMW-GS for both cultivars. The staining intensity and background streaking appeared to be stronger for Glenlea than for Katepwa. A-PAGE results (Fig. 28) showed that the residue fraction of both cultivars still contained substantial amounts of gliadins as well as lesser amounts of albumin and globulin proteins. Katepwa appeared to contain

Figure 25. SDS-PAGE of unreduced Osborne residue protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.

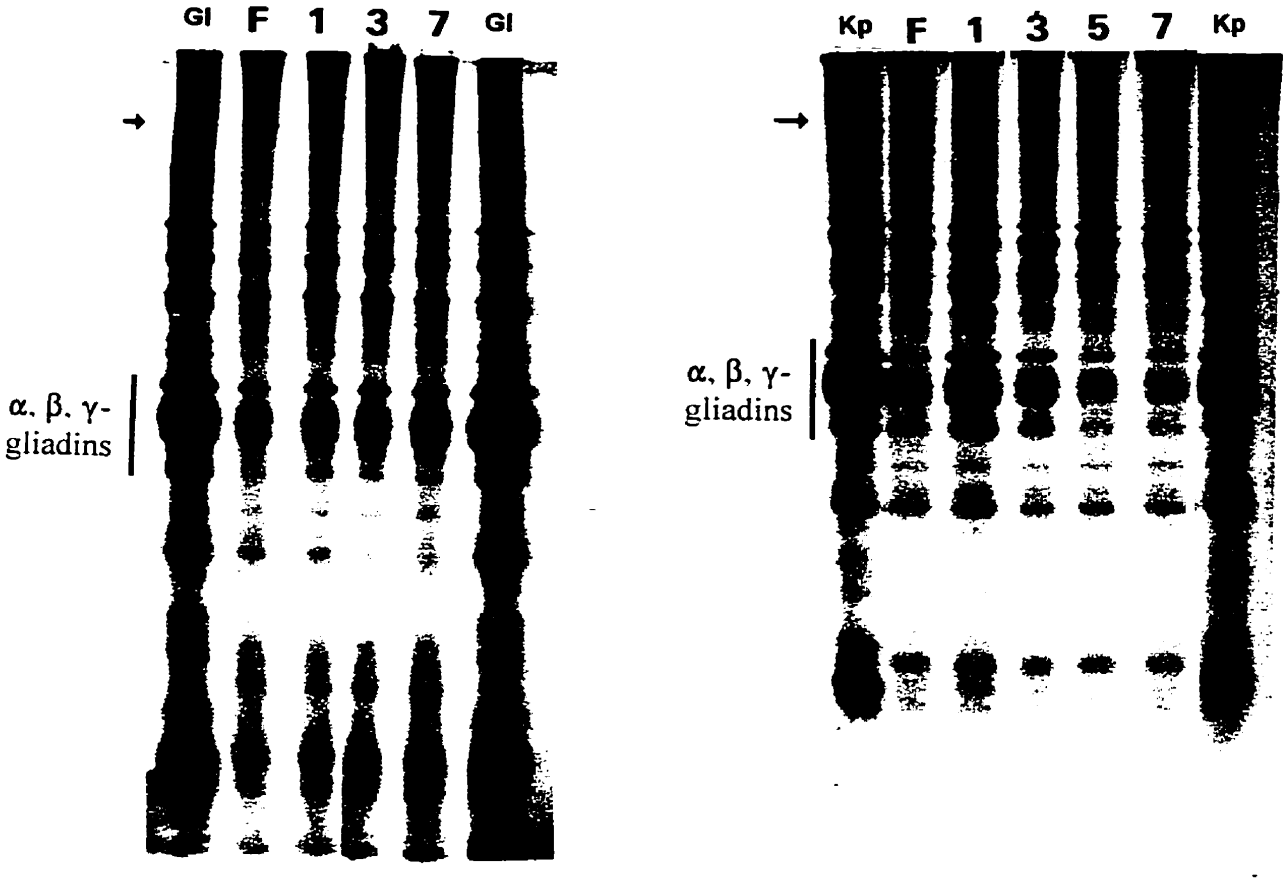
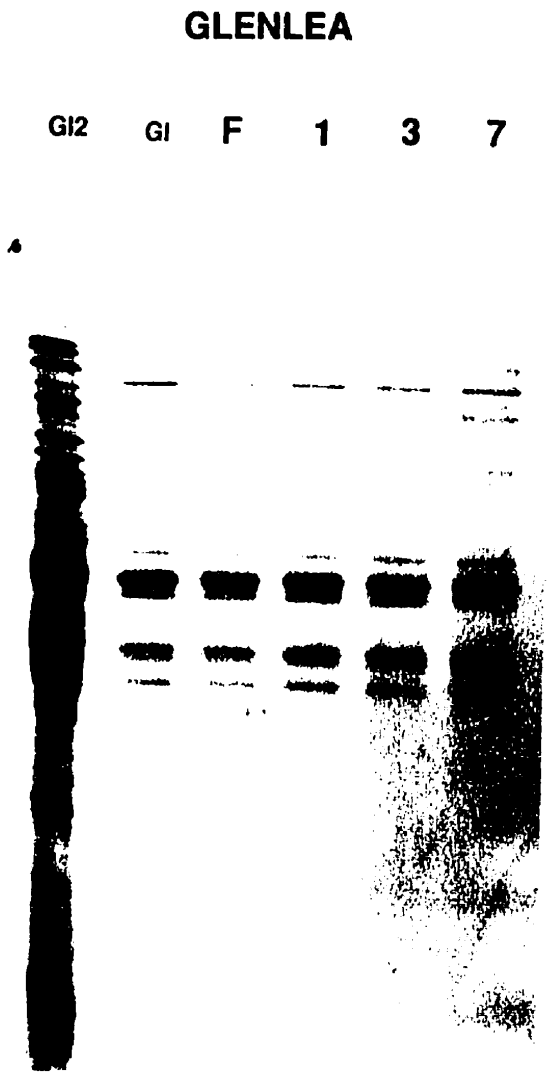

GLENLEA
Gl F 1 3 7 Gl
α, β, γ-gliadins
KATEPWA
Kp F 1 3 5 7 Kp
α, β, γ-gliadins

Figure 26. Two-step one dimensional SDS-PAGE of unreduced Osborne residue slot protein of flour and dough subjected to various mixing treatments. As defined in Table 2. F = flour, 1 = premix, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, slot protein of total protein extracts. Gl2 = Glenlea and Kp2 = Katepwa, total protein extracts.


GLENLEA
Gl2
Gl
F
1
3
7

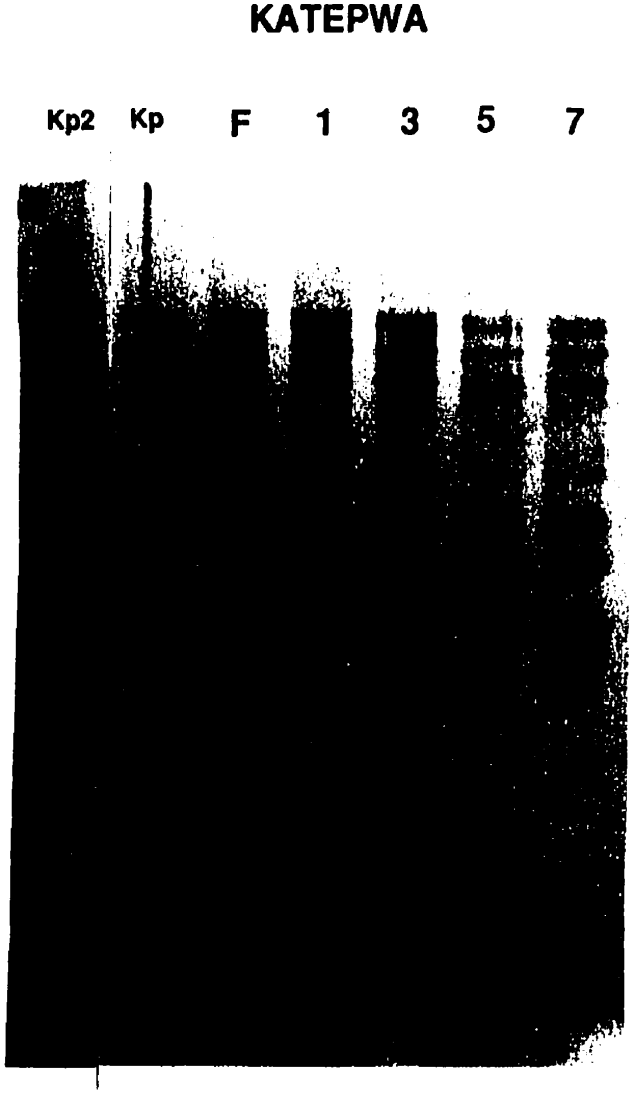
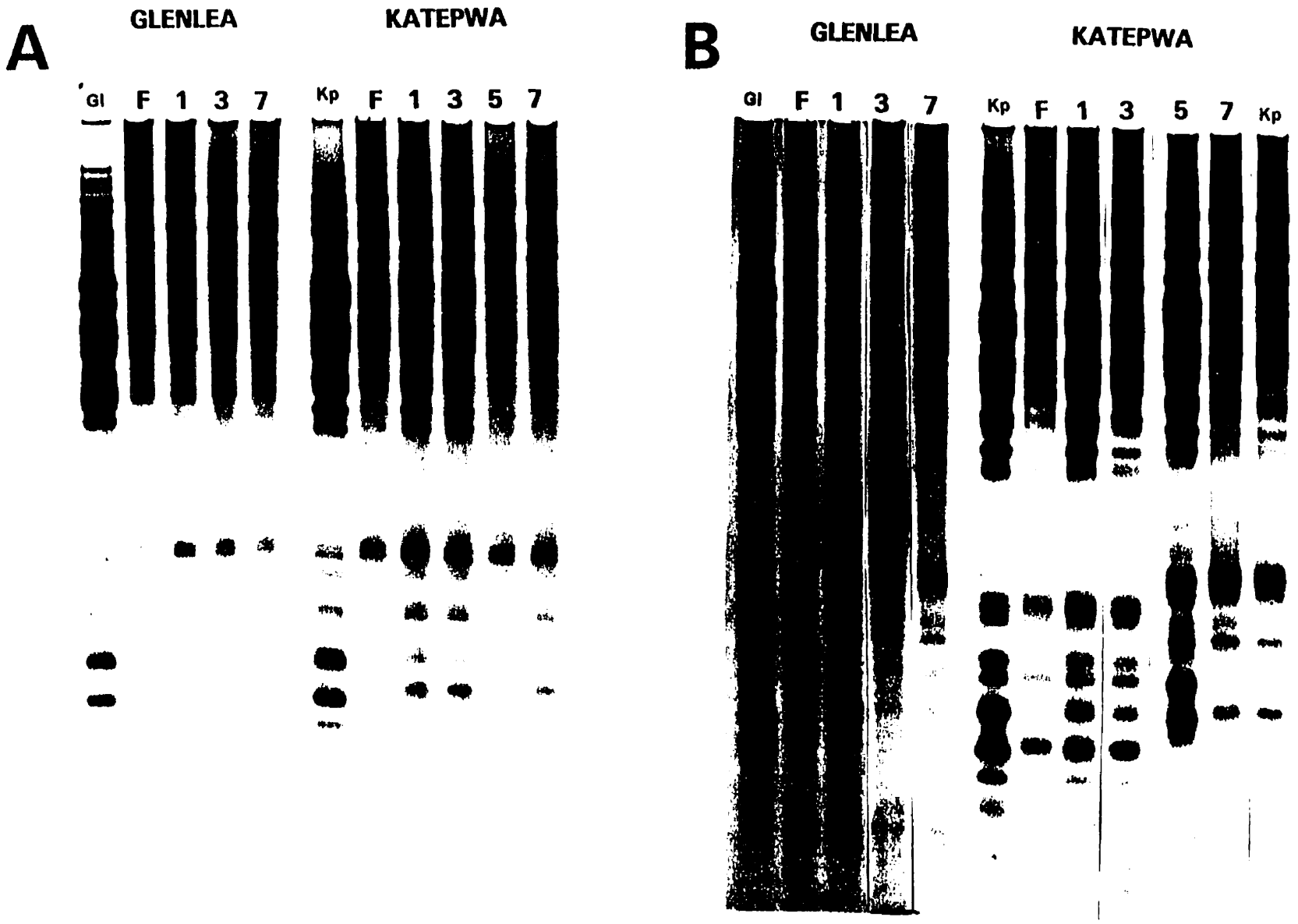

KATEPWA
Kp2
Kp
F
1
3
5
7

Figure 27. SDS-PAGE electrophoregrams of reduced Osborne residue protein of flour and dough subjected to various mixing treatments. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min. Gl = Glenlea and Kp = Katepwa, total protein extracts.

GLENLEA

KATEPWA

Figure 28. A-PAGE of Osborne residue protein of flour and dough subjected to various mixing treatments. The freeze-dried samples were resolubilized for electrophoresis in 70% ethanol (A) and 0.05 M acetic acid (B) solutions. As defined in Table 2, F = flour, 1 = undermixed, 3 = peak development, 5 = 15 min, 7 = 30 min.Gl = Glenlea and Kp = Katepwa, total protein extracts.


A
GLENLEA
KATEPWA
Gl
F
1
3
7
Kp
F
1
3
5
7
B
GLENLEA
KATEPWA
Gl
F
1
3
7
Kp
F
1
3
5
7
Kp

much more gliadin protein in the AAI residue fraction than Glenlea. Fig. 28 shows that 0.05 M acetic acid was more effective than 70% ethanol in solubilizing the residue proteins.

In terms of mixing related effects, SDS-PAGE of unreduced Osborne AAI protein provided results that were consistent with the quantitative results obtained for this protein fraction (Tables 5 and 6, and Fig. 7). For Katepwa dough mixed to 1 min, compared to corresponding results for Katepwa flour, electrophoregrams (Fig. 25B) showed a substantial increase in the concentration of all gliadin bands, as well as triplet band (triticin) protein in the HMW region of the gel. The latter also contained a polypeptide of lower and higher mobility (difficult to see in the photograph) than the main triticin band (Fig. 25, arrow) whose staining intensity also increased at 1 min of mixing. The staining intensity of both types of protein components decreased progressively with increasing mixing up to 15 min. A-PAGE of the Osborne AAI protein fraction of Katepwa (Fig. 28) also showed a similar trend.

Compared to the Katepwa results, SDS-PAGE of Glenlea unreduced Osborne AAI protein (Fig. 25), showed a similar, but less pronounced, increase in gliadin band concentration upon flour mixing to 1 min protein. For Glenlea, there was no decrease in the intensity of gliadin protein bands with increasing mixing, as was observed for Katepwa. However, for Glenlea, there was a comparable decrease in triticin protein with increasing dough mixing. As well, SDS-PAGE of reduced Glenlea AAI protein (Fig. 27) showed a noticeable decrease in the concentration of HMW-GS with increasing dough mixing time. No similar result was obtained for Katepwa HMW-GS in reduced AAI protein analyzed by SDS-PAGE.

E. Effects of Structural Relaxation of Doughs on Protein Solubility and Electrophoretic Composition

In the present study, the doughs were allowed to relax for 45 min at a controlled temperature (30 °C) and humidity (95-100% RH) after mixing to the required time. A 45 min rest period was chosen as the maximum period of relaxation based on the published results of Dempster et al (1952). In that study, the rheological properties of conventional, low-work doughs stabilized after approximately 45 min of rest. Frazier et al (1985) later confirmed the stable resistance of 45 min rest for dough mixing work levels ranging from 10-400 kJ/kg. A 45 min rest period also corresponds to typical rest periods given to a dough after moulding prior to baking. E.g. normal commercial bread production typically uses a 40-60 final proof period (Tipples 1982).

Previous publications reported to the effects of structural relaxation on the rheological behaviour of the dough, such as resistance to extension, and its behaviour when subjected to compression (Dempster et al 1952,1953, 1955, Frazier et al 1975, 1985). Effects of dough relaxation on protein solubility and composition have also been reported for SDS gel protein (**reference**). This section of the thesis examines the effects of structural relaxation of mechanically developed doughs in relation to protein solubility. The effects of dough relaxation (45 min) compared to control doughs (no relaxation) of Glenlea and Katepwa Osborne fractions are presented in Tables 9 and 10, respectively.

1. Salt-Soluble (0.5M NaCl) Protein.

The protein solubilities (% total flour protein) of the salt-soluble protein fractions of both Glenlea and Katepwa samples were not affected by dough relaxation at 45 min. Comparisons of

Table 9. Osborne protein fractions (% of total protein) of Glenlea at different mixing treatments (1, 15, and 30 min) derived from control doughs (no relaxation) and doughs rested for 45 min.

Osborne Fraction	1 min		15 min		30 min	
	0	45	0	45	0	45
Salt-soluble	9.6±1.2a	10.0±1.6a	8.9±0.9a	9.9±0.1a	8.1±0.4a	8.1±1.2a
Water-extractable	2.4±0.2a	2.2±0.1ba	1.9±0.2a	1.8±0.2a	2.1±0.0a	1.9±0.3a
Alcohol-soluble	32.7±3.1a	27.1±0.9b	32.8±2.7a	34.4±0.3a	32.1±1.5b	37.7±0.9a
Acetic acid-soluble	12.5±0.2b	14.4±1.4a	16.7±1.9a	15.2±1.4a	20.3±0.2a	19.6±2.9a
Residue	37.5±0.1a	37.1±1.5a	32.7±1.9a	34.9±1.1a	28.1±0.6a	28.6±0.4a
N recovery	94.7	90.8	93.0	96.2	90.7	95.9

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

Table 10. Osborne protein fractions (% of total protein) of Katepwa at different mixing treatments (1, 15, and 30 min) derived from control doughs (no relaxation) and doughs rested for 45 min.

Osborne Fraction	1 min		3.5 min		15 min		30 min	
	0	45	0	45	0	45	0	45
Salt-soluble	2.2±0.2a	2.2±0.1a	1.9±0.0a	2.0±0.0a	1.8±0.4a	1.8±0.1a	1.8±0.4a	1.9±0.3a
Water-extractable	11.4±0.3a	11.4±0.0a	11.5±0.7a	11.5±0.0a	11.5±0.9a	9.9±0.5a	11.1±0.9a	10.9±0.3a
Alcohol-soluble	25.2±0.5a	22.4±0.6b	29.4±0.8a	25.9±1.0b	36.7±0.6a	36.2±3.1a	36.2±1.5a	35.8±0.4a
Acetic acid-soluble	19.8±1.2a	20.8±0.6a	19.9±1.4a	22.4±0.0b	19.8±1.2a	19.3±1.4a	20.2±0.5a	19.2±1.9a
Residue	41.2±1.1a	38.6±1.1b	34.8±1.2a	37.9±3.9a	31.5±0.1a	32.1±1.4a	30.1±0.9a	29.5±0.0a
N recovery	99.8	95.4	97.5	99.7	101.3	99.3	99.4	97.3

Values in rows followed by the same letter are not significantly different ($p < 0.05$).

the SDS-PAGE patterns under reducing conditions, indicated that structural relaxation affected the protein composition of the salt-soluble fractions of Katepwa. Fig. 29 shows the presence of some HMW-GS extracted from doughs that were allowed to rest for 25 min (lanes 2,5,11). These observations were not evident in reduced SDS-PAGE patterns of Glenlea (results not shown).

Under non-reducing conditions, protein compositional differences were not observed between the control and rested doughs, for both cultivars (results not shown). No effects were observed when the samples were analyzed by two-step SDS-PAGE and A-PAGE (results not shown).

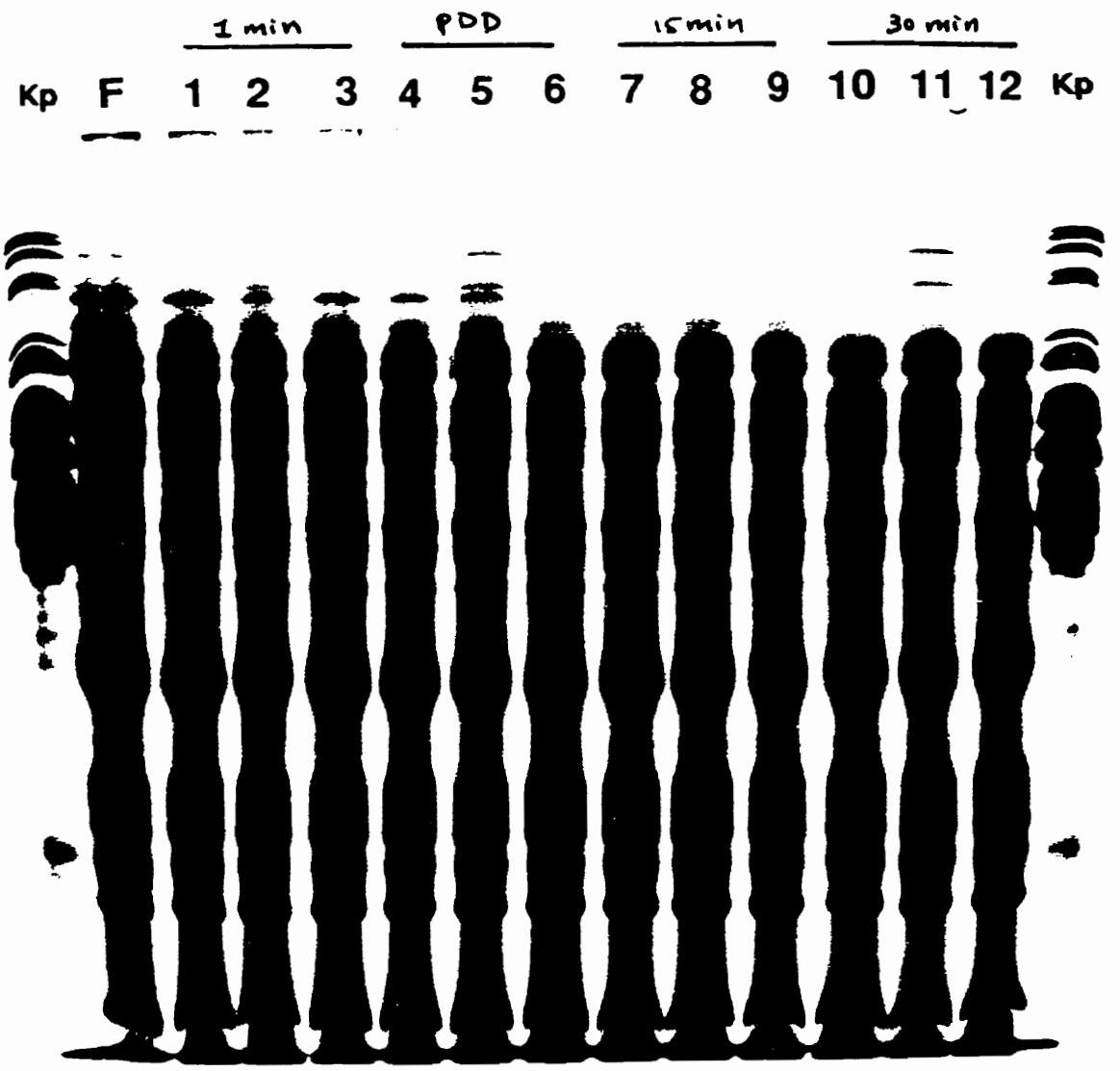
2. Water-Soluble Protein.

Structural relaxation of doughs had no significant effect on the amount of water-soluble proteins. No effects of relaxation on the protein composition by electrophoresis were observed (results not shown).

3. Alcohol-Soluble (70% ethanol) Protein.

The solubility of the ES protein from the Glenlea doughs mixed for decreased significantly upon relaxation (control = 32.7%, rested dough = 27.1%) (Table 9). This was in contrast with the overmixed sample which showed an increase in ES protein solubility when the dough was allowed to relax (control = 32.1%, rested dough = 37.7%). For Katepwa, the 1 and 3.5 (PDD) min mixed dough samples showed a relaxation effect; dough relaxation decreased the amount of ES by about 3-4% (Table 10). For the electrophoresis results, no significant change was observed between the control and rested samples (results not shown).

Figure 29. SDS-PAGE of reduced Osborne salt-soluble protein of Katepwa's flour and dough subjected to various mixing treatments and 25 and 45 min relaxation. F = flour. 1 = undermixed, no relaxation. 2 = undermixed, 25 min relaxation. 3 = undermixed, 45 min relaxation. 4 = PDD, no relaxation. 5 = PDD, 25 min relaxation. 6 = PDD, 45 min relaxation. 7 = 15 min mixing, no relaxation. 8 = 15 min mixing, 25 min relaxation. 9 = 15 min mixing, 45 min relaxation. 10 = 30 min mixing, no relaxation. 11 = 30 min mixing, 25 min relaxation. 12 = 30 min mixing, 45 min relaxation.Gl = Glenlea and Kp = Katepwa, total protein extracts.


1 min
PDD
15 min
30 min
Kp F 1 2 3 4 5 6 7 8 9 10 11 12 Kp

4. Acid-Soluble (0.05M acetic acid) Protein.

Structural relaxation effects were observed in the Glenlea dough mixed to 1 min (Table 9, control 12.5%, rested 14.4% .For Katepwa dough mixed to 3.5 min or PDD, the amount of AAS protein significantly increased from 19.9% to 22.4% when the dough was allowed to rest. No other significant change was observed in the solubility of this fraction for both cultivars. Electrophoresis results showed no significant change between the control and rested samples (results not shown).

5. Residue (0.05M acetic acid-insoluble) Protein.

AAI protein fractions of Glenlea showed no significant structural relaxation effects at any mixing stage. AAI protein fractions from Katepwa also showed an effect of structural relaxation for the 1 min mixed dough which contained less protein (38.6%) upon relaxation than the control sample (41.2% (Table 10). Electrophoresis results showed no effects of structural relaxation (results not shown).

V. CONCLUSIONS

The objective of this study was to obtain additional new information on the nature of the changes of protein composition that occur in wheat endosperm proteins during dough mixing. It was hypothesized that intercultivar differences exist in the response of the different wheat protein constituents to the effects of dough mixing and relaxation.

At the outset, the study showed that Glenlea and Katepwa doughs substantially differ in mixing strength. To attain PDD in a GRL-200 mixer, Glenlea flour required 15 min of mixing with a work input of 58 Whr/kg, whereas, Katepwa required only 3.5 min of mixing with a work input of 15 Whr/kg; however, equivalent work inputs were obtained at comparable mix times. The mixing curves of both cultivars reflect the expected mixing characteristics typical of each cultivar as shown in previous work (Paredes-Lopez 1980, Marchylo et al 1992, Dupuis et al 1996).

Consistent with earlier results of Orth and Bushuk (1972) and Orth and O'Brien (1976), and more recent results of Dupuis et al (1996), this study showed that Glenlea contained less AAS protein than Katepwa. For both cultivars, AAS protein increased progressively with increasing mixing time. Mixing Katepwa dough beyond 15 min did not extract more AAS protein, whereas significantly more AAS proteins was extracted for Glenlea. Glenlea and Katepwa were comparable in the effects of mixing on the AAI protein fraction. AAI protein content decreased with increasing mixing time. Overmixing (30 min) results showed that comparable amounts of AAI proteins were present in Glenlea and Katepwa. By this mixing stage, significantly more AAI protein was solubilized by mixing of the stronger dough (Glenlea) than the relatively weaker dough (Katepwa). These results indicate that, in the cultivars studied, intrinsic flour strength is

best differentiated by the amount of AAI proteins in flour or PDD doughs, rather than in doughs mixed beyond peak development.

The Osborne protein fractionation results showed considerable effects of dough mixing, and some compelling differences between Glenlea and Katepwa were found in response to dough mixing, most notably in the ES and AAS protein fractions.

Dough mixing had no significant effect on the amounts of the Osborne salt- and water-soluble protein fractions of Katepwa. For Glenlea, the amount of salt-soluble protein decreased significantly upon initial dough mixing to 1 min. As for Katepwa, dough mixing of Glenlea had no significant effects on Osborne water-soluble protein. It was noteworthy that the water-soluble protein fractions of both cultivars contained significant quantities of gliadin proteins, as shown by electrophoresis. This indicates that the modified Osborne procedure does not prevent the solubilization of gliadin, as may be widely believed. Accordingly, care must be taken in interpreting the significance of intercultivar differences in modified Osborne water-soluble protein. In this study, Katepwa contained significantly more water-extractable proteins than Glenlea flour and dough samples. However, the result may be due to differences in the amount of gliadin proteins in the fraction, rather than albumin proteins specifically.

Electrophoresis of Osborne salt soluble protein showed some effects related to dough mixing. The unreduced salt-soluble protein fractions of both cultivars, and especially Glenlea, showed triplet band (triticin) proteins and substantial streaking in the HMW region of the gel which decreased in staining intensities as mixing time increased. The salt-soluble protein fractions of Glenlea and Katepwa flour and dough samples both contained considerable amounts of slot proteins too large to enter the electrophoretic gel. However, analysis of reduced slot proteins by SDS-PAGE showed that Katepwa contained much greater quantities of resolved polypeptides

than was found in Glenlea. Why Glenlea flour and dough samples appeared to be deficient in salt-soluble slot protein after the reduction step is not clear; this interesting result warrants further study.

In contrast to the similarity of dough mixing effects on the Osborne salt- and water-soluble protein fractions of Glenlea and Katepwa flour, there were substantial and significant intercultivar differences, caused by dough mixing, in the pattern and amount of Osborne ethanol-soluble (ES) and Osborne AAS protein fractions. Initial mixing of Katepwa dough, essentially a flour hydration step, significantly affected the proportion of Osborne ES protein in an unexpected way. The amount of ES protein decreased (36% to 25%) when the Katepwa dough was mixed for 1 min and significantly increased back to 36% (ES protein in flour) with further mixing up to 15 min. Mixing beyond this time did not result in the extraction of more ES protein. This pattern of variation with mixing was not found for Glenlea ES protein; there was essentially no change in Glenlea ES protein from flour through to doughs mixed for 30 min. Because of the complex effect of dough mixing on Katepwa ES protein, compared to Glenlea, Katepwa had a significantly lower amount of ES protein at 1 min mixing, but a significantly higher amount of ES protein at 15 and 30 min of mixing.

SDS-PAGE under non-reducing condiions. However, SDS-PAGE of unreduced Katepwa Osborne AAI protein provided results that were closely (inversely) related to the quantitative ES protein results. For Katepwa dough mixed to 1 min, compared to corresponding results for Katepwa flour, these electrophoregrams showed a substantial increase in the concentration of all gliadin bands, as well as triticin protein in the HMW region of the gel. The staining intensity of all of these protein components decreased progressively with increasing mixing up to 30 min. A-PAGE of gliadins in the AAI protein fraction of Katepwa also showed a similar trend. Compared

to the Katepwa results, SDS-PAGE of unreduced AAI protein of Glenlea, showed a similar, but less pronounced, increase in gliadin band intesnsities upon flour mixing to 1 min, and a comparable decrease in triticin protein staining with increasing dough mixing. For Glenlea AAI protein, SDS-PAGE under reducing conditions showed a noticeable decrease in the concentration of HMW-GS with increasing dough mixing time. No similar result was obtained for Katepwa reduced AAI protein analyzed by SDS-PAGE.

In contrast to the pattern of variation that was found for the Katepwa Osborne ES protein fraction with mixing, a significant increase in Katepwa AAS protein was observed upon 1 min of mixing, with no significant change thereafter. SDS-PAGE of Katepwa unreduced AAS protein clearly showed an increase in the staining intensity of gliadin bands at 1 to 3.5 min of mixing, after which a substantial decrease in gliadin band intensity was observed.

The effect of dough mixing on Glenlea Osborne AAS protein was also different to that obtained for Glenlea ES protein, as well as being different from results obtained for Katepwa AAS protein. While there was no significant difference between Glenlea flour and dough mixed for 1 min, mixing Glenlea dough up to 30 min caused a steady and significant increase in the amount of Osborne AAS proteins. SDS-PAGE of unreduced Glenlea Osborne AAS protein also showed a progressive increase in streaking in the HMW gel region as mixing time increased. SDS-PAGE of reduced Glenlea AAS protein also showed an increase in staining of HMW-GS for 15 and 30 min mixed doughs. For Glenlea, these results indicate an increase in the solubility of glutenin (in dilute acetuic acid solution) with increasing dough mixing, which can be explained by polymer disaggregation and/or depolymerization.

The quantitative protein fractionation results for Katepwa flour and dough samples indicated that a significant effect on gliadin proteins (and/or possibly ethanol soluble glutenin) occurs upon

the initial transformation of flour into dough at 1 min of mixing; gliadin proteins (nominally defined as the Osborne ES protein fraction) and/or glutenin (soluble in 70% ethanol) appear to become partially insoluble or unextractable. This would account for the decrease in ES protein and the concomitant increase seen in AAS and AAI protein at 1 min of mixing. Because the latter two fractions are mainly composed of glutenin protein, it is possible that aggregation of gliadins with glutenin is the underlying reason for the decrease in extractability of gliadins upon initial dough mixing.

The aggregation may also be promoted by the exposure of gluten proteins to salt. In this study, doughs were mixed in the presence of 1% salt (flour basis, equivalent to ~ 0.17 M salt in the water added to the flours). Flour and dough samples were also treated with 0.5 M salt solution in the first step of Osborne fractionation. It is well known that salt can reduce the extractability of both gliadin and glutenin proteins in various aqueous solvents (Kim and Bushuk 1995, Fu et al 1996). Furthermore, it has been recently shown (Fu and Sapirstein 1996) that if salt solution is the first solvent used in the fractionation of flour proteins, as was the case in this study, subsequently fractionated glutenin is substantially contamination with gliadins and other monomeric proteins. Similarly, Dupuis et al (1996) showed that the Osborne AAS protein fraction contained considerable amounts of gliadins despite the use of 70% ethanol solution as a protein solvent in the previous fractionation step.

If gliadins are indeed aggregating with glutenin at the flour hydration stage, it seems reasonable that further mixing would be expected to result in a disaggregation of gliadins and glutenin, resulting in an increase in ES protein solubility, as was observed for Katepwa. The absence of a similar pattern of variation with Glenlea flour and mixed dough, especially the absence of any effects on the ES protein fraction, suggests that no similar aggregation of gliadin

and glutenin proteins occurs; dough mixing causes a gradual disaggregation of polymeric glutenin evidenced by the significant decrease and increase in Glenlea Osborne AAI and AAS protein, respectively, with increasing dough mixing.

The quantitative Osborne fractionation and electrophoresis results taken together indicate that there are considerable differences between Katepwa and Glenlea in the response of their respective endosperm proteins to dough mixing, but not dough relaxation. Differences which occur when flour is initially hydrated (1 min of mixing) appear to be critical to the resulting pattern of variation in protein composition caused by dough mixing. For Katepwa, a moderate mixing strength flour, a significant proportion of gliadin proteins became unextractable when flour was hydrated. With further dough mixing, gliadin extractability increased. No similar result was found in the case of the extra-strong Glenlea flour. For Katepwa, aggregation of gliadin and glutenin during flour hydration is a plausible explanation for this result. Such an aggregation event, which would tend to dilute or plasticize the polymeric glutenin protein, would also explain the lower dough mixing requirements of Katepwa flour compared to that of Glenlea (Dupuis et al 1996, Fu et al 1996). According to this hypothesis, dough mixing requirements are inversely related to the quantity of gliadin proteins aggregating with glutenin during the earliest stages of dough development.

The degree of aggregation may, in turn, be inversely related to the molecular size of glutenin. Sapirstein (1997) has proposed that extra strong dough mixing cultivars like Glenlea with glutenin of very large average molecular size, possess glutenin which is chemically "unfriendly", i.e. its specific surface area is low. As a result, Glenlea glutenin interacts relatively poorly with aqueous solvents (it is relatively insoluble), and other proteins like gliadin (gluten is therefore highly elastic). This would explain why Glenlea, showed no similar gliadin/glutenin

aggregation/disaggregation effects with dough mixing compared to those found for Katepwa.

VI. SUMMARY

1. Glenlea and Katepwa flours differed in mixing strength when mixed in a GRL-200 mixer, although at comparable mix times, comparable work inputs were required. To attain PDD, Glenlea flour required 15 min of mixing with a work input of 58 Whr/kg, whereas, Katepwa flour required only 3.5 min of mixing with a work input of 15 Whr/kg.
2. Glenlea contained significantly less directly extractable AAS protein than Katepwa. Dough mixing showed that AAS proteins increased progressively with increased mixing time. In contrast, Glenlea contained significantly more AAI protein than Katepwa (by direct extraction with acetic acid). Mixing showed that the AAI proteins progressively decreased with mixing time.
3. Flour hydration and dough mixing to 30 min produced the following results in the five Osborne protein fractions:
    a. For Glenlea, the amount of salt-soluble protein decreased upon initial dough mixing to 1 min. As well, no significant effects of dough mixing in the water-soluble protein fraction was found. Mixing had no effects on the salt-soluble and water-soluble fractions of Katepwa. Intercultivar differences were insignificant for salt-soluble protein but were significant for water-soluble protein fraction. Katepwa had more water-soluble protein in the 1 min, PDD, and 30 min dough samples compared to Glenlea .
    b. There were no significant differences in the amounts of Glenlea ES protein in response to mixing. With Katepwa, the amount of alcohol-soluble protein decreased substantially when doughs were initially mixed. Katepwa ES protein increased up to 15 min of mixing, and remained constant thereafter.

c. Glenlea showed a significant linear increase of AAS proteins as mixing time increased to 30 min. For Katepwa, a significant increase was observed only at 1 min of mixing for flour and undermixed samples.

d. For the AAI residue protein fraction, Glenlea showed a significant increase at 1 min of mixing followed by a significant decreased with further mixing to 30 min. The corresponding Katepwa fraction showed a similar but more pronounced pattern of variation with mixing especially at 1 min.

4. Mixing produced the following electrophoretic results in the five Osborne protein fractions:

a. SDS-PAGE of unreduced salt-soluble protein fractions of both cultivars, and especially Glenlea, showed triplet band (triticins) proteins and substantial streaking in the HMW region of the gel which decreased in staining intensities as mixing time increased. This protein fraction also contained considerable amount of slot protein observed in both cultivars. The reduced slot proteins by SDS-PAGE showed that Katepwa contained much greater quantities of resolved polypeptides than was found in Glenlea.

b. SDS-PAGE of the Osborne water-esctractable protein fraction of both Glenlea and Katepwa showed no significant effects due to dough mixing. A-PAGE result of this fraction showed the presence of considerable amounts of gliadin proteins in flour and mixed doughs.

c. SDS-PAGE of the Osborne ES protein fraction of both Glenlea and Katepwa did not show any clear effects due to dough mixing. SDS-PAGE of reduced ES slot protein of Katepwa flour and doughs contained considerably more HMW- and LMW-GS protein than Glenlea.

d. SDS-PAGE of Katepwa unreduced Osborne AAS protein showed an increase in the

staining intensity of gliadin bands at 1 to 3.5 min of mixing, after which a substantial decrease in band intensity was observed. SDS-PAGE of Glenlea unreduced Osborne AAS protein showed a progressive increase in streaking in the HMW region of the gel as mixing time increased. SDS-PAGE of Glenlea reduced AAS protein also showed an increase in staining of the HMW-GS for 15 and 30 min mixed doughs. These results indicate that for Glenlea, there is a progressive increase in the solubility of glutenin in acetic acid with increasing dough mixing likely due to polymer disaggregation.

e. SDS-PAGE of Katepwa unreduced Osborne AAI dough mixed to 1 min, showed a substantial increase in the concentration of all gliadin bands, as well as triticin protein in the HMW region of the gel, as compared to the corresponding results for Katepwa flour.The staining intensity of all of these protein bands decreased progressively with increased mixing. A-PAGE of gliadins in the AAI protein fraction of Katepwa also showed a similar trend. SDS-PAGE of Glenlea unreduced Osborne AAI residue also showed similar but less pronounced trend. SDS-PAGE of the reduced Glenlea fraction showed a decrease in the concentration of HMW-GS with increased dough mixing.

5. Dough relaxation up to 45 min showed no clear systematic effects, for either Glenlea and Katepwa, on quantitative protein solubility and their electrophoretic compositions.

6. For Katepwa flour and dough samples, a significant effect on gliadin proteins occurred upon flour hydration and dough mixing to 1 min. The gliadin proteins appear to become partially insoluble or unextractable accounting for the decrease in the ES protein and the concomitant increase in AAS and AAI protein at 1 min mixing. The decrease in extractability of gliadins upon initial dough mixing is possibly due to the aggregation of gliadins with glutenins. With further dough mixing, gliadin extractability increased. This aggregation hyphothesis can

explain the lower dough mixing requirements for Katepwa flour compared to that of Glenlea which showed no similar result with regards to gliadin-glutenin aggregation upon flour hydration.

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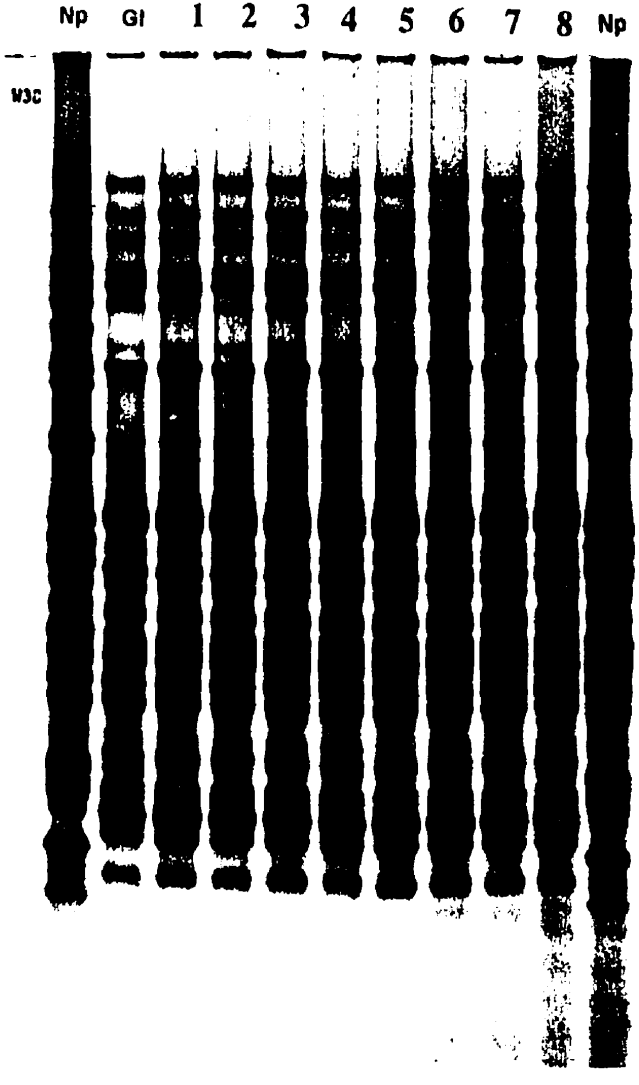
APPENDIX

Figure 30. A- PAGE of Glenlea and Katepwa wheat assessed for varietal purity and homogeneity. Np = Neepawa, Gl = Glenlea (100 kernels), Kp = Katepwa, 1-8 = single seeds.

GLENLEA



KATEPWA

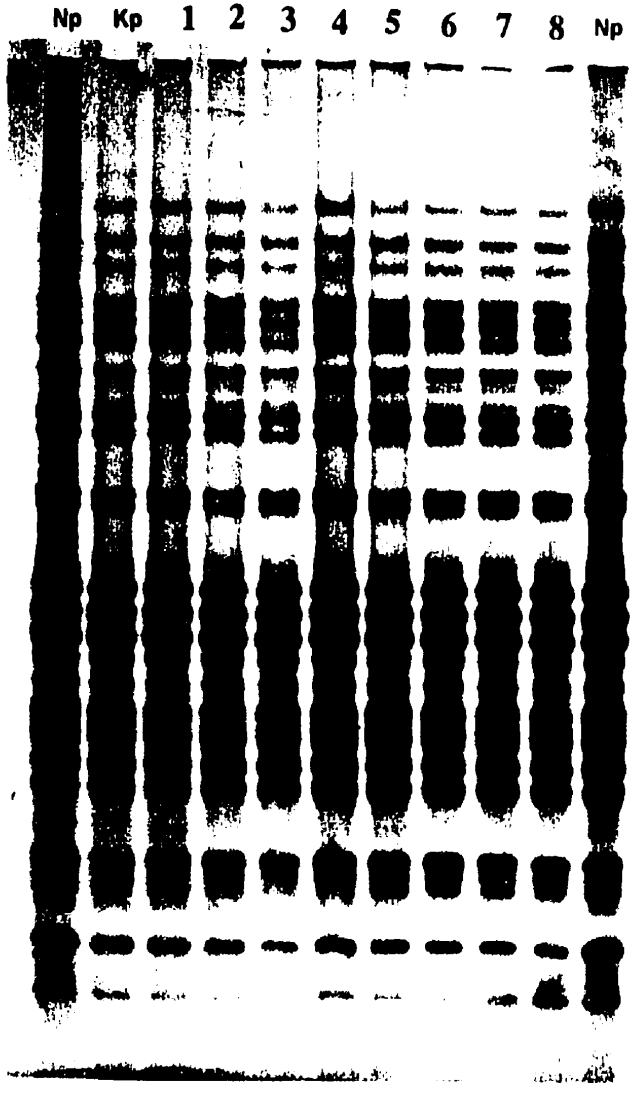
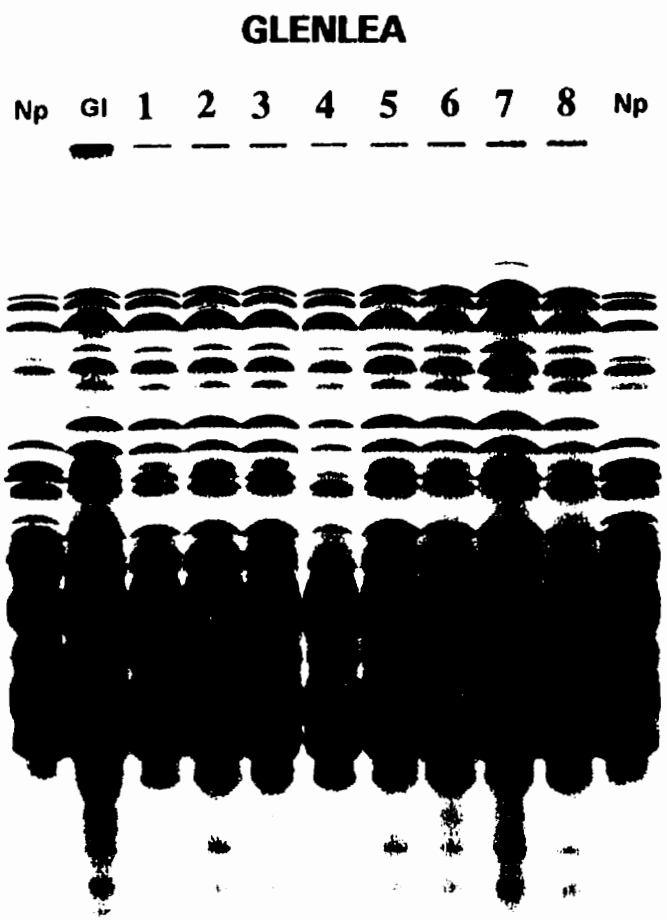


Figure 31. SDS-PAGE of Glenlea and Katepwa wheat assessed for varietal purity and homogeneity. Np = Neppawa, Gl = Glenlea (100 kernels), Kp = Katepwa, 1-8 = single seeds.


GLENLEA
Np Gl 1 2 3 4 5 6 7 8 Np

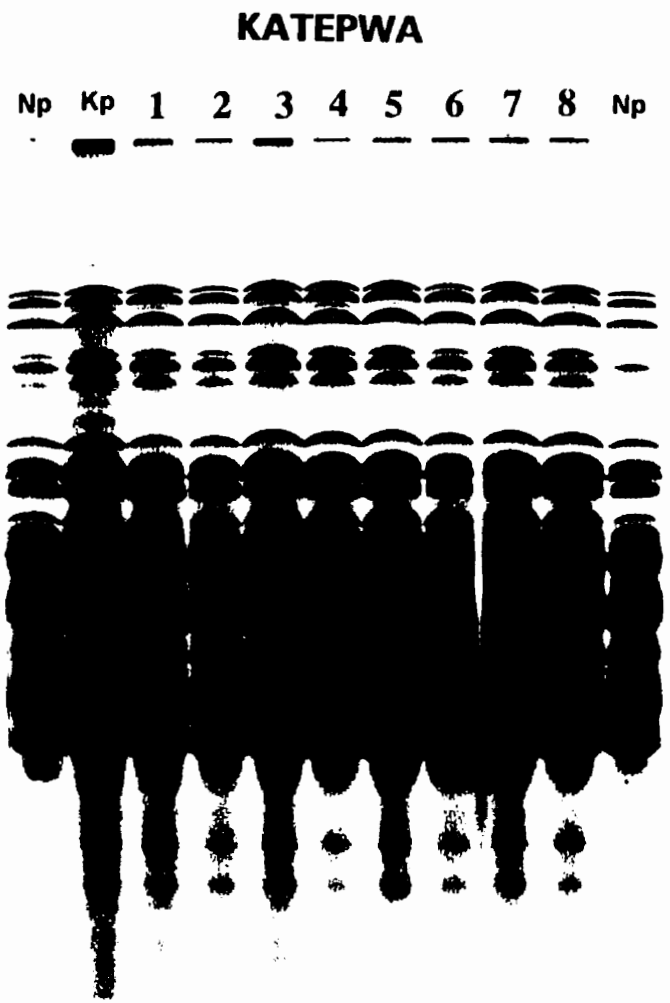
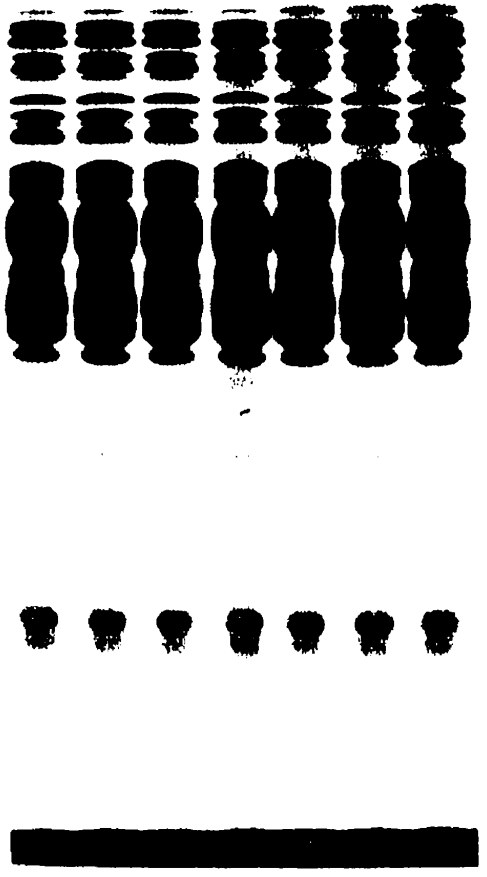

KATEPWA
Np Kp 1 2 3 4 5 6 7 8 Np

Figure 32. SDS-PAGE of total protein extracts of freeze-dried doughs mixed in a GRL-200 mixer. As defined in Table 2. F = flour, 1 = premix, no relaxation, 2 = premix, 45 min relaxation, 3 = peak development, no relaxation, 4 = peak development, 45 min relaxation, 5 = 15 min, no relaxation, 6 = 15 min, 45 min relaxation, 6 = 30 min, no relaxation, 7 = 30 min, 45 min relaxation. Gl = Glenlea and Kp = Katepwa, total protein extracts.

GLENLEA

Gl 1 2 3 4 7 8



KATEPWA

Kp 1 2 3 4 5 6 7 8

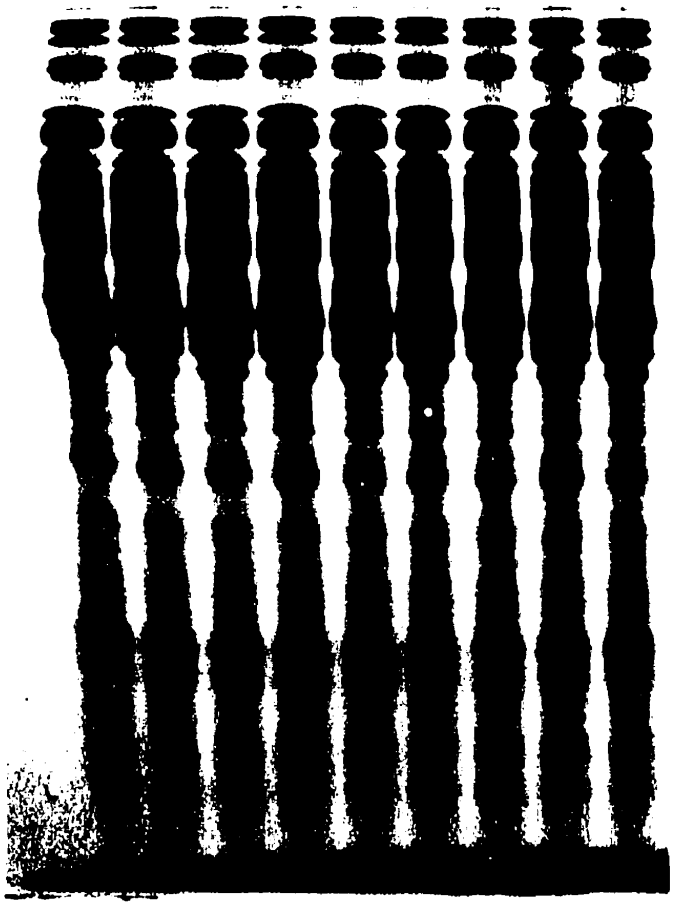


Figure 33. SDS-PAGE of total protein extracts of freeze-dried doughs mixed in a mixogram.F = flour, 1 = premix, no relaxation, 2 = premix, 45 min relaxation, 3 = peak development, no relaxation, 4 = peak development, 45 min relaxation, 5 = 20 min, no relaxation, 6 = 20 min, 45 min relaxation.Gl = Glenlea and Kp = Katepwa, total protein extracts.

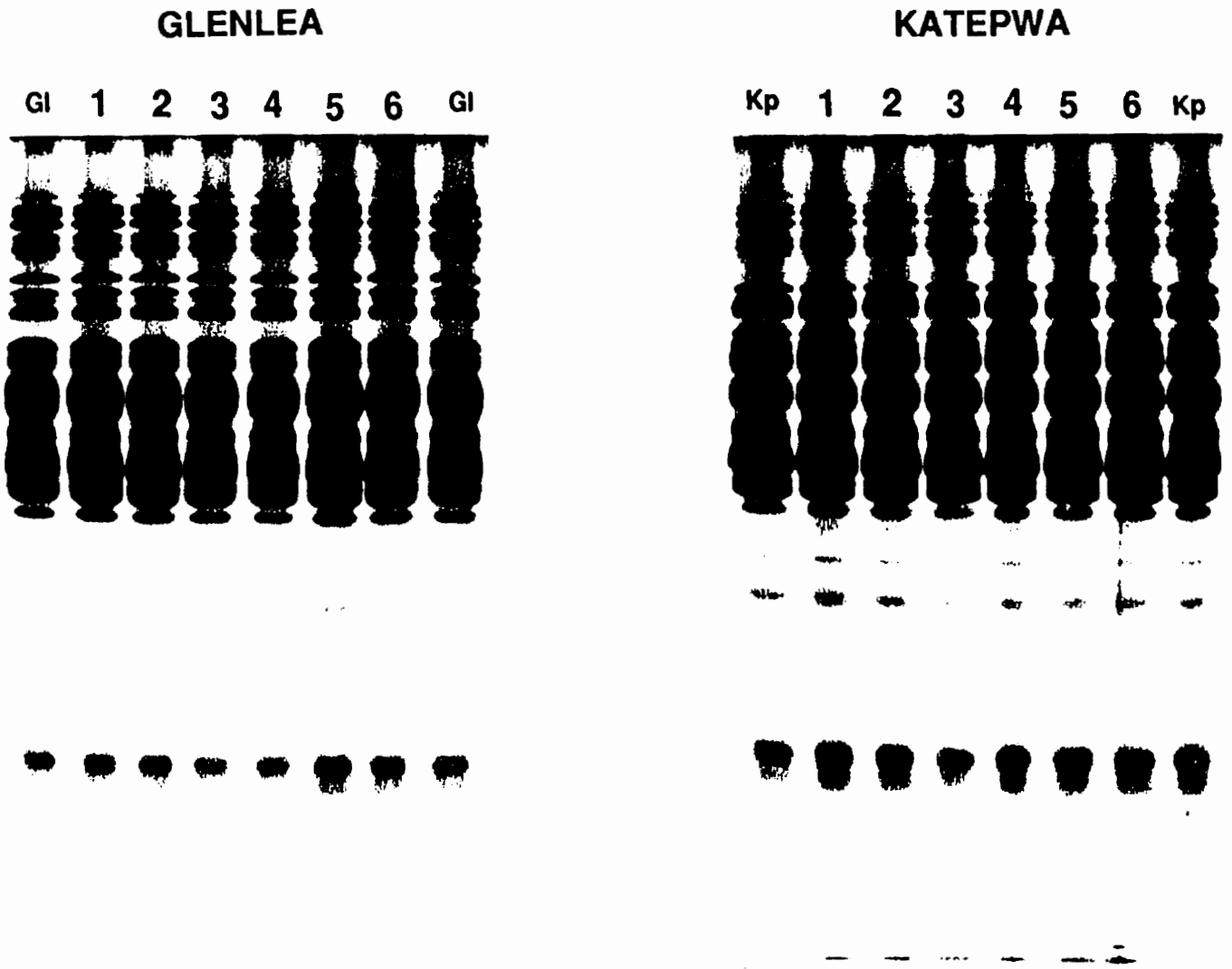
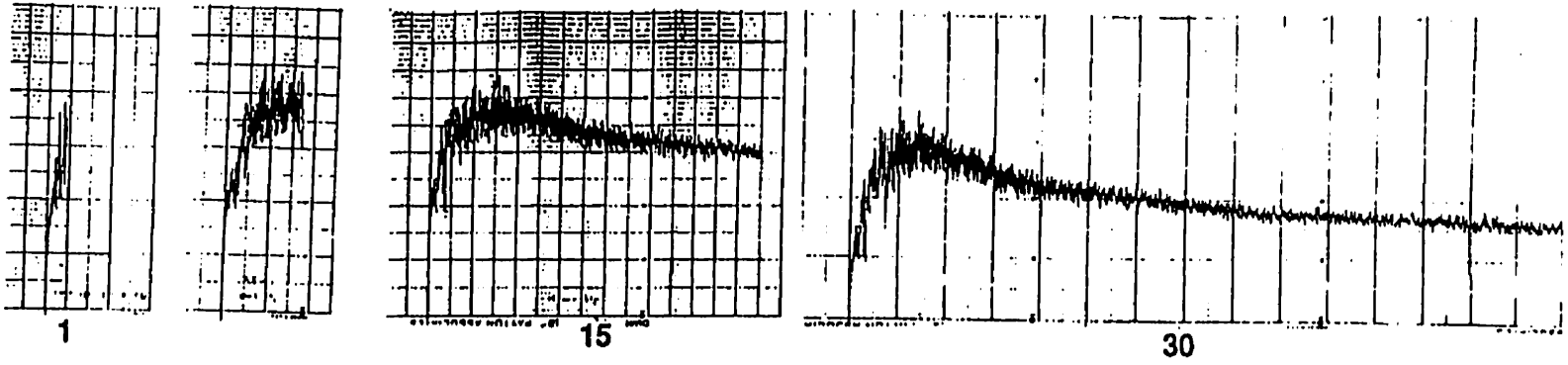
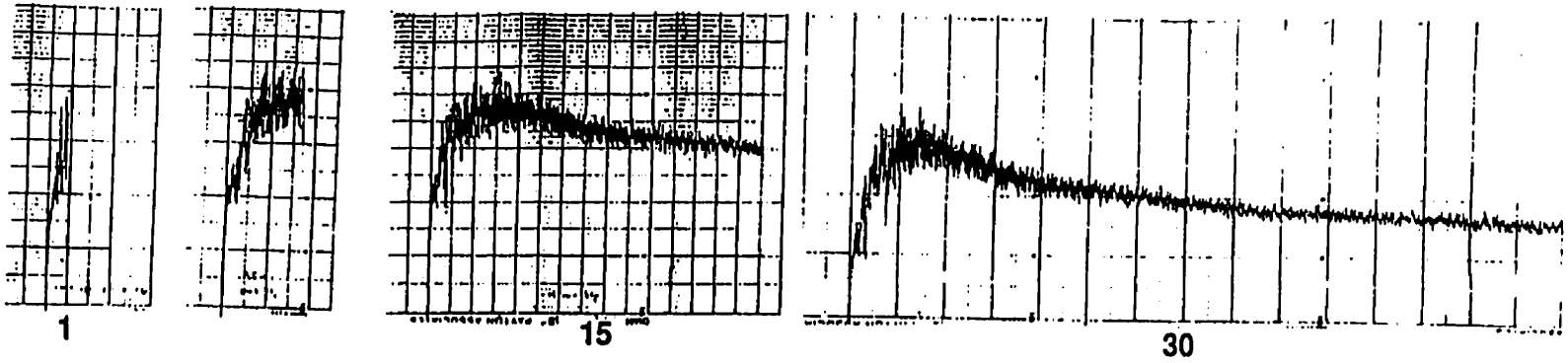

GLENLEA
Gl 1 2 3 4 5 6 Gl
KATEPWA
Kp 1 2 3 4 5 6 Kp

Figure 34. Mixing curves of Glenlea flour-water-1% salt doughs in air in a GRL-200 mixer for 1 min, 15 min (PDD), and 30 min.



Mixing Time (min)

Figure 35. Mixing curves of Katepwa flour-water-1% salt doughs mixed in air in a GRL-200 mixer for 1 min, 3 min (PDD), 15 min, and 30 min.



Mixing Time (min)

Figure 36. Farinograms of Glenlea and Katepwa.

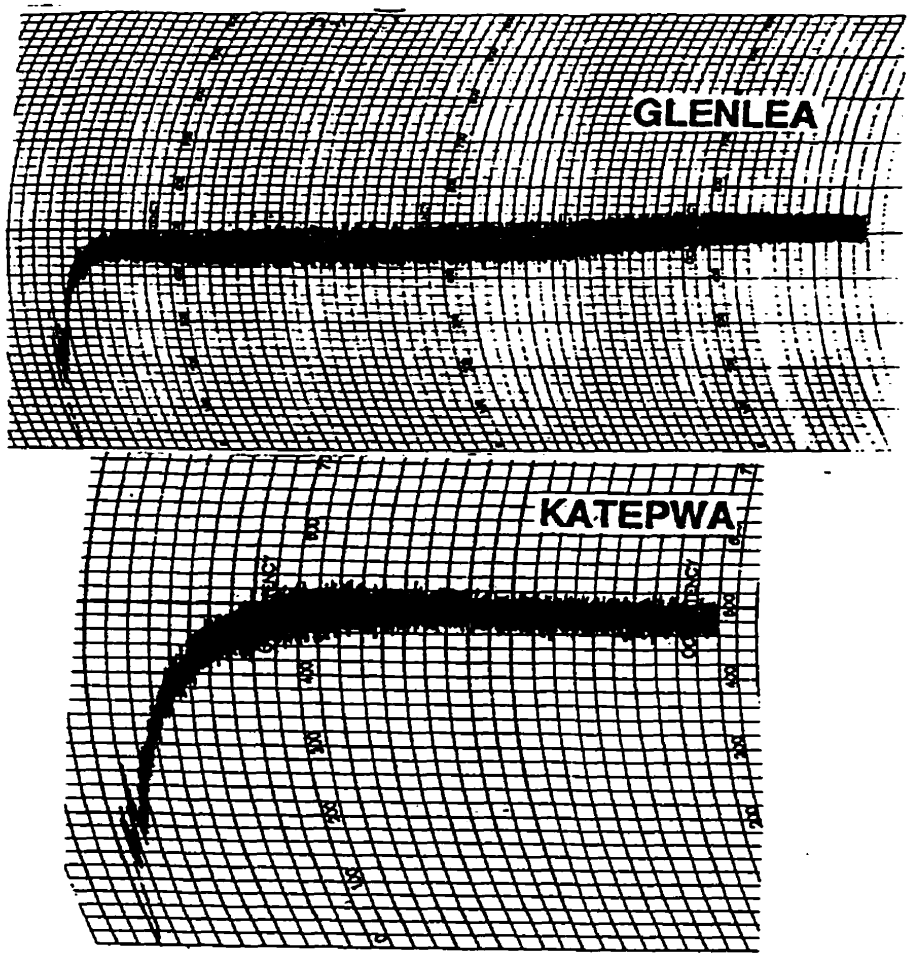
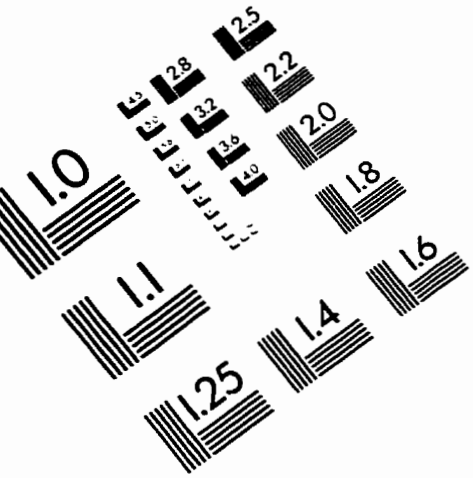
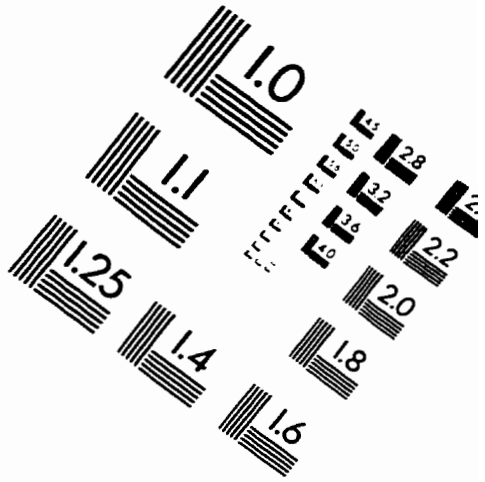
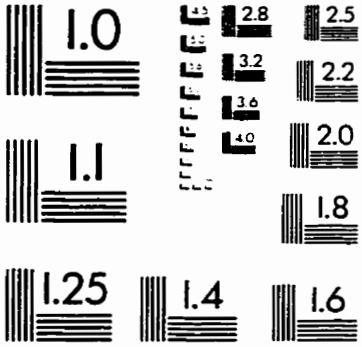
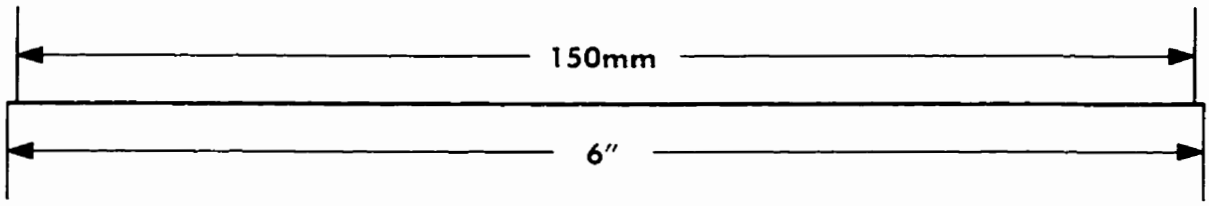

GLENLEA
KATEPWA

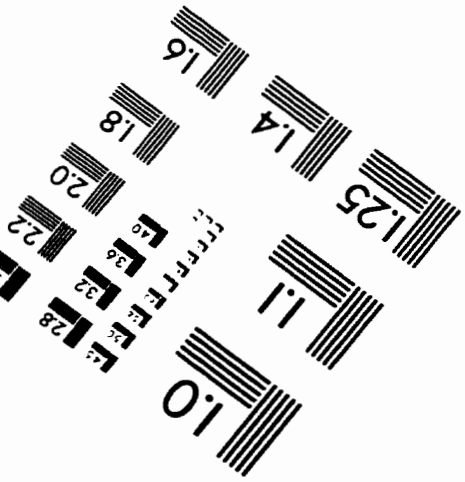
IMAGE EVALUATION
TEST TARGET (QA–3)

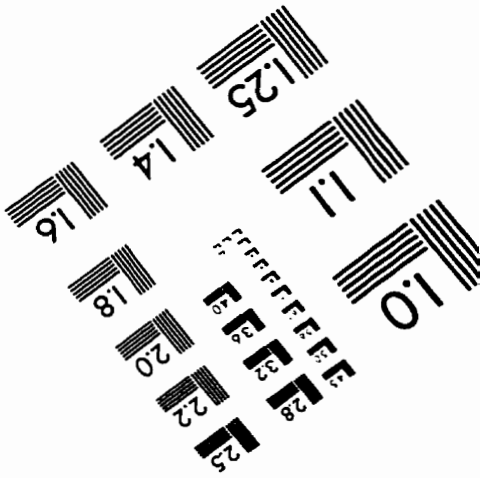












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