EFFECTS OF DOUGH MIXING ON GLUTEN PROTEINS

A Thesis

Submitted to the Faculty

of

Graduate Studies

The University of Manitoba

by

Brigitte Dupuis

In Partial Fulfillment of the

Requirements for the Degree

of

Doctor of Philosophy

Food and Nutritional Sciences

January 1999

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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BRIGITTE DUPUIS

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

DOCTOR OF PHILOSOPHY

À mon cher Grandpapa Gérard Dupuis,
et
À ceux qui donnent brillance à ma vie,
Merci.

ꕥ

In the midst of winter,
I found in me an invincible spring.

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You cannot acquire experience by making experiments.
You cannot create experience.
You must undergo it.

Albert Camus

ABSTRACT

Dupuis, Brigitte, Ph.D., The University of Manitoba, January 1999

Effects of Dough Mixing on Gluten Proteins

Advisor: Dr. Walter Bushuk

The gluten proteins, gliadin and glutenin, are mainly responsible for the viscoelastic properties unique to wheat flour dough. This study was undertaken to evaluate the changes occurring to the gluten proteins during dough mixing and to examine how these proteins, related to breadmaking quality, participate in the mechanism of dough development and breakdown.

Flours from four different cultivars were selected for their wide range of mixing strength. Flour-water doughs and doughs containing potassium iodate or N-ethyl-maleimide were undermixed, mixed to peak development, and overmixed. A small scale fractionation procedure, coupled with a selective precipitation method, was used to obtain six protein fractions: salt-soluble (SS), ethanol-soluble (ES) gliadin and glutenin, acetic acid-soluble (AS) gliadin and glutenin, and acetic acid-insoluble (AI) glutenin. The solubility distribution and composition of the fractions were monitored at each stage of mixing.

The presence of glutenin in the SS fraction and the formation of a foam layer during fractionation of doughs suggested that mixing altered the conformation of glutenin and/or induced gliadin-glutenin interaction to an extent sufficient to enhance the solubility and surface activity of some of the gluten proteins. The ES gliadin and glutenin increased

during mixing, but showed no cultivar-specific behaviour related to mixing strength. The quantity of AI glutenin was directly related to mixing strength and decreased during mixing. AS gliadin in the flours was inversely related to dough mixing strength and was attributed to genotype-specific gliadin-glutenin interaction.

Protein solubility distribution and electrophoretic results provided convincing evidence for the existence of genotype-specific gliadin-glutenin interaction. Results showed that all cultivars exhibited gliadin-glutenin interaction during mixing and the degree of interaction was inversely related to mixing strength. Intrinsic gliadin-glutenin interaction was measured by quantifying the amount of gliadin in the AS protein fraction of flours. Gliadin-glutenin interaction was manifested by an unusual drop in the AS gliadin during the very early stages of mixing of doughs. Strong cultivars (with long mixing requirements) exhibited the lowest degree of interaction and weak cultivars (with short mixing requirements) exhibited the highest.

Analysis by reversed-phase high-performance liquid chromatography of changes in subunit composition during mixing of three glutenin fractions revealed some variation in subunits related to quality. Allelic differences were most pronounced for the 1Dx subunits (1Dx2 versus 1Dx5) and much less evident for 1Ax and 1B subunits. The 1Ax and 1Bx subunits appeared to be less affected by the mixing process. The 1Dx and y-type (1B, 1D) subunits exhibited more dynamic behaviour throughout mixing, suggesting a more active role in the -SH/-SS- interchange reactions for these subunits. However, differences observed in the glutenin subunit composition of glutenins during mixing could not explain the large changes in dough properties and the inherent variation between cultivars.

Using size-exclusion high-performance liquid chromatography, the presence of glutenin comprised only of LMW-GS (LMW glutenin) was identified in the ES, AS and AI fractions of glutenin. LMW glutenin, like the gliadins, may be involved in interaction with the glutenin of the larger M_r. The absorption level used to mix a dough influenced the extent of glutenin breakdown (reduced M_r and enhanced solubility) and possibly the degree of gliadin-glutenin interaction (proportion of AS gliadin). Full formula ingredients and resting did not appear to have a major impact on the protein solubility distribution and glutenin subunit composition.

Results from this study provide additional support for glutenin breakdown and the sulphydryl-disulfide (-SH/-SS-) interchange reaction as important mechanisms in dough mixing and offer convincing evidence for gliadin-glutenin interaction as an additional mechanism. This study concludes that glutenin breakdown occurs by both depolymerization and disaggregation and that the -SH/-SS- interchange reaction, like gliadin-glutenin interaction, exerts its functional importance at a higher structural level than the subunit level.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Dr. W. Bushuk for having accepted to guide me through my graduate experience. I will always remember his quiet guidance, good humour, unspoken confidence in my abilities, and his patience in the last few years. I also wish to thank Dr. H.D. Sapirstein for the many helpful discussions and for his patience in explaining concepts or illustrating techniques in the laboratory. My sincere thanks to the other members of my advisory committee, Dr. B. Marchylo, for his support and help at the Grain Research Laboratory (GRL), and Dr. L. Malcolmson, for her mentorship and special guidance in things not found written in a textbook. I would also like to thank Dr. F. MacRitchie, Kansas State University, for accepting to serve as the external examiner on my examining committee.

I would like to acknowledge those that provided invaluable technical assistance: Dr. F. MacRitchie, for SE-HPLC data on the flours used for this study, Dr. F. Békés, CSIRO Division of Plant Industry, Sydney, Australia, for preparing a large number of full formula dough samples; Michael Nightingale, GRL, for his invaluable help with SE-HPLC analyses; Jean Mellish and Sandy Gowan, GRL, for sharing their expertise in electrophoresis and silver staining; Joan Morgan and Bruno Klassen, GRL, for help with the starch damage test; Rick Zillman, for help in the bake lab and especially for offering me his home when I suddenly needed a roof over my head; Al Patterson, Bob Talbot and Heather Warkentin, Imaging Services, for their photographic skills and friendly service; Wayne Johnson and Randy Roller, for their continuous technical assistance over the last

five years, but most of all for their patience and moral support, their humour and their friendship throughout the roller coaster ride we call graduate life.

To my friends and colleagues at the Department of Food Science, the Grain Research Laboratory, the Agriculture and Agri-Food Canada Cereal Research Centre, and the Canadian International Grains Institute, so many of you have touched my life in one way or another. I thank you all for your support, for the fun, the laughter, and the daily sharing.

Finally, I would like to gratefully acknowledge the financial assistance provided by: Natural Sciences and Engineering Research Council of Canada (1967 Science & Engineering Scholarship), University of Manitoba (Duff Roblin Graduate Fellowship, William B. Malchy Graduate Fellowship, Garson N. Vogel Memorial Award), American Association of Cereal Chemists (Rheology Division and A.E. Staley Mfg. Co.), International Wheat Gluten Association (Gluten Workshop, Sydney, AU), Canadian Institute of Food Science and Technology/International Union of Food Science and Technology (9th World Congress of Food Science and Technology, Budapest, Hungary), Canadian Federation of University Women Scholarship, and last but not least Mom and Dad.

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LIST OF ABBREVIATIONS

Abbreviation	Description
70PS	70% 1-propanol-soluble
70PI	70% 1-propanol-insoluble
AI	acetic acid-insoluble
AS	acetic acid-soluble
AS gliadin	acetic acid-soluble-propanol-soluble, ASPS
AS glutenin	acetic acid-soluble-propanol-insoluble, ASPI
ASPI	acetic acid-soluble-propanol-insoluble, AS glutenin
ASPS	acetic acid-soluble-propanol-soluble, AS gliadin
Do	AC Domain, variety of the Canada Western Red Spring class
ES	ethanol-soluble
ES gliadin	ethanol-soluble-propanol-soluble; ESPS
ES glutenin	ethanol-soluble-propanol-insoluble; ESPI
ESPI	ethanol-soluble-propanol-insoluble; ES glutenin
ESPS	ethanol-soluble-propanol-soluble; ES gliadin
Gl	Glenlea, variety of the Canada Western Extra Strong class
GS	glutenin subunit
HMW-GS	high molecular weight glutenin subunits
Ka	AC Karma, variety of the Canada Prairie Spring class
Kp	Katepwa, variety of the Canada Western Red Spring class
LMW-GS	low molecular weight glutenin subunits
M_r	relative molecular mass
MT	mixing time
MU	mixograph units
NEMI	N-ethylmaleimide
RP-HPLC	Reversed-phase high-performance liquid chromatography
SDS-PAGE	sodium dodecyl sulfate polyacrylamide gel electrophoresis
SEC	size exclusion chromatography
-SH	sulphydryl
-SS-	disulfide
SS	salt-soluble
%UPP	% unextractable polymeric protein

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FORWARD

The following publications or presentations have resulted from the studies reported in this thesis:

1. Dupuis, B. and Bushuk, W. 1996. Variation in high molecular weight glutenin subunit composition in various solubility fractions of flours of diverse dough strength. Pages 262-266 in: Gluten '96, Proc. 6th Int. Gluten Workshop, Sept. 2-4, 1996, Sydney, Australia. C.W. Wrigley (ed.), Royal Aust. Chem. Inst., N. Melbourne.

2. Dupuis, B., Bushuk, W. and Sapirstein, H.D. 1997. Changes in gluten proteins during mixing of flours of diverse breadmaking quality. Cereal Foods World 42: 659. AACC 82nd Annual Meeting.

3. Dupuis, B., Bushuk, W. and Sapirstein, H.D. 1999. Effects of dough mixing on the solubility distribution of gluten proteins: evidence for gliadin-glutenin interaction. (in preparation)

4. Dupuis, B., Bushuk, W. and Sapirstein, H.D. 1999. Effects of dough mixing on the subunit composition of three glutenin fractions of different solubility. (in preparation)

5. Dupuis, B., Bushuk, W. and Sapirstein, H.D. 1999. Effects of dough mixing on the quantity and distribution of glutenin comprised only of low molecular weight subunits. (in preparation)

1. INTRODUCTION

Wheat is one of the most important cereal grains worldwide, in terms of production and utilization. Wheat flour has the unique ability to form a viscoelastic dough when mixed with water. We have taken advantage of this unique property in the production of an enormous variety of baked products, one of which is bread. The storage proteins of wheat flour provide the unique viscoelastic properties that are essential to the development of a bread wheat dough suitable for breadmaking.

Since the discovery that breadmaking quality of wheat flours was related to protein content and quality (Finney and Barmore, 1948), research has focused on elucidating the molecular basis for the functionality of the storage proteins in relation to breadmaking quality. Approximately half of the storage proteins are gliadins, the monomeric proteins that confer viscous properties to bread doughs. The remaining 50% consists of glutenins, large polymers of disulfide-linked subunits with a M_r distribution ranging up into the tens of millions (Wrigley, 1996) that confer elasticity to bread doughs. Although the relationship of gliadins to breadmaking quality is still controversial, sufficient evidence has accumulated that shows a distinct and strong relationship between the glutenins and quality (recently reviewed by Weegels et al, 1996).

Correlative studies have shown that variation in the portion of glutenin insoluble in various solvents is related to the variation in dough strength or breadmaking performance. This fraction of glutenin, considered to be of high M_r, as well as the presence of specific subunits of glutenin, the quantity and the M_r distribution of glutenin have all been related to quality. Most of these studies, based on flour, have been used to evaluate the same

factors during dough development and breakdown. Protein solubility distribution, using a number of solvents, and protein composition have been most extensively studied in an effort to understand the mixing phenomenon. Much of the research on dough mixing was reported some decades ago. But renewed interest has resulted in the application of new fractionation methods and improved techniques (eg. electrophoresis, RP-HPLC, SE-HPLC) to the study of changes in the storage proteins during dough mixing. Much less attention has been given to the possible role of interactions between proteins, such as between gliadin and glutenin, in mixing behaviour. Because of the strong correlation between the insoluble fraction of glutenin and mixing strength, there has also been very little research on glutenins of low molecular weight.

In light of the above, the broad objective of this thesis was to obtain a better understanding of the mechanism of dough development and breakdown using flours of diverse strength. The first objective was to investigate the changes in solubility of flour proteins during mixing of flour-water doughs. A small-scale modified Osborne fractionation procedure was initially developed to reduce the time of fractionation and to increase sample size. This fractionation procedure produced three fractions of interest, all containing gliadins and/or glutenins: the ethanol-soluble, acetic acid-soluble, and acetic-acid-insoluble fractions. Because of significant cross-contamination in the two soluble fractions, a modified purification method was incorporated with the fractionation procedure to separate gliadins and glutenins. The changes occurring during mixing to the fractionated and purified fractions were evaluated. Doughs containing potassium iodate, an oxidizing agent, or N-ethylmaleimide (NEMI), a sulfhydryl-blocking agent, were similarly studied. Although the mechanisms are different, the reaction of these chemicals

with accessible sulfhydryl groups in dough would interfere with the sulfhydryl-disulfide interchange reaction thought to be of major importance in dough development. Effects of these treatments compared to control doughs might provide additional information on protein behaviour during dough mixing.

The second objective was to evaluate the glutenin subunit composition of the three glutenin fractions and to determine how they might be affected by mixing. More specifically, were there changes in the glutenin during mixing that involved a predominance of the high molecular weight glutenin subunits (HMW-GS) associated with quality? This objective was achieved by the use of reversed-phase high-performance liquid chromatography (RP-HPLC).

Other objectives of the thesis study included: monitoring of ω-gliadins that co-precipitate with glutenins during purification and evaluating whether changes during mixing were related to breadmaking quality (RP-HPLC); measuring the content of glutenin comprised only of LMW-GS (SE-HPLC) and evaluating the importance of the changes in these proteins during mixing; briefly evaluating (a) the mixing behaviour of full formula doughs versus flour-water doughs made from the same flours (b) the effect of resting after mixing on the storage proteins in full formula doughs. In addition to the effect of mixing, intervarietal differences, and interactions where appropriate, were also evaluated for all objectives discussed.

The hypotheses being tested were:

(1) Mixing causes depolymerization and disaggregation of glutenin. This reduction in M_r would be reflected in a change in protein solubility distribution.

(2) Glutenins respond differently to mixing depending on the subunit composition. This difference in response would be detected by variation in the glutenin subunit composition for each fraction and mixing time.

(3) Gliadin-glutenin interaction is an important factor influencing the mixing properties of a flour. Additional evidence in support of the hypothesis previously described (Almonte, 1998, Dupuis et al, 1996, Fu et al, 1996) would arise from the analysis of protein solubility distribution changes during mixing.

2. LITERATURE REVIEW

2.1. Introduction

The four major unit operations in the commercial production of bread are: mixing, fermentation, proofing, and baking. Regardless of the process or formulation used, dough mixing is considered the most critical step. Mixing is required to blend ingredients into a homogeneous mass and hydrate flour particles. Occlusion of air during dough mixing creates the nuclei for gas cells which will later expand during fermentation and set during baking. Sufficient work input, in the form of mixing, must be provided to develop a dough to optimum. An optimally developed dough must have the viscoelastic properties necessary to maintain expansion and gas holding capacity through the subsequent fermentation, proofing, and baking stages.

Dough development is thought to result from the disaggregation/ depolymerization of glutenin and the realignment and aggregation of glutenin and gliadin. Both processes of disruption and formation reach an optimum balance to form a continuous protein network of optimum functionality. The physical mechanism of development involves the scission of covalent bonds, specifically disulfide bonds, the disruption of noncovalent interactions, and the alignment of molecules by the shear created in mixing. Interchange reactions (-SH/-SS-) occur to relieve the stress exerted by mixing and are influenced by the presence of oxidants (eg. oxygen, iodate), reducing agents (eg. glutathione, L-cysteine-hydrochloride) and thiol-blocking agents (NEMI). Changes in protein solubility, molecular weight, and sulfhydryl content have been reported to occur as part of this proposed mechanism of dough development. However, despite these observations, little

experimental evidence exists to support the generally held view of dough development and the importance of interchange reactions and molecular weight distribution. The actual mechanisms involved in the development of optimum dough structure can only be hypothesized until further research provides additional information on the structure and interactions of the gluten proteins.

Two major problems continue to challenge cereal chemists in their investigation of the molecular basis for dough strength. Wheat storage proteins are difficult to solubilize and cross-contamination occurs in different solubility fractions. This is clearly evident with the modified Osborne procedure where gliadins and glutenins are mutually soluble to varying degrees in acqueous ethanol and dilute acetic acid solutions. Recently, an improved fractionation procedure involving selective precipitation with different solutions of 1-propanol was developed (Fu and Sapirstein, 1996) which can minimize or eliminate cross-contamination. The method involves more steps than a simple fractionation procedure, but allows for the relatively pure protein fractions to be correlated to quality parameters. A second problem, also related to solubility, is the very large size of native glutenin. Current techniques do not allow for the analysis of glutenin in its native state. But newer methods, such as multi-stacking SDS-PAGE (Khan and Huckle, 1992), field-flow fractionation (Stevenson and Preston, 1996), and techniques based on light scattering (Sutton, 1996) may overcome this problem.

This thesis focuses on the storage proteins responsible for the unique viscoelastic properties of wheat flours. This literature review will focus on those storage proteins, their composition, structure, and genetics. In particular, the relationship of the storage proteins to dough mixing, as one factor in breadmaking quality, will be reviewed.

2.2. Wheat Flour Proteins

2.2.1. Composition

Osborne (1907) classified wheat proteins into four groups based on solubility. Sequential extraction with water, salt solution (0.5M NaCl) and 70% ethanol produced four wheat flour protein fractions classified as: water-soluble albumins, salt-soluble globulins, alcohol-soluble gliadins and glutenins, protein insoluble in 70% ethanol. The modified Osborne fractionation method (Chen and Bushuk, 1970) further classified glutenins into dilute (0.05N) acetic acid-soluble and -insoluble fractions. The albumins are proteins of low relative molecular mass (M_r <30,000) constituting about 15% of total protein (Bushuk, 1993). Globulins, making up about 5% of total protein, have a greater range of M_r (10,000-98,000). Except for the high molecular weight (HMW) albumins (Gupta et al, 1991a) and the triticins (Singh et al, 1987), the albumins and globulins are metabolic proteins of a non-storage nature.

The storage proteins, gliadin and glutenin, comprise upto 80% of total flour protein and confer the viscoelastic properties unique to wheat flours. Gliadins, forming about 40% of total protein, are single chain polypeptides (M_r = 30,000-80,000) classified as α-, β-, γ-, and ω-gliadins, based on their relative mobility during acid-PAGE (Bushuk and Sapirstein, 1991). The α-, β-, and γ-gliadins (M_r = 36,000-44,000) can be classified as sulfur-rich, whereas the ω-gliadins (M_r = 50,000-75,000) are classified as sulfur-poor because they lack cysteine residues (Field et al, 1983a). The ω-gliadins have unusual amino acid compositions, with high levels of glutamine (40-50 mol%), proline (20-30 mol%) and phenylalanine (7-9 mol%), low levels of charged residues and no cysteine

(Shewry et al, 1994). The α-, β-, and γ-gliadins, together with the LMW glutenins, have the most variable amino acid composition, reflecting their highly heterogeneous nature compared to that of the ω-gliadins and HMW glutenins. Glutenins are very large polymeric storage proteins made up of disulfide-linked HMW (M_r 95,000-140,000) and low molecular weight (LMW, M_r 30,000-51,000) subunits (Payne and Corfield, 1979). The HMW-GS are rich in glutamine (35 mol%) and glycine (20 mol%) and low in proline (10 mol%) compared with other prolamin groups. The HMW-GS are sometimes designated as A-subunits and the LMW subunits are designated as B- and C-subunits, based on their mobility (slower to faster, respectively) in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). A group of LMW subunits with relative mobilities intermediate between the HMW and B-LMW subunits is designated as D-subunits. The D-subunits are mutated ω-gliadins containing only one cysteine residue (Masci et al, 1993, 1995). The glutenin subunits are cross-linked by disulfide bonds to form the largest proteins in nature, with M_r's estimated to range up into the millions (Sutton, 1996, Wrigley, 1996). These estimates are based on a new method known as field-flow fractionation (Gustavsson et al, 1994; Wahlund et al, 1996). Both the LMW-GS and the HMW-GS polymerize to form native glutenin, although studies of null lines have shown that glutenin polymers can be formed consisting only of LMW-GS (Gao and Bushuk, 1993) or HMW-GS (Gupta et al, 1995).

2.2.2. Structure and Genetics

Three distinct features are common to the primary structure of gliadins and glutenin subunits: a central repetitive domain flanked by non-repetitive N- and C-

domains. The α-/β-, and γ- gliadins, like the LMW glutenin subunits (LMW-GS), are characterized by a relatively short repetitive sequence in the central domain (89-137 residues) and long (up to 185 residues) C-terminal domains (Colot, 1990). The α-/β-, and γ- gliadins contain α-helix (30-35%) and β-sheet structures (10-20%). The high stability of the helical conformation is considered to be due in part to extensive hydrogen bonding as well as to the presence of disulfide bonds (Tatham and Shewry, 1985). The LMW-GS resemble the sulfur-rich gliadins in amino acid composition, secondary structure and cysteine content. The repetitive domain is rich in β-turns with short stretches of α-helix, while the non-repetitive C-terminal domain is richer in α-helix with regions of β-turns and β-sheet (Tatham et al, 1987). The C-terminal domains contain most or all of the cysteine residues (6-8), although one cysteine is found at the N-terminal of LMW-GS (Shewry et al, 1994).

In contrast, the ω-gliadins and HMW glutenin subunits (HMW-GS) have short N- and C-terminal domains and their sizes are governed by the relatively long central repetitive domains (285-666 residues). The ω-gliadins are rich in β-turns, which are probably interspersed with random coils. No α-helix or β-sheet is detectable and hydrophobic interactions are very important to conformational stability (Tatham and Shewry, 1985). The HMW-GS central repetitive domains are reported to be rich in β-reverse turns, while the nonrepetitive N- and C-terminal domains are predicted to consist predominantly of α-helical structure. Differences in subunit size result mainly from variation in the repeat structure and in particular from differences in the number of hexapeptide and tripeptide motifs (D'Ovidio et al, 1994). Two types of HMW-GS exist in

native glutenin: the high M_r x-type and the low M_r y-type. Tripeptide motifs are found in the x-type central domain, in addition to the hexa- and nonapeptide motifs common to both types of HMW-GS. These subunits vary in repeat motifs and cysteine content. Three (x-type) and five (y-type) cysteine residues are located in the N-terminal and one in the C-terminal of HMW-GS, and several have an extra cysteine residue in the central repetitive domain close to either terminal (Shewry et al, 1992). A cysteine substitution in the N-terminal domain of the subunit 1Dx5 is unique among HMW-GS and could result in a more highly cross-linked glutenin polymer when this subunit is present (Shewry et al, 1992).

The gliadins are genetically controlled by genes on the short arms of the homoeologous group 1 and 6 chromosomes. The loci of group 1 chromosomes (*Gli-1*) code for all ω-, most γ-, and a few β-gliadins. The group 6 chromosome loci (*Gli-2*) code for all α-, most β-, and some γ-gliadins. Gliadin genes are tightly linked to each other and those on group 1 chromosomes are also linked with the genes controlling the low molecular weight glutenin subunits (LMW-GS). Each gliadin locus controls the synthesis of several mutually inherited polypeptides, and 12-30 alleles have been recently assigned to each locus (Metakovsky, 1991).

Genes for the major polypeptides of glutenin, the LMW-GS, are located on the short arms of homoeologous group 1 chromosomes. There is some evidence that a few C subunits may be controlled by genes on group 6 chromosomes (Tao and Kasarda, 1989; Gupta and Shepherd, 1993). Gene loci for the LMW-GS (*Glu-3*) and the gliadins (*Gli-1*) are tightly linked. The *Glu-B3* and *Gli-B1* loci are reported to be 1.8-2.0 cM apart, whereas no recombination has been reported between *Glu-A3* and *Gli-A1* and between

Glu-D3 and *Gli-D1* (Dachkevitch et al, 1993). Any one cultivar can contain from 7 to 16 LMW-GS (MacRitchie, 1992). The LMW-GS genes are very similar (6-65% sequence identity in the large nonrepetitive domain) to the α-, β-, and γ-gliadin genes (Colot, 1990). Allelic patterns suggest that the LMW-GS are controlled by a cluster or block of genes (Gupta and Shepherd, 1993).

The HMW-GS are genetically controlled by genes on the long arms of group 1 chromosomes. Each *Glu-1* locus contains two tightly linked genes (*Glu-1-1* and *Glu-1-2*) which code for a high M_r x- and a lower M_r y-type subunit, respectively (Ng et al, 1989). Each variety contains between three and five subunits, two of which are controlled by *Glu-1* loci on chromosome 1D, one or two by chromosome 1B, and none or one by chromosome 1A (Orth and Bushuk, 1974; Payne et al, 1981a; Shewry et al, 1992). Sequence homology between alleles is very high (as much as 97%), with differences of only a few amino acids.

2.2.3. Relationship to Breadmaking Quality

The existence of a protein quality factor has been known for many years (Finney and Barmore, 1948). Fractionation studies have shown a distinct relationship between solubility and breadmaking quality. Flours of good quality have a greater proportion of protein insoluble in aqueous solutions of urea (Pomeranz, 1965), acetic acid (Orth and Bushuk, 1972), hydrochloric acid (MacRitchie, 1987), and sodium dodecyl sulfate (Gupta et al, 1993). The importance of glutenin as a protein quality factor in breadmaking potential was first demonstrated by Orth and Bushuk (1972). Since then, much research has focused on the possible relationship between the structure of glutenin and its functionality in breadmaking. Later studies provided further evidence for a solubility-

quality relationship (Field et al, 1983b; Gao and Bushuk, 1992; MacRitchie, 1987; Marchylo et al, 1990; Moonen et al, 1982; for a review refer to Schofield, 1994). Sapirstein and Johnson (1996) developed a simple spectrophotometric method to measure the content of insoluble glutenin in flour. The method involves the solubilization of insoluble glutenin with 50% 1-propanol and dithiothreitol (after removal of monomeric protein with 50% 1-propanol). A very strong relationship (R^2=0.85) was reported between the spectrophotometrically determined insoluble glutenin content and mixing strength for the samples of Canadian wheats studied. Bean et al (1998) later developed a simple and rapid procedure also based on extraction of flour with 50% 1-propanol but using nitrogen combustion analysis for the combined determination of insoluble glutenin plus residue protein content. Good correlations between dough strength parameters and absolute and relative amounts of insoluble protein were found for the samples of U.S. wheats studied. Wieser et al (1998) also reported on a small scale and rapid method for the quantitation of different protein fractions in wheat, although no correlation analysis was undertaken. Their method involved the Osborne fractionation procedure and RP-HPLC analysis; total integrated area as measured by absorbance at 214 nm was used in combination with a standard curve to determine the quantity of protein in each fraction. The shortcoming of this method is the significant, and sometimes genotypic, cross contamination of the acetic acid-soluble fraction (Dupuis et al, 1996).

Reconstitution studies by Lee and MacRitchie (1971) provided evidence that the molecular weight distribution of glutenin influenced quality as measured by dough properties. By reconstitution with suitable amounts of high molecular weight gluten (2M urea insoluble) and whole gluten, MacRitchie (1973) was able to produce a flour of strong

dough characteristics from a flour that was originally weak and very extensible. MacRitchie (1973) concluded that the strength of a flour was directly related to the molecular weight distribution of its gluten proteins. Based on reconstitution/interchange studies of paired weak and strong flours, MacRitchie (1978) reported that differences in the baking performance of bread wheats were related to the properties of the acetic acid-insoluble glutenin. In another study using sequential extraction with dilute HCl, glutenins exhibited a large influence on mixing requirements and loaf volume. Gliadins were reported to decrease mixing requirements but have only a small negative effect on loaf volume (MacRitchie, 1987). The relative weakening effect of gliadins was reported to be in the order: ω-1 > ω-2 $\approx$ α- $\approx$ β- > γ- (Fido et al, 1997) when added to flour-water doughs mixed in a mixograph.

Huebner and Wall (1976) obtained two fractions of glutenin by gel filtration of AUC-extracted proteins from flours of varying quality. They reported that the ratio of glutenin I, a very high molecular weight fraction, to glutenin II, a broad spectrum of lower molecular weight glutenins, was generally higher for flours of better breadmaking performance. Flours of weak mixing and baking characteristics usually contained less unextractable protein and glutenin I.

Further support for the relationship between solubility-baking quality and glutenin properties (molecular weight, size distribution, subunit composition and ratio of HMW-GS to LMW-GS) was provided by Graveland et al (1982, 1985). Glutenins were separated into fractions varying in subunit composition and molecular weight. Glutenin I, SDS-insoluble gel protein, was the protein fraction of largest molecular weight (estimated at several million) and contained subunits in the ratio of 3 HMW-GS to 3.4 B-type-LMW-

GS to 1.7 C-type-LMW-GS. Glutenin II, SDS-soluble and 70% ethanol-insoluble, had the same ratio of subunits but was of lower molecular weight than glutenin I. Glutenin III, SDS-soluble and 70% ethanol-soluble, was the lowest molecular weight fraction and contained only LMW-GS in a ratio of 3.4 B-type to 1.7 C-type. A good breadmaking wheat variety was reported to contain more glutenins overall, more glutenin I and less glutenin II than a poor quality variety. The role of Glutenin III in quality was not discussed, but this fraction was identical to previously isolated ethanol-soluble (Payne and Corfield, 1979) and high molecular weight gliadin (Bietz and Wall, 1980) fractions.

In a later study (Gupta et al, 1993), it was reported that protein unextractable in 0.5% SDS was very strongly positively correlated with dough strength. Size-exclusion high-performance liquid chromatography (SE-HPLC) showed that the unextractable protein contained a greater proportion of larger polymers and a significantly higher ratio of HMW-GS to LMW-GS than the extractable fraction.

The composition of protein fractions and hence their relationship to breadmaking quality will vary with the fractionation procedure and the starting material used. However, based on three fractionation procedures and reconstitution studies, Chakraborty and Khan (1988a, 1988b) reported that, regardless of the fractionation procedure, fractions containing larger amounts of glutenin gave the highest positive responses to loaf volume. The extent of loaf volume response, however, was dependent on the fractionation procedure.

That protein quality for breadmaking is an inherited trait is supported by strong evidence which suggests that the presence of certain HMW-GS is correlated positively with good breadmaking quality. Orth and Bushuk (1973b) reported that the presence or

absence of certain HMW-GS coded for by the D-genome had a significant effect on baking quality. Payne et al (1979) showed that breadmaking quality and the composition of the HMW-GS were related. A strong correlation was reported between the presence of HMW-GS 1 and quality. A strong correlation between HMW-GS 5 and 10 and baking quality was subsequently reported (Payne et al, 1981b) and later supported by Ng and Bushuk (1988). Payne et al (1987) developed a HMW-GS scoring system, mainly based on the effects of these subunits on the SDS-sedimentation test, an indirect measure of breadmaking quality. The Glu-1 score allowed the ranking of the major HMW-GS in order of quality and the statistical evaluation of the amount of variation in bread-making quality attributable to the HMW glutenin subunits. The Glu-1 scores accounted for 59-69% of the variation in bread-making quality of 67 Canadian cultivars studied (Lukow et al, 1989). Moonen et al (1983) reported similar associations of specific HMW-GS alleles with quality, but also stated that the effects were additive. Subsequent research has shown that the proportion of the intervarietal variation in breadmaking potential that can be attributed to differences in the composition of the HMW-GS fluctuates widely between 15% and 60% depending on the group of varieties analyzed (Kolster, 1992). Epistatic effects between alleles at the *Glu-1* loci also have an important effect on quality (Carillo et al, 1990; Kolster et al, 1991; Rousset et al, 1992).

Allelic variation of LMW-GS can also affect quality, whereas any correlation with gliadins is less definitive. The co-migration of LMW-GS with gliadins in SDS-PAGE has made the study of these proteins difficult. The development of a two-step SDS-PAGE method has shown significant variation of LMW-GS patterns among bread wheat varieties (Gupta and Shepherd, 1987). These authors also reported an additive effect on dough

resistance when a specific LMW-GS (Glu-A3m) was present with a HMW-GS (Glu-A1b; i.e. 2*). In another study (Gupta et al, 1991b), correlations between predicted and actual dough quality parameters were reported to be highest if both LMW-GS and HMW-GS were included. Metakovsky et al (1990) found a correlation of dough resistance to extension (R_{max}) with LMW-GS of the B genome and with gliadin composition associated with chromosome 6A (mainly α-gliadins). They reported that extensibility related best to specific gliadin blocks coded by chromosomes 1A, 6B and 6D and LMW-GS of the A and D genomes.

In addition to HMW-GS composition, the ratio of HMW-GS to LMW-GS was also an important factor in breadmaking quality. Varieties with greater dough strength were reported to have a higher HMW-GS:LMW-GS ratio (Gupta and MacRitchie, 1991). For a fixed glutenin content, an increase in the HMW-GS:LMW-GS ratio resulted in a concomitant increase in dough strength (Gupta et al, 1992). MacRitchie and Gupta (1993) investigated the effects of sulfur deficiency in the soil on protein composition and functionality. The amounts of relatively sulfur-poor HMW-GS increased and the sulfur-rich LMW-GS, HMW albumins and triticins decreased with decreasing sulfur level. These changes resulted in a higher HMW-GS:LMW-GS ratio, higher percentage of unextractable polymeric protein (considered to be of higher molecular weight), and hence greater dough strength. Khatkar et al (1995) studied the dynamic rheological properties of glutens and gluten subfractions (gliadin and glutenin) from wheats of good and poor breadmaking quality. The viscoelastic properties of the glutenin subfraction and the ratio of gliadin to glutenin were reported to be the main factors governing intercultivar variation in breadmaking quality.

Evidence that gliadins affect strength or baking quality is conflicting. Some studies report a positive correlation with quality (Branlard and Dardevet, 1994) while others report the contrary (Fido et al, 1997). Evaluation of the influence of gliadins on mixing strength or baking quality are hampered by their comigration with LMW-GS in electrophoresis and HPLC methods of analysis. The tightly linked genes (*Gli-1* and *Glu-3*) may confound effects of gliadins and LMW-GS when conducting statistically based correlation studies: correlations discovered for one particular group of proteins may in fact be due to the other group of proteins expressed by the tightly linked genes.

Hamer et al (1992) reported that breadmaking quality was not only governed by the quality of the HMW-GS, but also by other factors, such as the amount of glutenin proteins. Others had previously suggested that, in addition to HMW-GS quality and composition, the quantity of glutenin or HMW-GS was also important (MacRitchie, 1987; Ng et al, 1989). Predictive models based on HMW-GS composition alone showed no correlation (Hamer et al, 1992). Expanding these models to include other parameters related to breadmaking quality improved the positive correlation between predicted and measured loaf volume. However, much of the variation could still not be accounted for with these models, leading Hamer et al (1992) to speculate on the relative importance of other factors, including amount of glutenin proteins, protein composition, and gliadins.

Despite the fact that Kolster and Vereijken (1993) could only explain 20% of the variation in quality by differences in HMW-GS composition, the authors considered this level of variation caused by HMW-GS to be of interest. In addition to ranking the HMW-GS according to their effects on quality, Kolster and Vereijken (1993) also reported interactions between alleles. Such interactions would explain the lack of consistent

effectiveness of scoring systems that are based on additive effects. At a constant number of HMW-GS or at an identical HMW-GS composition, variation of the level of expression was reported. The presence of Glu-A1 alleles (1 or 2*) appeared to increase the proportion of subunits produced. The Glu-B1 alleles were ranked according to level of expression: (7+9) = (7+8) > 7 = (6+8). The ranking of Glu-B1 alleles with respect to quantity produced was reported to be identical to their ranking for quality (Kolster, 1992). In contrast, different Glu-D1 alleles (e.g. 5+10 or 2+12) produced identical amounts of subunits. The authors concluded that breadmaking quality was influenced by quantity (amount of HMW-GS) as well as quality (HMW-GS composition). Thus, the superior quality of alleles produced by Glu-A1 were probably a result of an increased proportion of HMW-GS. Both quantity and quality appeared to be involved in the alleles of Glu-B1. And the Glu-D1 alleles appeared to exert their effect solely by intrinsic quality.

Recent studies lend further support for the importance of quantity as well as quality of HMW-GS (Gupta and MacRitchie, 1994; Gupta et al, 1994a). Alleles were similarly ranked, additive and epistatic effects were reported, and the LMW-GS were observed to affect quality (Gupta et al, 1994b). These observations supported the conclusion by Hamer et al (1992) that Glu-1 quality scores, or HMW-GS composition alone, were insufficient to account for quality differences between bread wheat varieties. The very close relationship (R^2>0.80) between dough mixing requirements and the overall expression of total glutenin subunits in insoluble glutenin (Sapirstein and Fu, 1998, Sapirstein and Johnson, 1996) provides additional evidence for this view. Allelic effects on dough strength were attributed to variation in (i) the quantity and size distribution of polymeric protein, and (ii) the amount or types of subunits produced. The relative size

distribution of the polymeric protein was in turn governed by (i) the ratio of HMW-GS:LMW-GS, which depends on the quantity of individual subunits, and (ii) the polymerizing behavior of these subunits, which may be related to their basic size or structure (Gupta and MacRitchie, 1994).

2.3. Dough Mixing

During mixing, flour proteins and other constituents are first hydrated; hydrated protein aggregates are then disaggregated and reoriented by the shearing action of mixing, and form a protein (gluten) network (Tsen, 1969; Graveland et al, 1994). After appropriate mixing, the developed dough should have the optimum foam structure with appropriate viscoelastic properties required to sustain handling, and withstand expansion and retain gas during fermentation, proofing, and baking. Although most of the flour constituents are involved in dough development, the proteins are primarily responsible for the unique rheological properties of dough and, ultimately, the quality of the final baked bread. It has been postulated that two opposing processes occur during mixing; the gradual transformation of hydrated flour proteins into a continuous film (by a disaggregation/reaggregation mechanism) and the breakdown of this film (Paredes-Lopez and Bushuk, 1982b). The disaggregation of flour protein (most of which is in the form of protein bodies in flour particles) appears to be necessary before film formation can occur (Mecham et al, 1965; Graveland et al, 1994).

2.3.1. Role of sulfhydryl groups and disulfide bonds in mixing

As is common for most food systems, several molecular forces are involved in the complex reactions occurring between flour constituents, particularly the gluten proteins, during dough mixing. The most important covalent bond involved in dough mixing

reactions is the disulfide bond, providing stability and participating in interchange reactions with sulphydryl groups between protein molecules. The concept of sulfhydryl-disulfide interchange reactions was first suggested by Goldstein in 1957 (Bloksma, 1975). Low molecular weight thiol compounds interchange with the disulfide bonds made accessible by Brownian motion and alignment during mixing. The interchange reaction allows for the relief of stresses created during mixing and accommodates viscous flow. Although the level of cysteine residues is low in gluten proteins, evidence for the importance of disulfide (-SS-) bonds and sulfhydryl (-SH) groups includes effects on dough properties of oxidizing and reducing agents (Mecham et al, 1963) and effects of SH-blocking chemicals such as NEMI (Meredith and Bushuk, 1962). Only a small fraction of total -SH groups and -SS- bonds are considered to be rheologically effective (Bloksma, 1972). Loss of -SH groups during mixing has been reported while the -SS- content has been reported to remain constant or decrease very slightly (Schroeder and Hoseney, 1978; Tanaka and Bushuk, 1973c; Tsen and Bushuk, 1963).

During dough development, rheologically effective -SS- bonds facilitate alignment of protein chains to form a dough required for optimum bread quality (Bushuk and Kawka, 1990). During and after mixing, the -SH groups are slowly oxidized and become less numerous and the -SS- bonds then become important for the stabilization of the structure until setting during baking. Differences in mixing strength are partially due to differences in availability of -SS- bonds and differences in –SH content (Tanaka and Bushuk, 1973c). The content in flour of reactive -SH groups and -SS- bonds has been reported to be inversely related to dough strength (Tsen and Bushuk, 1968).

2.3.2. Role of noncovalent forces in mixing

Noncovalent interactions are also important in dough mixing. Hydrogen bonds contribute to the structure of dough. Indirect evidence for the contribution of hydrogen bonding to dough structure includes the significant effect on dough mixing properties reported when heavy water (D2O) is incorporated into doughs (Tkachuk and Hlynka, 1968). Deuterium bonds are considerably stronger than hydrogen bonds and result in a stronger dough. This observation, together with the high level of glutamine present in wheat storage proteins, provides strong evidence for the importance of hydrogen bonds.

Hydrophobic interactions are also important to dough structure. When stabilized by covalent cross-links (-SS- bonds), hydrophobic interactions, like hydrogen bonds, can contribute significantly to the elasticity and viscosity and thereby facilitate dough development (Bushuk and Kawka, 1990).

Despite the low level of ionizable residues in gluten proteins, ionic bonds play an important part in dough structure. This observation can be inferred from the effect that salt and pH have on dough properties. Salt increases mixing time and dough stability (Maher Galal et al, 1978). The increase of elasticity and reduction of extensibility observed (Bushuk and Kawka, 1990) when salt is incorporated into a dough is attributed to the loss of repulsive forces caused by charge masking. A similar effect is seen when the pH is altered and dough properties are measured with the mixograph. Lowering dough pH produces a weaker dough, presumably due to increased positive charge repulsions; increasing dough pH increases strength (Hoseney and Rogers, 1990).

2.3.3. Changes in protein solubility during mixing

In addition to the -SS-/-SH interchange reaction, changes in solubility and molecular size of glutenin, the high molecular weight component of flour proteins, are the two features most often reported to occur during dough development. There is a significant increase in protein solubility with dough mixing. This increase in solubility is attributed to disaggregation (Mecham et al, 1965; Tsen, 1969) or depolymerization (Tanaka and Bushuk, 1973c) of proteins. The amounts of water-soluble and salt-soluble protein fractions are not significantly altered during mixing (Mecham et al, 1963; Tanaka and Bushuk, 1973a), although Mecham et al (1963) reported a slight decrease in the water-soluble fraction when doughs were mixed in the presence of NEMI. A small increase in the ethanol-soluble protein occurs during mixing and is accentuated if mixing is done in the presence of NEMI (Mecham et al, 1963) or iodate (Tanaka and Bushuk, 1973a). A major shift in the solubility distribution on mixing occurs from the insoluble residue protein to the acetic acid-soluble protein, and under extended mixing to the ethanol-soluble protein (Tanaka and Bushuk, 1973a). The extent and rate of conversion from the insoluble to the soluble form varies among flours and is related to the mixing characteristics of the flours (Mecham et al, 1962; 1963). The extent of conversion of insoluble to soluble protein during mixing is greater for proteins of stronger flours and lesser for proteins of weaker flours (Parades-Lopez and Bushuk, 1982a). Addition of early fractions obtained from a successive protein fractionation procedure causes a decrease in mixing requirements, whereas the intermediate fractions containing higher proportions of glutenin cause a large increase in dough development time (MacRitchie, 1987). Thus, solubility changes predominantly involve the glutenin fraction. However, there is now

some evidence (Bushuk et al, 1997) that dough development involves changes in gliadin solubility also.

The most current research on changes in solubility during mixing has been based on an SDS solution (1.5% w/v) as extracting solvent. These studies (Wang et al, 1992; Weegels et al, 1993; 1997) support the previously reported increase in solubility of the insoluble protein fraction with mixing. In addition, repolymerization is suggested to occur during resting, as measured by the decrease in solubility observed. Weegels et al (1993, 1997) also analyzed changes in HMW-GS composition of the SDS-insoluble fraction (glutenin macropolymer or GMP). The content of HMW-GS in the GMP after mixing was reduced during mixing and increased with resting. Changes in subunit composition were also reported. Weegels et al (1993, 1997) observed a preferential incorporation of y-type HMW-GS during resting.

2.3.4. Changes in size distribution of glutenin during mixing

The molecular weight and size distribution of glutenin is also considered important to mixing properties of flour proteins. The shift in solubility of proteins from an insoluble form to a soluble form is considered to arise from a decrease in molecular weight of the protein (Lee and MacRitchie, 1971; Tanaka and Bushuk, 1973b; Tsen, 1967). Solubility appears to increase during mixing before a significant reduction in molecular size occurs. As measured by viscosity and gel filtration chromatography, protein solubilized during mixing retains a large molecular weight (Danno and Hoseney, 1982a; 1982b). This observation may result from the low resolving power of methods available for molecular weight measurement at the HMW range of flour proteins. The variation in response of different flours to extraction and mixing may be a result of differences in the molecular

size of protein aggregates (MacRitchie, 1973) or differences in lability to disaggregation (Tsen, 1967).

2.3.5. Gliadin-glutenin interactions during mixing

The idea of gliadin-glutenin interactions as an important factor influencing dough strength has been alluded to in the past (Branlard and Dardevet, 1985b; Eckert et al, 1993; Khan et al, 1989). Recent studies indicate that gliadin-glutenin interactions may influence dough mixing properties of flours and the extent of interaction may be genotype dependent (Bushuk et al, 1997; Dupuis et al, 1996, Fu et al, 1996). Bushuk et al (1997) examined changes in solubility and subunit composition of mechanically developed doughs. During mixing, gliadin solubility decreased initially then increased. The reverse was observed for the glutenin fraction. The authors suggested that the role of gliadins in gliadin-glutenin interaction was primarily a physical one. Dupuis et al (1996) examined the composition of the acetic acid-soluble (AS) and acetic acid-insoluble (AI) fractions of two flours of different mixing strength. The relative amount of gliadins in the AS fraction was significantly larger in the weaker flour and smaller in the stronger flour. It was hypothesized that the solubility of glutenin and gliadin was related to genotype-specific interaction between the two groups of proteins. The greater the interaction, the higher the amount of gliadin insoluble in ethanol but soluble in acetic acid, and the shorter the mixing time. Further support for the relationship between gliadin solubility and gliadin-glutenin interaction was provided by Fu et al (1996). In a study of the effect of salt on the water-solubility of gliadin and glutenin, the authors found that the gliadins in glutens of strong cultivars (longer mixing requirements) were much easier to extract; those of weaker cultivars (shorter mixing requirements) were more difficult to extract.

3. MATERIALS AND METHODS

3.1. Flours

Flours used in this study were milled from wheats of three Canadian classes grown in various locations (Katepwa and Glenlea, 1993 crop from Agassiz, MB; Glenlea, Roblin, and AC Domain, 1994 crop from Dauphin, MB; AC Karma, 1994 crop from Indian Head, SK). These flours were selected to provide a wide range in mixing behaviour. Glenlea is a hard red spring (HRS) wheat cultivar from the Canada Western Extra Strong (CWES) class with extra strong dough characteristics. Roblin, Katepwa and AC Domain are HRS wheat cultivars from the Canada Western Red Spring (CWRS) class with very strong (Roblin) and strong dough characteristics (Katepwa and AC Domain). AC Karma is a wheat cultivar from the Canada Prairie Spring-white (CPS-w) class with medium dough characteristics. All wheat samples were milled to straight grade flour on a Bühler pneumatic laboratory mill. Selected technological and quality parameters for each flour are listed in Table 1 and Table 2. Farinograms and mixograms for each flour are illustrated in Figure 1 and Figure 2, respectively. Flours are listed in order of increasing dough mixing strength. Falling Number values were obtained using AACC method 56-81B (1983). Dough mixing properties were measured using the farinograph method AACC 54-21 (1983) and using a 2-g direct drive computerized mixograph (National Manufacturing, Lincoln, NE).

3.2. Chemical Analyses

Moisture was determined according to AACC method 44-15A (AACC, 1983). The ash content of flours was determined according to the AACC method 08-01 (AACC, 1983). Protein content of flours, lyophilized doughs, and modified Osborne fractions were

Table 1. High Molecular Weight Subunit Composition, Flour Extraction, Moisture, Protein, Ash, Falling Number, and Starch Damage of Wheat Flours Used in Study

Flour	HMW-GS[1] (1A,1B,1D)	Extraction Rate[2]	Moisture	Protein[3]	Ash[4]	FN Value	Starch Damage[3,4]
Glenlea 94	2*,7+8,5+10	74.0	12.4	10.8	0.54	515	5.9
Glenlea 93	2*,7+8,5+10	75.2	12.5	13.6	0.42	484	5.8
Roblin	2*,7+8,5+10	75.5	13.8	14.6	0.46	610	4.1
Katepwa	2*,7*+9,5+10	72.4	12.6	13.3	0.45	484	5.1
AC Domain	2*,7*+9,5+10	75.7	13.4	14.3	0.46	626	5.0
AC Karma	1,7*+9,2+12	76.5	13.5	11.0	0.46	478	4.1

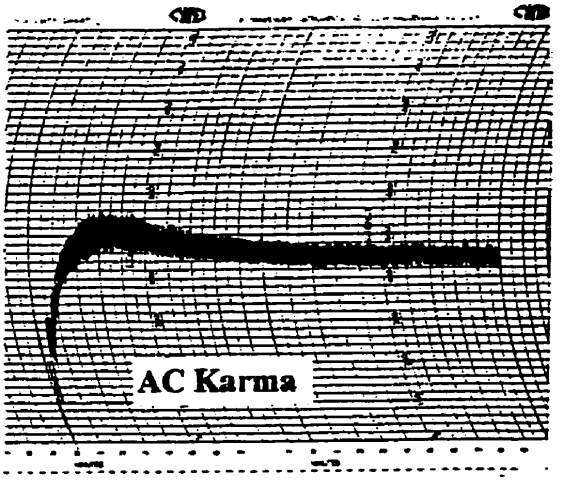
[1] High molecular weight glutenin subunit composition in order of 1A, 1B, 1D chromosome location
[2] As % of total recovered products (as is)
[3] 14% moisture basis
[4] as % flour weight

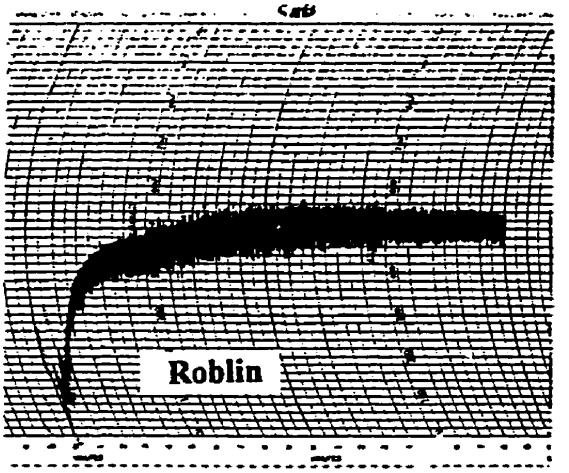
Table 2. Dough Mixing Properties of Flours Used in Study

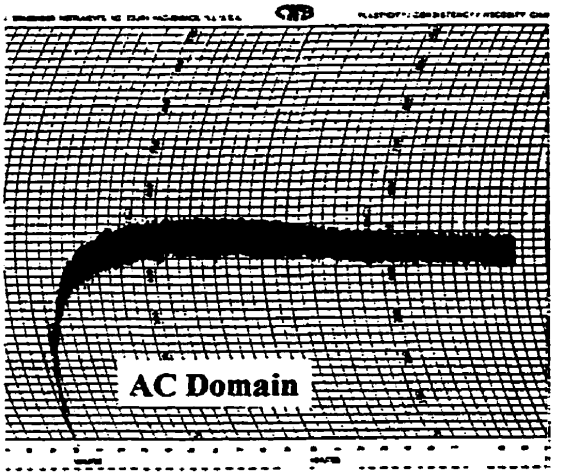
	Farinograph			Mixograph		
Flour[1]	FAb[2]	DDT[3]	MTI[4]	DDT[3]	PH[5]	MT[6]
Glenlea	58.7	2.0	40	6.7	42.5	0.23
Glenlea 93	61.8	23.0	10	5.2	64.7	0.54
Roblin	65.2	13.0	10	3.4	64.7	-0.96
Katepwa	64.8	6.5	15	3.4	49.0	-2.07
AC Domain	63.9	8.0	25	2.9	53.1	-1.26
AC Karma	58.3	4.0	25	2.5	41.6	-4.82

[1] ranking based on wheat class and decreasing mixing strength
[2] farinograph absorption,
[3] dough development time (min)
[4] mixing tolerance index
[5] peak height (mixograph units)
[6] mixing tolerance ("right of peak slope")

Figure 1. Farinograph curves of flours from five Canadian spring wheat varieties showing a range of mixing strength


AC Karma


Roblin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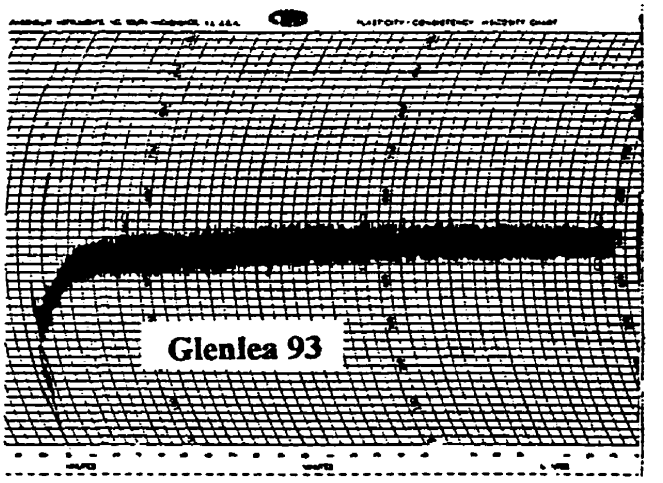
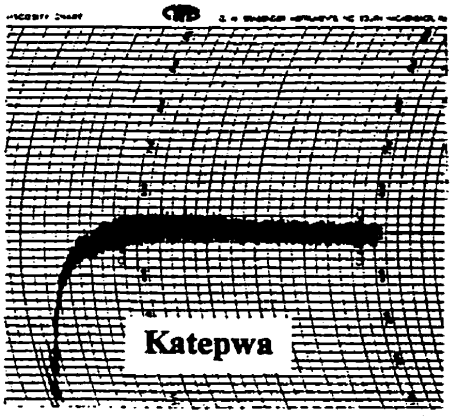

Glenlea 93


Katepwa

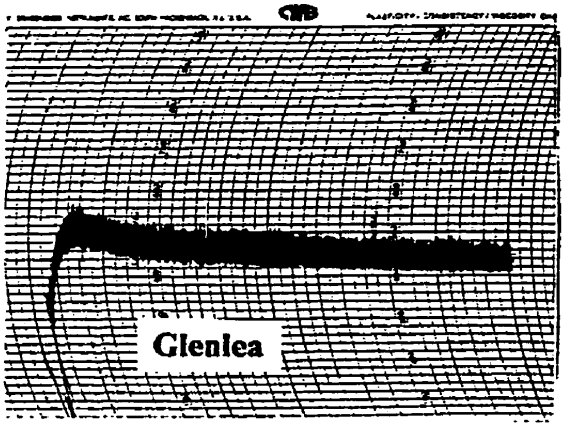
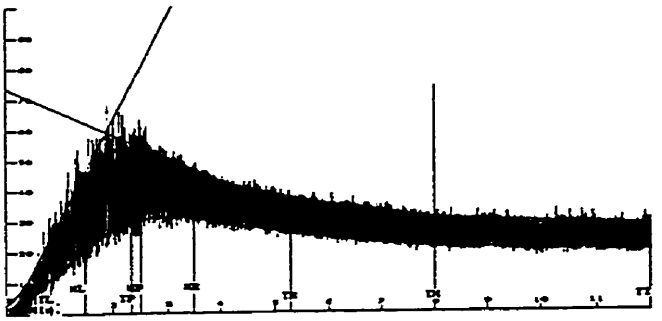
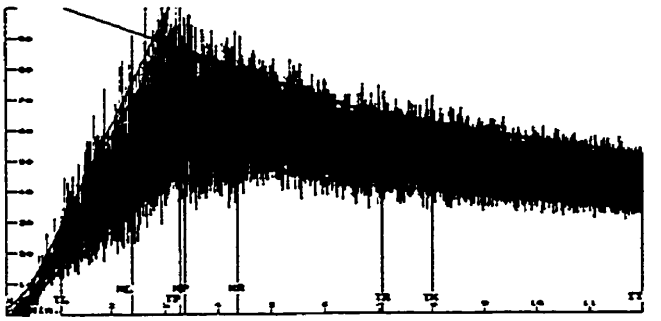

Glenlea

Figure 2. Mixograph curves (Farinograph absorption) of flours from five Canadian spring wheat varieties showing a range of mixing strength.

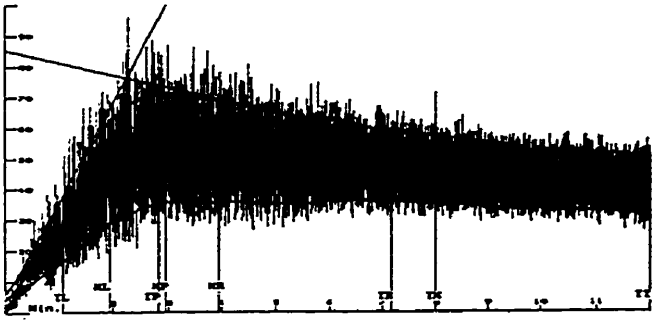
AC Karma



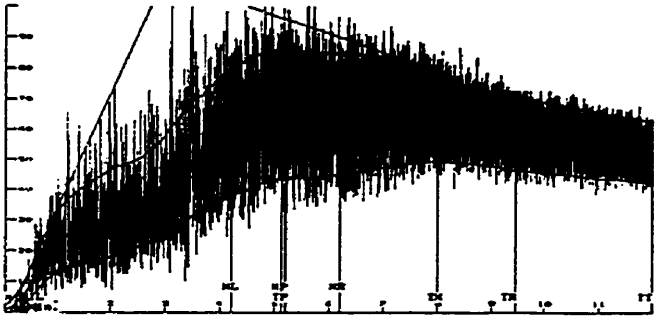
Roblin



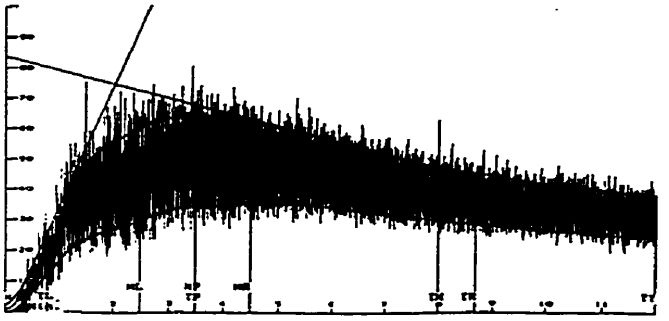
AC Domain



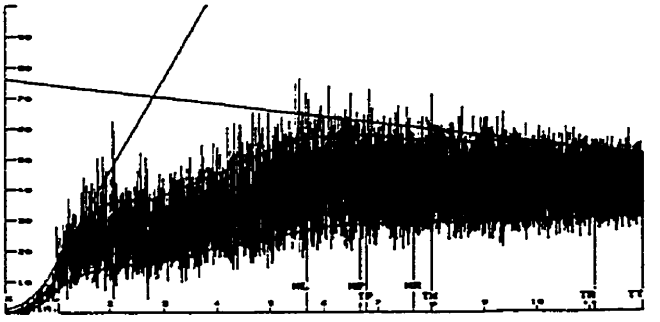
Glenlea 93



Katepwa



Glenlea



determined by a microKjeldahl procedure based on AACC (1983) methods 46-13 (microKjeldahl procedure) and 46-16 (improved copper sulfate-titanium dioxide procedure) with the following modifications. A copper sulfate-titanium dioxide catalyst mixture (Pope Kjeldahl Mixtures, Dallas, TX) was used instead of the more hazardous mercury catalyst. The pumice mixture contained potassium sulfate (10g), titanium dioxide (3g) and copper sulfate (2g). Saturated boric acid solution ($40g \cdot L^{-1}$) contained methyl red ($7.15\ ml \cdot L^{-1}$, 0.2%v/v) and methylene blue ($2.85ml \cdot L^{-1}$, 0.1% v/v) dyes as end-point indicators. Samples (10-100 mg) containing concentrated sulfuric acid (3 ml) were digested (450°C, 45-60 min) until solutions were clear. Cooled digests were diluted with water (20 ml). Sodium hydroxide solution (15 ml, 40% w/v) devoid of sodium thiosulfate (normally used to complex with mercury during distillation) was added to sample solution prior to distillation. Ammonia was distilled and captured in a 125-ml Erlenmeyer flask containing saturated boric acid solution (10 ml). Once a total volume of 50ml was collected, the solution was titrated with standardized HCl (0.0304 N). A nitrogen-to-protein conversion factor of 5.7 was used (Tkachuk, 1969).

Starch damage, as a percentage of flour weight, was determined using the AACC method 76-31 (AACC, 1983), with the following modifications. The supernatant obtained after incubation with fungal α-amylase and addition of dilute sulphuric acid was diluted twofold (50 µl supernatant plus 50 µl water) before addition of amyloglucosidase solution (0.1ml). Hexokinase (Boehringer Mannheim Canada, Laval, QC) was used in lieu of the glucose oxidase/peroxidase. Water (1.0 ml) and hexokinase solution (4.0 ml) were added to samples, blanks and standards. Absorbances were read at 340 nm within 30 minutes after addition of the hexokinase reagent. Calculation of percentage starch damage is as described in the AACC method, with the exception that the volume correction factor is 120 (50 µl taken to 6.0 ml).

3.3. Optimum Mixograph Water Absorption

Flour-water doughs (2g flour, 14% mb) were mixed, in triplicate, in a 2-g computerized mixograph (National Manufacturing, Lincoln, NE) at different water absorptions (%). Regression analysis (Microsoft®Excel 5.0) was performed for the mean values of mixing time (MT), mixograph peak height (PH), and work input (WI) against absorption. Figure 3 and Figure 4 illustrate that PH and absorption provide the highest correlation (regression for MT versus absorption not shown). Based on the regression analysis for PH, and using the regression equations obtained for each flour (Appendix, Table 1), an arbitrary value (40MU) was selected as the constant consistency from which the corresponding water absorptions (within normal breadmaking range) would be used to prepare all dough samples. This absorption will be referred to as the optimum absorption.

3.4. Preparation of Control Doughs

Optimum absorptions determined using the 2-g mixograph and the mixing times obtained by analyzing duplicates in the 35-g mixograph are listed for each sample in Table 3. Flours were mixed in a 35-g mixograph (Department of Plant Science, University of Manitoba) according to AACC method 54-40A (AACC, 1983) at room temperature (23°C). Mixing was stopped at the appropriate times. Doughs were quickly immersed in liquid nitrogen in the following manner. Fingers were lightly dusted with the corresponding flour to aid in handling. Doughs were alternately separated into pieces (approximately 14-18 g each) and flattened. Each piece was immersed in liquid nitrogen until the dough's exterior surface became white and cracking began throughout the dough piece. Although the time between the end of mixing and freezing was kept to a minimum, changes in dough properties with resting were observed progressively from the first to the third piece. Difficulties were

Figure 3. Regression analysis illustrating the effect of absorption on mixograph peak height (MU, mixograph units). AC Karma (KA), AC Domain (DO), Roblin (RO), Katepwa (KP), Glenlea 1993 (GL1), Glenlea 1994 (GL2).

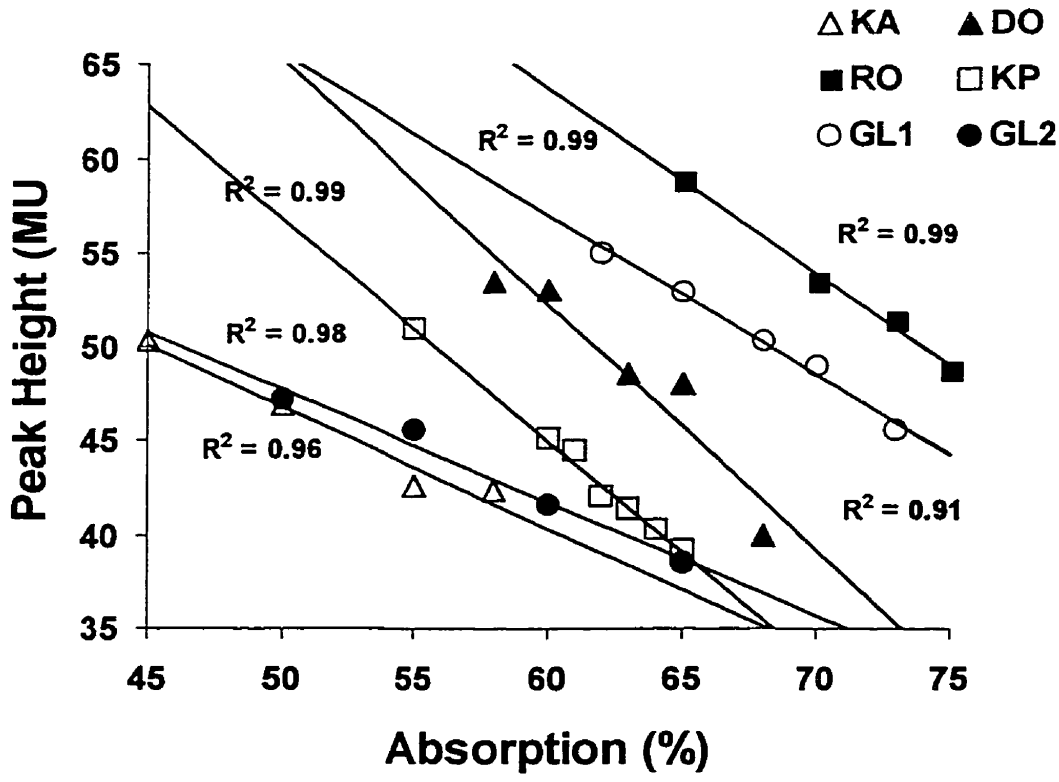
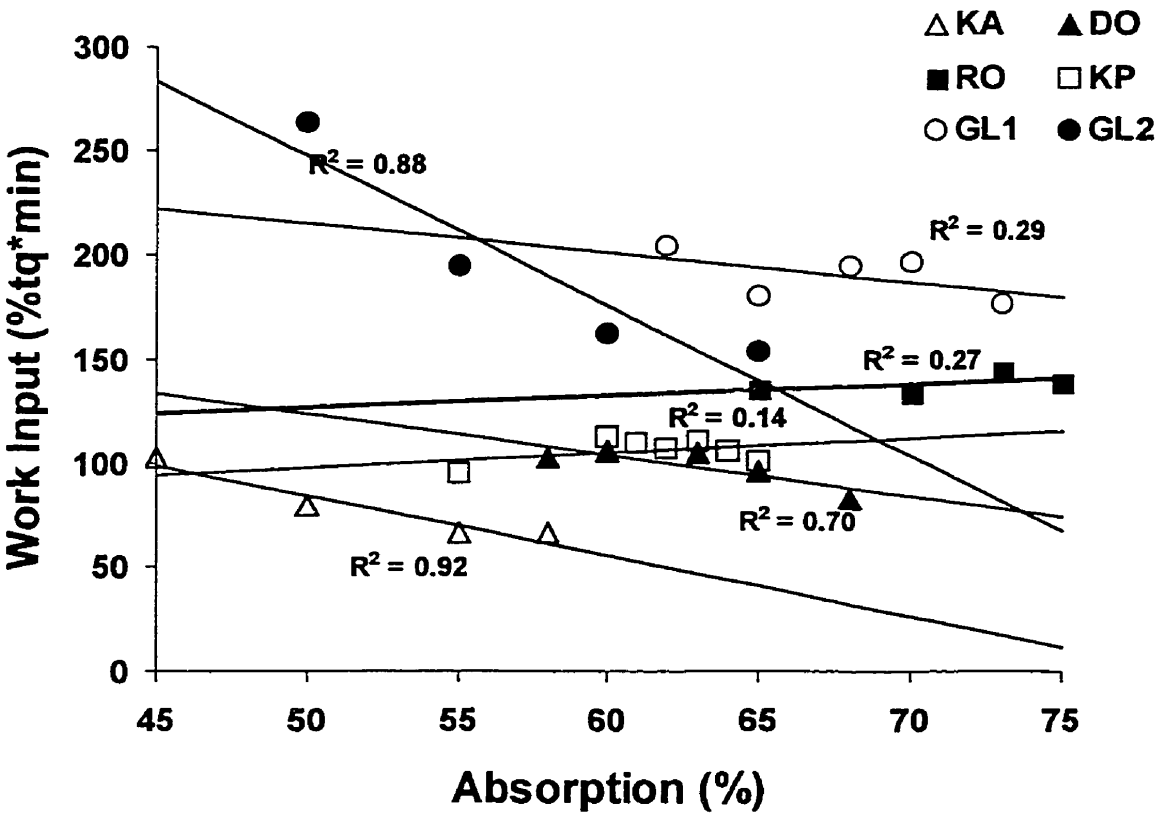

△KA
▲DO
■RO
□KP
○GL1
●GL2
R² = 0.99
R² = 0.99
R² = 0.99
R² = 0.98
R² = 0.96
R² = 0.91
Peak Height (MU
65
60
55
50
45
40
35
45
50
55
60
65
70
75
Absorption (%)

Figure 4. Regression analysis illustrating the effect of absorption on mixograph work input (%tq*min). AC Karma (KA), AC Domain (DO), Roblin (RO), Katepwa (KP), Glenlea 1993 (GL1), Glenlea 1994 (GL2).


△KA
▲DO
■RO
□KP
○GL1
●GL2
R² = 0.88
R² = 0.29
R² = 0.27
R² = 0.14
R² = 0.70
R² = 0.92
Work Input (%tq*min)
300
250
200
150
100
50
0
45
50
55
60
65
70
75
Absorption (%)

encountered in flattening overmixed dough samples and AC Karma dough samples, due to the extensibility and stickiness of the samples. Control doughs were lyophilized, ground in a coffee mill (Proctor-Silex Canada Inc., Picton, ON) and stored at room temperature in tightly sealed high-density polyethylene Nalgene containers (Nalge Company, Rochester, NY).

Table 3. Optimum absorptions and Mixing Times Selected for Control Doughs

Cultivar	Absorption (%)	50% MT	100% MT	200% MT
Glenlea (1994)	62.9	3.6	7.3	14.6
Katepwa	64.2	1.4	2.8	5.6
AC Domain	61.7	1.4	2.9	5.8
AC Domain	69.4	1.9	3.8	7.6
AC Karma	60.5	1.0	2.0	4.0

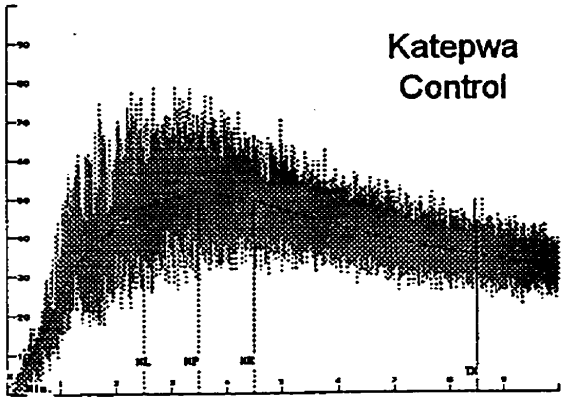
3.5. Preparation of Doughs Containing Potassium Iodate or N-ethylmaleimide

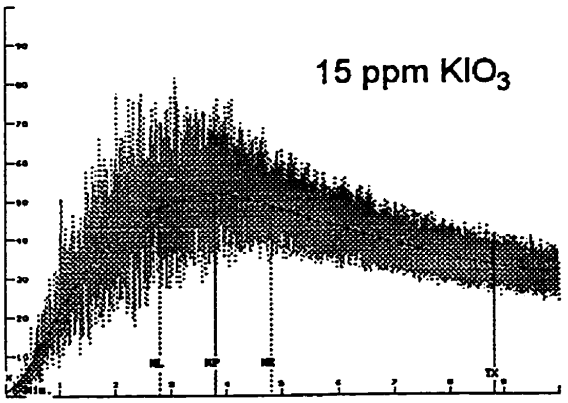
Doughs (2g, 14% mb) prepared from Katepwa flour at optimum absorption were mixed in a 2-g computerized mixograph (National Mfg., Lincoln, NE) using a range of concentrations of potassium iodate (15-120 ppm, KIO_3, Mallinckrodt Specialty Chemicals, Co., Paris, KY) and N-ethylmaleimide (30-240 ppm, NEMI, Sigma Chemical Co., St. Louis, MO). Concentrations for KIO_3 (Figure 5) and NEMI (Figure 6) were selected based on visual assessment of a range of mixograms prepared using concentrations cited in the literature. Doughs were initially mixed in duplicate in the 35-g mixograph to determine the appropriate mixing times (Table 4). Potassium iodate (60 ppm) and N-ethylmaleimide (120 ppm) were added to the flour samples as aqueous solutions. Samples were subsequently frozen and lyophilized as described for the control doughs.

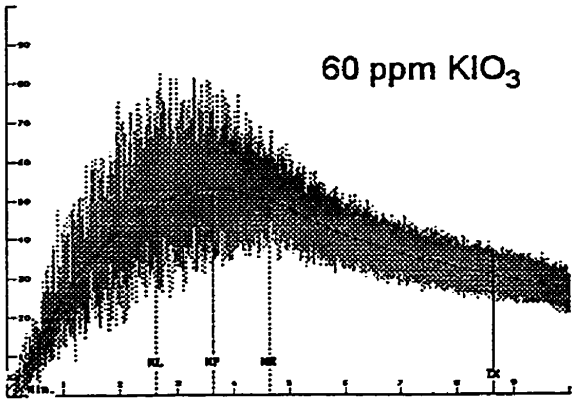
3.6. Preparation of Full Formula Doughs

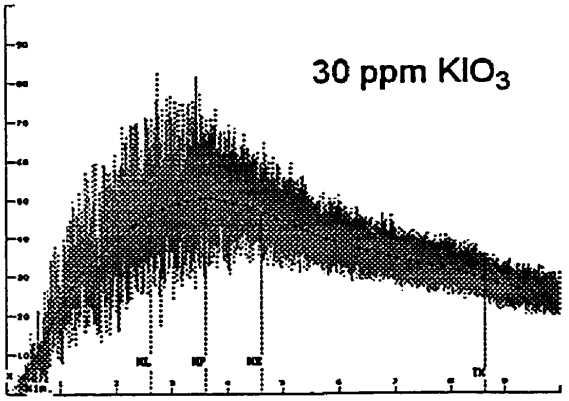
Full formula doughs were kindly provided by Dr. F. Békés, Grain Quality Research Laboratory, CSIRO, North Ryde, AU. The doughs were prepared using a small-scale

Figure 5. Effect of varying concentrations of KIO_3 on the mixing properties of Katepwa flour-water doughs (64.2% absorption) measured with a 2-g computerized mixograph


Katepwa
Control


15 ppm KIO_3


60 ppm KIO_3


30 ppm KIO_3

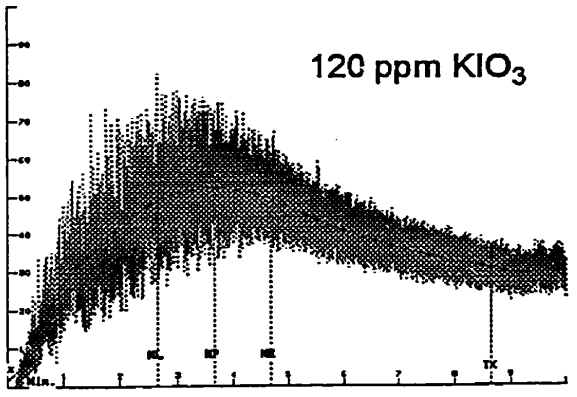
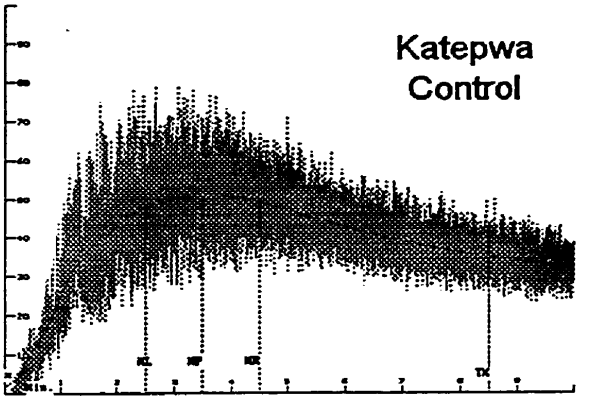
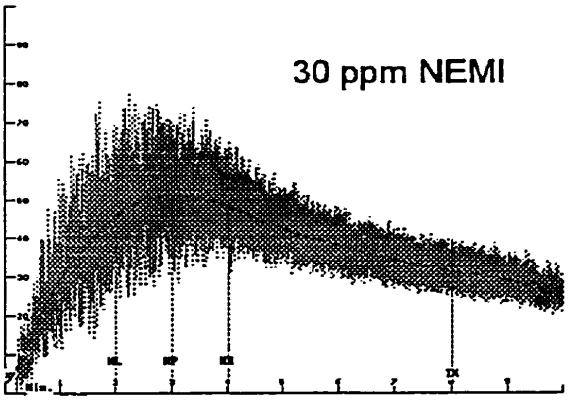
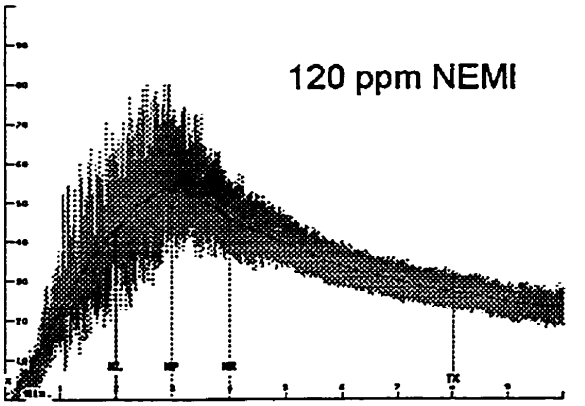
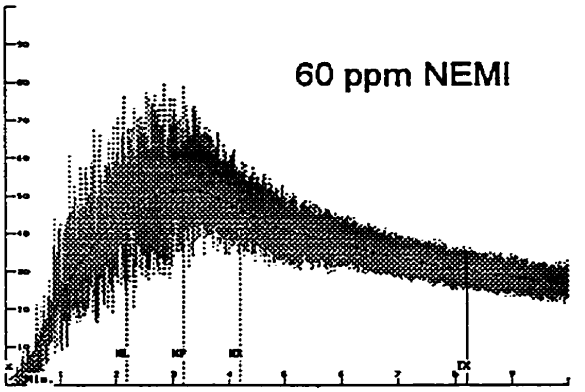

120 ppm KIO_3

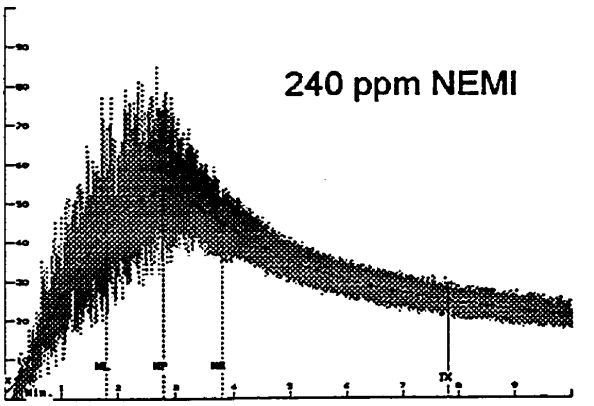
Figure 6. Effect of varying concentrations of NEMI on the mixing properties of Katepwa flour-water doughs (64.2% absorption) measured with a 2-g computerized mixograph


Katepwa
Control


30 ppm NEMI


120 ppm NEMI


60 ppm NEMI


240 ppm NEMI

procedure developed at the Grain Quality Research Laboratory. Samples of Glenlea (1994) and Katepwa flours used in this study (1.95 g) were mixed with an aqueous yeast solution (1 ml, containing 2% yeast and 0.6% BRI improver containing 0.6% malt and 100ppm ascorbic acid in 100 ml) and water (calculated based on the protein and moisture content of the flour). Doughs were allowed to rest 0, 2, and 4 hours, freeze-dried, stored in airtight containers and shipped to our laboratory for analysis.

Table 4. Optimum absorptions and Mixing Times Selected for Preparation of Doughs Containing Potassium Iodate (60ppm) or N-ethylmaleimide (120ppm)

Cultivar	Additive	Absorption (%)	50% MT	100% MT (min)	200% MT
Glenlea	KIO_3	62.9	3.8	7.7	15.4
	NEMI		2.7	5.4	10.8
Katepwa	KIO_3	64.2	1.6	3.2	6.4
	NEMI		1.3	2.6	5.2
AC Domain	KIO_3	69.4	2.2	4.4	8.8
	NEMI		1.6	3.3	6.6
AC Karma	KIO_3	60.5	1.2	2.4	4.8
	NEMI		1.0	2.0	4.0

3.7. Small-scale Modified Osborne Fractionation

The modified Osborne fractionation procedure (Chen and Bushuk, 1970) as modified and described by Dupuis et al (1996) was initially used to determine the protein distribution for flours used in this study. However, this large scale method is tedious and time-consuming. Only six fractionations can be performed over a 1-2 week period. The method is also limited by the small amount of acetic acid-soluble fraction that is produced. In addition, the limited amount of flour samples available and the large number of dough samples to be processed did not fit the standard method. Accordingly, a small-scale method was developed to overcome these limitations. Optimization was first performed using flours for which data for the large

scale modified Osborne method were available (Dupuis and Bushuk, 1996). Extraction times, sample-to-solvent ratio, and vortexing frequency were varied until the method produced results that were similar to those obtained by the large scale method. Other details will be discussed in the Results and Discussion section.

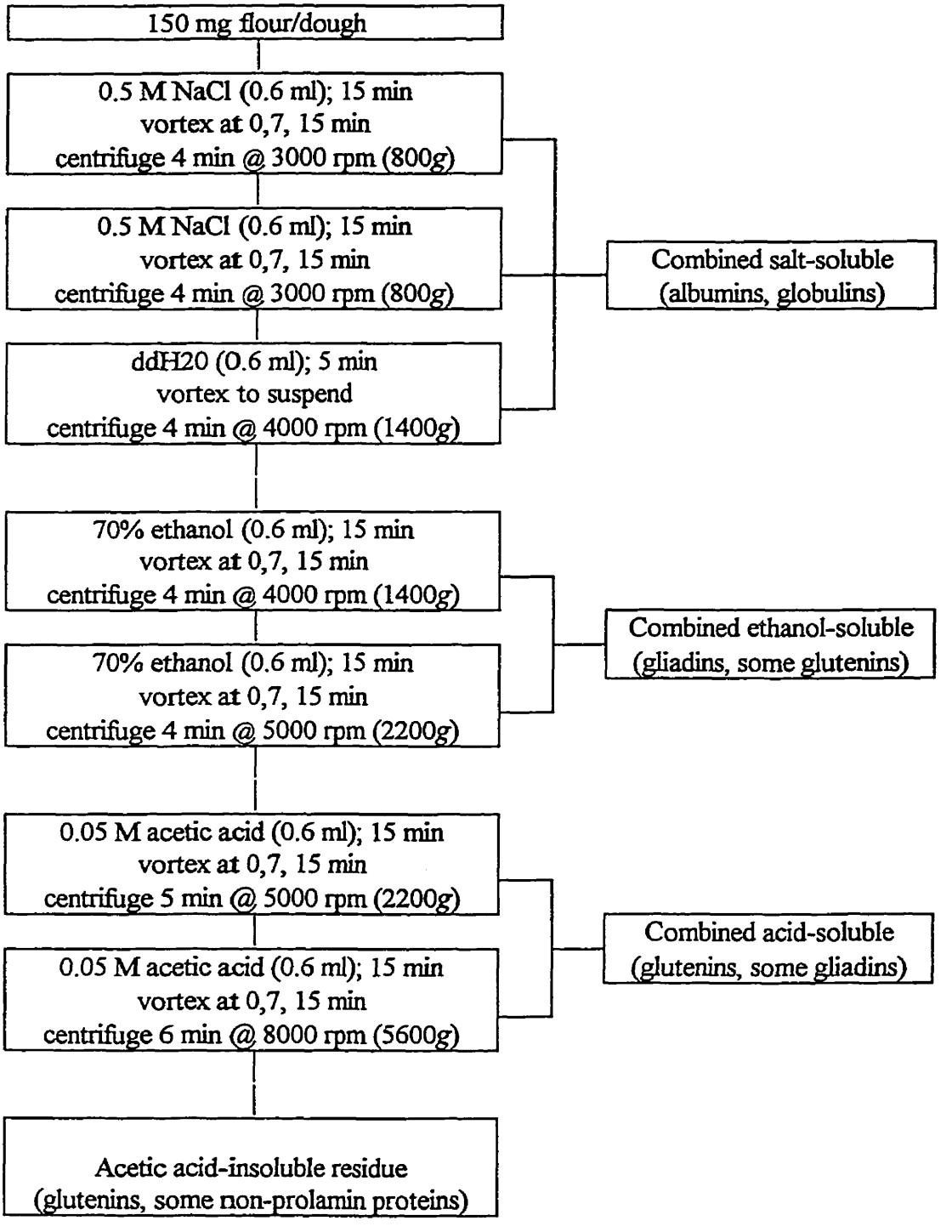
The small-scale procedure adopted for routine fractionation was as follows: flour or freeze-dried dough (150 mg) was extracted twice with salt solution (0.6 ml, 0.5N NaCl) in microcentrifuge tubes (1.5 ml; Fisherbrand, Pittsburgh, PA) for 15 min, with vortexing at 0, 7, and 15 min. The solution was centrifuged (4 min, 795*g*; Biofuge A tabletop microcentrifuge, Heraeus-Christ GmbH, Germany) and the supernatants transferred to a Kjeldahl digestion tube (for subsequent nitrogen determination) or a microcentrifuge tube (2.0 ml, for evaporation and subsequent analyses). The pellet was washed with distilled deionized water (0.6 ml, vortex until suspended) until fully re-suspended (approximately three minutes), centrifuged (4 min, 1413 *g*) and the supernatant pooled with the salt solution supernatants. This fraction was analyzed as such, without dialyzing to separate the water-soluble albumins from the salt-soluble globulins. The resulting pellet was extracted twice with aqueous ethanol solution (0.6 ml, 70%v/v), centrifuged (4 min, 1413*g* and 2208*g*, respectively) and the pooled supernatants transferred to Kjeldahl digestion tubes or microcentrifuge tubes (2.0 ml). The insoluble pellet was extracted twice with acetic acid solution (0.6ml, 0.05N), centrifuged (5 min at 2208*g*, 6 min at 5653*g*), and the supernatants were combined. The pellet was transferred to a Kjeldahl tube or dried in a vacuum evaporator (SpeedVac Concentrator, Refrigerated Condensation Trap RT490, VP100 High Vacuum Pump, Chemical Trap SCT-120, Filter Pump, Savant Instruments, Inc., Farmingdale, NY). After each fractionation step, quantitative transfer was achieved by careful aspiration of residual solvents using a disposable polyethylene transfer pipet

(FisherBrand®, Fisher Scientific, Pittsburgh, PA) with a pipet tip (200µl, FisherBrand®, Fisher Scientific, Pittsburgh, PA) attached. In the first extraction, the salt solution was added and then the flour/dough was suspended by immediate vortexing; this was repeated for each consecutive sample until all were prepared. The extraction was then timed for 15 minutes. In all subsequent extractions, the solvent was added to all samples, then all samples were vortexed before recording the extraction time. Protein distribution was determined for each pooled fraction and reported as a percentage of total flour or dough protein. The scheme for the small-scale modified Osborne protein fractionation procedure developed and used in this study is shown in Figure 7.

3.8. Purification by 70% (v/v) 1-propanol Precipitation

Ethanol-soluble (ES) and acetic acid-soluble (AS) fractions were further fractionated to separate the glutenins from gliadins. A modification to the 70% 1-propanol precipitation method of Fu and Sapirstein (1996) was required. The acidic environment of the AS fractions prevents the efficient precipitation of glutenin. Hence, a buffer was used to maintain neutral conditions. Evaporated fractions were suspended in buffered 50% (v/v) 1-propanol (Tris-HCl or phosphate; pH 7.5; 1.0ml for ES, 0.5ml for AS) and allowed to stand for 1 h with occasional vortexing. The solution was then brought to 70% (v/v) by addition of an appropriate volume of 100% 1-propanol (0.67ml for ES, 0.33ml for AS), allowed to stand for 1 h, and centrifuged (5 min, 15000*g*). The supernatant (ESPS or ASPS), comprising mainly gliadins, was immediately analyzed for protein content or evaporated for subsequent analyses. The 70% (v/v) 1-propanol-insoluble pellet (ESPI or ASPI), comprising mainly of glutenins and some ω-gliadins, was air dried overnight. According to Fu et al (1996), the ESPI and ASPI pellets can then be

Figure 7. Flowchart for the small-scale modified Osborne fractionation procedure


150 mg flour/dough
0.5 M NaCl (0.6 ml); 15 min
vortex at 0,7, 15 min
centrifuge 4 min @ 3000 rpm (800g)
0.5 M NaCl (0.6 ml); 15 min
vortex at 0,7, 15 min
centrifuge 4 min @ 3000 rpm (800g)
ddH20 (0.6 ml); 5 min
vortex to suspend
centrifuge 4 min @ 4000 rpm (1400g)
Combined salt-soluble
(albumins, globulins)
70% ethanol (0.6 ml); 15 min
vortex at 0,7, 15 min
centrifuge 4 min @ 4000 rpm (1400g)
70% ethanol (0.6 ml); 15 min
vortex at 0,7, 15 min
centrifuge 4 min @ 5000 rpm (2200g)
Combined ethanol-soluble
(gliadins, some glutenins)
0.05 M acetic acid (0.6 ml); 15 min
vortex at 0,7, 15 min
centrifuge 5 min @ 5000 rpm (2200g)
0.05 M acetic acid (0.6 ml); 15 min
vortex at 0,7, 15 min
centrifuge 6 min @ 8000 rpm (5600g)
Combined acid-soluble
(glutenins, some gliadins)
Acetic acid-insoluble residue
(glutenins, some non-prolamin proteins)

washed with sodium iodide solution (0.75M) to remove coprecipitated ω-gliadins (Fu et al, 1996). This step, however, was only performed for the fractions obtained by the large-scale flour fractionation/purification procedure. The NaI wash was eliminated from the small-scale scheme so that any changes in ω-gliadin solubility during mixing could be monitored.

The protein content of the 70PS fraction was determined by the spectrophotometric method of Sapirstein and Johnson (1996). An aliquot of freshly prepared supernatant (ESPS or ASPS) was diluted 10- or 100-fold with 50% (v/v) 1-propanol. A control blank comprising the same solvent proportions as the sample supernatants was similarly diluted and used as the blank against which absorbance was read. Protein concentration was determined using a standard curve of absorbance (214nm) versus protein concentration ($\mu g \cdot ml^{-1}$). The protein content of the purified glutenin fraction (ESPI or ASPI), expressed as a percentage of total flour or dough protein, was then obtained by difference:

$$\text{Protein}_{(\text{ES or AS})} - \text{Protein}_{(\text{ESPS or ASPS})} = \text{Protein}_{(\text{ESPI or ASPI})}$$

3.9. Reversed-phase High-Performance Liquid Chromatography

Reversed-phase high-performance liquid chromatography (RP-HPLC) was based in part on the procedures described by Singh et al (1991) and Marchylo et al (1989). Controls were prepared as follows: Katepwa flour (20 or 50mg) was extracted twice with 50% (v/v) 1-propanol (1 ml) for 30 min at room temperature, with intermittent vortexing, and centrifuged (3 min, 2200*g*). The residue was washed with 50% 1-propanol (1 ml), centrifuged, and the supernatant discarded. Residual solvent was removed using a disposable polyethylene transfer pipet with a pipet tip attached. This residue from Katepwa flour was used as a control in all RP-HPLC runs. Samples from all flours/doughs (purified ES and AS glutenin, AI residue) and

controls were chemically reduced (60 min, 55°C) with a buffer solution (100 µl) of Tris-HCl (0.08M, pH 7.5) containing 50% 1-propanol and 1% (w/v) dithiothreitol (Calbiochem, LaJolla, CA). The samples were subsequently alkylated (15 min, 55°C) with a buffer solution (100 µl) of Tris-HCl (0.08M, pH 7.5) containing 50% 1-propanol and 14% (v/v) 4-vinylpyridine (Sigma Chemical Co., St. Louis, MO). The samples were vortexed intermittently during reduction and alkylation, then centrifuged (5 min, 15000*g*). The supernatants were syringe filtered (45µm, 4mm Millex HV, Millipore Corporation, Bedford, MA) into microvials and immediately analyzed.

3.9.1. Samples Prepared by Large-Scale Modified Osborne Fractionation

Samples were analyzed using a Hewlett-Packard 1090M liquid chromatograph with a Zorbax®300SB-C8 (Rockland Technologies, Inc., Newport, DE) column (5 µm particle size, 300Å pore size, 150 mm x 4.6 mm I.D.) and cartridge guard column (12.5 mm x 4 mm, I.D.). The solvents, deaerated water (solvent A) and acetonitrile (solvent B, ACN, HPLC grade, Burdick and Jackson, Muskegon, NJ), both containing 0.1% trifluoroacetic acid (TFA, HPLC grade, Sigma Chemical Co., St. Louis, MO) were continuously sparged with helium during analyses. Solvent flow rate was maintained at 1.0 $ml \cdot min^{-1}$. After sample injection (5.0 µl) and an initial 3 min isocratic condition at 23% solvent B, proteins were eluted in an 82 min linear gradient from 23-44% solvent B. Solvent B was then reduced from 44% to 23% in the next minute for a final 4 min isocratic elution. The column was equilibrated at 23% solvent B for 7 min between runs. Column temperature was maintained at 50°C and the absorbance of column eluent was monitored at 214 nm with a HP 1090 diode array detector (Series II, 6mm path length, 8µl flow cell, 4nm slit assembly). System control, data acquisition and peak integration were executed using Hewlett-Packard HPLC Chemstation software.

3.9.2. Samples Prepared by Small-Scale Modified Osborne Fractionation

Samples prepared by the small-scale modified Osborne fractionation procedure were analyzed by RP-HPLC as described in Section 3.9.1, except for the following changes. A Zorbax®300SB-C8 (Rockland Technologies, Inc., Newport, DE) narrow bore column (5 μm particle size, 300Å pore size, 150 mm x 2.1mm I.D.) without a guard column was used for the analyses. Solvent flow rate was maintained at 0.25 ml·min^{-1}. After sample injection (1.6 μl) and an initial 3 min isocratic condition at 23% solvent B, proteins were eluted in an 83 min linear gradient from 23-45% solvent B. Solvent B was then reduced from 45% to 23% in the next minute for a final 4 min isocratic elution.

In both methods, integrated areas obtained for individual HMW-GS peaks and the LMW-GS region were used for calculations of relative amounts and ratios. Integrated areas of ω-gliadin peaks were excluded from glutenin quantitation, but they were used to determine the relative amount of ω-gliadins co-precipitated in the purified ES and AS fractions. Typical elution of ω-gliadins, HMW-GS and LMW-GS is shown in Fu and Sapirstein (1996).

3.10. Electrophoresis

Protein samples were analyzed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) with and without the reducing agent 2-mercaptoethanol (Sigma Chemical Co., St. Louis, MO) according to Ng and Bushuk (1987) with the following modifications. The stacking and separating gel concentrations were 3.0% and 14%, respectively. A dual cooled vertical slab gel electrophoresis unit (SE600-15-1.0, Hoefer Scientific Instruments, San Fernando, CA) with 15- or 20-tooth slot formers and 1.5mm or 1.0mm spacers was used. Electrophoresis was carried out at constant current (25-35mA per gel, 3-4 h) until the tracking dye migrated off the gel. Evaporated protein fractions obtained

from flour or dough (150mg) were extracted in a buffer solution (pH 6.8) containing Tris-HCl (0.06 M), 1.8% (w/v) SDS, 9% (v/v) glycerol, 0.01% (w/v) pyronin Y, and 5% (v/v) 2-mercaptoethanol when reducing conditions were desired. For Coomassie Brilliant (CBB G-250, Sigma Chemical Co., St. Louis, MO) staining, gels were fixed for at least 3 h in an aqueous solution of 10% (v/v) trichloroacetic acid (TCA) and 33% (v/v) methanol and stained overnight. The staining solution contained CBB G-250 (0.08% w/v), sulfuric acid (2.2% v/v), potassium hydroxide (9% v/v) and TCA (13% v/v). For silver staining, gels were fixed in an aqueous solution of 45% (v/v) ethanol, 12% (v/v) acetic acid and 37% formaldehyde (500 $\mu l \cdot L^{-1}$). The silver staining method of Blum et al (1987), as modified by Mellish and Tkachuk (1991), was followed with the exception that 45% (v/v) ethanol was used instead of 50% (v/v) ethanol.

Two-step one-dimensional SDS-PAGE was performed according to Sievert et al (1991) with some modifications. In step one, control and protein samples extracted under non-reducing conditions were loaded (25 µl) onto a gel (1.0 mm thick) and electrophoresed under the conditions described above. The top portion of the gel (approx. 0.5 cm) containing unreduced slot protein (glutenin) was carefully cut out and soaked for 2 h in Tris-HCl buffer (pH 6.8) containing 2-mercaptoethanol (5% v/v) at room temperature. For step two, the strip of reduced slot protein was carefully placed on top of a second gel (1.5 mm thick) prepared with a special slot former (11.5 cm central slot with 6-mm end slots on either side). A reduced control sample (Katepwa flour containing tracking dye) was loaded in a side slot to monitor migration of the proteins. The gel was electrophoresed and CBB stained as described above.

3.11. Size-Exclusion Chromatography

Size-exclusion chromatography (SEC) of flours used in this study was provided by Dr. Finlay MacRitchie of CSIRO Division of Plant Industry, Grain Quality Research Laboratory, North Ryde, Australia (Gupta et al, 1993). The percentage of unextractable polymeric protein (%UPP) is a measure of molecular size distribution and is positively correlated to quality parameters such as extensograph maximum resistance (MacRitchie and Gupta, 1993). For this analysis, the flours were extracted in 0.5% (w/v) SDS-0.05M Na-phosphate buffer (pH 6.9) for 5 min, centrifuged (20 min, 15,900 *g*), and the supernatant retained as extractable protein. The residue was then sonicated (30 s) in SDS-phosphate buffer to solubilize unextractable protein. Both protein fractions were filtered (45 μm) and fractionated by SEC. The percentages of extractable and unextractable protein were calculated as follows:

$$\frac{\text{Area of Peak 1 (extractable)}}{\text{Area of Peak 1 (total)}} \times 100 = \%\text{ extractable protein}$$

$$\frac{\text{Area of Peak 1 (unextractable)}}{\text{Area of Peak 1 (total)}} \times 100 = \%\text{ unextractable protein}$$

Where: Area of Peak 1 (total) = Σ Area of Peak 1 (extractable and unextractable)

SEC of 70PS samples was performed at the Grain Research Laboratory, Winnipeg, MB. Evaporated 70PS fractions were fully solubilized in phosphate buffer (pH 7.0) containing SDS (0.5%, 1.0ml for ES70PS, 0.5ml for AS70PS) and allowed to stand for 30 min at 60°C with occasional vortexing. Samples were filtered (45 μm) before transferring to vials. One sample was selected for fraction collection and subsequent SDS-PAGE analysis to identify the protein components present in the major peaks of interest.

Separation of 70PS fractions was performed using a Waters HPLC system, a Biosep-SEC-S 4000 guard column (7.8mm x 35mm, Phenomenex, Torrance, CA) and two Biosep-SEC-S 4000 analytical columns (7.8mm x 300mm) connected in series. The larger pore size

(400Å) of this column is reported to give better resolution of the proteins in the polymeric peak than the smaller pore size (300Å) columns (Larroque et al, 1997). Isocratic elution of protein (25 µl injection) was achieved using 0.1% TFA in 50% ACN. Flow rate was maintained at 0.5ml·min^{-1} for a total run time of 60 min. Column temperature was maintained at 30°C and column eluent was monitored by measuring UV absorption at 210 nm). System control, data acquisition and peak integration were executed using Waters Millennium software.

3.12. Statistical Analysis

All statistical analyses were executed using PC-based SAS software (The SAS System for Windows, Release 6.12, SAS Institute, Inc., Cary, NC) or Microsoft®Excel software (Version 5.0 or higher). Regression analysis or ANOVA with Duncan's multiple range test were performed to determine significant differences and interactions between all factors studied (mixing time, cultivar and treatment). All experiments were carried out at least in duplicate.

3.13. Chemicals and Reagents

HPLC grade 1-propanol was obtained from BDH, Inc. (Toronto, ON) and dithiothreitol from Calbiochem (La Jolla, CA). Ethanol was obtained from Commercial Alcohols Inc. (Toronto, ON). Sodium dodecyl sulfate was of electrophoresis grade and was obtained from Bio-Rad Laboratories (Hercules, CA). Acrylamide and bis-acrylamide were of electrophoresis grade and were obtained from Fisher Scientific (Fairlawn, NJ) and Sigma Chemical Co. (St. Louis, MO), respectively. All other chemicals not sourced in the methods were of reagent grade or better and were obtained from Mallinckrodt Specialty Chemicals Co. (Paris, KY), Sigma Chemical Co. (St. Louis, MO) or Fisher Scientific (Fairlawn, NJ). Water was distilled, deionized, purified and filtered (0.2µm) using a Milli-Q system (Millipore Corp., Marlborough, MA).

4. RESULTS

4.1. Protein Distribution and Glutenin Subunit Composition of Flours[1]

Results of the modified Osborne fractionation (large scale) of the five cultivars were consistent with the previously published (Orth and Bushuk, 1972) positive correlation of the AI fraction and the negative correlation of the AS fraction (Table 5) with breadmaking quality. The proportion of AI protein decreased from the strongest cultivar (Glenlea) to the weakest (AC Karma). Likewise, the content of AS protein was lowest for the stronger cultivar and highest for the weakest. The %UPP values also exhibited a positive correlation with mixing strength, following closely the trend seen with the AI fraction. The amount of gliadin in the AS fraction decreased significantly as dough strength increased. This relationship was reported previously (Dupuis et al, 1996) as the main cause for the negative correlation between the AS fraction and quality. The observed intercultivar variation in amount of gliadin in the AS fraction was explained on the basis of intercultivar variation in gliadin-glutenin interactions. ES glutenin ranged from 0.4 to 3.7% of total flour protein (1.0 to 10% of ES fraction), and AS glutenin ranged from 3.52 to 9.43% of total flour protein (41 to 66% of AS fraction). Although no clear intercultivar differences were found for the amount of ES or AS glutenin, the AS glutenin appeared to decrease with increasing dough strength. Also noteworthy was the

[1] Publication based on this chapter: Dupuis, B. and Bushuk, W. 1996. Pages 262-266 in: Gluten '96, Proc. 6th Int. Gluten Workshop, Sydney, Australia. C.W. Wrigley (Ed.), Royal Australian Chemical Institute, N. Melbourne, AU.

Table 5. Protein Distribution Obtained by Modified Osborne Fractionation and Purification of Glutenin in Ethanol-soluble and Acetic Acid-soluble Fractions (% of Total Flour Protein) and %UPP Obtained by SEC of Flours[1]

Cultivar	SS	ES			AS			AI	%UPP
		Total	Gliadin	Glutenin[2]	Total	Gliadin	Glutenin[2]		
Glenlea	18.6^{b}	38.0^{a}	34.4^{b}	3.7^{a}	5.4^{d}	1.86^{c}	3.52^{d}	35.9^{a}	59.2
Glenlea93	16.8^{c}	38.3^{a}	36.8^{a}	2.1^{b}	6.5^{d}	2.18^{c}	4.21^{cd}	36.7^{a}	60.8
Roblin	16.5^{c}	33.3^{b}	30.7^{c}	2.6^{ab}	13.8^{b}	7.85^{a}	5.97^{b}	32.7^{b}	54.6
Katepwa	19.1^{b}	37.0^{a}	34.6^{b}	0.4^{c}	9.8^{c}	5.80^{b}	4.30^{c}	31.1^{b}	48.7
AC Domain	17.1^{c}	32.0^{b}	28.8^{d}	2.0^{b}	13.6^{b}	7.94^{a}	5.70^{b}	32.2^{b}	53.9
AC Karma	20.5^{a}	29.0^{c}	26.9^{e}	1.5^{bc}	18.6^{a}	9.18^{a}	9.43^{a}	26.6^{c}	42.1

[1] Means of duplicates; letters indicate significant differences (a=0.05); average CV = 10%
SS: salt-soluble, ES: ethanol soluble, AS: acetic acid soluble, AI: acetic acid insoluble
[2] Glutenin contents obtained by difference (total ES protein - ES gliadin; total AS protein - AS gliadin)

apparent increase in ES gliadin with increasing dough strength. Recently Fu et al (1997) reported that when wet glutens isolated with 0.2% NaCl were subsequently extracted with water, the extent of disaggregation/solubilization of gliadins was significantly positively correlated to gluten strength. The observations reported in this study and that of Fu et al (1997) provide further support for the gliadin-glutenin interaction hypothesis (Dupuis et al, 1996, Fu et al, 1996).

The HMW-GS composition of each of the purified ES, AS and AI glutenins are shown in Table 6 and Table 7. All fractions contained both HMW-GS and LMW-GS. The most striking variation between the solubility fractions for all cultivars was in the amount of the 1Dx subunits, especially GS 5. The increment of variation between the ES and AS fractions appeared to be cultivar dependent. The smallest increase (14%) was noted for Karma (1Dx2) and ranged from 31% to 66% for the remaining cultivars (all contain 1Dx5), in increasing order of strength. The increment of variation (increase) between the AS and AI glutenins for GS 5 was also significant for all the cultivars except AC Karma (containing GS 2). The relative amount of subunits encoded by 1Dy alleles (10 or 12) was significantly greater in the AS glutenin than in the ES glutenin. AI glutenin contained only slightly higher amounts of these subunits for all cultivars except Glenlea, but the differences were insignificant. Previous results from this laboratory (Dupuis et al, 1996) showed that the AS and AI glutenins had similar HMW-GS composition, but that the content of subunits 5 and 10 was slightly higher in the AI glutenin. The larger number of cultivars and replicates used in this study have provided additional evidence for the differences detected for the Glu-1D subunits in glutenins of different solubility. Subunits

Table 6. Relative Amounts of HMW-GS 1Ax, 1Bx and 1By (% total HMW-GS) in ES, AS, and AI Glutenin Obtained by RP-HPLC[1]

	1Ax1/1Ax2*			1Bx7/1Bx7*			1By8/1By9		
Cultivar	ES	AS	AI	ES	AS	AI	ES	AS	AI
Glenlea	16.8^{a}	11.0^{b3}	11.7^{b2}	52.4^{a3}	45.2^{b2}	41.6^{c2}	7.5^{b2}	10.0^{a3}	10.7^{a2}
Glenlea93	14.4	12.2^{3}	12.1^{2}	54.4^{a3}	43.6^{b2}	40.4^{b2}	7.8^{b2}	11.3^{a2}	11.0^{a2}
Roblin	14.1	16.2^{12}	13.7^{12}	58.4^{a3}	44.7^{b2}	41.7^{b2}	7.9^{b2}	11.1^{a2}	11.3^{a2}
Katepwa	17.5^{a}	15.0^{b2}	13.8^{b12}	41.9^{a2}	31.3^{b1}	29.4^{b1}	11.9^{1}	14.4^{1}	14.2^{1}
AC Domain	16.2	18.1^{1}	15.6^{1}	40.6^{a2}	31.6^{b1}	29.2^{b1}	12.9^{1}	13.7^{1}	14.2^{1}
AC Karma	18.7	15.5^{12}	15.6^{1}	37.8^{a1}	30.5^{b1}	29.8^{b1}	10.8^{b1}	13.2^{a12}	13.6^{a1}

[1] Means of triplicates; average CV = 7.6%; significant differences (α=0.05) indicated by letters (between fractions within a cultivar) and numbers (between cultivars within a fraction)

Table 7. Relative Amounts of HMW-GS 1Dx and 1Dy (% total HMW-GS) and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Obtained by RP-HPLC[1]

	1Dx2/1Dx5			1Dy10/1Dy12			Ratio of HMW-GS to LMW-GS		
Cultivar	ES	AS	AI	ES	AS	AI	ES	AS	AI
Glenlea	12.2^{c3}	19.0^{b3}	21.9^{a3}	11.1^{b12}	14.8^{a35}	14.2^{a2}	0.37^{b2}	0.57^{a3}	0.52^{a2}
Glenlea93	11.7^{c3}	19.4^{b3}	22.7^{a3}	11.7^{12}	13.5^{45}	13.6^{2}	0.37^{b2}	0.54^{a3}	0.51^{a2}
Roblin	10.8^{c3}	15.6^{b4}	19.6^{a2}	8.8^{b2}	12.3^{a4}	13.7^{a2}	0.45^{b2}	0.66^{a4}	0.53^{b2}
Katepwa	14.6^{c2}	21.5^{b2}	24.6^{a1}	14.1^{b1}	17.8^{a2}	17.9^{a1}	0.39^{b2}	0.51^{a23}	0.42^{b1}
AC Domain	15.7^{c2}	20.5^{b23}	24.5^{a1}	14.6^{1}	16.2^{13}	16.5^{1}	0.37^{2}	0.40^{12}	0.43^{1}
AC Karma	21.4^{b1}	24.5^{a1}	24.5^{a1}	11.5^{b12}	16.3^{a1}	16.6^{a1}	0.26^{c1}	0.45^{a1}	0.37^{b1}

[1] Means of triplicates; average CV = 7.6%; significant differences (α=0.05) indicated by letters (between fractions within a cultivar) and numbers (between cultivars within a fraction)

1By8 and 1By9 exhibited quantitative differences between fractions similar to those found for the 1Dy subunits, a significantly greater proportion in the AS glutenin compared to the ES glutenin and relatively little difference between the AS and AI glutenin. The variation in proportion of 1By subunits between ES and AS glutenin was greater for cultivars containing subunit 8 (33-45%, Glenlea and Roblin) than for those containing subunit 9 (6-22%). The proportion of 1Bx subunits (7 or 7*) was significantly lower in AS glutenin when compared to the ES glutenin. The proportion of GS 7 or 7* in the AI glutenin was slightly but not significantly higher than in the AS glutenin. The increment of variation from the ES to AS to AI glutenin was similar for cultivars containing either subunit. It was 14-25% lower in the AS than the ES glutenin and 2-8% lower in the AI than in the AS glutenin. Quantitative variation in 1Ax subunits did not show any specific trend with solubility or quality (flour strength).

The ratio of HMW-GS to LMW-GS was significantly lower for the ES glutenin compared with the other two fractions for all cultivars except AC Domain. The ratios for AS and AI glutenins were similar. Similar results were obtained in our laboratory (Fu, 1996) for the glutenin soluble and insoluble in 50% 1-propanol. The author showed that for glutenins that are soluble and insoluble in 50% 1-propanol, the subunits controlled by the Glu-1D locus, especially subunit 5, were more prevalent in the insoluble glutenin, while 1Bx subunits were more prevalent in the soluble glutenin. Results obtained for the 1Ax subunits and the ratio of HMW-GS to LMW-GS were similar to those reported here. Our results showed that 1Dx subunits are present in greater proportion in the AI glutenin. The y-type subunits (1By8 or 1By9, and 1Dy10 or 1Dy12) are also present in greater

proportion in the AI glutenin, but not significantly different from the AS glutenin. Based on solubility results, the AI glutenin is presumed to be of higher molecular weight.

The three solubility fractions of glutenin examined (ES, AS and AI) showed substantial quantitative variation in HMW-GS composition. ES glutenin had the lowest HMW-GS to LMW-GS ratio, while AS glutenin had similar or sometimes higher ratios than AI glutenins. Intercultivar variation was noted for the ratio of HMW-GS to LMW-GS and occasionally for HMW-GS composition. ES glutenin contained more 1Bx subunits (7 or 7*), less 1By subunits (8 or 9) and less 1D subunits (2+12 and 5+10) than AS glutenin. AS glutenin contained slightly more 1Bx subunits (7 or 7*) and significantly less 1Dx5 than AI glutenin. The amount of 1Dx2 and 1Dy subunits (10 or 12) was similar for the AS and AI glutenin. The greater proportion of 1Dx5 in the insoluble glutenin may be attributed to the extra cysteine residue, which may confer an enhanced capacity to form larger, more insoluble, glutenin polymers (Hickman et al, 1994). The much higher proportion of 1Dx5 in the AI glutenin may also suggest that the variation in polymerization behaviour (Gupta et al, 1994) and quality (Payne et al, 1981b) between the two Glu-1D alleles (5+10 and 2+12) may in fact be due to differences residing essentially in subunit 5. In addition to the variation in the type of subunits among wheat cultivars, substantial intercultivar variation exists in the proportion of some subunits. The so-called strong mixing cultivars contained a larger proportion of HMW-GS which seem to promote formation of higher molecular weight glutenin polymers (e.g. 1Dx5) which, in turn, are less soluble in aqueous solvents and tend to form a gluten of higher elasticity. Based on the variation in HMW-GS composition in the three solubility fractions examined in this study, 1Dx subunits, especially subunit 5, and to a lesser extent, the y-type subunits

(1Dy and 1By), appear to be important in the formation of polymers of higher molecular weight. The 1Ax subunits, which do not exhibit any specific trend with solubility, and 1Bx subunits, which are present in greater proportion in the ES and AS glutenins, do not appear to be as important to intercultivar differences in quality. However, it should be noted that despite the greater proportion of 1Bx subunits in the more soluble fractions, overexpression of 1Bx7 (Glenlea and Roblin) may have an important impact on the overall size distribution of the larger, more insoluble glutenin.

4.2. Small Scale Modified Osborne Fractionation and Modified Purification Methods

4.2.1. Small scale modified Osborne fractionation

The classical protein fractionation procedure developed by Osborne (1907) separates wheat flour proteins into four groups: the water-soluble albumins, the salt-soluble globulins, the ethanol-soluble gliadins and the residue protein consisting of glutenins. The method was modified by Chen and Bushuk (1970) to extract acetic acid-soluble glutenin from the residue. Using this modified Osborne fractionation procedure, Orth and Bushuk (1972) were the first to report the significantly positive relationship between the amount of residue and loaf volume and the negative relationship between the amount of acetic acid-soluble fraction and loaf volume. Use of the modified Osborne fractionation procedure has declined over the years as other extractants, such as aqueous solutions of propanol or SDS, have become more popular. There are limitations to the Osborne procedure, but it continues to provide useful information on the structure-functionality of bread wheat proteins (Bushuk et al, 1997, Dupuis et al, 1996, Wieser et al, 1998). One of the major limitations to the Osborne procedure is the significant cross-contamination between the fractions. The acetic acid soluble fraction, which was considered to be composed of soluble glutenin contains a significant amount of gliadin (Dupuis et al, 1996, Orth and Bushuk, 1973a). Likewise, the alcohol-soluble or gliadin fraction contains glutenin (Bietz and Wall, 1973, Huebner and Bietz, 1993). The fractionation method, on a large scale, is also very tedious and time-consuming, taking weeks to produce a limited number of freeze-dried and purified fractions for analysis. The acetic acid soluble fraction in particular yields less than 100 mg quantities from a starting

flour weight of 20 g. Also of concern for this study was the limited quantity of some flour samples and the large sample size to be investigated. Accordingly, a small scale method was developed to overcome these limitations. The factors considered in the development of the method included: total extraction time, solvent-to-sample ratio, centrifugation speed, and equipment limitations.

Total fractionation time was considered as an important factor in selecting a suitable extraction time. The six extraction steps (see Materials and Methods) were initially timed for 30 minutes and the water wash for 15 minutes. Total fractionation time, including the centrifugation steps between each extraction, transfer of supernatants, and other experimental manipulations, exceeded 5 hours. This was considered too long to allow for same-day nitrogen determination, or supernatant evaporation and 70% (v/v) 1-propanol purification. Consequently, the extraction times were reduced to 15 minutes and the water wash step reduced to less than 5 minutes. Total fractionation time was considered more acceptable at just over 2 hours.

By scaling down the modified Osborne fractionation, certain conditions were introduced that can undoubtedly be considered additional sources of error. For example, the use of vortexing was required to suspend the samples in the much smaller volume microcentrifuge tubes. Vortexing may considerably increase the amount of shear exerted on the proteins when compared to the milder conditions of stirring with magnetic stir bars. Some of the variation observed between the large- and small-scale methods can be attributable to these differences in experimental error.

Solvent-to-sample ratios ranged from 10:1 to 4:1 for trial fractionations. Table 8 illustrates the effect of varying the ratio for one or more extraction steps. The salt-soluble

Table 8. Modified Osborne Protein Distribution (% of Total Flour Protein) Using the Large Scale and Several Small Scale Methods [1]

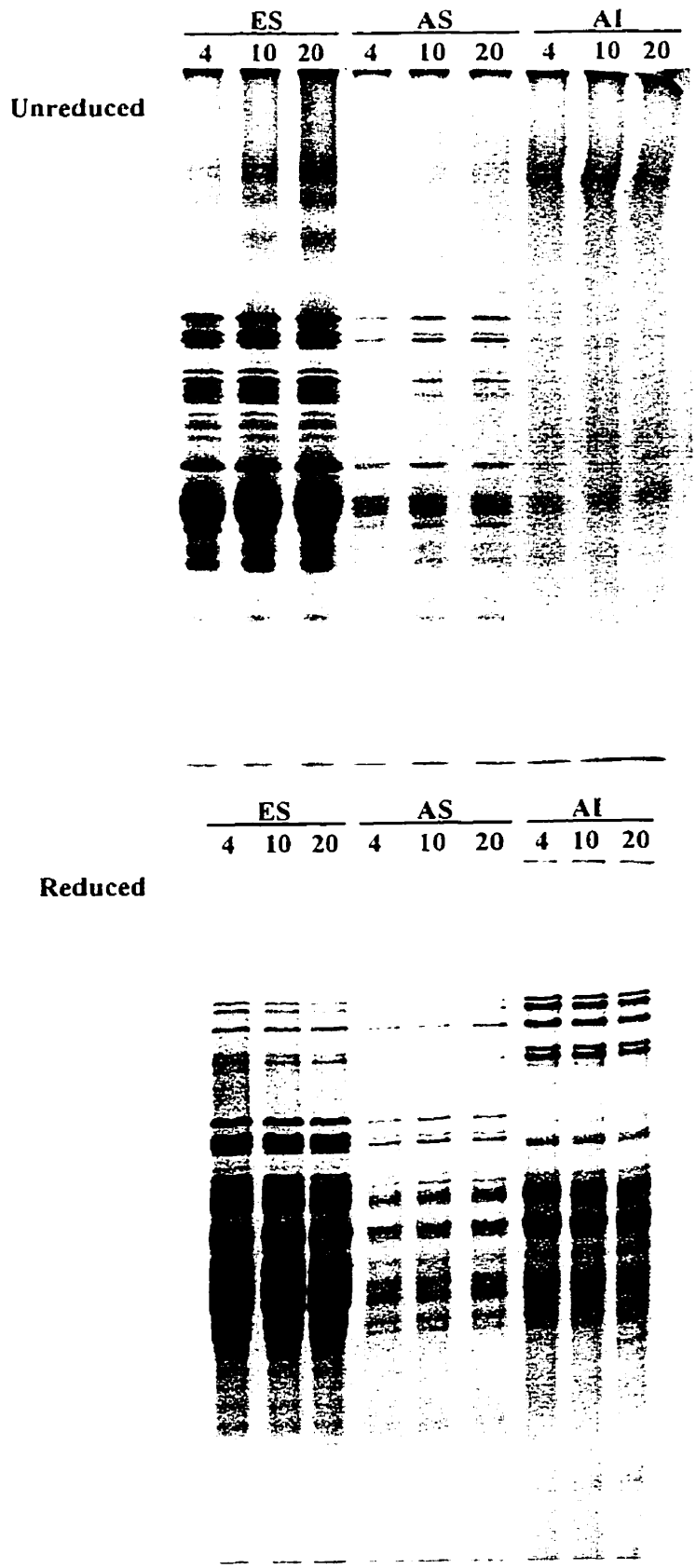
	Large	Small[2]	Small[3]	Small[4]	Small[5]	Small[6]
SS Protein						
Glenlea	18.6^{b}	18.8^{b}	20.3^{b*}	20.3^{b}	17.9^{b}	18.6^{b}
Katepwa	19.1^{b}	19.2^{b}	20.1^{bc}	20.3^{b}	17.9^{b*}	18.1^{b}
AC Domain	17.1^{c}	17.9^{b}	18.4^{c*}	18.4^{b*}	16.4^{c}	16.0^{c*}
AC Karma	20.5^{a}	21.3^{a}	22.8^{a*}	22.9^{a*}	20.1^{a}	20.3^{a}
ES Protein						
Glenlea	38.0^{a}	35.4^{b*}	35.3^{ns*}	37.9^{b}	34.8^{b*}	35.4^{ns*}
Katepwa	37.0^{a}	39.0^{ab}	36.9^{ns}	41.7^{a}	40.8^{a}	38.0^{ns}
AC Domain	32.0^{b}	39.6^{a*}	37.8^{ns*}	41.3^{a*}	38.2^{ab*}	38.4^{ns*}
AC Karma	29.0^{c}	42.8^{a*}	36.2^{ns*}	41.2^{a*}	35.9^{b*}	35.7^{ns*}
AS Protein						
Glenlea	5.4^{d}	5.9^{b}	6.4^{b}	5.8^{c}	9.7^{b*}	8.7^{c*}
Katepwa	9.8^{c}	10.1^{a}	12.4^{a}	9.1^{ab}	12.0^{ab}	12.6^{b*}
AC Domain	13.6^{b}	11.2^{a}	10.6^{ab*}	7.6^{bc*}	11.5^{ab}	13.6^{b}
AC Karma	18.6^{a}	11.7^{a*}	13.8^{a}	10.0^{a*}	15.3^{a}	18.0^{a}
AI Protein						
Glenlea	35.9^{a}	39.2^{a*}	38.6^{bc}	38.3^{a*}	39.1^{a*}	38.5^{a*}
Katepwa	31.1^{b}	32.9^{b*}	32.3^{ab}	31.2^{b}	31.7^{bc}	33.8^{b*}
AC Domain	32.2^{b}	33.2^{b}	33.6^{ns}	32.9^{b}	33.0^{b}	31.8^{b}
AC Karma	26.6^{c}	27.3^{c}	27.7^{ns}	29.3^{c}	29.4^{c}	27.0^{c}
Protein Recovery (%)						
Glenlea	97.2	99.3	100.6	102.4	101.6	101.2
Katepwa	95.4	100.0	101.7	102.2	102.3	102.5
AC Domain	94.7	99.8	100.5	100.2	99.1	99.8
AC Karma	95.0	103.1	100.5	103.3	100.7	101.1

[1] Letters indicate significant differences (α=0.05) between cultivars within a scale
Asterisk indicates significant difference (α=0.05) from the large scale method
[2] Solvent-to-sample ratio of 10:1 used throughout method
[3] Solvent-to-sample ratio 4:1 for water wash
[4] Solvent-to-sample ratio 4:1 for water wash and ethanol extractions
[5] Solvent-to-sample ratio 4:1 used throughout method
[6] Solvent-to-sample ratio 4:1 used throughout method and lower centrifugation speeds

(SS) fraction was least affected by changes in solvent-to-sample ratios. The ethanol-soluble (ES) fraction showed the greatest response to changes in solvent-to-sample ratio. The content of the ES protein was generally greater for the small scale method, the magnitude of which varied with cultivar. Decreasing the ratio from 10 to 4 reduced the amount of ES protein. However, it was not possible to reproduce the statistical relationship between cultivar and amount of ES protein observed in the large scale method. The amount of ES protein obtained by the large scale method significantly increased for cultivars with increasing dough strength. This relationship was less evident or nonexistent in the small scale methods. Regardless of the solvent-to-sample ratio used, the small scale method enhanced the extractability of proteins in ethanol, particularly for the weaker cultivars. The content of acetic acid-soluble (AS) protein increased or decreased with a change in ratio, depending on the cultivar. The negative correlation between the AS fraction and mixing strength was maintained, although less discriminating, regardless of the ratio used for the small scale. The acetic acid-insoluble (AI) protein content increased slightly for all cultivars, especially Glenlea. Protein recovery was greater for all small scale trials. This was expected since the loss incurred during freeze-drying, sample grinding and sample manipulation in the large scale method was eliminated in the small scale method.

Altering the solvent-to-sample ratio for the small scale method did not cause qualitative changes in the protein fractions, as shown by electrophoresis (Figure 8). More gliadin was extracted in the ethanol and acetic acid at higher ratios (unreduced gel). Glutenin solubility in acetic acid appeared to increase slightly, with a concomitant decrease in the glutenin remaining in the AI fraction (reduced gel). Despite the changes in protein

Figure 8. SDS-PAGE under non-reducing and reducing conditions of ES, AS and AI fractions of Katepwa flour obtained using three different solvent-to-sample ratios for the small scale modified Osborne fractionation procedure. 4 = 4:1, 10 = 10:1, 20 = 20:1.


ES
AS
AI
4 10 20 4 10 20 4 10 20
Unreduced
ES
AS
AI
4 10 20 4 10 20 4 10 20
Reduced

distribution caused by scaling down the modified Osborne fractionation, the positive relationship between the AI fraction and mixing strength was also maintained.

The final improvement to the small scale method involved reducing the centrifugation speeds used after the first ethanol and acetic acid extraction steps. This was required to improve resuspension of the pellet obtained after centrifugation. Changing the centrifugation speeds had the most influence on the AS and AI fractions (Table 8, compare last two columns). A general shift was observed in the protein distribution toward the more soluble fractions. Statistical evaluation of the large and small scale methods is reported in Table 9. The overall coefficient of variation (CV), a measure of the intercultivar variability, is slightly higher for the SS and AI fractions of the small scale method but much lower for the ES and AS fractions. Hence, the ability to detect cultivar differences is not as powerful with the small scale method. Precision, a measure of the intracultivar variability, is also reduced by scaling down the method. But the loss of precision is small and acceptable. Despite the loss of precision and increase in variation for the small scale method, the important relationship between the AS and AI fractions and mixing strength is maintained. The apparent relationship observed in the ES fraction from the large scale method (the content of ES protein increases with increasing mixing strength) is lost in the small scale method.

The small scale method is reproducible over time, but is sensitive to variations in experimental conditions. For example, Katepwa flour was being fractionated for analysis nine months after the method was finalized. The centrifugation speed after the first salt solution extraction was inadvertently changed from 3000*g* to 5000*g*. The fractionation was nevertheless completed to assess the effects of the change. A second sample was

Table 9. Statistical Comparison of the Modified Osborne Protein Distribution (as % of Total Flour Protein) for the Large and Small Scale Methods[1]

Cultivar	Large Scale	SD[2]	CV[3]	Small Scale	SD	CV
SS Protein						
Glenlea	18.6^{b}	0.8	4.2	18.6^{b}	1.4	7.4
Katepwa	19.1^{b}	0.6	3.3	18.1^{b}	0.3	1.8
AC Domain	17.1^{c*}	0.5	3.0	16.0^{c*}	0.5	2.9
AC Karma	20.5^{a}	0.6	2.7	20.3^{a}	0.9	4.7
Mean	18.8			18.2		
Range	4.6			5.9		
CV	7.4			9.7		
Precision	3.3			4.2		
ES Protein						
Glenlea	38.0^{a*}	0.6	1.5	35.4^{ns*}	1.0	2.7
Katepwa	37.0^{a}	2.7	7.2	38.0^{ns}	0.5	1.3
AC Domain	32.0^{b*}	1.6	5.1	38.4^{ns*}	3.3	8.7
AC Karma	29.0^{c*}	0.9	3.1	35.7^{ns*}	3.6	10.2
Mean	34.0			36.9		
Range	12.5			10.1		
CV	12.0			6.9		
Precision	4.2			5.7		
AS Protein						
Glenlea	5.4^{d*}	0.5	9.3	8.7^{c*}	0.9	10.1
Katepwa	9.8^{c*}	1.7	17.5	12.6^{b*}	0.6	5.0
AC Domain	13.6^{b}	0.9	6.3	13.6^{b}	1.9	14.3
AC Karma	18.6^{a}	1.7	9.0	18.0^{a}	1.8	10.2
Mean	11.9			13.2		
Range	15.7			11.4		
CV	43.4			27.7		
Precision	10.5			9.9		
AI Protein						
Glenlea	35.9^{a*}	1.0	2.8	38.5^{a*}	1.2	3.2
Katepwa	31.1^{b*}	1.0	3.2	33.8^{b*}	1.4	4.2
AC Domain	32.2^{b}	0.7	2.2	31.8^{b}	2.2	7.0
AC Karma	26.6^{c}	1.7	6.5	27.0^{c}	3.2	11.8
Mean	31.4			32.8		
Range	12.2			16.1		
CV	11.3			14.3		
Precision	3.7			6.5		

[1] Letters indicate significant differences (α=0.05) between cultivars within each scale
Asterisks indicate significant differences (α=0.05) between scale within a cultivar
[2] SD = standard deviation , [3] CV = coefficient of variation

fractionated with the correct centrifugation speed and the results compared to the original data (Table 10). After nine months, the method yielded results within the standard deviation range for all fractions. However, when the centrifugation speed was altered a distinct shift to the less soluble fractions was noted, especially the ES and AS fractions.

Table 10. Example of the Reproducibility and Sensitivity to Experimental Conditions of the Small Scale Modified Osborne Fractionation of Katepwa Flour (% of total flour protein)

Fraction	Small Scale 1997[a]	Small Scale 1998[b]	Small Scale 1998 Altered[c]
SS	18.1±0.3	17.9	17.0
ES	38.0±0.5	37.5	31.9
AS	12.6±0.6	12.0	15.5
AI	33.8±1.4	33.5	34.9

[a] Data obtained from finalized method

[b] Data obtained nine months later

[c] Data obtained nine months later, using a higher centrifugation speed after the first salt solution extraction (5000*g* versus 3000*g*)

The small scale method appears to be less precise and less discriminating in evaluating the effects of cultivar. But, it produces similar results to the large scale method (solubility-quality relationship) in a much shorter time, for a much larger sample size, and reduced amounts of reagents. The large scale method required almost 14 days to process 6 samples, while the small scale method required only 1-2 days to process 12 samples. The small scale method is particularly attractive because the loss in precision or discrimination against the enormous gain in time and decreased solvent use does not significantly compromise the important solubility-quality relationship - the basis for using the modified Osborne fractionation scheme for this thesis study.

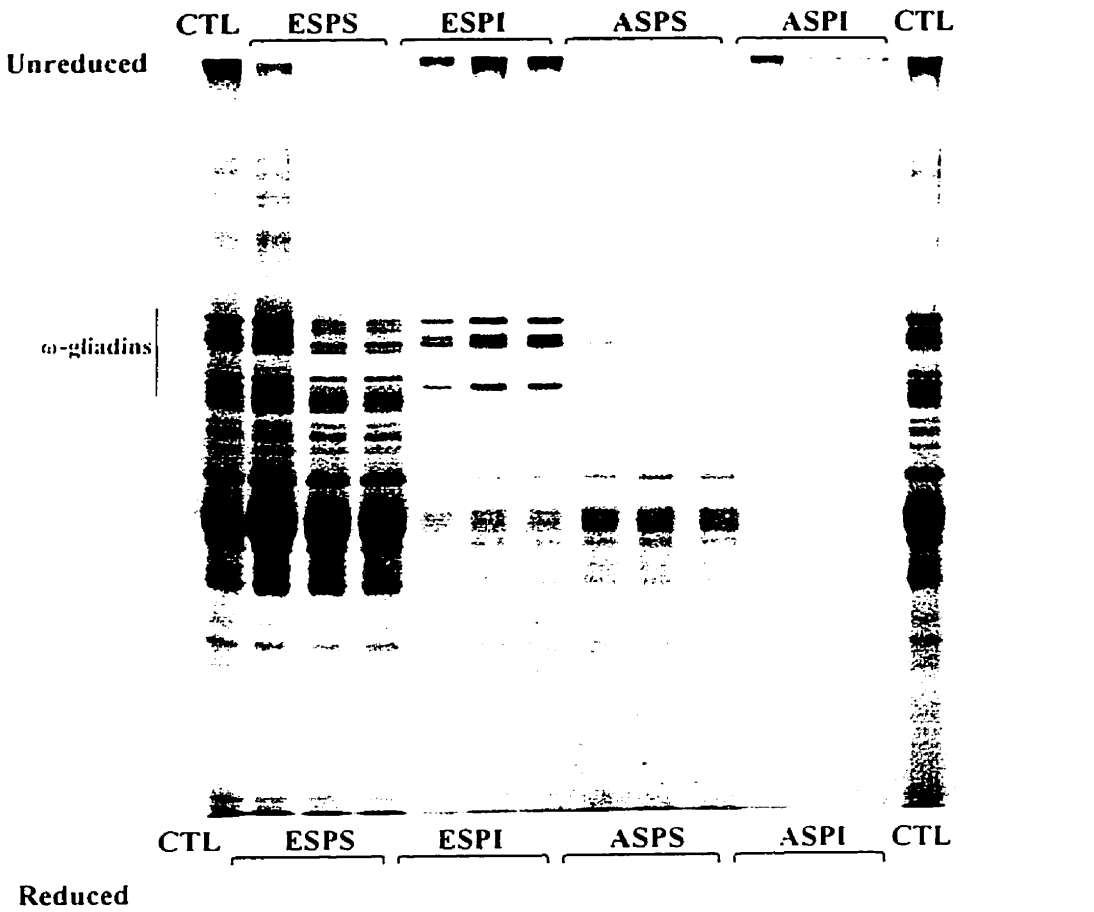
4.2.2. Purification of ES and AS fractions

As previously reported (Dupuis et al, 1996, Dupuis and Bushuk, 1996), the ES and AS fractions of the modified Osborne fractionation procedure are contaminated with glutenin and gliadin, respectively. A modification of the 70% 1-propanol purification procedure developed by Fu and Sapirstein (1996) was successful in separating the fractions into 70% 1-propanol-soluble (70PS) gliadin and 70% 1-propanol-insoluble (70PI) glutenin (with co-precipitated ω-gliadins). Buffered 50% 1-propanol was required to maintain a neutral environment for the effective precipitation of glutenins. A Tris buffer was used in the method when the samples were being purified for RP-HPLC analysis. A phosphate buffer was used when protein content was to be determined spectrophotometrically or by Kjeldahl analysis.

SDS-PAGE under non-reducing and reducing conditions of a sample purified in a Tris buffer, a phosphate buffer, and a phosphate buffer followed by a water wash is shown in Figure 9. The qualitative protein composition did not appear to be affected by the buffer system nor by the presence or absence of a water wash after precipitation in phosphate buffer. Quantitatively, the Tris buffer appears to be more efficient in separating gliadins from the precipitating glutenins, especially for the ES fraction. This difference in efficiency may be due to the ionic effect of the phosphate buffer system.

Samples are fractionated using the small scale method and the ES and AS fractions purified by 70% 1-propanol precipitation in an appropriate buffer system. To determine the protein contents of the purified fractions, the 70PS supernatants were initially subjected to Kjeldahl nitrogen determination and the protein contents of the 70PI glutenin precipitates were obtained by difference. A disadvantage encountered by using this

Figure 9. SDS-PAGE under non-reducing and reducing conditions of ESPI, ESPS, ASPS, and ASPI fractions obtained from Katepwa dough (mixed to 50% peak; 150 mg) by 70% 1-propanol purification in Tris buffer (first lane in each fraction), phosphate buffer with subsequent water wash (second lane in each fraction), and phosphate buffer without water wash (third lane in each fraction).


CTL
ESPS
ESPI
ASPS
ASPI
CTL
Unreduced
ω-gliadins
CTL
ESPS
ESPI
ASPS
ASPI
CTL
Reduced
HMW-GS

normally reliable and accurate method was the very small titration volumes resulting from such small samples. Titration volumes as low as 0.20 ml were encountered, with blank volumes as high as 0.18 ml. Protein contents were calculated to be as low as 0.24 mg in some cases. The error range for a standard 25-ml buret is ±0.06 ml (±0.15 mg protein). Thus, without sufficient confidence to accept the results obtained by the Kjeldahl method, an alternate method was selected. The spectrophotometric method developed and reported by Sapirstein and Johnson (1996) is simple, non-destructive, very sensitive (4 $\mu g \cdot ml^{-1}$ protein) and accurate. Other advantages of the spectrophotometric method over the Kjeldahl method are: the speed of analysis, reduced use of reagents, and the ability to analyze a large number of samples in a short time. The protein contents of ES and AS purified fractions obtained from flour samples and control dough samples were determined using both the Kjeldahl and the spectrophotometric methods. Statistical comparison of the two methods for all samples is included in the Appendix (Tables 2-5). The intercultivar variation is generally better with the spectrophotometric method, although the precision of the method is not always better. The AS gliadin fraction (and AS glutenin fraction, calculated by difference) is the fraction of most importance when considering the accuracy of the results. This fraction is the smallest and hence the most sensitive to variation. The precision of the spectrophotometric method was better for all the control dough samples but not the flours. The overall CV was slightly better for the spectrophotometric method than for the Kjeldahl method.

SDS-PAGE of all fractions obtained from the flours by the small scale method and the buffered 70% 1-propanol precipitation method are illustrated in Figure 10 (Glenlea and Katepwa) and Figure 11 (AC Domain and AC Karma). As with the large scale method,

Figure 10. SDS-PAGE under non-reducing and reducing conditions of small scale modified Osborne fractions obtained from Glenlea and Katepwa flours (150 mg). (1) Salt-soluble, (2) ESPS, (3) ESPI, (4) ASPS, (5) ASPI, and (6) AI.

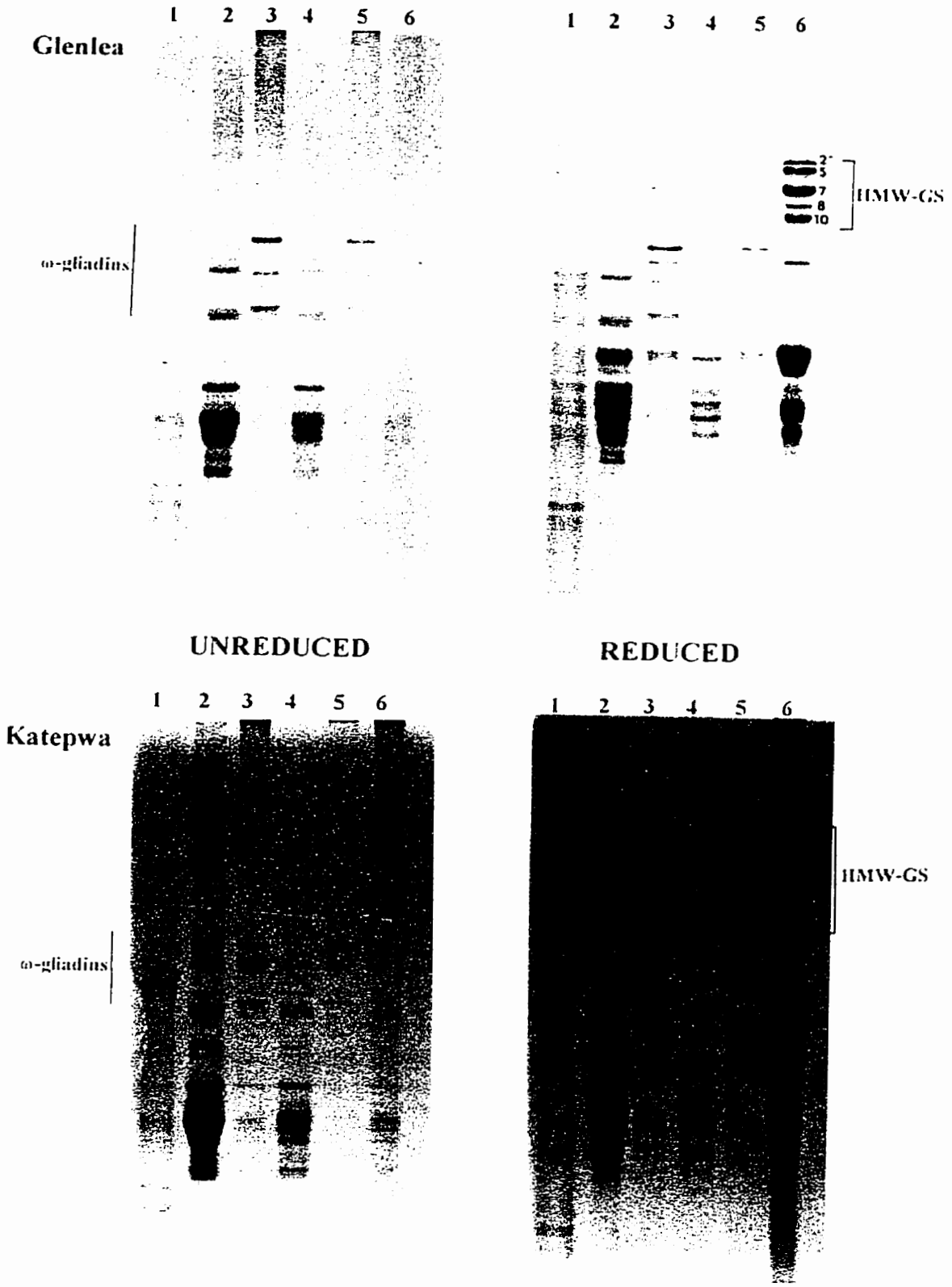
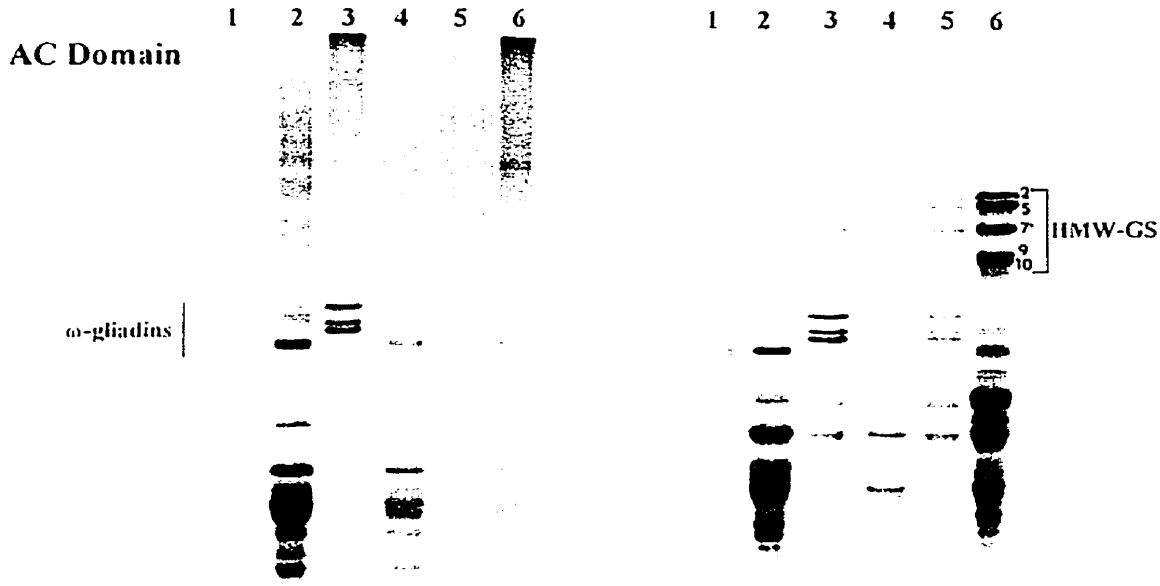
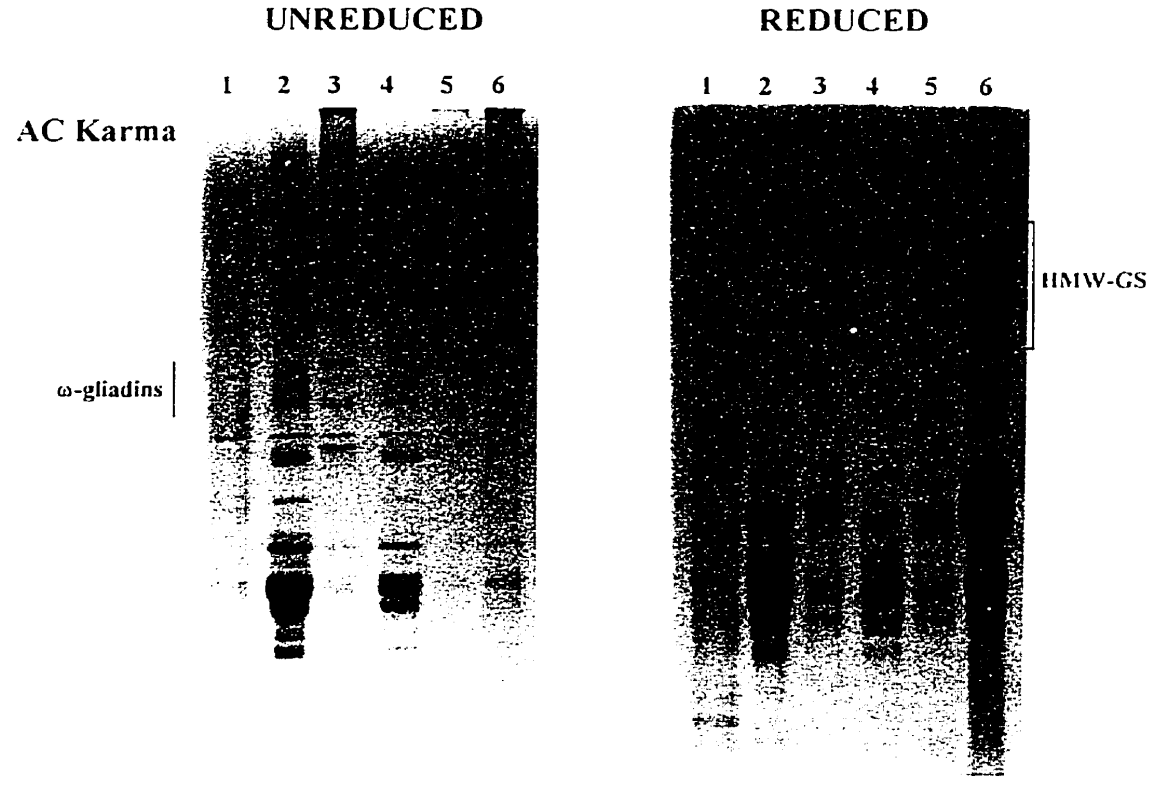

Glenlea
1 2 3 4 5 6
ω-gliadins
2*
3
7
8
10
HMW-GS
UNREDUCED
REDUCED
Katepwa
1 2 3 4 5 6
ω-gliadins
HMW-GS

Figure 11. SDS-PAGE under non-reducing and reducing conditions of small scale modified Osborne fractions obtained from AC Domain and AC Karma flours (150 mg). (1) Salt-soluble, (2) ESPS, (3) ESPI, (4) ASPS, (5) ASPI, and (6) AI.


AC Domain
1 2 3 4 5 6
ω-gliadins
1 2 3 4 5 6
2
5
7
9
10
HMW-GS


UNREDUCED
REDUCED
AC Karma
1 2 3 4 5 6
ω-gliadins
1 2 3 4 5 6
HMW-GS

gliadins (in the 70PS fractions) were effectively separated from the glutenins (in the 70PI fractions). The ω-gliadins that co-precipitate with the glutenins in the 70PI fraction (see lanes 3 and 5 of unreduced gels) are normally removed by a sodium iodide wash (0.75M; Fu et al, 1996). This step in the purification method was omitted to allow the investigation of the changes occurring with the ω-gliadins during mixing.

4.3. Changes in Protein Solubility Distribution During Mixing [2]

4.3.1. Selection of Optimum Absorption

Farinograph absorption is the most commonly used optimum water absorption level used when preparing doughs (Gupta et al, 1994b, Tanaka, 1972, Tsen and Bushuk, 1968, Weegels et al, 1997). Farinograph absorption is the amount of water added to a flour with 14% moisture to produce a maximum consistency of 500 Brabender units (Bloksma and Bushuk, 1988). When the water content of a dough is increased, the peak height of the mixing curve decreases and the mixing time increases (Bohn and Bailey, 1936). The opposite occurs when the water content of a dough is decreased. Variations of ±2% from 'optimum' absorption do not change the varietal pattern of a mixing curve except in height (Swanson, 1941). In addition to the Farinograph absorption, mixograph users have used fixed absorption and variable absorption based on moisture content and protein content (Hazelton et al, 1997).

Because the flour samples studied varied slightly in starch damage and particularly in protein content, optimum mixograph absorption was selected for this study. An arbitrary consistency (40 MU) was selected taking into account the need to keep water addition within a reasonable range for all the flours used. For instance, the range of absorptions used was 60-69% to obtain 40 MU consistency. In contrast, the range required to obtain a higher consistency (50 MU) would be 45-62% absorption. A comparison for one cultivar (AC Domain) of the effects of absorption (69.4% and 61.7%, based on consistencies of 40 MU and 50 MU, respectively) on the protein solubility

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[2] Presented in part at the 82nd Annual Meeting of the Am. Assoc. Cereal Chemists: Dupuis, B., Bushuk, W. and Sapirstein, H.D. 1997. Changes in gluten proteins during mixing of flours of diverse breadmaking quality. Cereal Foods World 42: 659.

distribution and glutenin subunit composition will be discussed in this and the following chapters.

4.3.2. Control Doughs

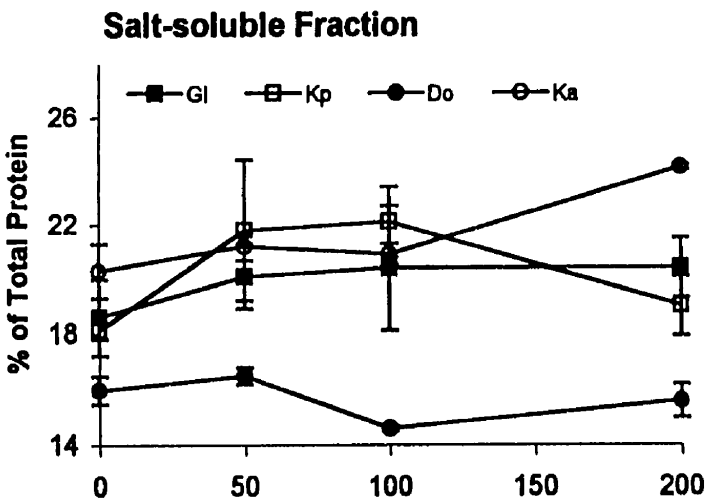
The glutenin fraction insoluble in a variety of solvents (e.g. aqueous solutions of acids, SDS, urea) is related to breadmaking quality, as measured by dough strength or loaf volume (Gupta et al, 1993, MacRitchie, 1973, 1987, Orth and Bushuk, 1972, Pomeranz, 1965). It has been frequently reported that glutenin solubility in different solvents changes during dough mixing and resting (Bushuk et al, 1997, Danno and Hoseney, 1982a, Tanaka and Bushuk, 1973a, Tsen, 1967, Wang et al, 1992, Weegels et al, 1997). Very little research has focused on the phenomenon of gliadin-glutenin interaction. Recent research suggests that gliadin-glutenin interaction is a factor in the solubility behaviour of the protein fractions during mixing (Almonte, 1998, Bushuk et al, 1997, Dupuis et al, 1996, Fu et al, 1997) and in the rheological properties of glutens (Khatkar et al, 1995). Dupuis et al (1996) suggested that gliadin-glutenin interaction could be responsible for differences in dough mixing time to optimum development. How does the solubility distribution of gliadin and glutenin change during mixing? Can evidence be found for the importance of gliadin-glutenin interaction in dough mixing behaviour? The changes occurring in the modified Osborne fractions during mixing have been presented graphically in this chapter for ease of discussion and interpretation. The reader should refer to the Appendix (Tables 6-17) for complete data tables.

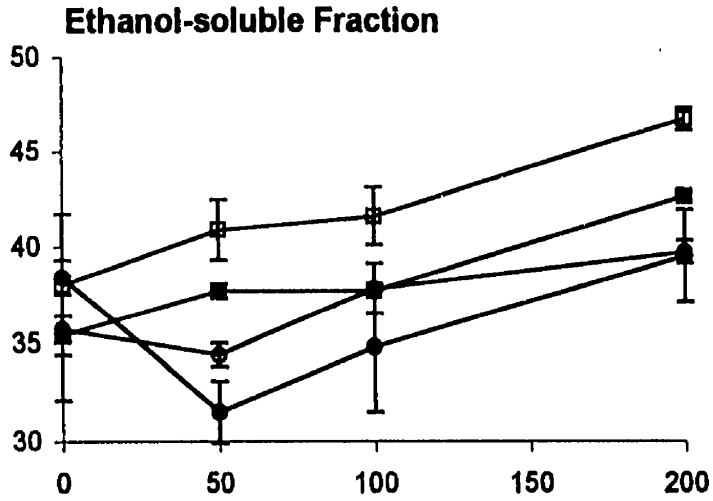
4.3.2.1.Salt-soluble fraction

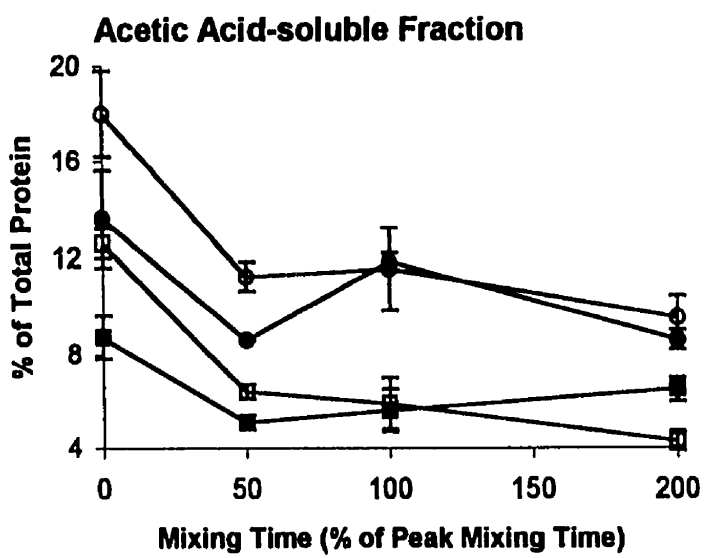
There was a slight increase in SS protein with mixing for all cultivars (Figure 12). This increase varied with cultivar but showed no relationship to dough strength. AC Karma contained the largest amount of SS protein, followed by Glenlea and Katepwa, and AC Domain with the least. Electrophoretic analysis of the SS fraction for each cultivar at each mixing time showed a slight increase in the monomeric protein with mixing (Figure 13, unreduced gel) and showed the presence of HMW-GS at all stages of mixing except in the flour (Figure 13, reduced gel). Band intensity was more pronounced for the *x*-type subunits than for the *y*-type subunits. The increase observed in SS protein can therefore be attributed, at least in part, to the release of monomeric protein and glutenin from the more insoluble fractions as mixing progresses.

Because the non-storage proteins (mostly water- and salt-soluble proteins) are not considered to be of major importance in breadmaking quality, the effects of mixing on the SS fraction were not closely followed. However, two observations deserve mentioning and may warrant further investigation: the presence of glutenin in the SS fraction of dough samples, but not of flours, and the development of a foam layer during salt extraction of dough samples. The presence of glutenin in the SS fraction was also reported by Almonte (1998). Dialysis of the SS fraction allowed the author to establish the presence of glutenin in the globulin (salt-soluble) fraction but not in the albumin (water-soluble) fraction. Almonte (1998) also found the *x*-type HMW-GS to predominate in the SS protein fraction.

Figure 12. Changes in modified Osborne fractions during mixing of control doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka) flours. Mixing time of 0 % corresponds to flour.


Salt-soluble Fraction
Gl
Kp
Do
Ka
% of Total Protein
26
22
18
14
0
50
100
150
200


Ethanol-soluble Fraction
50
45
40
35
30
0
50
100
150
200


Acetic Acid-soluble Fraction
% of Total Protein
20
16
12
8
4
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

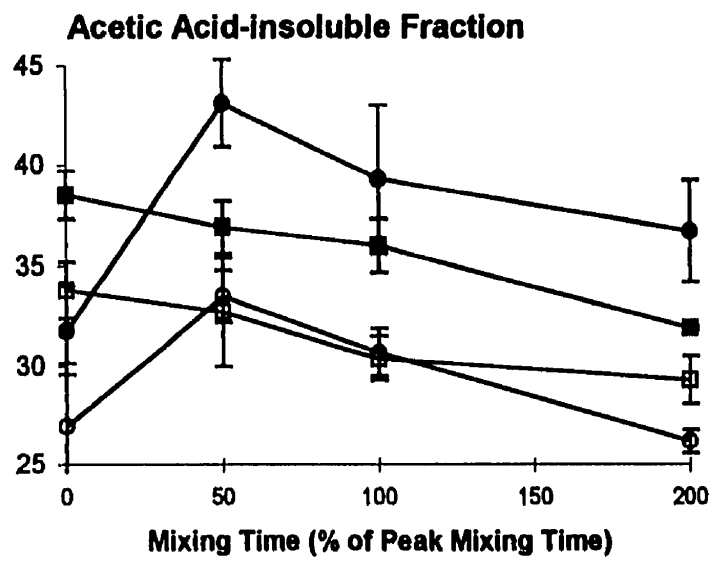
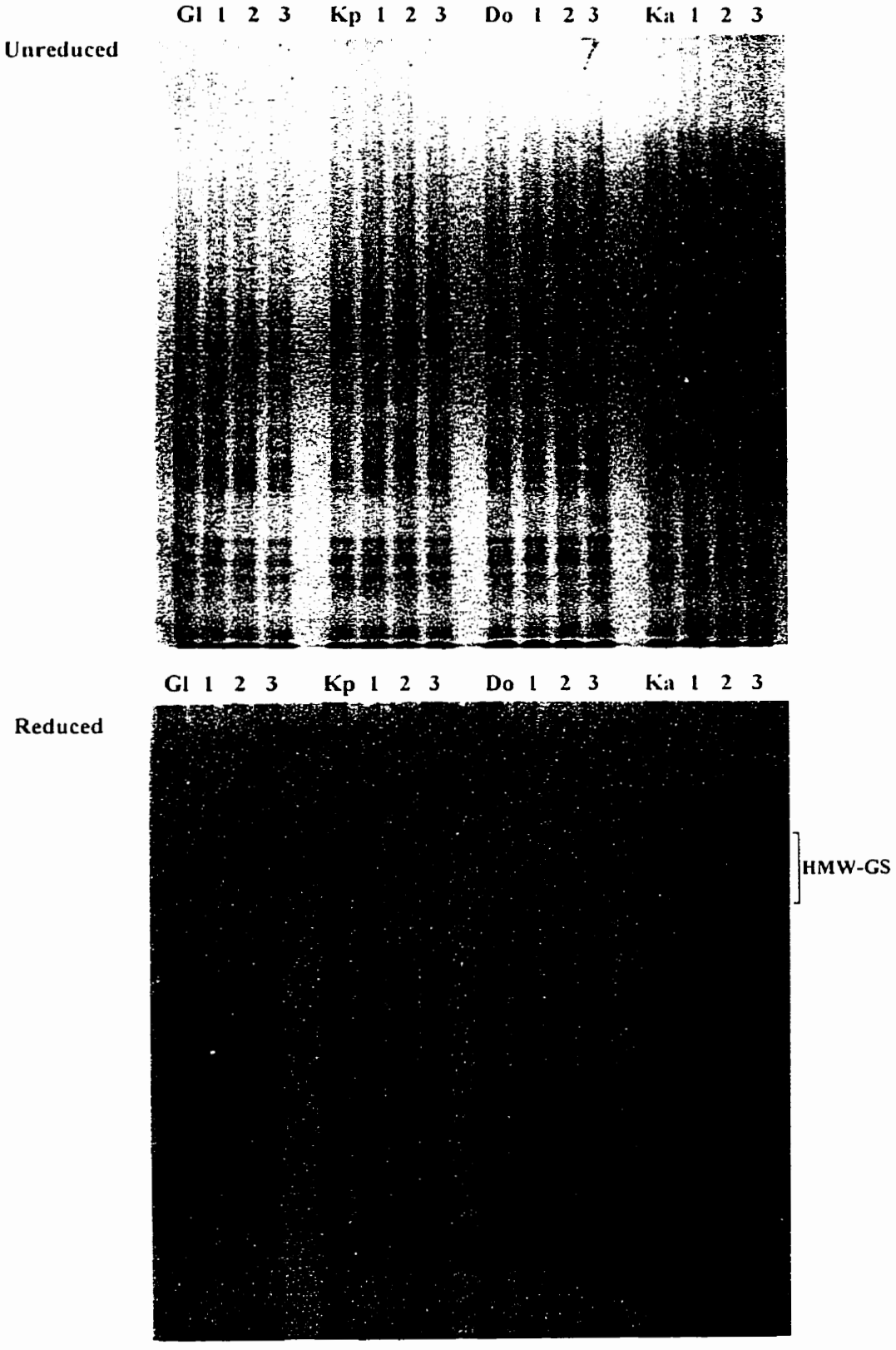

Acetic Acid-insoluble Fraction
45
40
35
30
25
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

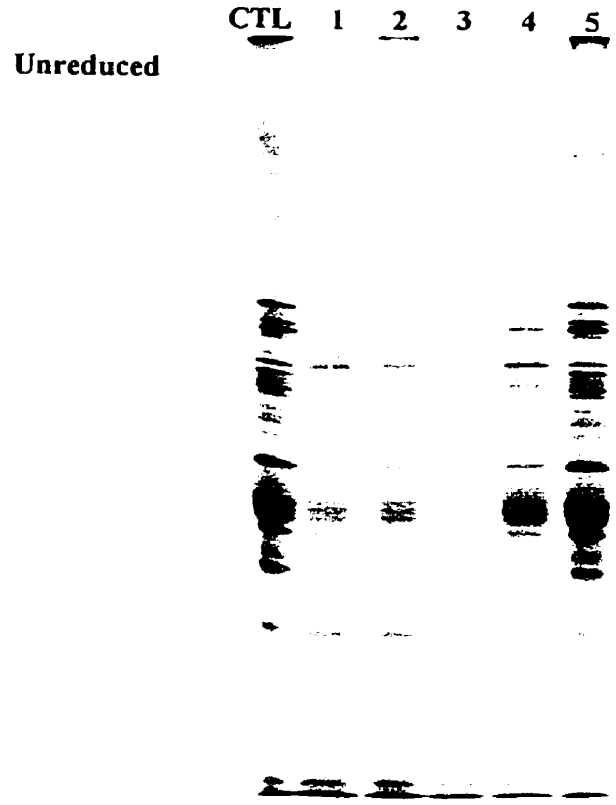
Figure 13. SDS-PAGE of unreduced and reduced salt-soluble fractions of flours and control doughs (150 mg). First lanes designated as (Gl) Glenlea, (Kp) Katepwa, (Do) AC Domain and (Ka) AC Karma correspond to fractions obtained from flours. Subsequent lanes correspond to fractions obtained from control doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.

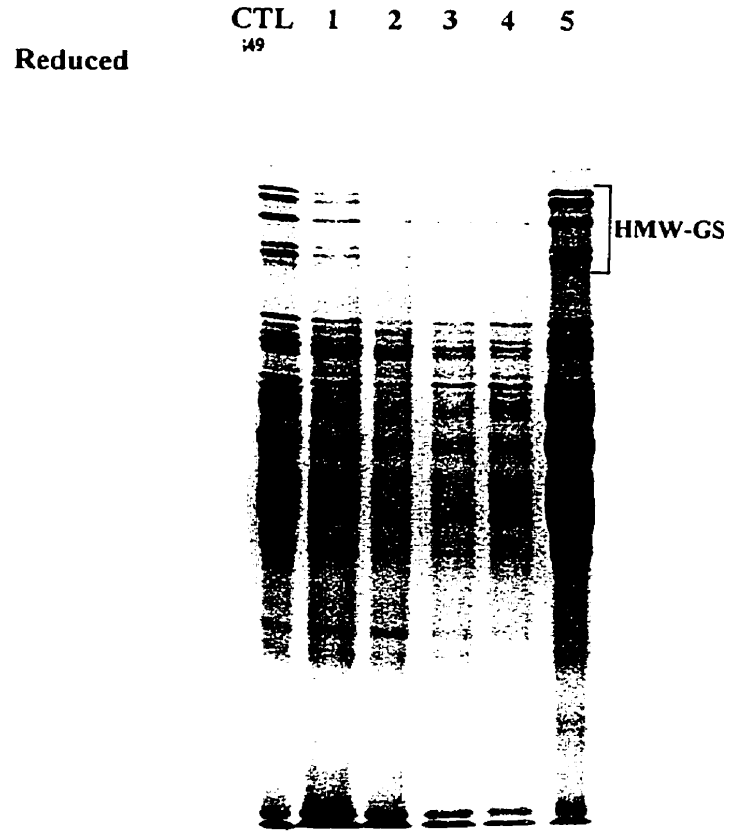

Gl 1 2 3 Kp 1 2 3 Do 1 2 3 Ka 1 2 3
Unreduced
Gl 1 2 3 Kp 1 2 3 Do 1 2 3 Ka 1 2 3
Reduced
HMW-GS

When dough samples were extracted with salt solution and subsequently centrifuged, a foam layer was observed on top of the supernatant for most samples. The amount of foam (by visual assessment) appeared to increase after the second salt extraction step and again after the water wash. There did not appear to be any consistent relationship between mixing time or cultivar and the amount of foam layer. However, it was noted that Glenlea dough samples produced very little or no foam layer. A foam layer was not observed during the salt extraction of flour samples. When transferring the supernatant for subsequent analyses, care was taken to keep the foam layer with the salt-insoluble pellet. The foam layer disappeared when samples were subsequently extracted with ethanol.

The identity of the foam layer, as well as the separate salt extractions, was investigated by SDS-PAGE under non-reducing and reducing conditions (Figure 14). Albumins, globulins and gliadins were present in the salt-soluble fraction (Figure 14, unreduced). Gliadins were found in greater proportion in the water wash (lane 4) and the foam layer (lane 5). Triticins were detectable only in the foam layer [bands appearing above the HMW-GS, lane 1 (CTL) and lane 5 (foam layer) of Figure 14, unreduced]. In addition to the monomeric proteins, glutenins were also solubilized (Figure 14, reduced). HMW-GS bands were evident in both salt-soluble fractions, the water wash, and the foam layer. This glutenin is in its polymeric form and large enough in M_r to be excluded from the gel under non-reducing conditions (slot protein). It appears that mixing induces conformational changes in the glutenin which allow solubilization of a small portion of this

Figure 14. SDS-PAGE under non-reducing and reducing conditions of the salt-soluble subfractions of Katepwa dough (mixed to 50% peak, 150mg). (CTL) Katepwa flour control, (1) total combined salt-soluble fraction, (2) supernatant from first salt extraction, (3) supernatant from second salt extraction, (4) water wash of salt-insoluble pellet, (5) foam layer.


CTL 1 2 3 4 5
Unreduced


CTL 1 2 3 4 5
Reduced
HMW-GS

protein in an aqueous salt solution while retaining sufficiently large M_r. This salt-soluble glutenin and foam created by the action of mixing was not studied further. The nature of this glutenin, although present in very small amounts (est. 2-4% total dough protein at the most), may warrant further investigation. Characterization of this small fraction of glutenin might provide clues to the mechanism(s) occurring during mixing to the larger, insoluble fraction of glutenin.

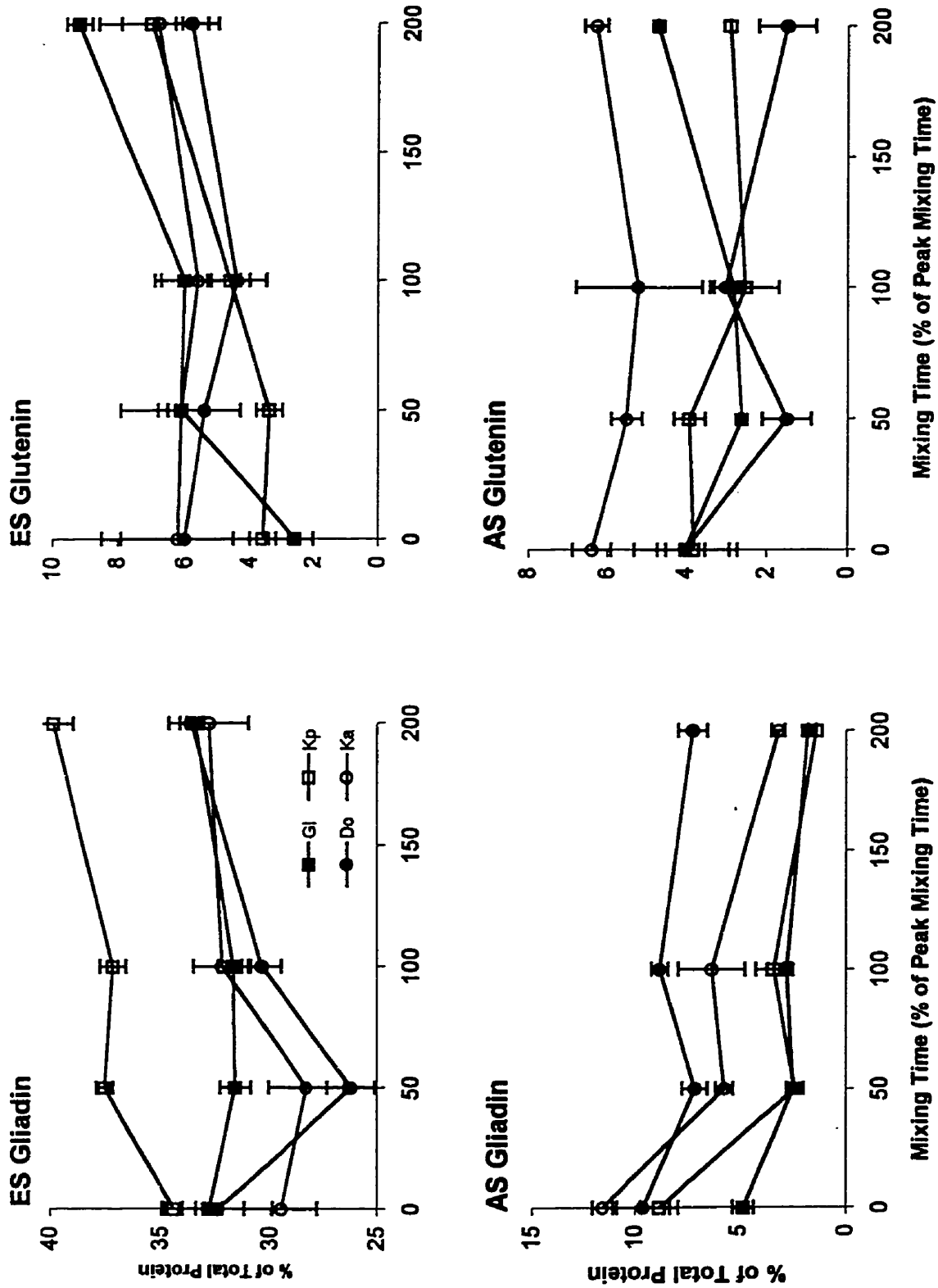
4.3.2.2. Ethanol-soluble fraction

No significant difference in ES protein content existed between cultivars for the flours. Differences in amount of ES protein occurred during mixing, but these differences were not related to strength. Two distinct trends in solubility were observed in the ES fraction during mixing. The ES fraction from the stronger cultivars Glenlea and Katepwa (Figure 12) showed an initial increase on mixing to 50% MT, remained stable, then increased again from peak to overmixing. In contrast, the ES protein from the weaker cultivars AC Domain and AC Karma decreased on initial mixing before slowly increasing after 50% MT. This unusual drop in ES protein during the initial stages of mixing appears to be specific to the weaker cultivars. Almonte (1998) reported similar behaviour for the ES protein of Katepwa mixed to 1 min, but not for that of Glenlea the stronger cultivar. Bushuk et al (1997) also observed the same behaviour for the ES protein obtained from doughs made from flours of four New Zealand and two Canadian cultivars. These two studies provide similar evidence for the behaviour of ES protein despite the preparation of doughs under different conditions: flour-water-1% salt doughs mixed in a GRL-200 mixer (Almonte, 1998) and full formula doughs mixed in a mechanical dough development mixer

(Bushuk et al, 1997). Doughs from three different flours mixed in a farinograph did not exhibit the same behaviour in the ES protein content unless mixed under nitrogen (Tanaka and Bushuk, 1973a)

The effect of mixing time and cultivar on the content of the purified ES fractions was also investigated. Except for Katepwa, the amount of ES gliadin generally decreased during the initial stages of mixing (Figure 15). From 50% MT to peak, the amount of ES gliadin remained relatively stable for the stronger cultivars Glenlea and Katepwa and increased for the weaker cultivars AC Domain and AC Karma. Katepwa was the only cultivar to show a significant increase in ES gliadin with overmixing. The ES glutenin comprised 7-17% of the ES fraction in flours, and up to 15-22% after overmixing. The relative amount (% of total protein) decreased or increased depending on the stage of mixing or the cultivar. There appeared to be an intercultivar difference for the ES glutenin content in the flours (Gl<Kp<Do<Ka). Sapirstein and Fu (1998) observed a similar trend for propanol-soluble glutenin. The relationship observed for the flours in this study was, however, not maintained during mixing. The amount of ES glutenin for Glenlea and Katepwa increased throughout the mixing process. Glenlea showed the sharpest increase during early mixing while the ES glutenin in Katepwa began to increase at 50%. Both cultivars showed similar rates of increase during overmixing. The amount of ES glutenin in AC Domain and AC Karma appeared to initially decrease until peak was reached, then increase after optimum development. But the changes occurring for the weaker cultivars were not statistically significant.

Figure 15. Changes in purified ES and AS fractions during mixing of control doughs prepared with Glenlea (Gl), Katepwa, (Kp), AC Domain (Do) and AC Karma (Ka) flours. Mixing time of 0% corresponds to flour.


ES Gliadin
ES Glutenin
AS Gliadin
AS Glutenin
% of Total Protein
Mixing Time (% of Peak Mixing Time)
Gl
Kp
Do
Ka
0
50
100
150
200
25
30
35
40
2
4
6
8
10
5
15

SDS-PAGE under non-reducing and reducing conditions was used to investigate any qualitative changes occurring during mixing for all cultivars (Figure 16 to Figure 19). No qualitative variation in the protein composition of the purified fractions was detected. The changes in amount of protein for the ES gliadin and ES glutenin reported in Figure 15 were not distinguishable by visual assessment of the electrophoregrams. Changes in band intensity corresponding to increases in gliadin content or increases in slot protein corresponding to glutenin were imperceptible in the unreduced gels. Increased band intensity for the glutenin subunits in the reduced gels were slightly detectable. An interesting observation noted for the ES gliadin fractions for all cultivars was the presence of up to five distinct shaded regions in the HMW region of the unreduced gels. Similar regions were detected in electrophoregrams of 50% and 70% 1-propanol-soluble fractions studied by Fu (1996). These "ladder-like bands" were also observed in the ES protein fraction studied by Almonte (1998). This oligomeric protein, thought to be glutenin comprised only of LMW-GS, was quantitated by SEC and will be discussed Section 4.6.

4.3.2.3.Acetic acid-soluble fraction

The AS fraction (Figure 12) showed similar behaviour for all cultivars. There was a relatively large and significant decrease in the initial stages of mixing. Any changes in AS protein content after 50% MT were only significant for AC Domain. These results contrast with those reported by Almonte (1998), in which Glenlea showed no significant change and Katepwa showed an increase in AS protein content during the initial stages of

Figure 16. SDS-PAGE of unreduced and reduced purified ES and AS fractions of Glenlea flour and control doughs (150 mg). First lanes designated as ESPS, ESPI, ASPS, ASPI correspond to flour fractions. Subsequent lanes correspond to control doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.

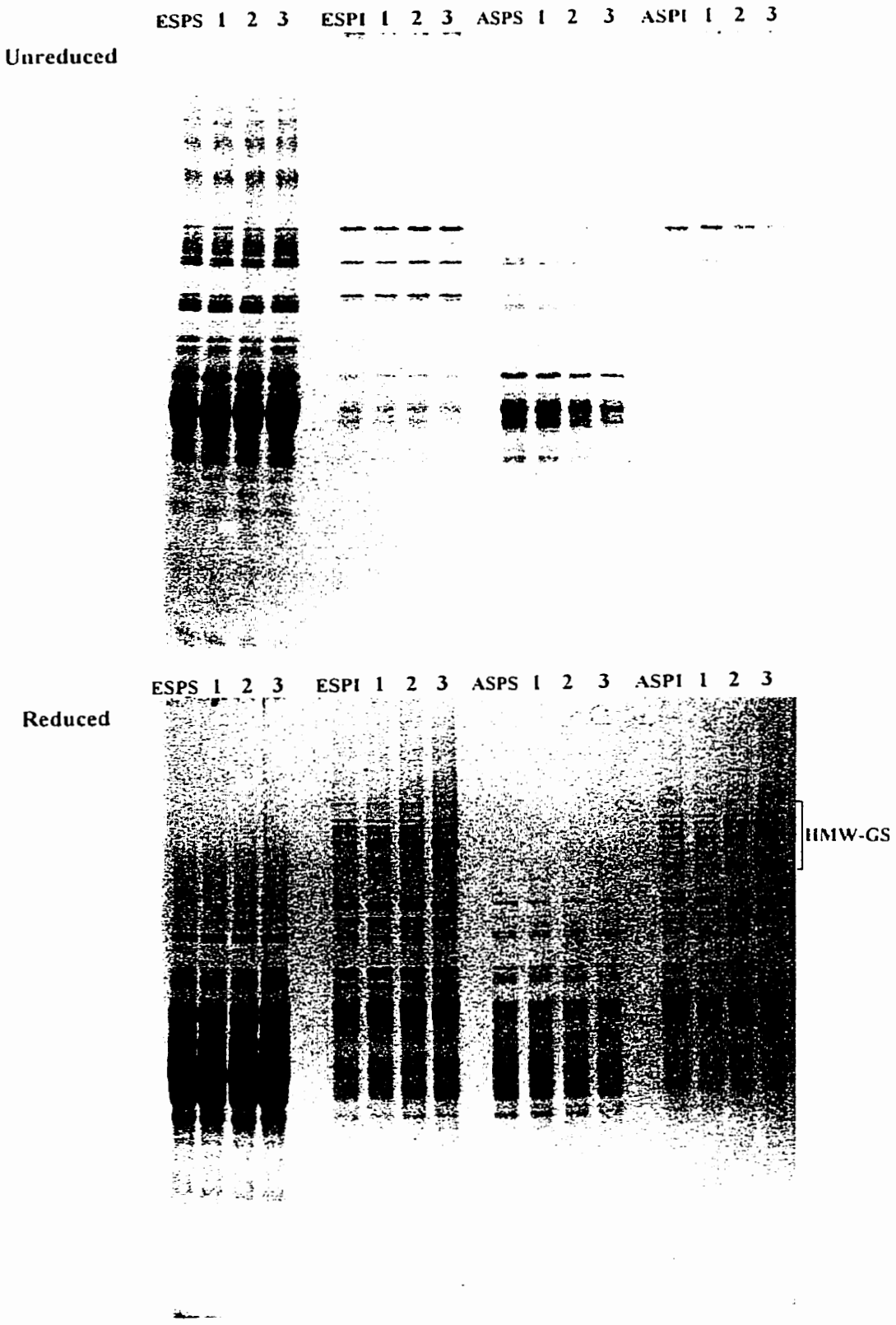

ESPS 1 2 3 ESPI 1 2 3 ASPS 1 2 3 ASPI 1 2 3
Unreduced
ESPS 1 2 3 ESPI 1 2 3 ASPS 1 2 3 ASPI 1 2 3
Reduced
HMW-GS

Figure 17. SDS-PAGE of unreduced and reduced purified ES and AS fractions of Katepwa flour and control doughs (150 mg). First lanes designated as ESPS, ESPI, ASPS, ASPI correspond to flour fractions. Subsequent lanes correspond to control doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.

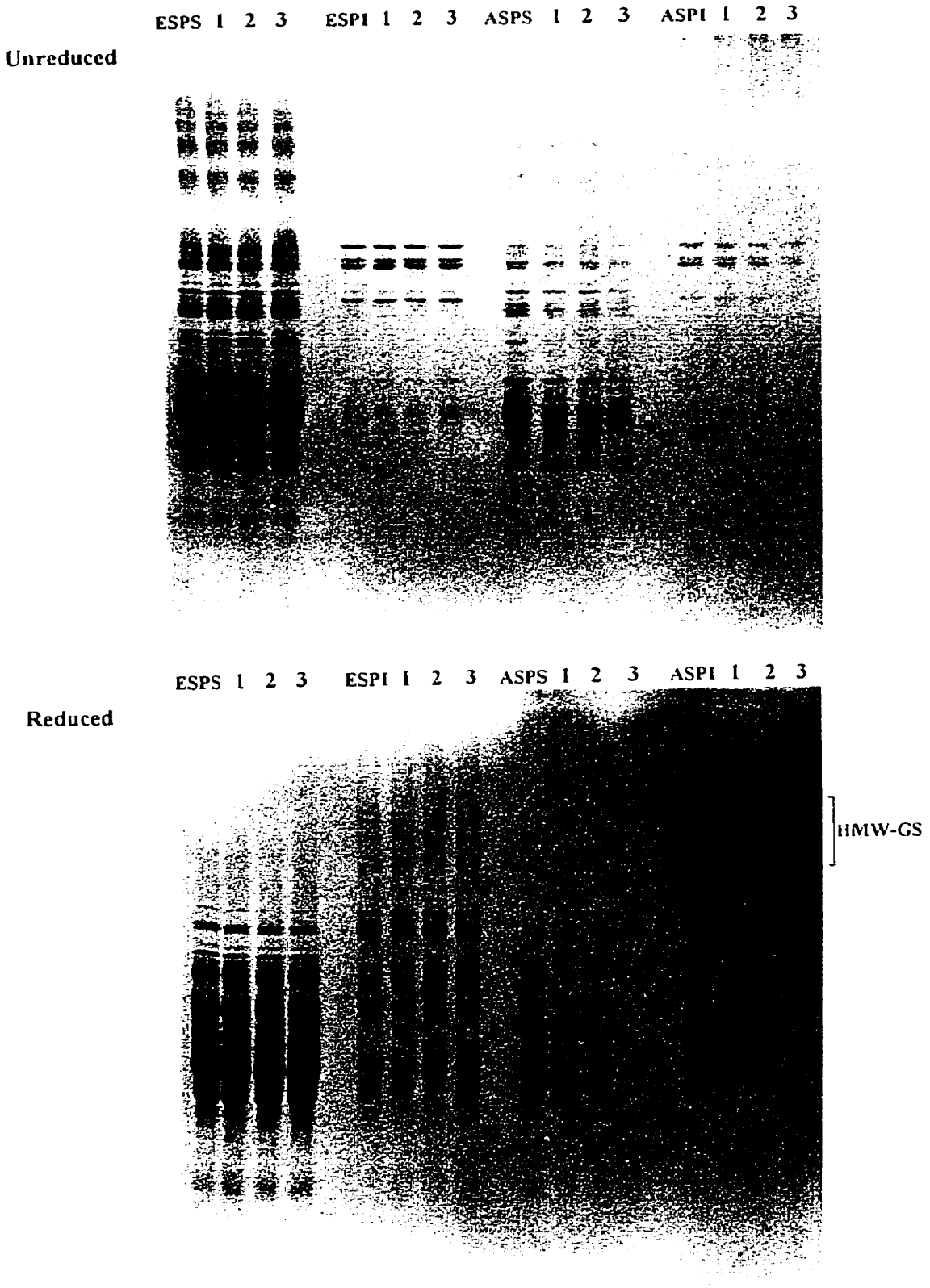

ESPS 1 2 3
ESPI 1 2 3
ASPS 1 2 3
ASPI 1 2 3
Unreduced
ESPS 1 2 3
ESPI 1 2 3
ASPS 1 2 3
ASPI 1 2 3
Reduced
HMW-GS

Figure 18. SDS-PAGE of unreduced and reduced purified ES and AS fractions of AC Domain flour and control doughs (150 mg). First lanes designated as ESPS, ESPI, ASPS, ASPI correspond to flour fractions. Subsequent lanes correspond to control doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.

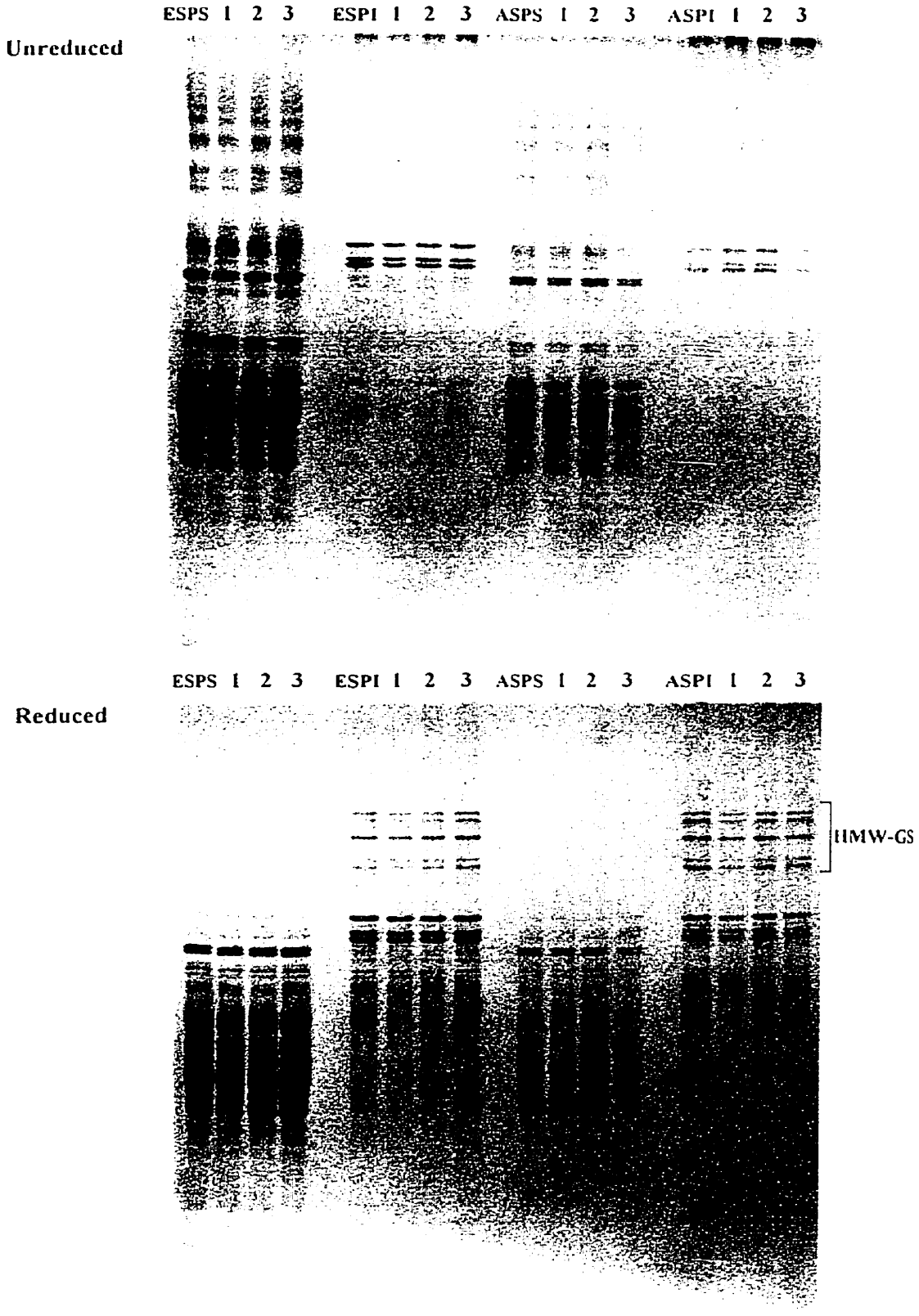
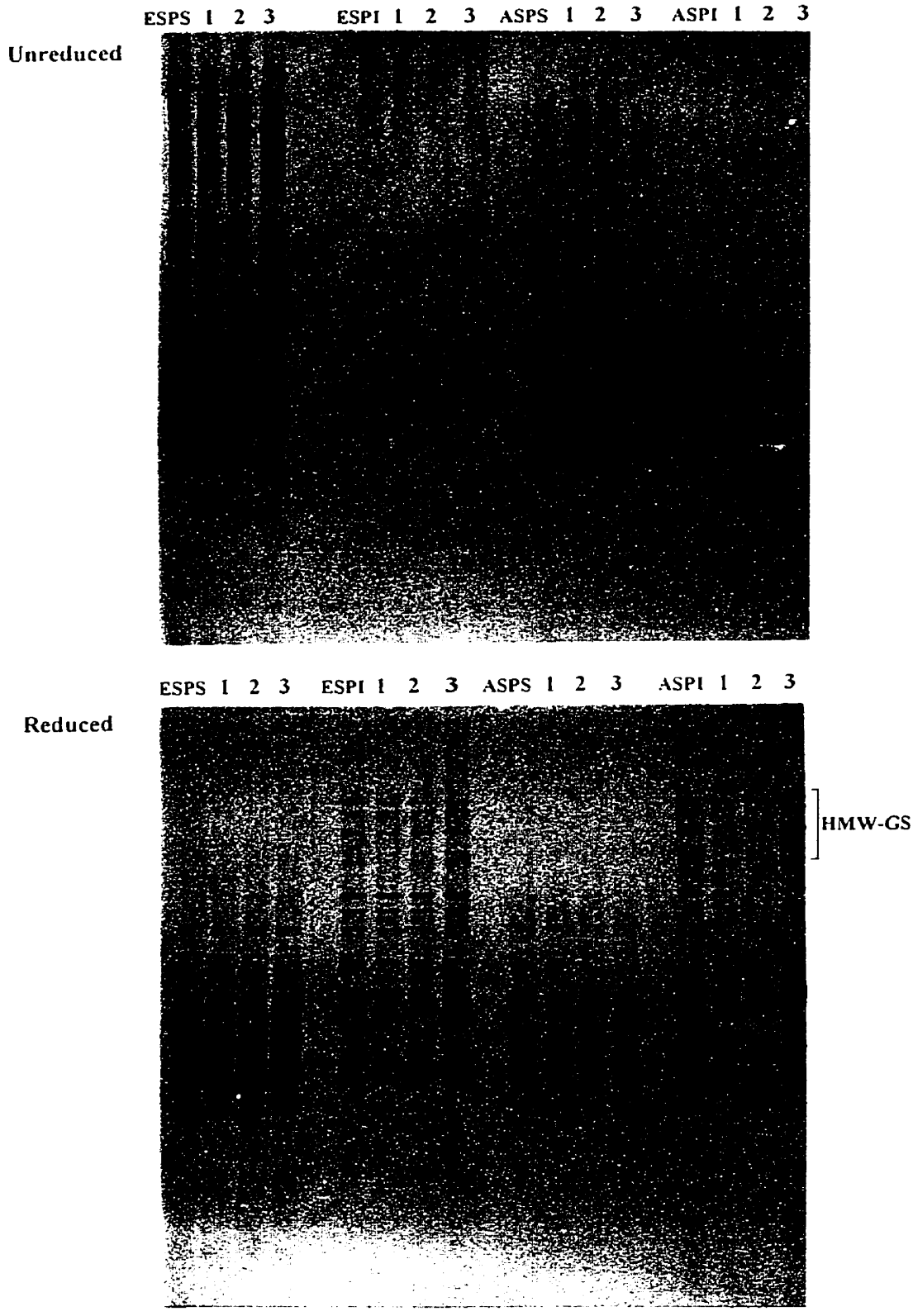

Unreduced
ESPS 1 2 3
ESPI 1 2 3
ASPS 1 2 3
ASPI 1 2 3
Reduced
ESPS 1 2 3
ESPI 1 2 3
ASPS 1 2 3
ASPI 1 2 3
HMW-GS

Figure 19. SDS-PAGE of unreduced and reduced purified ES and AS fractions of AC Karma flour and control doughs (150 mg). First lanes designated as ESPS, ESPI, ASPS, ASPI correspond to flour fractions. Subsequent lanes correspond to control doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.


ESPS 1 2 3
ESPI 1 2 3
ASPS 1 2 3
ASPI 1 2 3
Unreduced
ESPS 1 2 3
ESPI 1 2 3
ASPS 1 2 3
ASPI 1 2 3
Reduced
HMW-GS

mixing. On continued mixing, the amount of AS protein increased significantly for Glenlea and remained relatively stable for Katepwa. The quantities of AS protein were not comparable between the two studies, those of Almonte (1998) being significantly higher than in this study. Other researchers have also reported that mixing causes an increase in the amount of AS protein (Parades-Lopez and Bushuk, 1983, Tanaka and Bushuk, 1973).

It was previously reported that the amount of AS protein was negatively related to dough strength (Orth and Bushuk, 1973a). This relationship was maintained in this study from the flour through undermixing and mixing to peak. Glenlea contained the least (5.1-8.7%), Katepwa (5.9-12.6%) and AC Domain (8.6-13.6%) were intermediate, AC Karma contained the most (11.2-18.0%). The negative relationship was still evident at the overmixing stage for all cultivars, except Glenlea.

The AS fraction is the smallest of the four modified Osborne fractions and displays the highest level of variation, on a large or a small scale, especially when the fraction is purified into the 70PS gliadin and 70PI glutenin. These two factors result in data that are less easily evaluated statistically for the purified fractions, although improvement would be expected with greater replication. Despite the inherent limitations and the low level of statistical significance, some observations for the purified AS fraction were still considered important. There is a relatively large and significant decrease in AS gliadin during the initial stages of mixing (Figure 15) for all cultivars. This is followed by a small increase as the doughs are mixed to peak. After peak the amount of AS gliadin significantly decreases again. The negative relationship between the amount of AS protein and strength is reflected in this subfraction. The amount of AS gliadin in flours ranged from 4.8% for the longest mixing cultivar Glenlea to 11.6% for the shortest mixing cultivar AC Karma

(Figure 15). This relationship was maintained to a great extent through mixing to peak, but was less pronounced once the doughs were overmixed. The only cultivar exhibiting anomalous behaviour was AC Domain. In contrast to the flour, AC Domain doughs contained more AS gliadin than AC Karma, the weakest cultivar studied.

Dupuis et al (1996) had previously suggested that the negative correlation between the AS protein fraction and breadmaking quality was in fact due to the gliadins contaminating this Osborne fraction. The negative relationship between the amount of AS glutenin and mixing strength was less pronounced than with the AS gliadin, lending further support to their suggestion. The correlation between the two factors may be more difficult to assess for the AS glutenin fraction because of the large variation and low quantity for some of the samples. The results (Figure 15) indicated that the strongest cultivar Glenlea contained less AS glutenin than the weakest cultivar AC Karma throughout the mixing process (2.6-4.7% and 5.2-6.4%, respectively). With the exception of overmixed doughs, Katepwa and AC Domain were usually intermediate but with values closer to Glenlea. All cultivars, except Katepwa, showed a decrease in the amount of AS glutenin on mixing to 50% MT (Figure 15). The changes were less consistent for doughs mixed from 50% to 100% MT and all cultivars, except AC Domain, showed an increase in AS glutenin for overmixed doughs.

The decrease in AS gliadin was evident in the SDS-PAGE analysis under non-reducing and reducing conditions (Figure 16 to Figure 19). In the unreduced gels, the gliadin bands in the AS gliadin fraction and the ω-gliadin bands in the AS glutenin fraction diminished in intensity as mixing progressed. In the reduced gels, the intensity of the HMW-GS bands appeared to change according to the results illustrated in Figure 15. The

amount of AS glutenin decreases until 100% MT, then increases. The exception to this trend was AC Domain, but the high level of variation for three of the four mixing time data makes it difficult to evaluate the actual changes occurring in this AS glutenin fraction. The changes in ω-gliadin content in the ES and AS glutenin fractions will be discussed in Section 4.5.

4.3.2.4.Acetic acid-insoluble fraction

The amount of AI glutenin decreased throughout mixing for Glenlea and Katepwa control doughs (Figure 12). However, AC Domain and AC Karma exhibited a large increase in AI glutenin during the initial mixing stage. The amounts of AI glutenin peaked at 50% MT and decreased through breakdown for both weaker cultivars. The enhanced solubility of glutenin during mixing can be explained by the reduction in the M_r either by depolymerization or disaggregation. This would explain the decrease in AI glutenin observed during mixing. Numerous studies have shown that the amount of insoluble glutenin decreases with mixing (Bushuk et al, 1997, Danno and Hoseney, 1982a, Parades-Lopez and Bushuk, 1982a, Sievert et al, 1991, Tanaka and Bushuk, 1973a, Tsen, 1967, Wang et al, 1992). The increase in AI glutenin during the early stages of mixing observed for the weaker cultivars is due to the increase in gliadin insolubility, but also possibly some soluble glutenin. The unusual increase in AI glutenin observed for the weaker cultivars coupled with the unusual decrease in the ES and AS gliadin, is attributed to gliadin-glutenin interaction.

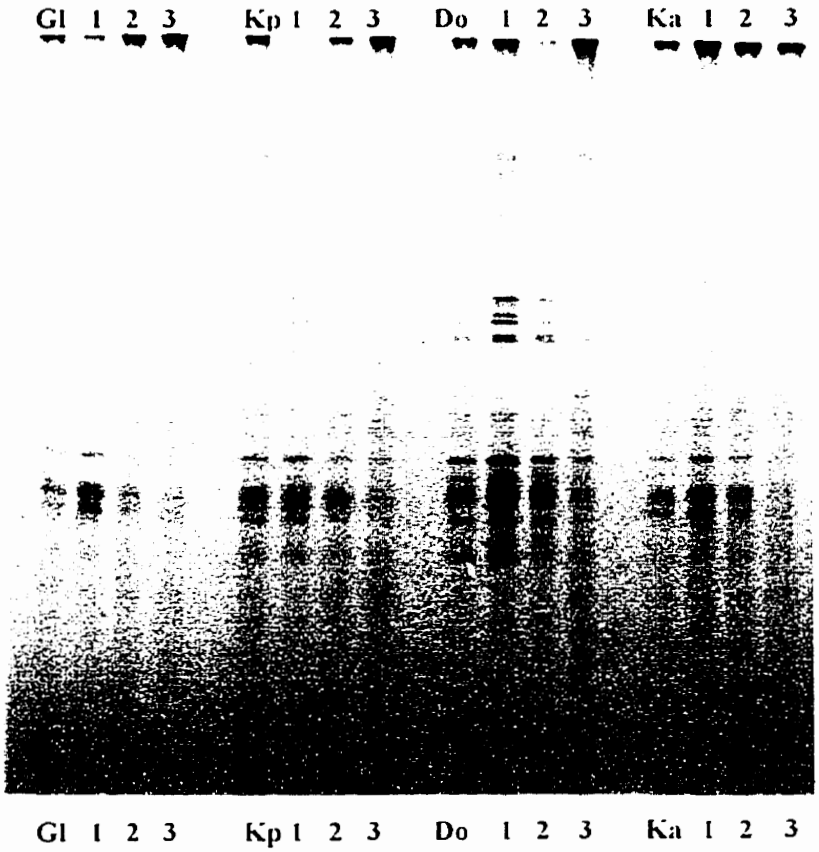
The SDS-PAGE gels for the unreduced and reduced AI fractions (Figure 20) illustrate both phenomena observed in the protein distribution data. The band intensities

for the monomeric proteins (unreduced gel) exhibit a distinct increase for the lanes corresponding to 50% MT when compared to the control lanes, then decrease for 100% and 200% MT. Also worth noting, the slot protein for each cultivar appears to increase with mixing, suggesting that some of the glutenin still present in the AI fraction is reduced in size but still remains insoluble. The solubilization of glutenin during mixing, measured as a decrease in band intensity, is more difficult to detect (reduced gel). Loss of band intensity is only barely perceptible. As with the electrophoretic analysis of the other modified Osborne fractions, there did not appear to be any qualitative changes in protein composition during mixing.

4.3.2.5. Gliadin-glutenin interaction

The total amount of gliadin (ES and AS) in flour ranged from 37.6% for Glenlea to 43.2% for Katepwa. AC Domain and AC Karma were intermediate with 42.0% and 41.1%, respectively. In contrast, total glutenin (ES, AS and AI) was highest for Glenlea (45.1%), lowest for AC Karma (39.5%), and intermediate for Katepwa (41.2%) and AC Domain (41.2%). Total gliadin showed no correlation to dough strength and the positive relationship indicated by total glutenin was due to the insoluble fraction. The negative correlation between the AS fraction and dough strength was also apparent for AS gliadin and total soluble glutenin (ES and AS, excluding AI). The amounts of these two soluble fractions were inversely related to mixing strength: for AS gliadin, Glenlea 4.8%, Katepwa 8.8%, AC Domain 9.6%, and AC Karma 11.6%; for total soluble glutenin, Glenlea 6.6%, Katepwa 7.4%, AC Domain 10.0%, and AC Karma 12.6%. In addition to the positive correlation between AI protein and dough strength and the negative correlation associated

Figure 20. SDS-PAGE of unreduced and reduced AI fractions of flours and control doughs (150 mg). First lanes designated as (Gl) Glenlea, (Kp) Katepwa, (Do) AC Domain and (Ka) AC Karma correspond to flour fractions. Subsequent lanes correspond to control doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.


Gl 1 2 3 Kp 1 2 3 Do 1 2 3 Ka 1 2 3
Unreduced
Gl 1 2 3 Kp 1 2 3 Do 1 2 3 Ka 1 2 3
Reduced
HMW-GS

with the AS protein (attributable to AS gliadin), total soluble glutenin also appears to be negatively associated with strength.

The observation that the amount of gliadin insoluble in ethanol but soluble in dilute acetic acid appeared to be genotype-specific, was attributed to gliadin-glutenin interaction (Dupuis et al, 1996). It was suggested that gliadin-glutenin interaction might play an important role in dough mixing and be an important factor in determining the mixing strength of a flour. Based on the unusual solubility results observed in this study, it appears that, during the very early stages of mixing, gliadin interacts with glutenin and becomes insoluble in ethanol or acetic acid. As mixing progresses, the gliadin is released along with soluble glutenin. Evidence of gliadin-glutenin interactions can be found in the changes occurring during mixing to the soluble glutenin and total gliadin (Table 11). It is very clear that gliadin solubility is reduced in both the ES and AS fractions during the early stages of mixing for all cultivars (the only increase observed was for Katepwa ES gliadin). As mixing progresses, gliadin solubility increases slightly for the stronger cultivars and more so for the weaker cultivars. Overmixing appears to enhance the solubility of gliadins in ethanol. Despite the apparent inverse relationship between the total soluble glutenin and dough strength, there does not appear to be any strong indication of interaction between the soluble and insoluble glutenin during mixing. Soluble glutenin content generally increases as mixing progresses, presumably due to the disaggregation or depolymerization of the insoluble glutenin.

Table 11. Changes in Proportion of Gliadin (ES, AS and total) and Glutenin (ES, AS and total soluble) During Mixing of Control Doughs.

	0-50% MT			50-100% MT			100-200% MT		
Gliadin	ES	AS	Total	ES	AS	Total	ES	AS	Total
Glenlea	-1.2	-2.3	**-3.5**	0.1	0.3	0.4	1.7	-1.0	0.7
Katepwa	3.1	-6.4	**-3.3**	-0.4	1.0	0.6	2.7	-2.0	0.7
AC Domain	-6.2	-2.5	**-8.7**	4.2	1.7	5.9	3.2	-1.6	1.6
AC Karma	-1.2	-5.9	**-7.1**	3.9	0.6	4.5	0.6	-3.1	-2.5
Glutenin									
Glenlea	3.5	-1.4	2.1	-0.1	0.2	0.1	3.2	1.9	5.1
Katepwa	-0.2	0.1	-0.1	1.2	-1.4	-0.2	2.4	0.4	2.8
AC Domain	-0.6	-2.5	-3.1	-1.0	1.5	0.5	1.4	-1.5	-0.1
AC Karma	-0.1	-0.9	-1.0	-0.5	-0.3	-0.8	1.2	1.1	2.3

4.3.3. Effect of Absorption

Control doughs from AC Domain flour were initially prepared using a lower absorption than the calculated optimum. The doughs were prepared again at the optimum absorption. This provided an opportunity to evaluate the effect of absorption on the changes in protein distribution during mixing. The effect of absorption on the changes in glutenin subunit composition during mixing will also be discussed in the Section 4.4.

To achieve the constant consistency of 40 MU used for this study, AC Domain required an absorption of 69.4%. Doughs were also prepared at 61.7% absorption to produce a consistency of 50 MU. The protein solubility distribution for AC Domain doughs prepared at these two absorptions (differing by 7.7%) is reported in Table 12. There was significantly more SS protein in the 62% doughs (mixed to 100% and 200% MT). Significantly less gliadin was found in the AS fraction obtained from the 62% dough, as well as less total gliadin. Less AS gliadin suggests that less gliadin-glutenin interaction occurs in the dough of lower absorption.

There was significantly more soluble glutenin (ES and AS) in the 62% dough. The increase in soluble glutenin as mixing progressed was much greater in the 62% dough than the 69% dough, indicating a more rapid rate of depolymerization of glutenin. When compared to the 69% doughs, the content of ES glutenin is lower in the 62% dough mixed to 50% MT, slightly higher once mixed to peak, and significantly higher when overmixed. The difference in the AS glutenin is more consistent throughout mixing. The 62% doughs have lower amounts of AI protein than the 69% doughs at all mixing times, although these differences (2.3-6.1% of total protein) were not significant.

When water is added to flour, 30-35% becomes bound (MacRitchie, 1986). The remaining free water is dispersed through the dough during mixing, becoming available as a solvent and providing a medium for chemical reactions between flour constituents. Changing the level of water addition, which ultimately changes the amount of free water, will alter the rheological properties of a dough. Absorption will influence dough stiffness and, hence, the work input required (Walker and Hazelton, 1996). Mixograph peak time, peak height, and curve width will be affected by changes in water absorption (as well as protein content and quality). These changes were apparent for the AC Domain doughs prepared at two levels of water absorption. Increasing water absorption from 61.7% to 69.4% decreased mixograph peak height from 50 MU to 40 MU and increased the mixograph dough development time from 2.9 to 3.8 min. The curve width, particularly in the ascending portion, was wider and more irregular in the dough made at the lower water absorption (results not shown).

Table 12. Comparison of Modified Osborne Protein Distribution for AC Domain Control Doughs Prepared at 62% and 69% Absorption

	50% Mixing Time	
	62%	69%
SS	16.4±0.5	16.5±0.3
ESPS (gliadin)	28.6±0.6	26.2±1.1
ESPI (glutenin)	4.1±0.6	5.4±1.1
ES	32.6±0.7	31.5±1.5
ASPS (gliadin)	6.2±0.1	7.1±0.6
ASPI (glutenin)	3.4*±0.1	1.5*±0.6
AS	9.6±1.1	8.6±0.0
AI	40.8±1.9	43.1±2.2
Recovery	99.4	99.7
	100% Mixing Time	
	62%	69%
SS	17.0*±0.3	14.5*±0.0
ESPS (gliadin)	29.6±0.8	30.4±0.9
ESPI (glutenin)	4.6±0.8	4.4±0.9
ES	34.2±0.8	34.8±3.3
ASPS (gliadin)	5.6*±0.4	8.8*±0.4
ASPI (glutenin)	4.1±0.4	3.0±0.4
AS	9.7±1.0	11.8±0.4
AI	37.0±0.6	39.3±3.7
Recovery	97.9	100.4
	200% Mixing Time	
	62%	69%
SS	17.9*±0.0	15.6*±0.5
ESPS (gliadin)	35.0±0.7	33.6±0.5
ESPI (glutenin)	9.2*±0.7	5.8*±0.5
ES	44.2±1.4	39.5±2.4
ASPS (gliadin)	3.0*±0.1	7.2*±0.7
ASPI (glutenin)	3.4±0.1	1.5±0.7
AS	6.3±0.7	8.7±0.3
AI	30.6±0.1	36.7±2.6
Recovery	99.0	100.5

* indicates significant difference (α=0.05) between absorption levels

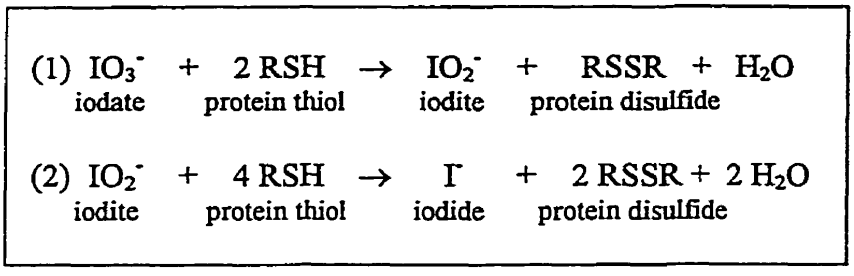
The changes observed in doughs at the macroscopic level must also be reflected at the protein molecular level. At lower water absorption, greater shear forces are exerted on the protein molecules, resulting in a larger extent of glutenin breakdown during mixing. This phenomenon is observed in the AI fraction. The dough prepared at 61.7% absorption contained less AI protein throughout mixing and exhibited a more severe rate of breakdown than the dough prepared at 69.4% absorption. The amount of AI protein in the dough of lower absorption decreased by 9.3% (50% MT to peak), then by 17.3% (peak to 200% MT) during mixing compared to 8.6% and 6.6% for the dough of higher absorption.

Evidence for the greater susceptibility of glutenin to breakdown in the dough of lower absorption is found in the lower amount of AI protein, but also in the presence of greater amounts of soluble glutenin. Interestingly, the dough made at lower absorption also contained significantly less AS gliadin when mixed to peak and beyond. When both doughs are mixed to optimum development, the dough of lower absorption contains glutenin of lower M_r and exhibits less gliadin-glutenin interaction, as measured by the amount of gliadin soluble in acetic acid, than the dough of higher absorption. In both cases, the doughs are supposedly optimally developed, but the proteins are in different states. Further investigation into the effects of absorption on mixing behaviour might provide additional information on the two mechanisms, reduction of glutenin M_r and gliadin-glutenin interaction, and their relative importance.

4.3.4. Iodate Doughs

Potassium bromate and to a lesser extent potassium iodate are two halogenates that have been used as bread improvers over the last 70 years (Fichett and Frazier, 1986).

It was recognized long ago that potassium iodate reacted more quickly than potassium bromate. (Sullivan et al, 1940). The mechanism of the oxidants' action was reported to have a rapid initial reaction followed by a slower secondary reaction (Bushuk and Hlynka, 1960). Tkackuk and Hlynka (1961) proposed a two-step reaction mechanism that would explain the different rates of reaction observed between iodate and bromate. The rate-limiting first step would be slow for bromate and fast for iodate:



The halogenates exert their major effect on the sulfhydryl-disulfide interchange system of the proteins in dough (for review see Dupuis, 1997). Potassium iodate (60 ppm) was used in this study to evaluate whether its fast-acting improving action could provide clues to specific changes occurring in the gluten proteins during mixing.

4.3.4.1.Salt-soluble fraction

The amount of SS protein varies during mixing, generally decreasing but occasionally increasing (Figure 21). No consistent changes were observed during mixing. Cultivar differences were observed throughout mixing, although the ranking was not related to quality. AC Karma had the greatest amount, followed by Glenlea, Katepwa and AC Domain.

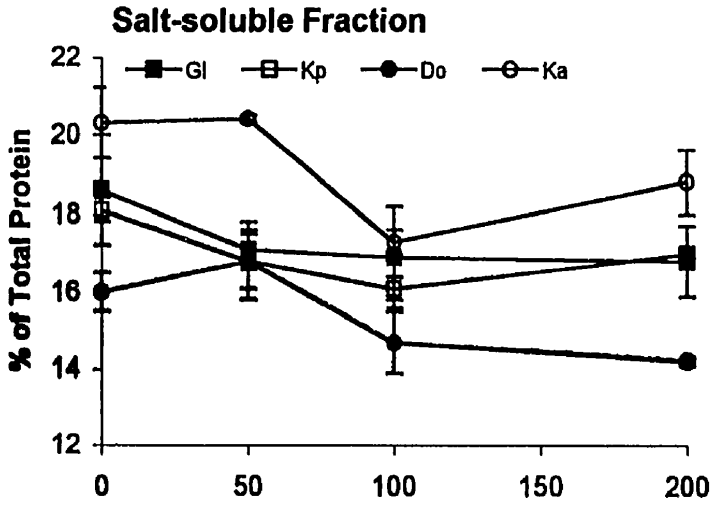
4.3.4.2.Ethanol-soluble fraction

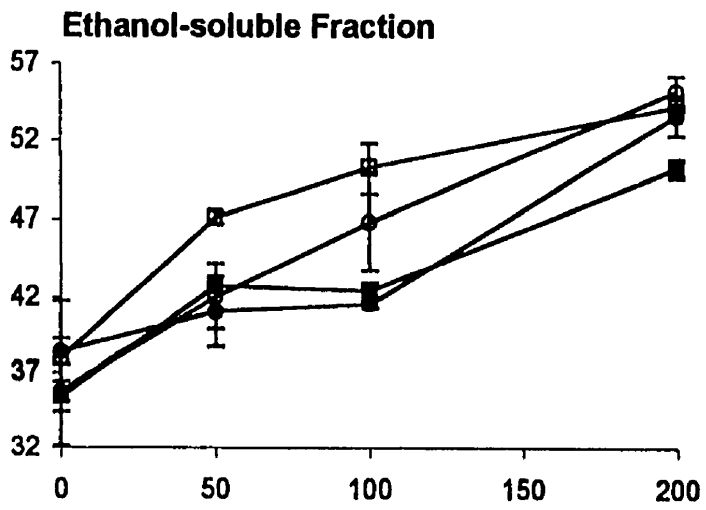
There was a large and significant increase in the amount of ES protein throughout mixing and for all cultivars (Figure 21). The largest increases were observed in the initial stages of mixing (to 50% MT) and during overmixing (after peak). The amount of ES protein and the changes in the amount during mixing were not genotype dependent, although Glenlea overmixed dough contained significantly less ES protein than the other overmixed doughs. The increase in the amount of ES protein was due to an increase in both gliadin and glutenin. ES gliadin content increased initially, dropped slightly, then increased again during overmixing (Figure 22). No relationship between cultivar and ES gliadin content was evident. The unusual decrease observed in the ES fraction of control doughs for the weaker cultivars was absent in the iodate doughs. The amount of ES glutenin increased throughout mixing for all cultivars. The apparent negative relationship between the content of ES glutenin in flour and mixing strength was lost during mixing.

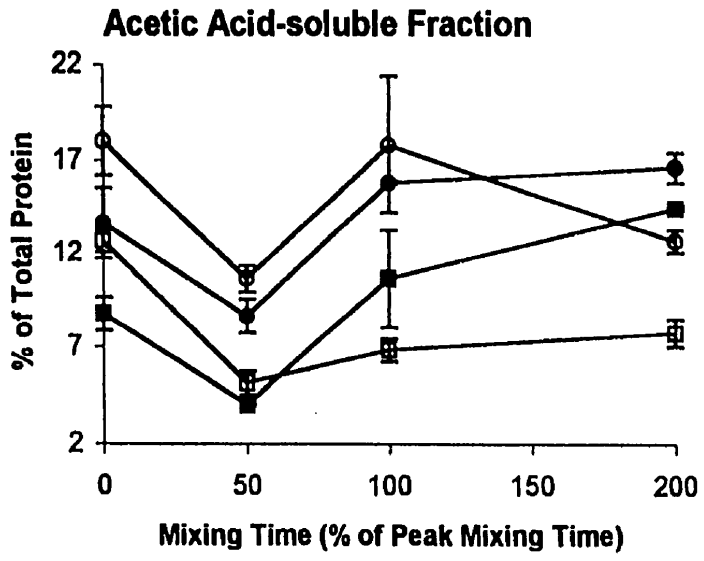
4.3.4.3.Acetic acid-soluble fraction

There was a significant drop in the AS protein during the initial stages of mixing followed by a significant increase between 50% and 100% MT (Figure 21). The change in AS protein during overmixing appeared to be cultivar dependent. Glenlea exhibited the largest increase (3.8%), Katepwa and AC Domain were intermediate (0.9% and 0.8%, respectively), and AC Karma exhibited a decrease (-5.2%) in AS protein. As with the control doughs, the content of AS protein was negatively related to mixing strength. This

Figure 21. Changes in modified Osborne fractions during mixing of iodate doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka) flours. Mixing time of 0 % corresponds to flour.


Salt-soluble Fraction
Gl
Kp
Do
Ka
% of Total Protein
22
20
18
16
14
12
0
50
100
150
200


Ethanol-soluble Fraction
57
52
47
42
37
32
0
50
100
150
200


Acetic Acid-soluble Fraction
% of Total Protein
22
17
12
7
2
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

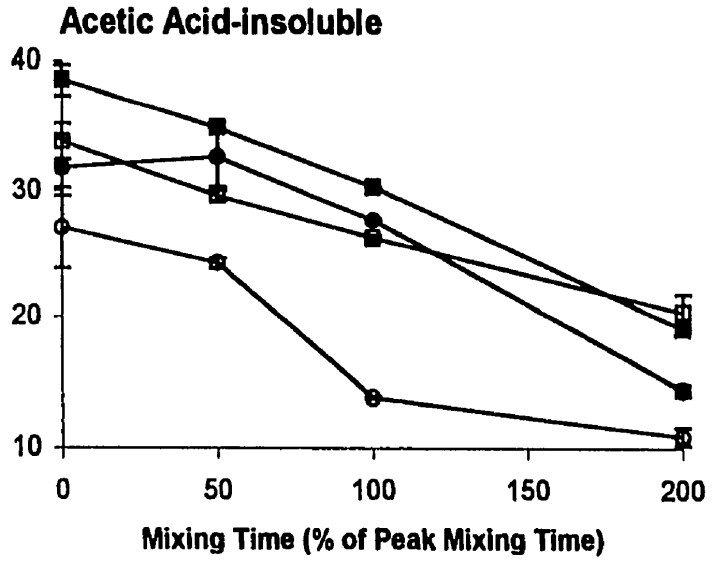
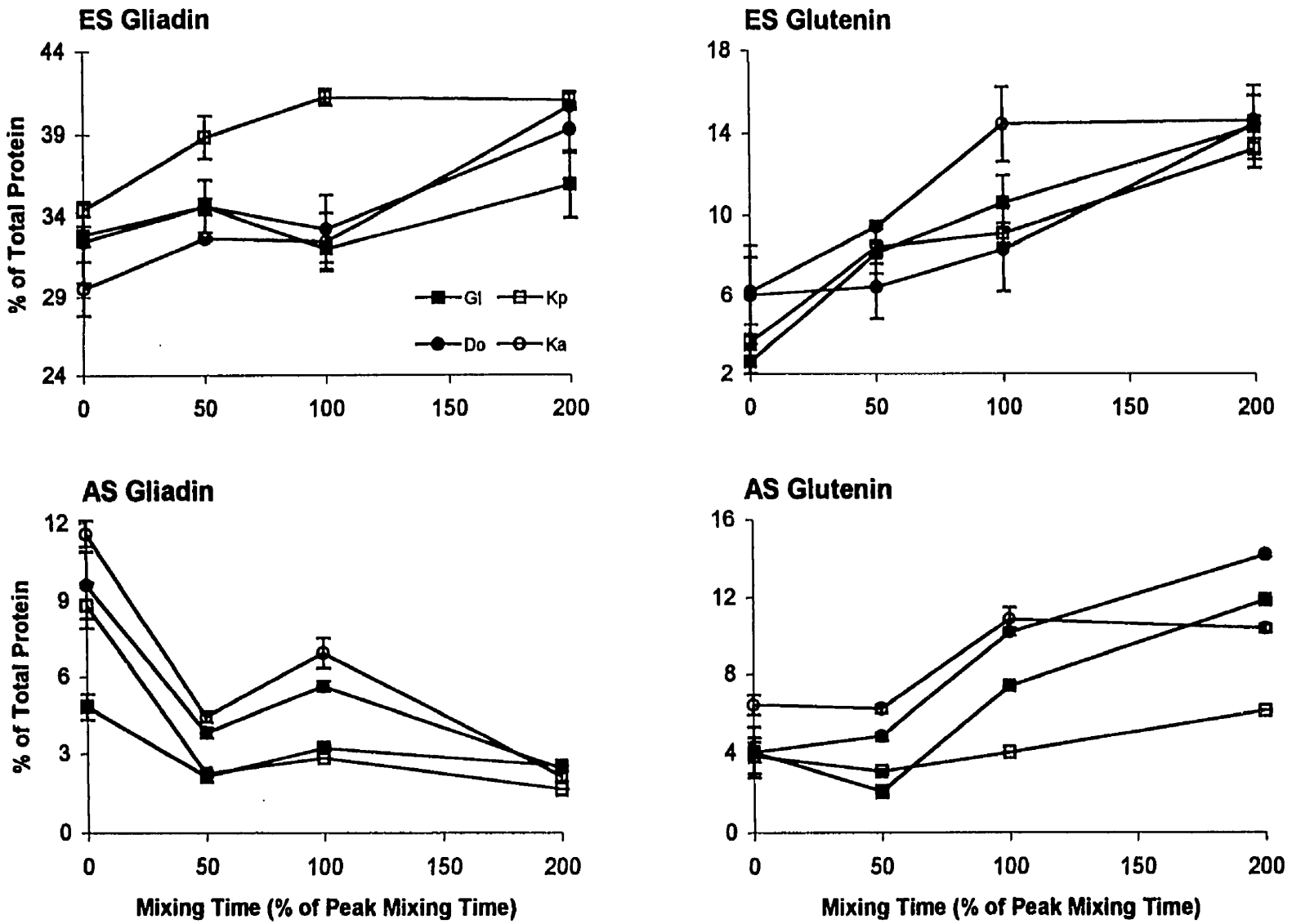

Acetic Acid-insoluble
40
30
20
10
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

Figure 22. Changes in purified ES and AS fractions during mixing of iodate doughs prepared with Glenlea (Gl), Katepwa, (Kp), AC Domain (Do) and AC Karma (Ka) flours. Mixing time of 0% corresponds to flour.

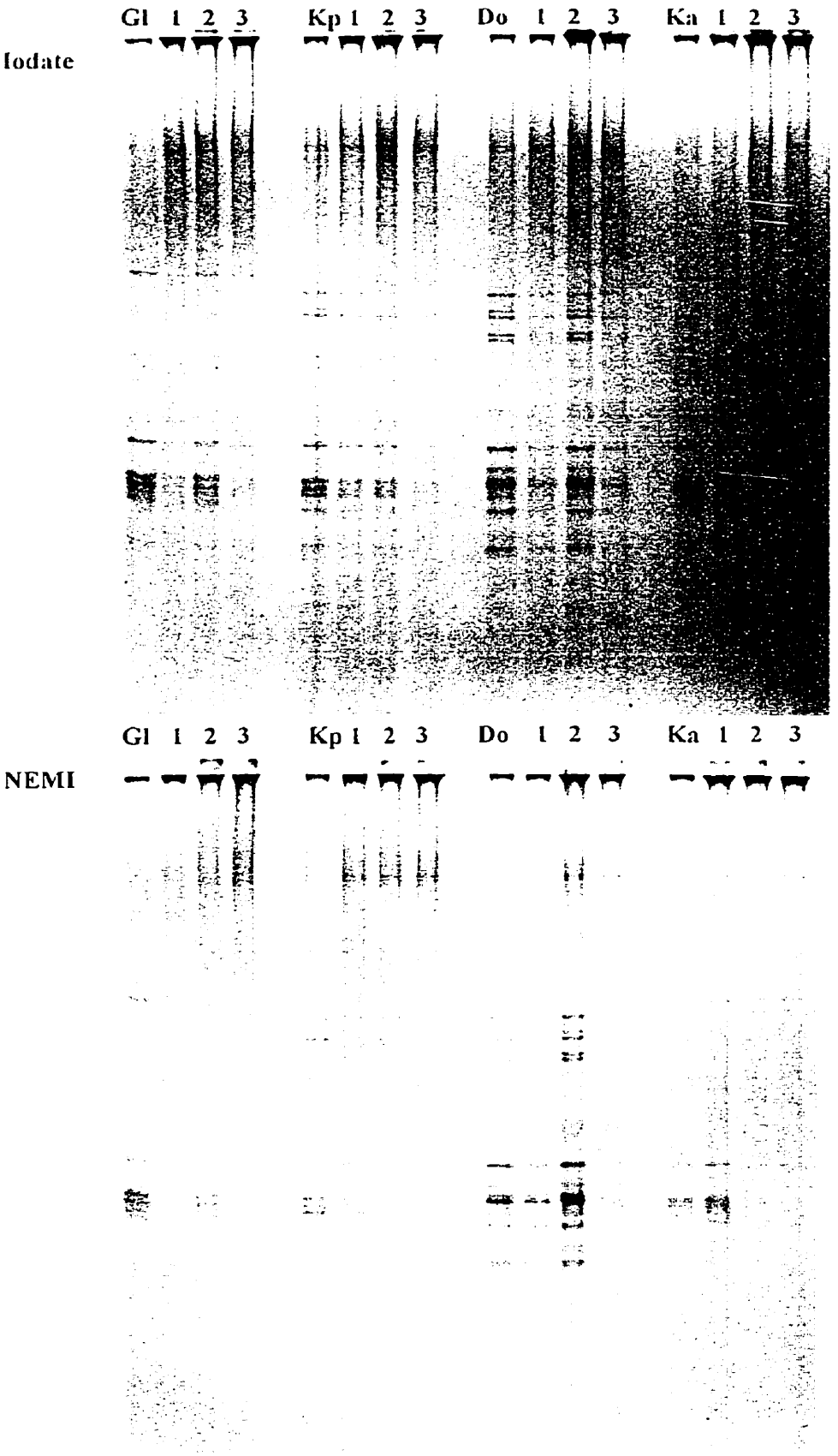

ES Gliadin
% of Total Protein
44
39
34
29
24
0
50
100
150
200
Gl
Kp
Do
Ka
ES Glutenin
18
14
10
6
2
AS Gliadin
12
9
6
3
0
Mixing Time (% of Peak Mixing Time)
AS Glutenin
16
12
8
4

relationship was observed from flour through mixing to peak, but was lost during overmixing. The initial loss of AS protein can be attributed to the loss of a significant amount of AS gliadin and a small but statistically insignificant amount of AS glutenin (Figure 22). There was a relatively large increase in the AS glutenin and a small increase in the AS gliadin for all cultivars mixed from 50% MT to peak. AS gliadin decreased during overmixing while AS glutenin continued to increase. The negative relationship between the amount of AS protein and mixing strength was evident for both the AS gliadin and the AS glutenin and was maintained until doughs had been mixed to peak. AC Karma contained the highest amount of both protein groups. Glenlea, and sometimes Katepwa, contained the least amount of AS gliadin and glutenin. However, this relationship was lost with overmixing.

4.3.4.4.Acetic acid-insoluble fraction

The amount of AI protein decreased throughout mixing for all cultivars (Figure 21). Unlike the control doughs, an increase in AI protein during the initial mixing stage was not observed for both weaker cultivars. However, AC Domain exhibited a small increase (0.8%) and the loss of AI protein for AC Karma was lower than for the stronger cultivars Glenlea and Katepwa. The amount of AI protein continued to be related to mixing strength, although Katepwa doughs contained less than AC Domain doughs when mixed to 50% and 100% MT, and as much as Glenlea when mixed to 200% MT. SDS-PAGE under non-reducing conditions (Figure 23) indicated that there were some changes occurring related to gliadin-glutenin interactions but these changes were masked by the net loss in the fraction. For all cultivars, there was a distinct increase in gliadin band intensities

Figure 23. SDS-PAGE under non-reducing conditions of AI fractions of flours and doughs (150 mg) prepared with iodate and NEMI. First lanes designated as (Gl) Glenlea, (Kp) Katepwa, (Do) AC Domain and (Ka) AC Karma correspond to flour fractions. Subsequent lanes correspond to doughs mixed to (1) 50%, (2) 100% and (3) 200% of peak.


Iodate
Gl 1 2 3
Kp 1 2 3
Do 1 2 3
Ka 1 2 3
NEMI
Gl 1 2 3
Kp 1 2 3
Do 1 2 3
Ka 1 2 3

at 100% MT, relative to the doughs mixed to 50% MT. This is in contrast to the control doughs where an increase in band intensity, relative to the flours (0% MT), and an increase in the amount of AI protein was observed at 50% MT.

4.3.4.5. Gliadin-glutenin interaction

Based on the unusual solubility results observed for the control doughs, it was suggested that during the very early stages of mixing gliadin interacts with glutenin and becomes insoluble in ethanol or acetic acid. As mixing progresses, the gliadin is released along with soluble glutenin. Evidence of gliadin-glutenin interactions for iodate doughs can be similarly found in the changes occurring during mixing in the soluble glutenin and total gliadin (Table 13). However, unlike the control doughs, gliadin solubility in iodate doughs decreased in the AS fraction, but not in the ES fraction, during the early stages of mixing for all cultivars. The magnitude of the loss of AS gliadin was smallest for Glenlea, intermediate for Katepwa and AC Domain, and greatest for AC Karma. This is in agreement with the gliadin-glutenin interaction model in which the extent of interaction is considered to be inversely related to mixing strength. As mixing progressed to peak and beyond, gliadin solubility increased. Soluble glutenin content increased as mixing progressed.

4.3.5. NEMI Doughs

The effect on dough properties of the sulfhydryl-blocking agent NEMI is very similar to that of doughs containing oxidants such as potassium iodate, although the mechanisms of action are different. Potassium iodate exerts its improving effect on doughs

Table 13. Changes in Gliadin (ES, AS and total) and Glutenin (ES, AS and total soluble) During Mixing of Iodate Doughs.

	0-50% MT			50-100% MT			100-200% MT		
Gliadin	ES	AS	Total	ES	AS	Total	ES	AS	Total
Glenlea	1.8	-2.7	**-0.9**	-2.6	1.1	-1.5	3.9	-0.7	3.2
Katepwa	4.4	-6.6	**-2.2**	2.4	0.6	3.0	-0.2	-1.2	-1.4
AC Domain	2.2	-5.8	**-3.6**	-1.4	1.8	0.4	6.0	-3.2	2.8
AC Karma	3.1	-7.2	**-4.1**	-0.2	2.5	2.3	8.2	-4.8	3.4
Glutenin									
Glenlea	5.5	-2.0	3.5	2.5	5.4	7.9	3.7	4.5	8.2
Katepwa	4.8	-0.8	4.0	0.7	1.0	1.7	4.1	2.1	6.2
AC Domain	0.4	0.8	1.2	1.9	5.4	7.3	6.1	4.0	10.1
AC Karma	3.2	-0.2	3.0	5.0	4.7	9.7	0.2	-0.5	-0.3

by the oxidation of -SH groups to disulfide bonds, or to higher oxidation products, while NEMI exerts its effect by blocking the -SH groups. The -SH/-SS- interchange reaction so important in dough is restricted in the presence of both chemicals, thus dough breakdown occurs more quickly.

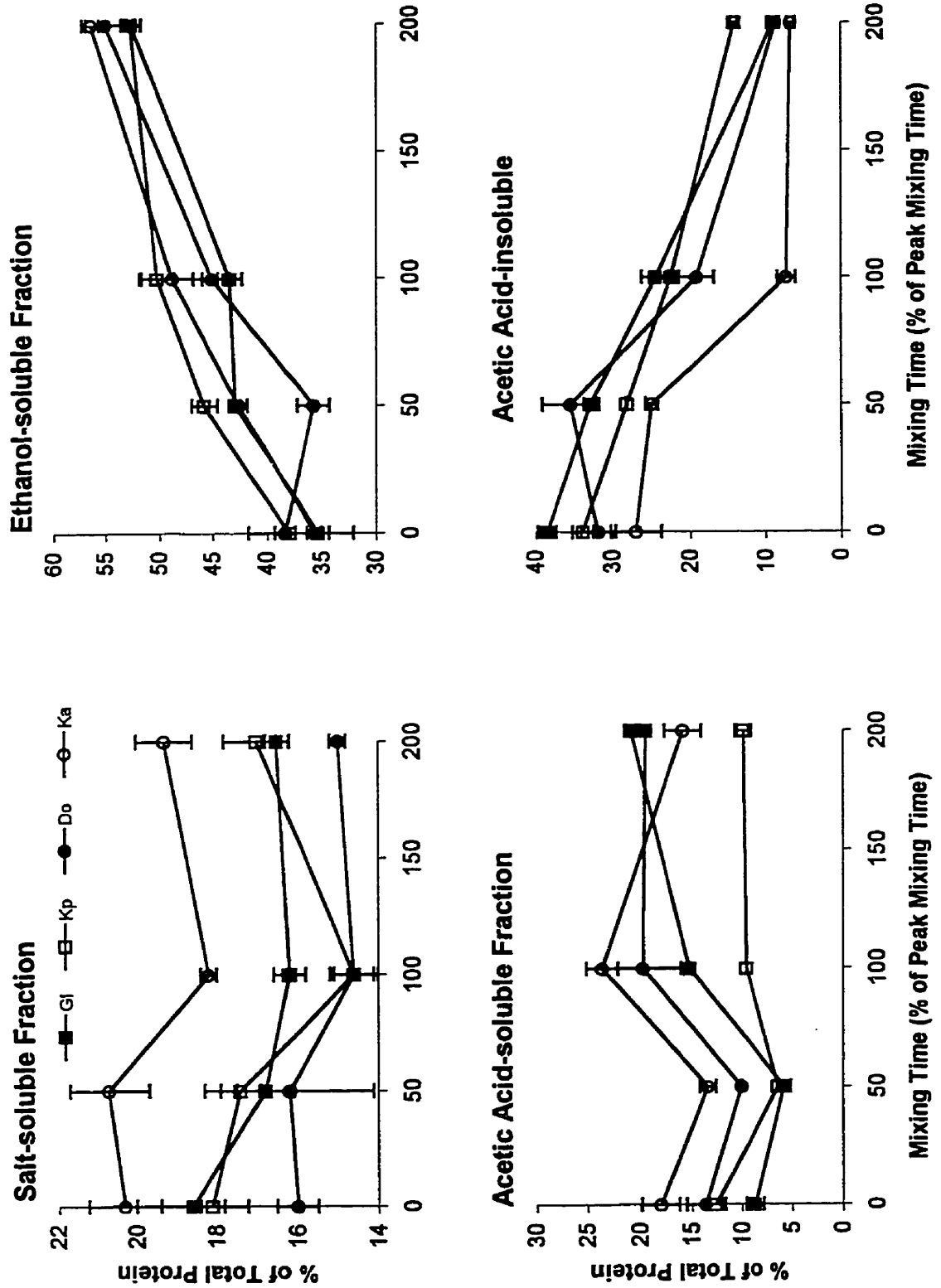
4.3.5.1.Salt-soluble fraction

The content of SS protein decreased significantly for doughs mixed from 50% MT to peak (Figure 24). After peak, all cultivars exhibited an increase in SS protein. The SS protein contents and changes during mixing varied with cultivar but showed no relationship to dough strength.

4.3.5.2.Ethanol-soluble fraction

The increase in the content of ES protein throughout mixing for all cultivars is large and significant (Figure 24). The initial drop in ES protein content characteristic of the control doughs made from the weaker cultivars was only evident for the AC Domain NEMI dough. Content of ES protein during mixing varied with cultivar but showed no

Figure 24. Changes in modified Osborne fractions during mixing of NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka) flours. Mixing time of 0 % corresponds to flour.

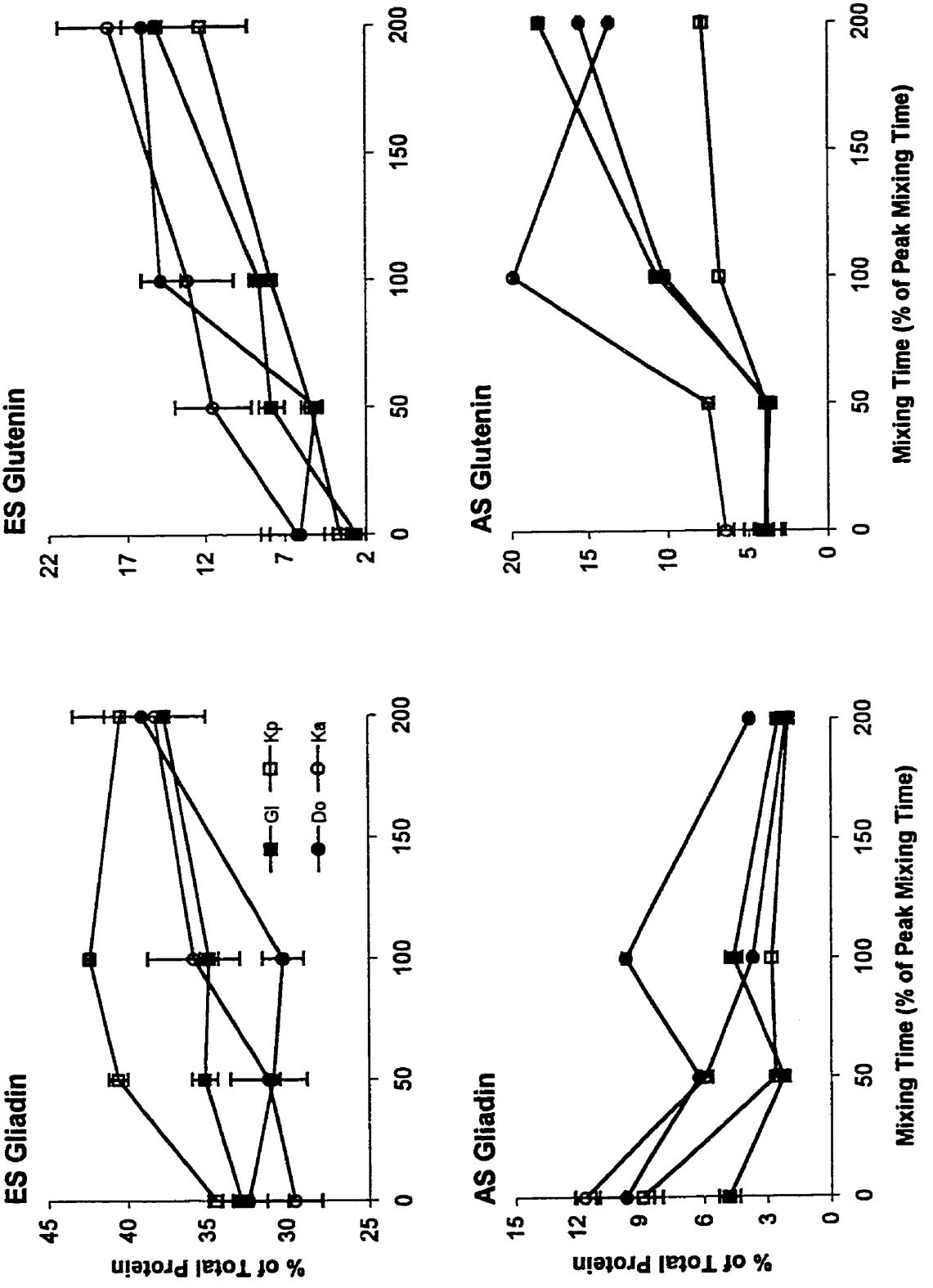

Salt-soluble Fraction
Gl
Kp
Do
Ka
% of Total Protein
Ethanol-soluble Fraction
Acetic Acid-soluble Fraction
Acetic Acid-insoluble
Mixing Time (% of Peak Mixing Time)

relationship to dough strength. Gliadin and glutenin contribute to the increase in ES protein (Figure 25). No relationship was found between the content of ES gliadin or ES glutenin during mixing and dough strength.

4.3.5.3.Acetic acid-soluble fraction

The changes in amount of AS protein during mixing were similar to those observed for the control and iodate doughs. An initial decrease occurred at 50% MT followed by an increase at peak for all cultivars (Figure 24). Overmixing caused a large increase in Glenlea (5.6%) and a large decrease in AC Karma (-7.8%), while the intermediate cultivars exhibited negligible changes. The inverse relationship between amount of AS protein and mixing strength was obvious for all NEMI doughs mixed to peak. The relationship was subesquently lost with overmixing. As with the control and iodate doughs, the AS gliadin content initially decreased for all cultivars. An increase up to peak followed by a decrease during overmixing were observed for Glenlea and AC Domain. But, Katepwa remained stable and AC Karma continued to decrease until doughs were overmixed (Figure 25). The content of AS gliadin which appeared to be cultivar-dependent for the flour did not show a similar relationship as doughs were mixed to peak. The amount of AS glutenin increased as mixing progressed. The degree of change was dependent on cultivar, but no trends related to quality were observed.

Figure 25. Changes in purified ES and AS fractions during mixing of NEMI doughs prepared with Glenlea (Gl), Katepwa, (Kp), AC Domain (Do) and AC Karma (Ka) flours. Mixing time of 0% corresponds to flour.


ES Gliadin
ES Glutenin
AS Gliadin
AS Glutenin
% of Total Protein
Mixing Time (% of Peak Mixing Time)
Gl
Kp
Do
Ka

4.3.5.4. Acetic acid-insoluble fraction

The amount of AI protein was reduced throughout mixing for the four cultivars (Figure 24). The NEMI doughs made from the two weaker cultivars exhibited behaviour similar to that observed in the iodate doughs. There was an initial increase in AI protein for AC Domain. The AI protein content for AC Karma did not increase, but decreased to a much lesser extent than Glenlea and Katepwa. With only two exceptions (AC Domain at 50% MT and Katepwa at 200% MT), the strong relationship between insoluble glutenin (AI) and dough strength was maintained throughout mixing. SDS-PAGE under non-reducing conditions of the AI fractions showed that gliadin content varied during mixing (Figure 23). The gliadin content of NEMI doughs appeared to increase at 50% MT (AC Karma) or 100% MT (other cultivars).

4.3.5.5. Gliadin-glutenin interaction

As with the control and iodate doughs, evidence for gliadin-glutenin interactions for NEMI doughs can be found in the changes occurring during mixing to the total gliadin (Table 14). Gliadin solubility in NEMI doughs decreased in the AS fraction, but not in the ES fraction, during the early stages of mixing for all cultivars except Katepwa. The loss of gliadin in the NEMI doughs is similar to, but slightly lower than, the observed loss for the iodate doughs. The losses were clearly lower for the treated doughs compared to the controls. Again a cultivar-dependent trend was observed, with Glenlea and Katepwa exhibiting little to no decrease in total gliadin and the weaker cultivars exhibiting greater losses. Soluble glutenin content increased as mixing progressed and did not appear to be important in mixing behaviour or gliadin-glutenin interactions.

Table 14. Changes in Gliadin (ES, AS and total) and Glutenin (ES, AS and total soluble) During Mixing of NEMI Doughs.

	0-50% MT			50-100% MT			100-200% MT		
Gliadin	ES	AS	Total	ES	AS	Total	ES	AS	Total
Glenlea	2.3	-2.6	**-0.3**	-0.3	2.4	2.1	2.8	-2.1	0.7
Katepwa	6.2	-6.2	**0.0**	1.8	0.2	2.0	-2.0	-0.8	-2.8
AC Domain	1.6	-3.4	**-1.8**	-0.6	3.4	2.8	8.8	-5.8	3.0
AC Karma	1.6	-5.7	**-4.1**	4.7	-2.2	2.5	2.4	-1.6	0.8
Glutenin									
Glenlea	5.2	-0.4	4.8	0.8	7.0	7.8	6.5	7.6	14.1
Katepwa	1.7	0.1	1.8	2.5	2.9	5.4	4.5	1.1	5.6
AC Domain	-1.0	-0.2	-1.2	9.8	6.4	16.2	1.2	5.4	6.6
AC Karma	5.3	1.1	6.4	1.6	12.4	14.0	5.1	-6.2	-1.1

4.3.6. Treatment Effects and Interactions

4.3.6.1. Treatment Effects

For ease of interpretation, the data representing treatment effects has been presented graphically in this section. Tables of means, standard deviations and statistical evaluation can be found in the Appendix (Tables 18-25). Compared to the control doughs, the amount of SS protein was lower for doughs treated with iodate or NEMI for all cultivars except AC Domain (Figure 26). The ES protein was found in significantly greater proportion in the iodate and NEMI doughs throughout mixing (Figure 27). There was no significant difference between the effects of iodate and NEMI treatment for both the SS and ES protein fractions. The distinct decrease in ES protein at 50% MT for AC Domain and AC Karma are clearly evident for the control doughs and the NEMI-treated AC Domain

Figure 26. Changes during mixing in the amount of SS protein obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.

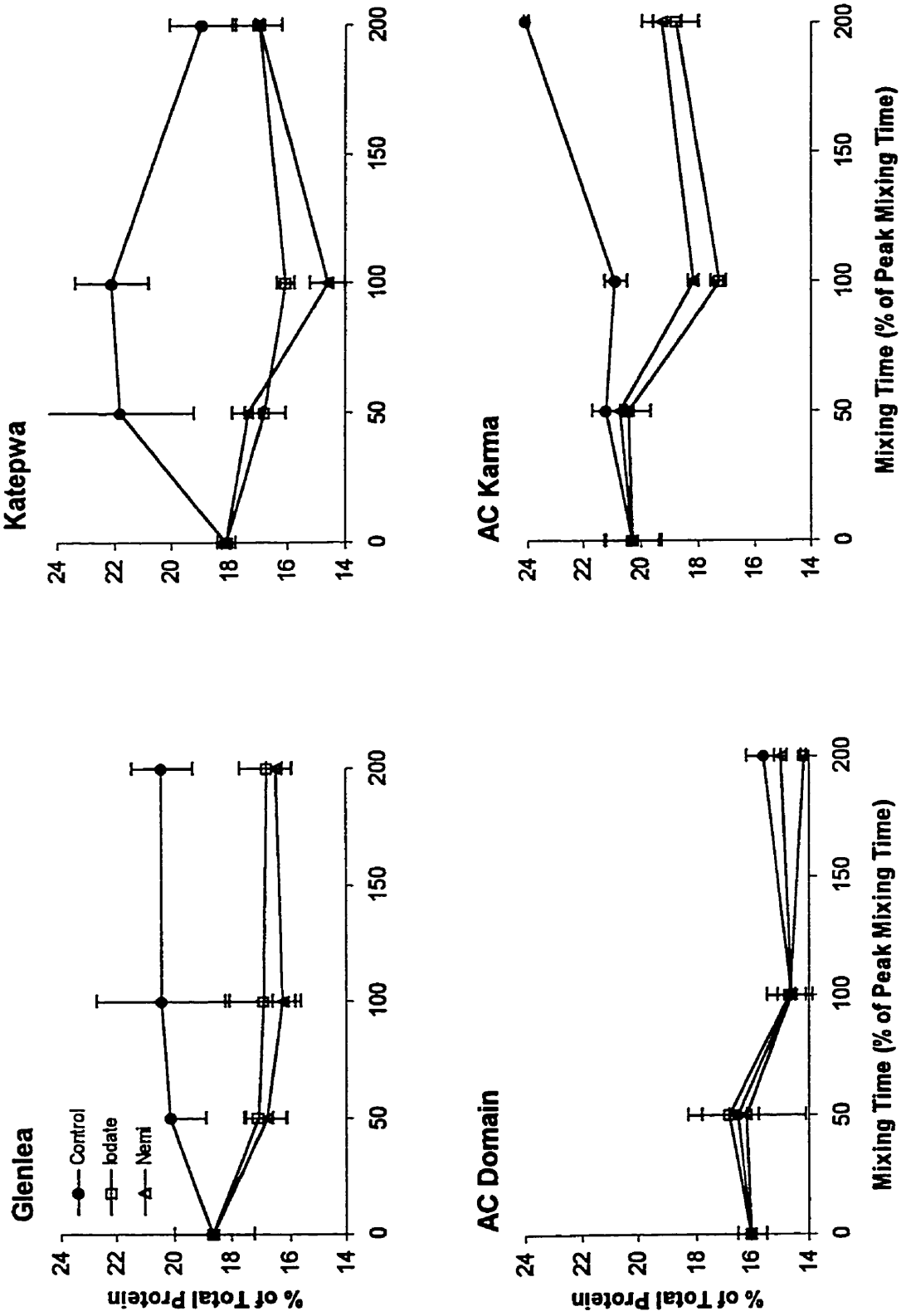
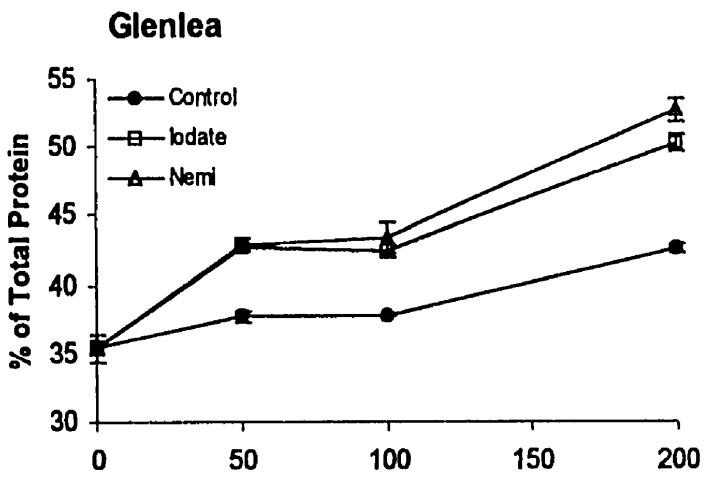
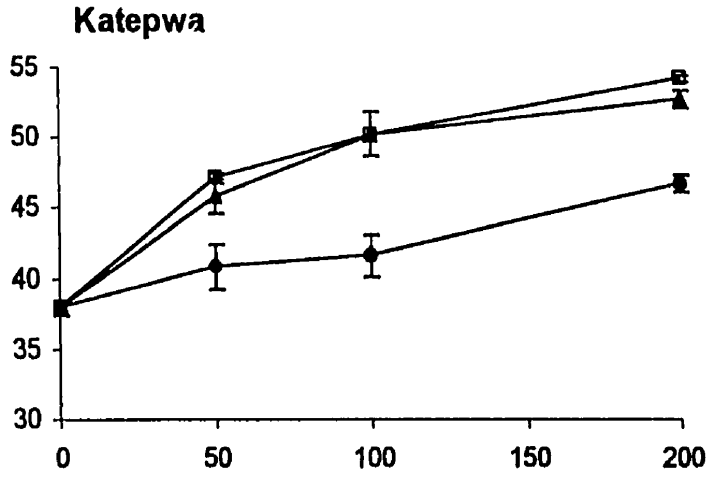
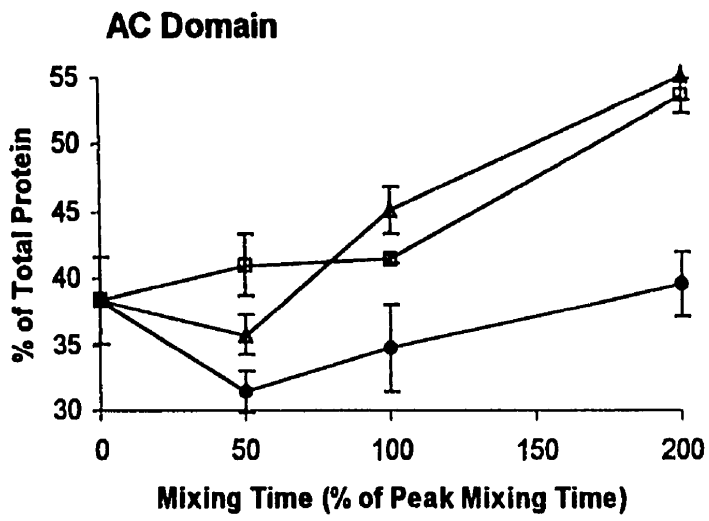
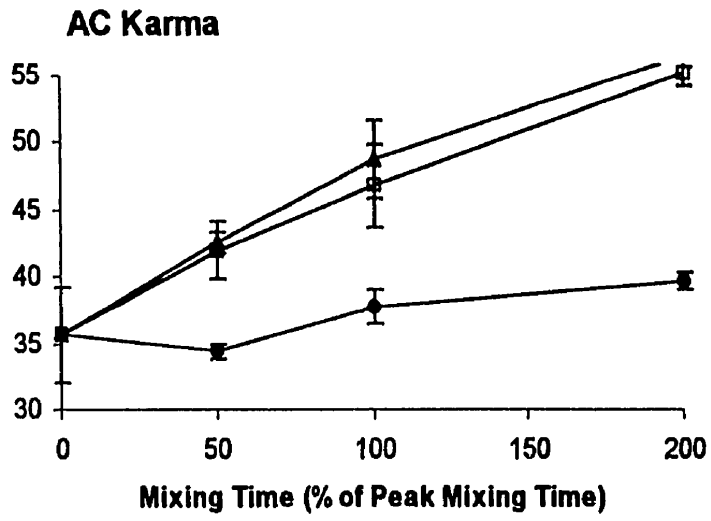

Glenlea
Katepwa
AC Domain
AC Karma
Control
Iodate
Nemi
% of Total Protein
Mixing Time (% of Peak Mixing Time)
24
22
20
18
16
14
0
50
100
150
200

Figure 27. Changes during mixing in the amount of ES protein obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.


Glenlea
Control
Iodate
Nemi
% of Total Protein
55
50
45
40
35
30
0
50
100
150
200


Katepwa
55
50
45
40
35
30
0
50
100
150
200

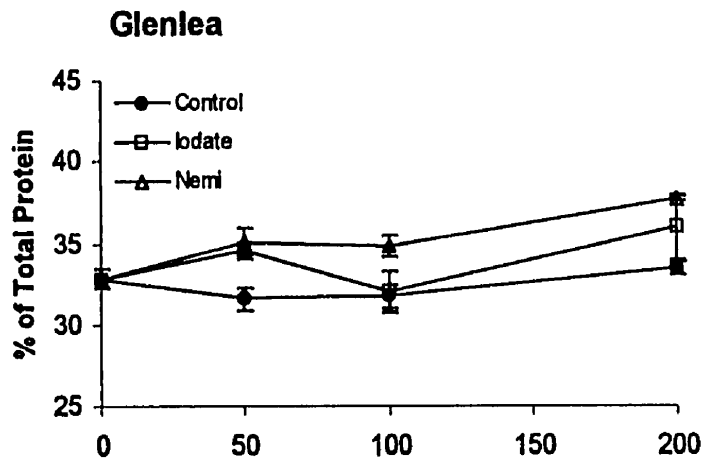

AC Domain
% of Total Protein
55
50
45
40
35
30
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

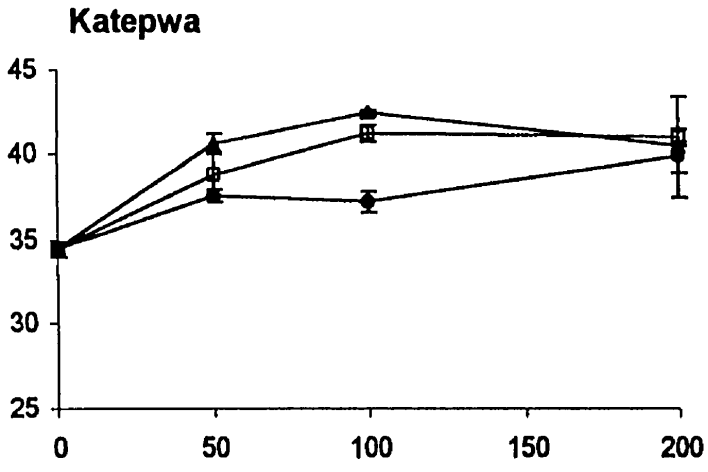

AC Karma
55
50
45
40
35
30
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

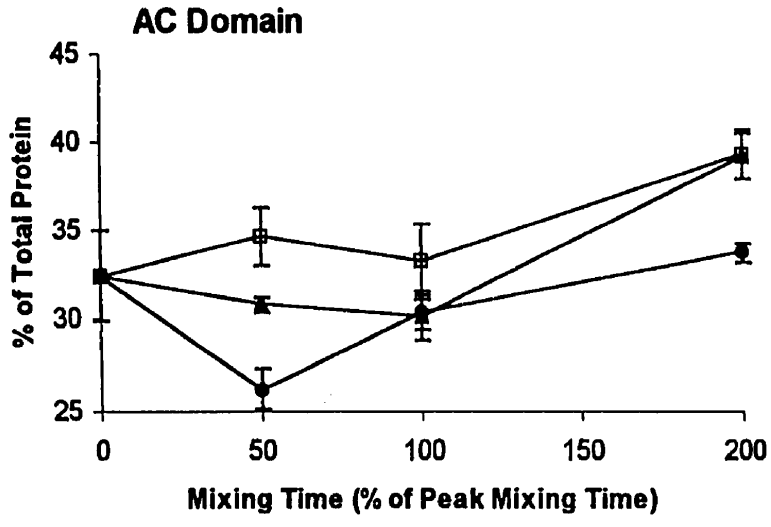
dough. There was generally a greater amount of ES gliadin (Figure 28) and ES glutenin (Figure 29) in the iodate and NEMI doughs and the treatment effects were generally not significantly different.

The treatments had a greater impact on the AS fraction (Figure 30) than the ES and SS fractions. In most cases, there was no difference between the control and treated doughs mixed to 50% MT. In contrast, significantly more AS protein was found at peak and overmixing in the treated doughs. NEMI treated doughs contained the highest amount of AS protein and iodate treated doughs contained intermediate amounts. The effect of the treatments also varied with cultivar. Glenlea exhibited an increase in AS protein from undermixing to overmixing. The difference between treatments was most accentuated for this culitvar as mixing progressed. Katepwa and AC Domain exhibited increases from undermixing to peak and then stabilized. The apparent drop in AS protein on overmixing observed for the control doughs of these two intermediate cultivars was absent in the treated doughs. The AS protein in the AC Karma treated doughs increased up to peak then decreased with overmixing. The effects of iodate and NEMI observed in the AS protein content were primarily exerted on the AS glutenin. There is an effect of treatment on the AS gliadin content, but this effect varies with cultivar and mixing time with no detectable trend (Figure 31). On the other hand, the AS glutenin shows the same treatment-dependent and cultivar-dependent behaviour observed in the total AS protein fraction (Figure 32). When doughs are mixed to peak and overmixed, treated doughs contain significantly more AS glutenin, and NEMI doughs contain significantly more than iodate doughs.

Figure 28. Changes during mixing in the amount of ES gliadin obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.


Glenlea
Control
Iodate
Nemi
% of Total Protein
45
40
35
30
25
0
50
100
150
200


Katepwa
45
40
35
30
25
0
50
100
150
200


AC Domain
% of Total Protein
45
40
35
30
25
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

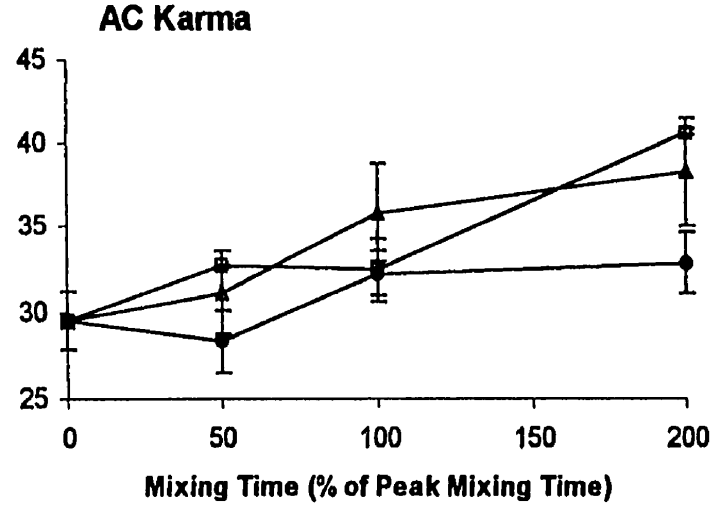

AC Karma
45
40
35
30
25
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

Figure 29. Changes during mixing in the amount of ES glutenin obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.

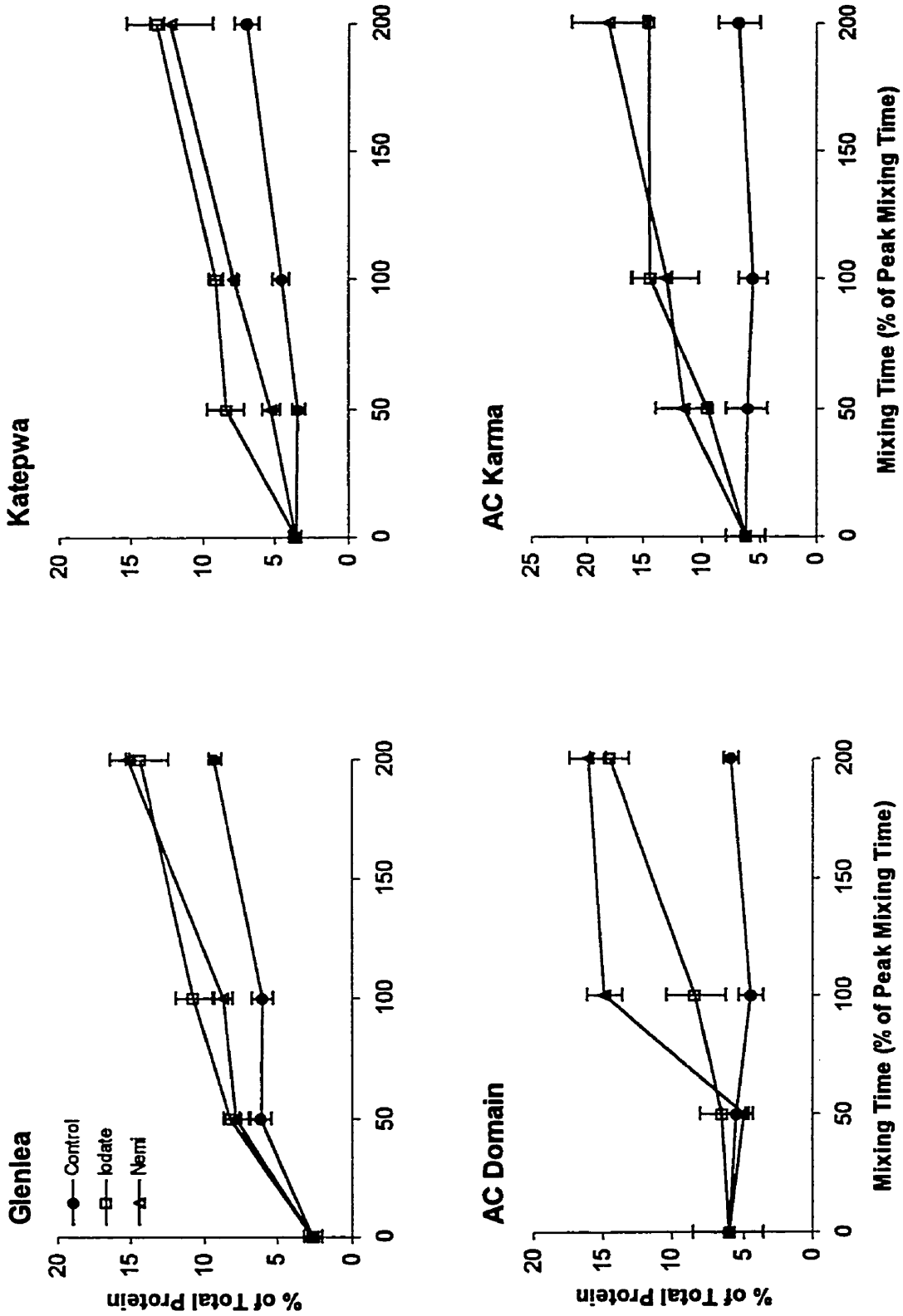

Glenlea
Katepwa
AC Domain
AC Karma
Control
Iodate
Nemi
% of Total Protein
Mixing Time (% of Peak Mixing Time)
0
5
10
15
20
25
50
100
150
200

Figure 30. Changes during mixing in the amount of AS protein obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.

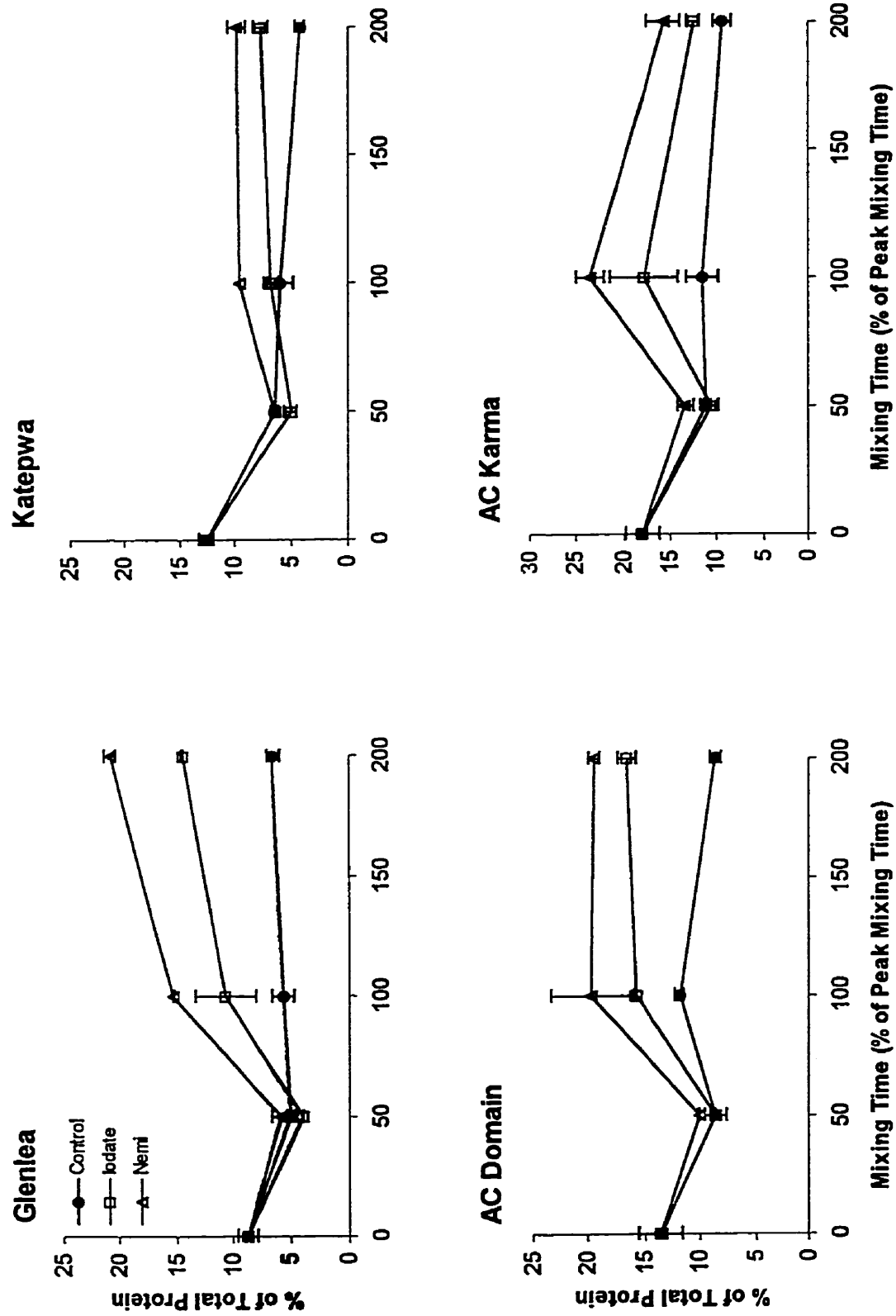
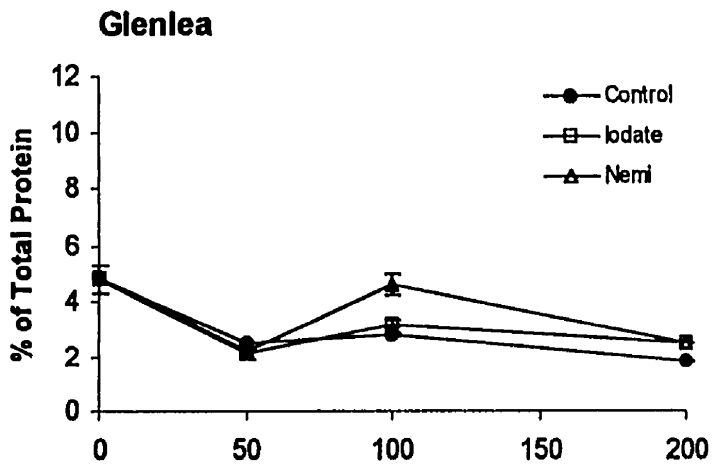
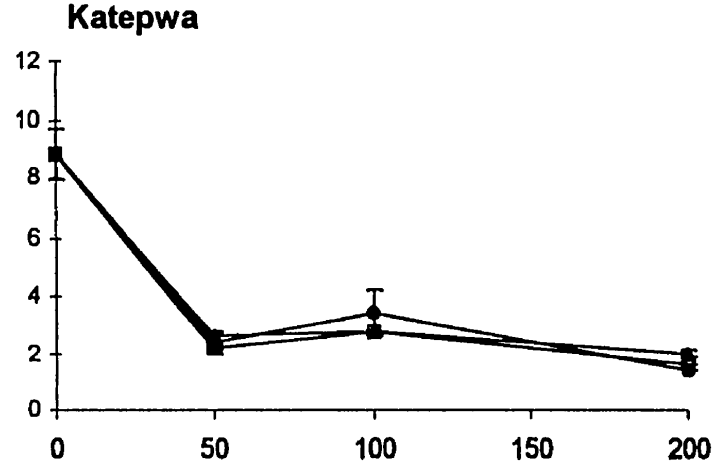
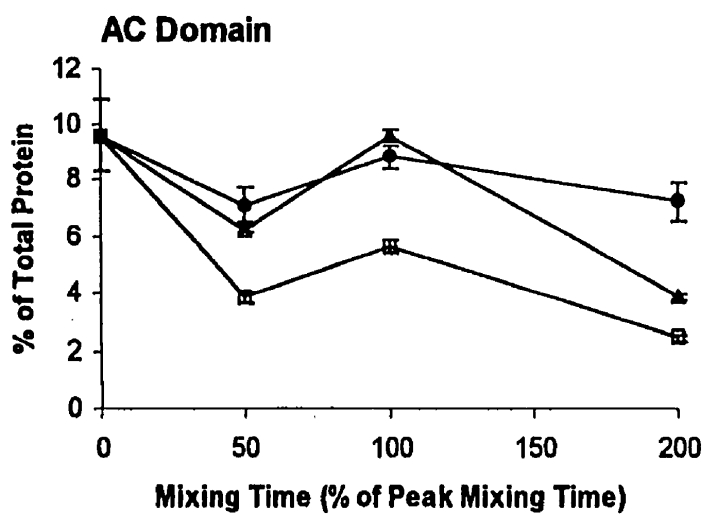

Glenlea
Katepwa
AC Domain
AC Karma
Control
Iodate
Nemi
% of Total Protein
Mixing Time (% of Peak Mixing Time)
0
5
10
15
20
25
30
50
100
150
200

Figure 31. Changes during mixing in the amount of AS gliadin obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.


Glenlea
Control
Iodate
Nemi
% of Total Protein
0
2
4
6
8
10
12
0
50
100
150
200


Katepwa
0
2
4
6
8
10
12
0
50
100
150
200


AC Domain
% of Total Protein
0
2
4
6
8
10
12
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

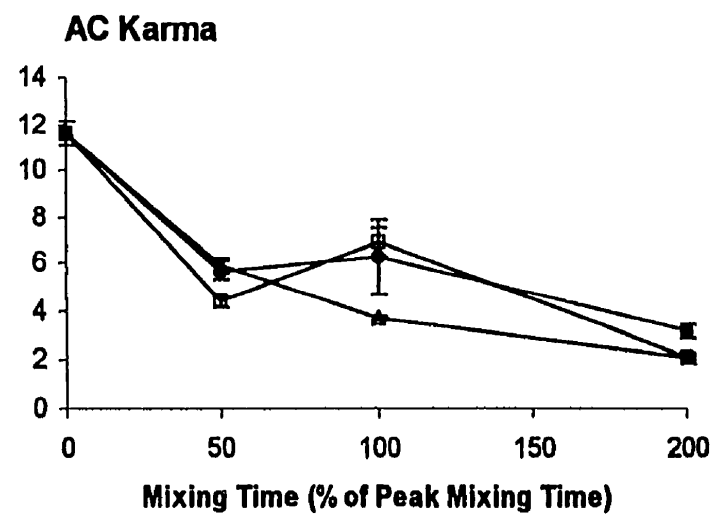
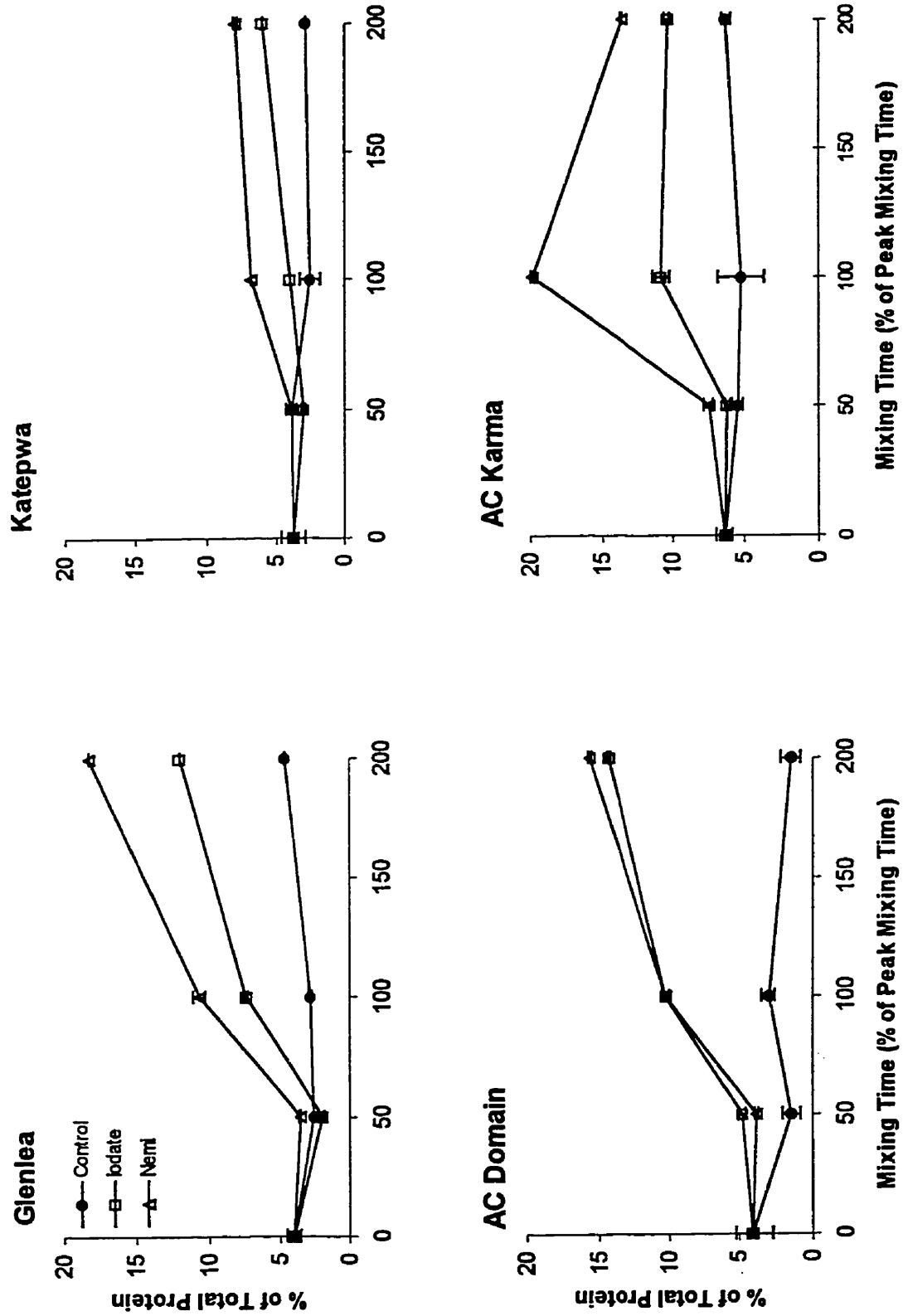

AC Karma
0
2
4
6
8
10
12
14
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

Figure 32. Changes during mixing in the amount of AS glutenin obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.

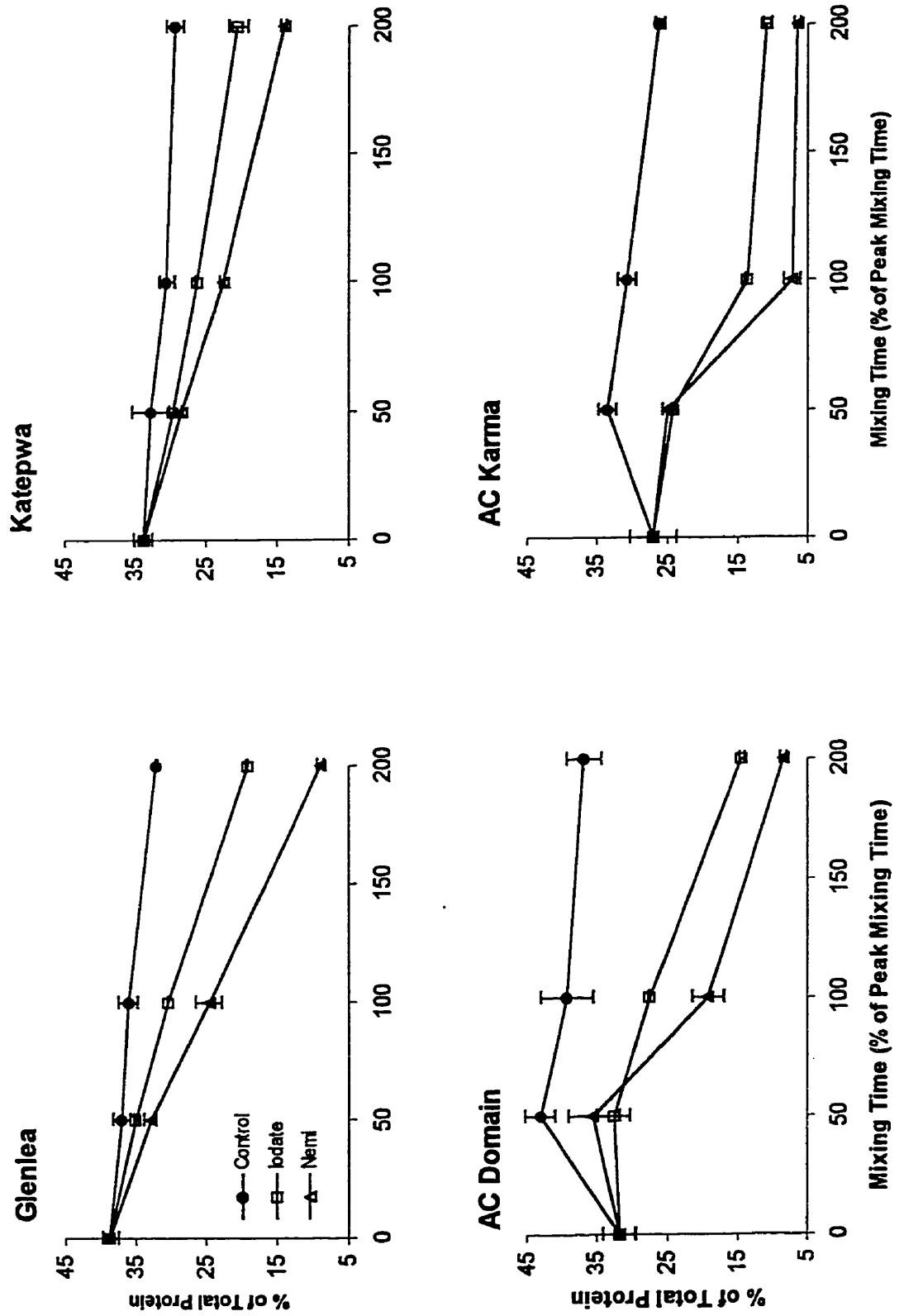

Glenlea
Katepwa
AC Domain
AC Karma
Control
Iodate
Nemi
% of Total Protein
Mixing Time (% of Peak Mixing Time)
0
5
10
15
20
50
100
150
200

The AI fraction provided the most dramatic results for treatment effects. AI glutenin significantly decreased for all cultivars. The amount of AI protein at 50% MT was higher for the controls when compared to the treated doughs. There was no significant difference in the amount of AI protein between iodate and NEMI treatments until doughs were mixed to peak and beyond. NEMI consistently had the greatest effect on the AI fraction, followed by iodate. The decrease in the amount of AI glutenin during mixing was virtually linear for Glenlea and Katepwa, for all treatments (Figure 33). The decline in Glenlea AI glutenin was much steeper than that of Katepwa, indicating a more rapid breakdown in the large polymers. AC Domain and AC Karma showed a more unique response to mixing. Figure 33 illustrates very well the initial increase in AI protein considered to result from gliadin-glutenin interactions. The rise was most significant for the control doughs, while the treated doughs exhibited more of a plateau (AC Domain) or small drop (AC Karma). This initial phase was then followed by a rapid loss of AI protein as doughs were mixed to peak and beyond.

4.3.6.2.Statistical Interactions

Statistical analysis of the data discussed in this section indicated some significant interactions. A summary table of significant main effects and interactions has been included in the Appendix (Table 26). Many of these interactions can be easily detected by visual assessment of the graphs presented. For example, the Cultivar-by-mixing time (CV×MT) interaction for the AI fraction obtained from control doughs as seen in Figure 12. The positive relationship between amount of AI glutenin and mixing strength was evident at 0% MT. This relationship was

Figure 33. Changes during mixing in the amount of AI protein obtained by modified Osborne fractionation of control, iodate, and NEMI doughs prepared from Glenlea, Katepwa, AC Domain and AC Karma flours. Mixing time of 0% corresponds to flour.


Glenlea
Katepwa
AC Domain
AC Karma
% of Total Protein
Mixing Time (% of Peak Mixing Time)
Control
Iodate
Nemi
45
35
25
15
5
0
50
100
150
200

altered at subsequent mixing times only by the change in AC Domain. The other cultivars maintained the relationship throughout mixing. A portion of the interaction occurring was also due to the unique increase in AI protein (attributed to gliadin-glutenin interaction) in AC Domain and AC Karma doughs. Thus the two main effects were much more important than the interactions. This was reflected in the much larger F-values for main effects compared to interactions tabulated in the Appendix (Table 26).

Another example can be found in Figure 15 for the ES glutenin. The CV×MT interaction reported as significant by statistical analysis simply illustrated that the increase in ES glutenin on mixing was not genotype dependent. The CV×MT interaction occurring in the AS fraction of iodate doughs appeared to indicate that the initial correlation between cultivar and amount of AS protein was lost after doughs were mixed to peak (Figure 21). Similar examples exist for the CV×TRTMT and MT×TRTMT interactions. For instance, the MT×TRTMT interaction often simply indicated that iodate and NEMI produced observable effects only after mixing past 50% MT.

If an attempt was made to interpret many of the interactions deemed significant by statistical analysis, the effort would add confusion and draw away from the important main effects. The interactions may be significant but may not be of relevance to the main effects being studied nor may they contribute any additional information. Hence most of the interactions have been disregarded. Those deemed relevant to the outcome of the data are discussed where appropriate. Due to the heterogeneous nature of the proteins in the dough systems being studied and the cumulative variation inherent in the complicated fractionation and purificiation scheme, it is likely that some of the interactions being identified by statistical analysis as significant are a reflection of these conditions. Unlike

other studies, one must consider when interactions should be emphasized and when they should be ignored, despite their statistical significance.

4.4. Changes During Mixing in the Glutenin Subunit Composition of Glutenins of Three Different Solubilities

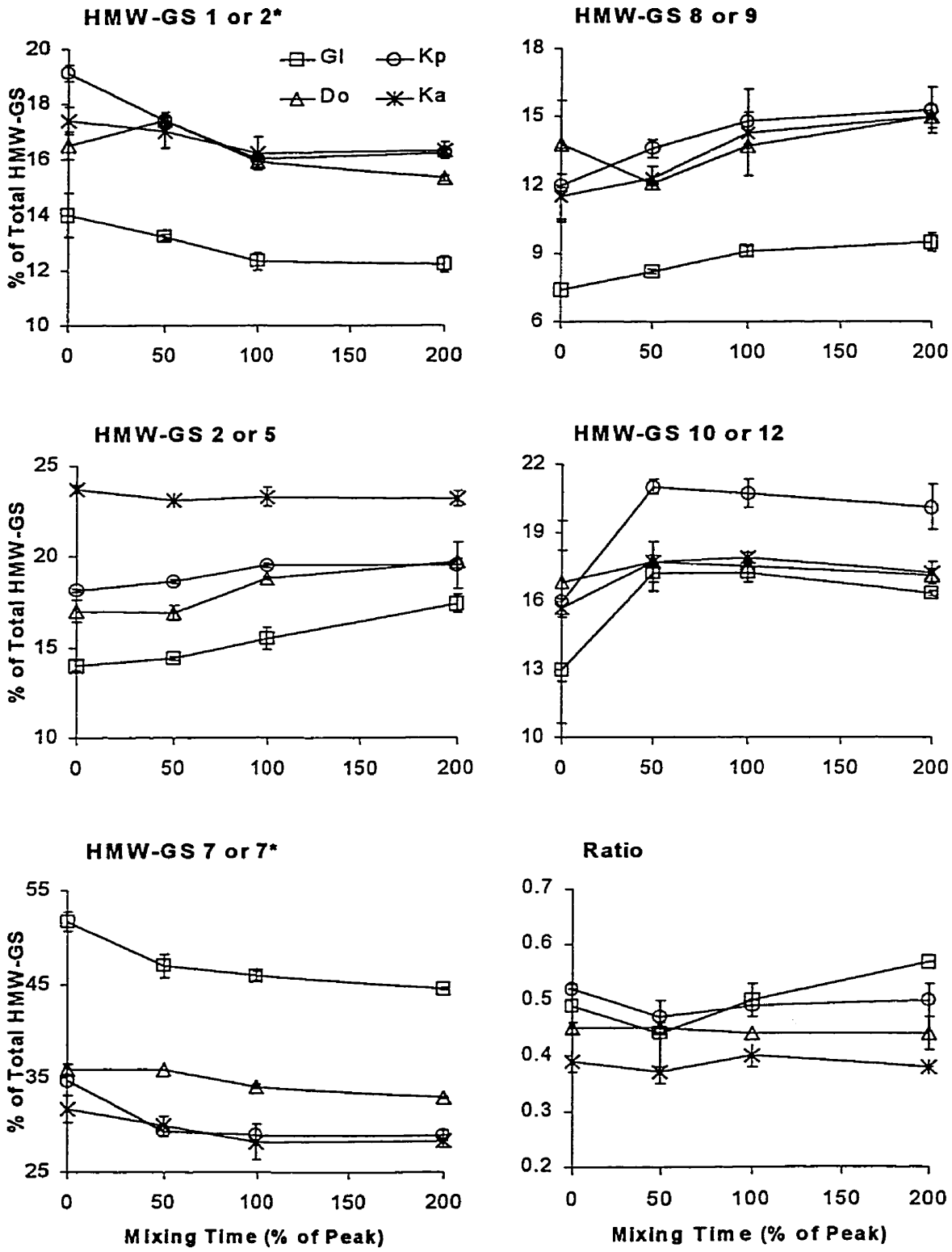
In the two previous chapters, the flours used in this study were characterized and the changes in protein solubility distribution during mixing were evaluated for control doughs and doughs treated with iodate or NEMI. The modified Osborne fractionation procedure and subsequent purification method produce three fractions of glutenin of different solubility, ES, AS and AI glutenin. The next step was to evaluate these three fractions of glutenin by RP-HPLC to determine the effects of mixing on HMW-GS composition and the ratio of HMW-GS to LMW-GS.

RP-HPLC chromatograms and SDS-PAGE electrophoregrams showed that no qualitative differences arose during mixing. Therefore, only sample chromatograms for each fraction of glutenin obtained from the flours of each cultivar have been included in the Appendix (Figure 1). Specific HMW-GS have been highly correlated with breadmaking quality parameters (Ng and Bushuk, 1988, Payne et al, 1979, Shewry et al, 1992). Also, the predominance in specific solubility fractions of HMW-GS related to quality has been reported (Dupuis et al, 1996, Fu, 1996). The ratio of HMW-GS to LMW-GS, a measure of the M_r of glutenin is also related to solubility fractions and quality parameters (Graveland et al, 1985, Gupta and MacRitchie, 1991, Payne and Corfield, 1979). The objective in this work was to investigate the relationship between changes in gluten proteins during mixing and the subunit composition of three glutenins of different solubilities. Once again, the results have been presented graphically and the comprehensive data tables can be found in the Appendix (Tables 27-48).

4.4.1. Control doughs

The proportion of individual HMW-GS in the three glutenin fractions changed significantly with mixing. For the ES glutenin, the changes that occurred were significant and generally similar for all cultivars (Figure 34). The relative amounts of 1Ax and 1Bx subunits (GS 2* and GS 7 or 7*, respectively) decreased until doughs were mixed to 50% MT or peak, then leveled off during overmixing. The relative amounts of 1Dx and 1By subunits (GS 5 and GS 8 or 9) increased with mixing. Both 1Dy subunit alleles (GS 10 and GS 12) exhibited a strong increase in relative amount during the initial stages of mixing and then remained relatively unchanged after 50% MT. Allelic variation was observed for the 1Ax and 1Dx subunits. The relative amount of GS 1 from AC Karma was not significantly changed by mixing, exhibiting a much smaller initial loss compared to the other cultivars containing the 1Ax2* subunit. The relative amount of GS 2 from AC Karma also showed no significant change with mixing compared to the other cultivars containing the 1Dx5 subunit. With the exception of Glenlea, the ratio of HMW-GS to LMW-GS (subsequently referred to simply as ratio) exhibited small but relatively insignificant changes during mixing. Glenlea's ratio increased after 50% MT while the ratios for the other cultivars remained fairly constant. Glutenin soluble in ethanol became enriched with y-type subunits (1By and 1Dy) and 1Dx5 as control doughs were mixed to optimum development. The ratio is considered a measure of the M_r of the glutenin polymer. Although little change in the ratios was observed with mixing, the relationship of high ratio and dough strength was statistically significant. Katepwa, Glenlea, and Domain had similarly high ratios in the flours and undermixed doughs. As mixing progressed to peak

Figure 34. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the ES fraction of glutenin during mixing of control doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

and beyond, the ratio for each cultivar varied more significantly with mixing strength: Glenlea had the highest ratio, Katepwa and AC Domain were intermediate, and AC Karma had the lowest.

The relative proportions of HMW-GS in the AS glutenin fraction also changed during mixing, but these changes were less significant (Figure 35). The 1By subunits (GS 8 or GS 9) increased significantly throughout mixing, while the 1Dy subunits (GS 10 or GS12) decreased after an initial increase at 50% MT. The relative amounts of 1Ax subunits (1 or 2*) and 1Bx subunits (7 or 7*) did not change significantly with mixing. The 1Dx2 and 1Dx5 subunits exhibited initial losses in relative proportion but subsequent increases were observed, except for AC Karma. The changes were only significant for AC Domain and AC Karma. Ratios were relatively unchanged due to mixing, except for slight increases followed by decreases after peak for Glenlea and AC Domain. The ratios were higher for this fraction than for the ES fraction, suggesting that the AS glutenin is of greater M_r. Mixing enriched the glutenin soluble in dilute acetic acid with y-type subunits. Subunits 1By8 and 1By9 increased continuously throughout mixing while subunits 1Dy10 or 1Dy12 decreased after 50% MT, but did not reach initial levels until the doughs were overmixed.

The relative amounts of individual HMW-GS in the AI glutenin (Figure 36) behaved similarily to the more soluble glutenins. However, the observed variation and lack of consistent trends for each subunit during mixing suggest that any relationships be considered with caution. The proportion of 1By9, but not 1By8, increased with mixing and that of 1Dy10, but not 1Dy12, initially increased then stabilized after 50% MT.

Figure 35. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AS fraction of glutenin during mixing of control doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).

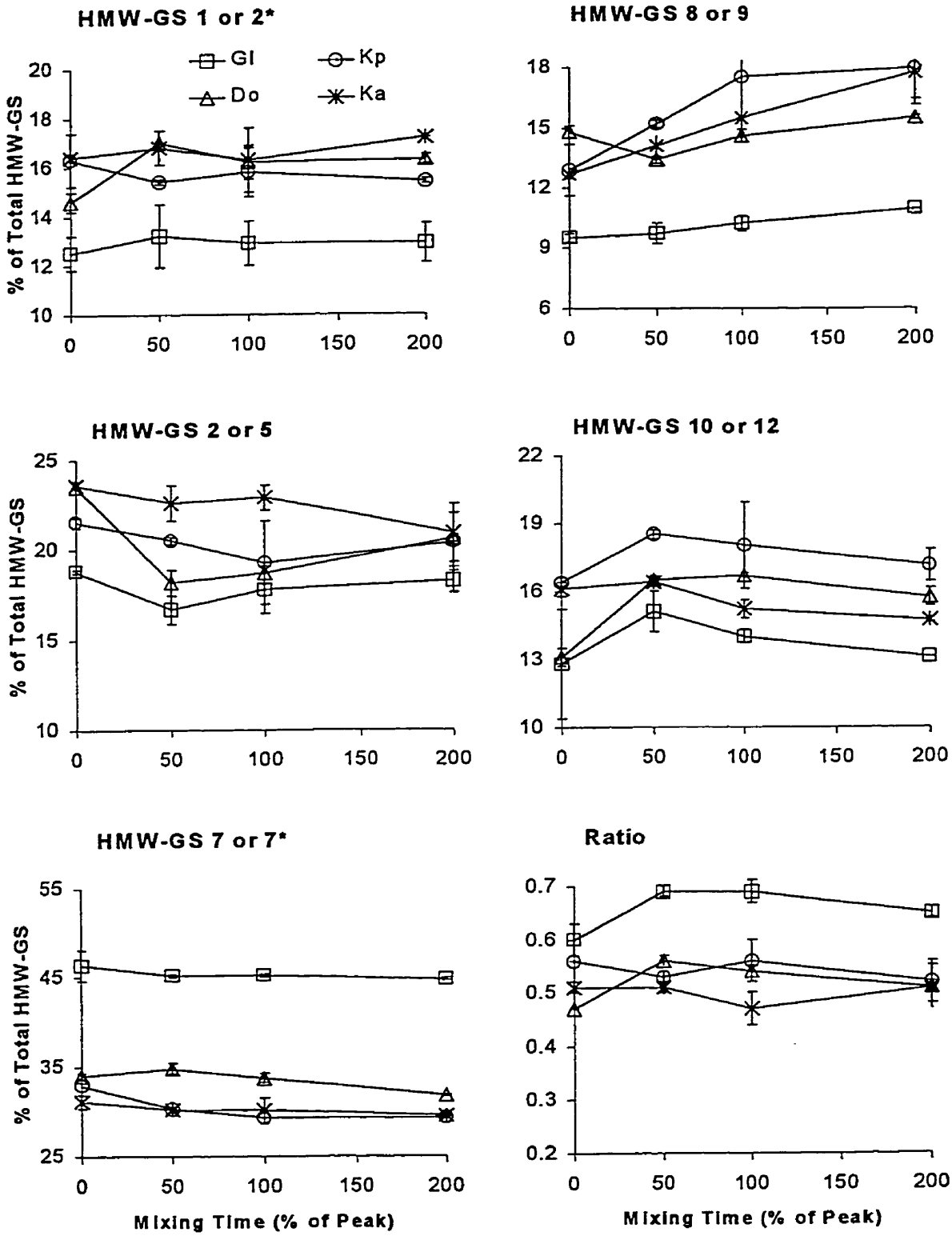
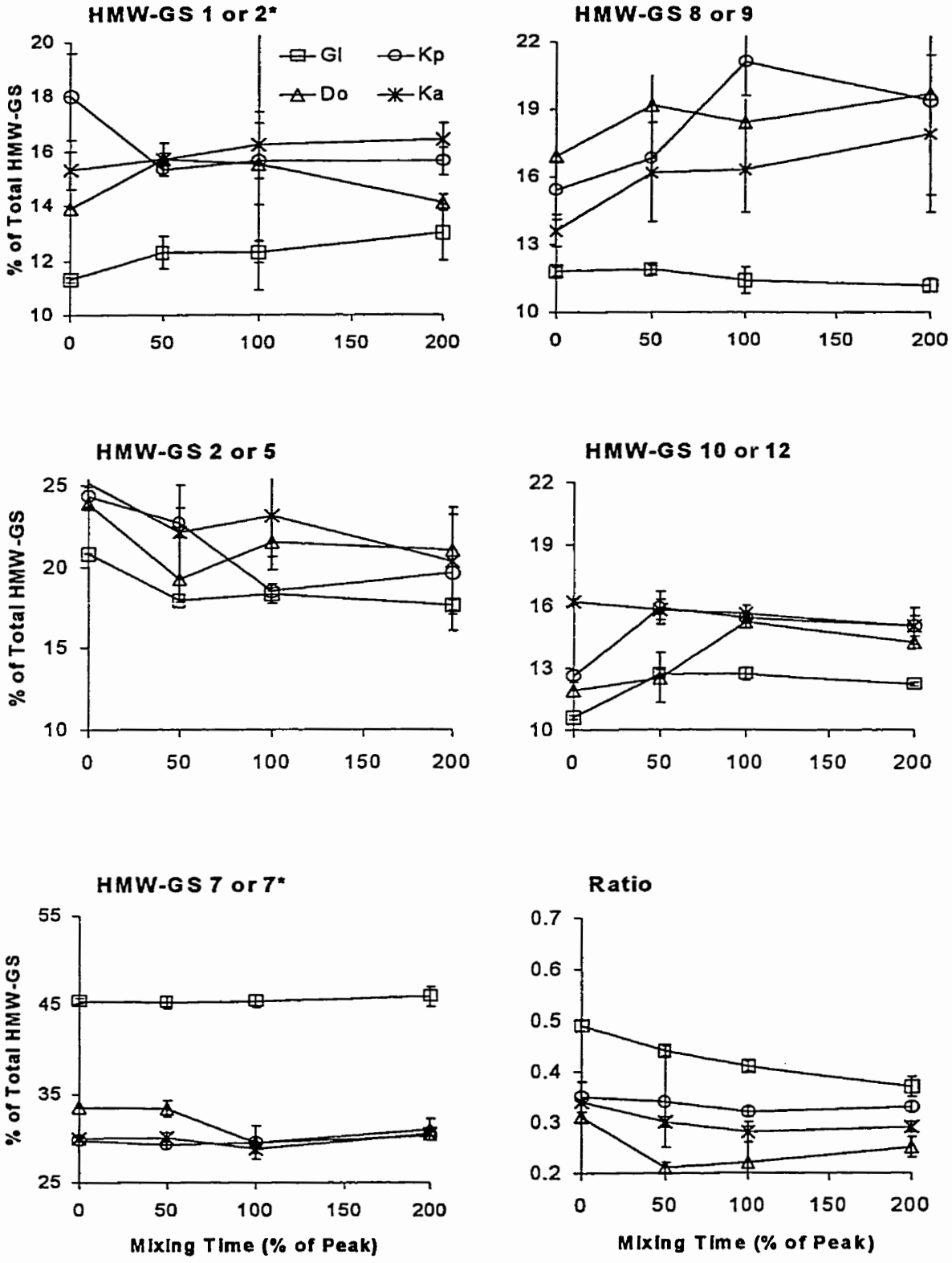

HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

Figure 36. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AI fraction of glutenin during mixing of control doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

However most of these changes were not significantly different. The 1Ax and 1Bx subunits exhibited very little change during mixing, although a very slight increase was evident. Again, statistically significant differences were small and few. The ratios provided the most significant changes during mixing. All cultivars exhibited a decrease in the ratio upon mixing. The largest decline was observed for Glenlea while the decreases for the remaining cultivars were less dramatic. Glenlea had the largest ratio throughout mixing and Katepwa the second largest. The ratios for AC Domain and AC Karma were similar but AC Domain was the lowest.

4.4.2. Effect of Absorption

The composition of glutenin subunits was affected by the absorption at which a dough is mixed. The subunit composition of glutenins obtained from AC Domain control doughs prepared at 61.7% and 69.4% absorption was investigated by RP-HPLC. The x-type subunits for the ES and AS glutenin fractions are presented in Figure 37. The HMW-GS 2* and 7* are present in greater proportion throughout mixing in the dough prepared at 69.4% absorption (69% dough). There was no significant difference for HMW-GS 5, except for the ES glutenin at 50% MT and the AS glutenin at 100% MT, where the 69% dough contained a lower proportion of HMW-GS 5.

The y-type subunits in the ES and AS glutenins were present in higher amounts for the dough prepared at 61.7% (62% dough), although the difference was only significant for HMW-GS 9 of the AS glutenin and HMW-GS 10 of the ES glutenin (Figure 38). Although only significant for the AS glutenin at 50% MT, the ratio was consistently higher for both ES and AS glutenin from the 69% dough. The effect of absorption on subunit

Figure 37. Changes during mixing in the relative amounts of the x-type subunits in ES and AS glutenin obtained from AC Domain control doughs prepared at 62% and at 69% absorption.

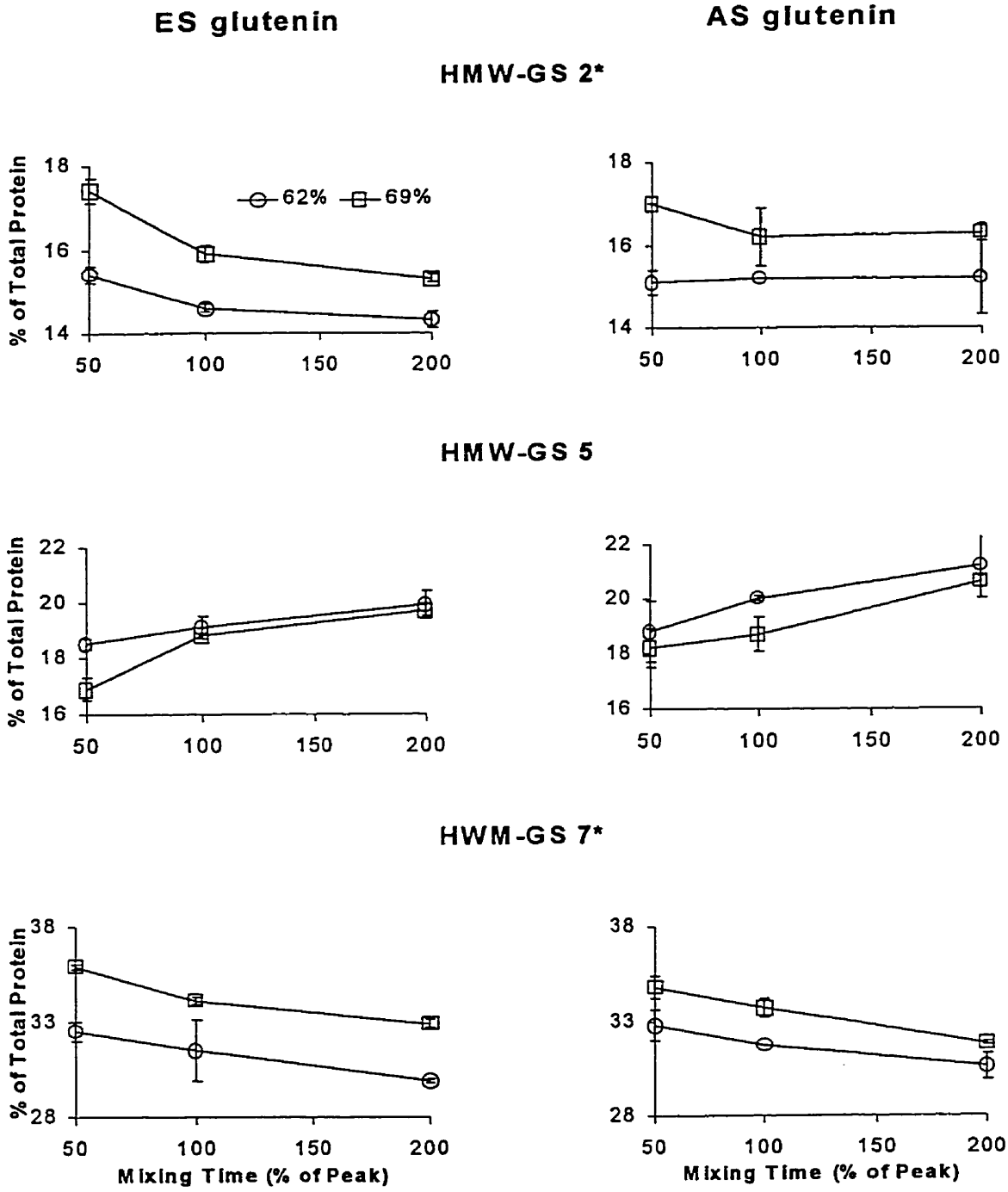
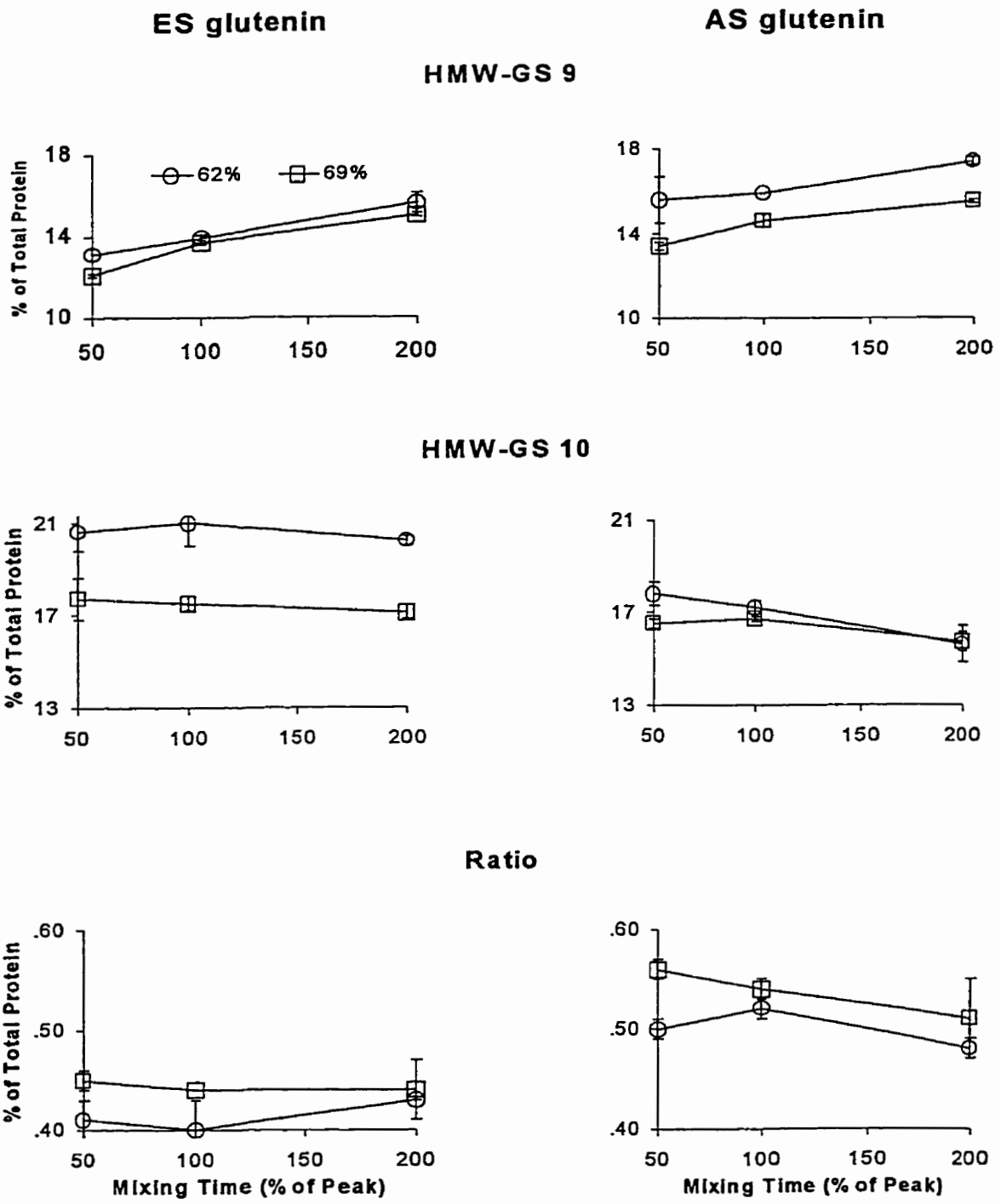

ES glutenin
AS glutenin
HMW-GS 2*
% of Total Protein
62%
69%
HMW-GS 5
HWM-GS 7*
Mixing Time (% of Peak)

Figure 38. Changes during mixing in the relative amounts of the y-type subunits and the ratio of HMW-GS to LMW-GS in ES and AS glutenin obtained from AC Domain control doughs prepared at 62% and 69% absorption.


ES glutenin
AS glutenin
HMW-GS 9
62%
69%
% of Total Protein
18
14
10
50
100
150
200
HMW-GS 10
21
17
13
Ratio
.60
.50
.40
Mixing Time (% of Peak)

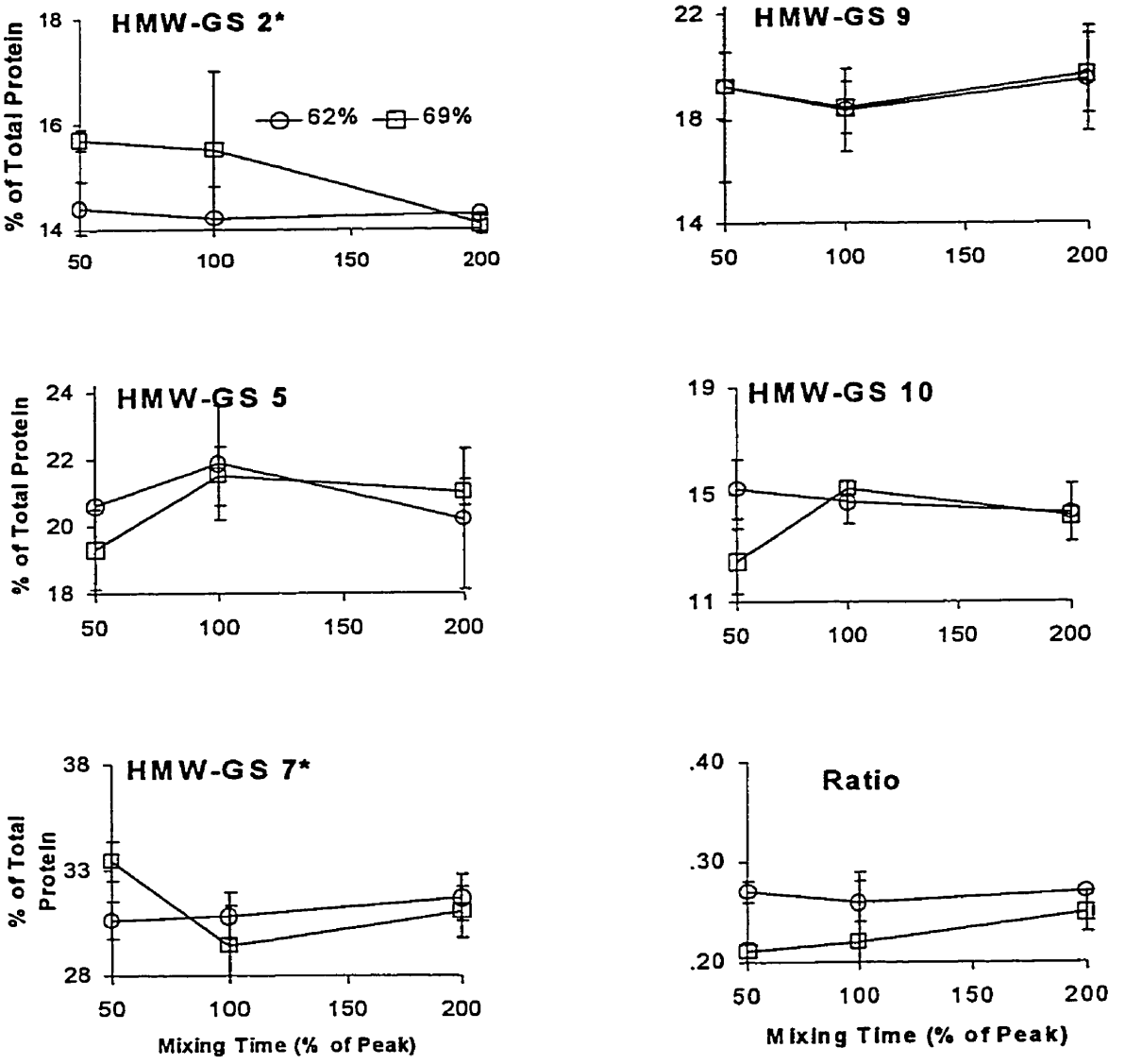
composition of the AI glutenin was much less significant (Figure 39). Subunits 5, 9 and 10 were virtually unaffected and subunits 2*, 7* and the ratio were affected during the early stages of mixing only. The proportion of subunits 2* and 7* was higher in the 69% dough mixed to 50% peak. The ratio was higher for the 62% dough.

In general, all fractions of 69% doughs are richer in the x-type subunits 2* and 7* whereas the fractions of 62% doughs are richer in the y-type subunits and to some extent HMW-GS 5. For doughs prepared at lower absorptions there is a greater amount of shear force exerted on the dough system and greater breakdown of the polymer. As a consequence, the mixing time is reduced. Because of this phenomenon, it would be expected that the ratio, a measure of the M_r of the glutenin polymer, of the AI fraction would be lower for a dough mixed at lower absorption as the polymer of reduced size becomes more soluble. During the initial stages of mixing, this was not observed for the AC Domain doughs. The 62% dough had a higher ratio than the 69% dough for the AI fraction. Conversely, the 69% dough had higher ratios for the soluble glutenin fractions than did the 62% doughs.

Subunit composition of control doughs examined earlier indicated that the y-type subunits for all fractions were found in increasing proportion during mixing. The y-type subunits were found in higher and similar proportion in the AI fraction for both absorptions. But more y-type subunits were released to the soluble fractions in the dough of lower absorption. Another possible explanation is that doughs at lower absorption exhibit a larger loss of x-type subunits to the SS fraction or the x-type subunits are remaining in the insoluble fraction as unreducable polymer. Further investigation into the SS glutenin and unreducible polymer would provide more information.

Figure 39. Changes during mixing in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS in AI glutenin obtained from AC Domain control doughs prepared at 62% and 69% absorption.

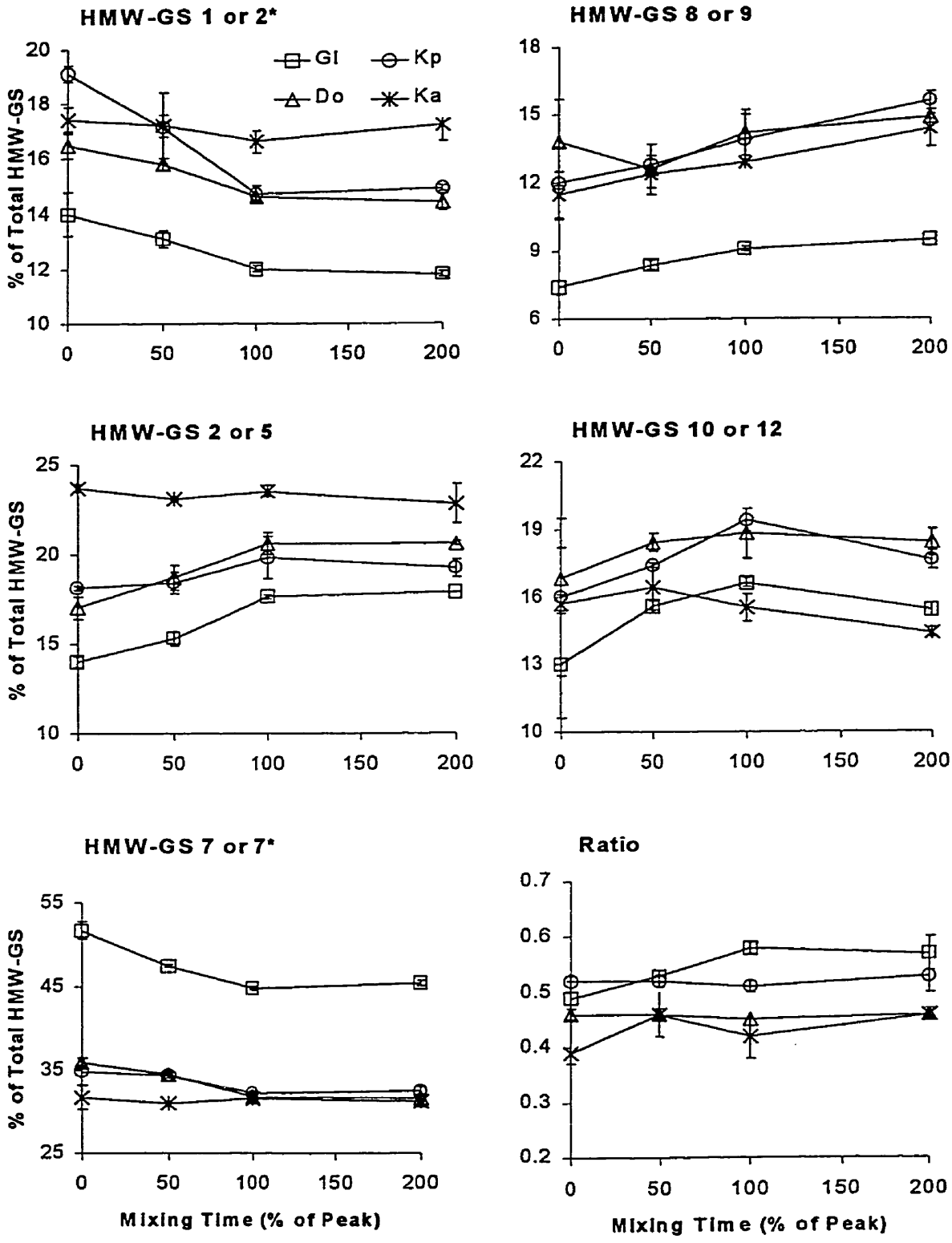
Al glutenin


HMW-GS 2*
% of Total Protein
18
16
14
62%
69%
50
100
150
200
HMW-GS 9
22
18
14
HMW-GS 5
24
22
20
18
HMW-GS 10
19
15
11
HMW-GS 7*
38
33
28
% of Total Protein
Mixing Time (% of Peak)
Ratio
.40
.30
.20
Mixing Time (% of Peak)

4.4.3. Iodate Doughs

The changes observed in the subunit composition of iodate doughs was comparable to those observed in the control doughs, with a few exceptions. For the ES glutenin (Figure 40), the relative amounts of HMW-GS 2* decreased until doughs were mixed to peak, then leveled off during overmixing. HMW-GS 1 did not significantly change over mixing time. The relative amounts of HMW-GS 7 or 7*, with the exception of AC Karma, were also observed to decrease as mixing progressed to peak, then leveled off. The relative amounts of 1Dx5 and 1By subunits (GS 8 or 9) increased with mixing. HMW-GS 1Dx2 did not exhibit any significant change due to mixing. The 1Dy subunits (GS 10 and GS 12) exhibited an increase in relative amount during the initial stages of mixing and then remained relatively unchanged (AC Domain) or decreased (AC Karma) after 50% MT. The increase observed for the stronger cultivars (Glenlea and Katepwa) continued up to peak. With the exception of Glenlea, the ratio remained relatively unchanged during mixing. Glenlea's ratio increased until peak was reached then stabilized while the ratios for the other cultivars were relatively unchanged. Glutenin soluble in ethanol became enriched with y-type subunits (1By and 1Dy) and 1Dx5 as iodate doughs were mixed to peak. Although little change was observed with mixing, the stronger cultivars retained the higher ratios and the weaker cultivars retained the lower ratios throughout.

Figure 40. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the ES fraction of glutenin during mixing of iodate doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

In the AS glutenin (Figure 41), the 1By subunits (GS 8 or GS 9) increased during mixing in a similar manner to the control dough. The 1Dy subunits (GS 10 or GS 12) decreased overall, with Glenlea and AC Domain exhibiting a similar initial rise in proportion observed in the control dough. The relative amounts of 1Bx subunits (7 or 7*) did not change significantly with mixing. In contrast to the same subunits in the control doughs and with the exception of Katepwa, the HMW-GS 1 and 2* increased in proportion until peak, then remained stable. The 1Dx2 and 1Dx5 subunits remained relatively unchanged. Ratios rose then declined as mixing progressed. Once again, the positive relationship between ratio and mixing strength was maintained throughout mixing. The ratios were higher for this fraction than for the ES fraction, suggesting that the AS glutenin was of greater M_r. Mixing enriched the glutenin soluble in dilute acetic acid with 1By and 1Ax subunits.

The changes in composition observed in the AI glutenin (Figure 42) were similar to those observed in the control doughs but fluctuated less. Except for a significant drop in the relative amount of HMW-GS 2* for Katepwa, the 1Ax (GS 1 and GS 2*) and 1Bx (GS 7 and 7*) subunits remained virtually unchanged during mixing. Glenlea and AC Domain exhibited some significant increases and decreases in the amount of HMW-GS 5, but the 1Dx subunits generally were not greatly affected by mixing. The proportion of 1By8 and 1By9 subunits appeared to increase with mixing but the change was not statistically significant. The proportion of 1Dy10 and 1Dy12 subunits was greater in the doughs than in the flour, except for AC Karma, and remained relatively stable during mixing. All cultivars except Katepwa exhibited significant decreases in the ratio of the AI glutenin during mixing.

Figure 41. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AS fraction of glutenin during mixing of iodate doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).

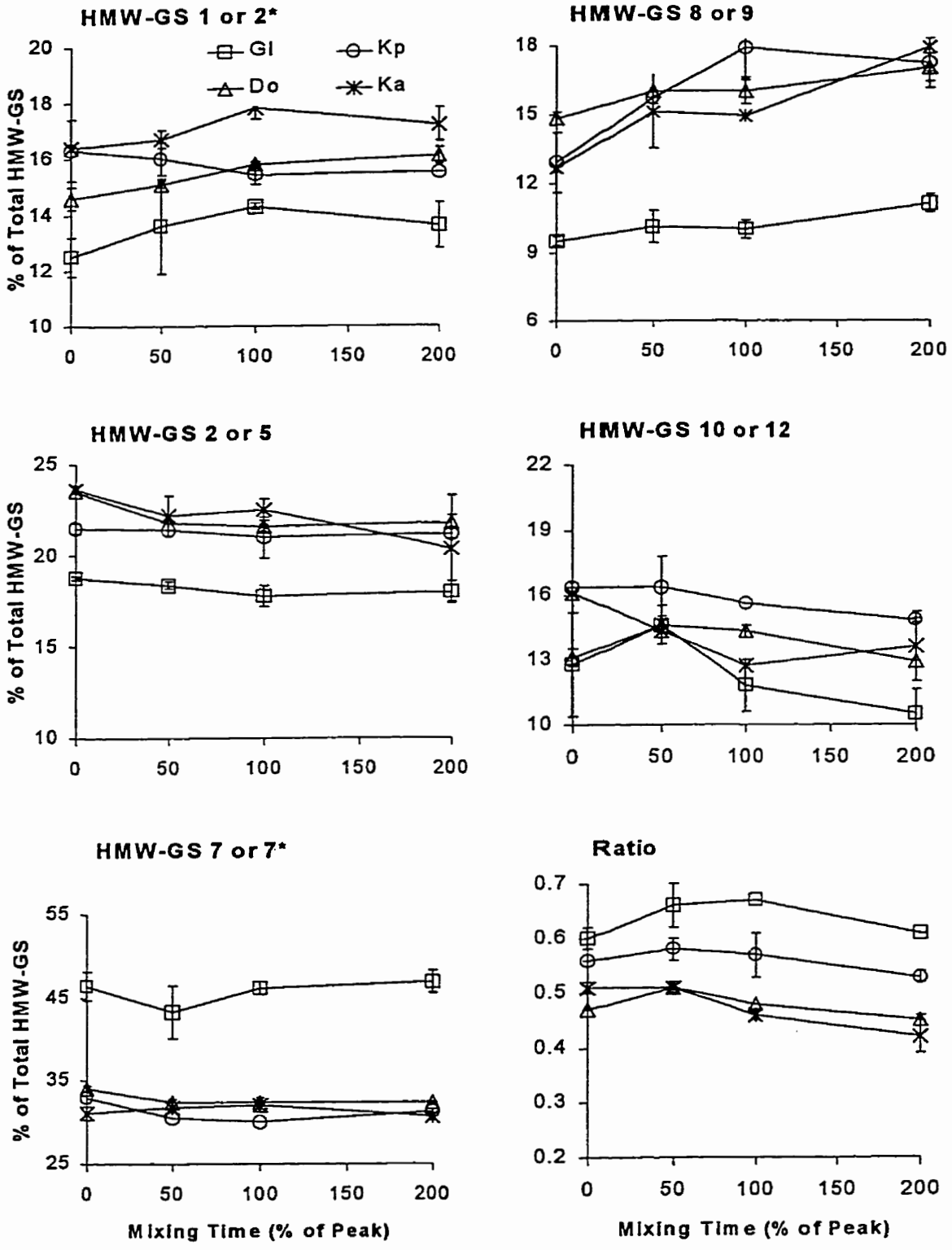
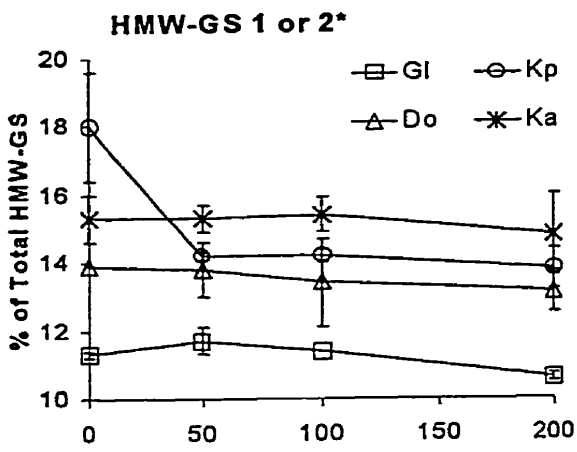
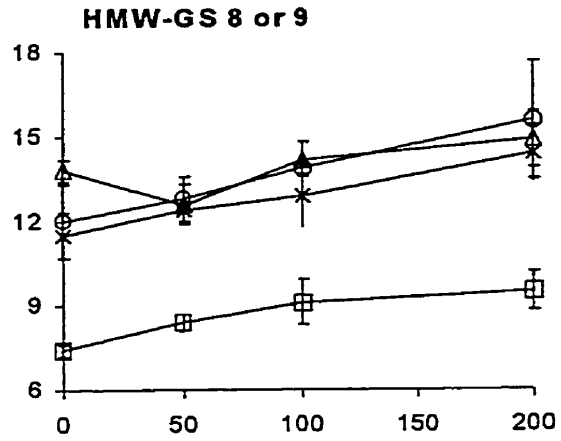
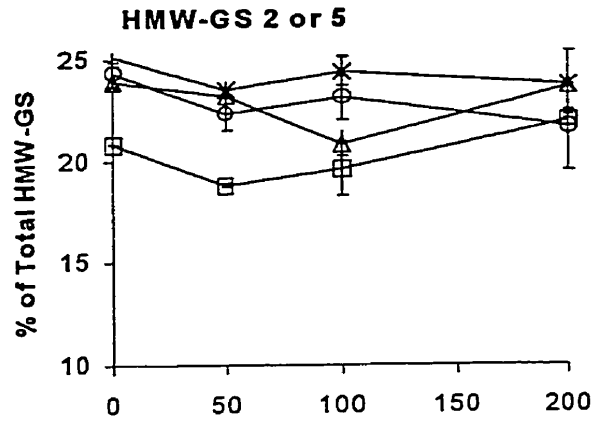
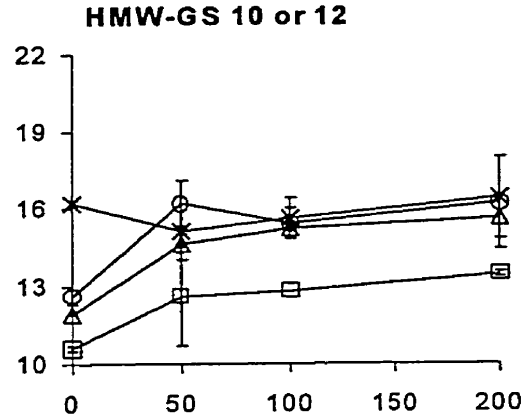

HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

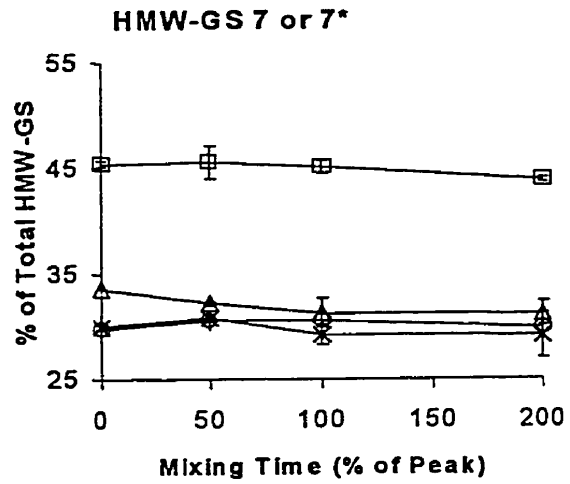
Figure 42. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AI fraction of glutenin during mixing of iodate doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).

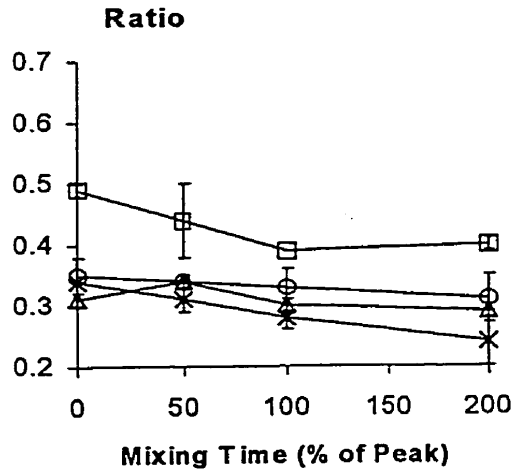

HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
20
18
16
14
12
10
0
50
100
150
200


HMW-GS 8 or 9
18
15
12
9
6
0
50
100
150
200


HMW-GS 2 or 5
% of Total HMW-GS
25
20
15
10
0
50
100
150
200


HMW-GS 10 or 12
22
19
16
13
10
0
50
100
150
200


HMW-GS 7 or 7*
% of Total HMW-GS
55
45
35
25
0
50
100
150
200
Mixing Time (% of Peak)


Ratio
0.7
0.6
0.5
0.4
0.3
0.2
0
50
100
150
200
Mixing Time (% of Peak)

Glenlea had the highest proportion throughout mixing while the other cultivars had similar values.

4.4.4. NEMI Doughs

The compositional changes occurring in the fractions obtained from NEMI doughs were similar to that of the control and iodate doughs, with a few exceptions. In the ES glutenin (Figure 43) little change was observed for the proportion of 1Ax subunits (1 and 2*), except for Katepwa during the initial stages of mixing. The proportion of 1Dx5 increased with mixing, while that of 1Dx2 did not. There was a significant decline in the proportion of 1Bx subunits for Glenlea, a small decline for AC Domain, and modest declines for Katepwa and AC Karma. The relative amounts of 1By subunits increased with mixing, but unlike the control and iodate doughs, the increase was only significant for Glenlea (GS 8) and AC Karma (GS 9). The proportion of 1Dy subunits initially increased, then declined after 50% MT. This change in proportion was similar to the control and iodate doughs, but only significant for AC Karma (GS 12). The ratio increased significantly for Glenlea and AC Karma.

No significant changes in the proportion of 1Ax and 1Bx subunits was observed in the AS glutenin (Figure 44). For the 1Dx subunits, the proportion of HMW-GS 5 was not significantly altered by mixing, while that of HMW-GS 2 fluctuated throughout mixing. The increase in relative amount of the 1By subunits with mixing that was observed for the control and iodate doughs was only somewhat significant for AC Domain NEMI treated dough. The proportion of 1Dy subunits declined after 50% MT, but was only significant

Figure 43. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the ES fraction of glutenin during mixing of NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).

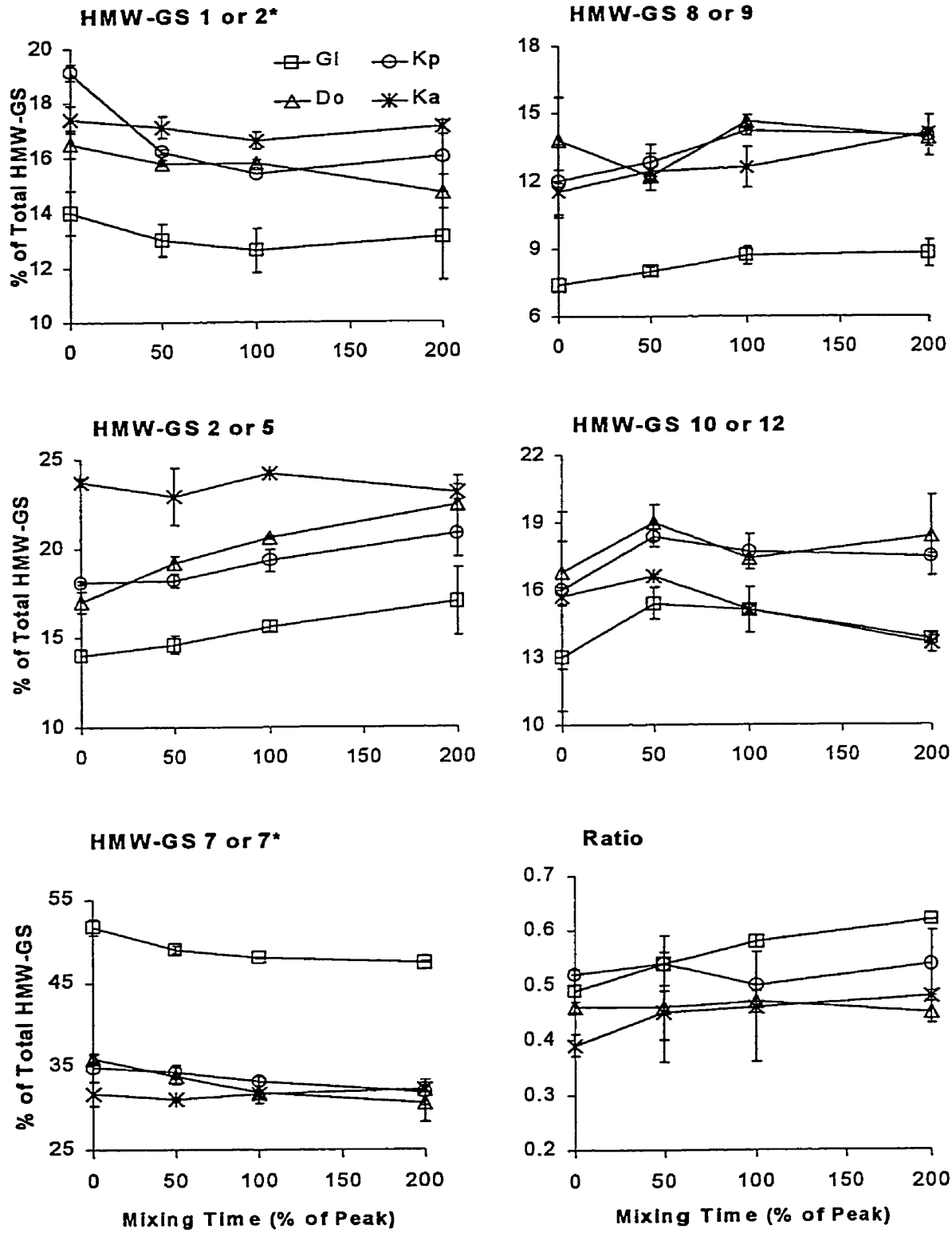
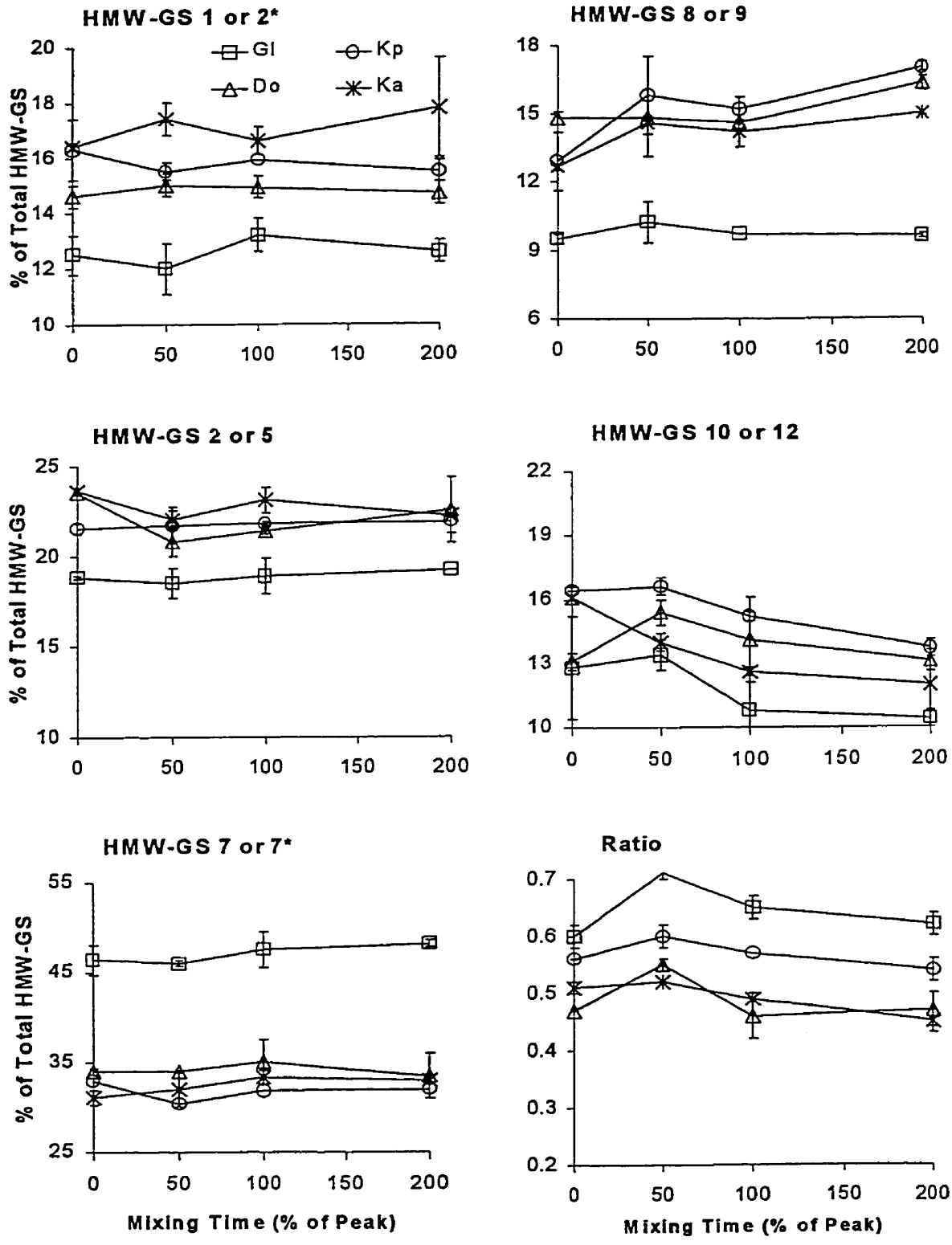

HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

Figure 44. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AS fraction of glutenin during mixing of NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

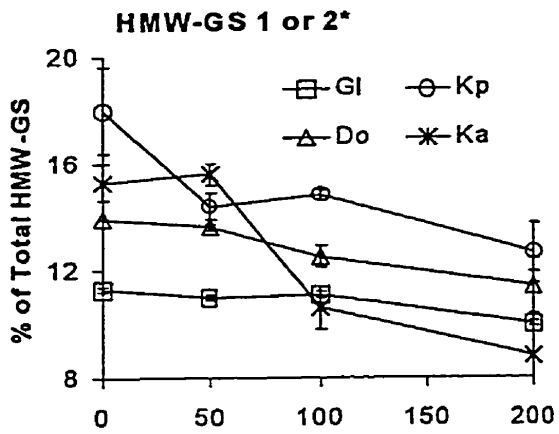
for AC Karma. The ratio also exhibited significant declines after 50% MT. The ratio was highest for Glenlea, intermediate for Katepwa, and lowest for AC Domain and AC Karma.

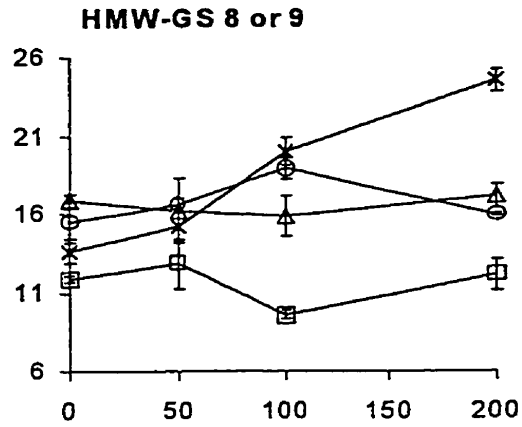
The proportions of HMW-GS in the AI glutenin fluctuated dramatically during mixing (Figure 45). The small decline in HMW-GS 1 and 2* observed in the iodate doughs is more significant in the NEMI doughs. The proportion of HMW-GS 7 and 7* was relatively unaffected by mixing. The variation and fluctuations (interactions) observed for the 1Dx and 1Dy subunits made it difficult to identify any trends in mixing behaviour for these subunits. For the 1Dx subunits, Glenlea and AC Domain exhibited increases after 50% MT. AC Karma appeared to decline but this decline was not significant. Katepwa initially declined, but rose again after peak. For the 1Dy subunits, there appeared to be an inital increase in the proportion to 50% MT or peak followed by an increase or decrease. The ratio for Glenlea declined in similar fashion to the ratio for control and iodate doughs. However, the other cultivars showed larger declines in ratio between 50% MT and peak. The direct relationship between ratio and mixing strength continued to be evident for the NEMI doughs throughout mixing.

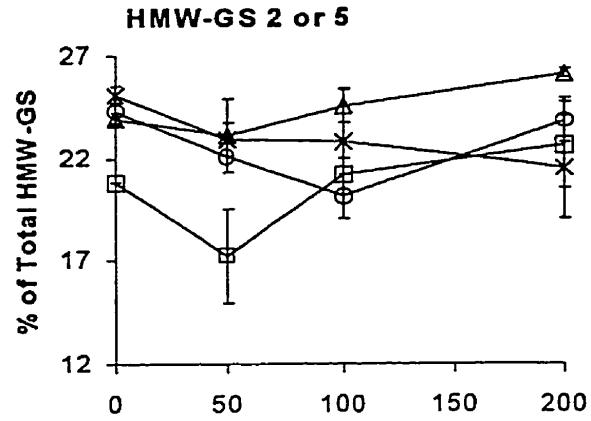
4.4.5. Treatment Effects and Interactions

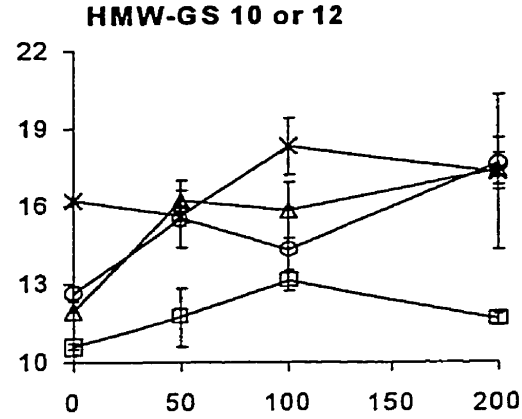
Complete tables for the effect of treatments on subunit composition during mixing are included in the Appendix (Tables 53-64). The most consistently significant effects (42-58% frequency) were observed for relative amounts of HMW-GS 7 or 7* and HMW-GS 10 or 12. With the exception of AC Domain, the proportion of HMW-GS 7 or 7* in the ES and AS glutenins was significantly lower for the control versus the treated doughs. The

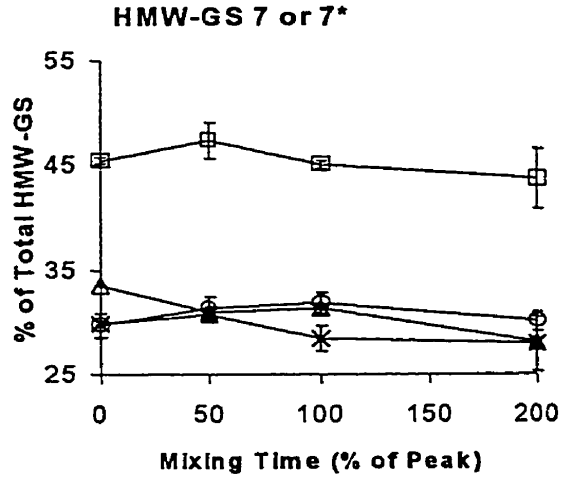
Figure 45. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AI fraction of glutenin during mixing of NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).

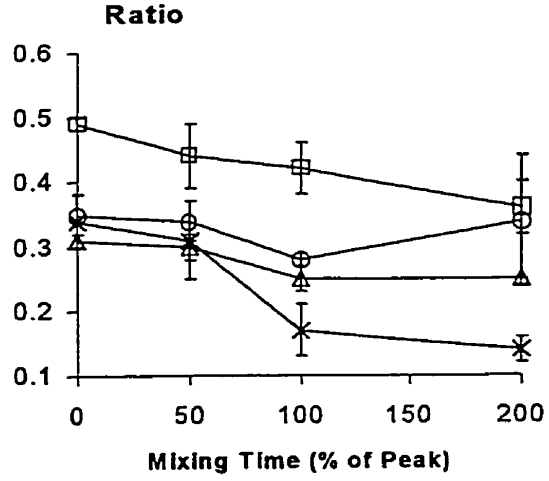

HMW-GS 1 or 2*
Gl
Kp
Do
Ka
% of Total HMW-GS
20
16
12
8
0
50
100
150
200


HMW-GS 8 or 9
26
21
16
11
6
0
50
100
150
200


HMW-GS 2 or 5
% of Total HMW-GS
27
22
17
12
0
50
100
150
200


HMW-GS 10 or 12
22
19
16
13
10
0
50
100
150
200


HMW-GS 7 or 7*
% of Total HMW-GS
55
45
35
25
0
50
100
150
200
Mixing Time (% of Peak)


Ratio
0.6
0.5
0.4
0.3
0.2
0.1
0
50
100
150
200
Mixing Time (% of Peak)

proportion of HMW-GS 10 or 12 in the ES and AS glutenins was significantly higher in the control versus the treated doughs. Significant effects were much less frequent for the other subunits and the ratio. The proportions of HMW-GS 2 or 5 and HMW-GS 1 or 2* in the AI glutenin were lower and higher in the control doughs, respectively, when compared to the iodate and NEMI doughs. The relative amount of HMW-GS 8 or 9 and the ratio also exhibited effects with less frequency, and they were less consistent in terms of effect and occurrence in specific fractions. In most cases, there was little difference between the effects of iodate or NEMI. When a difference was significant, NEMI was the more effective treatment. Other than the predominance of significant effects on HMW-GS 7/7* and HMW-GS 10/12 in the ES and AS glutenins, and to a lesser extent HMW-GS 2/5 and 1/2* in the AI glutenin, there did not appear to be any effects specific to one treatment or cultivar.

A table of F values for significant main effects and interactions between factors affecting the subunit composition and ratio has been included in the Appendix (Table 65). Because of the complexity of this group of variables and the lack of strong relationships, discussion of interactions was deemed of little significance to the overall results. The reader is referred to Table 65 in the Appendix for further information.

4.5. Changes During Mixing in the Content of ω-gliadins Co-precipitated with ES and AS Glutenins

The method for purification of the ES and AS modified Osborne fractions by 70% 1-propanol precipitation includes a sodium iodide wash (0.75M) of the 70PI pellet to remove ω-gliadins which co-precipitate with the glutenins. This step was omitted so that the content of contaminating ω-gliadins could be monitored during mixing. Figure 46 reports graphically the changes that occurred during mixing to the proportion of ω-gliadins (% of total fraction) in ES and AS glutenin for all treatments (for complete data, see Appendix, Tables 49-52). The proportion of ω-gliadins in ES glutenin (ranging from 12.7% to 41.5%) showed relatively consistent and significant decreases with mixing, especially for the iodate and NEMI doughs mixed from 50% MT to peak. There were cultivar differences but these were not related to quality. AC Karma had the lowest proportion of ω-gliadins in its ES glutenin, followed by Glenlea, AC Domain and Katepwa in order of increasing proportion. Any consistent changes in the proportion of ω-gliadins co-precipitated with the AS glutenin were less apparent. The proportion of ω-gliadins in the AS glutenin fraction (ranging from 2.8% to 23.1% with most below 14%) generally decreased as mixing progressed. Absorption did not appear to have any consistent or significant effect on the content of ω-gliadins present in the ES and AS glutenin fractions (results not shown).

The content of ω-gliadins was also reported as a percentage of total flour or dough protein (Figure 47). The content of ω-gliadins in the ES glutenin ranged from 0.9% to 5.2% while the content in the AS glutenin comprised less than 1.0% of total protein. An

Figure 46. Changes in the proportion of ω-gliadins (as % of ES and AS glutenin fractions) for control, iodate and NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).

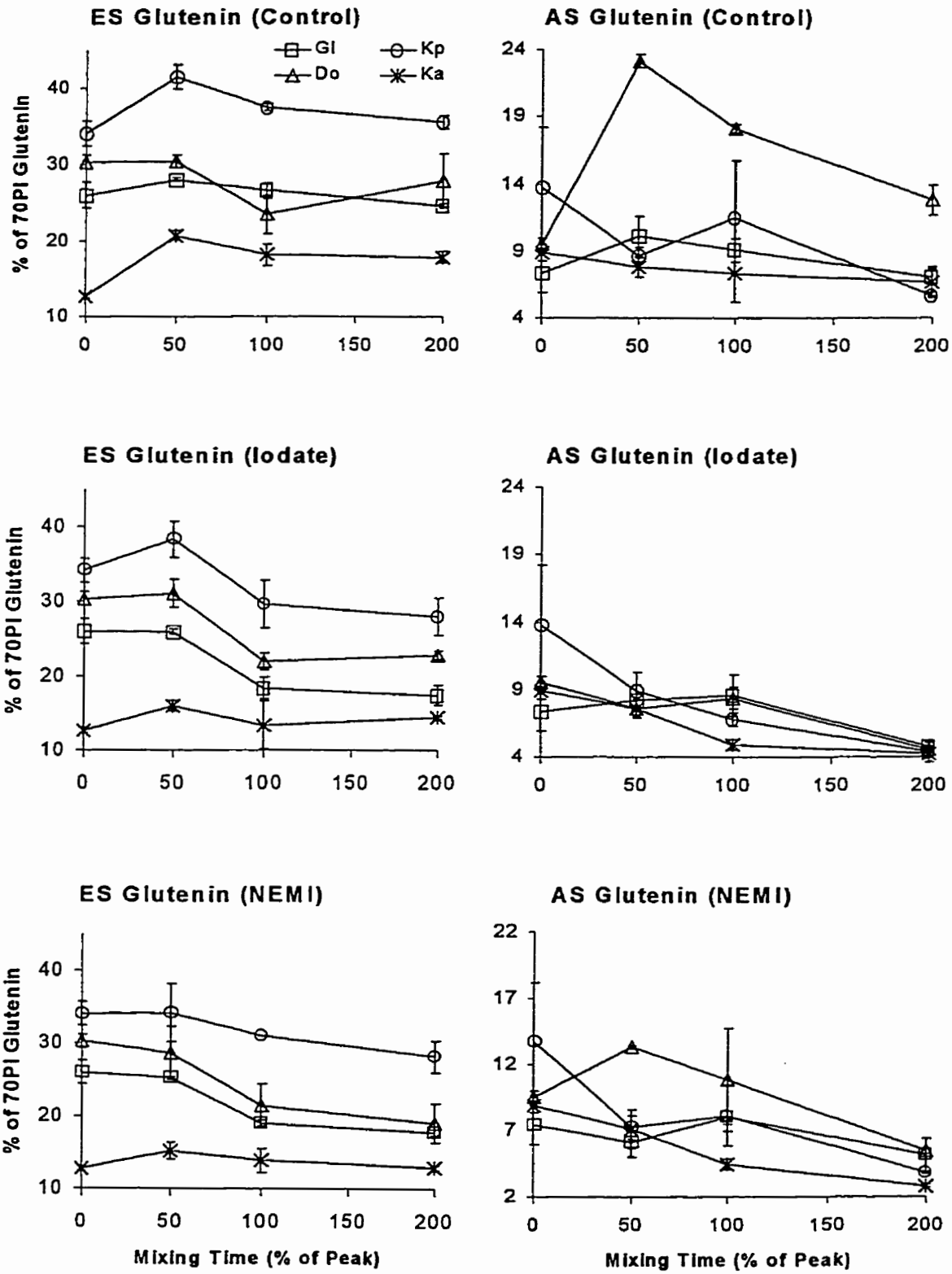
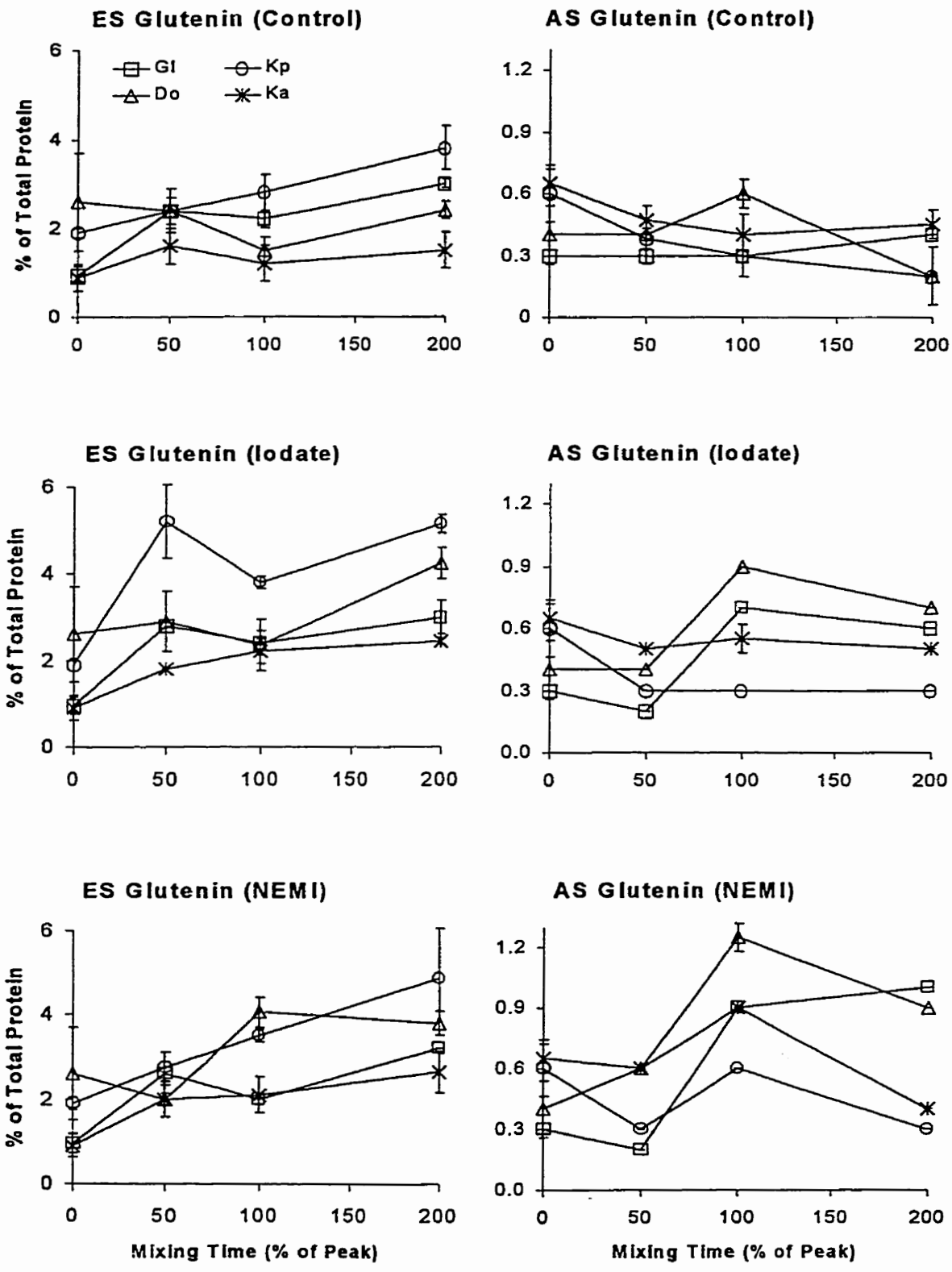

ES Glutenin (Control)
AS Glutenin (Control)
ES Glutenin (Iodate)
AS Glutenin (Iodate)
ES Glutenin (NEMI)
AS Glutenin (NEMI)
Gl
Kp
Do
Ka
% of 70PI Glutenin
Mixing Time (% of Peak)

Figure 47. Changes in the proportion of ω-gliadins (as % of total protein) in the ES and AS glutenin fractions for control, iodate and NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


ES Glutenin (Control)
AS Glutenin (Control)
ES Glutenin (Iodate)
AS Glutenin (Iodate)
ES Glutenin (NEMI)
AS Glutenin (NEMI)
% of Total Protein
Mixing Time (% of Peak)
Gl
Kp
Do
Ka
0
50
100
150
200
0
2
4
6
0.3
0.6
0.9
1.2
0.0

increase in the proportion of ω-gliadins was now observed in the ES glutenin as mixing progresses. For the control doughs, Glenlea and Katepwa, the stronger cultivars, exhibited significant increases while the changes for AC Domain and AC Karma were not significant. Some fluctuation in content occurred for the ES glutenin of iodate doughs. But with the exception of AC Domain, the cultivars exhibited an increase in the content of ω-gliadin in ES glutenin as mixing progressed. The same was true for the doughs treated with NEMI although a decline occurred for Glenlea and AC Domain at 50% and 100% MT, respectively.

Statistically, all the changes in the content of ω-gliadin in the AS glutenin were significant for all cultivars and treatments. However, because of the very low amount present, the changes were not considered of great significance to the role of gluten proteins in the mixing process.

Correcting the ES and AS gliadin and glutenin contents for the ω-gliadins does not diminish the evidence considered as support for the gliadin-glutenin interaction model. The loss of gliadin from the ES and AS fractions during the initial stages of mixing is slightly attenuated when the ω-gliadins are taken into account, but by no means eliminated. In fact, the cultivar-specific behaviour is accentuated. Glenlea and Katepwa appear to behave very differently from AC Domain and AC Karma. The loss in gliadin in the control doughs is much smaller for the stronger cultivars than the weaker cultivars. While in the iodate and NEMI doughs, the distinct loss is observed for the weaker cultivars only.

On its own, the fraction of ω-gliadins co-precipitated with glutenins in the purification procedure applied to the ES and AS fractions does not appear to be of major importance to dough mixing behaviour. Because of their rather low concentration (% of

total protein), these ω-gliadins may be an artifact of the purification process, or simply another indication of the inherent difficulties of solubility methods in producing pure fractions without cross-contamination. However, the quantity and behaviour of the ω-gliadins found in the ES glutenin suggest that interaction with glutenin is occurring and that as mixing progresses the ω-gliadins are released.

4.5.1. Treatment Effects and Interactions

Complete tables for the effect of treatments on ω-gliadin content during mixing are included in the Appendix (Tables 53-64). The proportion of ω-gliadins in the ES and AS glutenins was significantly higher in the control versus the treated doughs. In most cases, there was little difference between the effects of iodate or NEMI. When a difference was significant, NEMI was the more effective treatment.

A table of F values for significant main effects and interactions between factors affecting the ω-gliadin content has been included in the Appendix (Table 65). Because of the complexity of this group of variables and the lack of strong relationships, discussion of interactions was deemed of little significance to the overall results. The reader is referred to the table in the Appendix (Table 65) for further information.

4.6. Low Molecular Weight Glutenin: Presence in 70% 1-Propanol-soluble Fractions and Changes During Mixing

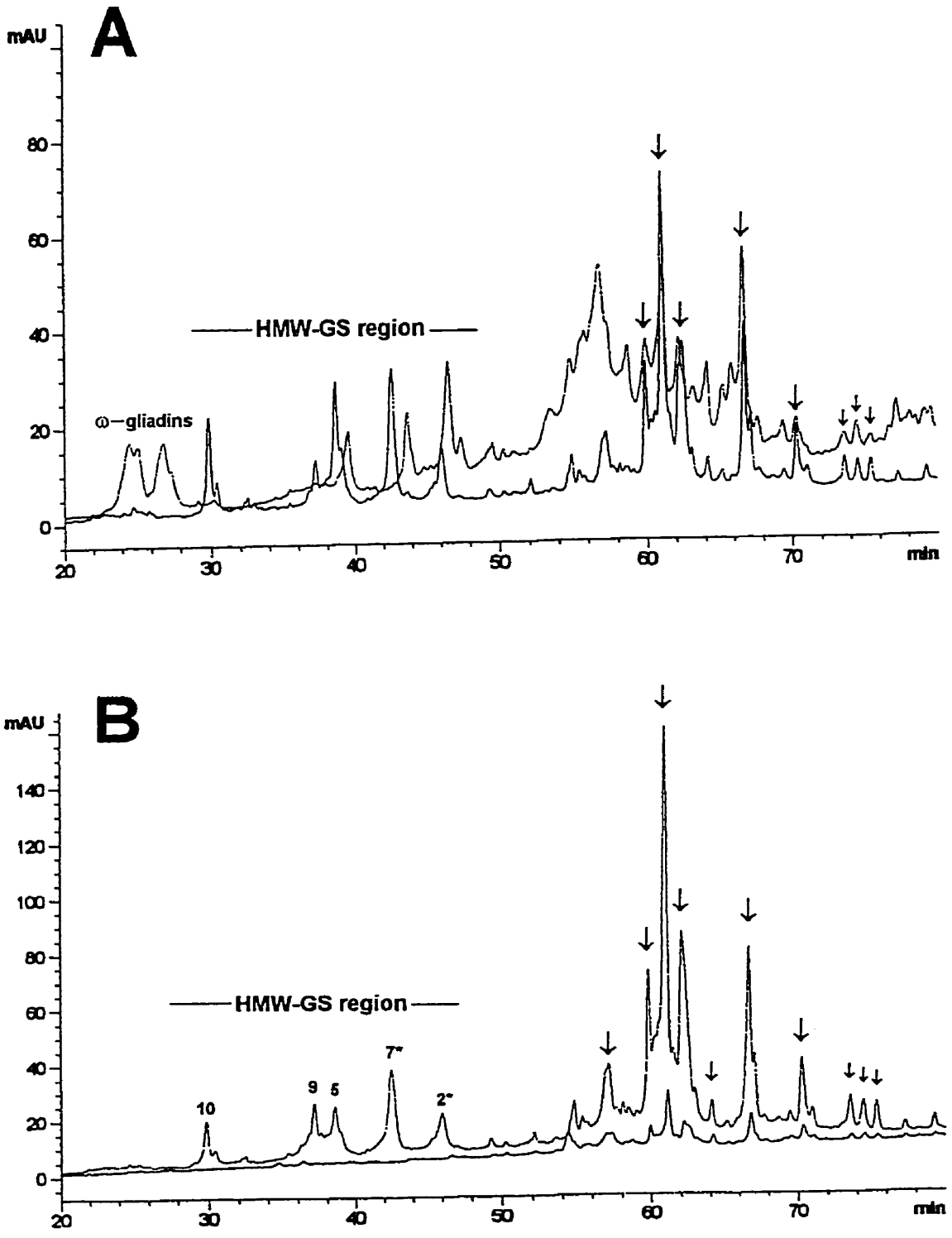
The ratio of HMW-GS to LMW-GS is considered to be an indicator of the relative molecular mass (M_r) of glutenin (Gupta et al, 1993). During the analysis of glutenin fractions, obtained from control doughs, by RP-HPLC it was noted that this ratio was higher for the glutenins soluble in 70% ethanol and dilute acetic acid than for the AI glutenin. This observation opposed the commonly held view that a larger ratio indicates a glutenin polymer of larger M_r. The possibility that incomplete reduction and alkylation of the AI glutenin could be the cause of the low ratio was investigated. (1) Reduction and alkylation must proceed under neutral to basic conditions. To investigate whether residual acetic acid was interfering with the reduction and alkylation, the AI residue was washed with water and dried prior to reduction and alkylation. (2) There is a large proportion of starch in the AI residue and the possibility that the starch was interfering was also investigated. After centrifugation, the gel-like AI fraction lies above the starchy residue. Once freeze-dried, most of the starchy residue was easily removed with a scalpel and the protein reduced and alkylated. (3) The concentrations of reducing and alkylating agents were doubled. None of these alterations in methodology affected the ratio of HMW-GS to LMW-GS (results not shown). What remained to be investigated was whether the source of the discrepancy in ratios was the more soluble fractions or the insoluble fraction.

The 70% 1-propanol (70P) purification method developed by Fu and Sapirstein (1996) provided electrophoretic evidence for the successful separation of monomeric protein from glutenin. Acid-PAGE indicated that most of the monomeric proteins, except for some ω-gliadins, were found in the 70PS supernatant. SDS-PAGE showed the

presence of HMW-GS only in the 70PI precipitate. The absence of HMW-GS in the SDS-PAGE patterns of the 70P supernatant was accepted as evidence that all the polymeric protein was precipitated by 70% 1-propanol. The presence of polymer comprised only of LMW-GS was not considered. In SDS-PAGE, the LMW-GS migrate in the same region as the monomeric gliadins and would not be discernible. Thus the successful separation of all glutenin polymers from the 70PS supernatant could not be categorically proven without investigating the presence or absence of a glutenin polymer lacking HMW-GS. In RP-HPLC, like in SDS-PAGE, the LMW-GS elute in the same region as the gliadins. In addition, the presence of ladder-like bands of ES, AS, or 70PS fractions in SDS-PAGE gels under non-reducing conditions (see Section 4.3 of this thesis, Almonte, 1998, Fu, 1996) suggest that glutenin oligomers and possibly larger polymers exist in the 70PS fraction. If indeed some glutenin comprising only LMW-GS remained in the 70PS supernatant, this glutenin would not be discovered without other methods of analysis, such as SEC or two-step electrophoresis. The ratios of the 70P purified ES and AS glutenins might be skewed to higher levels than expected because of the absence of these glutenins comprising only LMW-GS.

The quickest way to investigate the possibility that the 70P purification method was indeed leaving behind glutenin in the supernatant was to apply the method to the AI fraction. This fraction is normally analyzed as is because of the very low level of contaminating monomeric protein. Without the interference of gliadins and assuming that glutenin made up only of LMW-GS is also present in the AI fraction, 70P purification of the AI fraction followed by RP-HPLC analysis would reveal the presence of LMW-GS in the supernatant. Figure 48.A is a RP-HPLC chromatogram overlay of AS gliadin which

Figure 48. RP-HPLC chromatograms of fractions obtained from Katepwa flour (control) or dough (100% MT). (A) Control sample (50% 1-propanol-insoluble residue from Katepwa flour) overlaid with ASPS obtained by 70P purification of the AS fraction of Katepwa dough (100% MT). (B) 70PS and 70PI fractions obtained by purification of the AI glutenin fraction. Peaks considered to be LMW-GS in the control sample (A) and AIPI sample (B) but also present in the ASPS and AIPS samples (identical R_t) are highlighted with (↓).


A
mAU
80
60
40
20
0
HMW-GS region
ω−gliadins
20
30
40
50
60
70
min
B
mAU
140
120
100
80
60
40
20
0
HMW-GS region
10
9
5
7*
2*
20
30
40
50
60
70
min

was obtained by 70P purification of total AS protein and a control (50% 1-propanol-insoluble residue from Katepwa flour). The arrows indicate the presence of peaks in the AS gliadin which correspond to LMW-GS in the control (identical R_t). Figure 48.B is a RP- HPLC chromatogram overlay of the 70PS supernatant and 70PI precipitate obtained by 70P purification of the AI fraction of Katepwa dough. Arrows indicate the presence of peaks in the propanol soluble fraction corresponding to LMW-GS of the propanol-insoluble fraction. No peaks were discernible in the HMW-GS region.

Additional evidence for the presence of glutenin in the 70PS supernatant was provided by two-step SDS-PAGE. Katepwa flour and dough samples were subjected to SDS-PAGE under non-reducing conditions in the first step (Figure 49). The monomeric proteins migrate into the gel and the polymeric protein remains in the wells. The slot protein from the first gel is then cut away, incubated in a solution containing reducing agent, and applied to a second gel to analyze for the presence and composition of glutenin. The presence of LMW-GS but not HMW-GS was observed in the ESPS and ASPS fractions for the flour and doughs (Figure 50).

Final evidence was obtained by SDS-PAGE analysis of fractions collected from SEC of the ESPS fraction of Katepwa control dough (100% MT). Seven fractions were collected (Figure 51) and subjected to SDS-PAGE under non-reducing and reducing conditions (Figure 52). SDS-PAGE of the collected fractions (fractions 1-6) confirmed that the broad early eluting peak was glutenin containing only LMW-GS. The last fraction (fraction 7) corresponding to the largest peak in the chromatogram was identified as gliadin.

Figure 49. Two-step SDS-PAGE (Step 1). SDS-PAGE under non-reducing conditions of ESPS (ES) and ASPS (AS) fractions of Katepwa flour and control doughs (150 mg) mixed to 50%, 100% and 200% peak.

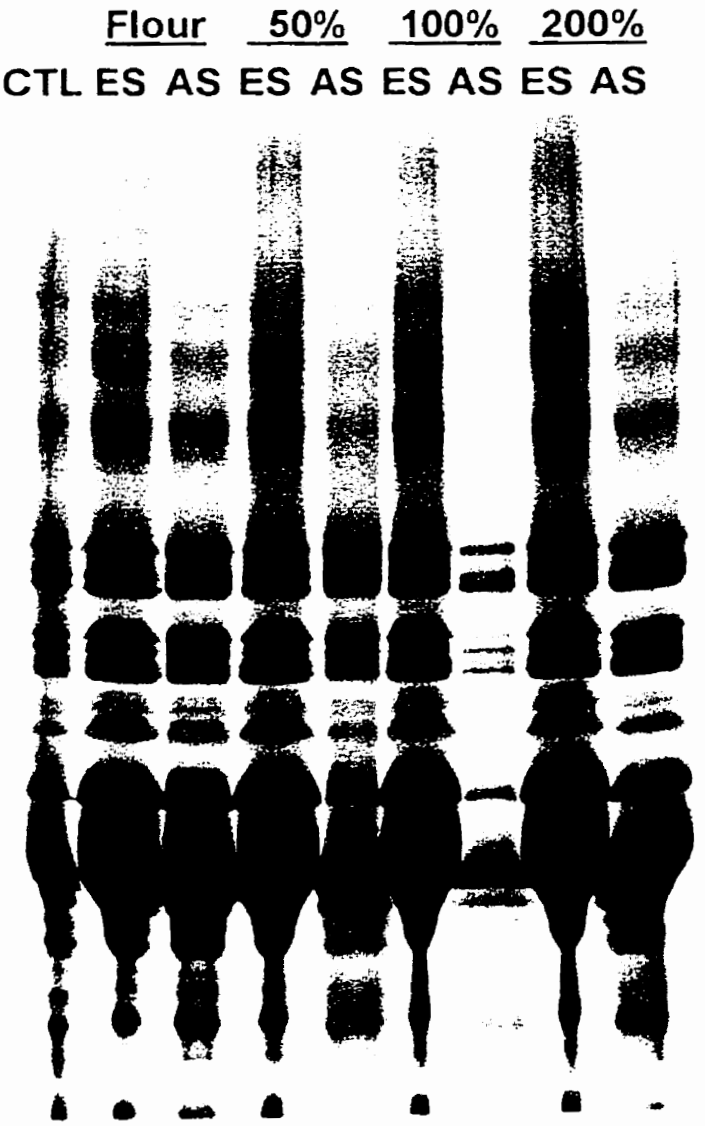

Flour 50% 100% 200%
CTL ES AS ES AS ES AS ES AS

Figure 50. Two-step SDS-PAGE (Step 2). SDS-PAGE under reducing conditions of the slot protein from Step 1 (Figure 49). ESPS (ES) and ASPS (AS) fractions of Katepwa flour and control doughs (150 mg) mixed to 50%, 100% and 200% peak.

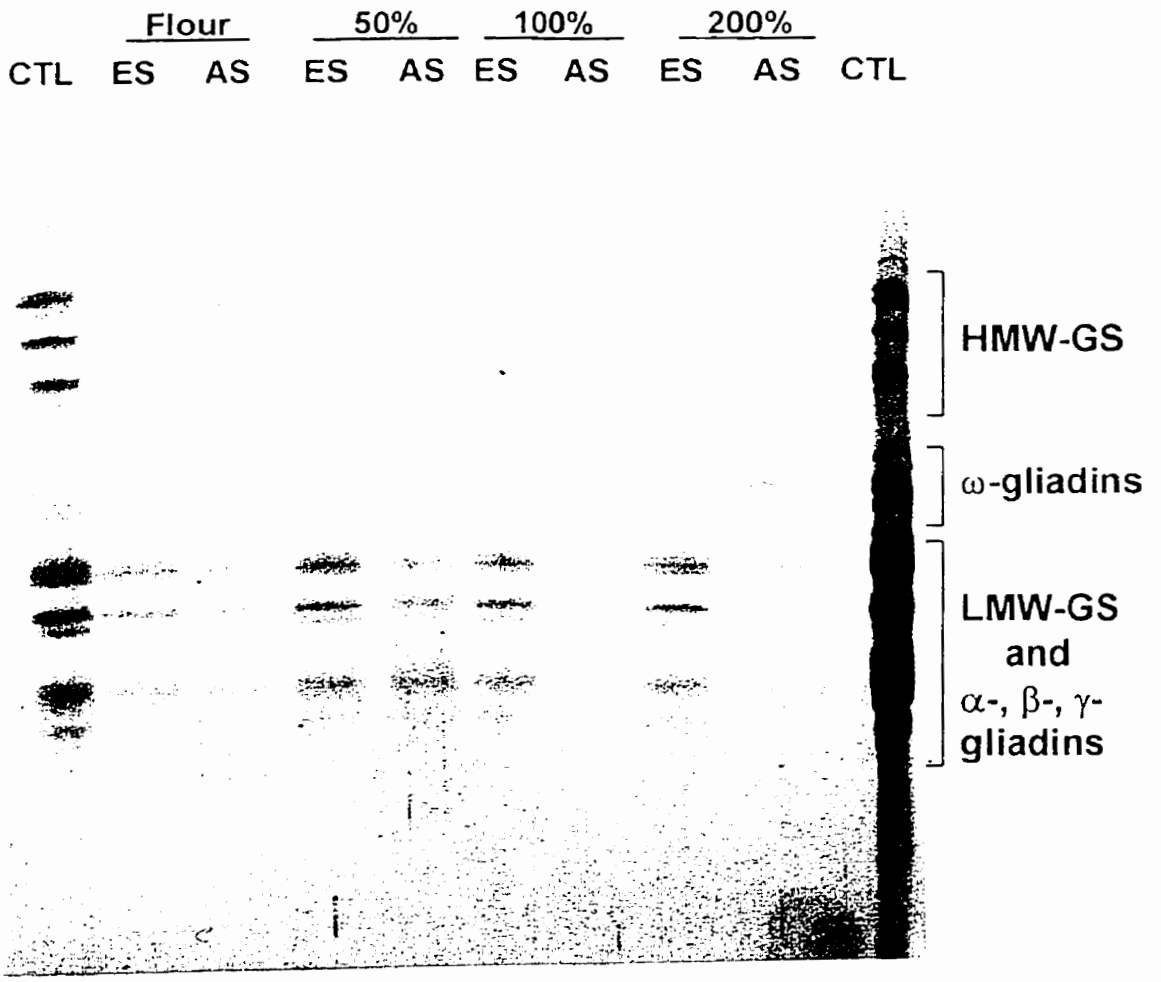

Flour
50%
100%
200%
CTL ES AS ES AS ES AS ES AS CTL
HMW-GS
ω-gliadins
LMW-GS and α-, β-, γ-gliadins

Figure 51. SE-HPLC chromatogram of a 70P supernatant fraction indicating the fractions collected for SDS-PAGE analysis.

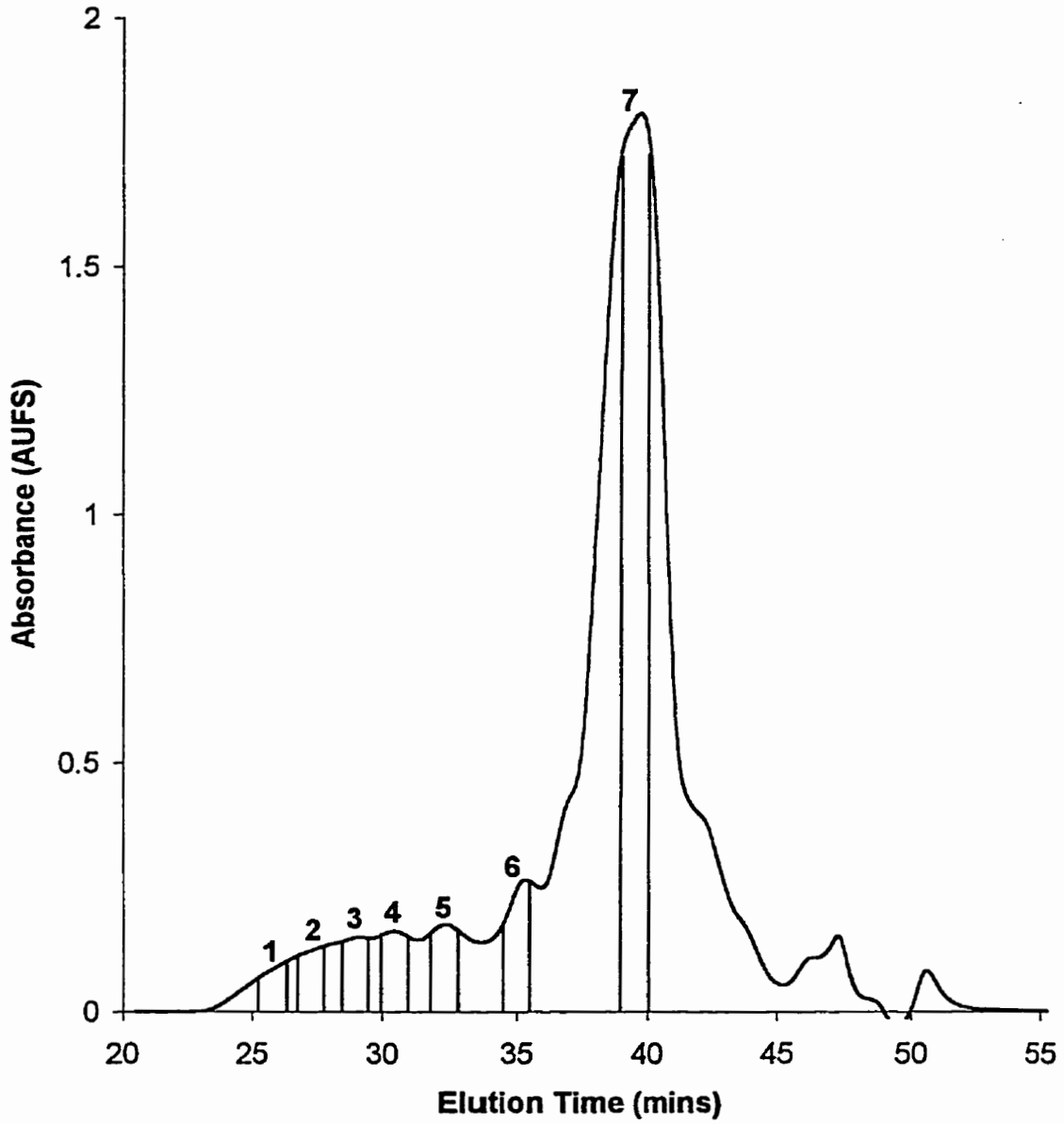
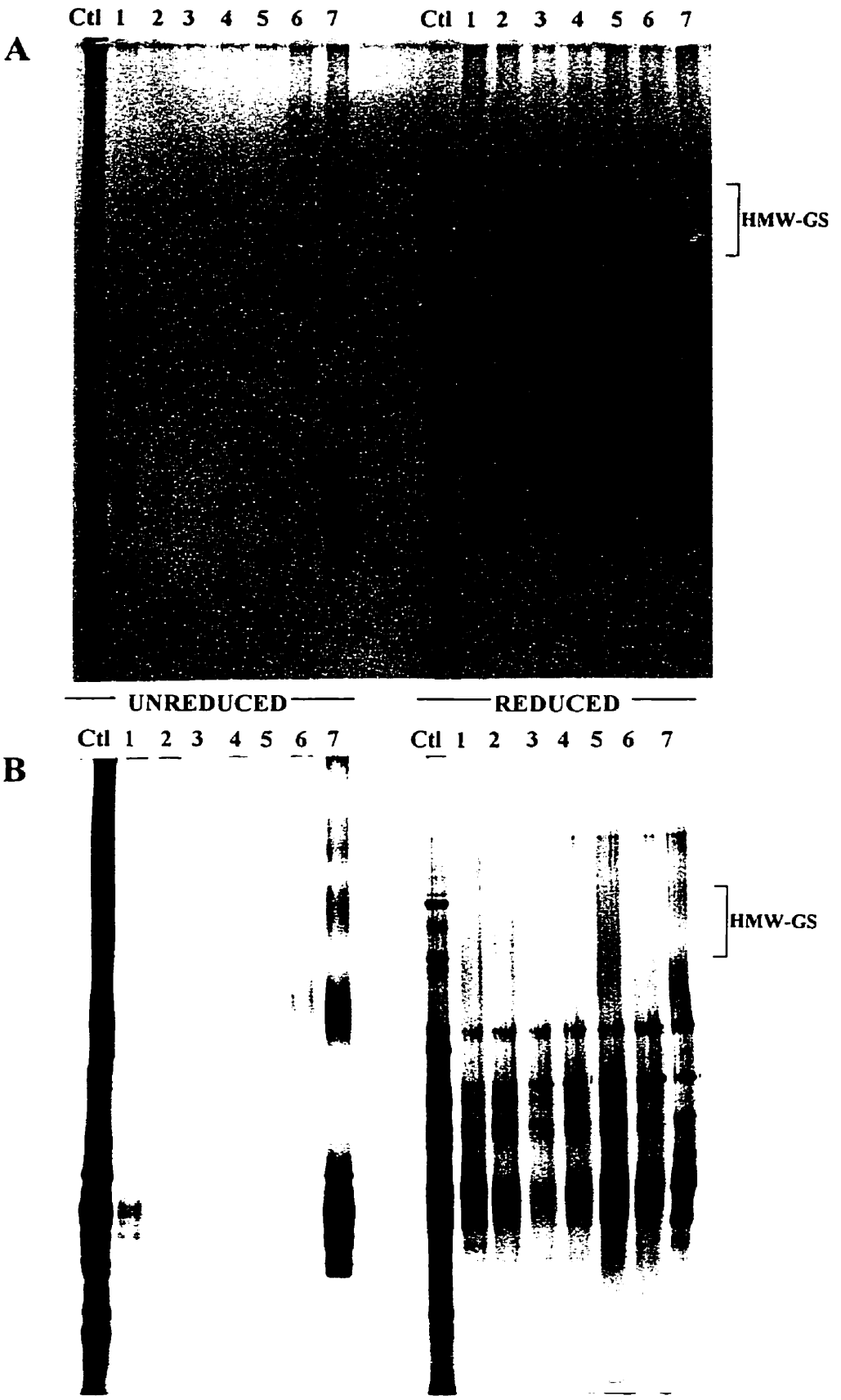

2
1.5
1
0.5
0
Absorbance (AUFS)
1
2
3
4
5
6
7
20
25
30
35
40
45
50
55
Elution Time (mins)

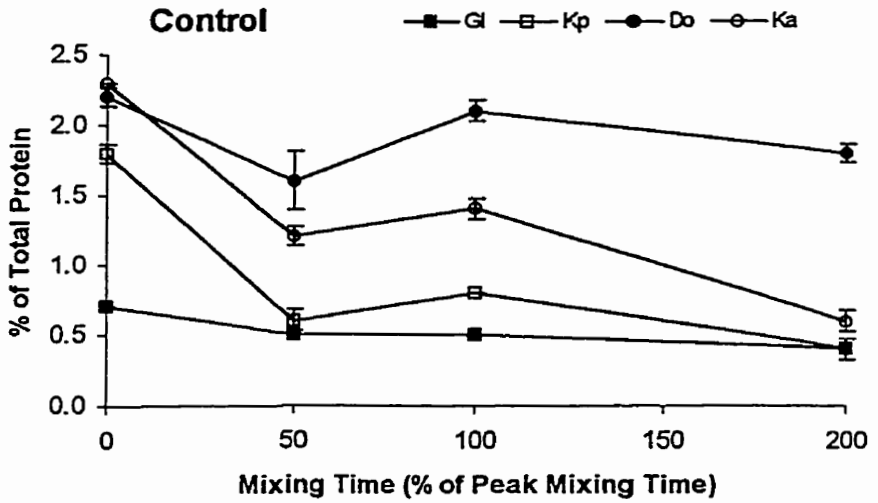
Figure 52. SDS-PAGE of unreduced and reduced fractions collected from SE-HPLC of the ESPS fraction of Katepwa control dough (100% MT). Numbered lanes (1-7, in order of decreasing M_r) correspond to numbered fractions in Figure 51. (A) Coomassie Brilliant Blue stain. (B) Silver stain.

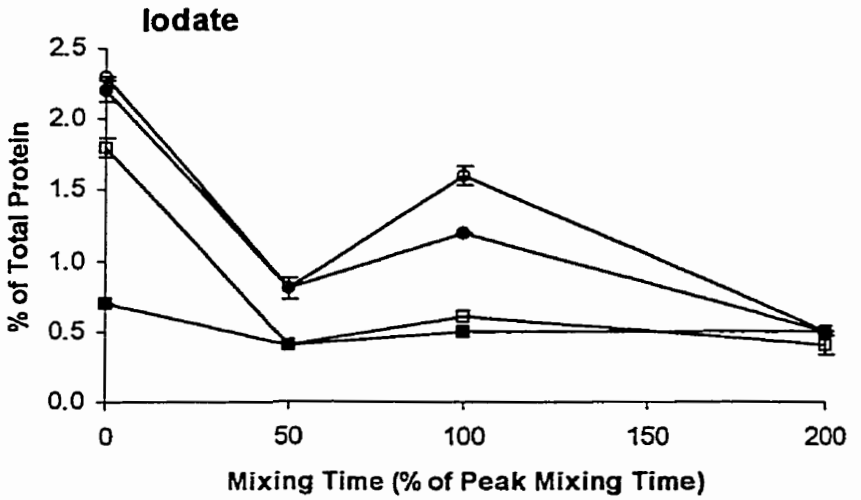

A
Ctl 1 2 3 4 5 6 7
Ctl 1 2 3 4 5 6 7
HMW-GS
UNREDUCED
REDUCED
B
Ctl 1 2 3 4 5 6 7
Ctl 1 2 3 4 5 6 7
HMW-GS

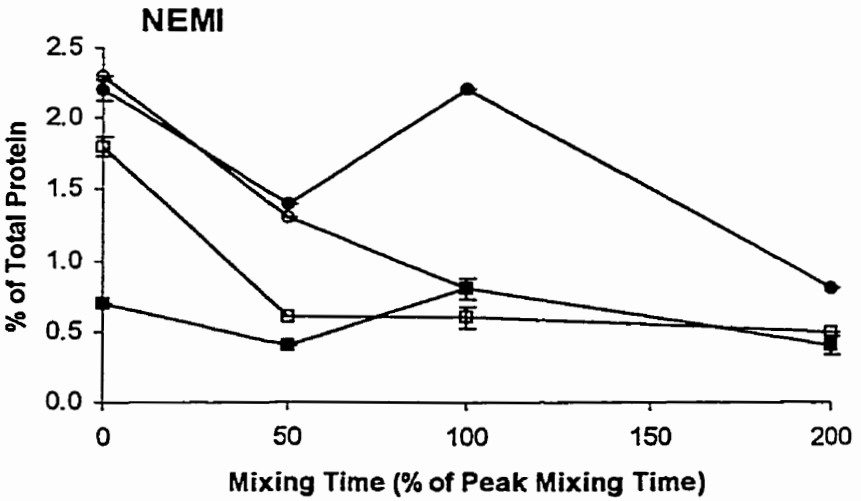
The effect of mixing on the content of LMW glutenin (glutenin comprising only LMW-GS and soluble in 70% 1-propanol) in the ES and AS fractions was evaluated. The peaks corresponding to glutenin (sample SE-HPLC chromatogram in Appendix (Figure 2) were integrated and the proportion of LMW glutenin in the ES and AS fractions was found to range between 16-30% and 17-28%, respectively (results not shown). Huebner and Bietz (1993) reported the proportion of ethanol-soluble glutenin recovered by Osborne fractionation and SE-HPLC ranged from 20-37% for six cultivars studied. The higher range reported by these authors may be due to the presence of ES glutenin containing HMW-GS as well as LMW-GS. The amount of glutenin in each fraction as a proportion of total flour or dough protein was then calculated and statistically evaluated (Appendix, Tables 66-71).

There was a sharp decrease in the quantity of LMW (AS) glutenin during the initial mixing period for all cultivars and treatments (Figure 53). This was followed by an increase as doughs were mixed to peak and a more gradual drop during overmixing. The quantity also appeared to be somewhat cultivar dependent, with Glenlea containing the least amount and AC Domain or AC Karma containing the most. The loss of ASPS glutenin was accentuated slightly by the presence of iodate. Though effects of treatment were significant (Appendix, Tables 70-71), the changes were small and showed no consistent trend. Despite the statistically significant cultivar ranking and changes occurring in this fraction of glutenin, the very low amounts present (less than 2.5% of total protein) suggest the importance to mixing behaviour and quality may be minimal. However, the initial drop in the content of ASPS glutenin characteristic of the ES and AS fractions should be kept in mind.

Figure 53. Changes in the ASPS glutenin (% of total protein) during mixing of control, iodate and NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


Control
Gl
Kp
Do
Ka
% of Total Protein
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

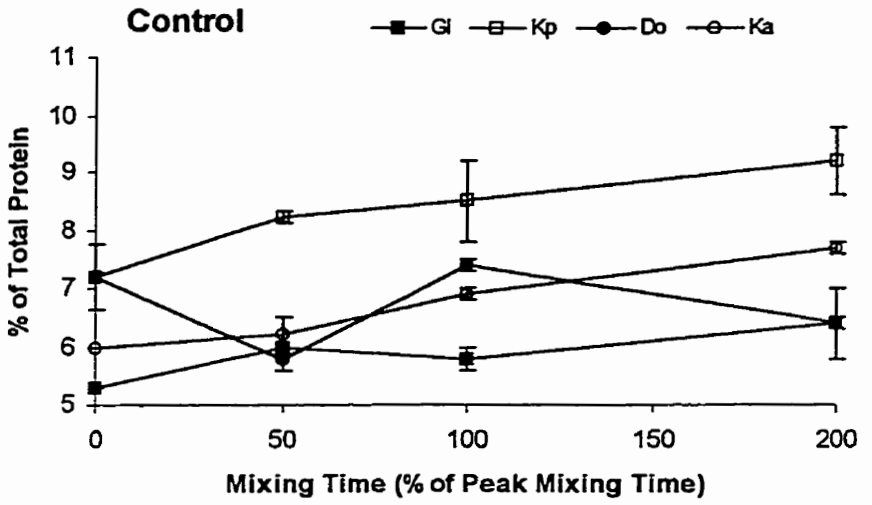

Iodate
% of Total Protein
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

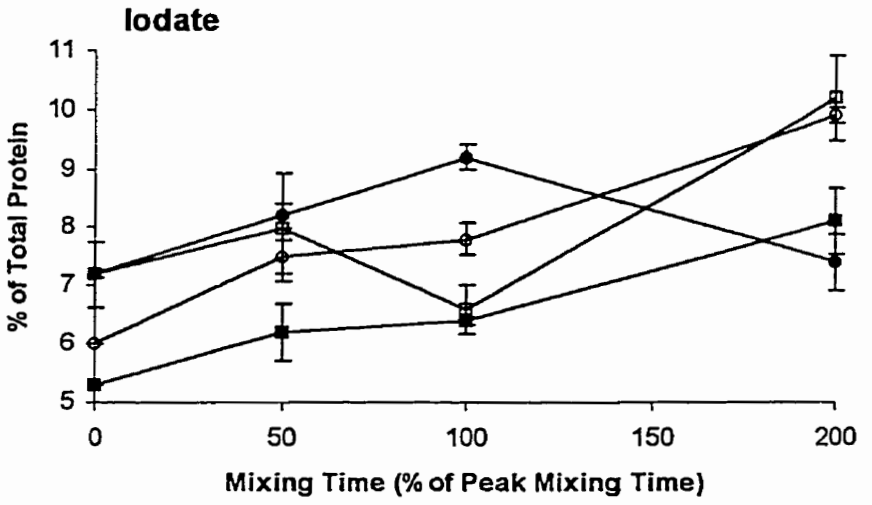

NEMI
% of Total Protein
2.5
2.0
1.5
1.0
0.5
0.0
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

The ESPS glutenin represents a much greater proportion of total protein, ranging between 5-11%. Graveland et al (1982) quantified the amount of what they termed glutenin III and reported intermediate amounts, 6% and 8% of total protein, for two different cultivars studied. The amount of ESPS glutenin generally increases as mixing progresses for all cultivars and all treatments (Figure 54). Interactions are more prevalent in this fraction and suggest that there is no relationship between cultivar and the amount or the mixing behaviour of ESPS glutenin.

After modified Osborne fractionation, 70% 1-propanol purification and SEC, most of the glutenin and gliadin was now purified and separated into several fractions (except the gluten proteins lost to the SS fraction or remaining unreducible in the AI fraction). Figure 55 is a graphical representation of total gliadin and total soluble glutenin for the four cultivars and three treatments studied. Total gliadin is the sum of the gliadin obtained by 70P purification and corrected for LMW glutenin. Total soluble glutenin includes the ESPI, ASPI fractions corrected for LMW glutenin. Changes in the two protein groups are very similar when corrected for ω-gliadins co-precipitated with glutenins (data not shown). What stands out the most in this figure is the mixing behaviour of the gliadins. The characteristic initial loss in gliadin content for the weaker cultivars indicating gliadin-glutenin interaction is now unmistakable, particularly for the control and NEMI doughs. The loss of gliadin was partially recovered as mixing progressed. In the case of the stronger cultivars, a similar loss of gliadin was observed but the magnitude of the loss and the ensuing recovery is much smaller. This supports the hypothesis that gliadin-glutenin interaction is of much greater significance in the weaker cultivars, but is nevertheless present in the stronger cultivars.

Figure 54. Changes in the ESPS glutenin (% of total protein) during mixing of control, iodate and NEMI doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


Control
Gi
Kp
Do
Ka
% of Total Protein
11
10
9
8
7
6
5
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)


Iodate
% of Total Protein
11
10
9
8
7
6
5
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

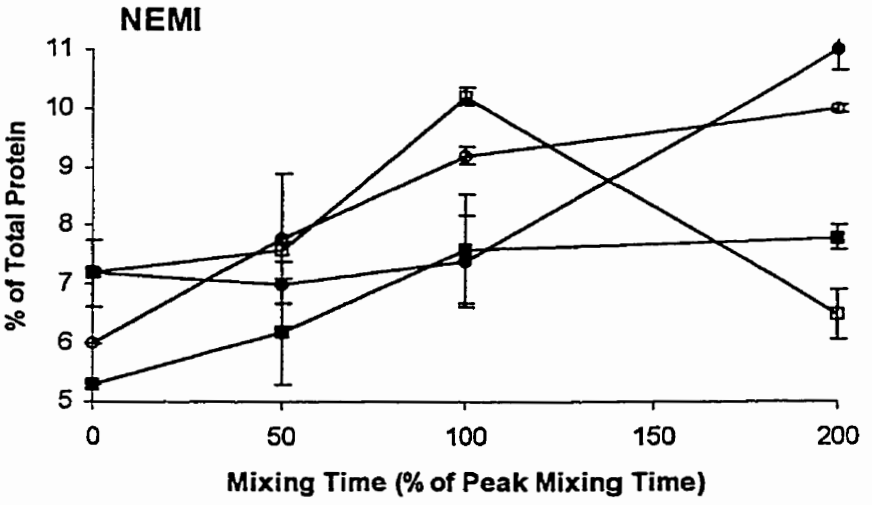
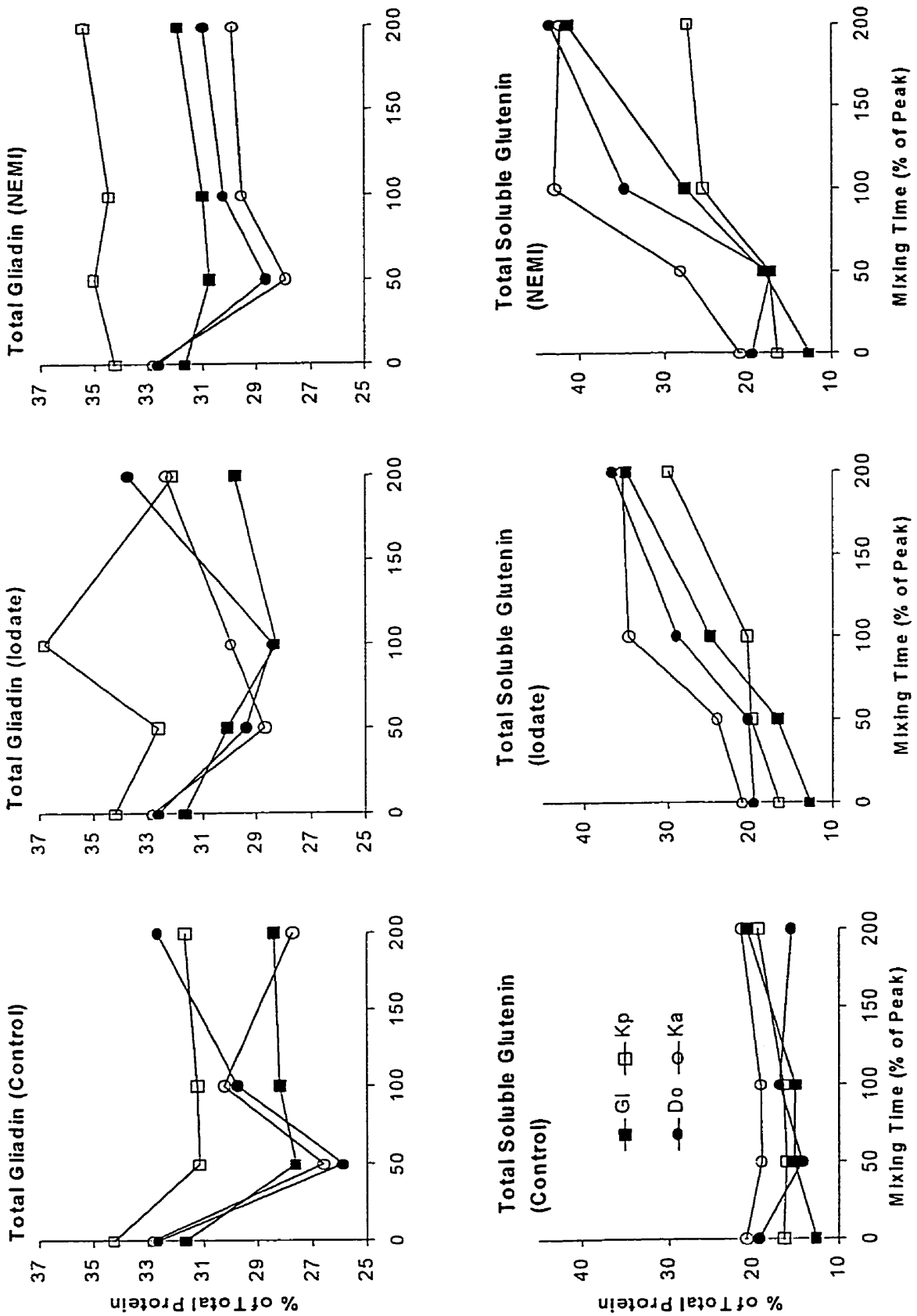

NEMI
% of Total Protein
11
10
9
8
7
6
5
0
50
100
150
200
Mixing Time (% of Peak Mixing Time)

Figure 55. Total gliadin (ES gliadin and AS gliadin minus 70PS glutenin, % of total protein) and total soluble glutenin (ES glutenin and AS glutenin plus 70PS glutenin, % of total protein) in flours and control doughs prepared from Glenlea (Gl), Katepwa (Kp), AC Domain (Do) and AC Karma (Ka).


Total Gliadin (Control)
Total Gliadin (Iodate)
Total Gliadin (NEM)
Total Soluble Glutenin (Control)
Total Soluble Glutenin (Iodate)
Total Soluble Glutenin (NEM)
% of Total Protein
Mixing Time (% of Peak)
Gl
Kp
Do
Ka

The existence of glutenin comprised only of LMW-GS has been previously reported in the literature. In most cases, this LMW glutenin was identified through the study of null lines (Gao and Bushuk, 1993, Gupta et al, 1995) and through the analysis of solubilized proteins by gel filtration chromatography (Bietz and Wall, 1973, Graveland et al, 1982, Tatham et al, 1987). Very little research has focused on the influence of this LMW glutenin on breadmaking quality or mixing behaviour. Huebner and Bietz (1993) showed that the subunit composition of ethanol-soluble glutenin varied with cultivar, as was to be expected. When this LMW glutenin was analyzed by SE-HPLC, the elution profiles varied in pattern and amount.

This study has shown that, like glutenin comprised of both HMW-GS and LMW-GS, the LMW glutenin is present in flour in a range of M_r, with peak distribution being skewed toward the more soluble fractions (viz. 70% ethanol, 70% 1-propanol) of lower M_r. As a percentage of total protein, 5.3-11.0% in the ES fraction, 0.4-2.3% in the AS fraction, and a small amount in the AI fraction, estimated at less than 2%, LMW glutenin comprised only of LMW-GS represents at least 8-15% of total flour protein.

Glutenin comprised only of LMW-GS represents a fair amount of protein in flour. The emphasis of quality, or strength, lies with the larger insoluble fraction of flour protein and the soluble glutenins and gliadins have attracted less attention from researchers. However, it appears that the soluble glutenins and gliadins may play an important role in mixing behaviour. Although at times conflicting, there are a number of reports relating gliadins and LMW-GS to quality parameters (Branlard and Dardevet, 1985a, Gupta and Shepherd, 1987, Metakovsky et al, 1997). The results reported here and in other chapters clearly illustrate that gliadins interact with glutenins during the inital stages of mixing. The

observed drop in ASPS glutenin during the initial stages of mixing is also noteworthy. The possibility that soluble LMW glutenins interact non-covalently with the larger glutenin polymer and gliadins to facilitate dough development should also be considered. Isolation and study of this LMW glutenin could provide an opportunity to study the conformations of native glutenin which may offer clues to the changes that occur during mixing.

4.7. Changes in Glutenin Subunit Composition and Ratio of HMW-GS to LMW-GS During Mixing of Full Formula Doughs With and Without Resting

The results reported in this thesis are based on studies with flour-water doughs. The effect of full formula ingredients and resting on changes in gluten proteins was evaluated for a select number of freeze-dried dough samples kindly provided by Dr. F. Békés of CSIRO, Sydney, Australia.

Because the full formula doughs included yeast, the protein solubility distribution, which is normally determined by Kjeldahl analysis, was not performed (interference of yeast nitrogenous matter). The subunit composition was investigated by RP-HPLC and the total area of the chromatograms used as a measure of the amount of each glutenin fraction investigated (ES, AS and AI). The subunit composition, ratio, and ω-gliadin content for the flour-water control doughs prepared in Winnipeg (mixed to 50%, 100% and 200% of peak) and the full formula doughs prepared in Australia (mixed to 67%, 100% and 150% of peak) were compared. Very few significant differences were found (results not shown), despite the variation in mixing times for the under- and overmixed doughs.

The changes occurring in the relative amount of each HMW-GS and the ratio for the ES glutenin (Figure 56), AS glutenin (Figure 57) and AI glutenin (Figure 58) were comparable to those observed in the flour-water control doughs reported in the previous section. No significant differences were found between doughs rested for 0 or 2 hours, with three minor exceptions (HMW-GS 9 in ES glutenin mixed to 150%, HMW-GS 7* in AS glutenin mixed to 100%, HMW-GS 7* in AI glutenin mixed to 150%). A few more significant differences were observed when doughs mixed to peak were rested for 4 hours (Appendix, Tables 72-76). But, in general, full formula treatment and resting time

Figure 56. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the ES fraction of glutenin during mixing of full formula doughs prepared from Glenlea, rested 0h (Gl-0) or 2 h (Gl-2), and from Katepwa, rested 0 h (Kp-0) or 2 h (Kp-2).

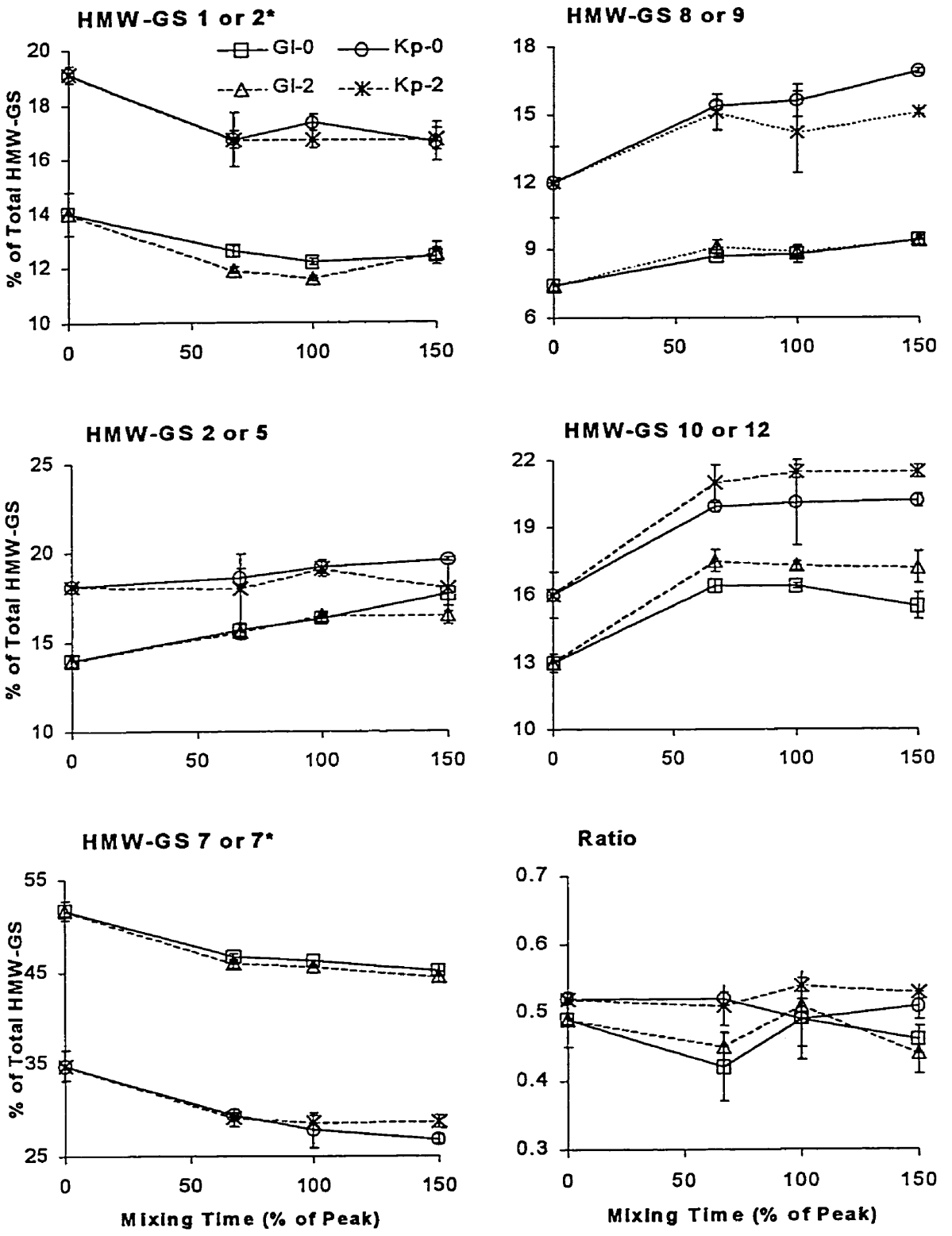

HMW-GS 1 or 2*
Gl-0
Kp-0
Gl-2
Kp-2
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

Figure 57. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AS fraction of glutenin during mixing of full formula doughs prepared from Glenlea, rested 0h (Gl-0) or 2 h (Gl-2), and from Katepwa, rested 0 h (Kp-0) or 2 h (Kp-2).

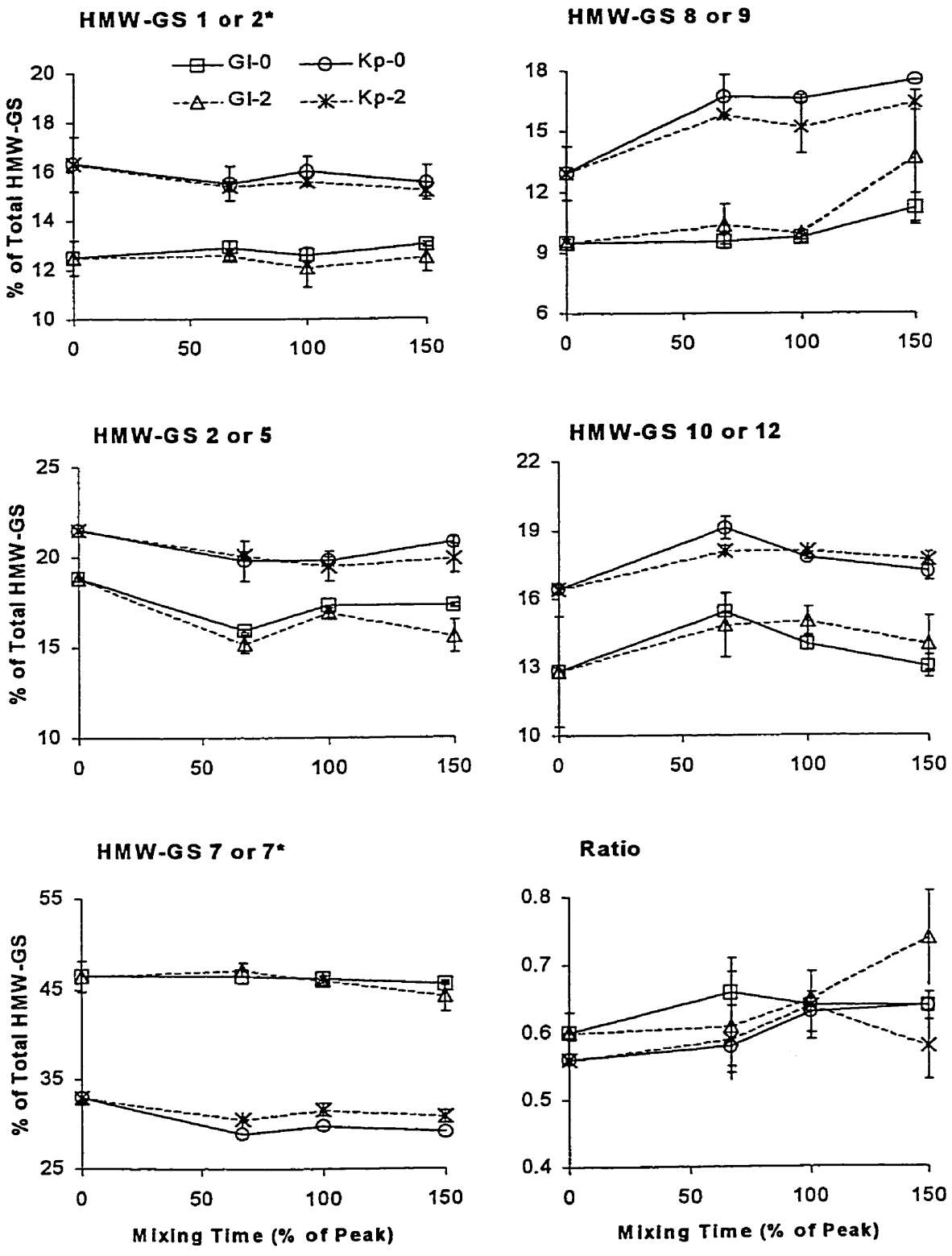
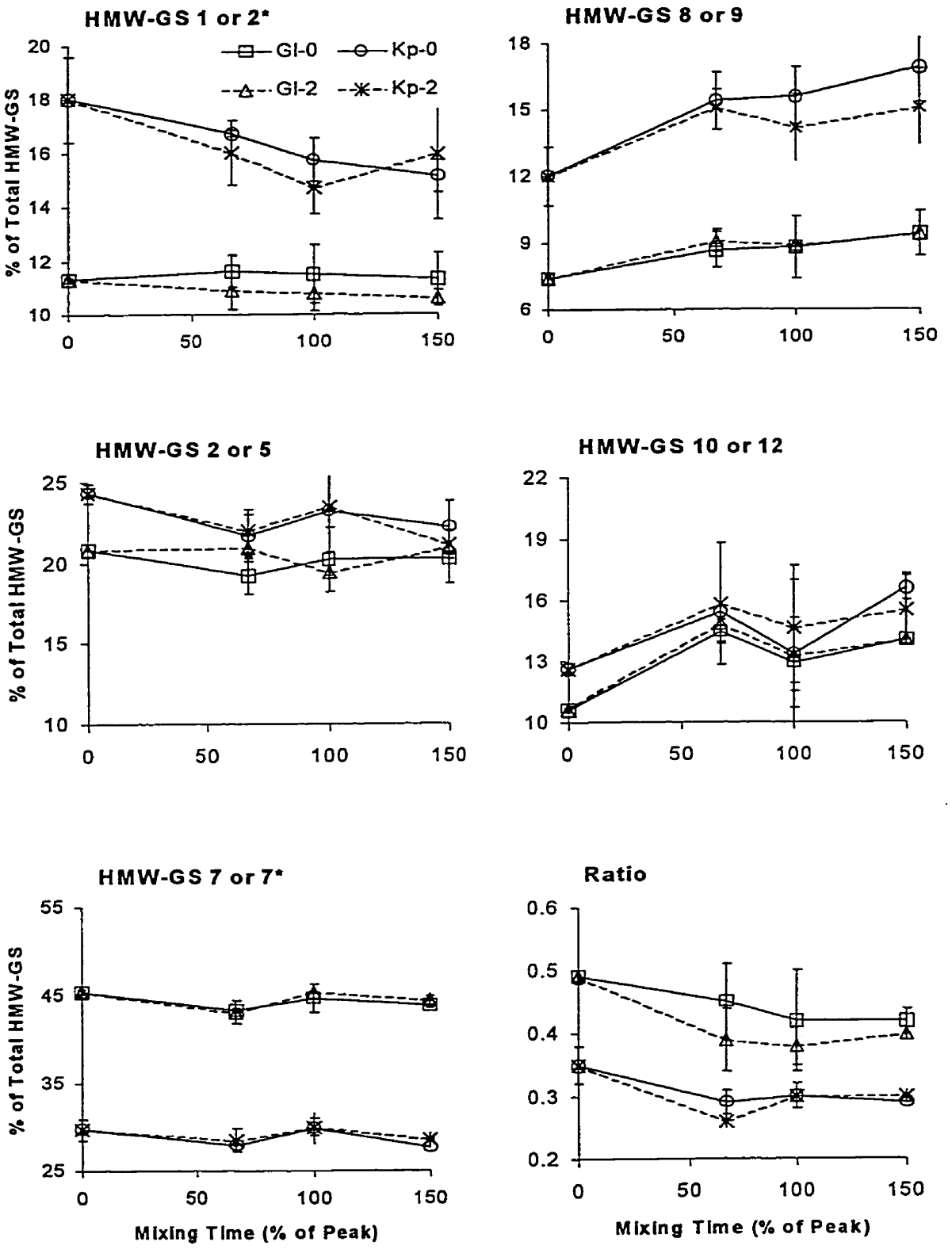

HMW-GS 1 or 2*
Gl-0
Kp-0
Gl-2
Kp-2
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

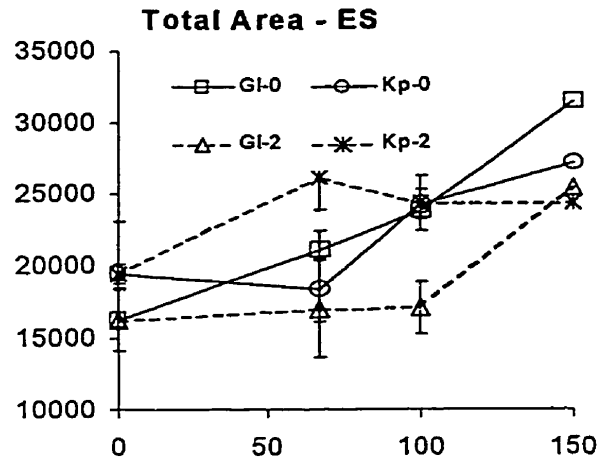
Figure 58. Changes in the relative amounts of the HMW-GS and the ratio of HMW-GS to LMW-GS of the AI fraction of glutenin during mixing of full formula doughs prepared from Glenlea, rested 0h (Gl-0) or 2 h (Gl-2), and from Katepwa, rested 0 h (Kp-0) or 2 h (Kp-2).

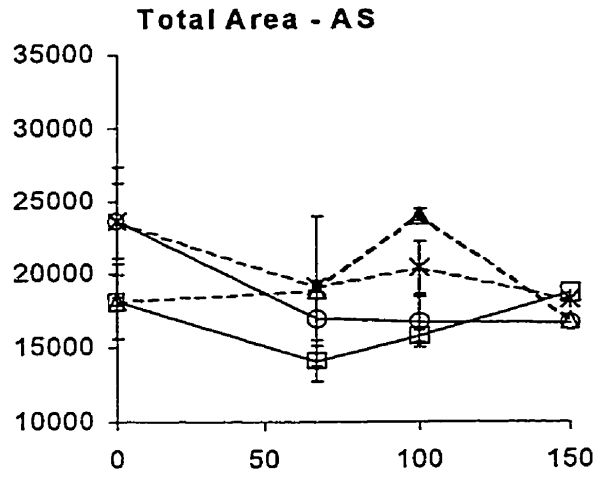

HMW-GS 1 or 2*
Gl-0
Kp-0
Gl-2
Kp-2
% of Total HMW-GS
HMW-GS 8 or 9
HMW-GS 2 or 5
HMW-GS 10 or 12
HMW-GS 7 or 7*
Ratio
Mixing Time (% of Peak)
Mixing Time (% of Peak)

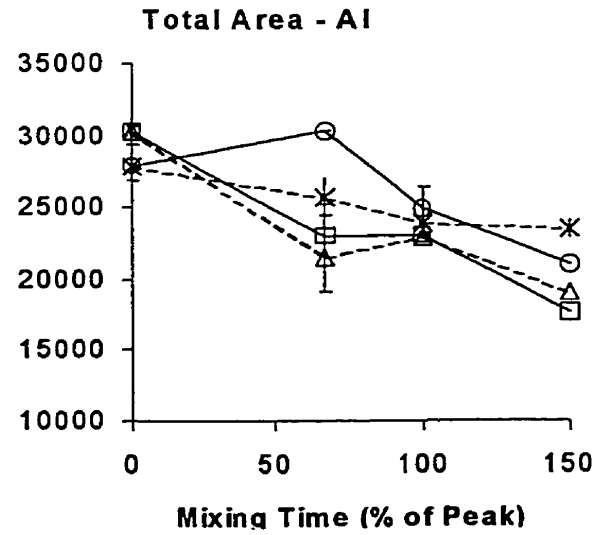
after mixing did not have a significant impact on the subunit composition or ratio of glutenins.

The effect of mixing on the amount of each glutenin fraction (as measured by total area under the RP-HPLC chromatogram) was as expected. The ES glutenin increased, the AI glutenin decreased, and the AS glutenin was intermediate (Figure 59). The effect of resting on the total area was only significant for Glenlea in the ES glutenin mixed to peak and beyond, and in the AS and AI glutenin which had been overmixed. Significant differences for Katepwa were observed in the AS and AI glutenin mixed to 67% of peak. Resting produced a significant decrease in the amount of ES glutenin for Glenlea dough mixed to peak and beyond. However, the resting effects observed in the AS and AI glutenins were different for each cultivar. These preliminary results on a small sample size suggest that full formula ingredients and resting do not have a major influence on the mixing behaviour of the gluten proteins.

Figure 59. Changes in the total area (mAU·sec) of the RP-HPLC chromatogram of the ES, AS and AI glutenin during mixing of full formula doughs prepared from Glenlea, rested 0h (Gl-0) or 2 h (Gl-2), and from Katepwa, rested 0 h (Kp-0) or 2 h (Kp-2).


Total Area - ES
35000
30000
25000
20000
15000
10000
Gl-0
Kp-0
Gl-2
Kp-2
0
50
100
150


Total Area - AS
35000
30000
25000
20000
15000
10000
0
50
100
150


Total Area - AI
35000
30000
25000
20000
15000
10000
0
50
100
150
Mixing Time (% of Peak)

5. SUMMARY

Among the flours produced from cereals, wheat flour has the unique ability to form a viscoelastic dough when mixed with water. Mixing represents a critical stage in the production of bread. Without sufficient water addition and energy input in the form of mixing, a dough cannot be developed to the optimum viscoelastic properties required to produce a satisfactory loaf of bread. Historically, the optimum point of development is subjectively selected by an experienced baker. Recording dough mixers, such as the mixograph used in this study, can be used to obtain an objective measurement of optimum mixing. This optimum is usually considered to be the point at which maximum dough consistency is reached. An optimally mixed dough will produce a loaf of high volume and acceptable texture. If a dough is undermixed or overmixed, the final product will not be of optimum quality.

Extensive research has been conducted on the proteins of wheat since the discovery that protein content and protein quality are major determinants of end-product quality. The storage proteins are mainly responsible for the viscoelastic properties unique to wheat. These proteins represent about 80% of total flour protein and consist of approximately equal amounts of gliadins, the monomeric proteins, and glutenins, the very large polymeric protein composed of disulfide cross-linked subunits. Research has focused on elucidating the biochemical basis for the functional properties of the storage proteins. Gliadins are responsible for the viscous component of wheat dough systems and glutenins provide the elastic component. Many studies have used solubility and molecular size-based

fractionation to separate and evaluate the gliadins and glutenins, both heterogeneous protein groups by nature.

Breadmaking quality has been shown to be strongly associated with the soluble and insoluble fractions of glutenin. Qualitative and quantitative variations in the glutenin subunit composition have also been related to breadmaking quality. Past research has reported on the effect of mixing on the soluble and insoluble fractions of glutenin, which are related to breadmaking quality. But very little research has been conducted on the effect of dough mixing on subunit composition or the gliadin-glutenin interaction. This study used an improved solubility-based fractionation procedure, coupled with RP-HPLC and SE-HPLC, in an effort to provide additional information on the effect of mixing on the gluten proteins and to evaluate how these proteins related to breadmaking quality participate in the mechanism of dough development and breakdown during mixing.

Flours from four different cultivars, from three different wheat classes, selected for their wide range of mixing strength, were used in this study to evaluate the changes occurring to the gluten proteins during mixing and to examine the significance of intervarietal variation in mixing behaviour. The effect of mixing on the protein solubility distribution and glutenin subunit composition was evaluated. In addition, the content of ω-gliadins contaminating soluble glutenin fractions and the content of native glutenin composed solely of LMW-GS were also monitored during mixing.

A small scale version of the modified Osborne fractionation (Chen and Bushuk, 1970) was developed and a selective precipitation method (Fu and Sapirstein, 1996) modified to separate flour proteins into six fractions. Scaling down the modified Osborne fractionation method resulted in a dramatic reduction in total fractionation time and

solvent use, and allowed for the preparation of a much larger number of samples. The small scale method was less precise and discriminating than the large scale method. But, the strong association of the AS and AI fractions with mixing strength was maintained. The method was reproducible, but sensitive to variation in experimental conditions. The selective precipitation method developed by Fu and Sapirstein (1996) was modified slightly for this study. The acidic environment of the AS protein solubilized in 50% 1-propanol prevented the efficient precipitation of glutenins from solution. The purification procedure was enhanced by selective precipitation in buffered 50% 1-propanol. A Tris buffer was used when samples were being prepared for RP-HPLC. The residual ionic strength from a phosphate buffer is high enough to precipitate reduced glutenin subunits during the reduction and alkylation step during RP-HPLC sample preparation. Tris buffer ions elute with the solvent peak and hence their absorbance at 214 nm does not interfere with the analysis. On the other hand, Tris buffer could not be used if samples were being prepared for Kjeldahl analysis (large interference of Tris nitrogen) or spectrophotometric analysis (significant absorbance at 214 nm). Use of a spectrophotometric method (Sapirstein and Johnson, 1996) to determine the protein content in purified fractions decreased further the total fractionation/purification time and allowed for analysis of a larger number of samples.

Despite the strong influence of experimental conditions (e.g. type and sequence of solvents, solvent-to-sample ratio, temperature, extraction time, intensity of mixing), solubility fractionation remains a necessity for the study of the complex group of proteins found in wheat flour. Despite the limitation (cross-contamination of protein groups) and the tedious nature of the modified Osborne fractionation procedure, the procedure if

coupled with the modified Fu and Sapirstein (1996) purification method, provides three fractions of glutenin and two of gliadin that can be studied.

In the first part of this thesis, the flours were fractionated on a large scale and the GS composition analyzed by RP-HPLC. As previously reported (Orth and Bushuk, 1972), the AS and AI fractions were strongly correlated with breadmaking quality, as measured by dough strength in this study. The relative amount of the AI fraction decreased significantly from the strongest (Glenlea) to the weakest cultivar (AC Karma). In contrast, the relative amount of the AS fraction increased from the strongest to the weakest cultivar. The most notable observation in the solubility distribution for the flours was the intercultivar variation in the amount of AS gliadin. An inverse relationship was observed, providing additional evidence for the genotype-specific variation of this AS gliadin reported previously (Almonte, 1998, Dupuis et al, 1996, Fu, 1996). The inverse relationship between AS gliadin and mixing strength was attributed to cultivar specific variation in gliadin-glutenin interaction, which in turn was suggested to influence the mixing time of flour doughs. Further evidence for this phenomenon was accumulated when doughs made from the same flours were studied. No other clear intervarietal differences were observed for the flours, although the AS glutenin also appeared to be inversely related to mixing strength.

RP-HPLC analysis of the GS composition was performed for the three glutenins (ES, AS, AI) of different solubility obtained from the flours. The most striking variation between the solubility fractions for all cultivars was in the amount of the subunit 1Dx5. This subunit was found in significantly greater proportion in the less soluble fractions (ES < AS < AI). The 1Ax subunits showed little variation between solubility groups. 1Bx

subunits were present in significantly greater amounts in the ES glutenin, but differences between the AS and AI glutenins were not significant. The 1By and 1Dy subunits were found in significantly greater proportion in the AS glutenin compared to the ES glutenin, but relatively little difference was observed between the AS and AI glutenin. The ratio of HMW-GS to LMW-GS was significantly lower for the ES glutenin compared with that of AS and AI glutenin, and similar for the AS and AI glutenin. These results suggest that 1Dx subunits, and to a lesser extent 1Dy and 1By subunits, are important in the formation of glutenin polymers of larger M_r.

The next step in the thesis study was to evaluate the changes in protein solubility distribution of the same flours during mixing. The small scale modified Osborne fractionation produced four fractions: SS, ES, AS and AI. The ES and AS fractions were purified by selective precipitation to yield two subgroups of gliadin and glutenin in each fraction. The SS fraction was not extensively studied, but some interesting observations were made. The presence of HMW-GS and the formation of a foam layer in the SS fraction of doughs, but not flours, suggested that conversion of a flour to a dough induces conformational changes, increasing the solubility and surface activity of some glutenin. The x-type HMW-GS appeared to be preferentially solubilized, as indicated by band intensity of SDS-PAGE electrophoregrams. Almonte (1998) reported similar observations for two flours fractionated using the large scale modified Osborne procedure. Further research would be needed to determine whether these subunits are more soluble by virtue of their position within the cross-linked glutenin polymer (susceptibility to shear forces during dough mixing and/or during solubility fractionation) or whether they are present in polymers of low M_r which are by nature more soluble. Except for Glenlea, the foam

produced during extraction with dilute salt solution did not appear to vary with cultivar. SDS-PAGE of the foam layer showed the presence of both glutenin and gliadin. Interestingly, virtually no foam was produced in the doughs prepared from Glenlea flour. This observation leads to the speculation that Glenlea glutenin, or glutenin from stronger mixing cultivars, is more hydrophobic. On the other hand, the gliadin-glutenin interaction hypothesis provides a plausible explanation for the creation of a foam. Although the SS glutenin and the foam layer constitute only a small fraction of total gluten protein, further investigation of this fraction might provide clues to the mixing behaviour of the larger, more insoluble glutenin.

The most obvious effect of mixing on the gluten proteins was the change in solubility of glutenin attributed to a reduction in size. Several studies have shown that mixing causes a decrease in the size of the large glutenin polymer and hence an increase in the amount of glutenin soluble by direct extraction with solutions of dilute acetic acid (Mecham et al, 1963, Tsen, 1967, Sievert et al, 1991) or sodium dodecyl sulfate (Danno and Hoseney, 1982a, Wang et al, 1992, Weegels et al, 1994) or by sequential fractionation procedures such as the modified Osborne method (Paredes-Lopez, 1982a, Tanaka and Bushuk, 1973a, Bushuk et al, 1997).

Solubility distribution and SDS-PAGE results in this study suggested that mixing caused the depolymerization and/or disaggregation of glutenin into smaller polymers of altered solubility. Mixing caused a decrease in the amount of AI glutenin and a concomitant increase in the amount of AS and ES glutenin. In addition, some glutenin appeared to be reduced in size by mixing, but remained large enough to be insoluble. This was suggested by the increase with mixing in intensity of slot protein observed in the SDS-

PAGE patterns of unreduced AI glutenin. The response of glutenin to mixing varied with cultivar. But this variation was caused by variation in gliadin-glutenin interaction between the cultivars. The rate of change in the amount of AI glutenin was very similar for all cultivars. Nor did there appear to be a cultivar-dependent change, related to quality, in the ES and AS glutenins with mixing. As reported by Bushuk et al (1997), differences in rate of solubility with mixing become apparent only when absolute mixing times are used for comparison of cultivars.

The changes in solubility distribution of the gluten proteins were more dramatic in the presence of iodate or NEMI. The response to oxidant or sulphydryl-blocking agent was generally more pronounced for the weaker cultivars and these chemicals clearly exert their effects on the glutenin. The interchange reaction is hindered by the oxidation or blocking of free thiol groups. As a result, the shear stress caused by mixing cannot be relieved, there is a more dramatic reduction in the size of glutenin and a concomitant increase in solubility. Iodate and NEMI exerted similar effects on the rate and extent of change in the ES fraction. But, NEMI produced greater changes in solubility than iodate for the AS and AI fractions, which is in agreement with other reports (Mecham et al, 1963, Tanaka and Bushuk, 1973a).

Gliadin-glutenin interaction was the second phenomenon observed to occur during dough mixing. In contrast to the well documented effect of mixing on insoluble glutenin fractions, investigation of the existence and relative importance of gliadin-glutenin interactions to mixing behaviour is scant. This study provides further evidence for the hypothesis presented earlier (Dupuis et al, 1996) that gliadin-glutenin interaction is a cultivar-specific phenomenon that influences mixing behaviour. In that study the modified

Osborne fractionation was used to analyze two flours of widely diverse mixing strength. The AS fraction, commonly referred to as the glutenin fraction (AS soluble glutenin), contained a significant proportion of gliadin, the amount of which appeared to be cultivar-dependent and inversely related to mixing strength. The gliadin that was unextractable in 70% ethanol but extractable in dilute acetic acid was considered to be involved in gliadin-glutenin interactions. Stronger cultivars containing less AS gliadin, i.e. exhibiting a lower degree of intrinsic gliadin-glutenin interaction, required longer mixing times to reach optimum development. Weaker cultivars containing more AS gliadin, i.e. exhibiting a greater degree of interaction, reached optimum development with shorter mixing times. The conclusions presented by Dupuis et al (1996) were consistent with two other studies which suggested that gliadin-glutenin interaction was a factor in the solubility behaviour of protein fractions during mixing (Bushuk et al, 1997) and in the dynamic rheological properties of glutens (Khatkar et al, 1995). Results recently obtained from this laboratory provided additional support for the hypothesis that gliadin-glutenin interaction influences the mixing behaviour of flours made from different cultivars. Fu et al (1997) studied the water solubility of gliadins from glutens made with varying amounts of salt. Gliadins from the gluten of a very strong cultivar were much easier to extract than those from a weaker cultivar, and gliadin extractability was intermediate for a cultivar of intermediate strength. This suggested that the intrinsic interaction of gliadin with glutenin, as measured by the extractability of gliadin in water, was inversely related to the mixing requirements of a flour. More definitive evidence that gliadin-glutenin interaction was indeed occurring during mixing was provided by Almonte (1998). During the early stages of mixing, an unusual drop in ES protein was observed for Katepwa, the weaker cultivar. Also, an

increase in the AI protein fraction was observed for both the extra strong cultivar Glenlea and the weaker Katepwa during the same initial mixing stage. The absence of unusual effects on the ES fraction of Glenlea was thought to be due to the absence of gliadin-glutenin interaction for this extra strong cultivar. However, the more comprehensive results obtained in this study suggest that Glenlea also exhibits gliadin-glutenin interaction, but to a lesser extent than weaker cultivars.

Although the mixing conditions and fractionation procedures differed, the unusual initial drop in ES protein and the increase in AI protein observed by Almonte (1998) were also evident for the weaker cultivars in this study. In contrast to Almonte's results, the AS protein fraction of all cultivars also showed an initial drop in relative proportion. The unusual changes in solubility distribution during the initial stage of mixing could clearly be attributed to gliadin-glutenin interaction. This was especially evident when total gliadin (ES and AS) was considered for all cultivars. All cultivars exhibited the solubility phenomenon attributed to gliadin-glutenin interaction and the degree of interaction (the extent of loss of gliadin solubility) varied with cultivar. Glenlea and Katepwa exhibited the smallest loss of gliadin solubility (lowest degree of interaction) and the weaker cultivars, AC Domain and AC Karma, exhibited more than double the loss of gliadin solubility (greater interaction). The change in total soluble glutenin during the initial stages of mixing suggested that this glutenin may also be involved in interactions with the larger insoluble glutenin. But the evidence was less definitive. As flour is hydrated and mixed during the initial stages of mixing, gliadins and glutenins aggregate, resulting in a decrease in gliadin solubility. As mixing progresses, gliadins are slowly released along with glutenin that has been reduced in size.

The loss in solubility of gliadin was reduced significantly in the presence of iodate and especially NEMI, suggesting that the oxidant and sulphydryl-blocking agent interfered with gliadin-glutenin interaction. A reduction in gliadin-glutenin interaction might in part explain the effects of these chemicals on dough mixing properties. This effect was greatest for the stronger cultivars and NEMI's effect was more pronounced for all cultivars except AC Karma.

RP-HPLC analysis of the GS composition was performed for the three glutenins (ES, AS, AI) of different solubility obtained from the flours and doughs. The 1Ax and 1Bx subunits were the least affected by mixing and appeared to be the least important with respect to quality. Both 1Ax and 1Bx subunits were found in greater proportion in the soluble fractions obtained from flours, but the proportions between fractions were similar upon mixing. The proportions of AC Karma subunits 1Dx2 and 1Dy12 also exhibited very little changes during mixing for each type of glutenin (ES, AS, AI). The most significant changes in GS composition were observed for subunits 1Dx5, 1Dy10, and both 1By subunits (8 and 9). Subunit 1Dx5 was found in greatest proportion in the AI glutenin and in least proportion in the ES glutenin, although this difference diminished with mixing. The proportion of 1Dx5 was also observed to increase in the soluble fractions as mixing progressed. The proportion of subunits 1By8 and 1By9 was also greater for the AS and AI glutenins compared to the ES glutenin. In contrast to 1Dx5, this solubility difference was maintained throughout mixing, despite the fact that the proportions of these subunits increased in the more soluble fractions as mixing progressed. The proportion of subunit 1Dy10 was similar in the ES and AS glutenin and greater in these soluble fractions than in

the AI glutenin throughout mixing. As with the 1Dx5 and 1By subunits, the proportion of 1Dy10 increased in the soluble fractions with mixing.

The GS composition results suggest that the y-type subunits and 1Dx5 play a more critical role in the interchange reactions occurring during dough mixing. One interesting feature of the composition analysis was the enrichment of y-type subunits in all glutenin fractions. This observation could be explained in part by the presence of predominantly x-type subunits in the salt-soluble protein fraction. But, this might also suggest that some AI glutenin, enriched in x-type subunits, was not effectively reduced and solubilized during analysis. Up to 18% of total flour protein has been reported to be insoluble in 50% 1-propanol containing dithiothreitol (Sapirstein and Fu, 1998). However, this residue protein was reported to be comprised mainly of Glu-1D subunits.

The effects of iodate and NEMI were not consistent nor frequently significant, and in most cases, there was little difference between the chemicals. When a difference was significant, NEMI was the more effective treatment. It is well known that specific subunits are associated with dough strength and that additives such as iodate and NEMI alter dough rheology. But, the lack of more definitive effects of mixing and of treatments on GS composition for three different glutenins reported in this study suggests that -SH/-SS- interchange reactions and the participation of specific subunits (e.g. 1Dx5 or 1By subunits), play are a more critical role at a higher structural level than the molecular one.

The effect of absorption on the mixing behaviour of gluten proteins was briefly examined using one cultivar (AC Domain). The glutenin from the dough of lower absorption was more susceptible to breakdown, resulting in less AI glutenin and more soluble glutenin. There appeared to be less gliadin-glutenin interaction since less AS

gliadin was present in the dough prepared at lower absorption. The GS composition was also affected by the absorption at which the dough was prepared. The enrichment of y-type subunits, and to some extent 1Dx5, observed in the soluble glutenin fractions for control doughs appeared to be accentuated if a lower absorption was used. The lower ratio observed for ES and AS glutenins from the dough prepared at lower absorption may indicate the presence of glutenins of smaller size than those found in the same fractions from dough prepared at higher absorption. These preliminary results suggest that lower absorption influences mixing behaviour by increasing the severity of mixing, hence promoting glutenin breakdown. Lower absorption appears to interfere with gliadin-glutenin interaction, possibly by limiting the water available for the necessary noncovalent reactions.

The fraction of ω-gliadins co-precipitated with glutenins in the purification procedure applied to the ES and AS fractions does not appear to be of major significance to dough mixing. Because of the very low concentration (% of total protein) the ω-gliadins of the AS fraction may simply be an artifact of the purification process. However, the quantity and behaviour of the ω-gliadins of the ES fraction suggest that interaction with glutenin may be occurring.

The selective precipitation method used to purify the ES and AS fractions effectively separated glutenins from gliadins, except for some glutenins comprised only of LMW-GS. Supporting evidence for the presence of glutenin comprised only of LMW-GS (LMW glutenin) in three fractions (ES, AS, AI) was obtained by RP-HPLC, SDS-PAGE and SE-HPLC. Quantitation of this LMW glutenin, which remained soluble with the gliadins in the 70% 1-propanol buffered solution, was carried out by SE-HPLC and the

changes occurring during mixing were monitored. A sharp drop in LMW glutenin from the AS fraction during initial mixing was observed for all cultivars and treatments. Glenlea showed the least response. Although LMW glutenin from the AS fraction constituted less than 2.5% of total protein, the unusual initial drop in quantity may be an indication that LMW glutenin also participates in interaction with the larger more insoluble glutenin. SDS-PAGE of the unreduced AS gliadin fraction revealed the presence of ladder-like bands very similar in pattern and mobility (M_r) to the same bands found in the ES gliadin. Interaction would be a plausible explanation for the differential solubility of LMW glutenin that is of similar M_r. The proportion of LMW glutenin from the ES fraction is much larger and exhibits an increase with mixing. But no cultivar-specific relationship to amount or mixing behaviour seemed apparent. When total gliadin (ES and AS) and soluble glutenin (ES and AS) were corrected for LMW glutenin, the characteristic drop in gliadin content during initial mixing was unmistakable, was observed for all cultivars, and varied inversely with mixing strength. Evidence for the involvement of LMW glutenin in interactions with larger glutenin is less apparent and would require further research.

The last step in this study was the evaluation of effects of full formula ingredients and resting on changes in gluten proteins. From the select number of samples analyzed by RP-HPLC, very few significant differences were found between the flour-water doughs used in the major part of this study and the full formula doughs. The changes in relative amounts of each HMW-GS and the ratio for glutenins of each solubility fraction were also comparable to the flour-water results. No significant effects on mixing could be attributed to the ingredients used in the full formula doughs. In general, resting time after mixing did

not have a significant impact on GS composition, ratio or solubility distribution. Similar results were reported by Almonte (1998) for doughs rested up to 45 minutes.

6. CONTRIBUTIONS TO KNOWLEDGE

The major contributions to knowledge resulting from the present study of four flours of diverse mixing strength are as follows:

1. A small scale version of the modified Osborne fractionation procedure (Chen and Bushuk, 1970) was developed and a selective precipitation method (Fu and Sapirstein, 1996) was modified to separate flour proteins into six fractions. Five of these were relatively pure fractions of gliadin (ES, AS) or glutenin (ES, AS, AI). Results obtained for protein solubility distribution and GS composition were comparable to the large scale method and offered the advantages of dramatically reduced fractionation/purification time and solvent use, and larger sample throughput.
2. The SS fraction of doughs contained glutenin, evidenced by the presence of HMW-GS bands in SDS-PAGE gels, comprising predominantly x-type subunits. A foam layer was also produced during the sequential extraction of doughs, twice with salt solution and then with water. The foam layer, which was present in notable amounts for all cultivars except Glenlea, contained both gliadin and glutenin. The presence of SS glutenin and the formation of a foam layer suggested that mixing (i) altered the conformation of glutenin and/or (ii) induced gliadin-glutenin interaction to an extent sufficient to enhance the solubility and cause foam formation of some of the gluten proteins.
3. The quantity of the AI glutenin and AS gliadin in the flours were directly and inversely related to dough mixing strength, respectively. The inverse relationship between the AS gliadin and mixing strength was attributed to genotype-specific gliadin-glutenin interaction.

4. Protein solubility distribution and electrophoretic results provided convincing evidence for the existence of genotype-specific gliadin-glutenin interaction. Results showed that all cultivars exhibited gliadin-glutenin interaction during mixing and the degree of interaction was inversely related to mixing strength. Intrinsic gliadin-glutenin interaction was measured by quantifying the amount of gliadin in the AS protein fraction of flours. Gliadin-glutenin interaction was manifested by an unusual drop in the AS gliadin during the very early stages of mixing of doughs. Strong cultivars (with long mixing requirements) exhibited the lowest degree of interaction and weak cultivars (with short mixing requirements) exhibited the highest.
5. Analysis of subunit composition by RP-HPLC of the glutenin fractions obtained from the flours suggested that 1Dx subunits, and to a lesser extent 1Dy and 1By subunits, are important in the formation of the insoluble glutenin (larger M_r) which is related to breadmaking quality.
6. Analysis by RP-HPLC of changes in subunit composition during mixing of three glutenin fractions revealed some variation in subunits related to quality. Allelic differences were most pronounced for the 1Dx subunits (1Dx2 versus 1Dx5) and much less evident for 1Ax and 1B subunits. In agreement with the results obtained for the flours, the 1Ax and 1Bx subunits appeared to play a minor role in the mixing process. The 1Dx and y-type (1B, 1D) subunits exhibited more dynamic behaviour throughout mixing, suggesting a more active role in the -SH/-SS- interchange reactions for these subunits. However, differences observed in the GS composition of glutenins during mixing could not explain the large changes in dough properties and the variation between cultivars.

7. The presence of glutenin comprised only of LMW-GS (LMW glutenin) was identified in three fractions of glutenin, including the insoluble fraction, with the peak distribution falling in the ES fraction (soluble and of low M_r). LMW glutenin may exist as discrete polydisperse polymers noncovalently associated with the larger, more insoluble glutenin. The amount of LMW glutenin increased with mixing and that found in the AS fraction exhibited an initial drop similar to the AS gliadin associated with gliadin-glutenin interaction. Thus, LMW glutenin, like the gliadins, may be involved in interaction with the glutenin of the larger M_r.
8. The absorption level used to mix a dough will influence the extent of glutenin breakdown (reduced M_r and enhanced solubility) and possibly the degree of gliadin-glutenin interaction (proportion of AS gliadin). The gluten proteins of doughs made from the same flour at different absorption levels and mixed to peak are not necessarily in identical states. These findings are based on results from one cultivar at two absorption levels.
9. Full formula ingredients and resting do not appear to have a major impact on the protein solubility distribution and GS composition. This finding is based on a limited number of samples from only two cultivars.
10. Results from this study provide additional support for glutenin breakdown and -SH/-SS- interchange as important mechanisms in dough mixing and offers convincing evidence for gliadin-glutenin interaction as an additional mechanism. This study concludes that glutenin breakdown occurs by both depolymerization and disaggregation and that the interchange reaction, like gliadin-glutenin interaction, exerts its functional importance at a higher structural level than the molecular one.

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APPENDIX

Table 1. Regression Analysis of Absorption Against Mixing Time (MT), Mixograph Peak Height (PH), or Work Input to Peak (WI) Determined with a 2-g Mixograph

Flour Sample	Abs (%)	Range	Regression Equation	R^2	Pr > F
Mixing Time		(min)			
Glenlea 93	62-73	5.6-7.2	MT= 0.1451A-3.4496	0.8783	0.019
Glenlea 95	50-65	6.3-7.9	MT= -0.0742A+11.151	0.4573	0.324
Roblin	65-75	3.8-5.3	MT= 0.1555A-6.4615	0.9344	0.030
Katepwa	55-65	3.0-4.0	MT = 0.0961A-2.1789	0.7814	0.008
AC Domain	58-68	3.1-3.4	MT= 0.0233A+1.8400	0.5575	0.147
AC Karma	45-58	2.53-3.1	MT= -0.0429A+4.9375	0.9186	0.042
Peak Height		(MU)			
Glenlea 93	62-73	55.1-45.6	PH= -0.8505A+108.07	0.9903	<0.001
Glenlea 95	50-65	47.2-38.6	PH= -0.5990A+77.655	0.9778	0.011
Roblin	65-75	58.8-48.7	PH= -0.9921A+123.39	0.9959	<0.001
Katepwa	55-65	50.9-39.2	PH = -1.1885A+116.33	0.9912	<0.001
AC Domain	58-68	53.5-40.0	PH= -1.3059A+130.60	0.9094	0.012
AC Karma	45-58	50.3-42.4	PH= -0.6500A+79.350	0.9627	0.019
Work Input		(%Tq*min)			
Glenlea 93	62-73	204-177	WI= -1.4366A+287.28	0.2858	0.353
Glenlea 95	50-65	263-154	WI= -7.2400A+609.80	0.8789	0.062
Roblin	65-75	136-138	WI= 0.5355A+99.754	0.2642	0.490
Katepwa	55-65	112-95	WI= 0.6909A+63.161	0.1437	0.402
AC Domain	58-68	~104-83	WI= -1.9729A+221.95	0.7048	0.075
AC Karma	45-58	102-66	WI= -2.9031A+229.33	0.9170	0.042

Table 2. Statistical Comparison of the Content of ES and AS Purified Fractions (as % of Total Flour Protein) Obtained from Flour by the Small Scale Method and Determined Spectrophotometrically or by Kjeldahl[1]

Cultivar	Kjeldahl	SD	CV	Spec	SD	CV
ES gliadin						
Glenlea	30.9^{b*}	0.6	1.9	32.8^{ab*}	0.6	1.9
Katepwa	33.6^{a}	0.7	2.1	34.4^{a}	0.4	1.0
AC Domain	30.9^{b}	0.7	2.4	32.4^{ab}	2.5	7.8
AC Karma	28.0^{c}	2.03	8.2	29.5^{b}	1.7	5.8
Mean	30.8			32.2		
Range	8.3			6.3		
CV	7.6			6.9		
Precision	3.6			4.1		
ES glutenin						
Glenlea	4.5^{b*}	0.6	13.2	2.6^{*}	0.6	24.0
Katepwa	4.4^{b}	0.7	16.1	3.6	0.4	9.7
AC Domain	7.5^{a}	0.7	9.8	6.0	2.5	42.4
AC Karma	7.7^{a}	2.3	29.7	6.2	1.7	27.4
Mean	6.0			4.6		
Range	6.0			5.6		
CV	32.8			43.5		
Precision	17.2			25.9		
AS gliadin						
Glenlea	4.5^{c}	0.3	7.5	4.8^{c}	0.5	10.4
Katepwa	7.5^{b*}	0.2	2.7	8.8^{b*}	0.9	10.4
AC Domain	7.1^{b}	1.0	14.7	9.6^{ab}	1.3	13.9
AC Karma	9.2^{a*}	0.1	1.5	11.6^{a*}	0.5	4.2
Mean	7.1			8.7		
Range	5.1			7.6		
CV	24.3			31.7		
Precision	6.6			9.7		
AS glutenin						
Glenlea	4.2^{d}	0.3	8.1	4.0^{ab}	0.5	12.5
Katepwa	5.1^{c*}	0.2	3.9	3.8^{b*}	0.9	24.5
AC Domain	6.5^{b}	1.0	15.9	4.0^{ab}	1.3	34.0
AC Karma	8.8^{a*}	0.1	1.5	6.4^{a*}	0.5	7.8
Mean	6.1			4.5		
Range	5.0			3.7		
CV	30.1			29.5		
Precision	7.4			19.7		

[1] Letters indicate significant differences (α=0.05) between cultivars within each method
Asterisks indicate significant differences (α=0.05) between methods within a cultivar
[2] SD = standard deviation , [3] CV = coefficient of variation

Table 3. Statistical Comparison of the Content of ES and AS Purified Fractions (as % of Total Flour Protein) Obtained from Dough (50%MT) by the Small Scale Method and Determined Spectrophotometrically or by Kjeldahl[1]

Cultivar	Kjeldahl	SD	CV	Spec	SD	CV
ES gliadin						
Glenlea	31.0^{b}	1.3	4.1	31.6^{b}	0.7	2.2
Katepwa	35.0^{a*}	0.8	2.2	37.5^{a*}	0.4	1.1
AC Domain	27.6^{c}	0.5	1.8	28.6^{c}	0.6	2.2
AC Karma	26.5^{c}	0.6	2.2	28.3^{c}	1.8	6.5
Mean	30.0			31.5		
Range	10.1			10.8		
CV	11.9			12.8		
Precision	2.6			3.0		
ES glutenin						
Glenlea	6.5^{ab}	1.0	15.5	6.1	0.7	11.6
Katepwa	6.2^{ab*}	1.0	16.0	3.4^{*}	0.4	12.5
AC Domain	5.2^{b}	0.7	13.4	4.1	0.6	15.7
AC Karma	7.8^{a}	0.5	6.9	6.1	1.8	30.1
Mean	6.4			4.9		
Range	3.6			4.3		
CV	18.7			30.9		
Precision	13.0			17.5		
AS gliadin						
Glenlea	3.3^{b}	0.6	18.9	2.5^{b}	0.0	0.0
Katepwa	4.7^{b*}	0.8	16.6	2.5^{b*}	0.4	16.3
AC Domain	7.1^{a}	1.1	15.7	6.2^{a}	0.1	2.3
AC Karma	7.0^{a}	0.9	12.9	5.7^{a}	0.4	7.1
Mean	5.5			4.0		
Range	5.5			4.3		
CV	33.3			44.9		
Precision	16.0			6.4		
AS glutenin						
Glenlea	1.8^{b}	0.5	26.9	2.6^{c}	0.0	0.0
Katepwa	1.8^{b*}	1.0	53.8	3.9^{b*}	0.4	10.3
AC Domain	2.8^{b}	0.7	26.5	3.4^{b}	0.1	4.2
AC Karma	4.4^{a*}	0.6	13.7	5.5^{a*}	0.4	7.3
Mean	2.7			4.0		
Range	3.8			3.3		
CV	45.4			27.8		
Precision	30.2			5.4		

[1] Letters indicate significant differences (α=0.05) between cultivars within each method
Asterisks indicate significant differences (α=0.05) between methods within a cultivar
[2] SD = standard deviation , [3] CV = coefficient of variation

Table 4. Statistical Comparison of the Content of ES and AS Purified Fractions (as % of Total Flour Protein) Obtained from Dough (100%MT) by the Small Scale Method and Determined Spectrophotometrically or by Kjeldahl[1]

Cultivar	Kjeldahl	SD	CV	Spec	SD	CV
ES gliadin						
Glenlea	30.5^{b}	0.5	1.6	31.7^{bc}	0.7	2.2
Katepwa	34.7^{a*}	0.7	2.0	37.1^{a*}	0.6	1.7
AC Domain	27.8^{c}	1.6	5.7	29.6^{c}	0.8	2.6
AC Karma	29.4^{bc}	0.8	2.8	32.2^{b}	1.3	4.2
Mean	30.6			32.6		
Range	9.1			8.5		
CV	9.2			9.2		
Precision	3.0			2.7		
ES glutenin						
Glenlea	7.2^{ab}	0.5	6.8	6.0	0.7	11.8
Katepwa	7.3^{ab*}	0.9	12.0	4.6*	0.6	14.0
AC Domain	6.2^{b}	1.0	16.7	4.6	0.8	16.7
AC Karma	8.0^{a}	0.9	11.4	5.6	1.3	23.8
Mean	7.2			5.2		
Range	3.5			2.5		
CV	13.9			18.4		
Precision	11.7			16.6		
AS gliadin						
Glenlea	3.7^{ab}	1.2	31.7	2.8^{c}	0.1	2.6
Katepwa	4.1^{ab}	0.3	7.4	3.4^{bc}	0.8	24.0
AC Domain	7.3^{b*}	0.6	7.6	5.6^{ab*}	0.4	6.2
AC Karma	5.1^{a}	1.4	26.5	6.3^{a}	1.6	25.3
Mean	5.1			4.6		
Range	4.9			5.4		
CV	32.7			39.2		
Precision	18.3			14.5		
AS glutenin						
Glenlea	2.2^{b}	1.1	50.7	2.8^{b}	0.1	2.5
Katepwa	1.5^{b}	1.2	80.2	2.5^{b}	0.8	31.9
AC Domain	2.6^{b*}	0.5	18.3	4.1^{ab*}	0.4	8.7
AC Karma	6.8^{b}	0.3	4.6	5.2^{a}	1.6	31.0
Mean	3.3			3.7		
Range	6.4			5.0		
CV	69.9			39.2		
Precision	38.4			18.5		

[1] Letters indicate significant differences (α=0.05) between cultivars within each method
Asterisks indicate significant differences (α=0.05) between methods within a cultivar
[2] SD = standard deviation , [3] CV = coefficient of variation

Table 5. Statistical Comparison of the Content of ES and AS Purified Fractions (as % of Total Flour Protein) Obtained from Dough (200%MT) by the Small Scale Method and Determined Spectrophotometrically or by Kjeldahl[1]

Cultivar	Kjeldahl	SD	CV	Spec	SD	CV
ES gliadin						
Glenlea	33.9^{b}	0.4	1.3	33.4^{b}	0.4	1.1
Katepwa	37.8^{a}	1.0	2.5	39.8^{a}	0.9	2.3
AC Domain	34.7^{b}	0.2	0.4	35.0^{b}	0.7	2.0
AC Karma	29.9^{c}	0.5	1.6	32.8^{b}	1.8	5.4
Mean	34.1			35.3		
Range	9.2			8.8		
CV	8.7			8.5		
Precision	1.4			2.7		
ES glutenin						
Glenlea	8.7	0.7	7.6	9.2	0.4	3.9
Katepwa	8.7	0.6	6.4	7.0	0.9	13.2
AC Domain	9.2	1.0	10.9	9.2	0.7	7.7
AC Karma	9.6	0.9	9.0	6.8	1.8	25.8
Mean	9.1			8.0		
Range	2.4			4.1		
CV	8.4			18.2		
Precision	8.5			12.6		
AS gliadin						
Glenlea	2.4^{b}	0.6	24.4	1.8^{b}	0.0	0.0
Katepwa	2.0^{b}	0.5	23.2	1.4^{b}	0.0	0.0
AC Domain	3.7^{a}	0.5	13.7	3.0^{a}	0.1	2.4
AC Karma	4.7^{a}	1.0	21.5	3.2^{a}	0.3	8.8
Mean	3.2			2.3		
Range	4.3			2.0		
CV	38.9			34.9		
Precision	20.7			2.8		
AS glutenin						
Glenlea	4.2^{a*}	0.2	4.7	4.7^{b*}	0.0	0.0
Katepwa	2.3^{b*}	0.2	9.1	2.9^{d*}	0.0	0.0
AC Domain	2.8^{b}	0.4	13.3	3.4^{c}	0.1	2.1
AC Karma	4.7^{a*}	0.3	6.7	6.3^{a*}	0.3	4.5
Mean	3.5			4.3		
Range	2.9			3.6		
CV	29.8			32.9		
Precision	8.4			1.6		

[1] Letters indicate significant differences (α=0.05) between cultivars within each method
Asterisks indicate significant differences (α=0.05) between methods within a cultivar
[2] SD = standard deviation , [3] CV = coefficient of variation

Table 6. Effect of Mixing on the Protein Fractions (as % of Total Flour Protein) Obtained by Modified Osborne Fractionation of Flour-Water Control Doughs Mixed to Various Times[1]

Fraction and Cultivar	Flour	Mixing Time (as % of peak time) 50	100	200
Salt-soluble				
Glenlea	18.6±1.4^{ns}	20.1±1.2^{ns}	20.4±2.3^{ns}	20.4±1.1^{ns}
Katepwa	18.1±0.3^{b}	21.8±2.6^{a}	22.1±1.3^{a}	19.0±1.1^{ab}
AC Domain	16.0±0.5^{a}	16.5±0.3^{a}	14.6±0.1^{b}	15.6±0.6^{a}
AC Karma	20.3±1.0^{b}	21.2±0.5^{b}	20.9±0.4^{b}	24.1±0.1^{a}
Ethanol-soluble				
Glenlea	35.4±1.0^{c}	37.7±0.4^{b}	37.7±0.0^{b}	42.6±0.3^{a}
Katepwa	38.0±0.5^{c}	40.9±1.6^{b}	41.6±1.5^{b}	46.7±0.6^{a}
AC Domain	38.4±3.3^{ab}	31.5±1.6^{b}	34.8±3.3^{ab}	39.5±2.4^{a}
AC Karma	35.7±3.6^{ns}	34.4±0.6^{ns}	37.8±1.3^{ns}	39.7±0.6^{ns}
Acetic acid-soluble				
Glenlea	8.7±0.9^{a}	5.1±0.3^{b}	5.6±0.9^{b}	6.5±0.5^{b}
Katepwa	12.6±0.6^{a}	6.4±0.3^{b}	5.9±1.1^{bc}	4.3±0.4^{c}
AC Domain	13.6±2.0^{a}	8.6±0.0^{b}	11.8±0.4^{a}	8.6±0.4^{b}
AC Karma	18.0±1.8^{a}	11.2±0.6^{b}	11.5±1.7^{b}	9.5±0.9^{b}
Acetic acid-insoluble				
Glenlea	38.5±1.2^{a}	36.9±1.3^{a}	36.0±1.3^{a}	31.9±0.1^{b}
Katepwa	33.8±1.4^{a}	32.7±2.7^{ab}	30.4±1.1^{ab}	29.3±1.2^{b}
AC Domain	31.8±2.2^{b}	43.1±2.2^{a}	39.3±3.7^{a}	36.7±2.5^{ab}
AC Karma	27.0±3.2^{b}	33.5±1.3^{a}	30.7±1.2^{ab}	26.2±0.6^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 7. Effect of Cultivar on the Protein Fractions (as % of Total Flour Protein) Obtained by Modified Osborne Fractionation of Flour-Water Control Doughs Mixed to Various Times[1]

Mixing Time (% of peak time)	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
Salt-soluble				
0	18.6±1.4^{b}	18.1±0.3^{b}	16.0±0.5^{c}	20.3±1.0^{a}
50	20.1±1.2^{ab}	21.8±2.6^{a}	16.5±0.3^{b}	21.2±0.5^{a}
100	20.4±2.3^{a}	22.1±1.3^{a}	14.6±0.1^{b}	20.9±0.4^{a}
200	20.4±1.1^{b}	19.0±1.1^{b}	15.6±0.6^{c}	24.1±0.1^{a}
Ethanol-soluble				
0	35.4±1.0^{ns}	38.0±0.5^{ns}	38.4±3.3^{ns}	35.7±3.6^{ns}
50	37.7±0.4^{b}	40.9±1.6^{a}	31.5±1.6^{c}	34.4±0.6^{c}
100	37.7±0.0^{ab}	41.6±1.5^{a}	34.8±3.3^{b}	37.8±1.3^{ab}
200	42.6±0.3^{b}	46.7±0.6^{a}	39.5±2.4^{b}	39.7±0.6^{b}
Acetic acid-soluble				
0	8.7±0.9^{c}	12.6±0.6^{b}	13.6±2.0^{b}	18.0±1.8^{a}
50	5.1±0.3^{d}	6.4±0.3^{c}	8.6±0.0^{b}	11.2±0.6^{a}
100	5.6±0.9^{b}	5.9±1.1^{b}	11.8±0.4^{a}	11.5±1.7^{a}
200	6.5±0.5^{b}	4.3±0.4^{c}	8.6±0.4^{a}	9.5±0.9^{a}
Acetic acid-insoluble				
0	38.5±1.2^{a}	33.8±1.4^{b}	31.8±2.2^{b}	27.0±3.2^{c}
50	36.9±1.3^{b}	32.7±2.7^{b}	43.1±2.2^{a}	33.5±1.3^{b}
100	36.0±1.3^{ab}	30.4±1.1^{b}	39.3±3.7^{a}	30.7±1.2^{b}
200	31.9±0.1^{b}	29.3±1.2^{bc}	36.7±2.5^{a}	26.2±0.6^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 8. Effect of Mixing on the Content of ES and AS Purified Fractions (% of Total Flour Protein) of Flour-Water Control Doughs Mixed to Various Times[1]

		Mixing Time (as % of peak time)[1]		
Cultivar	Flour	50	100	200
ES gliadin				
Glenlea	32.8±0.6^{ab}	31.6±0.7^{b}	31.7±0.7^{ab}	33.4±0.4^{a}
Katepwa	34.4±0.4^{c}	37.5±0.4^{b}	37.1±0.6^{b}	39.8±0.9^{a}
AC Domain	32.4±2.5^{a}	26.2±1.1^{b}	30.4±0.9^{a}	33.6±0.5^{a}
AC Karma	29.5±1.7	28.3±1.8	32.2±1.3	32.8±1.8
ES glutenin				
Glenlea	2.6±0.6^{c}	6.1±0.7^{b}	6.0±0.7^{b}	9.2±0.4^{a}
Katepwa	3.6±0.4^{b}	3.4±0.4^{b}	4.6±0.6^{b}	7.0±0.9^{a}
AC Domain	6.0±2.5	5.4±1.1	4.4±0.9	5.8±0.5
AC Karma	6.2±1.7	6.1±1.8	5.6±1.3	6.8±1.8
AS gliadin				
Glenlea	4.8±0.5^{a}	2.5±0.0^{b}	2.8±0.1^{b}	1.8±0.0^{c}
Katepwa	8.8±0.9^{a}	2.4±0.4^{bc}	3.4±0.8^{b}	1.4±0.0^{c}
AC Domain	9.6±1.3^{a}	7.1±0.6^{b}	8.8±0.4^{ab}	7.2±0.7^{b}
AC Karma	11.6±0.5^{a}	5.7±0.4^{b}	6.3±1.6^{b}	3.2±0.3^{c}
AS glutenin				
Glenlea	4.0±0.5^{b}	2.6±0.0^{c}	2.8±0.1^{c}	4.7±0.0^{a}
Katepwa	3.8±0.9^{ab}	3.9±0.4^{a}	2.5±0.8^{b}	2.9±0.0^{ab}
AC Domain	4.0±1.3^{a}	1.5±0.6^{b}	3.0±0.4^{ab}	1.5±0.7^{b}
AC Karma	6.4±0.5	5.5±0.4	5.2±1.6	6.3±0.3

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 9. Effect of Cultivar on the Content of ES and AS Purified Fractions (% of Total Flour Protein) of Flour-Water Control Doughs Mixed to Various Times[1]

Mixing Time (% of peak time)	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES gliadin				
0	32.8±0.6^{ab}	34.4±0.4^{a}	32.4±2.5^{ab}	29.5±1.7^{b}
50	31.6±0.7^{b}	37.5±0.4^{a}	26.2±1.1^{c}	28.3±1.8^{c}
100	31.7±0.7^{b}	37.1±0.6^{a}	30.4±0.9^{b}	32.2±1.3^{b}
200	33.4±0.4^{b}	39.8±0.9^{a}	33.6±0.5^{b}	32.8±1.8^{b}
ES glutenin				
0	2.6±0.6^{ns}	3.6±0.4^{ns}	6.0±2.5^{ns}	6.2±1.7^{ns}
50	6.1±0.7^{ns}	3.4±0.4^{ns}	5.4±1.1^{ns}	6.1±1.8^{ns}
100	6.0±0.7^{ns}	4.6±0.6^{ns}	4.4±0.9^{ns}	5.6±1.3^{ns}
200	9.2±0.4^{a}	7.0±0.9^{ab}	5.8±0.5^{b}	6.8±1.8^{ab}
AS gliadin				
0	4.8±0.5^{c}	8.8±0.9^{b}	9.6±1.3^{ab}	11.6±0.5^{a}
50	2.5±0.0^{c}	2.4±0.4^{c}	7.1±0.6^{a}	5.7±0.4^{b}
100	2.8±0.1^{c}	3.4±0.8^{c}	8.8±0.4^{a}	6.3±1.6^{b}
200	1.8±0.0^{c}	1.4±0.0^{c}	7.2±0.7^{a}	3.2±0.3^{b}
AS glutenin				
0	4.0±0.5^{ab}	3.8±0.9^{b}	4.0±1.3^{ab}	6.4±0.5^{a}
50	2.6±0.0^{c}	3.9±0.4^{b}	1.5±0.6^{d}	5.5±0.4^{a}
100	2.8±0.1^{ab}	2.5±0.8^{b}	3.0±0.4^{ab}	5.2±1.6^{a}
200	4.7±0.0^{b}	2.9±0.0^{c}	1.5±0.7^{d}	6.3±0.3^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 10. Effect of Mixing Time on the Protein Fractions (as % of Total Flour Protein) Obtained by Modified Osborne Fractionation of Iodate Doughs Mixed to Various Times[1]

		% of Total Flour Protein for Each Mixing Time[1]		
Cultivar	Flour	50	100	200
Salt-soluble				
Glenlea	18.6±1.4^{ns}	17.1±0.5^{ns}	16.9±1.3^{ns}	16.8±0.9^{ns}
Katepwa	18.1±0.3^{a}	16.8±0.7^{b}	16.1±0.3^{b}	17.0±0.1^{b}
AC Domain	16.0±0.5^{ab}	16.8±1.0^{a}	14.7±0.8^{bc}	14.2±0.1^{c}
AC Karma	20.3±0.9^{a}	20.4±0.1^{a}	17.3±0.3^{b}	18.8±0.8^{ab}
Ethanol-soluble				
Glenlea	35.4±1.0^{c}	42.7±0.0^{b}	42.4±0.5^{b}	50.2±0.6^{a}
Katepwa	38.0±0.5^{d}	47.1±0.4^{c}	50.2±1.6^{b}	54.2±0.2^{a}
AC Domain	38.4±3.3^{b}	41.0±2.3^{b}	41.5±0.3^{b}	53.6±1.3^{a}
AC Karma	35.7±3.6^{c}	42.0±2.1^{bc}	46.8±3.1^{b}	55.2±1.0^{a}
Acetic acid-soluble				
Glenlea	8.7±0.9^{b}	4.0±0.0^{c}	10.6±2.6^{b}	14.4±0.1^{a}
Katepwa	12.6±0.6^{a}	5.1±0.6^{c}	6.8±0.6^{b}	7.7±0.7^{b}
AC Domain	13.6±1.9^{a}	8.6±0.9^{b}	15.8±0.1^{a}	16.6±0.8^{a}
AC Karma	18.0±1.8^{a}	10.6±0.7^{b}	17.8±3.6^{a}	12.6±0.6^{b}
Acetic acid-soluble				
Glenlea	38.5±1.2^{a}	34.9±0.4^{b}	30.3±0.0^{c}	19.1±0.1^{d}
Katepwa	33.8±1.4^{a}	29.6±0.4^{b}	26.2±0.2^{c}	20.4±1.4^{d}
AC Domain	31.8±2.2^{ab}	32.6±2.3^{a}	27.6±0.1^{b}	14.4±0.4^{c}
AC Karma	27.0±3.2^{a}	24.2±0.4^{a}	13.8±0.1^{b}	10.9±0.7^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 11. Effect of Cultivar on the Protein Fractions (as % of Total Flour Protein) Obtained by Modified Osborne Fractionation of Iodate Doughs Mixed to Various Times[1]

Mixing Time (% of peak time)	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
Salt-soluble				
0	18.6±1.4^{b}	18.1±0.3^{b}	16.0±0.5^{c}	20.3±0.9^{a}
50	17.1±0.5^{b}	16.8±0.7^{b}	16.8±1.0^{b}	20.4±0.1^{a}
100	16.9±1.3^{ab}	16.1±0.3^{ab}	14.7±0.8^{b}	17.3±0.3^{a}
200	16.8±0.9^{b}	17.0±0.1^{b}	14.2±0.1^{c}	18.8±0.8^{a}
Ethanol-soluble				
0	35.4±1.0^{ns}	38.0±0.5^{ns}	38.4±3.3^{ns}	35.7±3.6^{ns}
50	42.7±0.0^{b}	47.1±0.4^{a}	41.0±2.3^{b}	42.0±2.1^{b}
100	42.4±0.5^{bc}	50.2±1.6^{a}	41.5±0.3^{c}	46.8±3.1^{ab}
200	50.2±0.6^{b}	54.2±0.2^{a}	53.6±1.3^{a}	55.2±1.0^{a}
Acetic acid-soluble				
0	8.7±0.9^{c}	12.6±0.6^{b}	13.6±1.9^{b}	18.0±1.8^{a}
50	4.0±0.0^{c}	5.1±0.6^{c}	8.6±0.9^{b}	10.6±0.7^{a}
100	10.6±2.6^{bc}	6.8±0.6^{c}	15.8±0.1^{ab}	17.8±3.6^{a}
200	14.4±0.1^{b}	7.7±0.7^{d}	16.6±0.8^{a}	12.6±0.6^{c}
Acetic acid-insoluble				
0	38.5±1.2^{a}	33.8±1.4^{b}	31.8±2.2^{b}	27.0±3.2^{c}
50	34.9±0.4^{a}	29.6±0.4^{b}	32.6±2.3^{ab}	24.2±0.4^{c}
100	30.3±0.0^{a}	26.2±0.2^{c}	27.6±0.1^{b}	13.8±0.1^{d}
200	19.1±0.1^{a}	20.4±1.4^{a}	14.4±0.4^{b}	10.9±0.7^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 12. Effect of Mixing on the Content of ES and AS Purified Fractions (% of Total Flour Protein) of Iodate Doughs Mixed to Various Times[1]

		Mixing Time (as % of peak time)[1]		
Cultivar	Flour	50	100	200
ES gliadin				
Glenlea	32.8±0.6^{ab}	34.6±0.5^{ab}	32.0±1.3^{b}	35.9±2.0^{a}
Katepwa	34.4±0.4^{c}	38.8±1.3^{b}	41.2±0.5^{a}	41.0±0.5^{a}
AC Domain	32.4±2.5^{b}	34.6±1.6^{ab}	33.2±2.1^{b}	39.2±1.4^{a}
AC Karma	29.5±1.7^{b}	32.6±0.1^{b}	32.4±1.8^{b}	40.6±0.2^{a}
ES glutenin				
Glenlea	2.6±0.6^{ns}	8.1±0.5^{ns}	10.6±1.3^{ns}	14.3±2.0^{ns}
Katepwa	3.6±0.4^{c}	8.4±1.3^{b}	9.1±0.5^{b}	13.2±0.5^{a}
AC Domain	6.0±2.5^{b}	6.4±1.6^{b}	8.3±2.1^{b}	14.4±1.4^{a}
AC Karma	6.2±1.7^{b}	9.4±0.1^{b}	14.4±1.8^{a}	14.6±0.2^{a}
AS gliadin				
Glenlea	4.8±0.5^{a}	2.1±0.2^{c}	3.2±0.1^{b}	2.5±0.0^{bc}
Katepwa	8.8±0.9^{a}	2.2±0.1^{b}	2.8±0.0^{b}	1.6±0.0^{b}
AC Domain	9.6±1.3^{a}	3.8±0.2^{bc}	5.6±0.2^{b}	2.4±0.1^{c}
AC Karma	11.6±0.5^{a}	4.4±0.2^{c}	6.9±0.6^{b}	2.1±0.2^{d}
AS glutenin				
Glenlea	4.0±0.5^{c}	2.0±0.2^{d}	7.4±0.1^{b}	11.9±0.0^{a}
Katepwa	3.8±0.9^{b}	3.0±0.1^{b}	4.0±0.0^{b}	6.1±0.0^{a}
AC Domain	4.0±1.3^{c}	4.8±0.2^{c}	10.2±0.2^{b}	14.2±0.1^{a}
AC Karma	6.4±0.5^{b}	6.2±0.2^{b}	10.9±0.6^{a}	10.4±0.2^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 13. Effect of Cultivar on the Content of ES and AS Purified Fractions (% of Total Flour Protein) of Iodate Doughs Mixed to Various Times[1]

Mixing Time (% of peak time)	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES gliadin				
0	32.8±0.6^{ab}	34.4±0.4^{a}	32.4±2.5^{ab}	29.5±1.7^{b}
50	34.6±0.5^{b}	38.8±1.3^{a}	34.6±1.6^{b}	32.6±0.1^{b}
100	32.0±1.3^{b}	41.2±0.5^{a}	33.2±2.1^{b}	32.4±1.8^{b}
200	35.9±2.0^{b}	41.0±0.5^{a}	39.2±1.4^{ab}	40.6±0.2^{a}
ES glutenin				
0	2.6±0.6^{ns}	3.6±0.4^{ns}	6.0±2.5^{ns}	6.2±1.7^{ns}
50	8.1±0.5^{ns}	8.4±1.3^{ns}	6.4±1.6^{ns}	9.4±0.1^{ns}
100	10.6±1.3^{ab}	9.1±0.5^{b}	8.3±2.1^{b}	14.4±1.8^{a}
200	14.3±2.0^{ns}	13.2±0.5^{ns}	14.4±1.4^{ns}	14.6±0.2^{ns}
AS gliadin				
0	4.8±0.5^{c}	8.8±0.9^{b}	9.6±1.3^{ab}	11.6±0.5^{a}
50	2.1±0.2^{b}	2.2±0.1^{b}	3.8±0.2^{a}	4.4±0.2^{a}
100	3.2±0.1^{c}	2.8±0.0^{c}	5.6±0.2^{b}	6.9±0.6^{a}
200	2.5±0.0^{a}	1.6±0.0^{c}	2.4±0.1^{ab}	2.1±0.2^{b}
AS glutenin				
0	4.0±0.5^{ab}	3.8±0.9^{b}	4.0±1.3^{ab}	6.4±0.5^{a}
50	2.0±0.2^{d}	3.0±0.1^{c}	4.8±0.2^{b}	6.2±0.2^{a}
100	7.4±0.1^{b}	4.0±0.0^{c}	10.2±0.2^{a}	10.9±0.6^{a}
200	11.9±0.0^{a}	6.1±0.0^{a}	14.2±0.1^{a}	10.4±0.2^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 14. Effect of Mixing Time on the Protein Fractions (as % of Total Flour Protein) Obtained by Modified Osborne Fractionation of NEMI Doughs Mixed to Various Times[1]

		% of Total Flour Protein for Each Mixing Time[1]		
Cultivar	Flour	50	100	200
Salt-soluble				
Glenlea	18.6±1.4^{a}	16.8±0.7^{ab}	16.2±0.4^{b}	16.5±0.3^{ab}
Katepwa	18.1±0.3^{a}	17.4±0.5^{a}	14.6±0.6^{b}	17.0±0.8^{a}
AC Domain	16.0±0.5^{ns}	16.2±2.1^{ns}	14.6±0.5^{ns}	15.0±0.2^{ns}
AC Karma	20.3±0.9^{ab}	20.7±1.0^{a}	18.2±0.2^{b}	19.3±0.7^{ab}
Ethanol-soluble				
Glenlea	35.4±1.0^{c}	42.9±0.4^{b}	43.4±1.1^{b}	52.6±0.9^{a}
Katepwa	38.0±0.5^{d}	45.8±1.2^{c}	50.2±1.6^{b}	52.7±0.6^{a}
AC Domain	38.4±3.3^{c}	35.8±1.5^{c}	45.1±1.7^{b}	55.0±1.8^{a}
AC Karma	35.7±3.6^{c}	42.6±0.8^{b}	48.8±2.8^{b}	56.4±0.8^{a}
Acetic acid-soluble				
Glenlea	8.7±0.9^{c}	5.8±0.7^{d}	15.2±0.1^{b}	20.8±0.6^{a}
Katepwa	12.6±0.6^{a}	6.4±0.1^{c}	9.6±0.0^{b}	9.9±0.8^{b}
AC Domain	13.6±1.9^{b}	10.1±0.1^{b}	19.7±3.7^{a}	19.4±0.5^{a}
AC Karma	18.0±1.8^{b}	13.4±0.8^{c}	23.6±1.5^{a}	15.8±1.8^{bc}
Acetic acid-insoluble				
Glenlea	38.5±1.2^{a}	32.8±0.9^{b}	24.5±1.8^{c}	9.0±0.5^{d}
Katepwa	33.8±1.4^{a}	28.2±0.1^{b}	22.5±0.4^{c}	14.2±0.4^{d}
AC Domain	31.8±2.2^{a}	35.4±3.7^{a}	19.2±2.3^{b}	8.6±0.5^{c}
AC Karma	27.0±3.2^{a}	25.0±0.8^{a}	7.2±1.2^{b}	6.6±0.5^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 15. Effect of Cultivar on the Protein Fractions (as % of Total Flour Protein) Obtained by Modified Osborne Fractionation of NEMI Doughs Mixed to Various Times[1]

Mixing Time (% of peak time)	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
Salt-soluble				
0	18.6±1.4^{b}	18.1±0.3^{b}	16.0±0.5^{c}	20.3±0.9^{a}
50	16.8±0.7^{b}	17.4±0.5^{ab}	16.2±2.1^{b}	20.7±1.0^{a}
100	16.2±0.4^{b}	14.6±0.6^{c}	14.6±0.5^{c}	18.2±0.2^{a}
200	16.5±0.3^{bc}	17.0±0.8^{b}	15.0±0.2^{c}	19.3±0.7^{a}
Ethanol-soluble				
0	35.4±1.0^{ns}	38.0±0.5^{ns}	38.4±3.3^{ns}	35.7±3.6^{ns}
50	42.9±0.4^{b}	45.8±1.2^{a}	35.8±1.5^{c}	42.6±0.8^{b}
100	43.4±1.1^{b}	50.2±1.6^{a}	45.1±1.7^{ab}	48.8±2.8^{a}
200	52.6±0.9^{b}	52.7±0.6^{b}	55.0±1.8^{ab}	56.4±0.8^{a}
Acetic acid-soluble				
0	8.7±0.9^{c}	12.6±0.6^{b}	13.6±1.9^{b}	18.0±1.8^{a}
50	5.8±0.7^{c}	6.4±0.1^{c}	10.1±0.1^{b}	13.4±0.8^{a}
100	15.2±0.1^{b}	9.6±0.0^{c}	19.7±3.7^{ab}	23.6±1.5^{a}
200	20.8±0.6^{a}	9.9±0.8^{c}	19.4±0.5^{a}	15.8±1.8^{b}
Acetic acid-insoluble				
0	38.5±1.2^{a}	33.8±1.4^{b}	31.8±2.2^{b}	27.0±3.2^{c}
50	32.8±0.9^{ab}	28.2±0.1^{bc}	35.4±3.7^{a}	25.0±0.8^{c}
100	24.5±1.8^{a}	22.5±0.4^{ab}	19.2±2.3^{b}	7.2±1.2^{c}
200	9.0±0.5^{b}	14.2±0.4^{a}	8.6±0.5^{b}	6.6±0.5^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 16. Effect of Mixing on the Content of ES and AS Purified Fractions (% of Total Flour Protein) of NEMI Doughs Mixed to Various Times[1]

		Mixing Time (as % of peak time)[1]		
Cultivar	Flour	50	100	200
ES gliadin				
Glenlea	32.8±0.6^{c}	35.1±0.8^{b}	34.8±0.6^{b}	37.6±0.1^{a}
Katepwa	34.4±0.4^{b}	40.6±0.6^{a}	42.4±0.2^{a}	40.4±3.0^{a}
AC Domain	32.4±2.5^{b}	30.8±0.4^{b}	30.2±1.3^{b}	39.0±1.3^{a}
AC Karma	29.5±1.7^{b}	31.1±2.4^{ab}	35.8±2.9^{ab}	38.2±3.2^{a}
ES glutenin				
Glenlea	2.6±0.6^{c}	7.8±0.8^{b}	8.6±0.6^{b}	15.1±0.1^{a}
Katepwa	3.6±0.4^{b}	5.3±0.6^{b}	7.8±0.2^{b}	12.3±3.0^{a}
AC Domain	6.0±2.5^{b}	5.0±0.4^{b}	14.8±1.3^{a}	16.0±1.3^{a}
AC Karma	6.2±1.7^{b}	11.5±2.4^{ab}	13.1±2.9^{ab}	18.2±3.2^{a}
AS gliadin				
Glenlea	4.8±0.5^{a}	2.2±0.1^{b}	4.6±0.4^{a}	2.5±0.0^{b}
Katepwa	8.8±0.9^{a}	2.6±0.1^{b}	2.8±0.0^{b}	2.0±0.1^{b}
AC Domain	9.6±1.3^{a}	6.2±0.1^{b}	9.6±0.2^{a}	3.8±0.1^{c}
AC Karma	11.6±0.5^{a}	5.9±0.3^{b}	3.7±0.1^{c}	2.1±0.1^{d}
AS glutenin				
Glenlea	4.0±0.5^{c}	3.6±0.1^{c}	10.6±0.4^{b}	18.2±0.0^{a}
Katepwa	3.8±0.9^{b}	3.9±0.1^{b}	6.8±0.0^{a}	7.9±0.1^{a}
AC Domain	4.0±1.3^{c}	3.8±0.1^{c}	10.2±0.2^{b}	15.6±0.1^{a}
AC Karma	6.4±0.5^{d}	7.5±0.3^{c}	19.9±0.1^{a}	13.7±0.1^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 17. Effect of Cultivar on the Content of ES and AS Purified Fractions (% of Total Flour Protein) of NEMI Doughs Mixed to Various Times[1]

Mixing Time (% of peak time)	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES gliadin				
0	32.8±0.6^{ab}	34.4±0.4^{a}	32.4±2.5^{ab}	29.5±1.7^{b}
50	35.1±0.8^{b}	40.6±0.6^{a}	30.8±0.4^{c}	31.1±2.4^{c}
100	34.8±0.6^{bc}	42.4±0.2^{a}	30.2±1.3^{c}	35.8±2.9^{b}
200	37.6±0.1^{ns}	40.4±3.0^{ns}	39.0±1.3^{ns}	38.2±3.2^{ns}
ES glutenin				
0	2.6±0.6^{ns}	3.6±0.4^{ns}	6.0±2.5^{ns}	6.2±1.7^{ns}
50	7.8±0.8^{b}	5.3±0.6^{b}	5.0±0.4^{b}	11.5±2.4^{a}
100	8.6±0.6^{bc}	7.8±0.2^{c}	14.8±1.3^{a}	13.1±2.9^{ab}
200	15.1±0.1^{ns}	12.3±3.0^{ns}	16.0±1.3^{ns}	18.2±3.2^{ns}
AS gliadin				
0	4.8±0.5^{c}	8.8±0.9^{b}	9.6±1.3^{ab}	11.6±0.5^{a}
50	2.2±0.1^{b}	2.6±0.1^{b}	6.2±0.1^{A}	5.9±0.3^{a}
100	4.6±0.4^{b}	2.8±0.0^{d}	9.6±0.2^{a}	3.7±0.1^{c}
200	2.5±0.0^{b}	2.0±0.1^{c}	3.8±0.1^{a}	2.1±0.1^{c}
AS glutenin				
0	4.0±0.5^{ab}	3.8±0.9^{b}	4.0±1.3^{ab}	6.4±0.5^{a}
50	3.6±0.1^{b}	3.9±0.1^{b}	3.8±0.1^{b}	7.5±0.3^{a}
100	10.6±0.4^{b}	6.8±0.0^{c}	10.2±0.2^{b}	19.9±0.1^{a}
200	18.2±0.0^{a}	7.9±0.1^{d}	15.6±0.1^{b}	13.7±0.1^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 18. Effect of Treatment on the Content of Salt-soluble Proteins Obtained by Modified Osborne Fractionation (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	20.1±1.2^{a}	17.1±0.5^{b}	16.8±0.7^{b}
	100	20.4±2.3^{ns}	16.9±1.3^{ns}	16.2±0.4^{ns}
	200	20.4±1.1^{a}	16.8±0.9^{b}	16.5±0.3^{b}
Katepwa	50	21.8±2.6^{ns}	16.8±0.7^{ns}	17.4±0.5^{ns}
	100	22.1±1.3^{a}	16.1±0.3^{b}	14.6±0.6^{b}
	200	19.0±1.1^{ns}	17.0±0.1^{ns}	17.0±0.8^{ns}
AC Domain	50	16.5±0.3^{ns}	16.8±1.0^{ns}	16.2±2.1^{ns}
	100	14.6±0.1^{ns}	14.7±0.8^{ns}	14.6±0.5^{ns}
	200	15.6±0.6^{a}	14.2±0.1^{b}	15.0±0.2^{ab}
AC Karma	50	21.2±0.5^{ns}	20.4±0.1^{ns}	20.7±1.0^{ns}
	100	20.9±0.4^{a}	17.3±0.3^{b}	18.2±0.2^{b}
	200	24.1±0.1^{a}	18.8±0.8^{b}	19.3±0.7^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 19. Effect of Treatment on the Content of Ethanol-soluble Proteins Obtained by Modified Osborne Fractionation (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	37.7±0.4^{b}	42.7±0.0^{a}	42.9±0.4^{a}
	100	37.7±0.0^{b}	42.4±0.5^{a}	43.4±1.1^{a}
	200	42.6±0.3^{c}	50.2±0.6^{b}	52.6±0.9^{a}
Katepwa	50	40.9±1.6^{b}	47.1±0.4^{a}	45.8±1.2^{a}
	100	41.6±1.5^{b}	50.2±1.6^{a}	50.2±1.6^{a}
	200	46.7±0.6^{b}	54.2±0.2^{a}	52.7±0.6^{a}
AC Domain	50	31.5±1.6^{b}	41.0±2.3^{a}	35.8±1.5^{ab}
	100	34.8±3.3^{b}	41.5±0.3^{ab}	45.1±1.7^{a}
	200	39.5±2.4^{b}	53.6±1.3^{a}	55.0±1.8^{a}
AC Karma	50	34.4±0.6^{b}	42.0±2.1^{a}	42.6±0.8^{a}
	100	37.8±1.3^{b}	46.8±3.1^{a}	48.8±2.8^{a}
	200	39.7±0.6^{b}	55.2±1.0^{a}	56.4±0.8^{a}

[1] Means ● standard deviation, means with different letters are significantly different (α=0.05)

Table 20. Effect of Treatment on the Content of Acetic Acid-soluble Proteins Obtained by Modified Osborne Fractionation (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	5.1±0.3^{ab}	4.0±0.0^{b}	5.8±0.7^{a}
	100	5.6±0.9^{b}	10.6±2.6^{ab}	15.2±0.1^{a}
	200	6.5±0.5^{c}	14.4±0.1^{b}	20.8±0.6^{a}
Katepwa	50	6.4±0.3^{a}	5.1±0.6^{b}	6.4±0.1^{a}
	100	5.9±1.1^{b}	6.8±0.6^{b}	9.6±0.0^{a}
	200	4.3±0.4^{c}	7.7±0.7^{b}	9.9±0.8^{a}
AC Domain	50	8.6±0.0^{ns}	8.6±0.9^{ns}	10.1±0.1^{ns}
	100	11.8±0.4^{b}	15.8±0.1^{ab}	19.7±3.7^{a}
	200	8.6±0.4^{c}	16.6±0.8^{b}	19.4±0.5^{a}
AC Karma	50	11.2±0.6^{ab}	10.6±0.7^{b}	13.4±0.8^{a}
	100	11.5±1.7^{b}	17.8±3.6^{ab}	23.6±1.5^{a}
	200	9.5±0.9^{b}	12.6±0.6^{ab}	15.8±1.8^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 21. Effect of Treatment on the Content of Acetic Acid-insoluble Proteins Obtained by Modified Osborne Fractionation (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	36.9±1.3^{a}	34.9±0.4^{ab}	32.8±0.9^{b}
	100	36.0±1.3^{a}	30.3±0.0^{b}	24.5±1.8^{c}
	200	31.9±0.1^{a}	19.1±0.1^{b}	9.0±0.5^{c}
Katepwa	50	32.7±2.7^{ns}	29.6±0.4^{ns}	28.2±0.1^{ns}
	100	30.4±1.1^{a}	26.2±0.2^{b}	22.5±0.4^{c}
	200	29.3±1.2^{a}	20.4±1.4^{b}	14.2±0.4^{c}
AC Domain	50	43.1±2.2^{a}	32.6±2.3^{b}	35.4±3.7^{ab}
	100	39.3±3.7^{a}	27.6±0.1^{b}	19.2±2.3^{c}
	200	36.7±2.5^{a}	14.4±0.4^{b}	8.6±0.5^{c}
AC Karma	50	33.5±1.3^{a}	24.2±0.4^{b}	25.0±0.8^{b}
	100	30.7±1.2^{a}	13.8±0.1^{b}	7.2±1.2^{c}
	200	26.2±0.6^{a}	10.9±0.7^{b}	6.6±0.5^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 22. Effect of Treatment on the Content of Ethanol-soluble Gliadin (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	31.6±0.7^{b}	34.6±0.5^{a}	35.1±0.8^{a}
	100	31.7±0.7^{b}	32.0±1.3^{ab}	34.8±0.6^{a}
	200	33.4±0.4^{b}	35.9±2.0^{ab}	37.6±0.1^{a}
Katepwa	50	37.5±0.4^{b}	38.8±1.3^{ab}	40.6±0.6^{a}
	100	37.1±0.6^{b}	41.2±0.5^{a}	42.4±0.2^{a}
	200	39.8±0.9^{ns}	41.0±0.5^{ns}	40.4±3.0^{ns}
AC Domain	50	26.2±1.1^{c}	34.6±1.6^{a}	30.8±0.4^{b}
	100	30.4±0.9^{ns}	33.2±2.1^{ns}	30.2±1.3^{ns}
	200	33.6±0.5^{b}	39.2±1.4^{a}	39.0±1.3^{a}
AC Karma	50	28.3±1.8^{ns}	32.6±0.1^{ns}	31.1±2.4^{ns}
	100	32.2±1.3^{ns}	32.4±1.8^{ns}	35.8±2.9^{ns}
	200	32.8±1.8^{b}	40.6±0.2^{a}	38.2±3.2^{ab}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 23. Effect of Treatment on the Content of Ethanol-soluble Glutenin (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	6.1±0.7^{ns}	8.1±0.5^{ns}	7.8±0.8^{ns}
	100	6.0±0.7^{b}	10.6±1.3^{a}	8.6±0.6^{ab}
	200	9.2±0.4^{b}	14.3±2.0^{a}	15.1±0.1^{a}
Katepwa	50	3.4±0.4^{b}	8.4±1.3^{a}	5.3±0.6^{b}
	100	4.6±0.6^{b}	9.1±0.5^{a}	7.8±0.2^{a}
	200	7.0±0.9^{b}	13.2±0.5^{a}	12.3±3.0^{ab}
AC Domain	50	5.4±1.1^{ns}	6.4±1.6^{ns}	5.0±0.4^{ns}
	100	4.4±0.9^{b}	8.3±2.1^{b}	14.8±1.3^{a}
	200	5.8±0.5^{b}	14.4±1.4^{a}	16.0±1.3^{a}
AC Karma	50	6.1±1.8^{ns}	9.4±0.1^{ns}	11.5±2.4^{ns}
	100	5.6±1.3^{b}	14.4±1.8^{a}	13.1±2.9^{a}
	200	6.8±1.8^{b}	14.6±0.2^{a}	18.2±3.2^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 24. Effect of Treatment on the Content of Acetic Acid-soluble Gliadin (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	2.5±0.0^{a}	2.1±0.2^{b}	2.2±0.1^{ab}
	100	2.8±0.1^{b}	3.2±0.1^{b}	4.6±0.4^{a}
	200	1.8±0.0^{b}	2.5±0.0^{a}	2.5±0.0^{a}
Katepwa	50	2.4±0.4^{ns}	2.2±0.1^{ns}	2.6±0.1^{ns}
	100	3.4±0.8^{ns}	2.8±0.0^{ns}	2.8±0.0^{ns}
	200	1.4±0.0^{b}	1.6±0.0^{b}	2.0±0.1^{a}
AC Domain	50	7.1±0.6^{a}	3.8±0.2^{b}	6.2±0.1^{a}
	100	8.8±0.4^{a}	5.6±0.2^{b}	9.6±0.2^{a}
	200	7.2±0.7^{a}	2.4±0.1^{c}	3.8±0.1^{b}
AC Karma	50	5.7±0.4^{a}	4.4±0.2^{b}	5.9±0.3^{a}
	100	6.3±1.6^{ab}	6.9±0.6^{a}	3.7±0.1^{b}
	200	3.2±0.3^{a}	2.1±0.2^{b}	2.1±0.1^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 25. Effect of Treatment on the Content of Acetic Acid-soluble Glutenin (% of Total Flour Protein) of Doughs Mixed to Various Times[1]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	2.6±0.0^{b}	2.0±0.2^{c}	3.6±0.1^{a}
	100	2.8±0.1^{c}	7.4±0.1^{b}	10.6±0.4^{a}
	200	4.7±0.0^{c}	11.9±0.0^{b}	18.2±0.0^{a}
Katepwa	50	3.9±0.4^{a}	3.0±0.1^{b}	3.9±0.1^{a}
	100	2.5±0.8^{b}	4.0±0.0^{b}	6.8±0.0^{a}
	200	2.9±0.0^{c}	6.1±0.0^{b}	7.9±0.1^{a}
AC Domain	50	1.5±0.6^{b}	4.8±0.2^{a}	3.8±0.1^{a}
	100	3.0±0.4^{b}	10.2±0.2^{a}	10.2±0.2^{a}
	200	1.5±0.7^{c}	14.2±0.1^{b}	15.6±0.1^{a}
AC Karma	50	5.5±0.4^{b}	6.2±0.2^{b}	7.5±0.3^{a}
	100	5.2±1.6^{c}	10.9±0.6^{b}	19.9±0.1^{a}
	200	6.3±0.3^{c}	10.4±0.2^{b}	13.7±0.1^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 26. F Values of Significant[1] Main Effects and Interactions Between Factors Affecting Response Variables (Modified Osborne Fractions and Purified ES and AS Fractions)

Modified Osborne Fractions			Purified ES and AS Fractions		
Response Variable[2]	Source[3]	F Value	Response Variable[2]	Source[3]	F Value
AI protein	CV	139.09	AS gliadin	CV	224.47
	MT	360.11		MT	461.04
	TRTMT	344.16		TRTMT	16.50
	CV×MT	12.46		CV×MT	31.87
	CV×TRTMT	11.14		CV×TRTMT	12.78
	MT×TRTMT	72.73		MT×TRTMT	2.72
	CV×MT×TRTMT	3.55		CV×MT×TRTMT	5.01
AS protein	CV	152.16	AS glutenin	CV	232.34
	MT	89.76		MT	476.99
	TRTMT	111.43		TRTMT	598.22
	CV×MT	24.02		CV×MT	48.21
	CV×TRTMT	3.68		CV×TRTMT	37.92
	MT×TRTMT	31.97		MT×TRTMT	154.40
	CV×MT×TRTMT	2.17		CV×MT×TRTMT	17.20
ES protein	CV	24.32	ES gliadin	CV	83.94
	MT	221.79		MT	60.57
	TRTMT	129.63		TRTMT	33.92
	CV×MT	7.35		CV×MT	9.12
	CV×TRTMT	2.80		CV×TRTMT	ns
	MT×TRTMT	21.85		MT×TRTMT	5.08
	CV×MT×TRTMT	ns		CV×MT×TRTMT	1.90
SS protein	CV	110.84	ES glutenin	CV	18.17
	MT	9.04		MT	118.68
	TRTMT	60.87		TRTMT	78.14
	CV×MT	ns		CV×MT	2.90
	CV×TRTMT	6.42		CV×TRTMT	3.58
	MT×TRTMT	9.33		MT×TRTMT	14.80
	CV×TRTMT×MT	2.66		CV×MT×TRTMT	2.14

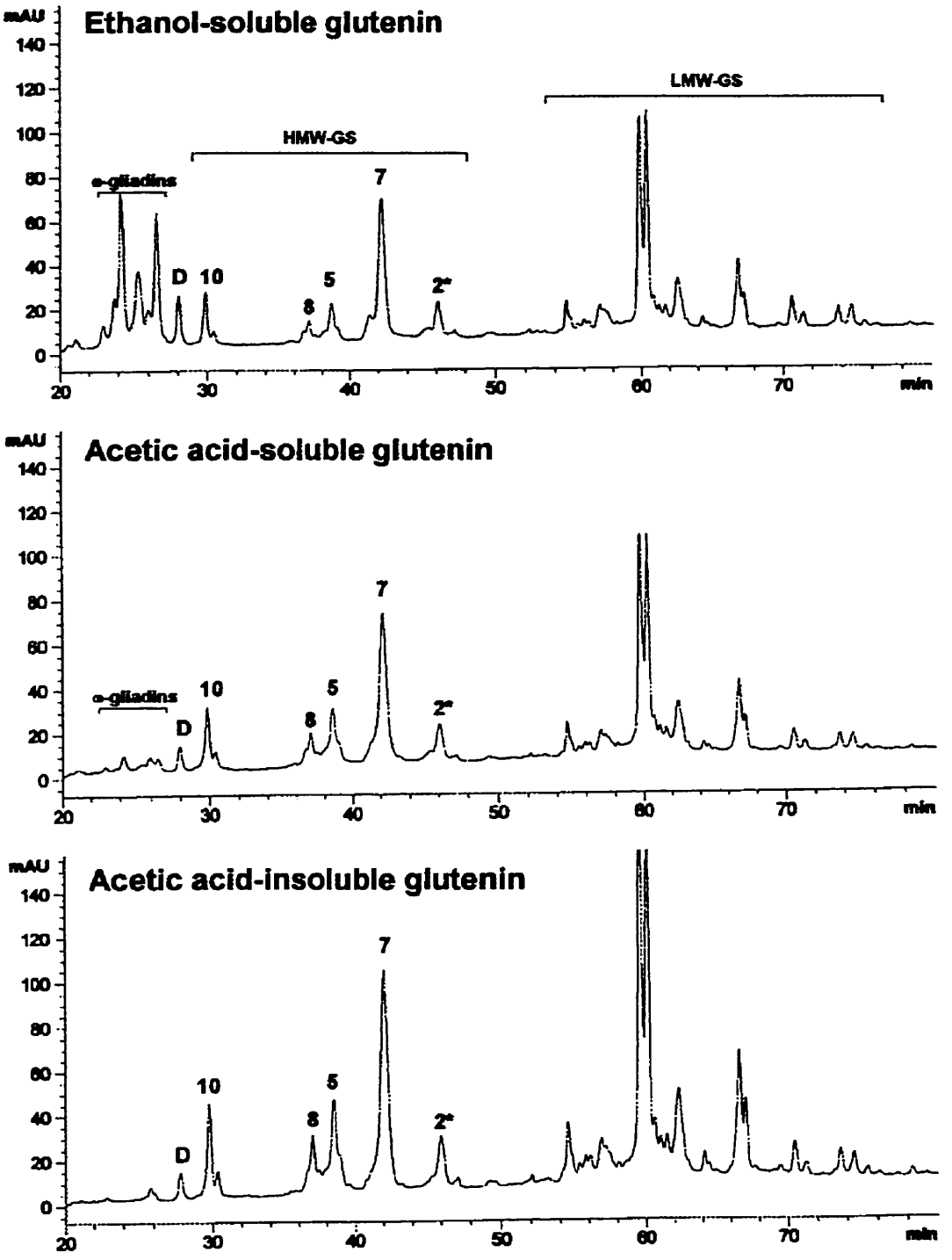
[1] Level of significance α=0.05

[2] as % of total flour/dough protein

[3] CV=cultivar, MT=mixing time, TRTMT=treatment (control, iodate, NEMI)

Figure 1. RP-HPLC chromatograms of ES, AS and AI glutenin for Glenlea, Katepwa, AC Domain and AC Karma

Glenlea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100
80
60
40
20
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30
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50
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HMW-GS
ω-gliadins
7
D
10
5
8
2*
Acetic acid-soluble glutenin
mAU
140
120
100
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ω-gliadins
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Acetic acid-insoluble glutenin
mAU
140
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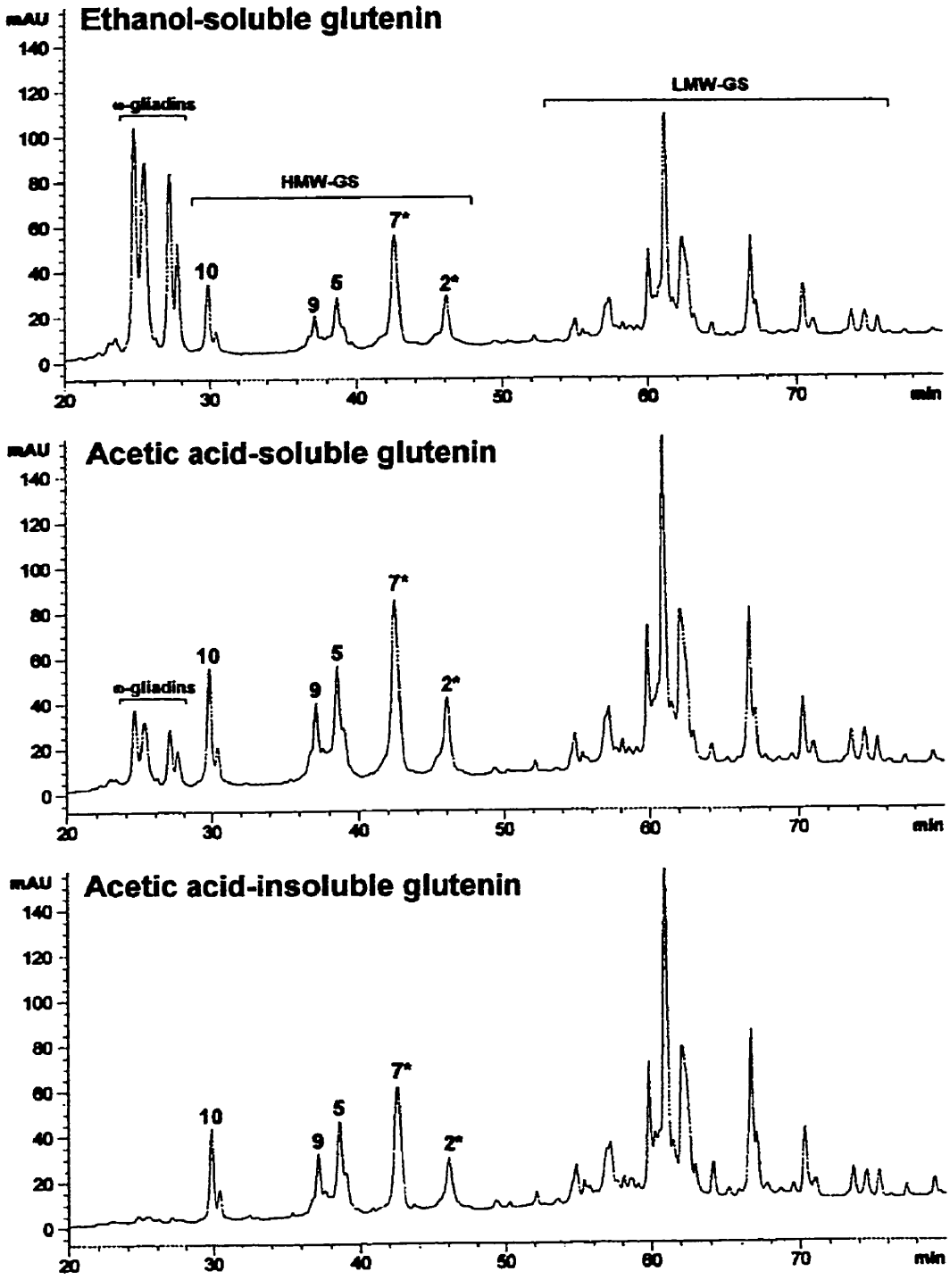
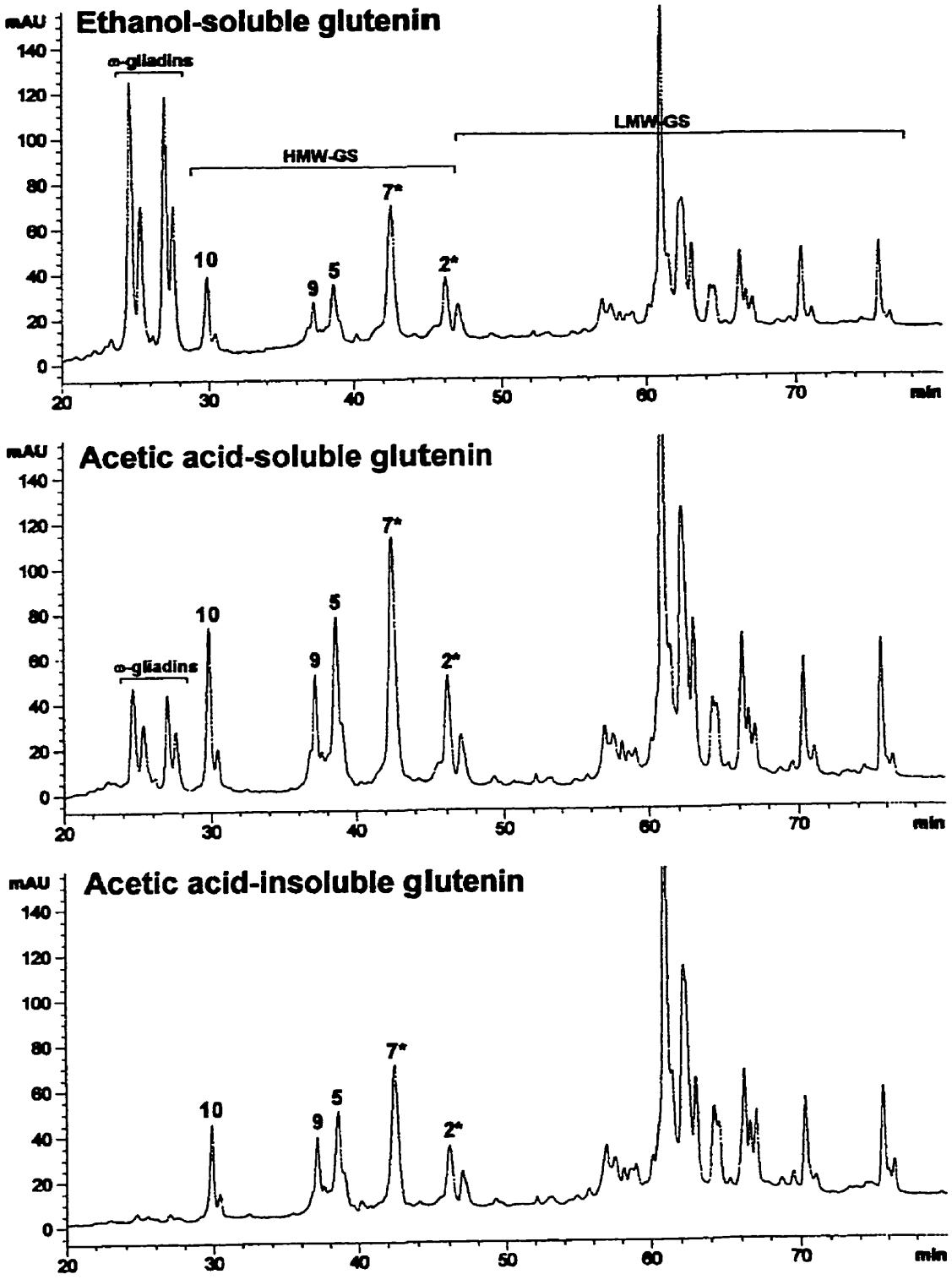
Katepwa


mAU
Ethanol-soluble glutenin
ω-gliadins
HMW-GS
LMW-GS
10
9
5
7*
2*
min
Acetic acid-soluble glutenin
Acetic acid-insoluble glutenin

AC Domain


Ethanol-soluble glutenin
mAU
ω-gliadins
HMW-GS
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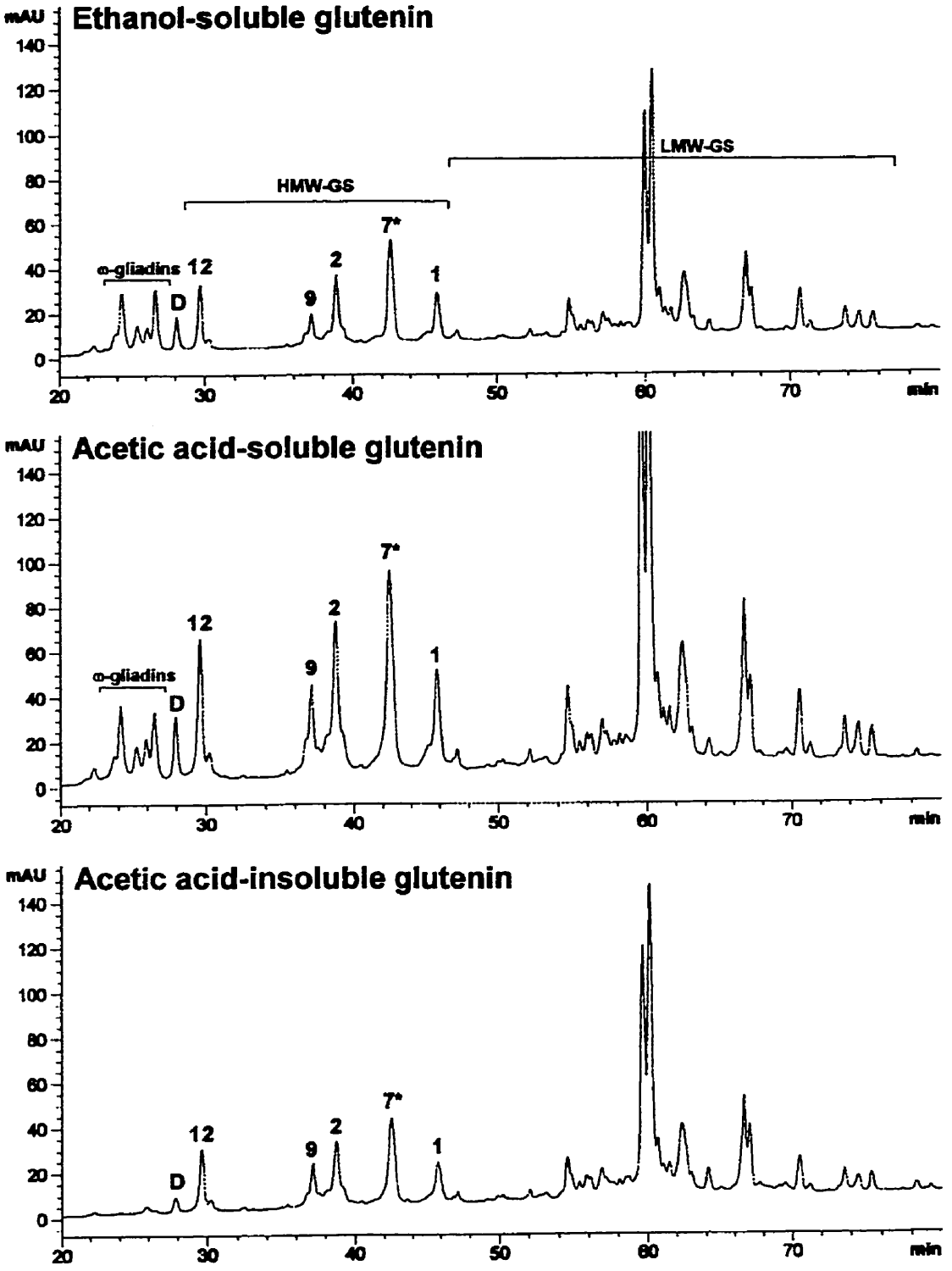
7*
2*
min
Acetic acid-soluble glutenin
ω-gliadins
Acetic acid-insoluble glutenin

AC Karma


Ethanol-soluble glutenin
mAU
140
120
100
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60
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HMW-GS
LMW-GS
ω-gliadins
12
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min
Acetic acid-soluble glutenin
ω-gliadins
12
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Acetic acid-insoluble glutenin
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Table 27. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Made From Glenlea Flour[1]

Subunit/Ratio	Flour	Flour-Water Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	14.0±0.8^{a}	13.2±0.1^{ab}	12.3±0.3^{b}	12.2±0.3^{b}
5	14.0±0.3^{c}	14.4±0.1^{bc}	15.5±0.6^{b}	17.4±0.5^{a}
7	51.7±1.0^{a}	47.0±1.2^{b}	45.9±0.5^{bc}	44.5±0.1^{c}
8	7.4±0.3^{c}	8.2±0.1^{b}	9.1±0.2^{a}	9.5±0.4^{a}
10	13.0±2.4^{b}	17.2±0.8^{a}	17.2±0.4^{a}	16.3±0.1^{ab}
Ratio	0.49±0.04^{ab}	0.44±0.06^{b}	0.50±0.03^{ab}	0.57±0.00^{a}
AS glutenin				
2	12.5±0.7^{ns}	13.2±1.3^{ns}	12.9±0.9^{ns}	12.9±0.8^{ns}
5	18.8±0.1^{ns}	16.7±0.8^{ns}	17.8±1.3^{ns}	18.3±0.7^{ns}
7	46.4±1.7^{ns}	45.2±0.2^{ns}	45.2±0.2^{ns}	44.8±0.1^{ns}
8	9.5±0.2^{b}	9.7±0.5^{b}	10.2±0.4^{ab}	10.9±0.0^{a}
10	12.8±2.4^{ns}	15.1±0.9^{ns}	14.0±0.3^{ns}	13.1±0.0^{ns}
Ratio	0.60±0.03^{c}	0.69±0.01^{a}	0.69±0.02^{ab}	0.65±0.01^{bc}
AI glutenin				
2	11.3±0.1^{ns}	12.3±0.6^{ns}	12.3±0.4^{ns}	13.0±1.0^{ns}
5	20.8±0.0^{a}	17.9±0.1^{b}	18.3±0.6^{b}	17.6±0.3^{b}
7	45.4±0.3^{ns}	45.2±0.7^{ns}	45.3±0.7^{ns}	45.9±1.1^{ns}
8	11.8±0.2^{ns}	11.9±0.2^{ns}	11.4±0.6^{ns}	11.2±0.3^{ns}
10	10.6±0.1^{b}	12.7±0.2^{a}	12.7±0.3^{a}	12.2±0.1^{a}
Ratio	0.49±0.01^{a}	0.44±0.01^{b}	0.41±0.01^{b}	0.37±0.02^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 28. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Made From Katepwa Flour[1]

Subunit/Ratio	Flour	Flour-Water Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	19.1±0.3^{a}	17.4±0.1^{b}	16.0±0.0^{c}	16.2±0.1^{c}
5	18.1±0.1^{ns}	18.6±0.1^{ns}	19.5±0.1^{ns}	19.5±1.3^{ns}
7	34.8±1.6^{a}	29.3±0.5^{b}	29.0±1.1^{b}	28.9±0.6^{b}
9	12.0±1.6^{b}	13.6±0.4^{ab}	14.8±0.4^{ab}	15.3±1.0^{a}
10	16.0±3.5^{ns}	21.0±0.3^{ns}	20.7±0.6^{ns}	20.1±1.0^{ns}
Ratio	0.52±0.00^{ns}	0.47±0.00^{ns}	0.49±0.00^{ns}	0.50±0.03^{ns}
AS glutenin				
2	16.3±1.1^{ns}	15.4±0.1^{ns}	15.8±1.0^{ns}	15.4±0.1^{ns}
5	21.5±0.3^{ns}	20.5±0.2^{ns}	19.3±2.3^{ns}	20.4±1.6^{ns}
7	32.9±0.7^{a}	30.3±0.3^{b}	29.3±0.7^{b}	29.3±0.6^{b}
9	12.9±1.3^{b}	15.2±0.2^{ab}	17.5±2.0^{a}	17.9±1.5^{a}
10	16.4±0.2^{ns}	18.5±0.2^{ns}	18.0±1.9^{ns}	17.1±0.7^{ns}
Ratio	0.56±0.00^{ns}	0.53±0.01^{ns}	0.56±0.04^{ns}	0.52±0.04^{ns}
AI glutenin				
2	18.0±1.6^{ns}	15.3±0.2^{ns}	15.6±4.7^{ns}	15.6±0.5^{ns}
5	24.3±0.6^{ns}	22.7±0.9^{ns}	18.5±0.0^{ns}	19.6±3.6^{ns}
7	29.7±1.2^{ns}	29.2±0.3^{ns}	29.5±0.2^{ns}	30.3±0.4^{ns}
9	15.4±1.3^{ns}	16.8±0.0^{ns}	21.1±1.5^{ns}	19.4±4.2^{ns}
10	12.6±0.3^{b}	15.9±0.8^{a}	15.4±0.1^{a}	15.0±0.5^{a}
Ratio	0.35±0.03^{ns}	0.34±0.09^{ns}	0.32±0.01^{ns}	0.33±0.01^{ns}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 29. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Made From AC Domain Flour[1]

Subunit/Ratio	Flour	Flour-Water Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	16.5±0.5^{b}	17.4±0.3^{a}	15.9±0.2^{bc}	15.3±0.1^{c}
5	17.0±0.6^{b}	16.9±0.4^{b}	18.8±0.1^{a}	19.7±0.2^{a}
7	35.9±0.6^{a}	35.9±0.0^{a}	34.1±0.2^{b}	32.9±0.1^{c}
9	13.8±1.9^{ab}	12.1±0.1^{b}	13.7±0.1^{ab}	15.0±0.1^{a}
10	16.8±1.4^{ns}	17.7±0.9^{ns}	17.5±0.3^{ns}	17.1±0.3^{ns}
Ratio	0.45±0.01^{ns}	0.45±0.01^{ns}	0.44±0.00^{ns}	0.44±0.03^{ns}
AS glutenin				
2	14.6±0.4^{b}	17.0±0.0^{a}	16.2±0.7^{a}	16.3±0.2^{a}
5	23.4±0.0^{a}	18.2±0.7^{c}	18.7±0.6^{c}	20.6±0.2^{b}
7	34.0±0.3^{a}	34.8±0.6^{a}	33.7±0.5^{a}	31.8±0.1^{b}
9	14.8±0.3^{b}	13.4±0.2^{c}	14.6±0.3^{b}	15.5±0.1^{a}
10	13.1±0.4^{c}	16.5±0.2^{ab}	16.7±0.1^{a}	15.7±0.4^{b}
Ratio	0.47±0.00^{b}	0.56±0.01^{a}	0.54±0.01^{a}	0.51±0.04^{ab}
AI glutenin				
2	13.9±0.0^{ns}	15.6±0.2^{ns}	15.5±1.5^{ns}	14.1±0.3^{ns}
5	23.9±0.3^{a}	19.2±1.2^{b}	21.5±0.9^{b}	21.0±0.4^{b}
7	33.5±0.0^{a}	33.4±0.9^{a}	29.5±1.9^{b}	31.0±1.2^{ab}
9	16.8±0.4^{ns}	19.2±1.3^{ns}	18.4±1.0^{ns}	19.7±1.5^{ns}
10	11.9±0.0^{c}	12.5±1.2^{bc}	15.2±0.3^{a}	14.2±0.3^{ab}
Ratio	0.31±0.01^{a}	0.21±0.01^{b}	0.22±0.07^{ab}	0.25±0.02^{ab}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 30. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Made From AC Karma Flour[1]

Subunit/Ratio	Flour	Flour-Water Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
1	17.4±0.5^{ns}	17.0±0.6^{ns}	16.2±0.4^{ns}	16.3±0.3^{ns}
2	23.7±0.2^{ns}	23.1±0.0^{ns}	23.3±0.5^{ns}	23.2±0.4^{ns}
7	31.7±1.4^{ns}	29.9±1.0^{ns}	28.2±1.9^{ns}	28.3±0.7^{ns}
9	11.5±1.0^{b}	12.3±0.5^{ab}	14.3±1.9^{ab}	15.0±0.5^{a}
12	15.7±0.4^{b}	17.7±0.0^{a}	17.9±0.2^{a}	17.2±0.5^{a}
Ratio	0.39±0.02^{ns}	0.37±0.02^{ns}	0.40±0.02^{ns}	0.38±0.00^{ns}
AS glutenin				
1	16.4±0.1^{ns}	16.8±0.7^{ns}	16.3±1.3^{ns}	17.2±0.0^{ns}
2	23.6±0.2^{a}	22.6±1.0^{ab}	22.9±0.7^{ab}	20.9±1.6^{b}
7	31.1±0.8^{ns}	30.2±0.3^{ns}	30.1±1.4^{ns}	29.5±0.1^{ns}
9	12.7±0.2^{c}	14.1±0.5^{bc}	15.5±1.0^{ab}	17.7±1.6^{a}
12	16.1±0.3^{a}	16.4±0.1^{a}	15.2±0.4^{b}	14.7±0.1^{b}
Ratio	0.51±0.01^{ns}	0.51±0.01^{ns}	0.47±0.03^{ns}	0.51±0.01^{ns}
AI glutenin				
1	15.3±0.7^{ns}	15.7±0.6^{ns}	16.2±1.2^{ns}	16.4±0.6^{ns}
2	25.1±0.4^{ns}	22.1±2.9^{ns}	23.1±3.3^{ns}	20.3±3.3^{ns}
7	29.9±0.2^{ab}	30.1±0.5^{a}	28.8±0.5^{b}	30.5±0.7^{a}
9	13.6±0.7^{ns}	16.2±2.2^{ns}	16.3±1.9^{ns}	17.9±3.5^{ns}
12	16.2±0.1^{ns}	15.8±0.5^{ns}	15.6±0.4^{ns}	15.0±0.9^{ns}
Ratio	0.34±0.00^{a}	0.30±0.01^{b}	0.28±0.02^{b}	0.29±0.01^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 31. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Flour[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	14.0±0.8^{c}	19.1±0.3^{a}	16.5±0.5^{b}	17.4±0.5^{b}
2/5	14.0±0.3^{d}	18.1±0.1^{b}	17.0±0.6^{c}	23.7±0.2^{a}
7	51.7±1.0^{a}	34.8±1.6^{bc}	35.9±0.6^{b}	31.7±1.4^{c}
8/9	7.4±0.3^{b}	12.0±1.6^{a}	13.8±1.9^{a}	11.5±1.0^{a}
10/12	13.0±2.4^{ns}	16.0±3.5^{ns}	16.8±1.4^{ns}	15.7±0.4^{ns}
Ratio	0.49±0.04^{a}	0.52±0.00^{a}	0.46±0.01^{ab}	0.39±0.02^{b}
AS glutenin				
1/2	12.5±0.7^{b}	16.3±1.1^{a}	14.6±0.4^{a}	16.4±0.1^{a}
2/5	18.8±0.1^{c}	21.5±0.3^{b}	23.5±0.0^{a}	23.6±0.2^{a}
7	46.4±1.7^{a}	32.9±0.7^{b}	34.0±0.3^{b}	31.1±0.8^{b}
8/9	9.5±0.2^{c}	12.9±1.3^{b}	14.8±0.3^{a}	12.7±0.2^{b}
10/12	12.8±2.4^{b}	16.4±0.2^{a}	13.1±0.4^{ab}	16.1±0.3^{ab}
Ratio	0.60±0.03^{a}	0.56±0.00^{b}	0.47±0.00^{c}	0.51±0.01^{c}
AI glutenin				
1/2	11.3±0.1^{c}	18.0±1.6^{a}	13.9±0.0^{b}	15.3±0.7^{b}
2/5	20.8±0.0^{c}	24.3±0.6^{ab}	23.9±0.3^{b}	25.1±0.4^{a}
7	45.4±0.3^{a}	29.7±1.2^{c}	33.5±0.0^{b}	29.9±0.2^{c}
8/9	11.8±0.2^{c}	15.4±1.3^{ab}	16.8±0.4^{a}	13.6±0.7^{bc}
10/12	10.6±0.1^{a}	12.6±0.3^{b}	11.9±0.0^{b}	16.2±0.1^{b}
Ratio	0.49±0.01^{a}	0.35±0.03^{b}	0.31±0.01^{b}	0.34±0.00^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 32. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	13.2±0.1^{b}	17.4±0.1^{a}	17.4±0.3^{a}	17.0±0.6^{a}
2/5	14.4±0.1^{d}	18.6±0.1^{b}	16.9±0.4^{c}	23.1±0.0^{a}
7	47.0±1.2^{a}	29.3±0.5^{c}	35.9±0.0^{b}	29.9±1.0^{c}
8/9	8.2±0.1^{c}	13.6±0.4^{a}	12.1±0.1^{b}	12.3±0.5^{b}
10/12	17.2±0.8^{b}	21.0±0.3^{a}	17.7±0.9^{b}	17.7±0.0^{b}
Ratio	0.44±0.06^{ab}	0.47±0.00^{a}	0.45±0.01^{ab}	0.37±0.02^{b}
AS glutenin				
1/2	13.2±1.3^{b}	15.4±0.1^{a}	17.0±0.0^{a}	16.8±0.7^{a}
2/5	16.7±0.8^{c}	20.5±0.2^{b}	18.2±0.7^{c}	22.6±1.0^{a}
7	45.2±0.2^{a}	30.3±0.3^{c}	34.8±0.6^{b}	30.2±0.3^{c}
8/9	9.7±0.5^{c}	15.2±0.2^{a}	13.4±0.2^{b}	14.1±0.5^{b}
10/12	15.1±0.9^{c}	18.5±0.2^{a}	16.5±0.2^{b}	16.4±0.1^{bc}
Ratio	0.69±0.01^{a}	0.53±0.01^{b}	0.56±0.01^{b}	0.51±0.01^{c}
AI glutenin				
1/2	12.3±0.6^{b}	15.3±0.2^{a}	15.6±0.2^{a}	15.7±0.6^{a}
2/5	17.9±0.1^{ns}	22.7±0.9^{ns}	19.2±1.2^{ns}	22.1±2.9^{ns}
7	45.2±0.7^{a}	29.2±0.3^{c}	33.4±0.9^{b}	30.1±0.5^{c}
8/9	11.9±0.2^{b}	16.8±0.0^{a}	19.2±1.3^{a}	16.2±2.2^{a}
10/12	12.7±0.2^{b}	15.9±0.8^{a}	12.5±1.2^{b}	15.8±0.5^{a}
Ratio	0.44±0.01^{a}	0.34±0.09^{b}	0.21±0.01^{d}	0.30±0.01^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 33. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	12.3±0.3^{b}	16.0±0.0^{a}	15.9±0.2^{a}	16.2±0.4^{a}
2/5	15.5±0.6^{c}	19.5±0.1^{b}	18.8±0.1^{b}	23.3±0.5^{a}
7	45.9±0.5^{a}	29.0±1.1^{c}	34.1±0.2^{b}	28.2±1.9^{c}
8/9	9.1±0.2^{b}	14.8±0.4^{a}	13.7±0.1^{a}	14.3±1.9^{a}
10/12	17.2±0.4^{b}	20.7±0.6^{a}	17.5±0.3^{b}	17.9±0.2^{b}
Ratio	0.50±0.03^{a}	0.49±0.00^{ab}	0.44±0.00^{bc}	0.40±0.02^{c}
AS glutenin				
1/2	12.9±0.9^{b}	15.8±1.0^{a}	16.2±0.7^{a}	16.3±1.3^{a}
2/5	17.8±1.3^{b}	19.3±2.3^{b}	18.7±0.6^{b}	22.9±0.7^{a}
7	45.2±0.2^{a}	29.3±0.7^{c}	33.7±0.5^{b}	30.1±1.4^{c}
8/9	10.2±0.4^{c}	17.5±2.0^{a}	14.6±0.3^{b}	15.5±1.0^{ab}
10/12	14.0±0.3^{c}	18.0±1.9^{a}	16.7±0.1^{ab}	15.2±0.4^{bc}
Ratio	0.69±0.02^{a}	0.56±0.04^{b}	0.54±0.01^{b}	0.47±0.03^{c}
AI glutenin				
1/2	12.3±0.4^{b}	15.6±4.7^{a}	15.5±1.5^{a}	16.2±1.2^{a}
2/5	18.3±0.6^{ns}	18.5±0.0^{ns}	21.5±0.9^{ns}	23.1±3.3^{ns}
7	45.3±0.7^{a}	29.5±0.2^{b}	29.5±1.9^{b}	28.8±0.5^{b}
8/9	11.4±0.6^{c}	21.1±1.5^{a}	18.4±1.0^{ab}	16.3±1.9^{b}
10/12	12.7±0.3^{b}	15.4±0.1^{a}	15.2±0.3^{a}	15.6±0.4^{a}
Ratio	0.41±0.01^{a}	0.32±0.01^{b}	0.22±0.07^{c}	0.28±0.02^{bc}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 34. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Control Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	12.2±0.3^{c}	16.2±0.1^{a}	15.3±0.1^{b}	16.3±0.3^{a}
2/5	17.4±0.5^{c}	19.5±1.3^{b}	19.7±0.2^{b}	23.2±0.4^{a}
7	44.5±0.1^{a}	28.9±0.6^{c}	32.9±0.1^{b}	28.3±0.7^{c}
8/9	9.5±0.4^{b}	15.3±1.0^{a}	15.0±0.1^{a}	15.0±0.5^{a}
10/12	16.3±0.1^{b}	20.1±1.0^{a}	17.1±0.3^{b}	17.2±0.5^{b}
Ratio	0.57±0.00^{a}	0.50±0.03^{b}	0.44±0.03^{c}	0.38±0.00^{d}
AS glutenin				
1/2	12.9±0.8^{c}	15.4±0.1^{b}	16.3±0.2^{ab}	17.2±0.0^{a}
2/5	18.3±0.7^{ns}	20.4±1.6^{ns}	20.6±0.2^{ns}	20.9±1.6^{ns}
7	44.8±0.1^{a}	29.3±0.6^{c}	31.8±0.1^{b}	29.5±0.1^{c}
8/9	10.9±0.0^{b}	17.9±1.5^{a}	15.5±0.1^{a}	17.7±1.6^{a}
10/12	13.1±0.0^{c}	17.1±0.7^{a}	15.7±0.4^{b}	14.7±0.1^{b}
Ratio	0.65±0.01^{a}	0.52±0.04^{b}	0.51±0.04^{b}	0.51±0.01^{b}
AI glutenin				
1/2	13.0±1.0^{b}	15.6±0.5^{a}	14.1±0.3^{b}	16.4±0.6^{a}
2/5	17.6±0.3^{ns}	19.6±3.6^{ns}	21.0±0.4^{ns}	20.3±3.3^{ns}
7	45.9±1.1^{a}	30.3±0.4^{b}	31.0±1.2^{b}	30.5±0.7^{b}
8/9	11.2±0.3^{b}	19.4±4.2^{a}	19.7±1.5^{a}	17.9±3.5^{ab}
10/12	12.2±0.1^{b}	15.0±0.5^{a}	14.2±0.3^{a}	15.0±0.9^{a}
Ratio	0.37±0.02^{a}	0.33±0.01^{b}	0.25±0.02^{c}	0.29±0.01^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 35. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Made From Glenlea Flour[1]

Subunit/Ratio	Flour	Iodate Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	14.0±0.8^{a}	13.1±0.3^{ab}	12.0±0.1^{b}	11.8±0.1^{b}
5	14.0±0.3^{c}	15.3±0.4^{b}	17.6±0.1^{a}	17.9±0.0^{a}
7	51.7±1.0^{a}	47.5±0.1^{b}	44.8±0.2^{c}	45.4±0.3^{c}
8	7.4±0.3^{c}	8.4±0.2^{b}	9.1±0.1^{a}	9.5±0.0^{a}
10	13.0±2.4^{b}	15.6±0.2^{ab}	16.6±0.2^{a}	15.4±0.3^{ab}
Ratio	0.49±0.04^{b}	0.53±0.01^{ab}	0.58±0.01^{a}	0.57±0.03^{a}
AS glutenin				
2	12.5±0.7^{ns}	13.6±1.7^{ns}	14.3±1.4^{ns}	13.6±0.8^{ns}
5	18.8±0.1^{ns}	18.4±0.2^{ns}	17.8±0.6^{ns}	18.0±0.6^{ns}
7	46.4±1.7^{ns}	43.3±3.2^{ns}	46.2±0.8^{ns}	46.9±1.3^{ns}
8	9.5±0.2^{b}	10.1±0.7^{ab}	10.0±0.4^{ab}	11.1±0.4^{a}
10	12.8±2.4^{ns}	14.6±0.9^{ns}	11.8±1.2^{ns}	10.5±1.1^{ns}
Ratio	0.60±0.02^{b}	0.66±0.04^{ab}	0.67±0.00^{a}	0.61±0.00^{ab}
AI glutenin				
2	11.3±0.1^{a}	11.7±0.4^{a}	11.4±0.0^{a}	10.6±0.1^{b}
5	20.8±0.0^{ab}	18.8±0.1^{c}	19.6±1.3^{bc}	21.9±0.3^{a}
7	45.4±0.3^{ns}	45.6±1.6^{ns}	45.1±0.6^{ns}	43.8±0.2^{ns}
8	11.8±0.2^{ns}	11.3±0.3^{ns}	11.2±0.8^{ns}	10.2±0.7^{ns}
10	10.6±0.1^{b}	12.6±1.9^{ab}	12.8±0.0^{ab}	13.4±0.1^{a}
Ratio	0.49±0.01^{a}	0.44±0.06^{ab}	0.39±0.00^{b}	0.40±0.01^{ab}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 36. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Made From Katepwa Flour[1]

Subunit/Ratio	Flour	Iodate Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	19.1±0.3^{a}	17.1±1.3^{b}	14.7±0.3^{c}	14.9±0.1^{c}
5	18.1±0.1^{ns}	18.4±0.6^{ns}	19.8±1.2^{ns}	19.2±0.5^{ns}
7	34.8±1.6^{a}	34.3±0.3^{ab}	32.2±0.3^{b}	32.5±0.4^{ab}
9	12.0±1.6^{b}	12.8±0.4^{ab}	13.9±1.1^{ab}	15.6±0.4^{a}
10	16.0±3.5^{ns}	17.4±0.1^{ns}	19.4±0.5^{ns}	17.6±0.4^{ns}
Ratio	0.52±0.00^{ns}	0.52±0.01^{ns}	0.51±0.01^{ns}	0.53±0.03^{ns}
AS glutenin				
2	16.3±1.1^{ns}	16.0±0.6^{ns}	15.4±0.1^{ns}	15.5±0.0^{ns}
5	21.5±0.3^{ns}	21.4±0.0^{ns}	21.0±1.1^{ns}	21.2±0.3^{ns}
7	32.9±0.7^{a}	30.5±1.0^{b}	30.1±0.3^{b}	31.3±0.7^{ab}
9	12.9±1.3^{b}	15.7±0.3^{ab}	17.9±1.4^{a}	17.2±1.1^{a}
10	16.4±0.2^{ns}	16.4±1.4^{ns}	15.6±0.0^{ns}	14.8±0.1^{ns}
Ratio	0.56±0.00^{ns}	0.58±0.02^{ns}	0.57±0.04^{ns}	0.53±0.01^{ns}
AI glutenin				
2	18.0±1.6^{a}	14.2±0.1^{b}	14.2±0.2^{b}	13.8±0.6^{b}
5	24.3±0.6^{ns}	22.3±0.8^{ns}	23.1±1.1^{ns}	21.6±2.1^{ns}
7	29.7±1.2^{ns}	30.5±0.8^{ns}	30.6±1.8^{ns}	29.8±1.1^{ns}
9	15.4±1.3^{ns}	16.9±0.8^{ns}	16.8±0.2^{ns}	18.4±2.1^{ns}
10	12.6±0.3^{b}	16.2±0.9^{a}	15.4±0.6^{ab}	16.2±1.8^{a}
Ratio	0.35±0.03^{ns}	0.34±0.01^{ns}	0.33±0.03^{ns}	0.31±0.04^{ns}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 37. Effect of Mixing Time on Changes in HMW-GS composition and ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Made From AC Domain Flour[1]

Subunit/Ratio	Flour	Iodate Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	16.5±0.5^{a}	15.8±0.2^{a}	14.6±0.1^{b}	14.4±0.3^{b}
5	17.0±0.6^{c}	18.7±0.7^{b}	20.6±0.6^{a}	20.6±0.1^{a}
7	35.9±0.6^{a}	34.4±0.4^{b}	31.8±0.6^{c}	31.6±0.4^{c}
9	13.8±1.9^{ns}	12.6±1.1^{ns}	14.2±1.0^{ns}	14.9±0.1^{ns}
10	16.8±1.4^{ns}	18.4±0.4^{ns}	18.8±1.1^{ns}	18.4±0.6^{ns}
Ratio	0.46±0.01^{ns}	0.46±0.01^{ns}	0.45±0.00^{ns}	0.46±0.01^{ns}
AS glutenin				
2	14.6±0.4^{b}	15.1±0.1^{b}	15.8±0.2^{a}	16.1±0.3^{a}
5	23.5±0.0^{a}	21.8±0.4^{b}	21.6±0.1^{b}	21.8±0.4^{b}
7	34.0±0.3^{a}	32.4±0.3^{b}	32.4±0.1^{b}	32.4±0.1^{b}
9	14.8±0.3^{b}	16.0±0.0^{ab}	16.0±0.6^{ab}	17.0±0.6^{a}
10	13.1±0.4^{b}	14.6±0.2^{a}	14.3±0.3^{a}	12.9±0.0^{b}
Ratio	0.47±0.00^{b}	0.51±0.00^{a}	0.48±0.00^{b}	0.45±0.01^{c}
AI glutenin				
2	13.9±0.0^{ns}	13.8±0.8^{ns}	13.4±1.3^{ns}	13.1±0.6^{ns}
5	23.9±0.3^{a}	23.2±0.4^{a}	20.8±0.6^{b}	23.6±1.2^{a}
7	33.5±0.0^{ns}	32.2±0.2^{ns}	31.3±1.4^{ns}	31.2±1.1^{ns}
9	16.8±0.4^{b}	16.2±0.7^{b}	19.2±0.6^{a}	16.6±1.0^{b}
10	11.9±0.0^{b}	14.6±0.6^{a}	15.2±0.1^{a}	15.6±0.8^{a}
Ratio	0.31±0.01^{b}	0.34±0.01^{a}	0.30±0.01^{bc}	0.29±0.00^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 38. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Made From AC Karma Flour[1]

Subunit/Ratio	Flour	Iodate Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
1	17.4±0.5^{ns}	17.2±0.4^{ns}	16.6±0.4^{ns}	17.2±0.6^{ns}
2	23.7±0.2^{ns}	23.1±0.1^{ns}	23.5±0.3^{ns}	22.8±1.1^{ns}
7	31.7±1.4^{ns}	31.0±0.0^{ns}	31.6±0.4^{ns}	31.2±0.6^{ns}
9	11.5±1.0^{b}	12.4±0.6^{ab}	12.9±0.1^{ab}	14.4±0.8^{a}
12	15.7±0.4^{ab}	16.4±1.1^{a}	15.5±0.6^{ab}	14.4±0.2^{b}
Ratio	0.39±0.02^{ns}	0.46±0.04^{ns}	0.42±0.04^{ns}	0.46±0.01^{ns}
AS glutenin				
1	16.4±0.1^{b}	16.7±0.3^{b}	17.8±0.1^{a}	17.2±0.6^{ab}
2	23.6±0.2^{ns}	22.2±1.1^{ns}	22.5±0.6^{ns}	20.4±2.9^{ns}
7	31.1±0.8^{ns}	31.8±0.6^{ns}	32.1±0.8^{ns}	30.7±0.4^{ns}
9	12.7±0.2^{b}	15.1±1.6^{b}	14.9±0.0^{b}	17.9±1.1^{a}
12	16.1±0.3^{a}	14.3±0.3^{ab}	12.7±0.1^{b}	13.6±1.6^{b}
Ratio	0.51±0.01^{a}	0.51±0.01^{a}	0.46±0.01^{ab}	0.42±0.03^{b}
AI glutenin				
1	15.3±0.7^{ns}	15.3±0.4^{ns}	15.4±0.5^{ns}	14.8±1.2^{ns}
2	25.1±0.4^{ns}	23.5±0.0^{ns}	24.4±0.7^{ns}	23.7±1.7^{ns}
7	29.9±0.2^{ns}	30.8±0.0^{ns}	29.2±1.0^{ns}	29.1±2.2^{ns}
9	13.6±0.8^{ns}	15.2±0.2^{ns}	15.4±1.1^{ns}	16.2±1.0^{ns}
12	16.2±0.1^{ns}	15.1±0.1^{ns}	15.6±0.8^{ns}	16.4±1.6^{ns}
Ratio	0.34±0.00^{a}	0.31±0.02^{a}	0.28±0.02^{ab}	0.24±0.04^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 39. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	13.1±0.3^{b}	17.1±1.3^{a}	15.8±0.2^{a}	17.2±0.4^{a}
2/5	15.3±0.4^{c}	18.4±0.6^{b}	18.7±0.7^{b}	23.1±0.1^{a}
7	47.5±0.1^{a}	34.3±0.3^{b}	34.4±0.4^{b}	31.0±0.0^{c}
8/9	8.4±0.2^{b}	12.8±0.4^{a}	12.6±1.1^{a}	12.4±0.6^{a}
10/12	15.6±0.2^{c}	17.4±0.1^{ab}	18.4±0.4^{a}	16.4±1.1^{bc}
Ratio	0.53±0.01^{ns}	0.52±0.01^{ns}	0.46±0.01^{ns}	0.46±0.04^{ns}
AS glutenin				
1/2	13.6±1.7^{b}	16.0±0.6^{ab}	15.1±0.1^{ab}	16.7±0.3^{a}
2/5	18.4±0.2^{b}	21.4±0.0^{a}	21.8±0.4^{a}	22.2±1.1^{a}
7	43.3±3.2^{a}	30.5±1.0^{b}	32.4±0.3^{b}	31.8±0.6^{b}
8/9	10.1±0.7^{b}	15.7±0.3^{a}	16.0±0.0^{a}	15.1±1.6^{a}
10/12	14.6±0.9^{ns}	16.4±1.4^{ns}	14.6±0.2^{ns}	14.3±0.3^{ns}
Ratio	0.66±0.04^{a}	0.58±0.02^{b}	0.51±0.00^{c}	0.51±0.01^{c}
AI glutenin				
1/2	11.7±0.4^{c}	14.2±0.1^{ab}	13.8±0.8^{b}	15.3±0.4^{a}
2/5	18.8±0.1^{b}	22.3±0.8^{a}	23.2±0.4^{a}	23.5±0.0^{a}
7	45.6±1.6^{a}	30.5±0.8^{b}	32.2±0.2^{b}	30.8±0.0^{b}
8/9	11.3±0.3^{c}	16.9±0.8^{a}	16.2±0.7^{ab}	15.2±0.2^{b}
10/12	12.6±1.9^{b}	16.2±0.9^{a}	14.6±0.6^{ab}	15.1±0.1^{ab}
Ratio	0.44±0.06^{a}	0.34±0.01^{b}	0.34±0.01^{b}	0.31±0.02^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 40. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	12.0±0.1^{c}	14.7±0.3^{b}	14.6±0.1^{b}	16.6±0.4^{a}
2/5	17.6±0.1^{c}	19.8±1.2^{b}	20.6±0.6^{b}	23.5±0.3^{a}
7	44.8±0.2^{a}	32.2±0.3^{b}	31.8±0.6^{b}	31.6±0.4^{b}
8/9	9.1±0.1^{b}	13.9±1.1^{a}	14.2±1.0^{a}	12.9±0.1^{a}
10/12	16.6±0.2^{b}	19.4±0.5^{a}	18.8±1.1^{a}	15.5±0.6^{b}
Ratio	0.58±0.01^{a}	0.51±0.01^{b}	0.45±0.00^{bc}	0.42±0.04^{c}
AS glutenin				
1/2	14.3±1.4^{b}	15.4±0.1^{b}	15.8±0.2^{ab}	17.8±0.1^{a}
2/5	17.8±0.6^{b}	21.0±1.1^{a}	21.6±0.1^{a}	22.5±0.6^{a}
7	46.2±0.8^{a}	30.1±0.3^{c}	32.4±0.1^{b}	32.1±0.8^{b}
8/9	10.0±0.4^{c}	17.9±1.4^{a}	16.0±0.6^{ab}	14.9±0.0^{b}
10/12	11.8±1.2^{c}	15.6±0.0^{a}	14.3±0.3^{ab}	12.7±0.1^{bc}
Ratio	0.67±0.00^{a}	0.57±0.04^{b}	0.48±0.00^{c}	0.46±0.01^{c}
AI glutenin				
1/2	11.4±0.0^{b}	14.2±0.2^{a}	13.4±1.3^{a}	15.4±0.5^{a}
2/5	19.6±1.3^{c}	23.1±1.1^{ab}	20.8±0.6^{bc}	24.4±0.7^{a}
7	45.1±0.6^{a}	30.6±1.8^{b}	31.3±1.4^{b}	29.2±1.0^{b}
8/9	11.2±0.8^{c}	16.8±0.2^{b}	19.2±0.6^{a}	15.4±1.1^{b}
10/12	12.8±0.0^{b}	15.4±0.6^{a}	15.2±0.1^{a}	15.6±0.8^{a}
Ratio	0.39±0.00^{a}	0.33±0.03^{b}	0.30±0.01^{b}	0.28±0.02^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 41. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Iodate Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	11.8±0.1^{c}	14.9±0.1^{b}	14.4±0.3^{b}	17.2±0.6^{a}
2/5	17.9±0.0^{c}	19.2±0.5^{bc}	20.6±0.1^{b}	22.8±1.1^{a}
7	45.4±0.3^{a}	32.5±0.4^{b}	31.6±0.4^{bc}	31.2±0.6^{c}
8/9	9.5±0.0^{c}	15.6±0.4^{a}	14.9±0.1^{ab}	14.4±0.8^{b}
10/12	15.4±0.3^{b}	17.6±0.4^{a}	18.4±0.6^{a}	14.4±0.2^{b}
Ratio	0.57±0.03^{a}	0.53±0.03^{a}	0.46±0.01^{b}	0.46±0.01^{b}
AS glutenin				
1/2	13.6±0.8^{c}	15.5±0.0^{b}	16.1±0.3^{ab}	17.2±0.6^{a}
2/5	18.0±0.6^{ns}	21.2±0.3^{ns}	21.8±0.4^{ns}	20.4±2.9^{ns}
7	46.9±1.3^{a}	31.3±0.7^{b}	32.4±0.1^{b}	30.7±0.4^{b}
8/9	11.1±0.4^{b}	17.2±1.1^{a}	17.0±0.6^{a}	17.9±1.1^{a}
10/12	10.5±1.1^{b}	14.8±0.1^{a}	12.9±0.0^{ab}	13.6±1.6^{a}
Ratio	0.61±0.00^{a}	0.53±0.01^{b}	0.45±0.01^{c}	0.42±0.03^{c}
AI glutenin				
1/2	10.6±0.1^{b}	13.8±0.6^{a}	13.1±0.6^{a}	14.8±1.2^{a}
2/5	21.9±0.3^{ns}	21.6±2.1^{ns}	23.6±1.2^{ns}	23.7±1.7^{ns}
7	43.8±0.2^{a}	29.8±1.1^{b}	31.2±1.1^{b}	29.1±2.2^{b}
8/9	10.2±0.7^{b}	18.4±2.1^{a}	16.6±1.0^{a}	16.2±1.0^{a}
10/12	13.4±0.1^{ns}	16.2±1.8^{ns}	15.6±0.8^{ns}	16.4±1.6^{ns}
Ratio	0.40±0.01^{a}	0.31±0.04^{b}	0.29±0.00^{b}	0.24±0.04^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 42. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Made From Glenlea Flour[1]

Subunit/Ratio	Flour	NEMI Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	14.0±0.8^{ns}	13.0±0.6^{ns}	12.6±0.8^{ns}	13.1±1.6^{ns}
5	14.0±0.3^{b}	14.6±0.5^{ab}	15.6±0.3^{ab}	17.0±1.9^{a}
7	51.7±1.0^{a}	49.0±0.4^{b}	48.0±0.1^{bc}	47.4±0.1^{c}
8	7.4±0.3^{b}	8.0±0.2^{ab}	8.7±0.4^{a}	8.8±0.6^{a}
10	13.0±2.4^{ns}	15.4±0.7^{ns}	15.1±1.0^{ns}	13.8±0.1^{ns}
Ratio	0.49±0.04^{c}	0.54±0.00^{bc}	0.58±0.01^{ab}	0.62±0.00^{a}
AS glutenin				
2	12.5±0.7^{ns}	12.0±0.9^{ns}	13.2±0.6^{ns}	12.6±0.4^{ns}
5	18.8±0.1^{ns}	18.5±0.8^{ns}	18.9±1.0^{ns}	19.2±0.0^{ns}
7	46.4±1.7^{ns}	46.0±0.3^{ns}	47.5±2.0^{ns}	48.2±0.4^{ns}
8	9.5±0.2^{ns}	10.2±0.9^{ns}	9.7±0.0^{ns}	9.6±0.1^{ns}
10	12.8±2.4^{ns}	13.4±0.7^{ns}	10.8±1.6^{ns}	10.4±0.1^{ns}
Ratio	0.60±0.02^{b}	0.71±0.01^{a}	0.65±0.02^{ab}	0.62±0.02^{b}
AI glutenin				
2	11.3±0.1^{a}	11.0±0.1^{a}	11.1±0.1^{a}	10.0±0.1^{b}
5	20.8±0.0^{ab}	17.2±2.3^{b}	21.2±0.8^{ab}	22.6±2.1^{a}
7	45.4±0.3^{ns}	47.3±1.7^{ns}	45.0±0.4^{ns}	43.7±2.8^{ns}
8	11.8±0.2^{ab}	12.8±1.6^{a}	9.6±0.3^{b}	12.1±1.0^{ab}
10	10.6±0.1^{b}	11.7±1.1^{ab}	13.1±0.4^{a}	11.6±0.2^{ab}
Ratio	0.49±0.01^{a}	0.44±0.05^{ab}	0.42±0.04^{ab}	0.36±0.04^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 43. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Made From Katepwa Flour[1]

Subunit/Ratio	Flour	NEMI Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	19.1±0.3^{a}	16.2±0.1^{b}	15.4±0.0^{b}	16.0±1.3^{b}
5	18.1±0.1^{b}	18.2±0.4^{b}	19.3±0.6^{ab}	20.8±1.3^{a}
7	34.8±1.6^{ns}	34.3±0.8^{ns}	33.2±0.6^{ns}	31.8±1.5^{ns}
9	12.0±1.6^{ns}	12.8±0.8^{ns}	14.2±0.2^{ns}	14.0±0.9^{ns}
10	16.0±3.5^{ns}	18.4±0.5^{ns}	17.7±0.8^{ns}	17.5±0.1^{ns}
Ratio	0.52±0.00^{ns}	0.54±0.05^{ns}	0.50±0.00^{ns}	0.54±0.06^{ns}
AS glutenin				
2	16.3±1.1^{ns}	15.5±0.3^{ns}	15.9±0.0^{ns}	15.5±0.4^{ns}
5	21.5±0.3^{ns}	21.7±1.0^{ns}	21.8±0.1^{ns}	21.9±0.7^{ns}
7	32.9±0.7^{a}	30.4±1.5^{b}	31.9±0.4^{ab}	32.0±0.4^{ab}
9	12.9±1.3^{b}	15.8±1.7^{ab}	15.2±0.5^{ab}	17.0±0.2^{a}
10	16.4±0.2^{a}	16.6±0.4^{a}	15.2±0.2^{b}	13.7±0.4^{c}
Ratio	0.56±0.00^{b}	0.60±0.02^{a}	0.57±0.00^{ab}	0.54±0.02^{b}
AI glutenin				
2	18.0±1.6^{a}	14.4±0.5^{b}	14.8±0.2^{b}	12.6±1.1^{b}
5	24.3±0.6^{a}	22.1±0.8^{ab}	20.1±1.1^{b}	23.8±1.1^{a}
7	29.7±1.2^{ns}	31.4±1.1^{ns}	31.8±1.0^{ns}	30.1±0.9^{ns}
9	15.4±1.3^{b}	16.6±0.4^{b}	18.9±0.7^{a}	15.9±0.1^{b}
10	12.6±0.3^{b}	15.5±1.1^{ab}	14.3±1.6^{b}	17.6±1.0^{a}
Ratio	0.35±0.03^{a}	0.34±0.03^{a}	0.28±0.01^{b}	0.34±0.1^{a}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 44. Effect of Mixing Time on Changes in HMW-GS composition and ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Made From AC Domain Flour[1]

Subunit/Ratio	Flour	NEMI Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
2	16.5±0.5^{a}	15.8±0.1^{ab}	15.8±0.1^{ab}	14.7±0.6^{b}
5	17.0±0.6^{c}	19.2±0.4^{bc}	20.6±0.1^{ab}	22.4±1.6^{a}
7	35.9±0.6^{a}	33.8±1.0^{ab}	31.8±0.1^{b}	30.6±2.3^{b}
9	13.8±1.9^{ns}	12.2±0.1^{ns}	14.6±0.3^{ns}	13.9±0.4^{ns}
10	16.8±1.4^{ns}	19.0±0.8^{ns}	17.4±0.1^{ns}	18.4±1.8^{ns}
Ratio	0.46±0.01^{ns}	0.46±0.1^{ns}	0.47±0.02^{ns}	0.45±0.02^{ns}
AS glutenin				
2	14.6±0.4^{ns}	15.0±0.4^{ns}	14.9±0.4^{ns}	14.7±0.4^{ns}
5	23.5±0.0^{ns}	20.8±0.8^{ns}	21.4±0.2^{ns}	22.5±1.8^{ns}
7	34.0±0.3^{ns}	34.0±0.1^{ns}	35.1±2.5^{ns}	33.5±2.5^{ns}
9	14.8±0.3^{b}	14.8±0.1^{b}	14.6±0.1^{b}	16.3±0.3^{a}
10	13.1±0.4^{ns}	15.4±0.6^{ns}	14.1±2.0^{ns}	13.1±0.5^{ns}
Ratio	0.47±0.00^{b}	0.55±0.01^{a}	0.46±0.04^{b}	0.47±0.03^{b}
AI glutenin				
2	13.9±0.0^{a}	13.6±0.1^{a}	12.5±0.4^{ab}	11.4±1.0^{b}
5	23.9±0.3^{ab}	23.1±1.8^{b}	24.5±0.8^{ab}	26.1±0.2^{a}
7	33.5±0.0^{a}	31.0±0.5^{b}	31.4±0.2^{b}	28.1±0.3^{c}
9	16.8±0.4^{ns}	16.2±2.1^{ns}	15.8±1.3^{ns}	17.1±0.8^{ns}
10	11.9±0.0^{b}	16.2±0.8^{a}	15.8±1.1^{a}	17.4±0.6^{a}
Ratio	0.31±0.01^{a}	0.30±0.02^{a}	0.25±0.02^{b}	0.25±0.00^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 45. Effect of Mixing Time on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Made From AC Karma Flour[1]

Subunit/Ratio	Flour	NEMI Dough Mixing Time (% of peak)		
		50	100	200
ES glutenin				
1	17.4±0.5^{ns}	17.1±0.4^{ns}	16.6±0.3^{ns}	17.1±0.3^{ns}
2	23.7±0.2^{ns}	22.9±1.6^{ns}	24.2±0.1^{ns}	23.1±0.4^{ns}
7	31.7±1.4^{ns}	31.0±0.2^{ns}	31.6±1.0^{ns}	32.2±0.4^{ns}
9	11.5±1.0^{b}	12.4±0.8^{ab}	12.6±0.9^{ab}	14.1±0.0^{a}
12	15.7±0.4^{b}	16.6±0.1^{a}	15.1±0.2^{b}	13.6±0.4^{c}
Ratio	0.39±0.02^{b}	0.45±0.05^{ab}	0.46±0.1^{ab}	0.48±0.01^{a}
AS glutenin				
1	16.4±0.1^{ns}	17.4±0.6^{ns}	16.6±0.5^{ns}	17.8±1.8^{ns}
2	23.6±0.2^{a}	22.0±0.5^{b}	23.1±0.7^{ab}	22.2±0.1^{b}
7	31.1±0.8^{ns}	32.0±1.9^{ns}	33.4±0.7^{ns}	33.0±0.4^{ns}
9	12.7±0.2^{ns}	14.6±1.5^{ns}	14.2±0.7^{ns}	15.0±0.1^{ns}
12	16.1±0.3^{a}	14.0±0.4^{b}	12.6±0.2^{bc}	12.0±1.3^{c}
Ratio	0.51±0.01^{ab}	0.52±0.00^{a}	0.49±0.00^{b}	0.45±0.02^{c}
AI glutenin				
1	15.3±0.7^{a}	15.6±0.4^{a}	10.6±0.8^{ab}	8.8±3.1^{b}
2	25.1±0.4^{ns}	22.9±0.8^{ns}	22.8±2.6^{ns}	21.5±2.5^{ns}
7	29.9±0.2^{ns}	30.7±0.7^{ns}	28.4±1.2^{ns}	27.9±2.6^{ns}
9	13.6±0.8^{c}	15.2±0.9^{c}	20.0±0.9^{b}	24.5±0.7^{a}
12	16.2±0.1^{ns}	15.6±0.4^{ns}	18.3±1.1^{ns}	17.3±3.0^{ns}
Ratio	0.34±0.00^{a}	0.31±0.06^{a}	0.17±0.04^{b}	0.14±0.02^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 46. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	13.0±0.6^{c}	16.2±0.1^{ab}	15.8±0.1^{b}	17.1±0.4^{a}
2/5	14.6±0.5^{c}	18.2±0.4^{b}	19.2±0.4^{b}	22.9±1.6^{a}
7	49.0±0.4^{a}	34.3±0.8^{b}	33.8±1.0^{b}	31.0±0.2^{c}
8/9	8.0±0.2^{b}	12.8±0.8^{a}	12.2±0.1^{a}	12.4±0.8^{a}
10/12	15.4±0.7^{b}	18.4±0.5^{a}	19.0±0.8^{a}	16.6±0.1^{b}
Ratio	0.54±0.00^{ns}	0.54±0.05^{ns}	0.46±0.1^{ns}	0.45±0.05^{ns}
AS glutenin				
1/2	12.0±0.9^{c}	15.5±0.3^{b}	15.0±0.4^{b}	17.4±0.6^{a}
2/5	18.5±0.8^{b}	21.7±1.0^{a}	20.8±0.8^{a}	22.0±0.5^{a}
7	46.0±0.3^{a}	30.4±1.5^{b}	34.0±0.1^{c}	32.0±1.9^{bc}
8/9	10.2±0.9^{b}	15.8±1.7^{a}	14.8±0.1^{a}	14.6±1.5^{a}
10/12	13.4±0.7^{c}	16.6±0.4^{a}	15.4±0.6^{ab}	14.0±0.4^{bc}
Ratio	0.71±0.01^{a}	0.60±0.02^{b}	0.55±0.01^{c}	0.52±0.00^{c}
AI glutenin				
1/2	11.0±0.1^{c}	14.4±0.5^{b}	13.6±0.1^{b}	15.6±0.4^{a}
2/5	17.2±2.3^{b}	22.1±0.8^{a}	23.1±1.8^{a}	22.9±0.8^{a}
7	47.3±1.7^{a}	31.4±1.1^{b}	31.0±0.5^{b}	30.7±0.7^{b}
8/9	12.8±1.6^{ns}	16.6±0.4^{ns}	16.2±2.1^{ns}	15.2±0.9^{ns}
10/12	11.7±1.1^{b}	15.5±1.1^{a}	16.2±0.8^{a}	15.6±0.4^{a}
Ratio	0.44±0.05^{a}	0.34±0.03^{ab}	0.30±0.02^{b}	0.31±0.06^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 47. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	12.6±0.8^{b}	15.4±0.0^{a}	15.8±0.1^{a}	16.6±0.3^{a}
2/5	15.6±0.3^{d}	19.3±0.6^{c}	20.6±0.1^{b}	24.2±0.1^{a}
7	48.0±0.1^{a}	33.2±0.6^{b}	31.8±0.1^{b}	31.6±1.0^{b}
8/9	8.7±0.4^{c}	14.2±0.2^{a}	14.6±0.3^{a}	12.6±0.9^{b}
10/12	15.1±1.0^{b}	17.7±0.8^{a}	17.4±0.1^{a}	15.1±0.2^{b}
Ratio	0.58±0.01^{a}	0.50±0.00^{b}	0.47±0.02^{bc}	0.46±0.1^{c}
AS glutenin				
1/2	13.2±0.6^{c}	15.9±0.0^{ab}	14.9±0.4^{b}	16.6±0.5^{a}
2/5	18.9±1.0^{b}	21.8±0.1^{a}	21.4±0.2^{a}	23.1±0.7^{a}
7	47.5±2.0^{a}	31.9±0.4^{b}	35.1±2.5^{b}	33.4±0.7^{b}
8/9	9.7±0.0^{b}	15.2±0.5^{a}	14.6±0.1^{a}	14.2±0.7^{a}
10/12	10.8±1.6^{b}	15.2±0.2^{a}	14.1±2.0^{ab}	12.6±0.2^{ab}
Ratio	0.65±0.02^{a}	0.57±0.00^{b}	0.46±0.04^{c}	0.49±0.00^{c}
AI glutenin				
1/2	11.1±0.1^{bc}	14.8±0.2^{a}	12.5±0.4^{b}	10.6±0.8^{c}
2/5	21.2±0.8^{ns}	20.1±1.1^{ns}	24.5±0.8^{ns}	22.8±2.6^{ns}
7	45.01±0.4^{a}	31.8±1.0^{b}	31.4±0.2^{b}	28.4±1.2^{c}
8/9	9.6±0.3^{c}	18.9±0.7^{a}	15.8±1.3^{b}	20.0±0.9^{a}
10/12	13.1±0.4^{b}	14.3±1.6^{b}	15.8±1.1^{ab}	18.3±1.1^{a}
Ratio	0.42±0.04^{a}	0.28±0.01^{b}	0.25±0.02^{bc}	0.17±0.04^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 48. Effect of Cultivar on Changes in HMW-GS Composition and Ratio of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From NEMI Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Cultivar			
	Glenlea	Katepwa	AC Domain	AC Karma
ES glutenin				
1/2	13.1±1.6^{b}	16.0±1.3^{ab}	14.7±0.6^{ab}	17.1±0.3^{a}
2/5	17.0±1.9^{b}	20.8±1.3^{ab}	22.4±1.6^{a}	23.1±0.4^{a}
7	47.4±0.1^{a}	31.8±1.5^{b}	30.6±2.3^{b}	32.2±0.4^{b}
8/9	8.8±0.6^{b}	14.0±0.9^{a}	13.9±0.4^{a}	14.1±0.0^{a}
10/12	13.8±0.1^{b}	17.5±0.1^{a}	18.4±1.8^{a}	13.6±0.4^{b}
Ratio	0.62±0.00^{a}	0.54±6.3^{ab}	0.45±0.02^{b}	0.48±0.01^{b}
AS glutenin				
1/2	12.6±0.4^{c}	15.5±0.4^{ab}	14.7±0.4^{bc}	17.8±1.8^{a}
2/5	19.2±0.0^{b}	21.9±0.7^{ab}	22.5±1.8^{a}	22.2±0.1^{a}
7	48.2±0.4^{a}	32.0±0.4^{b}	33.5±2.5^{b}	33.0±0.4^{b}
8/9	9.6±0.1^{d}	17.0±0.2^{a}	16.3±0.3^{b}	15.0±0.1^{c}
10/12	10.4±0.1^{b}	13.7±0.4^{a}	13.1±0.5^{a}	12.0±1.3^{ab}
Ratio	0.62±0.02^{a}	0.54±0.02^{b}	0.47±0.03^{c}	0.45±0.02^{c}
AI glutenin				
1/2	10.0±0.1^{ns}	12.6±1.1^{ns}	11.4±1.0^{ns}	8.8±3.1^{ns}
2/5	22.6±2.1^{ns}	23.8±1.1^{ns}	26.1±0.2^{ns}	21.5±2.5^{ns}
7	43.7±2.8^{a}	30.1±0.9^{b}	28.1±0.3^{b}	27.9±2.6^{b}
8/9	12.1±1.0^{c}	15.9±0.1^{b}	17.1±0.8^{b}	24.5±0.7^{a}
10/12	11.6±0.2^{b}	17.6±1.0^{a}	17.4±0.6^{a}	17.3±3.0^{a}
Ratio	0.36±0.04^{a}	0.34±0.1^{a}	0.25±0.00^{b}	0.14±0.02^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 49. Effect of Mixing Time on Changes in ω-gliadin Content (% of fraction) in ES and AS Glutenin Obtained From Control, Iodate and NEMI Doughs[1]

Fraction		Mixing Time (% of Peak)			
Treatment	Cultivar	0	50	100	200
ES Glutenin					
Control	Glenlea	26.0±1.7^{ab}	28.0±0.2^{a}	26.6±1.0^{ab}	24.6±0.2^{b}
	Katepwa	34.1±1.6^{b}	41.5±1.6^{a}	37.5±0.5^{b}	35.5±0.9^{b}
	AC Domain	30.3±0.9^{a}	30.4±0.8^{a}	23.5±2.5^{b}	27.8±0.3^{ab}
	AC Karma	12.7±0.3^{c}	20.7±0.6^{a}	18.2±1.4^{ab}	17.9±0.8^{b}
Iodate	Glenlea	26.0±1.7^{a}	25.9±0.4^{a}	18.4±1.4^{b}	17.4±1.3^{b}
	Katepwa	34.1±1.6^{ab}	38.3±2.4^{a}	29.6±3.2^{b}	27.9±2.5^{b}
	AC Domain	30.3±0.9^{a}	31.0±1.9^{a}	22.0±1.1^{b}	22.8±0.6^{b}
	AC Karma	12.7±0.3^{ns}	16.0±0.7^{ns}	13.4±3.3^{ns}	14.4±0.2^{ns}
NEMI	Glenlea	26.0±1.7^{a}	25.2±0.1^{a}	19.0±0.4^{b}	17.7±0.4^{b}
	Katepwa	34.1±1.6^{ns}	34.2±4.0^{ns}	31.0±0.0^{ns}	28.2±2.2^{ns}
	AC Domain	30.3±0.9^{a}	28.7±3.7^{ab}	21.4±2.9^{bc}	19.0±2.7^{c}
	AC Karma	12.7±0.3^{ns}	15.1±1.1^{ns}	13.8±1.6^{ns}	12.8±0.4^{ns}
AS Glutenin					
Control	Glenlea	7.4±1.5^{ab}	10.1±1.5^{a}	9.1±0.9^{ab}	7.1±0.6^{b}
	Katepwa	13.7±4.5^{ns}	8.7±0.6^{ns}	11.5±4.2^{ns}	5.7±0.2^{ns}
	AC Domain	9.5±0.5^{c}	23.1±0.5^{a}	18.1±0.3^{b}	12.8±0.1^{c}
	AC Karma	8.9±0.6^{ns}	7.8±0.7^{ns}	7.4±2.2^{ns}	6.7±1.1^{ns}
Iodate	Glenlea	7.4±1.5^{ab}	8.2±1.2^{ab}	8.6±1.5^{a}	4.8±0.4^{b}
	Katepwa	13.7±4.5^{a}	8.9±1.4^{ab}	6.8±0.5^{b}	4.4±0.1^{b}
	AC Domain	9.5±0.5^{a}	7.6±0.7^{b}	8.4±0.8^{ab}	4.5±0.6^{c}
	AC Karma	8.9±0.6^{a}	7.6±0.7^{a}	4.9±0.4^{b}	4.2±0.6^{b}
NEMI					
	Glenlea	7.4±1.5^{ns}	6.1±1.1^{ns}	8.0±0.5^{ns}	5.1±1.3^{ns}
	Katepwa	13.7±4.5^{a}	7.2±0.9^{ab}	8.1±2.3^{ab}	3.8±0.1^{b}
	AC Domain	9.5±0.5^{ab}	13.3±0.1^{a}	10.8±3.9^{ab}	5.4±0.2^{b}
	AC Karma	8.9±0.6^{a}	7.1±1.5^{a}	4.4±0.4^{b}	2.8±0.1^{b}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 50. Effect of Mixing Time on Changes in ω-gliadin Content (% of total protein) in ES and AS Glutenin Obtained From Control, Iodate and NEMI Doughs

Fraction		Mixing Time (% of Peak)			
Treatment	Cultivar	0	50	100	200
ES Glutenin					
Control	Glenlea	1.0±0.2^{c}	2.4±0.3^{ab}	2.2±0.2^{b}	3.0±0.1^{a}
	Katepwa	1.9±0.1^{b}	2.4±0.3^{b}	2.8±0.4^{b}	3.8±0.5^{c}
	AC Domain	2.6±1.1^{ns}	2.4±0.5^{ns}	1.5±0.3^{ns}	2.4±0.2^{ns}
	AC Karma	0.9±0.3	1.6±0.4	1.2±0.4	1.5±0.4
Iodate	Glenlea	1.0±0.2^{b}	2.8±0.1^{a}	2.4±0.3^{a}	3.0±0.4^{a}
	Katepwa	1.9±0.1^{c}	5.2±0.8^{a}	3.8±0.1^{b}	5.2±0.2^{a}
	AC Domain	2.6±1.1^{ns}	2.9±0.7^{ns}	2.4±0.6^{ns}	4.2±0.4^{ns}
	AC Karma	0.9±0.3^{c}	1.8±0.0^{b}	2.2±0.3^{ab}	2.4±0.1^{a}
NEMI	Glenlea	1.0±0.2^{d}	2.6±0.3^{b}	2.0±0.1^{c}	3.2±0.0^{a}
	Katepwa	1.9±0.1^{b}	2.8±0.4^{b}	3.5±0.1^{ab}	4.8±1.2^{a}
	AC Domain	2.6±1.1^{ab}	2.0±0.1^{b}	4.1±0.4^{a}	3.8±0.3^{a}
	AC Karma	0.9±0.3^{b}	2.0±0.4^{ab}	2.1±0.4^{b}	2.6±0.5^{b}
AS Glutenin					
Control	Glenlea	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}	0.4±0.0^{a}
	Katepwa	0.6±0.1^{a}	0.4±0.1^{b}	0.3±0.1^{b}	0.2±0.0^{b}
	AC Domain	0.4±0.1^{ab}	0.4±0.1^{ab}	0.6±0.1^{a}	0.2±0.1^{b}
	AC Karma	0.6±0.1^{a}	0.5±0.1^{b}	0.4±0.1^{b}	0.4±0.1^{b}
Iodate	Glenlea	0.3±0.0^{c}	0.2±0.0^{d}	0.7±0.0^{a}	0.6±0.0^{b}
	Katepwa	0.6±0.1^{a}	0.3±0.0^{b}	0.3±0.0^{b}	0.3±0.0^{b}
	AC Domain	0.4±0.1^{c}	0.4±0.0^{c}	0.9±0.0^{a}	0.7±0.0^{b}
	AC Karma	0.6±0.1^{a}	0.5±0.0^{b}	0.6±0.1^{ab}	0.5±0.0^{b}
NEMI					
	Glenlea	0.3±0.0^{c}	0.2±0.0^{d}	0.9±0.0^{b}	1.0±0.0^{a}
	Katepwa	0.6±0.1^{a}	0.3±0.0^{b}	0.6±0.0^{a}	0.3±0.0^{b}
	AC Domain	0.4±0.1^{c}	0.6±0.0^{c}	1.2±0.1^{a}	0.9±0.0^{b}
	AC Karma	0.6±0.1^{b}	0.6±0.0^{b}	0.9±0.0^{a}	0.4±0.0^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 51. Effect of Cultivar on Changes in ω-gliadin Content (% of fraction) in ES and AS Glutenin Obtained From Control, Iodate and NEMI Doughs[1]

Fraction		Cultivar			
Treatment	Mixing Time	Glenlea	Katepwa	AC Domain	AC Karma
ES Glutenin					
Control	0	26.0±1.7^{c}	34.1±1.6^{a}	30.3±0.9^{b}	12.7±0.3^{d}
	50	28.0±0.2^{b}	41.5±1.6^{a}	30.4±0.8^{b}	20.7±0.6^{c}
	100	26.6±1.0^{b}	37.5±0.5^{a}	23.5±2.5^{b}	18.2±1.4^{c}
	200	24.6±0.2^{b}	35.5±0.9^{a}	27.8±0.3^{b}	17.9±0.8^{c}
Iodate	50	25.9±0.4^{c}	38.3±2.4^{a}	31.0±1.9^{b}	16.0±0.7^{c}
	100	18.4±1.4^{bc}	29.6±3.2^{a}	22.0±1.1^{b}	13.4±3.3^{c}
	200	17.4±1.3^{c}	27.9±2.5^{a}	22.8±0.6^{b}	14.4±0.2^{c}
NEMI	50	25.2±0.1^{b}	34.2±4.0^{a}	28.7±3.7^{ab}	15.1±1.1^{c}
	100	19.0±0.4^{b}	31.0±0.0^{a}	21.4±2.9^{b}	13.8±1.6^{c}
	200	17.7±0.4^{b}	28.2±2.2^{a}	19.0±2.7^{b}	12.8±0.4^{c}
AS Glutenin					
Control	0	7.4±1.5^{ns}	13.7±4.5^{ns}	9.5±0.5^{ns}	8.9±0.6^{ns}
	50	10.1±1.5^{b}	8.7±0.6^{b}	23.1±0.5^{a}	7.8±0.7^{b}
	100	9.1±0.9^{b}	11.5±4.2^{b}	18.1±0.3^{a}	7.4±2.2^{b}
	200	7.1±0.6^{b}	5.7±0.2^{b}	12.8±0.1^{a}	6.7±1.1^{b}
Iodate	50	8.2±1.2^{ns}	8.9±1.4^{ns}	7.6±0.7^{ns}	7.6±0.7^{ns}
	100	8.6±1.5^{a}	6.8±0.5^{ab}	8.4±0.8^{a}	4.9±0.4^{b}
	200	4.8±0.4^{ns}	4.4±0.1^{ns}	4.5±0.6^{ns}	4.2±0.6^{ns}
NEMI	50	6.1±1.1^{b}	7.2±0.9^{b}	13.3±0.1^{a}	7.1±1.5^{b}
	100	8.0±0.5^{ns}	8.1±2.3^{ns}	10.8±3.9^{ns}	4.4±0.4^{ns}
	200	5.1±1.3^{a}	3.8±0.1^{ab}	5.4±0.2^{a}	2.8±0.1^{b}

[1] Means ● standard deviation, means with different letters are significantly different (α=0.05)

Table 52. Effect of Cultivar on Changes in ω-gliadin Content (% of total protein) in ES and AS Glutenin Obtained From Control, Iodate and NEMI Doughs[1]

Fraction		Cultivar			
Treatment	Mixing Time	Glenlea	Katepwa	AC Domain	AC Karma
ES Glutenin					
Control	0	1.0±0.2^{ns}	1.9±0.1^{ns}	2.6±1.1^{ns}	0.9±0.3^{ns}
	50	2.4±0.3^{ns}	2.4±0.3^{ns}	2.4±0.5^{ns}	1.6±0.4^{ns}
	100	2.2±0.2^{ab}	2.8±0.4^{a}	1.5±0.3^{bc}	1.2±0.4^{c}
	200	3.0±0.1^{ab}	3.8±0.5^{a}	2.4±0.2^{bc}	1.5±0.4^{c}
Iodate	50	2.8±0.1^{b}	5.2±0.8^{a}	2.9±0.7^{b}	1.8±0.0^{b}
	100	2.4±0.3^{b}	3.8±0.1^{a}	2.4±0.6^{b}	2.2±0.3^{b}
	200	3.0±0.4^{c}	5.2±0.2^{a}	4.2±0.4^{b}	2.4±0.1^{c}
NEMI	50	2.6±0.3^{ns}	2.8±0.4^{ns}	2.0±0.1^{ns}	2.0±0.4^{ns}
	100	2.0±0.1^{b}	3.5±0.1^{a}	4.1±0.4^{a}	2.1±0.4^{b}
	200	3.2±0.0^{ab}	4.8±1.2^{a}	3.8±0.3^{ab}	2.6±0.5^{b}
AS Glutenin					
Control	0	0.3±0.0^{b}	0.6±0.1^{ab}	0.4±0.1^{ab}	0.6±0.1^{a}
	50	0.3±0.0^{b}	0.4±0.1^{ab}	0.4±0.0^{ab}	0.5±0.1^{a}
	100	0.3±0.0^{b}	0.3±0.1^{b}	0.6±0.1^{a}	0.4±0.1^{b}
	200	0.4±0.0^{ab}	0.2±0.0^{b}	0.2±0.1^{b}	0.4±0.1^{a}
Iodate	50	0.2±0.0^{d}	0.3±0.0^{c}	0.4±0.0^{b}	0.5±0.0^{a}
	100	0.7±0.0^{b}	0.3±0.0^{d}	0.9±0.0^{a}	0.6±0.1^{c}
	200	0.6±0.0^{b}	0.3±0.0^{d}	0.7±0.0^{a}	0.5±0.0^{c}
NEMI	50	0.2±0.0^{d}	0.3±0.0^{c}	0.6±0.0^{a}	0.6±0.0^{b}
	100	0.9±0.0^{b}	0.6±0.0^{c}	1.2±0.1^{a}	0.9±0.0^{b}
	200	1.0±0.0^{a}	0.3±0.0^{d}	0.9±0.0^{b}	0.4±0.0^{c}

[1] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 53. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Glenlea Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	13.2±0.1	13.1±0.3	13.0±0.6
2/5	14.4±0.1	15.3±0.4	14.6±0.5
7	47.0±1.2	47.5±0.1	49.0±0.4
8/9	8.2±0.1	8.4±0.2	8.0±0.2
10/12	17.2±0.8	15.6±0.2	15.4±0.7
Ratio	0.44±0.06	0.53±0.01	0.54±0.00
Omegas	28.0±0.2[a]	25.9±0.4[b]	25.2±0.1[b]
AS glutenin			
1/2	13.2±1.3	13.6±1.7	12.0±0.9
2/5	16.7±0.8	18.4±0.2	18.5±0.8
7	45.2±0.2	43.3±3.2	46.0±0.3
8/9	9.7±0.5	10.1±0.7	10.2±0.9
10/12	15.1±0.9	14.6±0.9	13.4±0.7
Ratio	0.69±0.01	0.66±0.04	0.71±0.01
Omegas	10.1±1.5[a]	8.2±1.2[ab]	6.1±1.1[b]
AI glutenin			
1/2	12.3±0.6	11.7±0.4	11.0±0.1
2/5	17.9±0.1	18.8±0.1	17.2±2.3
7	45.2±0.7	45.6±1.6	47.3±1.7
8/9	11.9±0.2	11.3±0.3	12.8±1.6
10/12	12.7±0.2	12.6±1.9	11.7±1.1
Ratio	0.44±0.01	0.44±0.06	0.44±0.05

[1] Letters indicate significant differences (α=0.05) between treatments

Table 54. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Glenlea Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	12.3±0.3	12.0±0.1	12.6±0.8
2/5	15.5±0.6^{b}	17.6±0.1^{a}	15.6±0.3^{b}
7	45.9±0.5^{b}	44.8±0.2^{c}	48.0±0.1^{a}
8/9	9.1±0.2	9.1±0.1	8.7±0.4
10/12	17.2±0.4^{a}	16.6±0.2^{ab}	15.1±1.0^{b}
Ratio	0.50±0.03^{a}	0.58±0.01^{b}	0.58±0.01^{b}
Omegas	26.6±1.0^{a}	18.4±1.4^{b}	19.0±0.4^{b}
AS glutenin			
1/2	12.9±0.9	14.3±1.4	13.2±0.6
2/5	17.8±1.3	17.8±0.6	18.9±1.0
7	45.2±0.2	46.2±0.8	47.5±2.0
8/9	10.2±0.4	10.0±0.4	9.7±0.0
10/12	14.0±0.3^{a}	11.8±1.2^{ab}	10.8±1.6^{b}
Ratio	0.69±0.02	0.67±0.00	0.65±0.02
Omegas	9.1±0.9	8.6±1.5	8.0±0.5
AI glutenin			
1/2	12.3±0.4	11.4±0.0	11.1±0.1
2/5	18.3±0.6	19.6±1.3	21.2±0.8
7	45.3±0.7	45.1±0.6	45.01±0.4
8/9	11.4±0.6	11.2±0.8	9.6±0.3
10/12	12.7±0.3	12.8±0.0	13.1±0.4
Ratio	0.41±0.01	0.39±0.00	0.42±0.04

[1] Letters indicate significant differences (α=0.05) between treatments

Table 55. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Glenlea Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	12.2±0.3	11.8±0.1	13.1±1.6
2/5	17.4±0.5	17.9±0.0	17.0±1.9
7	44.5±0.1^{c}	45.4±0.3^{b}	47.4±0.1^{a}
8/9	9.5±0.4	9.5±0.0	8.8±0.6
10/12	16.3±0.1^{a}	15.4±0.3^{b}	13.8±0.1^{c}
Ratio	0.57±0.00	0.57±0.03	0.62±0.00
Omegas	24.6±0.2^{a}	17.4±1.3^{b}	17.7±0.4^{b}
AS glutenin			
1/2	12.9±0.8	13.6±0.8	12.6±0.4
2/5	18.3±0.7	18.0±0.6	19.2±0.0
7	44.8±0.1^{b}	46.9±1.3^{ab}	48.2±0.4^{a}
8/9	10.9±0.0^{a}	11.1±0.4^{a}	9.6±0.1^{b}
10/12	13.1±0.0^{a}	10.5±1.1^{b}	10.4±0.1^{b}
Ratio	0.65±0.01	0.61±0.00	0.62±0.02
Omegas	7.1±0.6	4.8±0.4	5.1±1.3
AI glutenin			
1/2	13.0±1.0^{a}	10.6±0.1^{b}	10.0±0.1^{b}
2/5	17.6±0.3^{b}	21.9±0.3^{a}	22.6±2.1^{a}
7	45.9±1.1	43.8±0.2	43.7±2.8
8/9	11.2±0.3	10.2±0.7	12.1±1.0
10/12	12.2±0.1^{b}	13.4±0.1^{a}	11.6±0.2^{c}
Ratio	0.37±0.02	0.40±0.01	0.36±0.04

[1] Letters indicate significant differences (α=0.05) between treatments

Table 56. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Katepwa Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	17.4±0.1	17.1±1.3	16.2±0.1
2/5	18.6±0.1	18.4±0.6	18.2±0.4
7	29.3±0.5[b]	34.3±0.3[a]	34.3±0.8[a]
8/9	13.6±0.4	12.8±0.4	12.8±0.8
10/12	21.0±0.3[a]	17.4±0.1[b]	18.4±0.5[b]
Ratio	0.47±0.00	0.52±0.01	0.54±0.05
Omegas	41.5±1.6	38.3±2.4	34.2±4.0
AS glutenin			
1/2	15.4±0.1	16.0±0.6	15.5±0.3
2/5	20.5±0.2	21.4±0.0	21.7±1.0
7	30.3±0.3	30.5±1.0	30.4±1.5
8/9	15.2±0.2	15.7±0.3	15.8±1.7
10/12	18.5±0.2	16.4±1.4	16.6±0.4
Ratio	0.53±0.01[b]	0.58±0.02[a]	0.60±0.02[a]
Omegas	8.7±0.6	8.9±1.4	7.2±0.9
AI glutenin			
1/2	15.3±0.2[a]	14.2±0.1[b]	14.4±0.5[ab]
2/5	22.7±0.9	22.3±0.8	22.1±0.8
7	29.2±0.3	30.5±0.8	31.4±1.1
8/9	16.8±0.0	16.9±0.8	16.6±0.4
10/12	15.9±0.8	16.2±0.9	15.5±1.1
Ratio	0.34±0.09	0.34±0.01	0.34±0.03

[1] Letters indicate significant differences (α=0.05) between treatments

Table 57. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Katepwa Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	16.0±0.0^{a}	14.7±0.3^{b}	15.4±0.0^{a}
2/5	19.5±0.1	19.8±1.2	19.3±0.6
7	29.0±1.1^{b}	32.2±0.3^{a}	33.2±0.6^{a}
8/9	14.8±0.4	13.9±1.1	14.2±0.2
10/12	20.7±0.6^{a}	19.4±0.5^{ab}	17.7±0.8^{b}
Ratio	0.49±0.00^{b}	0.51±0.01^{a}	0.50±0.00^{ab}
Omegas	37.5±0.5^{a}	29.6±3.2^{b}	31.0±0.0^{b}
AS glutenin			
1/2	15.8±1.0	15.4±0.1	15.9±0.0
2/5	19.3±2.3	21.0±1.1	21.8±0.1
7	29.3±0.7^{b}	30.1±0.3^{b}	31.9±0.4^{a}
8/9	17.5±2.0	17.9±1.4	15.2±0.5
10/12	18.0±1.9	15.6±0.0	15.2±0.2
Ratio	0.56±0.04	0.57±0.04	0.57±0.00
Omegas	11.5±4.2	6.8±0.5	8.1±2.3
AI glutenin			
1/2	15.6±4.7	14.2±0.2	14.8±0.2
2/5	18.5±0.0^{b}	23.1±1.1^{a}	20.1±1.1^{b}
7	29.5±0.2	30.6±1.8	31.8±1.0
8/9	21.1±1.5^{a}	16.8±0.2^{b}	18.9±0.7^{ab}
10/12	15.4±0.1	15.4±0.6	14.3±1.6
Ratio	0.32±0.01	0.33±0.03	0.28±0.01

[1] Letters indicate significant differences (α=0.05) between treatments

Table 58. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From Katepwa Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	16.2±0.1	14.9±0.1	16.0±1.3
2/5	19.5±1.3	19.2±0.5	20.8±1.3
7	28.9±0.6^{b}	32.5±0.4^{a}	31.8±1.5^{ab}
8/9	15.3±1.0	15.6±0.4	14.0±0.9
10/12	20.1±1.0^{a}	17.6±0.4^{b}	17.5±0.1^{b}
Ratio	0.50±0.03	0.53±0.03	0.54±6.3
Omegas	35.5±0.9^{a}	27.9±2.5^{b}	28.2±2.2^{b}
AS glutenin			
1/2	15.4±0.1	15.5±0.0	15.5±0.4
2/5	20.4±1.6	21.2±0.3	21.9±0.7
7	29.3±0.6^{b}	31.3±0.7^{a}	32.0±0.4^{a}
8/9	17.9±1.5	17.2±1.1	17.0±0.2
10/12	17.1±0.7^{a}	14.8±0.1^{b}	13.7±0.4^{b}
Ratio	0.52±0.04	0.53±0.01	0.54±0.02
Omegas	5.7±0.2^{a}	4.4±0.1^{b}	3.8±0.1^{c}
AI glutenin			
1/2	15.6±0.5^{a}	13.8±0.6^{ab}	12.6±1.1^{b}
2/5	19.6±3.6	21.6±2.1	23.8±1.1
7	30.3±0.4	29.8±1.1	30.1±0.9
8/9	19.4±4.2	18.4±2.1	15.9±0.1
10/12	15.0±0.5	16.2±1.8	17.6±1.0
Ratio	0.33±0.01	0.31±0.04	0.34±0.1

[1] Letters indicate significant differences (α=0.05) between treatments

Table 59. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From AC Domain Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	17.4±0.3^{a}	15.8±0.2^{b}	15.8±0.1^{b}
2/5	16.9±0.4^{b}	18.7±0.7^{a}	19.2±0.4^{a}
7	35.9±0.0^{a}	34.4±0.4^{ab}	33.8±1.0^{b}
8/9	12.1±0.1	12.6±1.1	12.2±0.1
10/12	17.7±0.9	18.4±0.4	19.0±0.8
Ratio	0.45±0.01	0.46±0.01	0.46±0.1
Omegas	30.4±0.8	31.0±1.9	28.7±3.7
AS glutenin			
1/2	17.0±0.0^{a}	15.1±0.1^{b}	15.0±0.4^{b}
2/5	18.2±0.7^{b}	21.8±0.4^{a}	20.8±0.8^{a}
7	34.8±0.6^{a}	32.4±0.3^{b}	34.0±0.1^{a}
8/9	13.4±0.2^{c}	16.0±0.0^{a}	14.8±0.1^{b}
10/12	16.5±0.2^{a}	14.6±0.2^{b}	15.4±0.6^{ab}
Ratio	0.56±0.01^{a}	0.51±0.00^{b}	0.55±0.01^{a}
Omegas	23.1±0.5^{a}	7.6±0.7^{c}	13.3±0.1^{b}
AI glutenin			
1/2	15.6±0.2^{a}	13.8±0.8^{b}	13.6±0.1^{b}
2/5	19.2±1.2	23.2±0.4	23.1±1.8
7	33.4±0.9^{a}	32.2±0.2^{ab}	31.0±0.5^{b}
8/9	19.2±1.3	16.2±0.7	16.2±2.1
10/12	12.5±1.2^{b}	14.6±0.6^{ab}	16.2±0.8^{a}
Ratio	0.21±0.01^{b}	0.34±0.01^{a}	0.30±0.02^{a}

[1] Letters indicate significant differences (α=0.05) between treatments

Table 60. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From AC Domain Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	15.9±0.2[a]	14.6±0.1[b]	15.8±0.1[a]
2/5	18.8±0.1[b]	20.6±0.6[a]	20.6±0.1[a]
7	34.1±0.2[a]	31.8±0.6[b]	31.8±0.1[b]
8/9	13.7±0.1	14.2±1.0	14.6±0.3
10/12	17.5±0.3	18.8±1.1	17.4±0.1
Ratio	0.44±0.00	0.45±0.00	0.47±0.02
Omegas	23.5±2.5	22.0±1.1	21.4±2.9
AS glutenin			
1/2	16.2±0.7	15.8±0.2	14.9±0.4
2/5	18.7±0.6[b]	21.6±0.1[a]	21.4±0.2[a]
7	33.7±0.5	32.4±0.1	35.1±2.5
8/9	14.6±0.3[b]	16.0±0.6[a]	14.6±0.1[b]
10/12	16.7±0.1	14.3±0.3	14.1±2.0
Ratio	0.54±0.01[a]	0.48±0.00[ab]	0.46±0.04[b]
Omegas	18.1±0.3[a]	8.4±0.8[b]	10.8±3.9[ab]
AI glutenin			
1/2	15.5±1.5	13.4±1.3	12.5±0.4
2/5	21.5±0.9[b]	20.8±0.6[b]	24.5±0.8[a]
7	29.5±1.9	31.3±1.4	31.4±0.2
8/9	18.4±1.0	19.2±0.6	15.8±1.3
10/12	15.2±0.3	15.2±0.1	15.8±1.1
Ratio	0.22±0.07	0.30±0.01	0.25±0.02

[1] Letters indicate significant differences (α=0.05) between treatments

Table 61. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From AC Domain Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	15.3±0.1	14.4±0.3	14.7±0.6
2/5	19.7±0.2	20.6±0.1	22.4±1.6
7	32.9±0.1	31.6±0.4	30.6±2.3
8/9	15.0±0.1^{a}	14.9±0.1^{a}	13.9±0.4^{b}
10/12	17.1±0.3	18.4±0.6	18.4±1.8
Ratio	0.44±0.03	0.46±0.01	0.45±0.02
Omegas	27.8±0.3^{a}	22.8±0.6^{ab}	19.0±2.7^{b}
AS glutenin			
1/2	16.3±0.2^{a}	16.1±0.3^{a}	14.7±0.4^{b}
2/5	20.6±0.2	21.8±0.4	22.5±1.8
7	31.8±0.1	32.4±0.1	33.5±2.5
8/9	15.5±0.1^{b}	17.0±0.6^{a}	16.3±0.3^{ab}
10/12	15.7±0.4^{a}	12.9±0.0^{b}	13.1±0.5^{b}
Ratio	0.51±0.04	0.45±0.01	0.47±0.03
Omegas	12.8±0.1^{a}	4.5±0.6^{b}	5.4±0.2^{b}
AI glutenin			
1/2	14.1±0.3^{a}	13.1±0.6^{ab}	11.4±1.0^{b}
2/5	21.0±0.4^{c}	23.6±1.2^{b}	26.1±0.2^{a}
7	31.0±1.2^{ab}	31.2±1.1^{a}	28.1±0.3^{b}
8/9	19.7±1.5	16.6±1.0	17.1±0.8
10/12	14.2±0.3^{b}	15.6±0.8^{ab}	17.4±0.6^{a}
Ratio	0.25±0.02^{b}	0.29±0.00^{a}	0.25±0.00^{b}

[1] Letters indicate significant differences (α=0.05) between treatments

Table 62. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From AC Karma Doughs Mixed to 50% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	17.0±0.6	17.2±0.4	17.1±0.4
2/5	23.1±0.0	23.1±0.1	22.9±1.6
7	29.9±1.0	31.0±0.0	31.0±0.2
8/9	12.3±0.5	12.4±0.6	12.4±0.8
10/12	17.7±0.0	16.4±1.1	16.6±0.1
Ratio	0.37±0.02	0.46±0.04	0.45±0.05
Omegas	20.7±0.6[a]	16.0±0.7[b]	15.1±1.1[b]
AS glutenin			
1/2	16.8±0.7	16.7±0.3	17.4±0.6
2/5	22.6±1.0	22.2±1.1	22.0±0.5
7	30.2±0.3	31.8±0.6	32.0±1.9
8/9	14.1±0.5	15.1±1.6	14.6±1.5
10/12	16.4±0.1[a]	14.3±0.3[b]	14.0±0.4[b]
Ratio	0.51±0.01	0.51±0.01	0.52±0.00
Omegas	7.8±0.7	7.6±0.7	7.1±1.5
AI glutenin			
1/2	15.7±0.6	15.3±0.4	15.6±0.4
2/5	22.1±2.9	23.5±0.0	22.9±0.8
7	30.1±0.5	30.8±0.0	30.7±0.7
8/9	16.2±2.2	15.2±0.2	15.2±0.9
10/12	15.8±0.5	15.1±0.1	15.6±0.4
Ratio	0.30±0.01	0.31±0.02	0.31±0.06

[1] Letters indicate significant differences (α=0.05) between treatments

Table 63. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From AC Karma Doughs Mixed to 100% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	16.2±0.4	16.6±0.4	16.6±0.3
2/5	23.3±0.5	23.5±0.3	24.2±0.1
7	28.2±1.9	31.6±0.4	31.6±1.0
8/9	14.3±1.9	12.9±0.1	12.6±0.9
10/12	17.9±0.2[a]	15.5±0.6[b]	15.1±0.2[b]
Ratio	0.40±0.02	0.42±0.04	0.46±0.1
Omegas	18.2±1.4	13.4±3.3	13.8±1.6
AS glutenin			
1/2	16.3±1.3	17.8±0.1	16.6±0.5
2/5	22.9±0.7	22.5±0.6	23.1±0.7
7	30.1±1.4[b]	32.1±0.8[ab]	33.4±0.7[a]
8/9	15.5±1.0	14.9±0.0	14.2±0.7
10/12	15.2±0.4[a]	12.7±0.1[b]	12.6±0.2[b]
Ratio	0.47±0.03	0.46±0.01	0.49±0.00
Omegas	7.4±2.2	4.9±0.4	4.4±0.4
AI glutenin			
1/2	16.2±1.2[a]	15.4±0.5[a]	10.6±0.8[b]
2/5	23.1±3.3	24.4±0.7	22.8±2.6
7	28.8±0.5	29.2±1.0	28.4±1.2
8/9	16.3±1.9[ab]	15.4±1.1[b]	20.0±0.9[a]
10/12	15.6±0.4[b]	15.6±0.8[b]	18.3±1.1[a]
Ratio	0.28±0.02[a]	0.28±0.02[a]	0.17±0.04[b]

[1] Letters indicate significant differences (α=0.05) between treatments

Table 64. Effect of Treatment on Changes in HMW-GS Composition, Ratio and ω-gliadin Content of HMW-GS to LMW-GS in ES, AS, and AI Glutenin Fractions Obtained From AC Karma Doughs Mixed to 200% Peak[1]

Subunit/Ratio	Treatment		
	Control	Iodate	NEMI
ES glutenin			
1/2	16.3±0.3	17.2±0.6	17.1±0.3
2/5	23.2±0.4	22.8±1.1	23.1±0.4
7	28.3±0.7^{b}	31.2±0.6^{a}	32.2±0.4^{a}
8/9	15.0±0.5	14.4±0.8	14.1±0.0
10/12	17.2±0.5^{a}	14.4±0.2^{b}	13.6±0.4^{b}
Ratio	0.38±0.00^{b}	0.46±0.01^{a}	0.48±0.01^{a}
Omegas	17.9±0.8^{a}	14.4±0.2^{b}	12.8±0.4^{b}
AS glutenin			
1/2	17.2±0.0	17.2±0.6	17.8±1.8
2/5	20.9±1.6	20.4±2.9	22.2±0.1
7	29.5±0.1^{c}	30.7±0.4^{b}	33.0±0.4^{a}
8/9	17.7±1.6	17.9±1.1	15.0±0.1
10/12	14.7±0.1	13.6±1.6	12.0±1.3
Ratio	0.51±0.01^{a}	0.42±0.03^{b}	0.45±0.02^{b}
Omegas	6.7±1.1^{a}	4.2±0.6^{b}	2.8±0.1^{b}
AI glutenin			
1/2	16.4±0.6^{a}	14.8±1.2^{a}	8.8±3.1^{b}
2/5	20.3±3.3	23.7±1.7	21.5±2.5
7	30.5±0.7	29.1±2.2	27.9±2.6
8/9	17.9±3.5^{b}	16.2±1.0^{b}	24.5±0.7^{a}
10/12	15.0±0.9	16.4±1.6	17.3±3.0
Ratio	0.29±0.01^{a}	0.24±0.04^{a}	0.14±0.02^{b}

[1] Letters indicate significant differences (α=0.05) between treatments

Table 65. F Values of Significant[1] Main Effects and Interactions Between Factors Affecting Response Variables Glutenin Subunit Composition (% of total HMW-GS), Ratio of HMW-GS to LMW-GS, and ω-Gliadin Content (% of total glutenin fraction), for ES, AS and AI glutenin

		F Values		
Variable[2]	Interaction[3]	AI	AS	ES
GS 1 or 2*	CV	78.12	117.41	292.35
	MT	15.17	ns	65.57
	TRTMT	35.44	ns	4.16
	CV×MT	5.64	ns	7.84
	CV×TRTMT	3.44	2.34	2.98
	MT×TRTMT	7.84	ns	ns
	CV×MT×TRTMT	2.00	ns	ns
GS 2 or 5	CV	22.26	91.87	572.55
	MT	9.35	12.32	57.10
	TRTMT	10.44	11.34	5.48
	CV×MT	2.26	3.77	10.60
	CV×TRTMT	ns	ns	2.81
	MT×TRTMT	2.50	ns	ns
GS 7 or 7*	CV	1157.50	1135.22	1812.82
	MT	8.74	4.51	83.86
	TRTMT	ns	15.89	16.82
	CV×MT	3.36	3.31	6.68
	CV×TRTMT	ns	ns	13.77
	MT×TRTMT	3.26	4.07	2.30
	CV×MT×TRTMT	ns	ns	2.25
GS 8 or 9	CV	87.19	238.19	196.73
	MT	10.21	42.86	31.83
	TRTMT	3.86	5.91	ns
	CV×MT	5.32	6.45	2.60
	CV×TRTMT	4.45	ns	ns
	MT×TRTMT	ns	2.68	ns
	CV×MT×TRTMT	2.50	ns	ns
GS 10 or 12	CV	82.17	61.40	32.42
	MT	31.54	19.16	15.49
	TRTMT	5.70	38.16	8.14
	CV×MT	5.67	5.22	ns
	CV×TRTMT	2.64	ns	2.64
	MT×TRTMT	ns	4.78	ns
ω-gliadin	CV	10.38	27.82	486.40
	MT	15.49	28.62	56.98
	TRTMT	ns	31.05	52.46
	CV×MT	5.48	8.47	9.47
	CV×TRTMT	4.48	7.96	ns
	MT×TRTMT	5.19	3.45	7.93
Ratio	CV	194.91	314.59	100.79
	MT	50.44	32.71	8.03
	TRTMT	6.62	6.27	22.13
	CV×MT	4.01	6.22	5.19
	CV×TRTMT	7.10	3.79	ns
	MT×TRTMT	3.29	3.67	3.01
	CV×MT×TRTMT	2.31	ns	ns

[1] Level of significance α=0.05

[2] GS = glutenin subunit, % of total HMW-GS [3] CV=cultivar, MT=mixing time, TRTMT=treatment

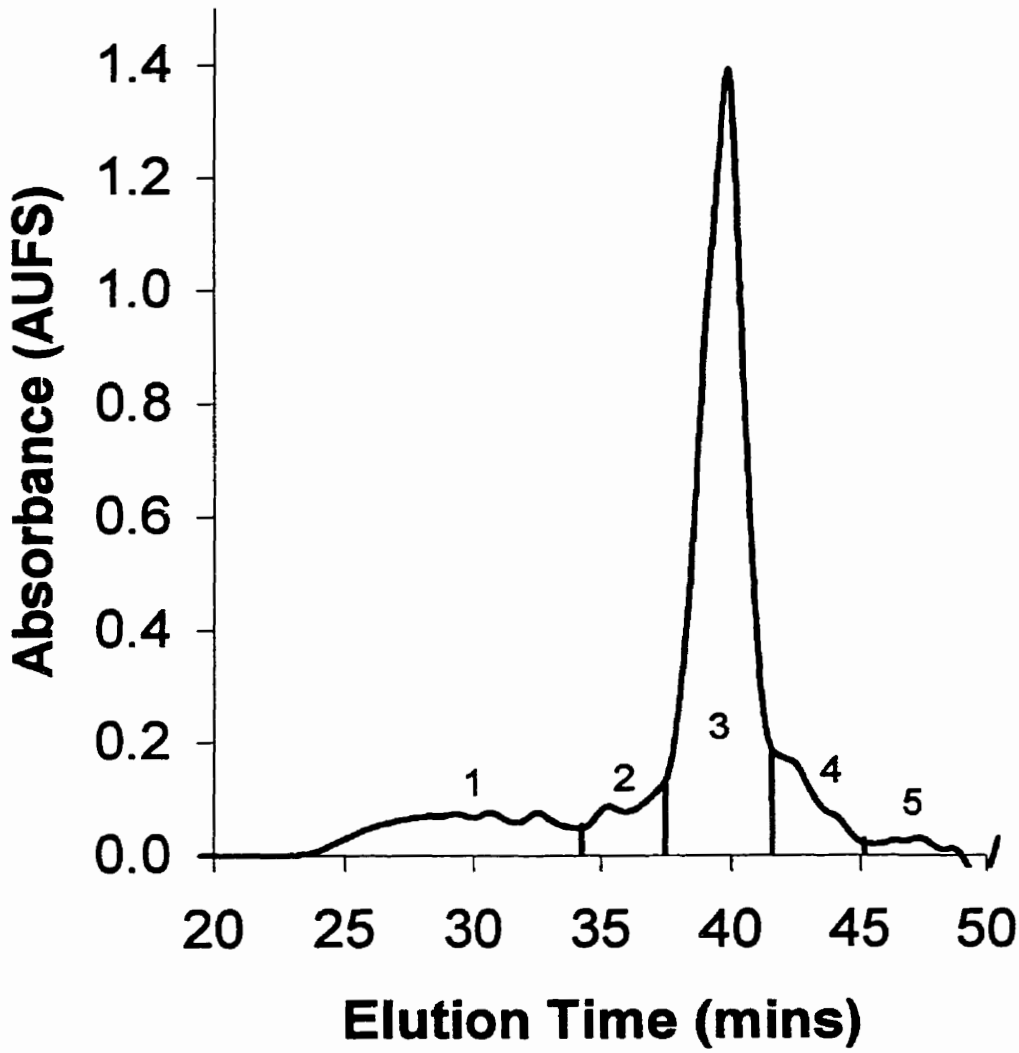

Figure 2. SE-HPLC chromatogram of a 70% 1-propanol-soluble protein sample. Areas 1 and 2 integrated to obtain the proportion of soluble glutenin comprising only LMW-GS.

Table 66. Effect of Cultivar on glutenin content[1] (% total flour or dough protein) of AS70PS samples fractionated by SEC[2]

Treatment	Mixing Time	Glenlea	Katepwa	AC Domain	AC Karma
Control	0	0.7±0.0^{d}	1.8±0.1^{c}	2.2±0.1^{b}	2.3±0.0^{a}
	50	0.5±0.0^{c}	0.6±0.1^{c}	1.6±0.2^{a}	1.2±0.1^{b}
	100	0.5±0.0^{d}	0.8±0.0^{c}	2.1±0.1^{a}	1.4±0.1^{b}
	200	0.4±0.1^{c}	0.4±0.1^{c}	1.8±0.1^{a}	0.6±0.1^{b}
Iodate	50	0.4±0.0^{b}	0.4±0.0^{b}	0.8±0.1^{a}	0.8±0.1^{a}
	100	0.5±0.0^{d}	0.6±0.0^{c}	1.2±0.0^{b}	1.6±0.1^{a}
	200	0.5±0.0^{a}	0.4±0.1^{b}	0.5±0.0^{a}	0.5±0.0^{a}
NEMI	50	0.4±0.0^{d}	0.6±0.0^{c}	1.4±0.0^{a}	1.3±0.0^{b}
	100	0.8±0.1^{b}	0.6±0.1^{c}	2.2±0.0^{a}	0.8±0.1^{b}
	200	0.4±0.1^{b}	0.5±0.0^{b}	0.8±0.0^{a}	0.4±0.1^{b}

[1] Glutenin = Peak 1 and Peak 2 combined

[2] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 67. Effect of Cultivar on glutenin content[1] (% total flour or dough protein) of ES70PS samples fractionated by SEC[2]

Treatment	Mixing Time	Glenlea	Katepwa	AC Domain	AC Karma
Control	0	5.3±0.0^{b}	7.2±0.6^{a}	7.2±0.1^{a}	6.0±0.0^{b}
	50	6.0±0.0^{b}	8.2±0.1^{a}	5.8±0.2^{b}	6.2±0.3^{b}
	100	5.8±0.2^{c}	8.5±0.7^{a}	7.4±0.1^{b}	6.9±0.1^{b}
	200	6.4±0.6^{c}	9.2±0.6^{a}	6.4±0.1^{c}	7.7±0.1^{b}
Iodate	50	6.2±0.5^{b}	8.0±0.9^{a}	8.2±0.2^{a}	7.5±0.3^{ab}
	100	6.4±0.1^{c}	6.6±0.4^{c}	9.2±0.2^{a}	7.8±0.3^{b}
	200	8.1±0.6^{b}	10.2±0.7^{a}	7.4±0.5^{b}	9.9±0.1^{a}
NEMI	50	6.2±0.9^{ns}	7.6±0.2^{ns}	7.0±0.7^{ns}	7.8±1.1^{ns}
	100	7.6±0.9^{bc}	10.2±0.1^{a}	7.4±0.8^{c}	9.2±0.1^{ab}
	200	7.8±0.2^{c}	6.5±0.4^{d}	11.0±0.4^{a}	10.0±0.1^{b}

[1] Glutenin = Peak 1 and Peak 2 combined

[2] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 68. Effect of Mixing Time on glutenin content[1] (% total flour or dough protein) of AS70PS samples fractionated by SEC[2]

		Mixing Time			
Cultivar	Treatment	0	50	100	200
Glenlea	Control	0.7±0.0^{a}	0.5±0.0^{b}	0.5±0.0^{b}	0.4±0.1^{c}
	Iodate	0.7±0.0^{a}	0.4±0.0^{d}	0.5±0.0^{c}	0.5±0.0^{b}
	NEMI	0.7±0.0^{b}	0.4±0.0^{c}	0.8±0.1^{a}	0.4±0.1^{c}
Katepwa	Control	1.8±0.1^{a}	0.6±0.1^{c}	0.8±0.0^{b}	0.4±0.1^{d}
	Iodate	1.8±0.1^{a}	0.4±0.0^{c}	0.6±0.0^{b}	0.4±0.1^{c}
	NEMI	1.8±0.1^{a}	0.6±0.0^{b}	0.6±0.1^{b}	0.5±0.0^{b}
AC Domain	Control	2.2±0.1^{a}	1.6±0.2^{b}	2.1±0.1^{a}	1.8±0.1^{ab}
	Iodate	2.2±0.1^{a}	0.8±0.1^{c}	1.2±0.0^{b}	0.5±0.0^{d}
	NEMI	2.2±0.1^{a}	1.4±0.0^{b}	2.2±0.0^{a}	0.8±0.0^{c}
AC Karma	Control	2.3±0.0^{a}	1.2±0.1^{c}	1.4±0.1^{b}	0.6±0.1^{d}
	Iodate	2.3±0.0^{a}	0.8±0.1^{c}	1.6±0.1^{b}	0.5±0.0^{d}
	NEMI	2.3±0.0^{a}	1.3±0.0^{b}	0.8±0.1^{c}	0.4±0.1^{d}

[1] Glutenin = Peak 1 and Peak 2 combined

[2] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 69. Effect of Mixing Time on glutenin content[1] (% total flour or dough protein) of ES70PS samples fractionated by SEC[2]

		Mixing Time			
Cultivar	Treatment	0	50	100	200
Glenlea	Control	5.3±0.0^{b}	6.0±0.0^{ab}	5.8±0.2^{ab}	6.4±0.6^{a}
	Iodate	5.3±0.0^{b}	6.2±0.5^{b}	6.4±0.1^{b}	8.1±0.6^{a}
	NEMI	5.3±0.0^{b}	6.2±0.9^{ab}	7.6±0.9^{b}	7.8±0.2^{b}
Katepwa	Control	7.2±0.6^{b}	8.2±0.1^{ab}	8.5±0.7^{ab}	9.2±0.6^{a}
	Iodate	7.2±0.6^{b}	8.0±0.9^{b}	6.6±0.4^{b}	10.2±0.7^{a}
	NEMI	7.2±0.6^{b}	7.6±0.2^{b}	10.2±0.1^{a}	6.5±0.4^{b}
AC Domain	Control	7.2±0.1^{a}	5.8±0.2^{c}	7.4±0.1^{a}	6.4±0.1^{b}
	Iodate	7.2±0.1^{c}	8.2±0.2^{b}	9.2±0.2^{a}	7.4±0.5^{bc}
	NEMI	7.2±0.1^{b}	7.0±0.7^{b}	7.4±0.8^{b}	11.0±0.4^{a}
AC Karma	Control	6.0±0.0^{c}	6.2±0.3^{c}	6.9±0.1^{b}	7.7±0.1^{a}
	Iodate	6.0±0.0^{c}	7.5±0.3^{b}	7.8±0.3^{b}	9.9±0.1^{a}
	NEMI	6.0±0.0^{c}	7.8±1.1^{b}	9.2±0.1^{ab}	10.0±0.1^{a}

[1] Glutenin = Peak 1 and Peak 2 combined

[2] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 70. Effect of Treatment on glutenin content[1] (% total dough protein) of AS70PS samples fractionated by SEC[2]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	0.5±0.0^{a}	0.4±0.0^{b}	0.4±0.0^{c}
	100	0.5±0.0^{b}	0.5±0.0^{b}	0.8±0.1^{a}
	200	0.4±0.1^{ns}	0.5±0.0^{ns}	0.4±0.1^{ns}
Katepwa	50	0.6±0.1^{a}	0.4±0.0^{b}	0.6±0.0^{a}
	100	0.8±0.0^{a}	0.6±0.0^{b}	0.6±0.1^{b}
	200	0.4±0.1^{ns}	0.4±0.1^{ns}	0.5±0.0^{ns}
AC Domain	50	1.6±0.2^{a}	0.8±0.1^{b}	1.4±0.0^{a}
	100	2.1±0.1^{b}	1.2±0.0^{c}	2.2±0.0^{a}
	200	1.8±0.1^{a}	0.5±0.0^{c}	0.8±0.0^{b}
AC Karma	50	1.2±0.1^{a}	0.8±0.1^{b}	1.3±0.0^{a}
	100	1.4±0.1^{a}	1.6±0.1^{a}	0.8±0.1^{b}
	200	0.6±0.1^{a}	0.5±0.0^{ab}	0.4±0.1^{b}

[1] Glutenin = Peak 1 and Peak 2 combined

[2] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 71. Effect of Treatment on glutenin content[1] (% total dough protein) of ES70PS samples fractionated by SEC[2]

Cultivar	Mixing Time	Treatment		
		Control	Iodate	NEMI
Glenlea	50	6.0±0.0^{ns}	6.2±0.5^{ns}	6.2±0.9^{ns}
	100	5.8±0.2^{b}	6.4±0.1^{ab}	7.6±0.9^{a}
	200	6.4±0.6^{b}	8.1±0.6^{a}	7.8±0.2^{ab}
Katepwa	50	8.2±0.1^{ns}	8.0±0.9^{ns}	7.6±0.2^{ns}
	100	8.5±0.7^{b}	6.6±0.4^{c}	10.2±0.1^{a}
	200	9.2±0.6^{a}	10.2±0.7^{a}	6.5±0.4^{b}
AC Domain	50	5.8±0.2^{b}	8.2±0.2^{a}	7.0±0.7^{ab}
	100	7.4±0.1^{b}	9.2±0.2^{a}	7.4±0.8^{b}
	200	6.4±0.1^{b}	7.4±0.5^{b}	11.0±0.4^{a}
AC Karma	50	6.2±0.3^{ns}	7.5±0.3^{ns}	7.8±1.1^{ns}
	100	6.9±0.1^{c}	7.8±0.3^{b}	9.2±0.1^{a}
	200	7.7±0.1^{b}	9.9±0.1^{a}	10.0±0.1^{a}

[1] Glutenin = Peak 1 and Peak 2 combined

[2] Means ± standard deviation, means with different letters are significantly different (α=0.05)

Table 72. Effect of Mixing on Changes in HMW-GS composition (% total HMW-GS), ratio of HMW-GS to LMW-GS, ω-gliadin content (% total fraction), and total area (mAU.s) in ES, AS, and AI glutenin fractions obtained from full formula doughs made from Glenlea flour

	Mixing Time (% of Peak)			
Subunit/Ratio	0	67	100	150
ES glutenin				
2	14.0±0.8^{a}	12.6±0.2^{b}	12.2±0.0^{b}	12.4±0.3^{b}
5	14.0±0.3^{c}	15.7±0.4^{b}	16.3±0.2^{b}	17.7±0.0^{a}
7	51.7±1.0^{a}	46.6±0.4^{b}	46.2±0.0^{b}	45.1±0.7^{b}
9	7.4±0.3^{b}	8.7±0.1^{a}	8.8±0.4^{a}	9.4±0.2^{a}
10	13.0±2.4	16.4±0.3	16.4±0.0	15.5±0.6
Ratio	0.49±0.04	0.42±0.5	0.49±0.04	0.46±0.02
Omegas	26.0±1.7^{b}	31.2±0.2^{a}	27.6±2.4^{ab}	27.0±0.6^{ab}
Total area	16271±40^{c}	21039±2063^{b}	23783±1319^{b}	31477±568^{a}
AS glutenin				
2	12.5±0.7	12.9±0.3	12.6±0.3	13.0±0.1
5	18.8±0.1^{a}	15.9±0.1^{c}	17.3±0.0^{b}	17.3±0.6^{b}
7	46.4±1.7	46.1±0.0	46.2±0.2	45.5±0.6
9	9.5±0.2^{b}	9.6±0.4^{b}	9.8±0.3^{b}	11.2±0.7^{a}
10	12.8±2.4	15.4±0.0	14.0±0.3	13.0±0.5
Ratio	0.60±0.03	0.66±0.5	0.64±.00	0.64±0.02
Omegas	7.4±1.5^{c}	16.9±1.1^{a}	13.3±3.1^{ab}	10.2±0.6^{bc}
Total area	18126±385^{ab}	14077±2566^{b}	15754±1359^{ab}	18721±408^{a}
AI glutenin				
2	11.3±0.1	11.6±0.6	11.5±1.1	11.3±1.0
5	20.8±0.0	19.2±1.1	20.2±2.0	20.3±1.6
7	45.4±0.3	43.3±0.2	44.7±1.6	43.9±0.4
9	11.8±0.2	11.5±0.8	10.7±1.4	10.5±1.0
10	10.6±0.1^{b}	14.4±0.6^{a}	12.9±2.2^{ab}	14.0±0.0^{a}
Ratio	0.49±0.01	0.45±0.06	0.42±0.08	0.42±0.02
Total area	30220±669^{a}	22951±428^{b}	22957±2222^{b}	17647±73^{c}

[1] No significant differences unless indicated by letters (α=0.05)

Table 73. Effect of Mixing on Changes in HMW-GS composition (% total HMW-GS), ratio of HMW-GS to LMW-GS, ω-gliadin content (% total fraction), and total area (mAU.s) in ES, AS, and AI glutenin fractions obtained from full formula doughs made from Katepwa flour

	Mixing Time (% of Peak)			
Subunit/Ratio	0	67	100	150
ES glutenin				
2	19.1±0.3^{a}	16.7±0.3^{b}	17.3±0.3^{b}	16.6±0.6^{b}
5	18.1±0.1^{c}	18.6±0.5^{bc}	19.2±0.4^{ab}	19.6±0.0^{a}
7	34.8±1.6^{a}	29.4±0.3^{b}	27.8±1.9^{b}	26.8±0.5^{b}
9	12.0±1.6^{b}	15.4±0.2^{a}	15.6±0.7^{a}	16.9±0.1^{a}
10	16.0±3.5	19.9±0.2	20.1±1.9	20.2±0.3
Ratio	0.52±0.00	0.52±0.01	0.49±0.06	0.51±0.02
Omegas	34.1±1.6^{b}	38.8±0.1^{a}	37.3±1.2^{a}	36.6±1.0^{ab}
Total area	19444±320^{b}	18320±684^{b}	24220±2186^{a}	27106±1034^{a}
AS glutenin				
2	16.3±1.1	15.5±0.7	16.0±0.6	15.5±0.7
5	21.5±0.3	19.8±1.1	19.8±0.2	20.8±0.3
7	32.9±0.7^{a}	28.9±1.1^{b}	29.7±0.2^{b}	29.1±0.0^{b}
9	12.9±1.3^{b}	16.7±1.1^{a}	16.6±0.2^{a}	17.5±0.0^{a}
10	16.4±0.2^{c}	19.1±0.5^{a}	17.8±0.1^{b}	17.2±0.4^{bc}
Ratio	0.56±0.00^{b}	0.58±0.03^{ab}	0.63±0.03^{a}	0.57±0.01^{ab}
Omegas	13.7±4.5	19.7±11.9	16.6±4.4	11.7±1.8
Total area	23614±8696	16954±2573	16716±1856	16560±1694
AI glutenin				
2	18.0±1.6^{a}	16.7±0.1^{ab}	15.7±0.8^{ab}	15.1±0.6^{b}
5	24.3±0.6	21.7±1.3	23.2±0.0	22.2±1.6
7	29.7±1.2	27.9±0.4	29.8±1.7	27.6±0.0
9	15.4±1.3	18.3±1.3	18.0±1.3	18.5±1.6
10	12.6±0.3	15.4±0.3	13.3±3.7	16.6±0.6
Ratio	0.35±0.03^{a}	0.29±0.02^{b}	0.30±0.02^{ab}	0.29±0.00^{b}
Total area	27864±2172^{ab}	30373±433^{a}	24957±20^{b}	21057±1381^{c}

[1] No significant differences unless indicated by letters (α=0.05)

Table 74. Effect of Resting on Changes in HMW-GS composition (% total HMW-GS), ratio of HMW-GS to LMW-GS, ω-gliadin content (% total fraction), and total area (mAU.s) in ES, AS, and AI glutenin fractions obtained from full formula doughs made from Glenlea and Katepwa flour, mixed to 67% peak, and allowed to rest [1]

Subunit/Ratio	Full Formula Dough (mixed to 67% peak) Rest Period (h)			
	Glenlea		Katepwa	
	0	2	0	2
ES glutenin				
2	12.6±0.2	11.9±0.1	16.7±0.3	16.7±1.0
5	15.7±0.4	15.6±0.4	18.6±0.5	18.0±1.9
7	46.6±0.4	45.9±0.5	29.4±0.3	29.1±0.9
9	8.7±0.1	9.1±0.3	15.4±0.2	15.1±0.8
10	16.4±0.3	17.5±0.5	19.9±0.2	21.0±0.8
Ratio	0.42±0.5	0.45±0.00	0.52±0.01	0.51±0.03
Omegas	31.2±0.2	30.8±2.3	38.8±0.1[b]	40.1±0.1[a]
Total area	21039±2063	17036±2176	18320±684	26111±3660
AS glutenin				
2	12.9±0.3	12.6±0.2	15.5±0.7	15.4±0.2
5	15.9±0.1	15.2±0.5	19.8±1.1	20.1±0.2
7	46.1±0.0	47.1±0.8	28.9±1.1	30.6±0.2
9	9.6±0.4	10.4±1.0	16.7±1.1	15.8±0.0
10	15.4±0.0	14.8±1.4	19.1±0.5	18.1±0.2
Ratio	0.66±0.5	0.61±0.08	0.58±0.03	0.59±0.05
Omegas	16.9±1.1	19.6±1.1	19.7±11.9	15.2±4.8
Total area	14077±2566	18856±268	16954±2573	19155±3706
AI glutenin				
2	11.6±0.6	10.9±0.7	16.7±0.1	16.0±1.1
5	19.2±1.1	20.9±0.8	21.7±1.3	22.0±1.3
7	43.3±0.2	43.1±1.3	27.9±0.4	28.5±1.3
9	11.5±0.8	10.3±0.5	18.3±1.3	17.6±0.8
10	14.4±0.6	14.8±0.9	15.4±0.3	15.8±3.0
Ratio	0.45±0.06	0.39±0.5	0.29±0.02	0.26±0.00
Total area	22951±428	21502±859	30373±433[a]	25738±1008[b]

[1] No significant differences unless indicated by letters (α=0.05)

Table 75. Effect of Resting on Changes in HMW-GS composition (% total HMW-GS), ratio of HMW-GS to LMW-GS, ω-gliadin content (% total fraction), and total area (mAU.s) in ES, AS, and AI glutenin fractions obtained from full formula doughs made from Glenlea and Katepwa flour, mixed to 100% peak, and allowed to rest [1]

Subunit/ Ratio	Full Formula Dough (mixed to 100% peak) Rest Period (h)					
	Glenlea			Katepwa		
	0	2	4	0	2	4
ES						
2	12.2±0.0	11.6±0.1	12.1±0.8	17.3±0.3^{a}	16.7±0.3^{ab}	16.0±0.1^{b}
5	16.3±0.2	16.5±0.1	15.3±0.8	19.2±0.4	19.1±0.4	18. 5±1.8
7	46.2±0.0	45.6±0.2	45.0±0.6	27.8±1.9	28.6±0.7	28.7±1.4
9	8.8±0.4	8.9±0.2	9.9±0.4	15.6±0.7	14.2±1.8	15.2±0.7
10	16.4±0.0^{b}	17.3±0.2^{ab}	17.8±0.5^{a}	20.1±1.9	21.5±0.3	21.5±0.3
Ratio	0.49±0.04	0.51±0.02	0.45±0.01	0.49±0.06	0.54±0.02	0.56±0.11
Omegas	27.6±2.4	28.9±0.4	31.7±1.5	37.3±1.2^{b}	38.3±0.0^{b}	40.9±0.4^{a}
Total area	23783±1319^{a}	17068±3346^{b}	22595±377^{ab}	24220±2186	24326±2290	27654±526
AS						
2	12.6±0.3	12.1±0.8	11.5±0.2	16.0±0.6^{a}	15.6±0.0^{a}	14.3±0.2^{b}
5	17.3±0.0^{b}	16.9±0.3^{b}	18.0±0.2^{a}	19.8±0.2	19.5±0.8	20.4±0.1
7	46.2±0.2	46.0±0.4	45.5±0.7	29.7±0.2^{b}	31.6±0.7^{a}	30.1±0.6^{ab}
9	9.8±0.3	10.0±0.1	10.3±0.1	16.6±0.2	15.2±1.3	17.2±0.8
10	14.0±0.3	15.0±0.6	14.6±0.3	17.8±0.1	18.1±0.1	17.9±0.0
Ratio	0.64±.00	0.65±0.01	0.64±0.02	0.63±0.03	0.64±0.05	0.58±0.02
Omegas	13.3±3.1	19.4±6.3	16.4±0.6	16.6±4.4	22.3±7.1	14.2±2.6
Total area	15754±1359	24008±5071	25569±5115	16716±1856^{b}	20377±359^{a}	20928±155^{a}
AI						
2	11.5±1.1	10.8±0.7	12.5±1.3	15.7±0.8	14.7±1.0	14.4±1.0
5	20.2±2.0	19.4±0.2	19.1±0.7	23.2±0.0	23.4±2.7	21.8±0.5
7	44.7±1.6	45.4±0.9	39.1±8.0	29.8±1.7	30.0±1.0	30.1±0.2
9	10.7±1.4	11.2±0.0	12.8±1.6	18.0±1.3	17.4±1.5	17.6±1.5
10	12.9±2.2	13.2±1.3	16.5±4.3	13.3±3.7	14.6±3.1	16.2±0.2
Ratio	0.42±0.08	0.38±0.03	0.36±0.01	0.30±0.02	0.30±0.01	0.29±0.01
Total area	22957±2222	22867±2439	15548±6224	24957±20	23871±1302	23197±1602

[1] No significant differences unless indicated by letters (α=0.05)

Table 76. Effect of Resting on Changes in HMW-GS composition (% total HMW-GS), ratio of HMW-GS to LMW-GS, ω-gliadin content (% total fraction), and total area (mAU.s) in ES, AS, and AI glutenin fractions obtained from full formula doughs made from Glenlea and Katepwa flour, mixed to 150% peak, and allowed to rest[1]

Subunit/Ratio	Full Formula Dough (mixed to 150% peak) Rest Period (h)			
	Glenlea		Katepwa	
	0	2	0	2
ES glutenin				
2	12.4±0.3	12.5±0.4	16.6±0.6	16.7±0.4
5	17.7±0.0	16.5±0.5	19.6±0.0	18.0±1.3
7	45.1±0.7	44.4±0.6	26.8±0.5	28.7±0.7
9	9.4±0.2	9.4±0.0	16.9±0.1[a]	15.1±0.1[b]
10	15.5±0.6	17.2±0.7	20.2±0.3	21.5±0.3
Ratio	0.46±0.02	0.44±0.03	0.51±0.02	0.53±0.01
Omegas	27.0±0.6[b]	31.6±1.0[a]	36.6±1.0	39.1±0.3
Total area	31477±568[a]	25415±1799[b]	27106±1034	24279±1959
AS glutenin				
2	13.0±0.1	12.5±0.6	15.5±0.7	15.2±0.3
5	17.3±0.6	15.6±1.0	20.8±0.3	19.9±0.8
7	45.5±0.6	44.2±1.7	29.1±0.0	30.8±0.7
9	11.2±0.7	13.7±3.3	17.5±0.0	16.4±0.4
10	13.0±0.5	14.0±1.2	17.2±0.4	17.7±0.3
Ratio	0.64±0.02	0.74±0.07	0.57±0.01	0.58±0.05
Omegas	10.2±0.6[b]	14.2±1.0[a]	11.7±1.8	14.5±6.3
Total area	18721±408[a]	16794±446[b]	16560±1694	18162±1821
AI glutenin				
2	11.3±1.0	10.6±0.3	15.1±0.6	15.9±2.4
5	20.3±1.6	20.9±0.4	22.2±1.6	21.1±0.9
7	43.9±0.4	44.4±0.5	27.6±0.0[b]	28.6±0.1[a]
9	10.5±1.0	10.2±0.3	18.5±1.6	19.0±1.7
10	14.0±0.0	14.0±0.0	16.6±0.6	15.5±1.8
Ratio	0.42±0.02	0.40±0.01	0.29±0.00	0.30±0.00
Total area	17647±73[b]	18997±33[a]	21057±1381	23491±687

[1] No significant differences unless indicated by letters (α=0.05)