Effects of Genotype and Environment on the Breadmaking Quality of Canada Western Extra Strong Red Spring Wheat Cultivars

BY

Christian Andrew Lukie

A Thesis
Submitted to The Faculty of Graduate Studies
In Partial Fulfillment of the Requirements
for the Degree of

MASTER OF SCIENCE

Department of Food Science
University of Manitoba
Winnipeg, Manitoba

THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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ACKNOWLEDGEMENTS

I would like to acknowledge the following people and entities for their support.

Thanks to Dr. Harry Sapirstein for being my advisor and mentor in the years that I did my Masters Thesis. His guidance, ideas and wisdom helped me to accomplish a great deal in this educational experience.

Thank you to The Canadian Wheat Board for the research fellowship, which funded most of my existence during my graduate years. Without this money there would have been no graduate degree for me.

Thank you to the Natural Sciences and Engineering Research Council of Canada for funding a Strategic Project grant, which made this study possible.

Thank you to Drs. Gavin Humphreys & Fred Townley-Smith (Agriculture & Agri-Food Canada Cereal Research Centre) for coordinating the sample growth and collection of the entire sample set.

Thank you to Randy Roller for his extensive help, above and beyond the call of duty. Thank you to Wayne Johnson for his work in the cereals laboratory, and afternoon grounding sessions.

Thank you to the Department of Food Science of the University of Manitoba, which was my home for many days, weekends and years. The friendships that I gained there will last a lifetime.

Finally, thanks to my family and friends who always were behind me through the rough and easy times and understood even though they did not understand. Thank you for sticking in there even when I had no time. This is yours.

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AS	ascending slope (g/s)
Au	absorbance units
BW	band width (%Torque)
CAP	curve area to peak (g*s)
CD	cell density (cells/cm^2)
CPC	constant protein content (10%)
CU	cell uniformity
CWES	Canada Western Extra Strong
CWRS	Canada Western Red Spring
CWT	cell wall thickness (μm)
DSL	dough sheet length (in)
E	environment effect
ffMT	full formula mixing time (min)
FPC	flour protein content (14% moisture basis)
G	genotype effect
GMP	glutenin macropolymer
GL	gray level
GS	glutenin subunits
HMW-GS	high molecular weight glutenin subunits (mAu)
IGC	50% 1-propanol insoluble glutenin content (%)
LMW-GS	low molecular weight glutenin subunits (mAu)
LV	loaf volume (cc)
MT	mixing time (min)
PDR	peak dough resistance (%Torque)
PF	peak force (g)
PT	peak time (g)
RPC	50% 1-propanol insoluble residue protein content (%)
RP-HPLC	reversed-phase high performance liquid chromatography
SPC	50% 1-propanol soluble protein content (%)
TGS	total glutenin subunits (Au)
WIP	work input to peak (%Torque)

1.0 INTRODUCTION

In 1995, The Canadian Wheat Board established a collaborative working group of researchers from the Canadian Grain Commission's Grain Research Laboratory, Agriculture and Agri-Food Canada's Cereal Research Centre, and the University of Manitoba's Department of Food Science to investigate the breadmaking quality of the CWES wheat class. The specific issues this group was to investigate the uniformity of cultivars within the class in terms of breadmaking quality, and the consistency of quality for each cultivar grown in different locations.

In 1995, the CWES class comprised three registered cultivars, Glenlea (first licensed in 1972 as a Utility class wheat), Bluesky and Wildcat (both registered in 1987). Descriptions of these three cultivars were published by Evans et al. (1972) and Clarke et al. (1994a and 1994b, respectively). In addition, the wheat line PT754, which had undergone three years of testing in the Parkland Wheat Co-operative Trial by the Prairie Registration Recommending Committee for Grain, was expected to be registered. In 1996, PT754 received a three-year interim registration, and was given the name Laser, thus bringing the total number of cultivars in the CWES class to four.

Buyers of CWES wheat expressed concern that the class was no longer performing as well as when Glenlea was the only cultivar within the class for various products, e.g. bread. It was suggested that one or more of the cultivars Bluesky, Wildcat and Laser possessed weaker dough properties compared to those of the established standard CWES wheat cultivar Glenlea. Further, it was not known whether the apparent

differences in quality were genetic and/or environmental in nature. This thesis project was undertaken in 1997 to comprehensively examine these questions.

CWES wheats are characteristically unique in dough physical properties and protein composition. The flour can be distinguished by very long dough mixing times and relatively high contents of so-called "unextractable" glutenin protein, i.e. glutenin of relatively high average molecular size. This wheat class was founded upon the cultivar Glenlea that was developed at the U of M and was originally licensed into the Canada Western Utility wheat class. In 1993, CWES became the new name of the wheat class to better reflect the functional quality of the cultivars within this class. Commercial interest in CWES wheat increased significantly in the 1990s as its gluten attributes became more widely known and studied. These attributes relate to its use as a blending wheat in a variety of applications where there is a need for increased gluten strength in the dough (Bushuk 1980). For example, to carry weaker and less costly wheats in white pan bread production, as a vital gluten replacer in whole wheat, high-fibre and hearth breads, and in frozen doughs to mitigate the damaging effects of extended freezing and thawing.

This study evaluated a sample set of 36 flours derived from six different wheat genotypes- four CWES, and two CWRS- grown in six different locations in Saskatchewan and Alberta. The CWES genotypes were Glenlea, Bluesky, Wildcat, and Laser. The CWRS genotypes were Katepwa and Laura. The three objectives of the study were as follows:

- To quantify genotypic differences in dough protein composition, dough mixing properties, and bread baking potential across locations
- To similarly quantify the magnitude of location effects

- To establish which tests (protein composition, mixing, and/ or baking) best characterize the unique properties of CWES wheat.

2.0 LITERATURE REVIEW

2.1 Introduction

The quality of wheat for breadmaking is dependent on many factors, but is mainly related to the uniqueness of starch and especially the protein component of the endosperm tissue. It has been well established that genotypic differences in wheat used for breadmaking can be explained mainly by differences in the gluten proteins, gliadin and glutenin. While the impact of gluten-related genotype effects on quality have been extensively studied, in-depth studies on the environmental influences on quality have been far less frequent with few solid conclusions. What is clear is that wheat cultivars of identical or similar genetic makeup, with similar qualitative protein composition, can have very different breadmaking properties when grown at different locations or in different years. These differences in breadmaking properties presumably arise due to the effects of varying climate, soil type, and agronomic practices on the quantitative variation in gluten protein composition, i.e. relative amounts of gliadin and glutenin proteins and the molecular size distribution of glutenin. This variation in quality is a concern to millers and bakers who require uniformity in raw materials to manufacture end products of consistent quality.

This study examined the impact of genotype and environment on protein composition, dough mixing and baking properties of strong mixing wheats from the CWES and CWRS wheat classes. This literature review examines these wheat cultivars from the standpoint of protein composition, genotype and environment effects and history.

2.2 Protein Content and Protein Quality as Quality Factors for Wheat Products

2.2.1 Composition

Depending on genotype and environmental factors, wheat protein constitutes approximately 7-17% of the total kernel composition. Protein content and composition is recognized as the most important biochemical component of the grain kernel for breadmaking quality (Bietz 1988). An early study by Larmour (1931) showed a linear relationship between protein content and loaf volume in a study of 665 samples of three different wheat cultivars grown in Saskatchewan. A linear correlation (r =0.63) between loaf volume and flour protein content in the range of 7-14.9% for these samples was reported. This was one of the earliest results that showed the usefulness of flour protein content for predicting bread quality.

Proteins were classified in the early 20th century by Osborne (1907). These fractions were classified according to solubility in various solvents. This method separated wheat protein into four distinct fractions by following a sequential extraction on one sample of flour. The first fraction was extracted with water (i.e. water soluble) and labeled water-soluble albumins. The next fraction was extracted with 0.5M NaCl and was called globulins, or salt-soluble proteins. The third fraction was extracted with 70% ethanol and named gliadins. Gliadins, also called prolamins, are the major portion of the functional breadmaking protein component gluten. The final fraction of the Osborne method is the alcohol-insoluble proteins glutenins. This fraction is the minor component in gluten, but is no less functional. These four fractions are classified as either non-storage or storage proteins.

Non-storage proteins include non-gluten or soluble proteins, and are used by the wheat kernel for the various metabolic requirements of the seed. These physiologically active proteins are mostly found in the albumin and globulin groups of protein (Hoseney 1994b) that are predominantly located in the aleurone cells, bran (pericarp), and germ, and minor amounts in the endosperm. These two protein fractions are believed to be comprised mostly of enzymes and other protein components relevant to the functioning of the wheat grain, and comprise approximately 20% of the kernel's protein content. Albumins are the minor protein component composed mainly of low molecular weight (LMW, Mr> 30,000) single chain polypeptides (Bushuk 1993). In wheat, they are more abundant than globulins, and make up about 10% of the total protein (Wrigley and Bietz 1988). On the other hand, globulins are usually larger proteins (Mr =10,000- 98,000), and represent about 5% of the non-storage protein content. Most non-storage proteins are enzymes and have thus far shown no importance in explaining intercultivar differences in breadmaking quality.

The storage proteins, gliadins and glutenins, are present in the wheat kernel as protein deposits in the starchy endosperm (Shewry et al. 1986), and comprise the majority of the wheat kernel's protein content (80%). The storage proteins, found exclusively in the endosperm, are used by the emerging embryo during germination (Hoseney 1994b), but are also considered the essential component for breadmaking ability. Gliadins are single chain polypeptides that are relatively small and compact (Mr =30,000-80,000), are partly responsible for dough extensibility (Khatkar and Schofield 1997), and viscous properties (Uthayakumaran 1999). Gliadins can be further classified according to their mobility in acid-polyacrylamide gel electrophoresis (PAGE) by

Bushuk and Sapirstein (1991) as α, β, γ, and ω groups. The α, β, and γ gliadins have a molecular weight range of 36,000-44,000 and are "sulfur-rich", while the ω gliadins are larger (Mr = 50,000-75,000) and "sulfur-deficient" due to a lack of the amino acid cysteine (Field et al. 1983). The sulfur-rich characterization of these proteins refers to the ability of the higher number of cysteine residues, and their ability to form intramolecular disulphide bonds that contribute to stability of the protein structure. They do not form intermolecular bonds, which prevent the gliadins from making large polymers, like the glutenins. Gliadins are the major storage protein fraction, and are present in nearly twice the amount as glutenins (Hoseney 1994b).

Glutenins are large polymeric proteins made up of disulphide bonded (intra and intermolecular) subunits with molecular weights ranging from 30,000-140,000. They are classified into LMW (Mr =30, 000-51, 000) and HMW (Mr = 95, 000-140, 000) storage proteins (Payne and Corfield 1979). Glutenins can reach molecular weights into the millions, because of their ability to form intra- and inter-molecular disulphide bonds, which result in glutenin macropolymers (GMP). These interactions both within and between glutenin subunits (GS) cause the GMP to be the largest protein chains to exist in nature (Wrigley 1996). The subunits, although overlapping the other protein groups in molecular weight, are very soluble in solvents such as 0.05N acetic acid (Chen and Bushuk 1970) or 0.1% DTT (Sapirstein and Johnson 2000). The polymers vary in solubility depending on molecular size (Gao and Bushuk 1993); larger polymers are less soluble than smaller polymers of essentially the same composition. Glutenins are less abundant in the protein of wheat, but they are a highly functional protein that contributes to the elasticity of mixed dough.

Gliadin and glutenin are the predominant constituents of gluten, which is the residue of dough after starch washes out of flour (i.e. wet gluten test). Although this is a simplistic description, gluten is not, and is responsible for overall quality of wheat for breadmaking (Finney 1943). It is the balance of gliadin and glutenin that is responsible for the formation of viscoelastic dough, which when transformed by yeast leavening and baking, becomes bread. The quantities of gliadin and glutenin found in this balance is hardly even. Sapirstein and Fu (1998) reported a range of 48-52% monomeric proteins (consisting mainly of propanol-soluble gliadins) and 10-28% glutenins (propanol-soluble and –insoluble glutenins) for a set of wheat cultivars having poor to very good breadmaking capabilities. The focus of this study centres on the relationships and functionality of these protein fractions to dough mixing and baking properties.

2.2.2 Protein Effect on Breadmaking Quality

The relationship between dough mixing and breadmaking requirements and the protein components in wheat have been extensively researched with varying results on their impact and requirements for end-use quality. According to a review by Weegels et al (1996a), research on the quality of proteins follows one of four routes. The first involves quantifying protein fractions to determine statistical relationships with quality. The second involves protein fractionation and reconstitution, or fortification studies to assess the contribution of fractions to breadmaking quality. The third path uses genetics to study the effect on quality of the addition or deletion of protein components. The last route to study protein quality involves the determination of how structure-function relationships between proteins change during processing and how they affect end-quality.

This route is concerned with the effects of protein quality on the physical and compositional properties of wheat cultivars. The present study is concerned with the first and last of these areas.

Bushuk et al. (1969) undertook a study that evaluated bread wheats based on protein quantity and quality. They used three varieties, Manitou, FW-136, and 11-463A, that were considered strong, weak and very strong by Farinograph curves. At a common native protein content, Manitou gave a significantly higher Remix-loaf volume (LV) than the other two varieties, with the strongest variety having the lowest volume. This was repeated at various protein contents. They suggested that the very strong variety required more time to develop than the standard 2.5 minutes to achieve its potential in LV. The conclusion was that the variety 11-463A did not achieve its full potential because of undermixing. This result can most definitely be attributed to the unique protein content of this very strong variety.

Tanaka and Bushuk (1972) studied the effect of protein content and wheat variety on the solubility and electrophoretic properties of flour proteins. They utilized five varieties of varying dough strength and protein content (10.5-14.2%) to evaluate the properties of the varieties based on their protein composition. An important finding was that the quality of the protein was not affected by the quantity. This was based on the fact that there was a similar protein composition among the varieties regardless of the amount of native protein in the flour. In addition, flour with a longer Farinograph development time contained less acetic-acid soluble protein and more insoluble residue protein (modified Osborne fractionation). In this study, the cultivar that contained insoluble residue was 11-463A, which later became known as Glenlea. A conclusion of

this study was that protein content of the flour did not have any qualitative effects on the protein.

Orth and Bushuk (1972) were the first workers to find a strong relationship ($r>0.6$) between breadmaking quality and the modified-Osborne glutenin fraction. In the study involving 26 wheat cultivars grown in Saskatchewan and Alberta, it was discovered that both the glutenins and residue fraction had a direct effect on baking performance. In addition, Osborne acetic acid soluble (i.e. LMW glutenin) and insoluble proteins (i.e. HMW glutenins) were positively and negatively correlated with loaf volume (LV), respectively. Variation was attributed to the molecular size of glutenins, where larger polymers achieved better quality bread with higher loaf volume. In addition to this study, Orth and Bushuk (1973) determined that the presence of specific HMW-GS, coded for by the D-genome, had a significant effect on the baking quality of a cultivar. This study took tetraploid and hexaploid wheat varieties and reduced and analysed them by sodium dodecyl sulphate (SDS)-PAGE. Further, common bread wheat varietiess contained HMW-GS that were not present in the durum samples, which substantiated their important for breadmaking quality of a variety. Arising from this research was considerable work focused on the glutenin component.

A study by MacRitchie (1978) determined that differences in baking potential were a result of the protein fraction insoluble in hydrochloric acid. Where there was an abundance of this protein fraction, there was also a high quality loaf of bread. This protein fraction was believed to be a grouping of HMW glutenin in the polymeric form. A study by Kurowska and Bushuk (1988) found that strong flours (i.e. Glenlea) contained a higher quantity of protein insoluble in the solvent solution cetyltrimethylammonium

bromide (0.01M); this fraction was glutenin. They suggested that glutenin was potentially very important for breadmaking quality.

Dupuis et al. (1996) set out to characterize the acetic acid soluble and insoluble fractions of glutenin from two bread wheats (Katepwa and Glenlea) with similar protein contents, but different protein quality. Both cultivars contained similar amounts of acetic acid soluble protein, but Katepwa contained significantly more gliadin. On the other hand, both Glenlea fractions contained significantly more HMW-GS 7. It was suggested that the acetic acid insoluble fraction appeared to be related to the higher incidence of subunit 7 and weaker interactions with gliadins (the acetic acid soluble fraction). They also determined that the quantity of HMW-GS and the ratio of HMW to LMW-GS did not aid in explaining differences in the solubility of glutenin.

Huang (1997) studied the relationship between quantities of HMW-GS and breadmaking quality of 12 hard red spring wheat cultivars with variable end-product capabilities. Three fractions, low-, medium-, and HMW-GS, were separated according to SDS-PAGE. They found significant relationships between the HMW-GS and the rheological properties and breadmaking quality. This supported the conclusion that quantity of HMW-GS (SDS-insoluble proteins) determined protein quality differences in hard red spring wheat cultivars.

Uthayakumaran et al. (1999) determined that the protein content and glutenin-to-gliadin ratio independently affected dough and baking properties. The impact of protein on mixing properties was studied using the 2 g mixograph for evaluating a group of diverse breadmaking quality cultivars from Australia. It was reported that with increasing flour protein content but constant glutenin:gliadin ratio, and constant flour

protein content and increasing glutenin:gliadin ratio, the mixing time, peak dough resistance, and extensibility of dough increased. But, with increasing glutenin:gliadin ratio, the extensibility decreased. Therefore, with a greater quantity of glutenin, the dough became less elastic and potentially of lower breadmaking quality.

Faergestad et al. (2000) studied the effect of protein content and quality on the characteristics of hearth bread based on a group of seventeen wheat samples from Norway. They ranged in protein content from 10.6-13.0%, and contained a variety of HMW-GS (1, 2*, 5+10, 2+12, 6+8, and 7+9). They determined that protein quality (based on HMW-GS) positively affected the form ratio (dimensions of the loaf) of the hearth bread, while protein content had no significant effect. For cultivars with variable protein quality, an adjustment in proofing time (higher quality cultivars receive longer proving time) would provide adequate processing to obtain a loaf within the optimum range of loaf ratio.

Glutenin and its subunit composition have received much attention as the principal factors underlying differences in breadmaking quality among different wheats. It is well-established that the glutenin fraction contributes more to bread-making quality than the gliadin fraction (Sapirstein and Fu 1996; Weegels et al. 1996a; MacRitchie et al. 1990). There have been many reviews and in-depth studies on this fraction of wheat protein, including Shewry et al. (1992) on LMW-GS, Weegels et al. (1996b) on HMW-GS, Southan and MacRitchie (1999) on the molecular weight distribution of proteins, and Lindsay and Skerritt (1999) on aspects of the glutenin macropolymer.

2.2.3 Protein Quality of Extra Strong Wheat

The cultivar Glenlea belongs to a unique class of wheat that has special properties, most of which can be attributed to its protein composition and quality. Its composition is unique and therefore has unique properties.

Sapirstein (1997) reported three main reasons for Glenlea's uniqueness. First, Glenlea has a functional grouping of HMW-GS that directly impact quality of end-product and dough strength. Next, it has a high ratio of HMW/LMW-GS, which is also important for quality. Finally, the quantity of subunits is abundant and concentrated in only a few found in the HMW group. The elasticity of glutenin is considered a response to the large amount of insoluble glutenin in the glutenin component of Glenlea. Although this concentration is tiny compared to the overall composition of wheat (1-4% of flour), it can dominate the technological properties of dough.

Fu and Sapirstein (1996a) extracted four fractions of protein from two CWRS wheat cultivars (Katepwa and Glenlea) based on differential solubility in aqueous solutions of 50% 1-propanol. They separated the total flour protein into monomeric proteins (albumins, globulins, and gliadins), soluble glutenin, insoluble glutenin, and residue protein. The monomeric proteins and a large amount of the polymeric glutenins (soluble glutenin) made 57-70% of the total flour protein, while a relatively constant amount (14-18%) remained in the residue fraction. The glutenin fraction, both soluble and insoluble, comprised 9.6-14.9% and 12-28% of the total flour protein content, respectively. They determined that the 50% 1-propanol insoluble glutenin fraction, which was mostly pure HMW-GS and LMW-GS, was highly correlated to the mixing requirements of Glenlea dough.

In addition, Fu and Sapirstein (1996b) determined that dough mixing requirements and loaf volume (i.e. breadmaking quality) were positively correlated with insoluble glutenin, but not with other fractions of protein. Both cultivars contained similar quantities of total polymeric protein (50%) and gliadins soluble in 70% 1-propanol (30%) but varied in other fractions. Glenlea contained 21% more propanol-insoluble glutenin and 30% less soluble glutenin. The ratio of these two fractions was much larger for Glenlea than Katepwa (4.5 and 2.8, respectively), but both fractions had the same subunit composition and similar HMW/ LMW-GS.

Sapirstein and Johnson (2000) developed a spectrophotometric method for measuring the soluble and insoluble glutenin content of flours based on solubility in 50% 1-propanol and the reducing solvent dithiothreitol. They reported a very strong relationship (r^2=0.85) between the insoluble glutenin content and mixing strength for a number of Canadian wheat samples. The quality of Glenlea seems to be largely a function of its glutenin composition. This latter study will be explained in greater depth in the thesis.

2.3 Genotype and Environment

2.3.1 Effects of Genotype and Environment

The effect of environment on the biochemical composition and physicochemical properties of wheat cultivars has long been studied by cereal chemists. Understanding genetic and environmental influences responsible for variation in end-product quality is important to millers and bakers who want to produce a consistent product. However,

explaining the exact nature and extent of genotypic and environmental effects on wheat quality is challenging.

The issue of environment was first addressed by LeClerc (1910) who observed that "wheat of one variety from one source and absolutely alike in chemical and physical characteristics, when grown in different localities, possessing different climatic conditions, yields crops of widely different appearance, and very different in chemical composition." Swanson (1939) agreed and suggested that mixograph characteristics of different varieties should not be compared unless grown under similar environmental conditions.

The environmental conditions and how they impact wheat quality is a large area of interest. Mangels (1925) compiled a list of potential factors that included rainfall/ irrigation, temperature during growing season, and length of growing season to help explain the impact of environment. Finney and Fryer (1957) determined that genotypes with longer mixing requirements were more tolerant of the effects of field temperature during the grain-filling phase of kernel development. Therefore, temperature during grain-filling became important as well. Quality is also highly compromised in the period of the crop cycle prior to harvest when the grain is near-full maturity and frost and wet weather can have significant impacts on the starch-hydrolysing enzyme activity (Watson et al. 1967) and gluten quality (Dexter et al. 1981). Further research has added to this list of factors to include temperature during grain filling, precipitation distribution across the entire growing cycle, frost, duration of grain fill, soil conditions, and pests (Peterson et al. 1992). All these factors can affect wheat protein composition, and cause changes in overall wheat quality. However, whether environment or genotype is a more important

determinant of quality depends on the nature of the environmental effects and genotypes under investigation.

Mixing, baking, physical, and compositional parameters were shown by many researchers to be more dependent on environment than genotype for most parameters (Finney and Barmore 1948; Fowler and de la Roche 1975; Kolster et al. 1991; Peterson et al. 1998). Finney and Barmore (1948) reported that variation in protein content was a critical environmental response variable affecting end-product quality. They used the response variable of loaf volume to show that similar wheat genotypes had a varying response to final loaf volume that was highly dependent on their protein content.

Fowler and de la Roche (1975) examined the individual effects of genotype, environment, and interactions on the quality of Canadian wheat varieties. They found that with Canadian spring and winter wheat classes, environmental effects significantly influenced crop yield, protein content and protein composition. The genotypic effects were of little importance, which suggested the high impact of environment on end-quality of varieties.

Baker and Kosmolak (1977) found similar results in a study of two hard red spring bread wheat varieties (Central and Western bread wheat composites) of 20-30 lines each grown at multiple locations throughout Western Canada. They reported that environmental effects were present for all parameters and were much greater than either genotype effects or interactions, for flour protein content and yield. Nevertheless, they did report a significant genotype by environment (GxE) interaction effect for both mixograph mixing time and remix loaf volume, but least important for flour protein

content. In this study, the environmental and genotypic effects were not so clearly defined.

In a study of bran mineral composition of 27 cultivars from 14 countries, protein quantity of wheat, and the component parts of protein (i.e. gliadin and glutenin), Peterson et al. (1986) found that GxE interactions were significant. This meant that the protein components and composition were impacted by both environment and genotype together. However, interactions although significant were much less important compared to the greater effects of the environment and genotype. Again, environmental effects dominated the genotypic effects.

Lukow and McVetty (1991) similarly found GxE interactions to be significant (4-30% total variation in parameters), but quantitatively much less important compared to the genotypic (53-94% total variation in parameters) effects when comparing rank correlations of breadmaking quality parameters in a large sample set of eight genetically diverse hard spring wheat cultivars. One observation they had was that limited sampling and compositing of samples at grain handling points (i.e. grain elevators) decreased purity of samples, and therefore wrongly estimated wheat quality characteristics from an environmental perspective. In other words, compositing lead to misrepresentations on the extent of genotypic effects on the quality characteristics of the cultivars, and essentially eliminated any environmental effects. This was substantiated by Bergman et al. (1998) who stated that sample compositing lead to results for quality parameters that may not be true for the population at large.

Kolster et al. (1991) studied the effects of four Dutch wheat genotypes and six Dutch growing locations on the HMW fraction of glutenin as determined by SDS-PAGE.

They reported that the environmental impact on quantity of HMW-GS varied considerably for a genotype at different locations, even for those with similar flour protein contents. They also showed that the variation within a location was much smaller, which suggested very little in the way of genotypic effects.

Peterson et al. (1992) reported that environmental effects on quality characteristics were larger than genotypic effects and GxE interaction effects for a group of eighteen hard red winter wheats grown at seven locations in the USA. Interaction effects were similar in magnitude to genotypic effects for a few physical parameters, mixing tolerance and kernel hardness, but smaller for flour protein concentration, mixograph mixing time and SDS sedimentation volume. Both genotypic and GxE interaction effects were significantly smaller than the environmental effects. This study concluded with a recommendation that environmental influences on end-product quality should be an important consideration for improving cultivars of hard red winter wheat.

Graybosch et al. (1996) studied the impact of genotype and environment on wheat flour composition in relation to the end-use quality on a sample set of 30 hard red winter wheat genotypes grown throughout Nebraska, USA. Mixing properties and protein quantity and composition were evaluated by SDS sedimentation volume, and size exclusion high performance liquid chromatography (SE-HPLC). They determined that total flour protein content and quantity of gliadin were most sensitive to growing location, while quantity of glutenin was almost completely genotype dependent.

Peterson et al. (1998) studied baking quality as influenced by the response of cultivars to environments in Nebraska. Thirty hard red winter wheat cultivars were grown at 10 locations in Nebraska over two growing seasons. Protein composition (SE-

HPLC), SDS sedimentation volume, mixing properties (2 g mixograph) and baking quality (straight dough) were evaluated to determine the extent of the influences of environment. They found that most mixing and baking parameters were more influenced by the environment than genotype. They concluded that studying and monitoring meteorological data better predicted wheat end-use quality.

Definitive conclusions cannot be made on the effect of environment and genotype on overall quality, composition, and physical properties of wheat. This thesis sheds some light on this topic with the various treatments and samples used.

2.3.1 Starch Dilution as a Method of Removing the Environmental Effect

Environment causes significant changes to the properties of an individual cultivar. Removing the effect of environment by experimental treatment was considered a useful means for isolating genotypic effects. The standard method to accomplish this was introduced in the 1940's and involved diluting samples with starch to remove flour protein content differences. Theoretically, a constant protein content (CPC) removes the effect of protein quantity (which was highly environmentally dependent), and allows the study of protein quality alone. The assumption in this approach was that protein composition remained constant regardless of protein content. However, there was research that showed this not to be the case.

Sandstedt and Ofelt (1940) used wheat starch without the amylo-dextrin fraction, as prepared by Sandstedt et al. (1939), to adjust four flour samples (flour protein contents ranging from 9.9-16.1%), to a CPC for the evaluation of dough strength among a variety of cultivars. They determined that high protein content did not always relate to good

breadmaking quality, and that differences between cultivars based on loaf volume increased with starch dilution. The beneficial effect of dough strength was clearly shown with this type of treatment; the weak cultivars became weaker with protein dilution and the stronger cultivars maintained loaf volume more readily. The study also indicated that there was wide variation in protein quality between varieties, and that protein quality within a variety varied depending on the naturally occurring protein level.

Ofelt and Sandstedt (1941) studied the effect of starch-diluted flour on mixing properties of eighteen southwestern USA wheat cultivars; these samples were similar to the ones used in the previous study (Sandstedt and Ofelt 1940). They found that mixograph mixing curves treated with starch to a constant protein content were similar to mixing curves of the same protein content without any treatment. In addition, there was no significant change in mixograph mixing time with the diluted samples, because mixing time was largely a varietal characteristic and not a protein related property. Therefore, they determined that protein content and quality depended on environment, while mixing properties were largely genotypic in nature.

Sandstedt and Fortmann (1944) studied six hard winter wheat varieties grown at 14 locations throughout the state of Nebraska to determine the effect of environment and genotype on mixing and baking properties of starch-diluted flour samples. They found genotypic differences to be relatively inconsequential compared to the differences caused by growing location. Specifically, they reported that loaf volume and dough handling properties were highly dependent on the growing location and very much independent of protein content. Further, they concluded that starch was useful as a diluent for

eliminating protein quantity effects of dough handling properties, crumb grain characteristics and loaf volume potential.

Harris et al. (1944) did a mixing study involving eight hard red spring wheat cultivars grown at four locations throughout North Dakota over two crop years. Significant differences in mixing properties were found among varieties and locations, and varietal characteristics were similar over crop years suggesting an insignificant environment/ year effect. Variation in mixograms of CPC samples concluded that varietal differences in mixing properties were related to the protein quality and not the protein content or starch component.

Harris et al. (1945) followed up their research with a baking study involving the same sample set from Harris et al. (1944). They found that the influences of genotype and environment were significantly larger when the protein levels were not manipulated with starch to a CPC of 12%. Adding starch did not expand the range of variation, but did cause significant differences among varieties and locations. In addition, flour protein content and loaf volume were more dependent on growing location than genotype.

There was a lull in this type of research methodology until the 1990's. Roels et al. (1993) studied the bread volume potential of flours mixed at a CPC. Using response surface methodology (RSM) they found that the six wheat cultivars in the study required different baking absorptions and mixing time requirements to yield manageable doughs. Further, that flours of different breadmaking quality, but with similar mixing requirements, absorption and flour protein, yielded loaves of equal volume.

Khatkar et al. (1996) reported the most recent work in this area. They used a National 2 g mixograph for a mixing study involving gluten fractionated and

reconstituted flour samples. The sample set of 13 cultivars was taken from France, Britain and Canada; the cultivars from Canada included Glenlea, Laura and Katepwa. Glutens of different wheats were isolated and then reconstituted into a common sample that had its gluten removed. The reconstituted samples (12% CPC) had significantly higher MMT, PDR, and work input to peak (WIP) values compared to the non-fractionated samples. The conclusion was that the differences in characteristics among weak and extra-strong cultivars were mainly due to differences in gluten protein quality, and flour protein content played only a minor role.

Although the treatment of starch addition to achieve a constant protein content has been shown as a very useful treatment for research purposes, there are opposing views on its effectiveness and reliability. Kolster et al. (1991) disagreed with use of starch as a treatment for evaluating dough strength and other differences among wheat genotypes. They maintained that since the environment affected variation in protein quality, it stood to reason that a cultivar grown at a different location would perform quite differently than the same cultivar at another location, even with the same protein content. They suggested that the environmental effect was dominant regardless of any reported knowledge on the influence of genotype. In other words, they believed that this form of research was not going to introduce any new significant knowledge. The effect of environment and genotype will be addressed in this thesis.

2.4 History of Utility Wheat and the Canada Western Extra Strong Wheat class

In March 1972, eighteen metric tons of seed were distributed to a select group of seed growers from across Manitoba, Saskatchewan and Alberta, to establish the

beginning of the CWES wheat class that was then called Canada Utility (Irvine 1983). The Canada Utility wheat class was comprised of the high-yielding wheat cultivars Glenlea and Pitic 62 that were perceived to be of non-bread wheat quality. These wheats were easily distinguishable from Marquis wheat quality (Irvine 1983), and were produced primarily for non-milling commercial uses, i.e. feed (Evans et. al. 1972). Glenlea wheat was characterised as having lower flour protein content, and higher alpha amylase activity than CWRS varieties (GRL 1978). The CWES wheat class, of which Glenlea is the standard, was first established in 1993, however CWES-type wheat cultivars have been in production since 1965.

In autumn of 1993, the designation "Canada Utility" was dropped when this wheat class was renamed CWES wheat as proposed by the Canadian Grain Commission. The class was given two grade-levels (1CW and 2CW) similar to the former Canada Utility class of wheat. When experimental work was initiated for this thesis (1998), the class contained three cultivars: Glenlea, Wildcat, and Bluesky. A fourth cultivar, Laser, was given three-year interim registration in 1997. Compared to the CWRS class of wheat, the common features of this class include harder and larger kernels; lower milling quality, and most notably a uniquely strong gluten and dough.

This class originated more than thirty years ago with line 11-463A (later registered as Glenlea) which was bred at the Department of Plant Science at the University of Manitoba. Glenlea arose as a result of the cross of (Pembina x Bage) x CB100 (Evans et. al. 1972); Pembina was the strong mixing parent. The result was a high yielding variety with very strong dough mixing characteristics. As a consequence of its dough strength, it was the general opinion that Glenlea possessed relatively low

breadmaking potential and so was considered a "non-bread" wheat variety. It was licensed into the Canada Utility wheat class, the other member being Pitic 62, a weak mixing Soft White Spring (SWS) wheat. One of the earliest studies that included this genotype reported that 11463-A (i.e. Glenlea) had very strong farinograph mixing properties, and high loaf volumes by the Remix test baking procedure (Bushuk et al. 1969). In fact, the loaf volume of Glenlea rivalled that of traditional bread wheat cultivars.

Early research indicated that Glenlea was an overly-strong mixing variety because of its high ratio of insoluble to soluble glutenin (Orth and Bushuk 1972). These flour protein fractions were collected by the modified Osborne procedure (Chen and Bushuk 1970). The study incorporated 26 common wheat cultivars grown in Canada, but of international parentage. It was found that the proportion of glutenin was negatively correlated (r=-0.67) with loaf volume quality (i.e. loaf volume per unit protein), but residue protein was positively correlated (r=+0.82). The residue protein contained the insoluble glutenin, which were glutenins of high average molecular size. Further, the ratio of gliadins to glutenins was positively correlated with loaf volume per unit protein. The distinct protein composition of Glenlea was apparently responsible for lower loaf volumes when mixing with constant work input, because there was insufficient time or energy available for proper dough development (Bushuk 1980). However, when Glenlea doughs were prepared under optimum mixing conditions, the resulting bread volume was comparable to that of the CWRS cultivar Manitou (Bushuk et al. 1969). The commercial potential of Glenlea was revealed when it was mixed in a 50:50 blend with a weak mixing flour (Bushuk 1980); the blend resulted in higher loaf volumes than those

obtained with Glenlea alone. Accordingly, the "carrying capacity" of Glenlea was discovered.

Subsequent to these earlier studies, Glenlea was included in numerous reports because of its unique mixing and baking characteristics. Paredes-Lopez and Bushuk (1982a and 1982b) studied the effect of dough mixing development and undevelopment on the physicochemical properties of Glenlea and two other wheat cultivars, the CWRS variety Neepawa and a Soft Wheat, Frederick, with intermediate and weak mixing properties, respectively. Glenlea was shown to have much higher tolerance to abusive mixing cycles. This research provided evidence of the high tolerance to overmixing that is a characteristic of Glenlea and other present day CWES cultivars.

Evidence also existed that Glenlea dough was tolerant of sprout damage. Glenlea was used in two studies (Lukow and Bushuk 1984a, 1984b) that examined the influence of germination on wheat breadmaking quality. It was determined that Glenlea tolerated significantly higher levels of alpha-amylase activity than Neepawa as evaluated by bread volume. Interestingly enough, Glenlea performed slightly better with loaf volume when small amounts of germinated kernels were present compared to none at all. Underlying the baking results was protein compositional data that showed Glenlea maintained a significantly higher level of all Osborne protein fractions compared to Neepawa, even though the protein fraction was reduced as a result of the germination.

Modified Osborne fractionation of the gluten was the focus of a study by Dupuis et al (1996). They found that breadmaking potential was proportional to the acetic acid insoluble fraction (i.e. glutenin), which in turn was proportional to the incidence and quantity of HMW-GS 7. This subunit was found in abundance in Glenlea, but was likely

not proportionately higher compared to other HMW-GS. This study also suggested that the interaction of gliadin and glutenin was a key to dough strength, and therefore Glenlea was important and linked to high strength. This was shown by the presence of both glutenin and gliadin in the mixed dough samples.

Sapirstein and Fu (1996) isolated gliadin and glutenin protein fractions from wheat cultivars of different dough strengths. These fractions were then added to an extra strong (Glenlea) and weak (Harus, an eastern soft white Winter wheat) mixing base flour, respectively. Results clearly showed that with increasing levels of enrichment, glutenin increased the mixing requirements, while gliadin caused decreased mixing requirements. Interestingly, only the glutenin enrichment experiment showed cultivar differences, while the residue and monomeric proteins did not, i.e. the stronger wheat produced a greater increase in mixing requirements compared to the weaker wheat where the increase was only slight. Skerritt et al. (1996) obtained a similar result in their study on the effects of gliadin and glutenin fractions on dough mixing properties in a range of different solvents.

Numerous studies have examined the protein composition of wheat cultivars, and occasionally Glenlea was included as an example for extra strong mixing properties. These studies included sensitivity of acetic acid extractable protein to salt and changes in properties of glutenin fractions treated with the reducing agent dithiothreitol (Kim and Bushuk 1995), and extractability of storage protein in mixing (Bushuk et al. 1997). These studies found that Glenlea-type wheat cultivars had protein components (i.e. gliadins and glutenins) that were less soluble and therefore more stable than weaker wheat cultivars. Glenlea characteristically has higher insoluble glutenin, and lower soluble and monomeric protein (Sapirstein and Fu 1998). This was determined by using

50% 1-propanol to extract gliadins and soluble glutenins. This fraction was further fractionated by increasing the concentration to 70% propanol. Glenlea and extra strong wheat cultivars have large potential for further research in the areas of protein composition and quality.

3.0 MATERIALS AND METHODS

3.1 Flours

The sample set comprised 36 flours of varying grade and end-use quality derived from six wheat cultivars grown in six different locations in 1996 (Table 1). Included in this set were four CWES cultivars, Glenlea, Bluesky, Wildcat, and Laser, and two CWRS cultivars, Katepwa and Laura. Wheat cultivars were collected from six growing locations in Western Canada from the 1996 crop year. The locations (Figure 1) in Saskatchewan were Regina, Saskatoon, Swift Current, and in Alberta they were Edmonton, Lacombe, and Beaverlodge. The wheat was milled on a Buhler pilot scale mill at the Canadian International Grains Institute in Winnipeg to approximately 75-76% extraction rate.

3.2 Protein Fractionation and Quantification

3.2.1 Sample Preparation

Flour Protein Content (FPC) was determined using the Kjeldahl method (AACC 1983). Further protein fractionation followed the method of Sapirstein and Johnson (2001); 50% 1-propanol soluble protein content (SPC), 50% 1-propanol insoluble glutenin content (IGC), and residue protein content (RPC) were evaluated. 100 mg of sample was placed into a microfuge tube along with 1 mL of 50% 1-propanol and was mixed with intermittent vortexing. After the first extraction, the mixture was centrifuged for 3 min at 2,200 x g with a tabletop centrifuge. The supernatant was used to quantify SPC, or discarded. Solvent remaining in the tube was removed with a Pasteur pipette without disturbing the pellet, which was then resuspended in another 1 mL of solution

Table 1

Flour protein content, grade, and degrading factors of full sample set.

Cultivar	Location	FPC (14%mb)	Grade	Degrading Factors
Katepwa	Swift Current	11.58	1CW	
Laura	Swift Current	11.45	1CW	
Laser	Swift Current	11.58	1CW	
Wildcat	Swift Current	11.11	1CW	
Bluesky	Swift Current	10.97	1CW	
Glenlea	Swift Current	10.89	1CW	
Katepwa	Saskatoon	12.48	1CW	
Laura	Saskatoon	11.49	1CW	
Laser	Saskatoon	12.32	1CW	
Wildcat	Saskatoon	12.08	1CW	
Bluesky	Saskatoon	12.06	1CW	
Glenlea	Saskatoon	11.69	1CW	
Katepwa	Edmonton	14.24	2CW	Frost
Laura	Edmonton	13.84	3CW	Frost
Laser	Edmonton	14.31	2CW	Immature
Wildcat	Edmonton	14.11	2CW	Immature
Bluesky	Edmonton	12.41	2CW	Mildew
Glenlea	Edmonton	12.89	1CW	
Katepwa	Beaverlodge	12.70	2CW	Frost
Laura	Beaverlodge	12.51	2CW	Frost
Laser	Beaverlodge	12.73	1CW	
Wildcat	Beaverlodge	12.70	1CW	
Bluesky	Beaverlodge	12.39	2CW	Frost, Green
Glenlea	Beaverlodge	12.02	2CW	Frost, Green
Katepwa	Regina	15.39	2CW	Frost
Laura	Regina	13.91	3CW	Frost
Laser	Regina	16.57	1CW	8% midge
Wildcat	Regina	16.04	1CW	
Bluesky	Regina	14.34	2CW	Frost
Glenlea	Regina	13.15	2CW	Frost
Katepwa	Lacombe	13.16	3CW	Frost
Laura	Lacombe	12.50	3CW	Frost
Laser	Lacombe	13.30	1CW	
Wildcat	Lacombe	12.94	2CW	Immature, Frost, Green
Bluesky	Lacombe	12.80	2CW	Frost, Green
Glenlea	Lacombe	12.16	2CW	Frost, Green

FPC= flour protein content

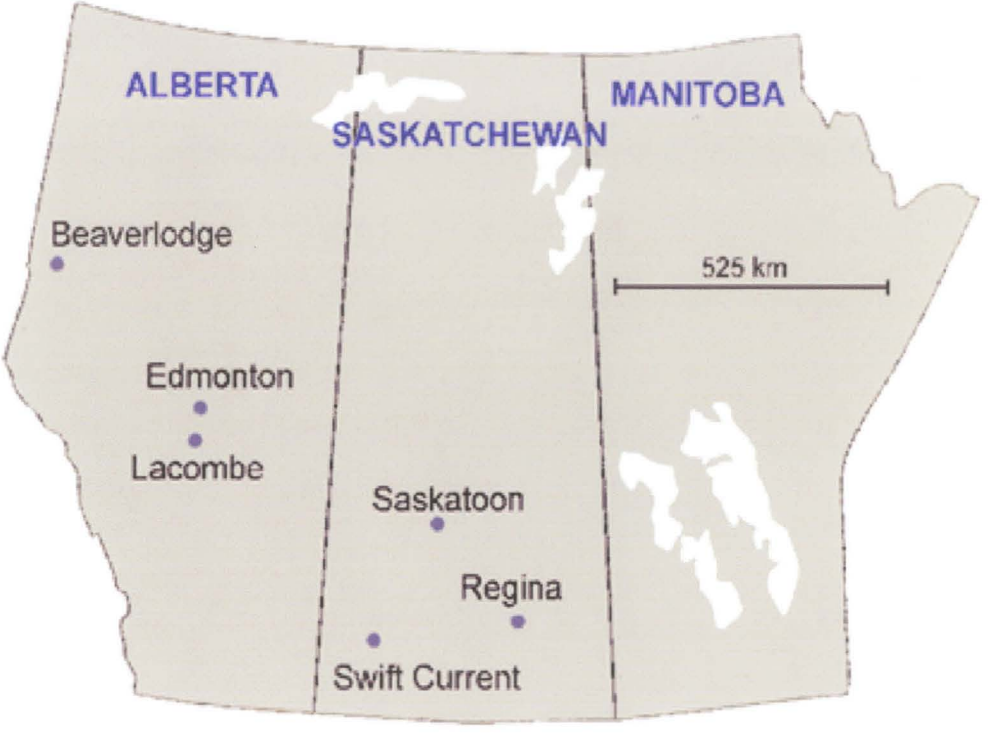


Figure 1. Map of Canada's Prairie Provinces with Locations of the Sample Set.

'A'. A micro-spatula was used to facilitate disruption of the dense pellet and its resuspension. After this second extraction, the mixture was centrifuged for 3 min at 15,000 x g. The supernatant was decanted and any liquid remaining in the tube was again extracted with a Pasteur pipette. The pooled supernatants contained monomeric protein and soluble glutenin (Fu and Sapirstein 1996a). To quantify this fraction, it was necessary to dilute it 100-fold before analysis by spectrophotometry (214 nm) which largely measured peptide bond absorbance.

The pellet, containing the 50% propanol insoluble protein, was extracted with 1 mL solution 'A' containing 0.1% (w/v) dithiothreitol (DTT). This concentration of DTT was not sufficient to produce glutenin subunits, but was adequate to effectively solubilize the propanol insoluble glutenin by partial reduction. The mixture was extracted for 30 min at 55 °C in a heating block and vortexed at 2 min, and then 10 min intervals thereafter. Vortexing samples after being heated for 2 min was critical to facilitate complete suspension of the pellet. After 30 minutes, the mixture was centrifuged for 3 min at 15,000 x g at room temperature. This extraction yielded the fraction for quantifying insoluble glutenin content (IGC). The microcentrifuge tube was inverted once before dilution (100-fold) to obtain a homogeneous supernatant (i.e. no liquid stratification) of IG. It is important to note that this dilution should take place within 30 minutes of centrifugation to avoid reaggregation and precipitation of glutenin isolated from strong mixing wheats such as Glenlea (Sapirstein and Johnson 2001).

3.2.2 Spectrophotometric Analysis

A 1 mL aliquot of solution 'A' provided the blank for the spectrophotometry. Samples were then read in random sequence against the blank. Absorbance of samples, based on a 10 mm path length cuvette, ranged from 0.250-0.700 depending on the sample. This corresponded to protein concentration in the range of 15-35 mg/L or 1.5-3.5% flour (14% mb). Final protein concentrations were reported in two ways: a percent flour basis (% flour), or normalized per unit protein, e.g. IGC/FPC. Residue protein content (RPC) was determined by difference, i.e. FPC less (SPC+IGC).

3.3 Reversed Phase High Performance Liquid Chromatography of Reduced Insoluble Glutenin

3.3.1 Sample Preparation

Samples were prepared as described by Fu and Sapirstein (1996a). The procedure required four stock solutions: "A"- 50% (v/v) 1-propanol; "B"-0.08M Tris-HCl, pH 7.5, containing 50% 1-propanol; "C"- solution "B" containing 1% (w/v) DTT; and "D"- solution "B" containing 14% (v/v) 4-vinylpyridine. Samples (100 mg) were twice extracted with 1 mL solution "A" for 30 minutes at room temperature with intermittent vortexing. Both extracts were centrifuged at 2200 g for 3 minutes. The pellet, containing propanol insoluble protein (i.e. insoluble glutenin) was reduced with 0.1 mL solution "C" (freshly prepared daily) for 1 hour at 60 °C with intermittent vortexing. The sample was then treated with 0.1 mL of solution "D" for a further 15 minutes at the same temperature, with minimal vortexing. Centrifugation at 15,000 x g for 5 minutes yielded

a supernatant, which was subsequently syringe filtered using a 0.45 micron sieve (Millex HV) into microvials that were analysed on the HPLC.

3.3.2 RP-HPLC Protocol for Reduced and Alkylated 50% Propanol Insoluble Protein

Samples were analysed with a Hewlett Packard 1090M liquid chromatograph that incorporates a DR5 solvent delivery system, autosampler, and heated column compartment (maintained at 50 °C for all analysis). The column used was a Zorbax 300 SB-C8 (Rockland Technologies, Inc., Newport, DE) with a 300 angstrom pore size, 5μm particle size, a length of 15 cm, and internal diameter of 4.6 mm. Solvents used were distilled-deionised-deaerated water (solvent A), and acetonitrile (solvent B). Both solvents contained 0.1% (v/v) trifluoroacetic acid, and were constantly, but slowly, sparged with helium. Sample running time was 82 minutes at a flow rate of 1 mL/minute with a linear gradient of 23-44% Solvent B. Eluent was monitored at 214 nm by the 1090M diode array detector-series II that incorporated a 6 mm path length, 8 μL flow cell, and 4 nm slit assembly. Hewlett Packard Chemstation software (version A.05)was used to quantify and analyse the chromatograms.

The chromatogram was divided into regions based on retention times that separated the protein into HMW-GS and LMW-GS (Sapirstein and Fu 1996a). Quantities were determined using the Chemstation software and reported in milli-Absorbance Units (mAu).

3.4 Mixing Experiment

3.4.1 Mixing Formulation and Protocol

The 2 g direct-drive computerized mixograph (National Manufacturing Company, Lincoln, NE) was used to determine dough mixing characteristics. Mixing conditions for each sample were as follows: 2 g flour (14% mb), constant absorption (60%), temperature (25 °C), and mixer speed (88 rpm). Constant absorption provided dough mixing based on constant mass for all samples. Water (distilled) was added directly on top of the sample in the mixing bowl with a 2 mL pipette that was calibrated daily using an analytical balance.

3.4.2 Mixograph Settings and Parameters

Computerized data acquisition and analysis for the mixograms was performed using Mixsmart software version 3.73 (Walker and Walker 1990) with the following settings: top, middle, and bottom filters were all 160; number of filter stages was 3; minimum and maximum torque standard readings were 63 and 900, respectively; peak fit windows for top and middle curve were each set to 10%; and the top-line of the mixogram envelope was used for all analysis. These data acquisition settings resulted in a smoother curve that clearly represented mixing properties of the samples used in this study.

The top-line, as opposed to the default middle-line of the mixogram envelope, was used as the basis for all mixogram analysis, as opposed to the default middle-line. This was an important change, because computed results based on the middle-line sometimes gave erroneous mixing times for CWES cultivars. For example, Glenlea

doughs normally exhibit an extended middle-line plateau stretching over one minute of mixing time, whereas the top-line covered only about 15 seconds (Figure 2A). Accordingly, the top-line yielded a more defined (hence more accurate) dough development and breakdown profile. This analysis protocol did not seem to influence the CWRS samples, e.g. Katepwa, because they routinely showed a well-defined mixing curve (Figure 2B). For very strong mixing samples, analysis based on the middle-line lead to misrepresentation of the mixing characteristics.

Mixogram parameters that were evaluated were as follows: mixograph mixing time to peak dough resistance (MT), band width at peak dough resistance (BW), peak dough resistance (PDR), and work input to peak dough development (WIP). WIP was represented as %Torque in the software, but it is power consumption of the mixograph that is actually determined as the measure of torque transferred to the mixing bowl.

3.4.3 Mixing Treatments

This study utilized traditional flour and water mixograms as well as two additional treatments. The mixing treatments were: 1) addition of wheat starch to a dilution level of 10% constant protein content (CPC), and 2) NaCl (% db). The starch, Whetstar-4, was manufactured by ADM/ Ogilvie Milling Co. (155 Iberia Ave., Candiac, PQ, J5R 3H1, 05/05/1998). Enzyme grade NaCl was obtained from Fisher Scientific.

The amount of starch added to each sample was calculated with a simple formula. Dividing the CPC level (i.e. 10%) and the native FPC resulted in a percentage that was then multiplied by the original flour mass of the native FPC (14% mb) samples. This

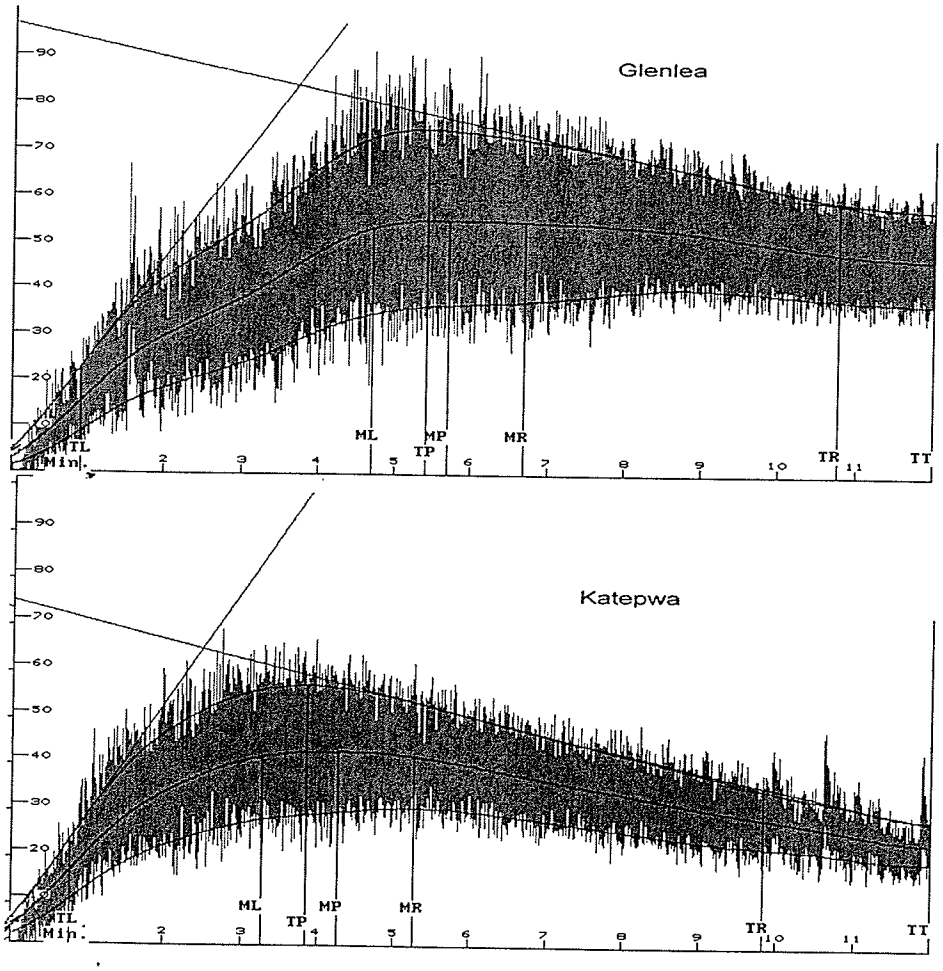


Figure 2. Mixograms of Glenlea and Katepwa with Middle-line and Top-line Curves shown.

adjusted flour mass was subtracted from the original flour mass to give the quantity of starch required to dilute each sample to 10% FPC. For example, the starch requirements for a 12% FPC and 14% moisture sample was calculated as follows:

- 10 divided by 12= 83%
- 83% of 2 g= 1.67 g sample flour
- 2 g – 1.67 g= 0.34 g starch

Therefore, this sample would comprise 1.66 g flour, 0.34 g starch, and 1.2 mL water added in the mixing bowl.

The quantities of SPC, IGC, and RPC as determined by the spectrophotometric method, required correction for starch for precise protein composition determination and for proper and correct correlation analysis among the measured parameters. This was done by multiplying the flour requirements for the individual sample by the quantity of protein then dividing by 100; all of these values are shown in Table 2. For example, the SPC of Glenlea at Swift Current was 6.32%, and its quantity of flour (14% mb) required for a 100 g bake was 91.8 g. Therefore, (6.32%*91.8 g)/ 100 = 5.8% SPC corrected for starch.

3.5 Experimental Breadmaking

3.5.1 Baking Formulation and Processing Steps

A modified-GRL-Chorleywood bake test (Kilborn and Tipples 1981) was used. For 100 g pup loaves, the standard formulation (Chamberlain et al. 1965 and 1962) was modified as follows: flour (corrected to 14% mb, 100%), sugar (2.5%), salt (2%), malt (0.6%), ammonium phosphate (0.1%), Fleischman's compressed yeast (3.2%, fresh

Table 2

Flour and Starch Requirements of the Sample Set for the Mixing and Baking Studies.

Cultivar	Location	Mixograph[1]			Baking[2]		
		Flour (g)	Starch (g)	Corrected Flour	Flour (g)	Starch (g)	Corrected Flour
Katepwa	Swift Current	1.972	0.27	1.703	98.590	13.45	85.143
Laura	Swift Current	1.980	0.25	1.730	99.021	12.52	86.505
Laser	Swift Current	1.966	0.27	1.698	98.297	13.41	84.890
Wildcat	Swift Current	1.969	0.20	1.771	98.449	9.87	88.574
Bluesky	Swift Current	1.969	0.17	1.795	98.438	8.67	89.767
Glenlea	Swift Current	1.972	0.16	1.811	98.601	8.06	90.539
Katepwa	Saskatoon	1.952	0.39	1.565	97.616	19.37	78.247
Laura	Saskatoon	1.964	0.25	1.709	98.213	12.74	85.470
Laser	Saskatoon	1.971	0.37	1.600	98.528	18.53	80.000
Wildcat	Saskatoon	1.969	0.34	1.630	98.449	16.95	81.500
Bluesky	Saskatoon	1.963	0.33	1.628	98.146	16.75	81.400
Glenlea	Saskatoon	1.940	0.28	1.660	97.005	14.02	82.988
Katepwa	Edmonton	1.964	0.59	1.379	98.196	29.25	68.942
Laura	Edmonton	1.969	0.55	1.423	98.466	27.32	71.149
Laser	Edmonton	1.951	0.59	1.363	97.533	29.37	68.166
Wildcat	Edmonton	1.949	0.57	1.381	97.428	28.39	69.037
Bluesky	Edmonton	1.956	0.38	1.577	97.811	18.98	78.833
Glenlea	Edmonton	1.957	0.44	1.519	97.861	21.93	75.930
Katepwa	Beaverlodge	1.965	0.42	1.547	98.258	20.89	77.369
Laura	Beaverlodge	1.965	0.39	1.570	98.230	19.71	78.524
Laser	Beaverlodge	1.947	0.42	1.530	97.357	20.87	76.482
Wildcat	Beaverlodge	1.958	0.42	1.543	97.922	20.79	77.131
Bluesky	Beaverlodge	1.961	0.38	1.583	98.034	18.89	79.145
Glenlea	Beaverlodge	1.947	0.33	1.619	97.357	16.38	80.972
Katepwa	Regina	1.969	0.69	1.279	98.454	34.50	63.959
Laura	Regina	1.971	0.55	1.417	98.562	27.69	70.872
Laser	Regina	1.930	0.77	1.165	96.499	38.26	58.241
Wildcat	Regina	1.947	0.73	1.214	97.340	36.64	60.698
Bluesky	Regina	1.954	0.59	1.362	97.694	29.57	68.120
Glenlea	Regina	1.959	0.47	1.490	97.933	23.45	74.488
Katepwa	Lacombe	1.971	0.47	1.498	98.573	23.67	74.906
Laura	Lacombe	1.965	0.39	1.572	98.241	19.66	78.585
Laser	Lacombe	1.959	0.49	1.473	97.967	24.33	73.638
Wildcat	Lacombe	1.955	0.44	1.511	97.749	22.19	75.557
Bluesky	Lacombe	1.960	0.43	1.531	98.006	21.44	76.570
Glenlea	Lacombe	1.957	0.35	1.610	97.844	17.36	80.483

[1] Based on 2 g flour basis.

[2] Based on 100 g flour basis.

weekly), shortening (3%) and ascorbic acid (125 ppm). Farinograph absorption was a guide for the baking absorption, but adjustments were made during mixing and panning to obtain an optimum level. Mixing was done on a 100 g prototype computerized National pin mixer.

The breadmaking procedure was as follows. Salt and sugar, malt, ammonium phosphate, and water were premixed in a beaker. Flour was added to the mixing bowl of the National pin mixer. The premixed solutions were added, followed by yeast then ascorbic acid. Mixing was controlled at 30°C by a circulating water bath flowing into the water jacket of the bowl, and all solutions (yeast, salt and sugar, water, malt and ascorbic acid) were also maintained at this temperature in the water bath. Mixing continued until the optimum mixing time (i.e. time at PDR plus 10%) was reached. Dough was fermented in a proofing cabinet for 25 minutes at 35 °C and 88% RH, followed by sheeting, moulding and panning. The dough sheet length (DSL) was recorded for further analysis, by measuring the length of the sheeted dough-piece after its third and final pass. Panned doughs were proofed for one hour, proof height was recorded, and then doughs were baked for 25 minutes at 230 °C. Bread was cooled for 30 minutes before weight and loaf volume (by rapeseed displacement) were recorded. Cooled loaves were double-bagged in polyethylene wrap and stored overnight in an incubator. Crumb grain was quantified the following day by digital image analysis (Sapirstein et al. 1994). As well, the texture of the bread crumb was determined using a TA.XT2 texture analyzer.

3.5.2 Prototype Computerised 100 g National Mixer

A prototype computerized National 100 g mixer was developed by National Manufacturing (Figure 3). Mixing was monitored by computer software that was developed in the Department of Food Science by Roller and Sapirstein (not published). The software monitored digital output of a torque recording sensor mounted beneath the bowl. This software automatically determined optimum mixing time as 10% past time at peak dough resistance. Accordingly, this mixer provided a more precise method (compared to visual assessment of a recorded dough curve) for obtaining optimally mixed doughs using a laboratory-scale dough mixer.

3.5.3 Baking Treatments

The two treatments used in the baking experiment were: 1) added starch (flour dilution to 10% FPC, and 2) added starch without shortening.

3.6 High Resolution Digital Image Analysis

The configuration of the prototype digital image analysis (DIA) system is shown in Figure 4. The various components of this system are briefly described below.

3.6.1 Digital Line Scan Camera and Image Resolution

The camera was a high-performance EG&G Reticon unit featuring a 2048 pixel line sensor, pixel size of 14 x 14 μm, maximum pixel rate of 33 MHz, and 10-bit analogue-to-digital output. Focus was provided by a conventional 50 mm F-mount lens. For typical commercial bread product, image resolution was $(73\ \mu m)^2$/pixel or 186

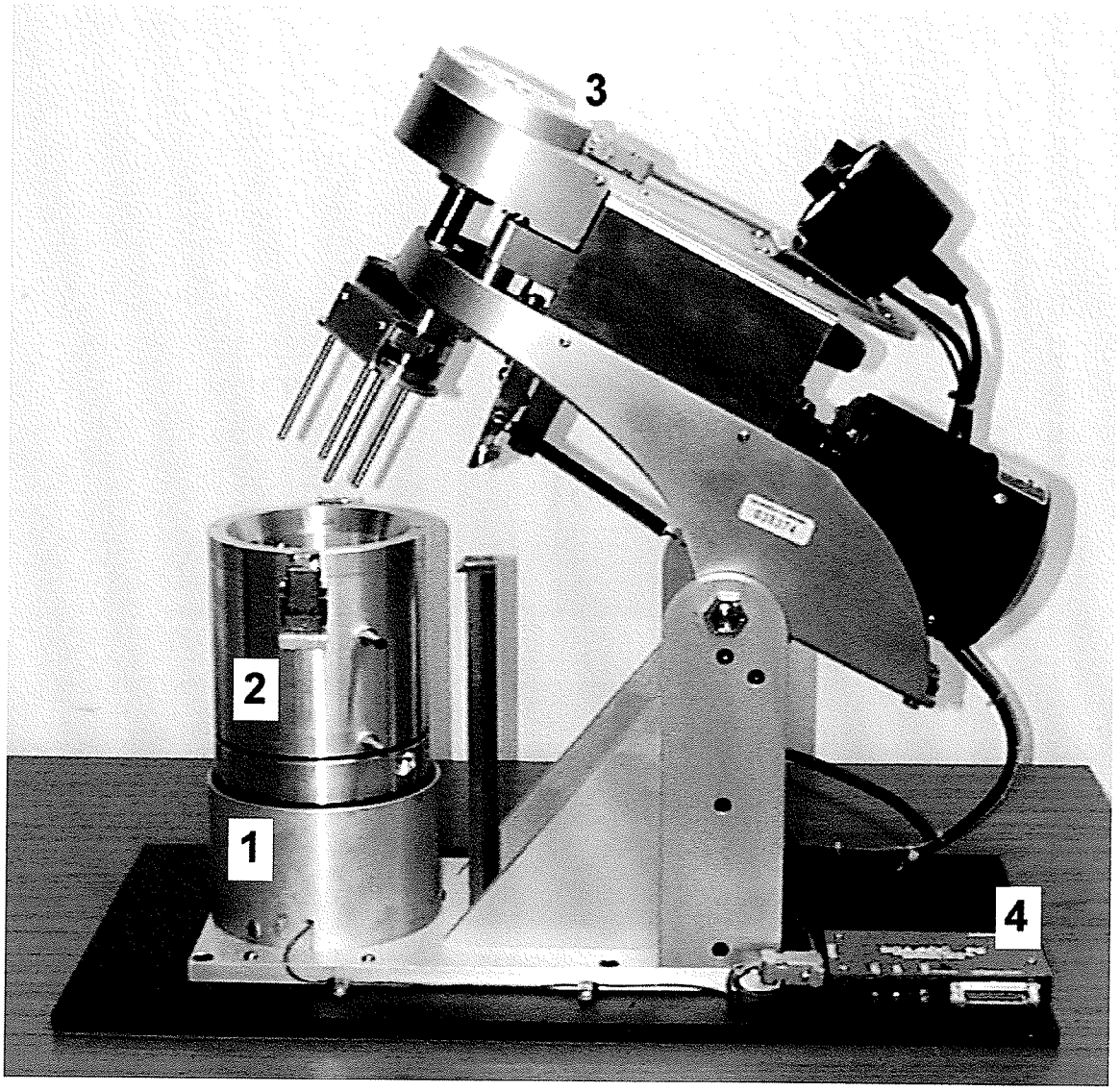


Figure 3. Prototype Computerised 100 g National Pin Mixer: 1) Strain Gauge Housing, 2) Water Jacketed Mixing Bowl, 3) Encoder for Data Acquisition and 4) A/D Converter.

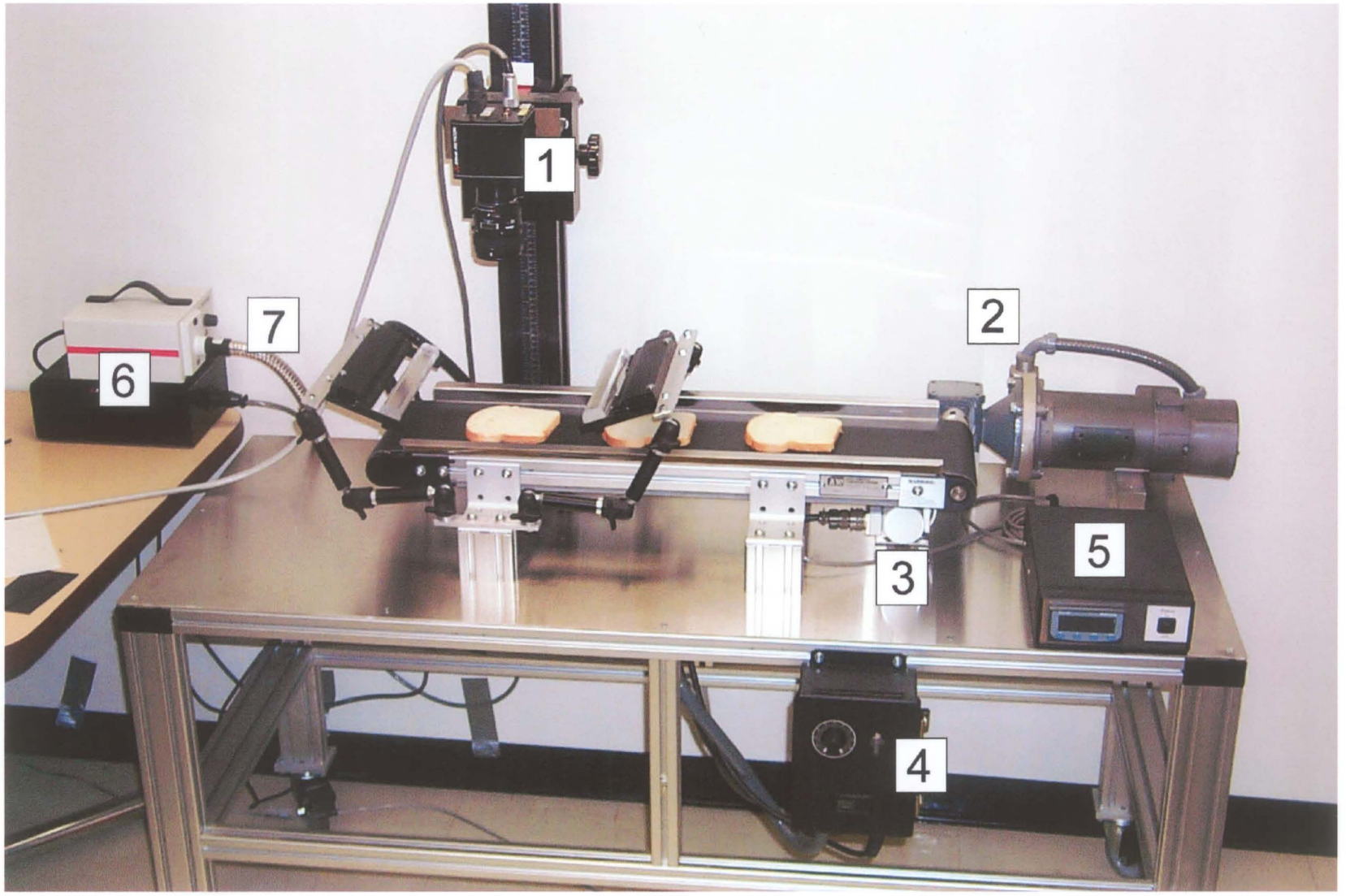


Figure 4. High Resolution Digital Imaging System. ((1) digital line-scan camera, (2) end-drive conveyor as well as DC motor, (3) encoder, (4) digital tachometer, (5) pulse counter, (6) quartz halogen light source and camera power supply, (7) fiber-optic cable, (8) fiber-optic light line w/cylindrical lens.)

pixels/mm^2. For experimental bread of smaller dimensions, e.g. 140 g "pup" loaves, the camera working distance can be shortened to provide resolution of (60 μm)^2/pixel or 273 pixels/mm^2. The image resolution of the new line scan system is almost 50% greater than that obtained with our predecessor area sensor counterpart (Sapirstein et al. 1994) with a 10-fold increase in area field of view for analysis of complete slices of commercial product.

3.6.2 Sample Conveyor System

The conveyor subsystem was an integral component of the line scan imaging system as digital images were formed by the accumulation of 1000-2000 image lines as product passes across the ultra-narrow scan field (< 0.1 mm at product surface) of the line scan camera. From a practical perspective, the conveyor supplies the hardware capability to conveniently acquire sequential images of baked product placed on the conveyor in single file by the operator. The conveyor system was comprised of three specialized components: DC end-drive motor, integrated solid-state motor controller and tachometer, and encoder and pulse counter (see Figure 4). To facilitate accurate imaging, an important feature of the conveyor system, was constant sample transport rate. A digital tachometer interfaced to the motor controller provided a visual display of motor speed that the user conveniently controlled. The last component of the conveyor system was a pulse-generating encoder (wheel) mounted under the conveyor that rotated with the conveyor belt, and a pulse counter that connected between the encoder and the digital image acquisition board. This wheel precisely controlled the image acquisition rate in order to obtain images with the desired aspect ratio (i.e. square pixels).

3.6.3 Lighting System

Sample illumination was a critical component of the imaging system. The requirements included the need for high lighting uniformity, stability, intensity, and a correct angle of incidence to provide a quality of scattered light. The optimum angle optimally exposed the 3-dimensional cellular structure of bread in 2-dimensional images. A lensed fiber-optic illumination system was implemented to satisfy these requirements. The system comprised dual 25 cm light lines connected to a single 150 W DC regulated halogen light source with a randomized common-end fiber optic cable to ensure equal light intensity output. An added benefit of the fiber optic lighting was the "cool" nature of the illumination. This helped to minimize drying of the product during image acquisition, and permitted measurement of bread texture immediately following image acquisition. Light line output was focused to a narrow 17 mm strip, and intensified (≈10x) using special cylindrical lenses. The intensified light permitted the use of relatively high lens f-stop settings (e.g. f/16). That, in turn, facilitated image focus over relatively large depths of field that substantially enhanced image quality. The geometry of the lighting (30° incident angle) was established by previous experiments (Sapirstein 1993 and 1995) to obtain images with maximum clarity and contrast with no need for image enhancement of any kind. Overall, the lighting system supplied a quality of illumination that resulted in bread crumb images of outstanding quality (Figure 5).

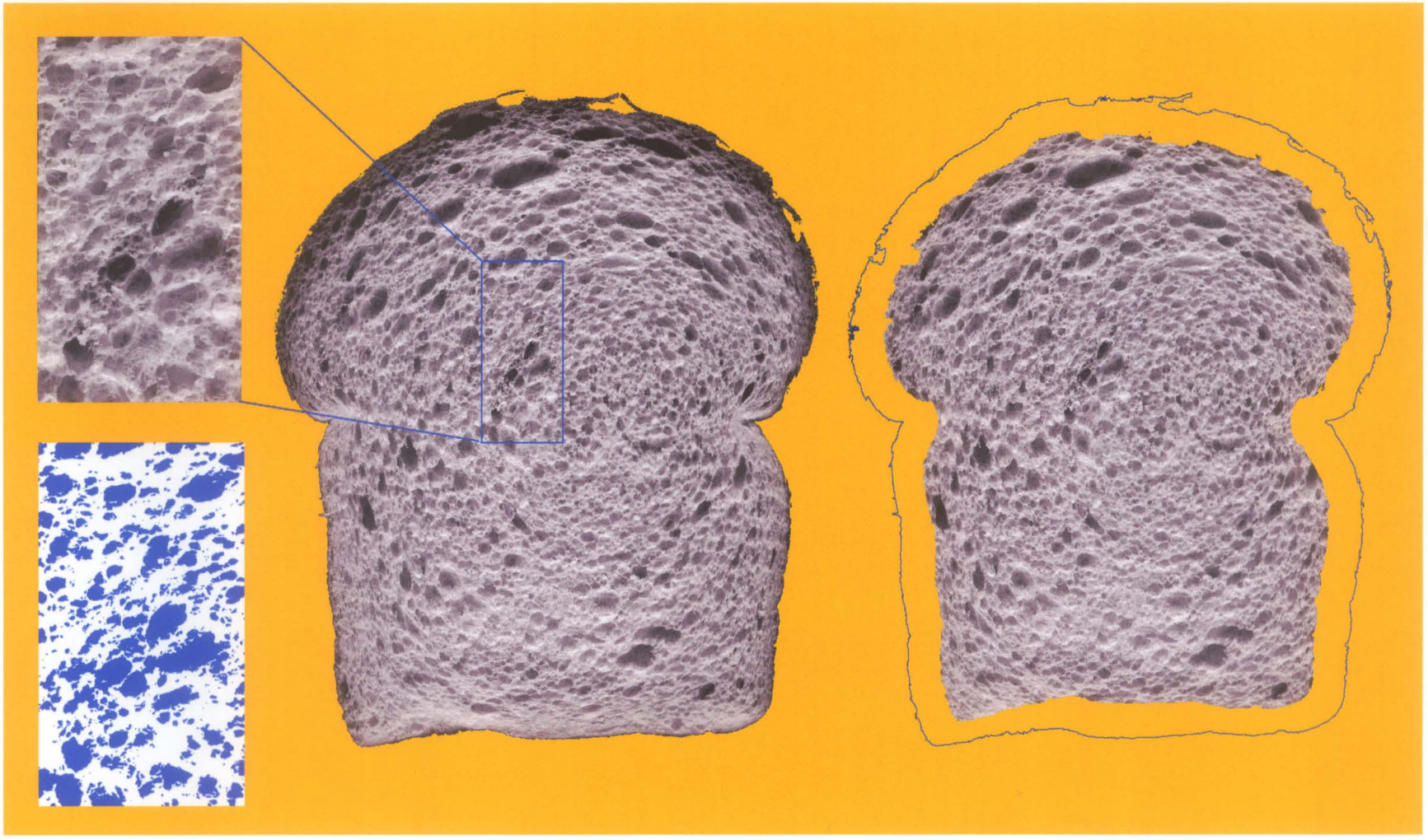


Figure 5. Digital Image of Full Slice of Bread, and Digital Erosion of Crust.

3.6.4 Image Acquisition and Analysis Software

The image acquisition and analysis software was developed in the C++ language that runs on a desktop PC with Windows 9x or NT. Tools are available to calibrate the system, acquire single or multiple images from the frame grabber, and store them to disk. Image analysis software was adapted from that originally described (Sapirstein et al. 1994) with several substantial enhancements especially at the front end of the image analysis process. Those front end modifications relate to the capability of the prototype line scan DIA system to acquire and analyze full bread slices (minus the crust) in contrast to relatively small (e.g. 4 x 6 cm) rectangular fields of view in the original area scan image system.

3.6.5 Digital Image Analysis Applied

An example of a representative digital image of a bread slice and a processed image after dynamic erosion analysis is shown in Figure 5. The crumb grain analysis software was a key feature of the imaging system. This DIA set-up implements the use of the K-means algorithm for image segmentation. This fast algorithm enables the computer to optimize the determination of bread structure relative to variations in product brightness caused by variations in processing conditions and treatments. In the absence of this or analogous optimization, substantially erroneous results are generated. In addition, the analysis software was capable of computing technologically relevant parameters that were directly interpretable by the PC. Measured crumb grain parameters include slice brightness (GL), crumb fineness (CD, cells/cm^2), cell wall thickness (CWT, μm), cell size uniformity (CU, number of small to large cells), and many others.

3.6.6 Imaging Methodology

Full-slices of bread were imaged after loaves were stored overnight in the incubator. The imaging system was focused and calibrated daily to ensure constant illumination. Checking the dimensions of a digitally imaged Canadian ten-cent coin monitored precise analysis for structural integrity. Conveyor speed was adjusted to ensure equal dimensions of the dime (18.4 x 18.4 mm).

Digital imaging of crumb grain was carried out on the day following baking. Loaves were first sliced into 12 mm thick slices using an Oliver Model 797 Gravity Feed Bread Slicer (Oliver Products Company, Grand Rapids, MI, USA). This yielded approximately 10 measurable slices per loaf of which the five central slices were used for analysis. Slices were placed on the conveyor and each was scanned. Images were saved into the computer and analyzed. Bread slices were returned to their bags, and stored overnight for texture analysis the following day.

3.7 Texture Analysis

The TA.XT2 texture analyzer (Figure 6; Texture Technologies, New York) was used to quantify the strength of each slice based on a destructive compression test. Individual slices were placed on an elevated carriage apparatus beneath a 3/4 inch diameter steel ball probe. Two aluminum plates (both 12 cm x 12 cm x 0.5 cm) were placed below the bread slice and one placed on top. The point of penetration on the slice was in the area slightly above the crease in the loaf associated with the lip of the baking pan. The ball probe moved through the slice at 1 mm/s to a maximum penetration depth

Figure 6. TAXT2 Texture Analyser and Bread Holding Apparatus.

of 35 mm (i.e. three times the thickness of the slice) before returning to the starting point above the bread slice.

Curves were analysed by a macro designed for determining the strength of a bread slice (Figure 7). The macro quantified peak force (PF, force at maximum stress), peak time (PT, time to reach maximum stress), ascending slope (AS, rate of increase in force), and curve area to peak (CAP, area under the curve from zero-time to PT).

3.8 Statistical Analysis

Results were analyzed by SAS version 7.0 (SAS Institute 1998). ANOVA and LSD tests were performed to determine significant differences. ANOVA effects were genotype and location and both were considered random effects. Error was estimated based on the flour by location interaction term. Coefficients of variation were calculated by the formula: 100*standard deviation of set/ average value of the set, where the set refers to the six average values of either locations or genotypes for a given parameter. Variances of genotype (G) and environment (E) were calculated in Microsoft Excel (Windows 98 Version) and reported as G/E variance ratios. These values were calculated by dividing the variance associated with the genotype with that associated with environment (i.e. variance G/variance E). For values greater than one, the effect was genotypic, and when the value was less than one it was environmental. This thesis used both environment and growing location interchangeably to describe the geographical locations. All experiments followed a completely randomized design generated by an experiment builder function in JMP IN Statistical Software (Version 2). There was a

minimum of two determinations for protein compositional analysis and mixing, and three replications for baking.

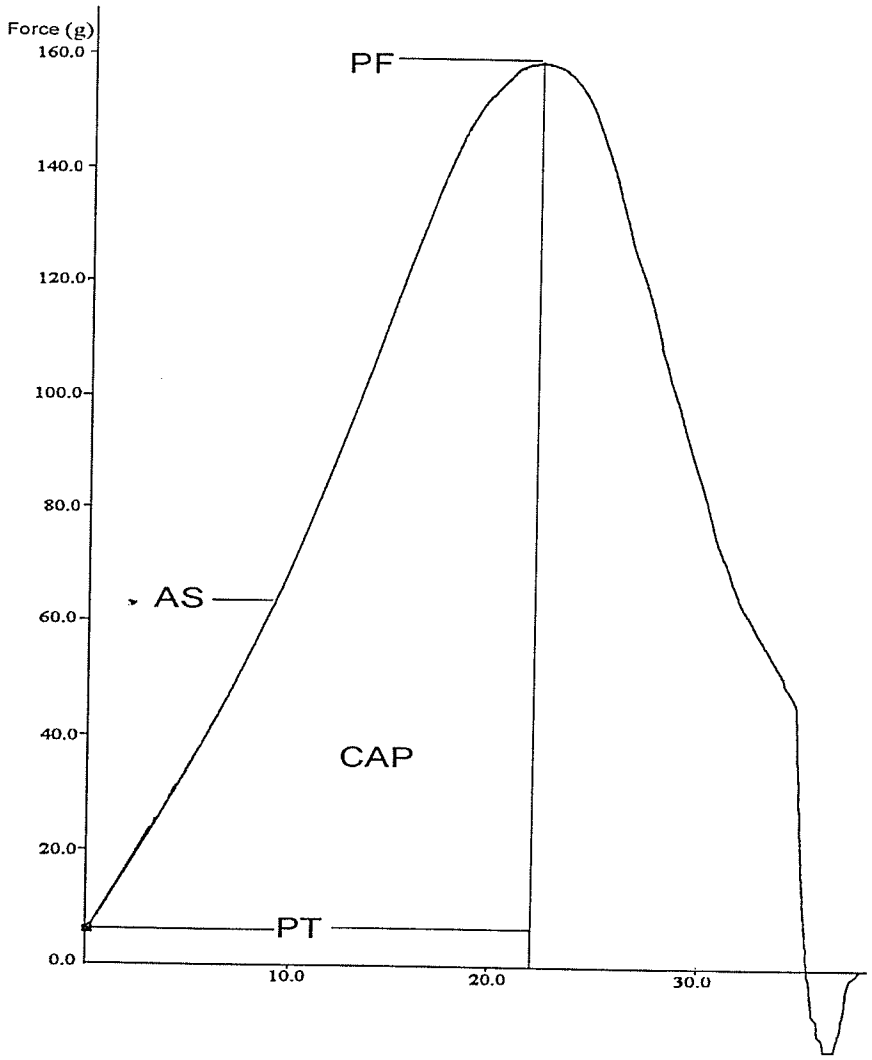


Figure 7. Texture Analysis Curve showing Peak Time (PT), Peak Force (PF), Ascending Slope (AS), and Curve Area to Peak (CAP).

4.0 RESULTS

4.1 Variation in Biochemical Composition of Strong-Mixing Wheat Cultivars

4.1.1 Flour Protein Content

There was small but significant variation in flour protein content (FPC) among the cultivars (Figure 8A), which ranged from 12.1 to 13.5% on average. The size of the standard deviation bars suggested considerable variation in FPC for individual cultivars regardless of location. Glenlea and Bluesky had the lowest FPC, while Katepwa, Laser and Wildcat had the highest. Compared to the relatively narrow 1.4% range in mean genotype FPC levels, the range of location FPC values was 3.6% from 11.3 to 14.9% (Figure 8B). Further, the range of the full sample set in FPC was 10.9 to 16.6%, a range of 5.7%. Harris et al (1945) also reported larger environmental effects than genotypic effects in their study of eight North Dakota Hard Red Spring wheat varieties, and protein ranges for environment and genotype of 2.9 and 1.2%, respectively. The coefficients of variation (CV) for genotype, location and the full sample set of FPC were 4.1, 9.9, and 10.5%, respectively (Table 3). Clearly, location effects on FPC were substantial and were reflected in the very low ratio of G/E variance of 0.17 (Figure 9). This also helps explain why it is challenging for plant breeders to increase FPC.

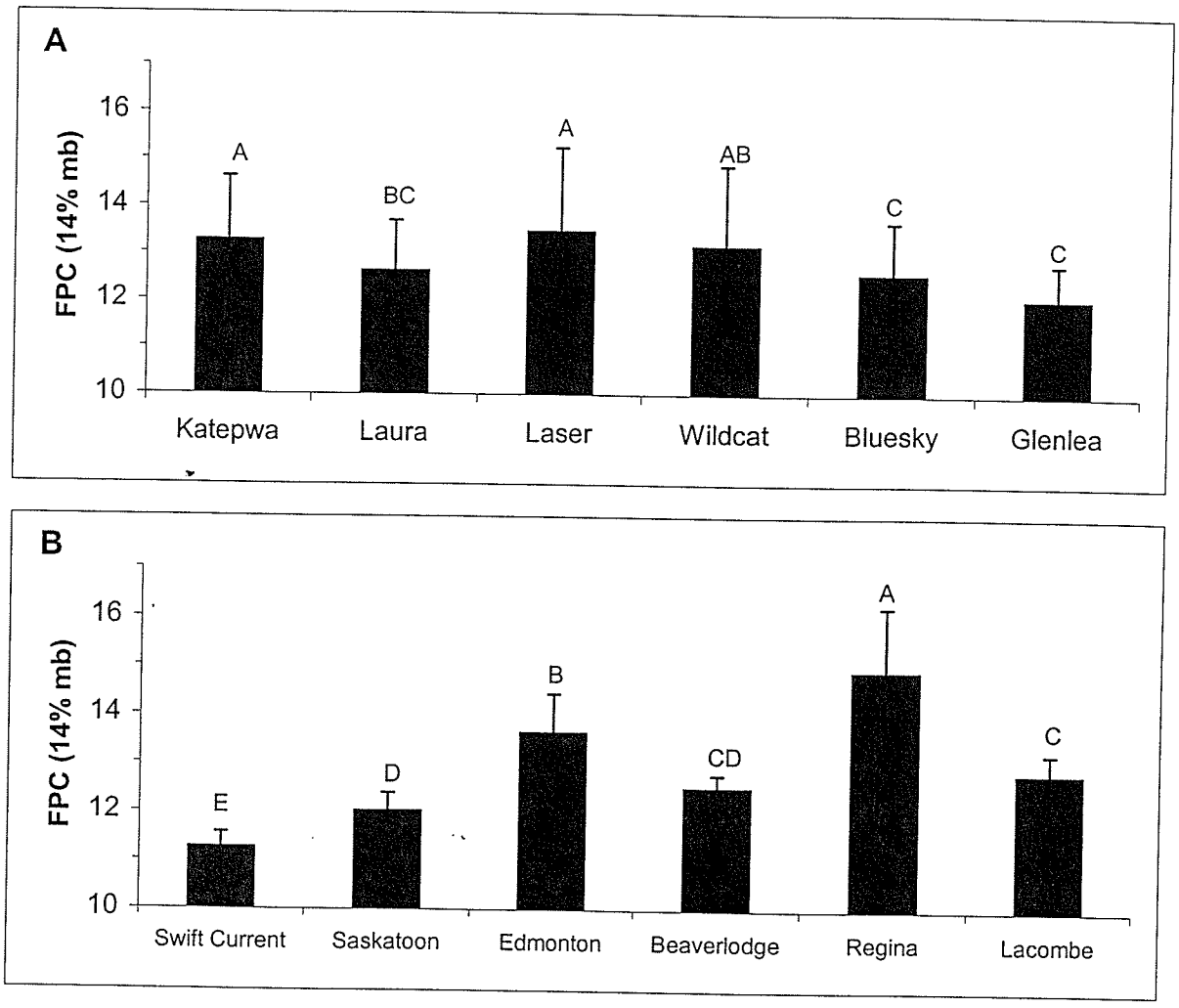


Figure 8. Average Flour Protein Content (FPC) of Cultivars (A) and Locations (B).

Table 3

Coefficients of variation (CV) of protein compositional parameters for effects of genotype, environment and full sample set.

Parameter*	Coefficients of Variation (%)		
	Genotype	Environment	Entire Set
FPC	4.1	9.9	10.4
SPC (%flour)	7.0	9.0	11.5
SPC/ FPC	4.7	3.6	5.9
IGC (%flour)	9.4	10.3	13.4
IGC/ FPC	11.2	6.5	12.5
RPC (%flour)	9.0	20.4	21.9
RPC/ FPC	5.0	14.4	15.2
IGC/ SPC	14.9	5.4	15.5
HMW GS	12.8	11.8	17.0
HMW GS/ FPC	13.8	6.4	14.9
LMW GS	10.1	10.5	14.6
LMW GS/ FPC	10.8	6.1	12.7
TGS	10.8	10.5	14.8
TGS/ FPC	11.6	6.1	13.1
HMW GS/ LMW GS	9.1	8.5	12.3

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits

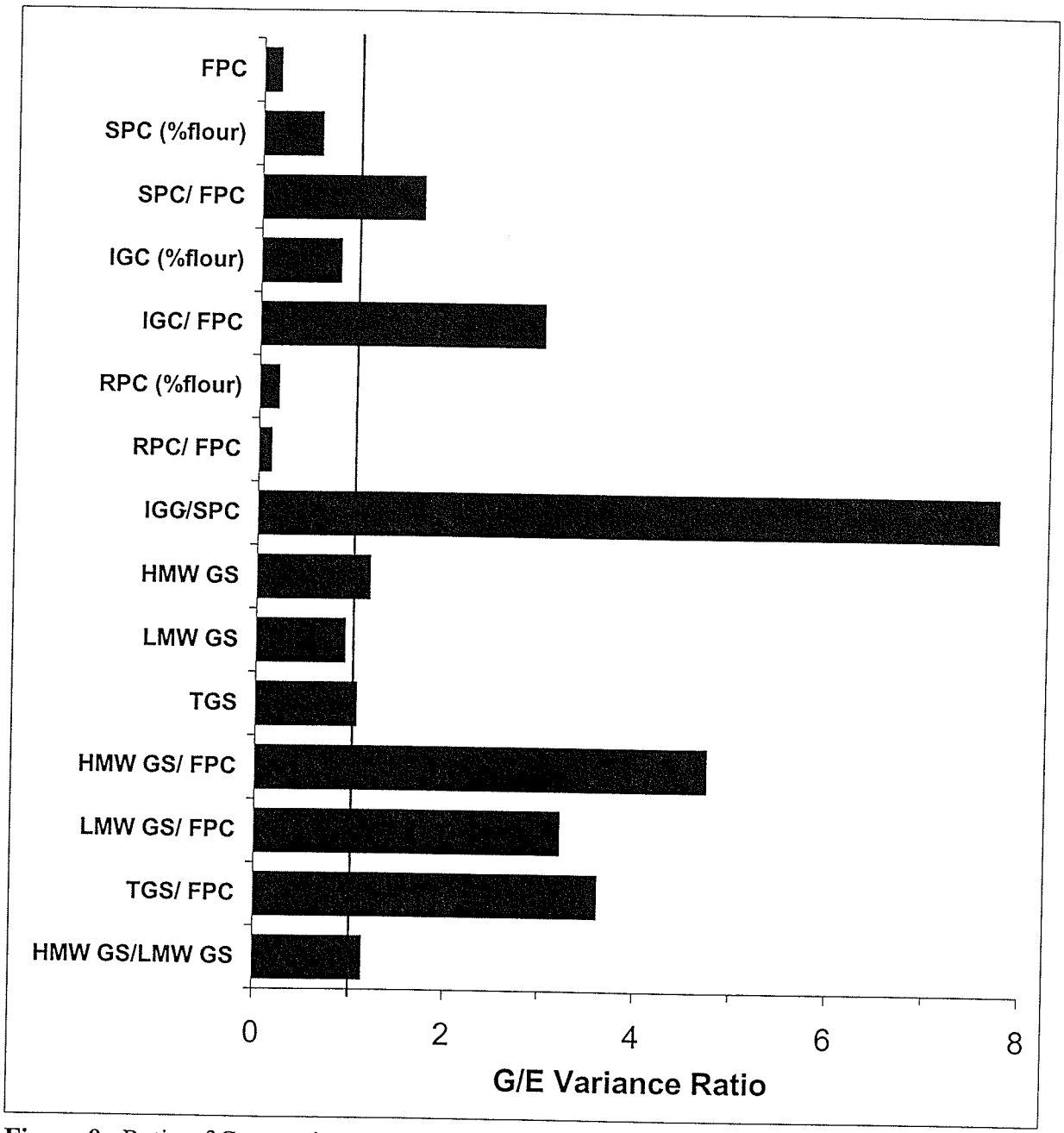


Figure 9. Ratio of Genotypic to Environmental (G/E) Variance for Protein Compositional Parameters. Equal Contributions of Genotype and Environmental Influence designated by Vertical Line.

4.1.2 50% 1-Propanol Soluble Protein Content

The 50% 1-propanol soluble protein content (SPC) consisted of a concentration of monomeric proteins (gliadins, albumins and globulins) and lower molecular weight glutenin (Fu and Sapirstein 1996a). This parameter varied significantly for the genotypes and ranged from 6.7 to 8.1%. Glenlea and Bluesky had the lowest amounts of SPC (% flour), whereas the CWRS cultivars and remaining two CWES cultivars had significantly higher concentrations of SPC (Figure 10A). The locations had a range of SPC from 6.6 - 8.3% (Figure 10B). The range of SPC for the full sample set was 6.2 – 9.5%. The CV values for genotype, location, and full sample set were 7.0, 9.0, and 11.5%, respectively (Table 3). The G/E variance ratio was 0.61 (Figure 9), indicating that SPC was influenced more by environment than genotype effects. Interestingly, the pattern of variation for SPC was similar to that of FPC for locations, but not so similar for genotypes.

When SPC was normalized (i.e. divided by the FPC), the pattern of variation shifted. The genotypic effect became larger than that of the location (Figure 10C and 10D). As a group, the CWRS cultivars had significantly higher SPC/ FPC values compared to the CWES genotypes. The CVs for SPC/ FPC were 4.7, 3.6, and 5.9% for genotype, location, and the full sample set, respectively (Table 3). This normalisation resulted in decreased variation and an increase in the G/E variance ratio from 0.61 to 1.5 (Figure 9). This result was expected because normalizing SPC should minimize the large location effects that significantly influenced the FPC.

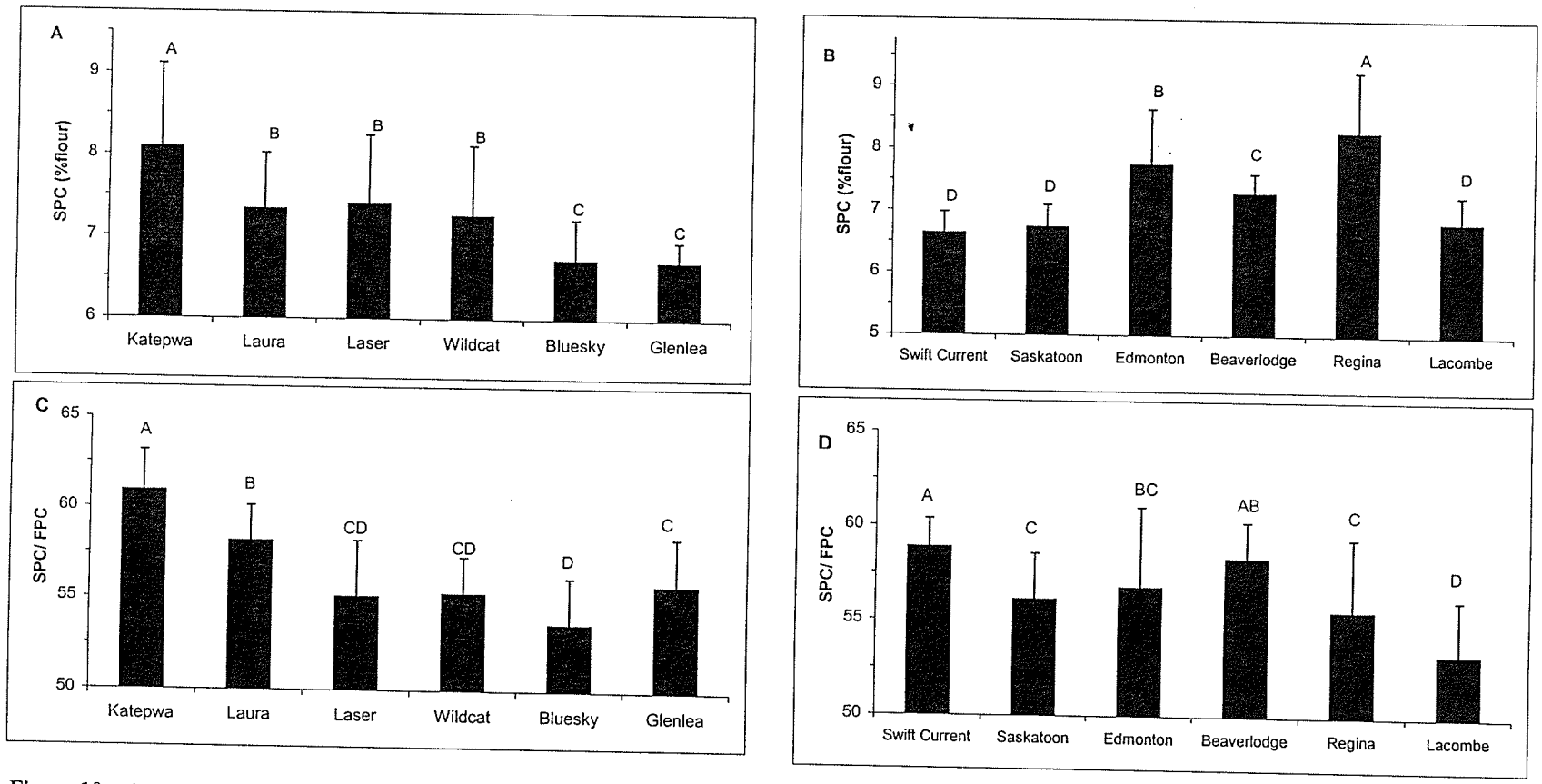


Figure 10. Average 50% 1-Propanol Soluble Protein Content (SPC) of Cultivars (A, C) and Locations (B, D). Top (A, B) and Bottom (C, D) Figures Expressed as % Flour and Normalised by FPC (x/FPC), respectively.

4.1.3 50% 1-Propanol Insoluble Glutenin Protein Content

There were significant differences in the protein quality parameter 50% propanol insoluble content, or insoluble glutenin content (IGC; Fu and Sapirstein 1996b), that allowed for more pronounced separation of the CWRS and CWES genotypes compared to FPC and SPC; clear discrimination between the CWRS and CWES classes existed. On average, the IGC (% flour) for genotypes ranged from 2.3 to 3.0% (Figure 11A), and for locations, it ranged from 2.6 to 3.3% (Figure 11B). The full sample set ranged from 2.2 to 3.5%. CV values for the genotype, location and full sample set were 9.4, 10.3, and 13.4%, respectively (Table 3). It was difficult to infer that IGC was more dependent on location than genotype, because their variation was so similar in magnitude.

Normalizing IGC resulted in the parameter becoming highly genotype dependent (Figure 9), again due to the removal of FPC. The CV values for genotype increased and the CV for locations decreased (Table 3). IGC/ FPC results for genotypes maintained the same ranking as IGC (% flour), but a significant split in the CWES class was established (Figure 11C); Glenlea and Bluesky had significantly larger quantities of IGC/ FPC compared to Laser and Wildcat. There was now a distinct separation of the CWES cultivars into "weaker" and "stronger" cultivars. This ranking of the CWES cultivars reflected the dough strength of the sample set, where Glenlea and Bluesky having the greatest quantity of IGC/ FPC and Katepwa the lowest. The locations (Figure 11D) Edmonton and Regina had significantly higher IGC, SPC and FPC than other locations. No plausible explanation can be offered since this issue is beyond the scope of this thesis.

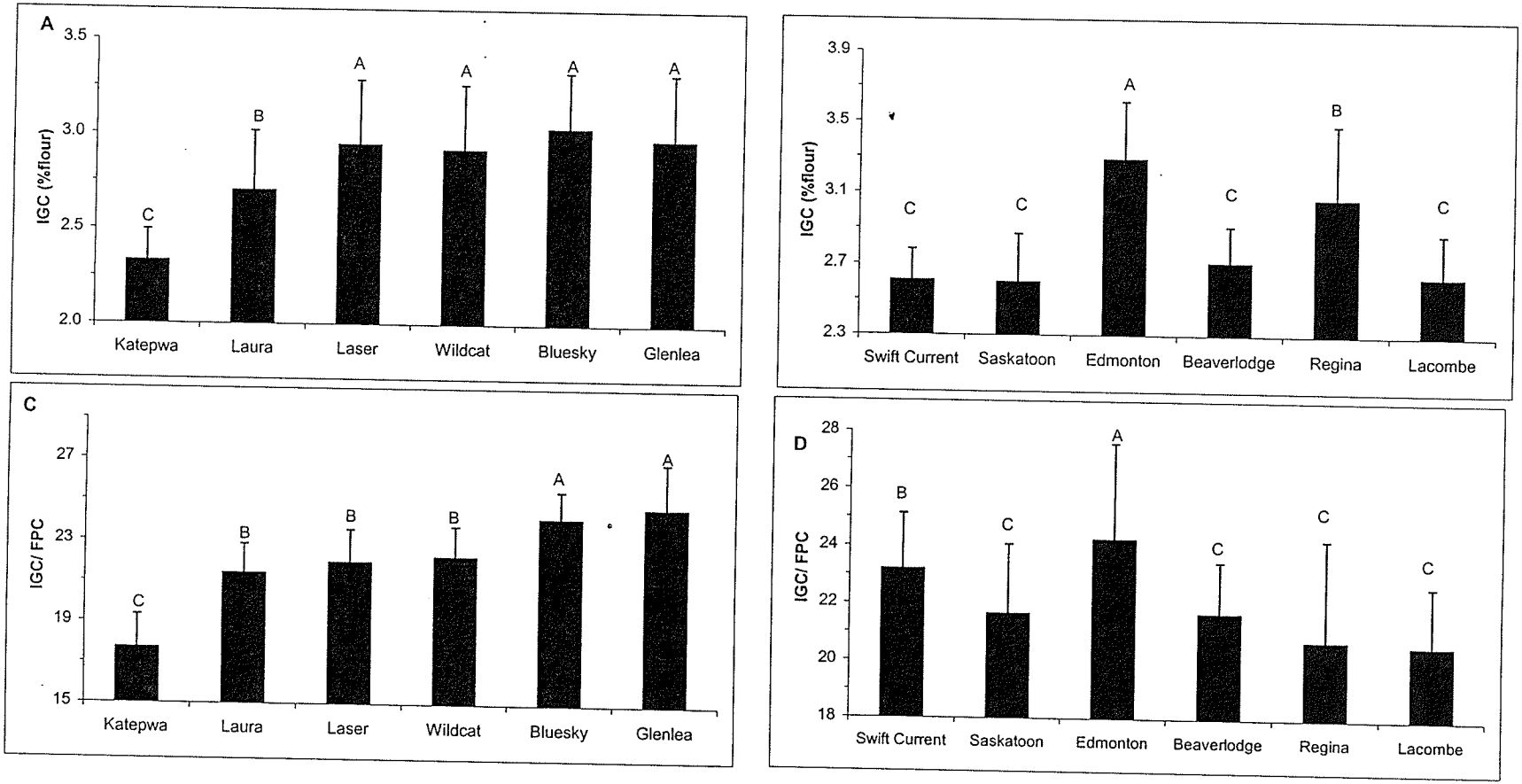


Figure 11. Average 50% 1-Propanol Insoluble Glutenin Protein Content (IGC) of Cultivars (A, C) and Locations (B, D). Top (A, B) and Bottom (C, D) Figures Expressed as % Flour and Normalised by FPC (x/FPC), respectively.

4.1.4 50% Propanol Insoluble Residue Protein Content

The pattern of variation for residue protein content (RPC) of genotypes was similar to FPC. The average RPC for genotypes ranged from 2.5 to 3.1% (Figure 12A), whereas the average values for locations were 2.0 to 3.6% (Figure 12B). The full sample set had a range of RPC from 1.8 to 4.3%. CV values were much higher than the previous parameters with 9.0, 20.4, and 21.9% for genotypes, locations and full sample set, respectively (Table 3). RPC was highly influenced by location effects as inferred by the G/E variance ratio of 0.19 (Figure 9).

For RPC/ FPC values, the rankings of the cultivars did not change (Figure 12C), but the CV values decreased substantially (Table 3). Like RPC, RPC/FPC was influenced by the environment (Figure 9), and locations showed significant differences (Figure 12D). With the exception of RPC, all other protein parameters became highly affected by genotype after normalization. At this point, it is uncertain why RPC did not shift like the other protein parameters.

4.1.5 Ratio of 50% 1-propanol Insoluble Glutenin to Soluble Protein Content

The IGC/ SPC provided a very important protein quality parameter particularly in relation to dough mixing properties. The averages for genotypes ranged from 0.29 to 0.45 (Figure 13A), while the locations ranged from 0.37 to 0.43 (Figure 13B). The range of the full sample set was 0.24 to 0.52. Although the range was narrow, IGC/ SPC provided enough variation to show a large impact of genotype on protein quality. IGC/ SPC also showed a clear separation of the CWES class into two strengths, similar to that observed for IGC/ FPC. The CV values for genotype, location and the full sample set

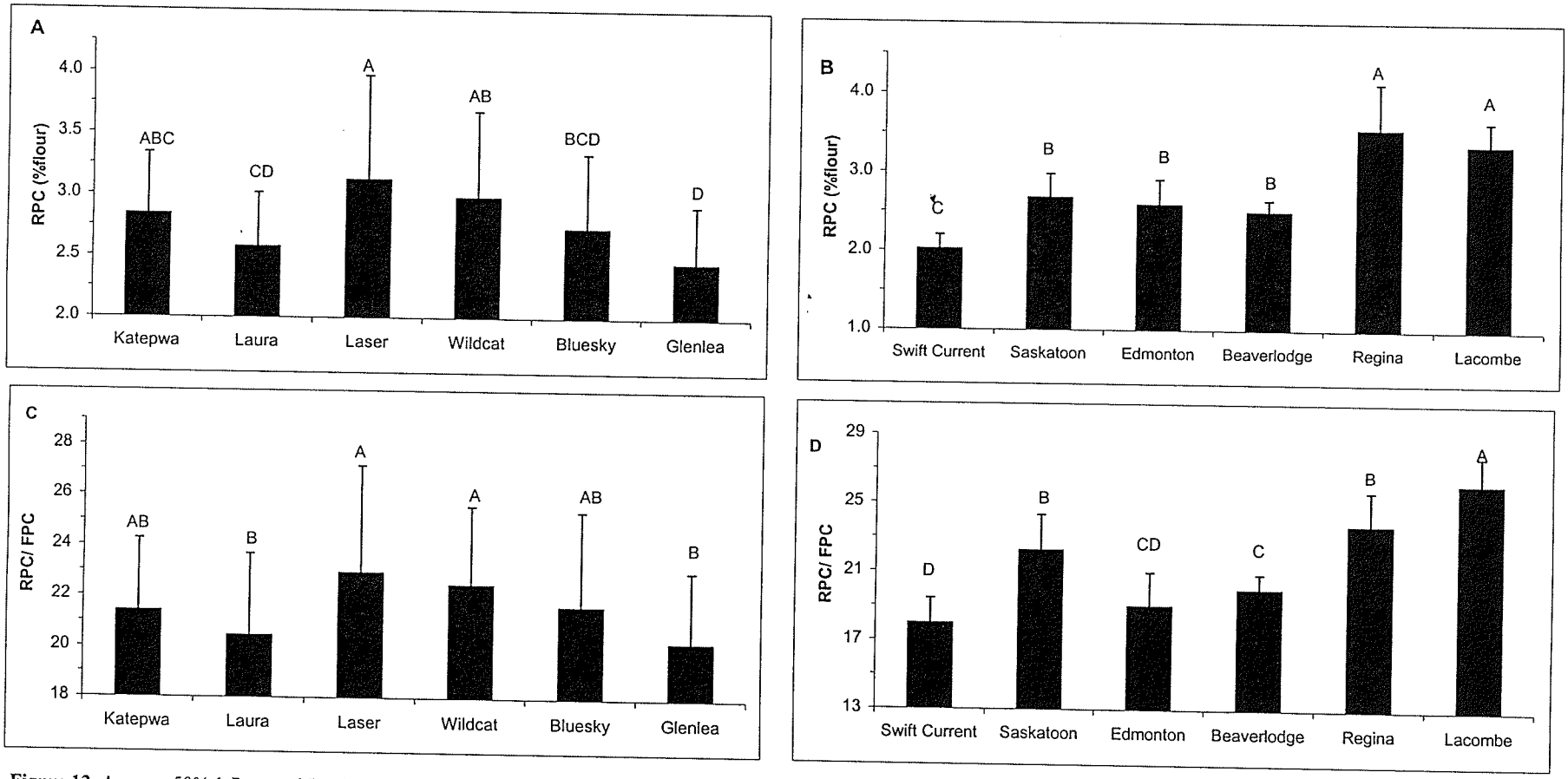


Figure 12. Average 50% 1-Propanol Insoluble Residue Protein Content (RPC) of Cultivars (A, C) and Locations (B, D). Top (A, B) and Bottom (C, D) Figures Expressed as % Flour and Normalised by FPC (x/FPC), respectively.

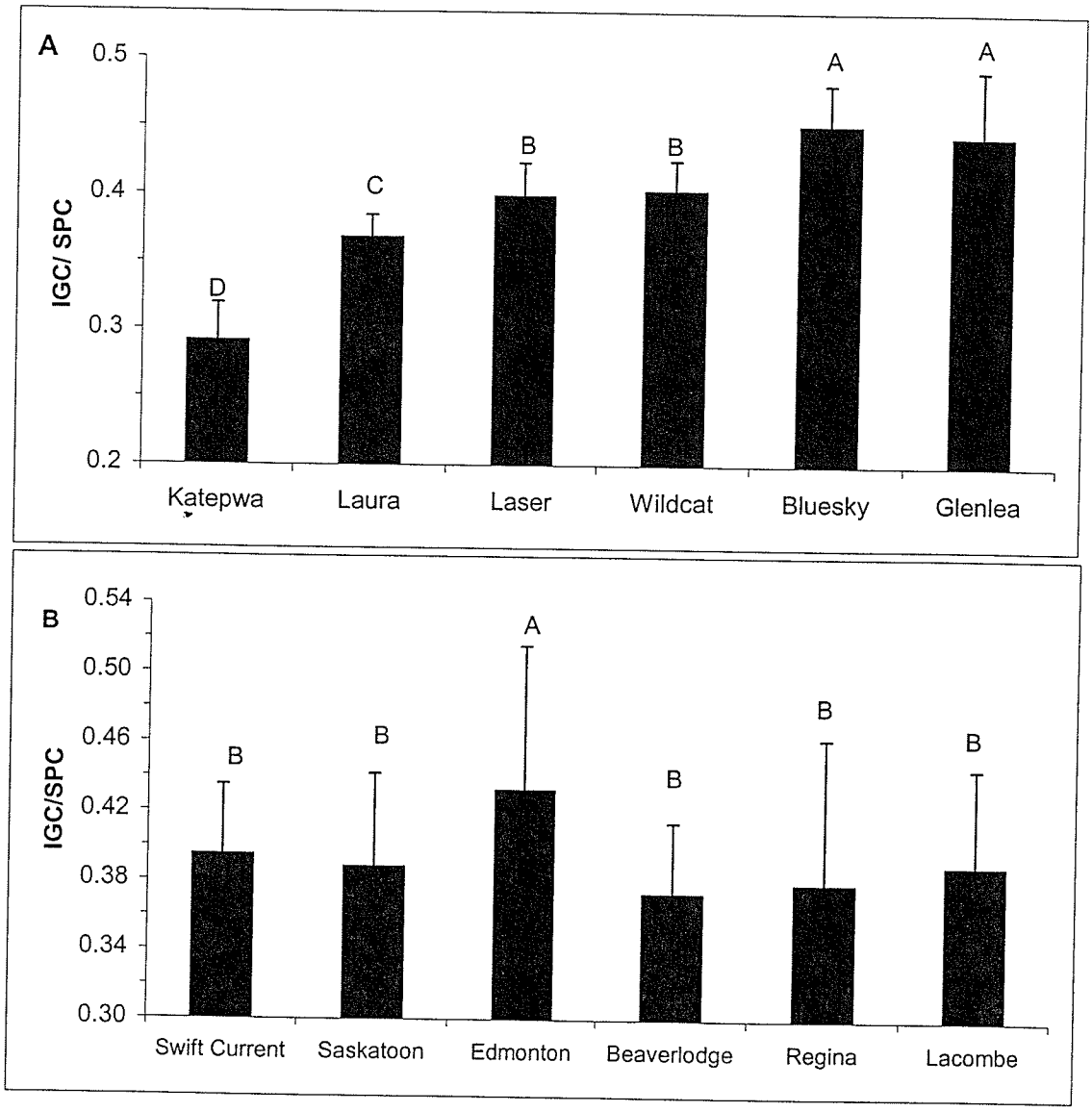


Figure 13. Average IGC/ SPC of Cultivars (A) and Locations (B).

were 14.9, 5.4, and 15.5%, respectively (Table 3). This parameter, like the other normalized parameters, were more affected by genotype than environment as shown by the significant G/E variance ratio value of 7.73 (Figure 9). This was the highest G/E variance ratio of the protein compositional parameters, and left no doubt that the protein quality of these cultivars was highly genotype dependent.

4.1.6 RP-HPLC of 50% 1-Propanol Insoluble Glutenin

There was significant variation for all the HPLC parameters for both genotypes and locations, and good separation of the CWES from the CWRS. Typical chromatograms for the six cultivars are shown in Figure 14. The average HMW-GS values for genotypes ranged from 5198 to 7481 Au (Figure 15A), while the locations ranged from 6146 to 7994 Au (Figure 16A); full sample set range was 9066 to 4458 Au. LMW-GS ranged from 10,537 to 13,190 Au for genotypes (Figure 15B), and 11,066 to 13,588 Au for locations (Figure 16B); full sample set range was 8949 to 15,530 Au. The sum total (T)GS ranged from 15,555 to 20,569 Au for genotypes (Figure 15C), and 17,231 to 21,583 Au for locations (Figure 16C); full sample set range was 13,407 to 24,009 Au. The range for HMW/ LMW-GS was 0.5 to 0.62 for genotypes (Figure 15D), and 0.53 to 0.59 for locations (Figure 16D); the full sample set range was 0.40 to 0.64. The genotype and location CV values were similar to each other and were low (Table 3). Except for HMW/ LMW-GS, which was clearly influenced by genotype, the other three HPLC parameters had G/E variance ratios that were very near one (Figure 9); there was clearly no dominating genotypic or environmental influence on the HPLC parameters on a percent flour basis.

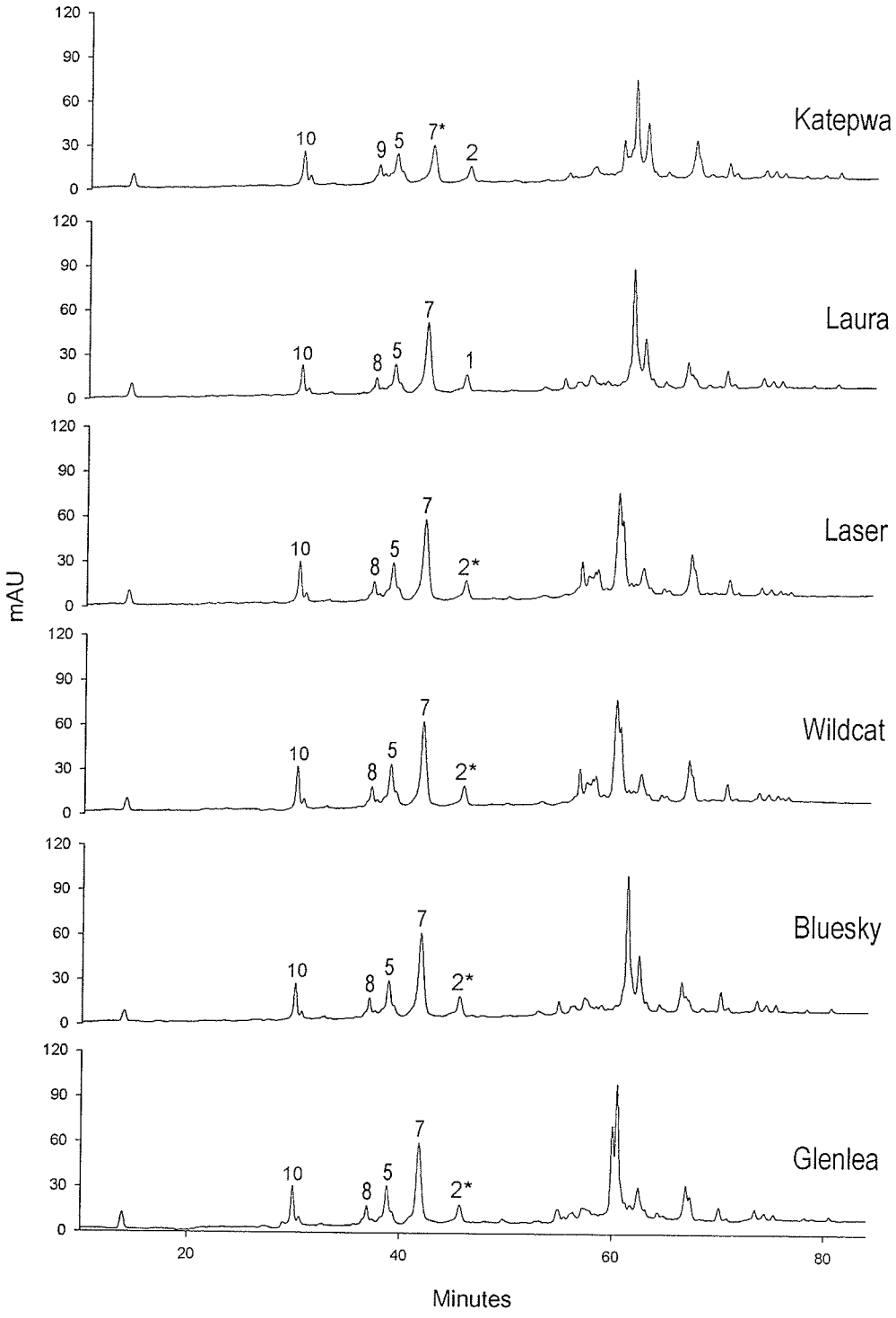


Figure 14. Typical RP-HPLC chromatograms of the cultivars in the sample set. The HMW glutenin subunit composition of these cultivars are indicated; the CWES cultivars have identical HMW-GS composition.

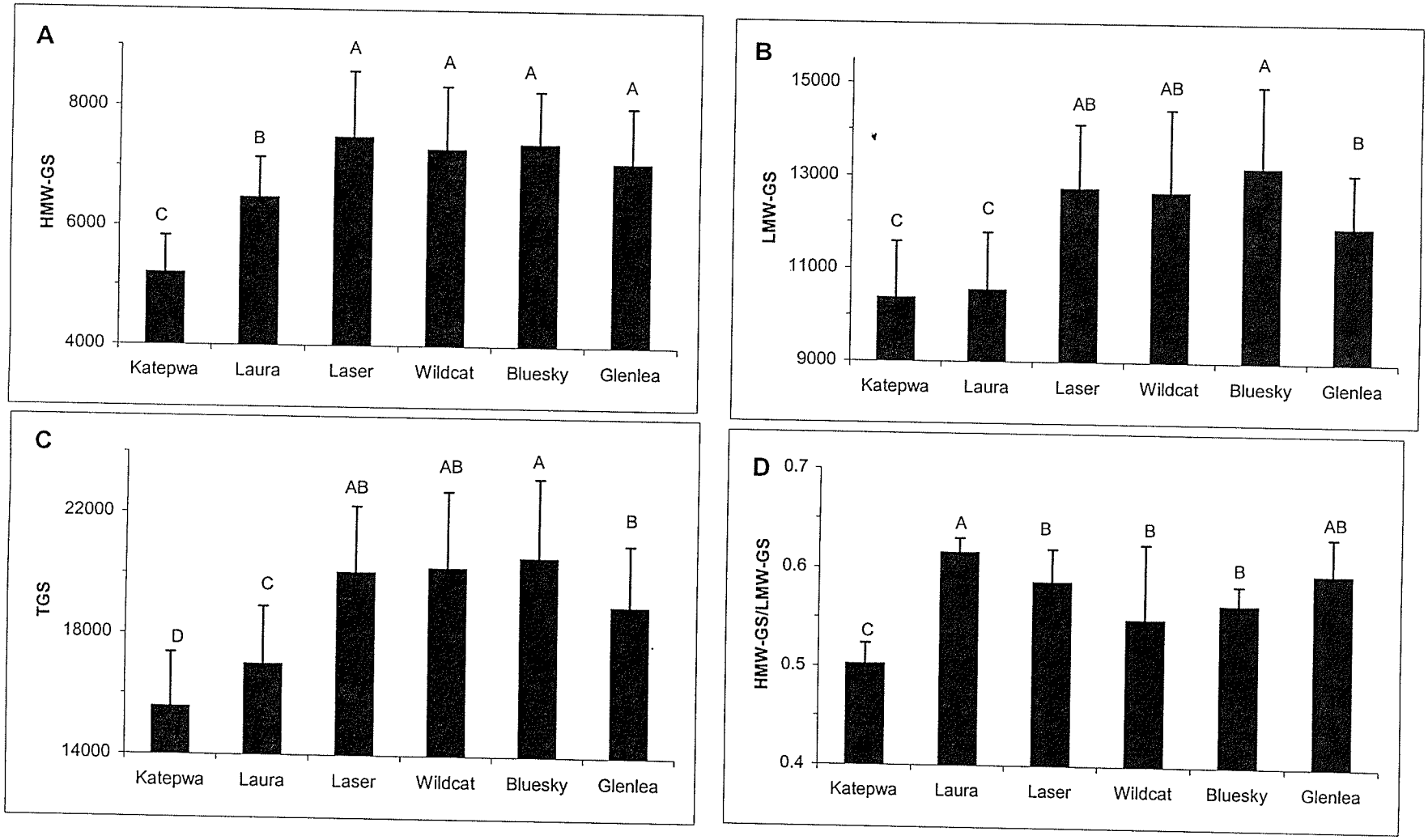


Figure 15. Average HMW GS (A), LMW GS (B), TGS (C), and HMW/ LMW GS (D) Contents of Cultivars.

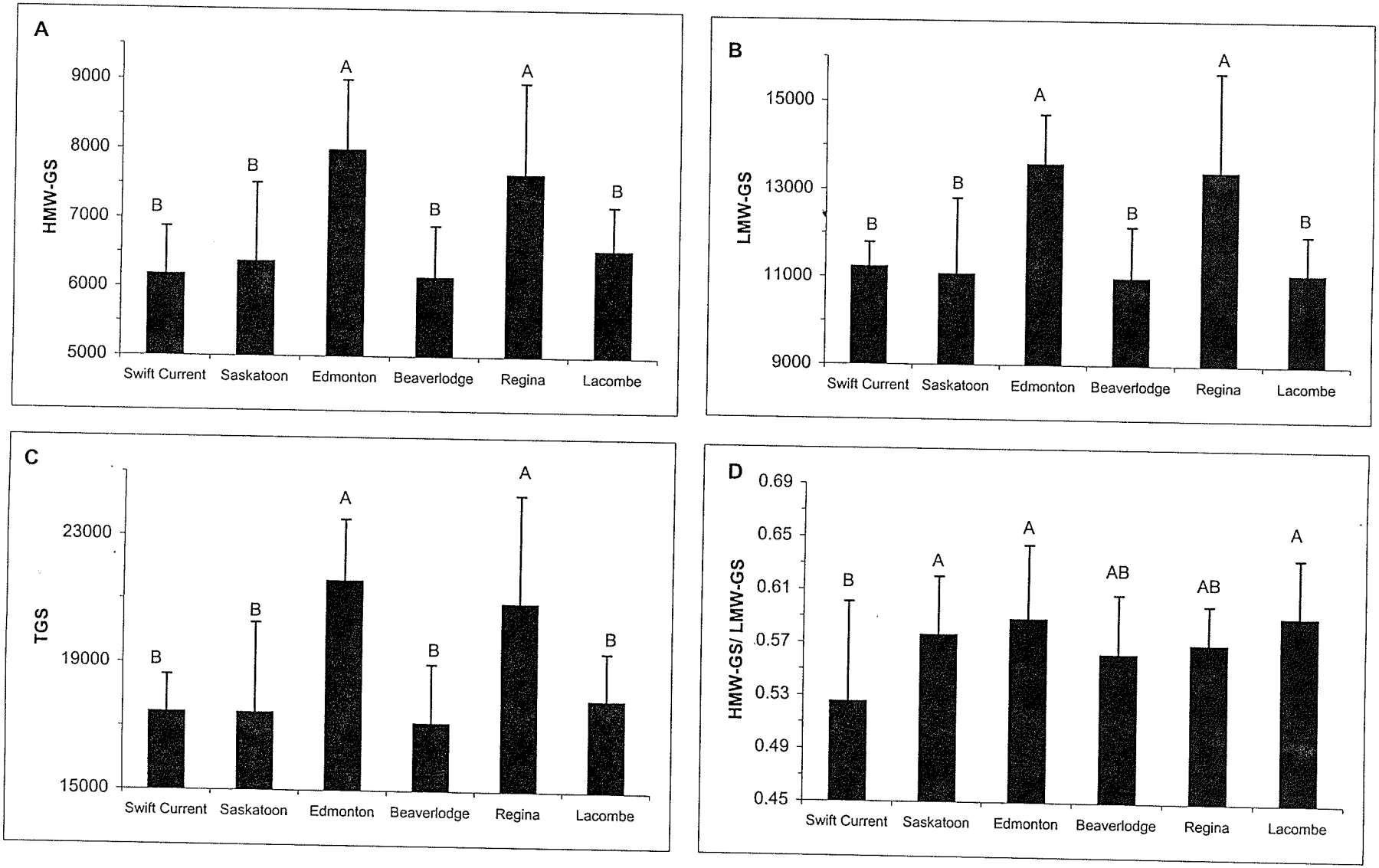


Figure 16. Average HMW GS (A), LMW GS (B), TGS (C), and HMW/ LMW GS (D) Contents of Locations.

The CWES cultivars had significantly more glutenin than the CWRS cultivars on a % flour basis. HMW-GS had virtually identical trends to that of IGC, which was understandable considering that the fraction was essentially the same. In addition, the locations Edmonton and Regina had a greater concentration of LMW, HMW, and TGS; this same result was shown with IGC. No explanation for this finding can be offered. Within the CWES class, Bluesky was significantly greater than all the other cultivars including Glenlea for LMW-GS and TGS, and equal to Glenlea for HMW-GS and HMW/LMW-GS. Bluesky had more glutenin than Glenlea. This implied that Glenlea had a slightly lower molecular size of glutenin than Bluesky, which may translate into a difference in functionality in future testing.

All of these parameters had significant separation of the CWES from the CWRS cultivars except HMW/LMW-GS, where all of the cultivars were statistically equal. It was obvious, from the previously discussed protein compositional parameter results that Laura was definitely not of the same quality as Glenlea, yet it was equal in quantity for this parameter. This alluded to the idea that protein quantity, regardless of which type of protein, may not be as important for quality and characteristics. It was interesting to note that Katepwa was significantly different from the rest of the genotypes. This result implied that molecular size distribution was significantly lower for Katepwa glutenin compared to Laura, and the entire CWES class.

Normalised values of all the HPLC parameters showed virtually no differences in ranking or significance (Figures 17A-D and 18A-D). As shown with other protein compositional parameters, the CV values for genotypes all increased slightly while the location CV values decreased significantly (Table 3). The increase in variance resulted in

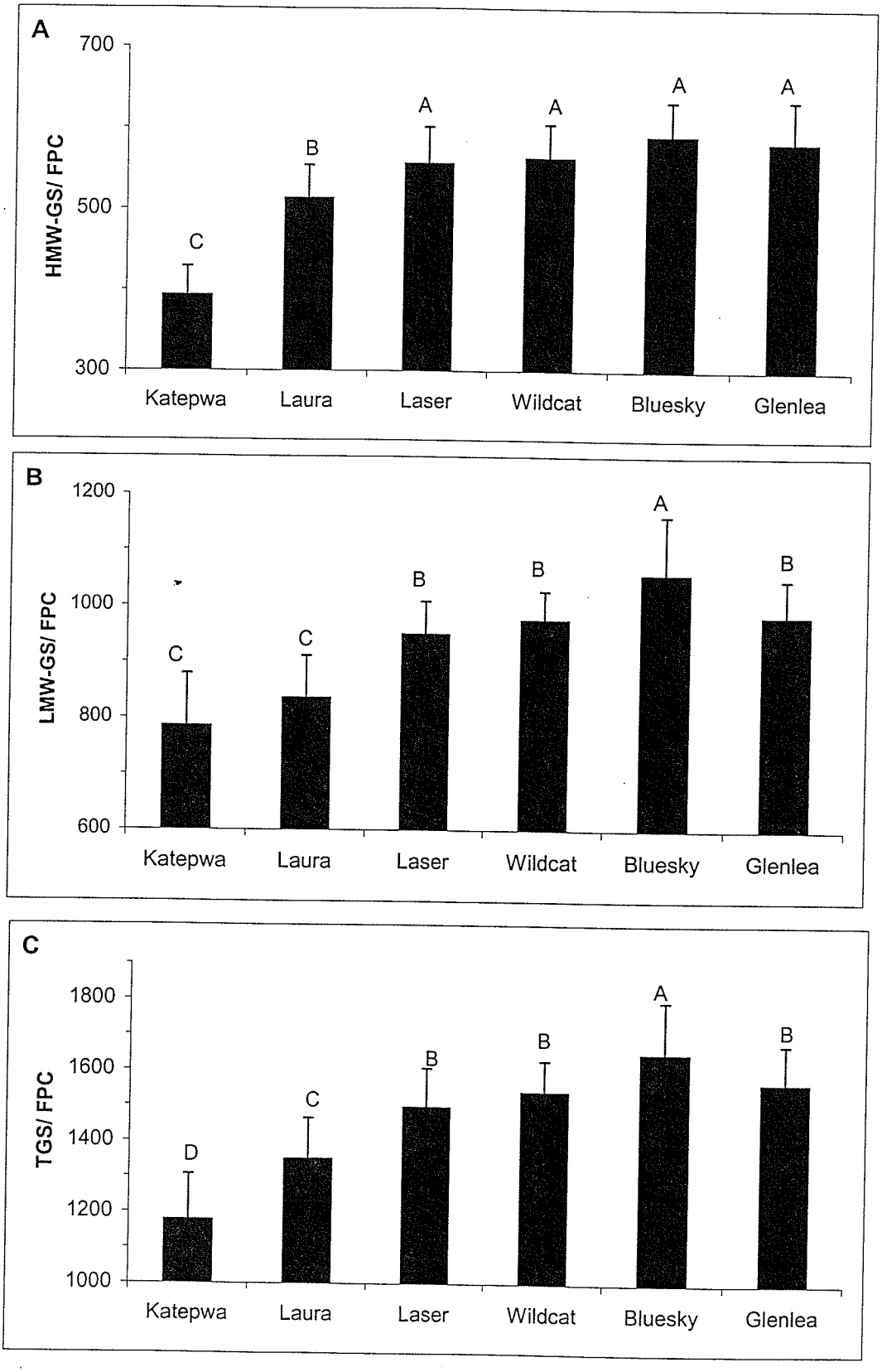


Figure 17. Normalised Average HMW GS (A), LMW GS (B), and TGS (C) Contents of Genotypes.

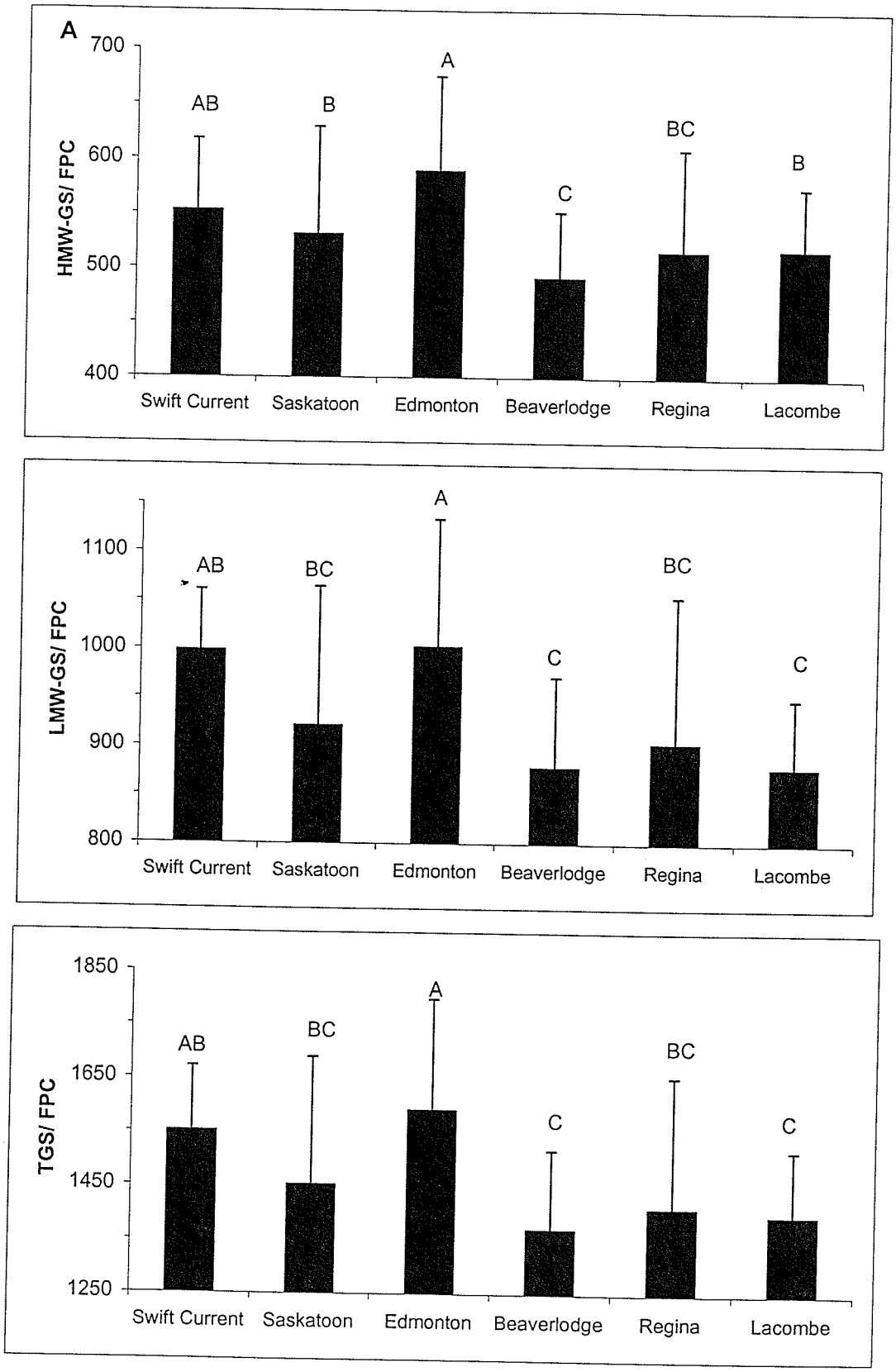


Figure 18. Normalised Average HMW GS (A), LMW GS (B), and TGS (C) Contents of Locations.

a substantial shift in G/E variance ratios towards significant genotypic influences on all parameters (Figure 9).

4.2. Mixing Properties of Strong-Mixing Wheat Cultivars

A representative mixogram of each cultivar is shown in Figure 19. Laura and Katepwa were the weaker mixing cultivars and were not very similar to the CWES cultivars. The CWES cultivars were also different although they were in the same class of wheat. The evaluation of mixograms provided the information for the mixing study.

4.2.1. Mixing Time

The mixing time (MT) of a sample was characterized as the time required for mixing to the peak dough resistance of a sample (see Figure 2). Significant variation for both cultivars and locations was seen. Values ranged from 2.8 to 5.0 minutes for the cultivars (Figure 20A), while the range for locations was 3.3 to 4.8 minutes (Figure 21A). The full sample set ranged from 1.9 to 5.9 minutes. There was distinct separation of the cultivars according to class, and within the CWES class; Glenlea and Bluesky were significantly greater than Wildcat and Laser. The CV values for genotype, location and full sample set were 23.4, 13.6, and 26.3%, respectively (Table 4). MT was the most variable mixing parameter when the flour samples remained untreated. Clearly, genotypic effects were substantial with this sample set, as verified by the high G/E variance ratio of 3.0 (Figure 22).

Starch addition caused an increase in MT of 5% for all cultivars over the untreated samples, but no changes in ranking. Khatkar et al. (1996) also reported an

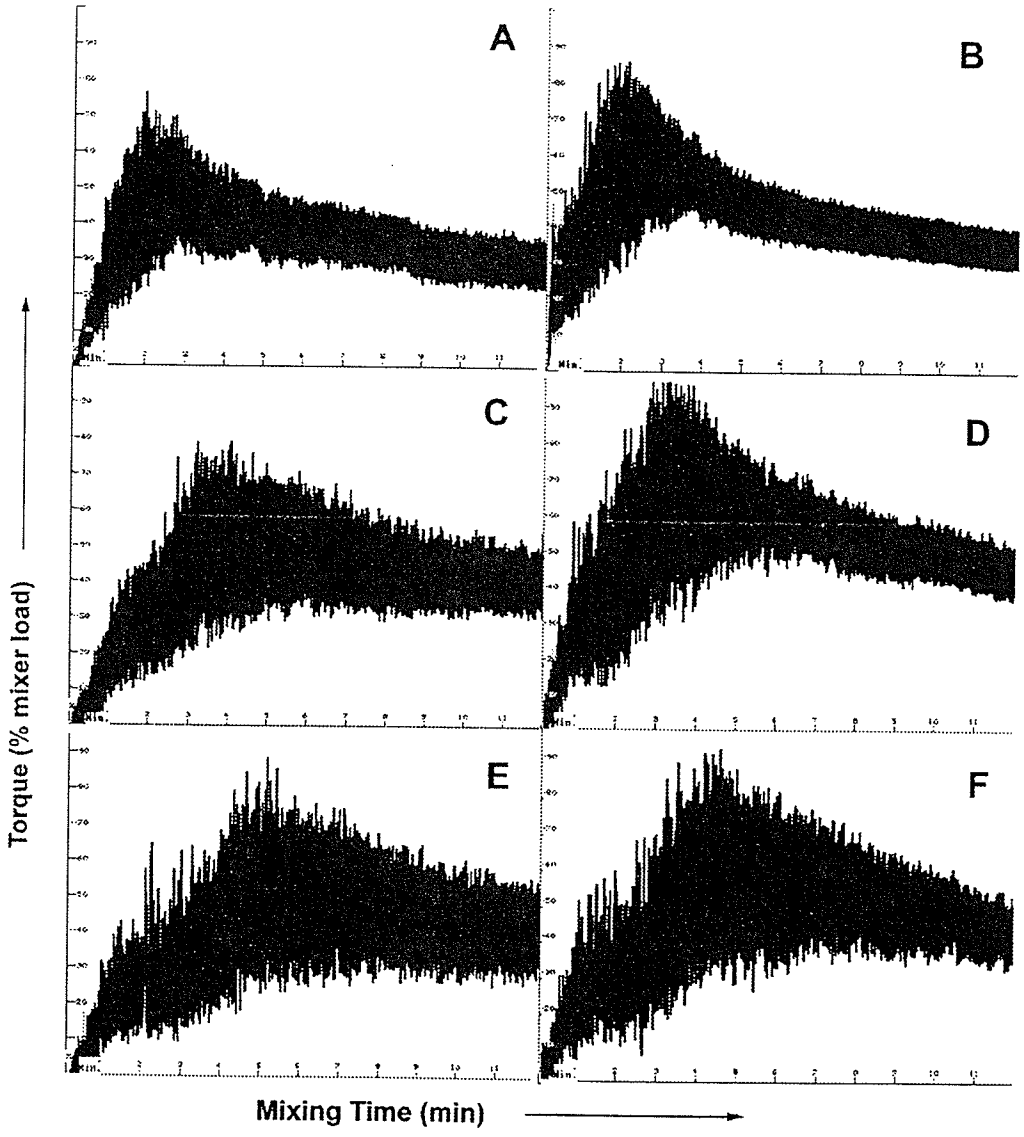


Figure 19. Typical 2 g Mixograms of the Six Cultivars of the Samples Set: Katepwa (A), Laura (B), Laser (C), Wildcat (D), Bluesky (E), and Glenlea (F).

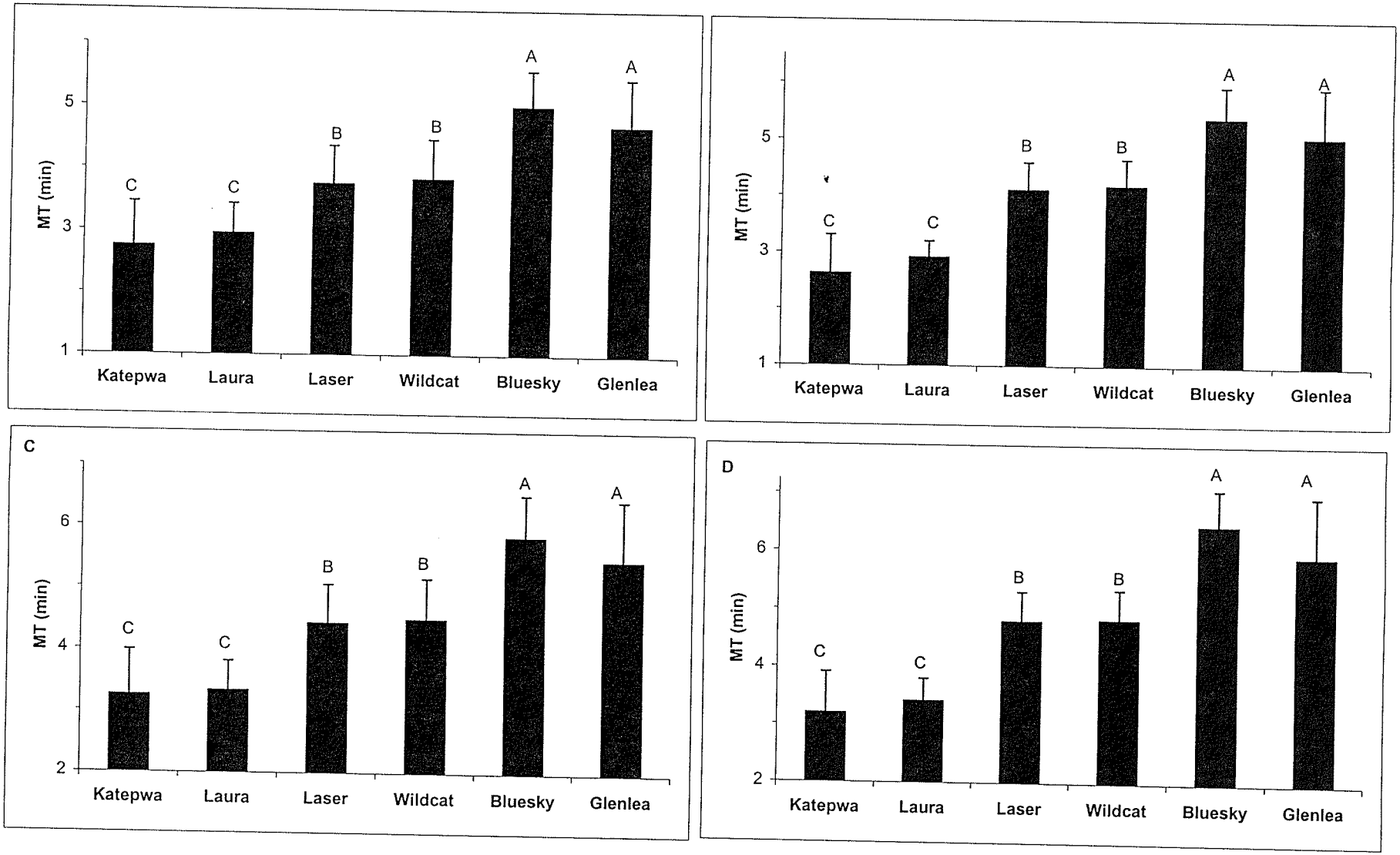


Figure 20. Average Mixograph Mixing Time (MT) of Cultivars: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

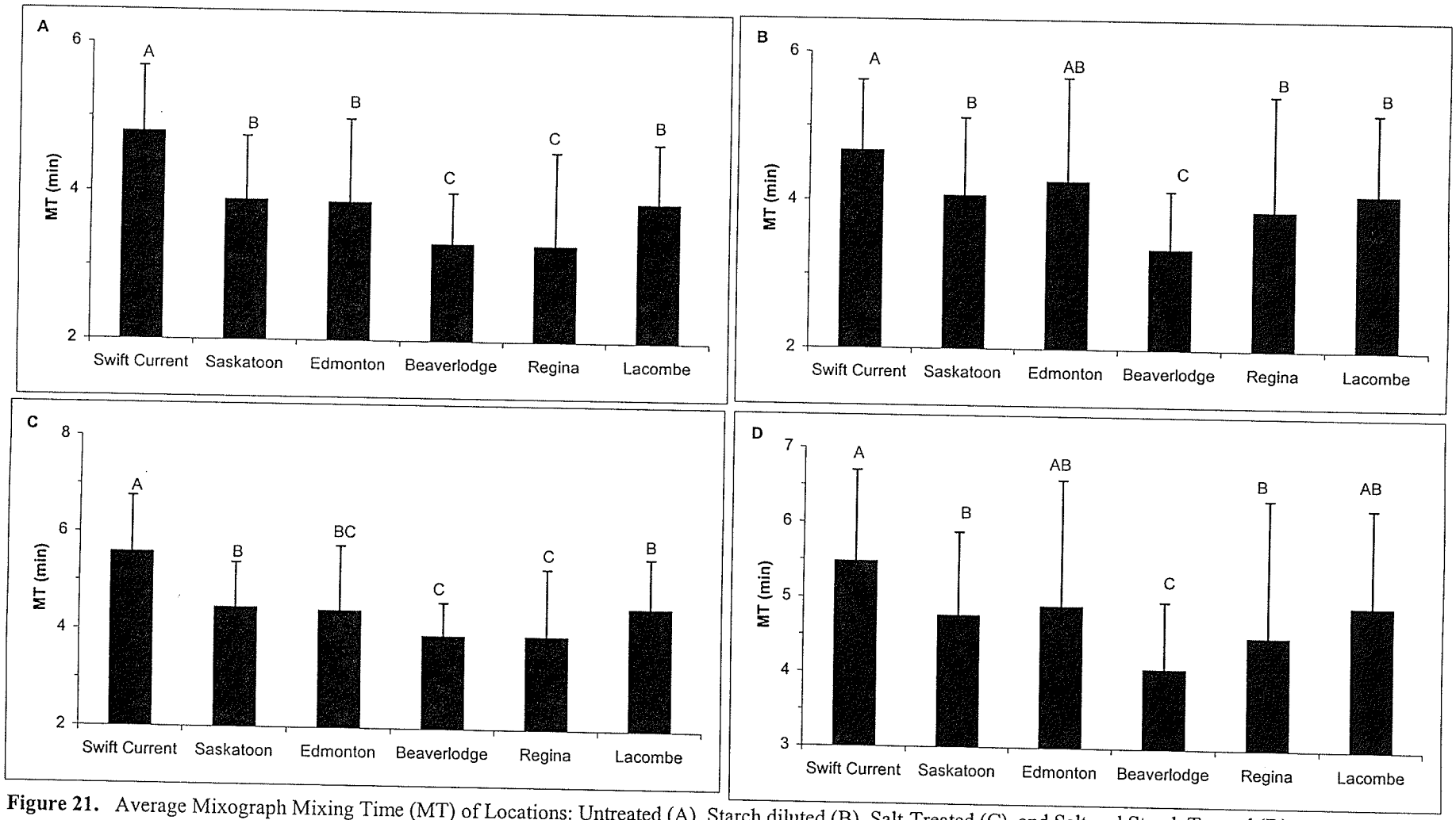


Figure 21. Average Mixograph Mixing Time (MT) of Locations: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

Table 4

Coefficients of variation (CV) of mixing parameters for effects of genotype, environment and the full sample set.

		Coefficients of Variation (%)		
Treatment	**Parameter***	**Genotype**	**Location**	**Full Set**
Untreated	MT	23.40	13.61	26.33
	BW	10.10	7.26	12.44
	PDR	8.11	8.98	12.08
	WIP	21.38	7.19	22.59
Starch	MT	27.20	10.85	28.58
	BW	12.44	8.08	14.42
	PDR	10.46	7.99	13.08
	WIP	27.56	12.10	29.08
Salt	MT	23.89	13.25	26.40
	BW	8.60	8.77	12.29
	PDR	7.19	10.14	12.17
	WIP	18.90	6.48	20.23
Starch and Salt	MT	27.05	9.94	28.22
	BW	9.78	8.11	12.75
	PDR	8.75	7.45	11.54
	WIP	25.09	12.34	27.24

*MT= mixing time, BW= band width, PDR= peak dough resistance, WIP= work input to peak

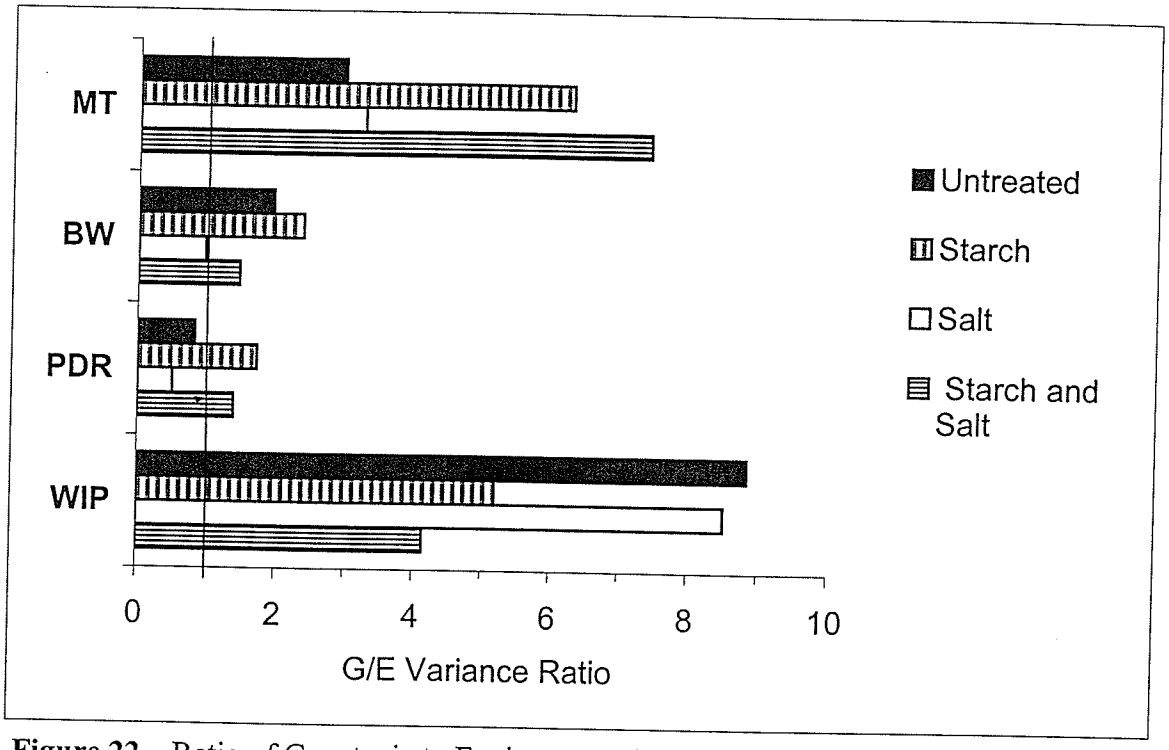


Figure 22. Ratio of Genotypic to Environmental (G/E) Variance for Mixing Parameters. Equal Contributions of Genotype and Environmental Influence designated by Vertical Line.

increase in MT in their mixing study involving fractionated and reconstituted flours being mixed at a CPC. The CWES samples in this thesis increased in MT, while the CWRS samples did not. The CVs for genotype, location and the full sample set were 27.2, 10.9, and 28.6%, respectively (Table 4). The genotypic effect maintained its dominance over that of location, as reflected by the G/E variance ratio (Figure 22). The CPC treatment had the same clear separation of the classes of wheat and within the CWES class as the untreated samples (Figure 20B). The separation for the locations was not as clear, because of significant variation.

Salt caused an increase in MT by an average of 16%, which was a significant increase for all cultivars. This was in agreement with earlier findings on the discovery that salt increased MT, BW, and PDR in a variety of wheats (Hlynka 1962; Bakhoum and Ponte Jr. 1982; and Lang et al. 1992). Although salt increased the values (Figures 20C and 21C), it did not alter the rankings from the order set by the untreated samples. The effect of genotype was greater than location as shown by the G/E variance ratio of 3.3 (Figure 22), but less than the genotypic effect of starch. The range of values remained essentially the same as reflected by the CV values, which were 23.9, 13.3, and 26.4% for genotype, location and the full sample set, respectively (Table 4). MT was the most variable among all samples treated with salt.

The combination of both salt and starch resulted in significant variation in MT, but no new findings compared to the other treatments. The samples had values that were intermediate to the untreated and salt treated values. The genotype and location averages showed similar results with only slight changes in magnitude (Figure 20D and 21D). Overall, the results from this combination of salt and starch resembled the salt treated

results; this was essentially the same result for all the mixing parameters and will be shown in each below. The CV values for genotype, location and full sample set were 27.1, 9.9, and 28.2% (Table 4). The magnitude of the CV values fully substantiated the genotypic influence of this mixing parameter. The G/E variance ratio was 7.4 (Figure 22), which was approximately equal to the starch treated G/E. The native samples were as useful as the treated samples for grouping the sample set according to mixing strength; treating the samples with starch and salt did not add any new information for ranking cultivars and locations. MT was useful for separating the cultivars according to strength, especially the CWES class.

4.2.2. Band Width at Peak

The band width at peak (BW) of a sample was measured by the thickness of the envelope at the peak dough resistance (see Figure 2). BW provided significant results for both locations and genotypes. The BW for genotypes ranged from 25.2 to 33.1 %Torque (Figure 23A), and the range for locations was 27.3 to 34.1 % Torque (Figure 24A). The range for the full sample set was 28.1 to 38.6 %Torque. There was good separation of the two classes of wheat, but further separation within the CWES class was not as distinct as with MT. CV values for genotype, location and the full sample set were 10.1, 7.3, and 12.4%, respectively (Table 4). The G/E variance ratio of 1.93 suggested a greater influence by genotype than location (Figure 22).

The addition of starch to remove the protein quantity effect caused a significant decrease in BW for cultivars and locations. The range of values decreased but the ranking of the cultivars (Figure 23B) and locations (Figure 24B) remained. The CV

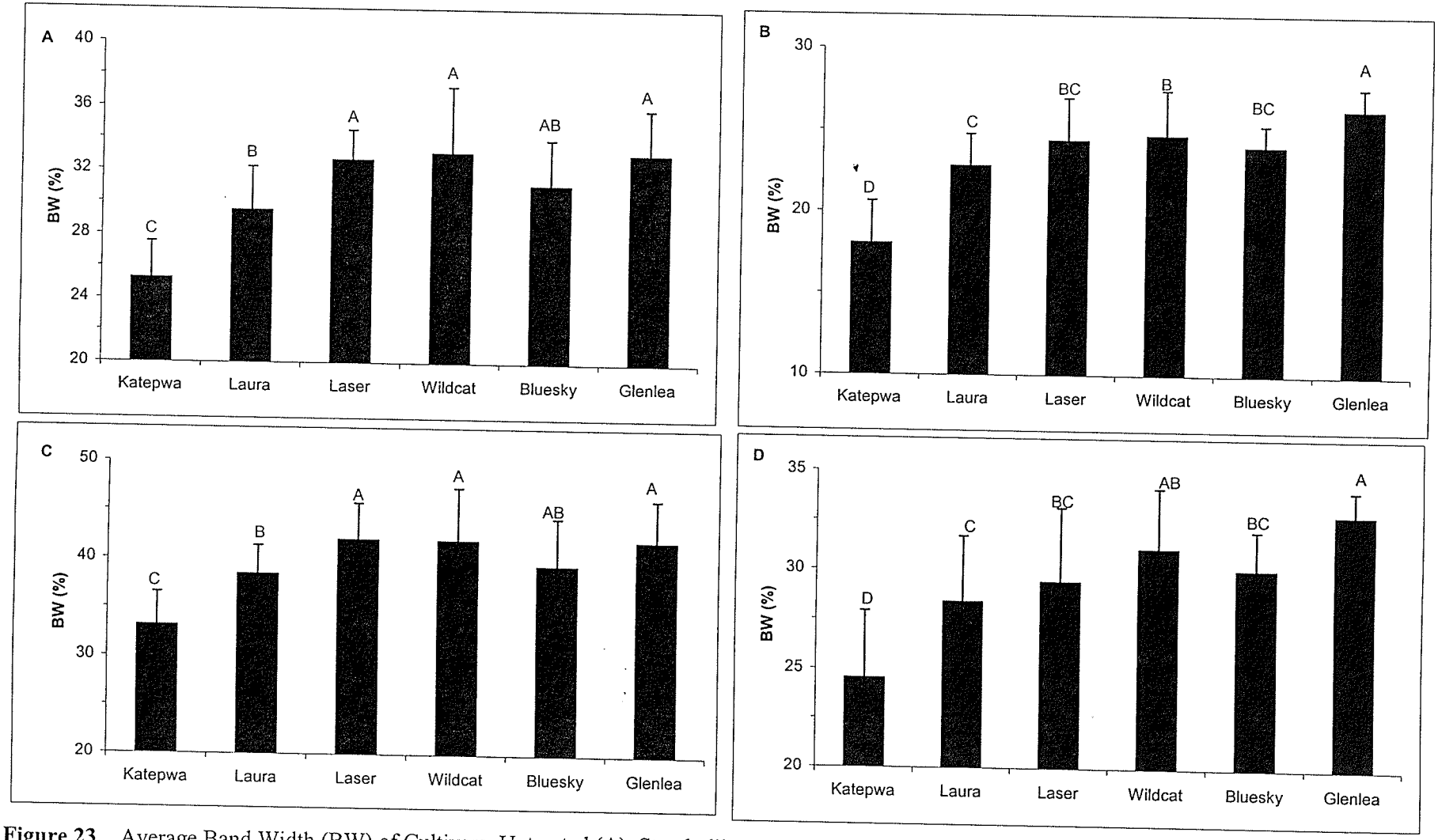


Figure 23. Average Band Width (BW) of Cultivars: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

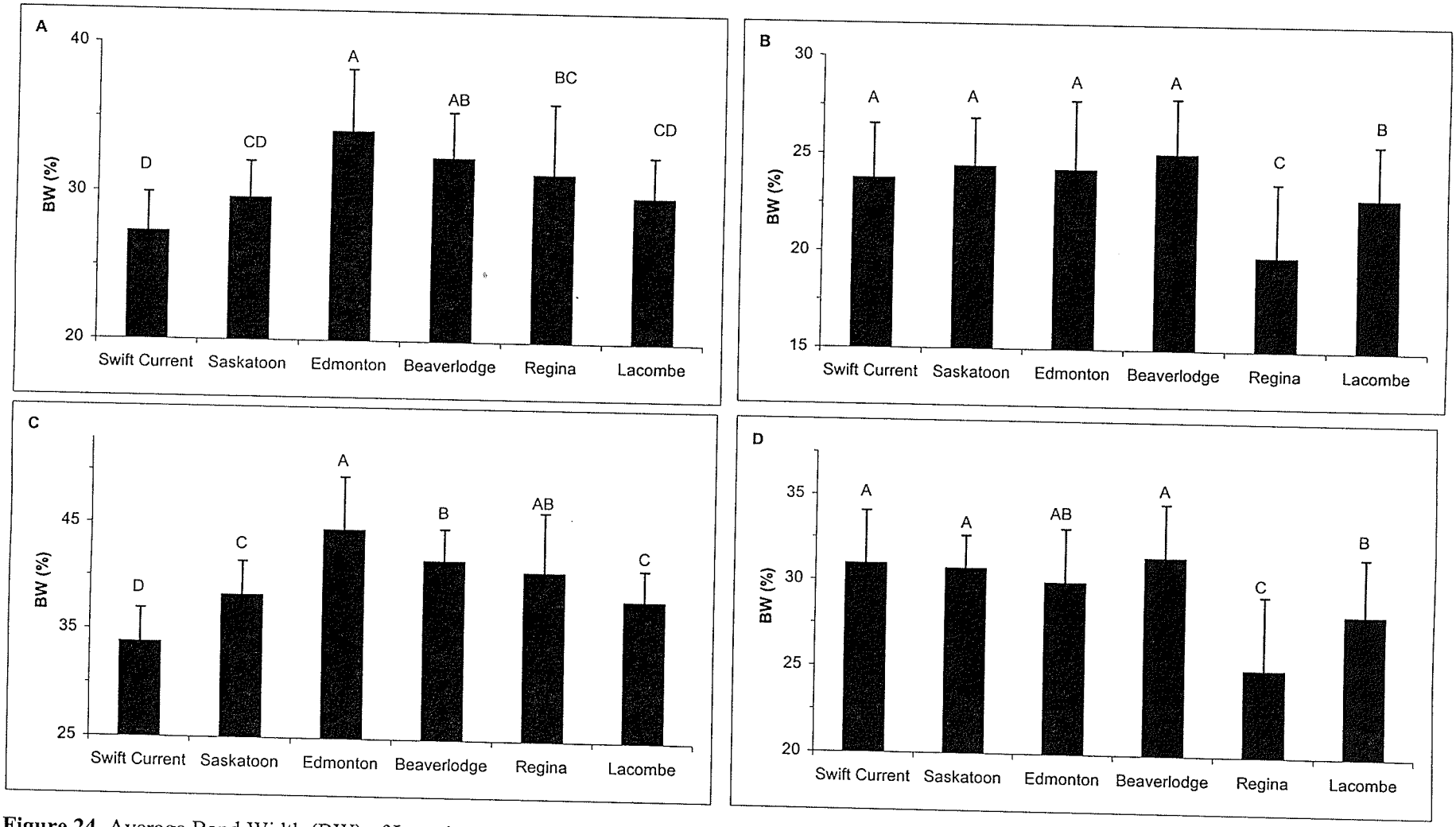


Figure 24. Average Band Width (BW) of Locations: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

values for genotype, location and the full sample set all increased, and were 12.4, 8.1, and 14.4%, respectively (Table 4). The magnitude of the genotypic effect increased slightly as shown by the increase in the G/E variance ratio from 1.9 to 2.4 (Figure 22).

Salt increased the BW values by approximately 28% with absolutely no change in the ranking of cultivars or locations (Figures 23C and 24C). This was in agreement with Hlynka (1970) on his study on the effect of salt concentration on mixing properties of wheat cultivars. The CV values for genotype, location and the full sample set decreased significantly to 8.6, 8.8, and 12.3%, respectively (Table 3). The decrease in range was caused by the CWRS cultivars increasing more than the CWES cultivars. This verified past research by Casutt et al. (1984) who also reported a greater increase in the weaker varieties with the addition of salt. They found that dough with higher inherent strength had less reaction to salt, but weaker cultivars show larger and more variable effects. It suggested that stronger cultivars already have a near maximum BW. In addition, the G/E variance ratio declined substantially to 0.96 (Figure 22). Although this value suggested environmental effects, it was very difficult to state that the BW was significant but location dependent. The comparison was much too close to commit to a conclusion of location being the stronger influence. The CV values decreased for genotype and the full sample set, but increased for locations (Table 4).

The result of the combination of salt and starch on BW resembled the starch treated samples alone. Overall, ranking of cultivars and locations did not significantly change, although the values and groupings changed slightly in magnitude (Figure 23D and 24D). The CV values were 9.8, 8.1 and 12.8% for genotype, location and the full sample set, respectively (Table 4). The CV values did not change substantially from the

control samples, but the location range did. The increase was not large because the G/E variance ratio still favoured the genotypic effect (Figure 22). The mixing treatments did not change BW much from the untreated sample, and did not provide highly distinct separation among the classes. BW was useful in separating the CWRS from the CWES, but not for separating within the CWES, which was an objective of the study.

4.2.3. Peak Dough Resistance

The peak dough resistance was measured as the height of the peak when the MT was reached (see Figure 2). PDR had significant variation among both the genotypes and locations. The range of PDR for genotypes was 57.2 to 70.2 %Torque (Figure 25A), and the locations ranged from 57.3 to 72.6% Torque (Figure 26A). The separation of the cultivars did not follow any pattern as the previous parameters, and there was no clear distinction between classes. Although it did show significant separation of Glenlea and Bluesky, PDR resulted in Katepwa as the equal of Bluesky, and Laura equal to Glenlea and the other CWES cultivars. These pairings made it difficult to believe that this measurement would be useful to distinguish between weak and strong wheats. The CV values for genotype, location and the full sample set were 8.1, 9.0, and 12.1%, respectively (Table 4). This close proximity variation, and the G/E variance ratio of 0.82 (Figure 22), made it difficult to conclude that location influenced PDR more than genotype.

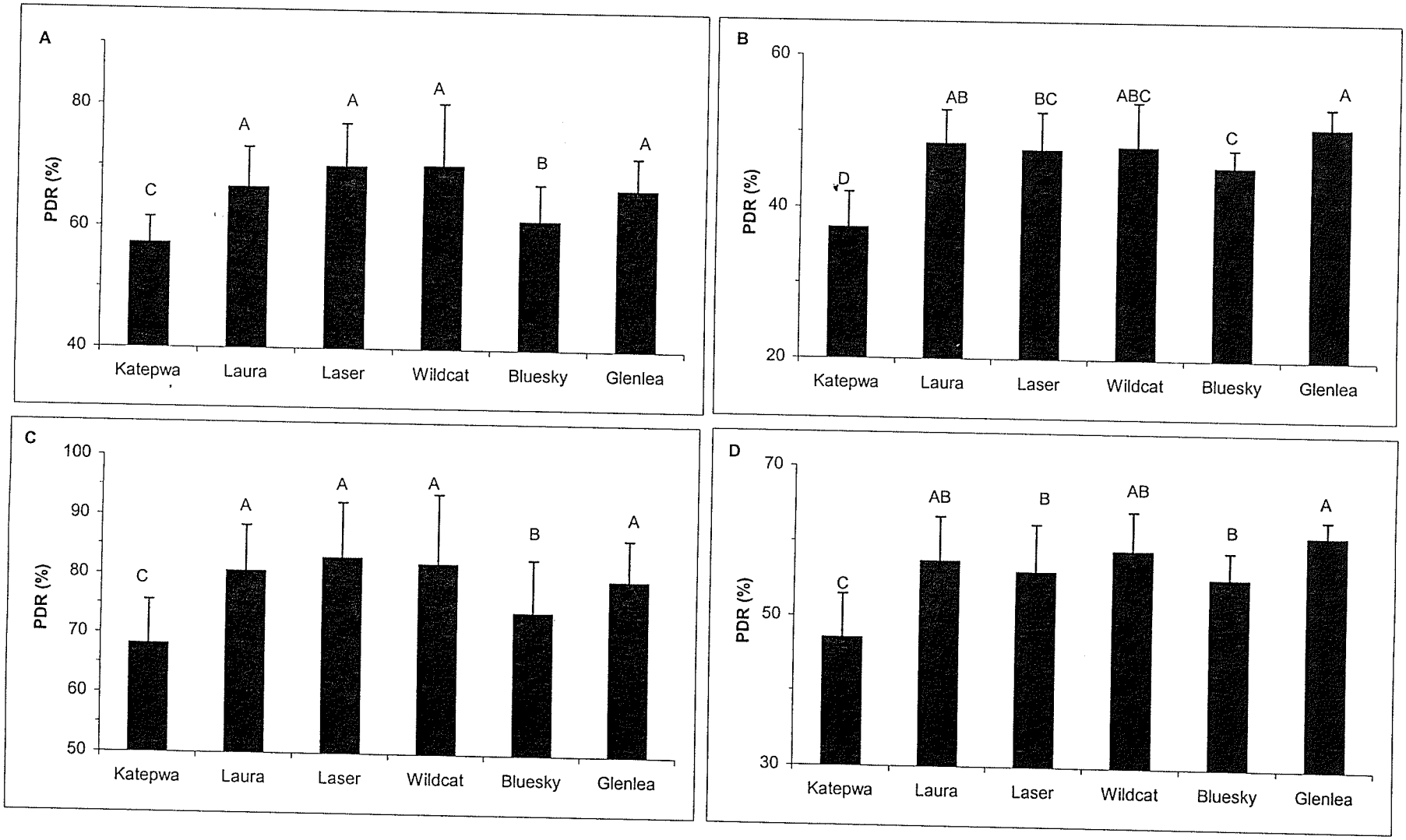


Figure 25. Average Peak Dough Resistance (PDR) of Cultivars: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

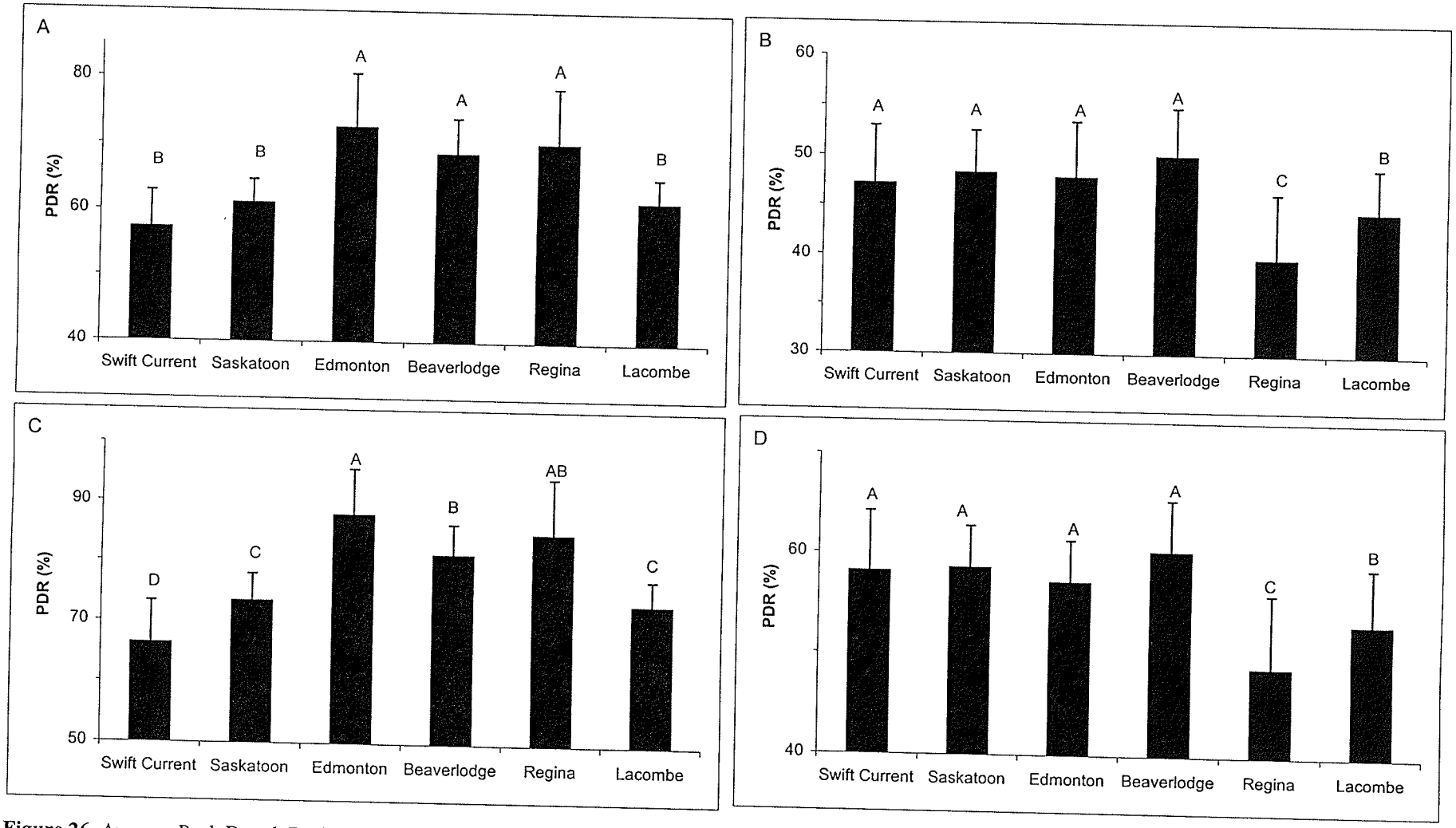


Figure 26. Average Peak Dough Resistance (PDR) of Locations: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

The effect of a CPC on the PDR of the cultivars and locations was a significant decline in PDR. There was virtually no change in the significance of the cultivars (Figure 25B), but the locations did shift slightly (Figure 26B). The range of values changed with an increase in the genotypic CV and a drop in location CV (Table 4). Glenlea had the greatest PDR of all cultivars at the CPC, but was statistically equal to Laura, Laser, and Wildcat. This alteration was important because the starch treated PDR became genotype dependent according to the G/E variance ratio (Figure 22). This was expected because mixing at the CPC essentially removed the location effects, as it had done for previous parameters.

Salt increased PDR over the untreated and starch treated samples. Although the magnitude of the values changed significantly, the total impact on ranking did not change dramatically. Glenlea was still equal to Laura, Wildcat and Laser, while Bluesky and Katepwa were significantly lower in value and no longer equal (Figure 25C). The locations showed significant and small variation (Figure 26C). The CV values for genotype, location and the full sample set were 7.2, 10.1 and 12.2%, respectively (Table 4). The location effect was greater than the genotypic effect as concluded by the significant G/E variance ratio of 0.5 (Figure 22).

Combining starch and salt together yielded variable results. The results for both genotypes and locations resembled the starch treatment (Figures 25D and 26D). The CV values for genotype, location, and the full sample set were 8.8, 7.5 and 11.5%, respectively (Table 4). The G/E variance ratio was low at 1.4 (Figure 22), and the genotype effect was only slightly greater than that of location. PDR did not change much with the treatments, but it was very useful for differentiating between Glenlea and

Bluesky. No other mixing parameter could distinguish between the strongest cultivars in the CWES class.

4.2.4. Work Input to Peak Dough Development

Work input to peak development (WIP) was the product of the PDR and MT of the sample (Figure 2). There was significant variation associated with the cultivars and distinct levels of strength for WIP. The range of values for WIP were 79.3 to 140.3 %Torque for cultivars (Figure 27A), and 107.6 to 127.0 %Torque for locations (Figure 28A). This parameter was clearly more influenced by genotype than location, based on those ranges and the G/E variance ratio of 8.8 (Figure 22). Like MT, WIP showed significant CWRS and CWES class distinction as well as the separation of Glenlea and Bluesky from Laser and Wildcat in the CWES class. The CV values for genotype, location and the full sample set were 21.4, 7.2, and 22.6%, respectively (Table 4).

Starch treatment caused a few important changes to WIP values for genotypes and locations. Although the values decreased significantly, the rankings for cultivars and locations did not (Figures 27B and 28B). The range of values increased with starch addition as shown by the CV values 27.6, 12.1, and 29.1% for genotype, location and the full sample set, respectively (Table 4). The G/E variance ratio decreased substantially to 5.2, but this value still translated into a commandingly genotypic influence (Figure 22).

The effect of salt resulted in a significant increase in WIP, and virtually no change in variation; salt increased WIP by an average of 29%. Glenlea and Bluesky were still significantly greater than Wildcat and Laser, and Laura and Katepwa (Figure 27C), and the locations were virtually identical to the untreated samples (Figure 28C). Salt caused a

tightening of the values, where the CV values for genotype, location and the full sample set decreased to 18.9, 6.5, and 20.2% (Table 4). The G/E variance ratio was high at 8.5 (Figure 22), which further showed how large the genotypic effect was.

Salt and starch in combination provided results that resembled the treatments alone, but did not significantly alter the values from the controls. In this instance, salt and starch did not add anything new to the analysis. The genotypes (Figure 27D) did not show any new levels of significance. The locations (Figure 28D) showed stronger relationships similar to the starch results. The CV values for genotype, location and the full sample set were 25.1, 12.3, and 27.2%, respectively (Table 4). The G/E variance ratio for this pair of treatments was 4.1 (Figure 22). The treatments did not add any new distinctions among the cultivars; therefore the untreated WIP values were a useful indicator for determining differences within the sample set, especially between the CWES cultivars.

4.3 Baking Quality of Strong-Mixing Wheat Cultivars

The baking data consisted of three separate sets of parameters: baking process parameters (i.e. those taken during or immediately after the baking process), textural and structural/ digital image analysis (DIA).

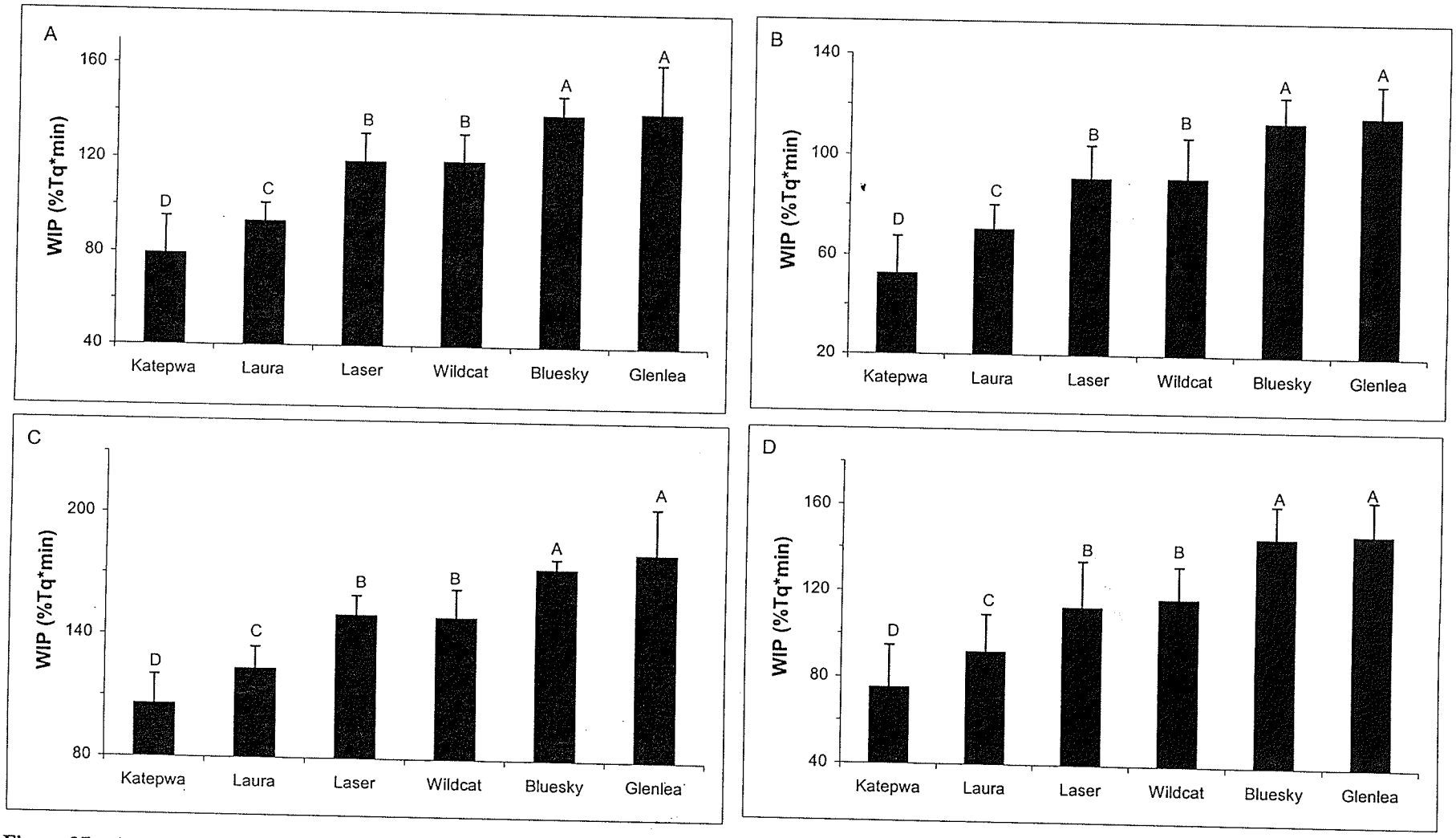


Figure 27. Average Work Input to Peak (WIP) of Cultivars: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

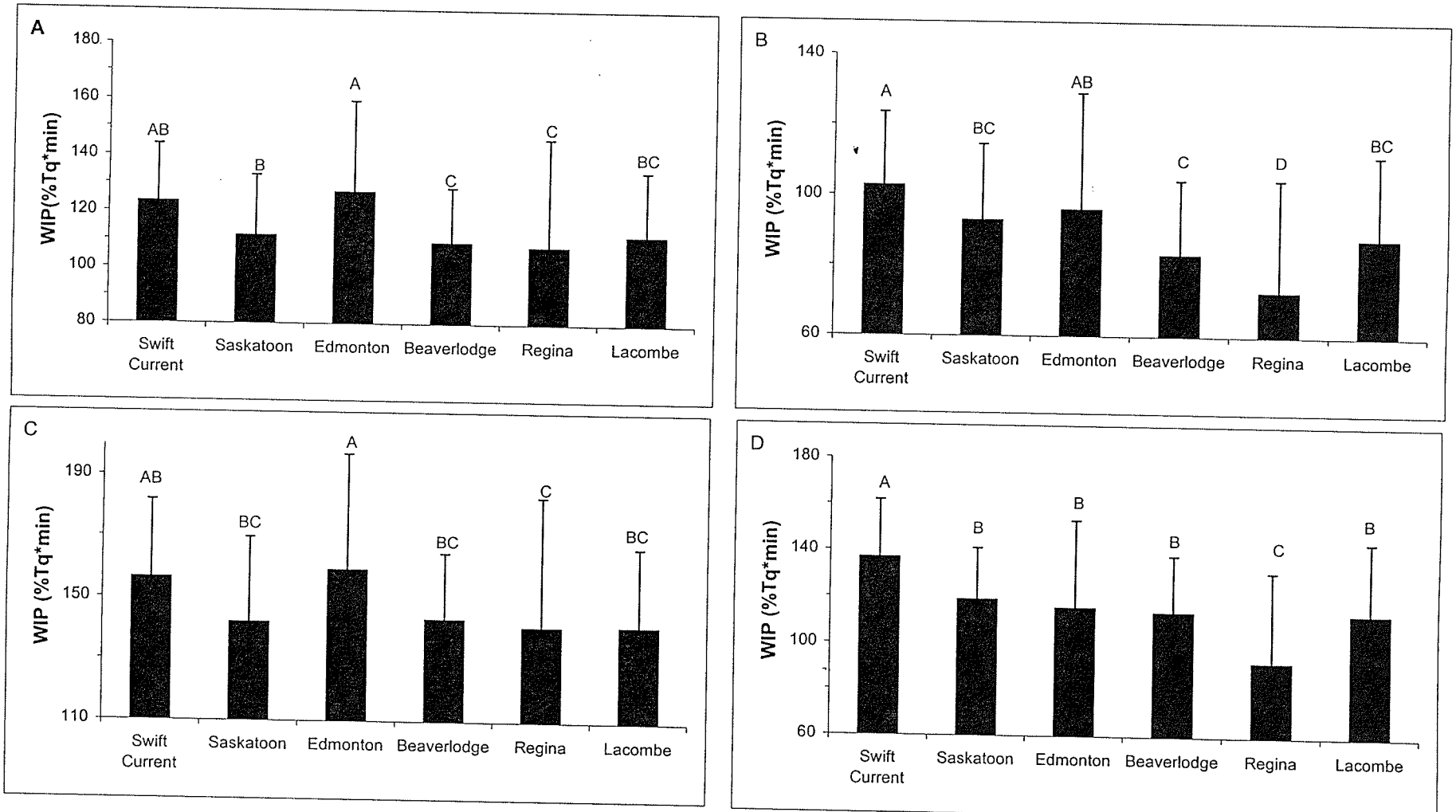


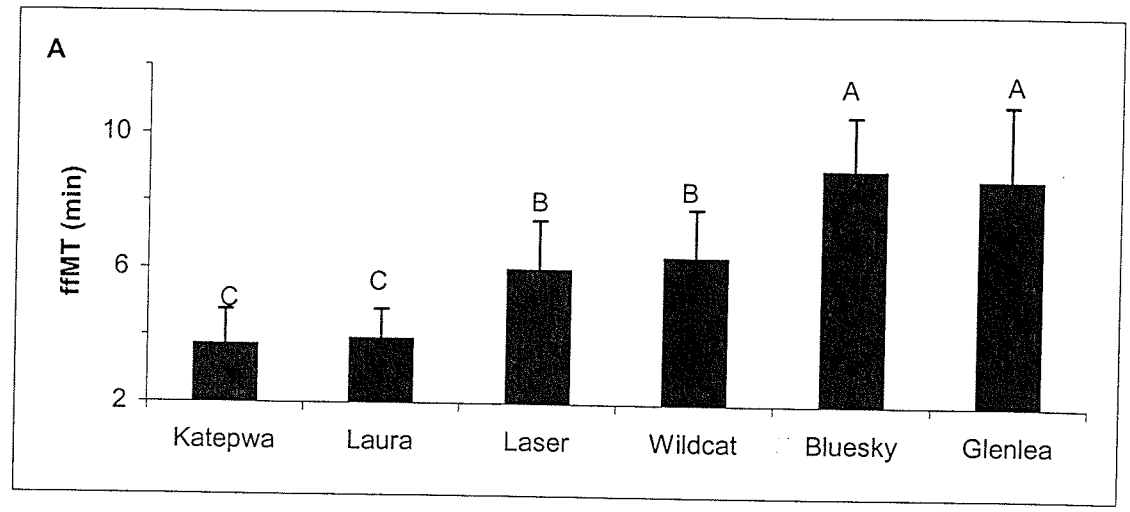
Figure 28. Average Work Input to Peak (WIP) of Locations: Untreated (A), Starch diluted (B), Salt-Treated (C), and Salt and Starch Treated (D).

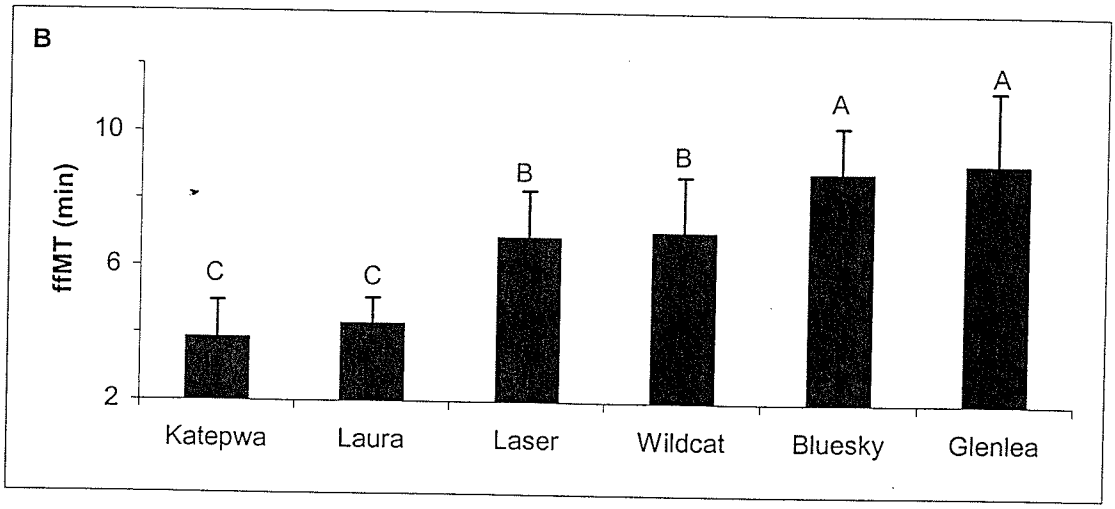
4.3.1 Baking Process Parameters

4.3.1.1 Full Formula Mixing Time

The full formula mixing time (ffMT) was measured as the peak mixing time plus 10%. ffMT provided a useful means for showing large genotypic and environmental effects, as well as significant separation of cultivars. The range in ffMT for genotypes was 3.7 to 9.0 minutes (Figure 29A), and the range for locations was 4.7 to 7.8 minutes (Figure 30A). These results were virtually identical to the mixograph mixing time result shown earlier (refer to Figures 20A and 21A). There was a clear separation of the cultivars into three significant levels separated by class (CWRS and CWES), and then again within the CWES class. The CV values associated with genotype, location and the full sample set were 36.1, 18.1 and 40.2%, respectively (Table 5). These were the highest CV values in the entire study. This large amount of variation was mostly attributed to the genotypic effects as was apparent by the G/E variance ratio of 4.0 (Figure 31).

Mixing at a CPC caused an increase in ffMT, which was also the same result as the Mixograph MT. All values of MT increased for both genotypes (Figure 29B) and locations (Figure 30B), but the ranking did not shift. The CV values for genotype, location and the full sample set decreased for both effects to 33.2, 16.2, and 37.1%, respectively (Table 5). The G/E variance ratio increased slightly to 4.2 (Figure 31), thus maintaining the large influence of genotype. All ffMTs decreased with the removal of shortening. The cultivars Glenlea and Bluesky decreased more than the other cultivars, but managed to maintain significantly higher ffMTs (Figure 29C). The location values also declined, but the separation was more clear with less overlap among cultivars





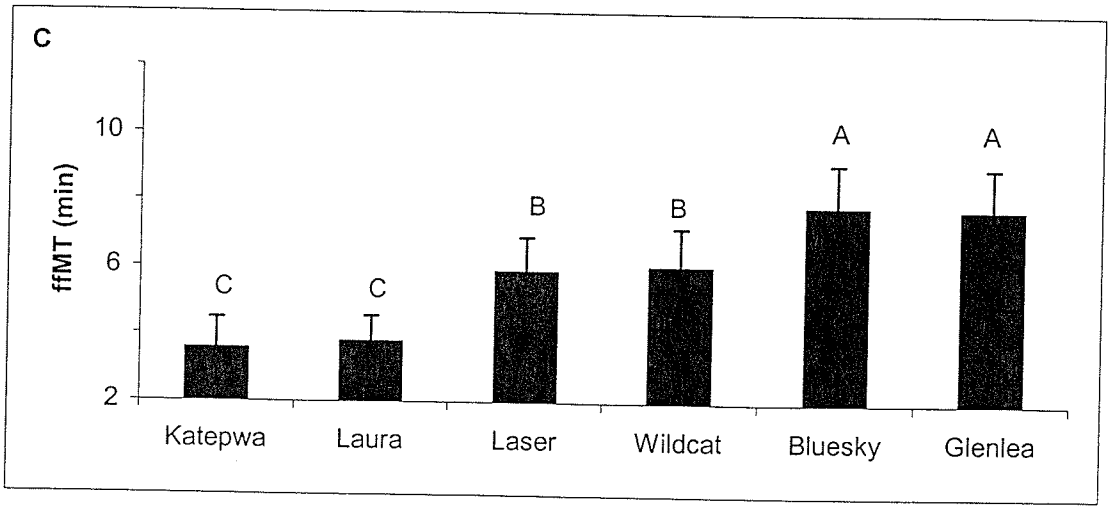


Figure 29. Average Full-Formula Mixing Time (ffMT) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

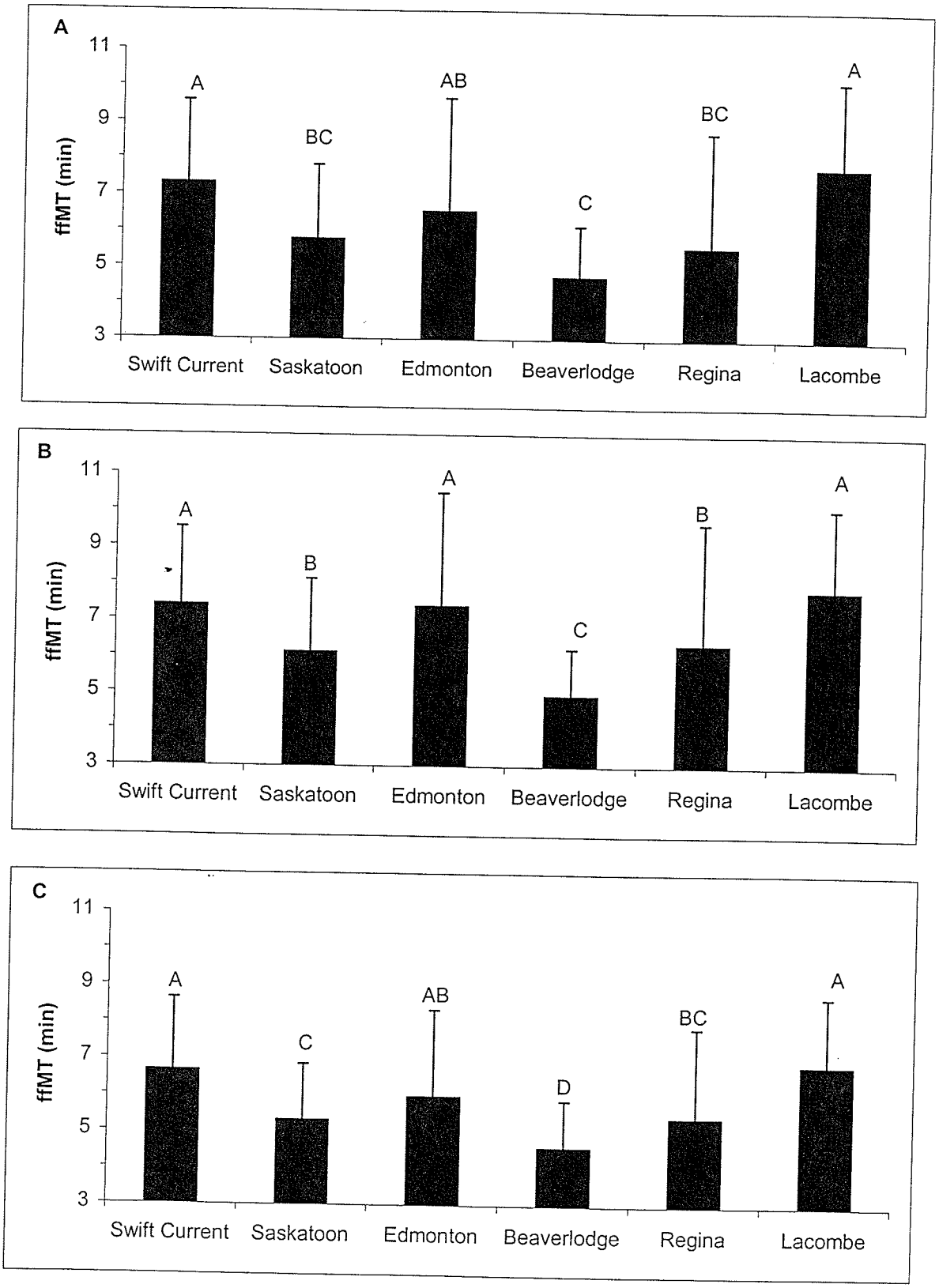


Figure 30. Average Full-Formula Mixing Time (ffMT) of Locations: Untreated (A), Starch diluted (B), and Starch Diluted Without Shortening (C).

Table 5

Coefficients of variation (CV) of baking process parameters for effects of genotype, environment and full sample set.

		Coefficients of Variation (%)		
Treatment	Parameter*	Genotype	Location	Full Set
Control	ffMT	36.05	18.11	40.17
	DSL	14.43	7.87	16.89
	LV	1.40	1.79	3.33
Starch	ffMT	33.20	16.15	37.14
	DSL	11.71	8.07	15.15
	LV	2.88	3.52	4.77
Starch without Shortening	ffMT	31.95	14.91	34.34
	DSL	12.85	7.38	14.73
	LV	5.04	3.49	6.07

*ffMT= full-formula mixing time, DSL= dough sheet length, LV= loaf volume

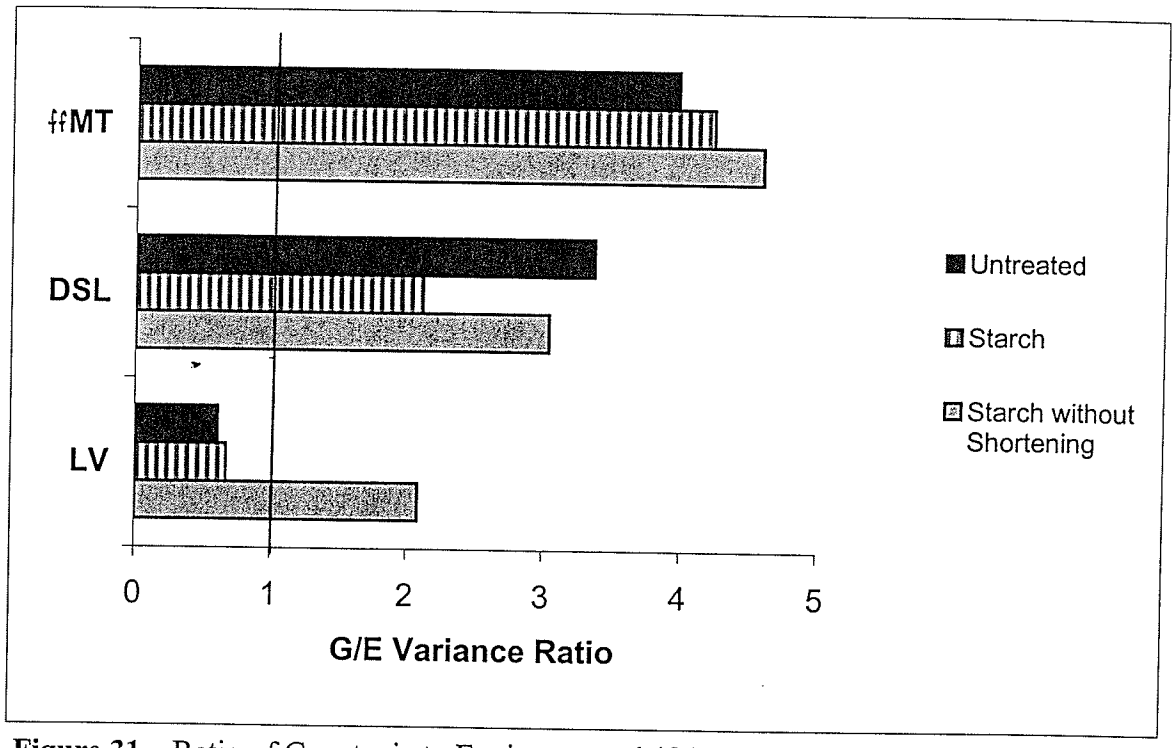


Figure 31. Ratio of Genotypic to Environmental (G/E) Variance for Baking Process Parameters. Equal Contributions of Genotype and Environmental Influence designated by Vertical Line.

(Figure 30C). The CV values decreased to the lowest level for genotype, location and the full sample set (Table 5), but the G/E variance ratio increased again (Figure 31). The ffMT did not provide more new information than the mixograph MT, but like MT, ffMT was very useful for separating cultivars and distinguishing between and within the two classes of wheat. ffMT was useful for determining differences between cultivars.

4.3.1.2 Dough Sheet Length

Dough sheet length (DSL) was an unsophisticated measurement of the relative extensibility of a sample, and was based on the length of the dough sheet after dough make-up. It showed significant variation for cultivars and locations. The range of DSL for genotypes was 11.6 to 17.1 inches (Figure 32A), and for locations, 12.9 to 15.9 inches (Figure 33A). The weakest cultivars, Katepwa and Laura, had the longest sheets and the stronger cultivars had the shortest, i.e. the CWES class. This was a virtually identical relationship to SPC/FPC (Figure 11C). For this parameter, it was Bluesky and not Glenlea that had the shortest length. It was the only parameter, other than mixograph PDR, which significantly differentiated between Glenlea and Bluesky. CV values for DSL were 14.4, 7.9, and 16.9% for genotype, location, and the full sample set, respectively (Table 5). DSL was highly influenced by genotypic effects as was evident from the G/E variance ratio of 3.4 (Figure 31).

Using a CPC caused an overall increase in sheet length. This was not a significant increase, and it did not improve the separation of the cultivars or locations (Figures 32B and 33B). In fact, the addition of starch caused a significant overlap in the

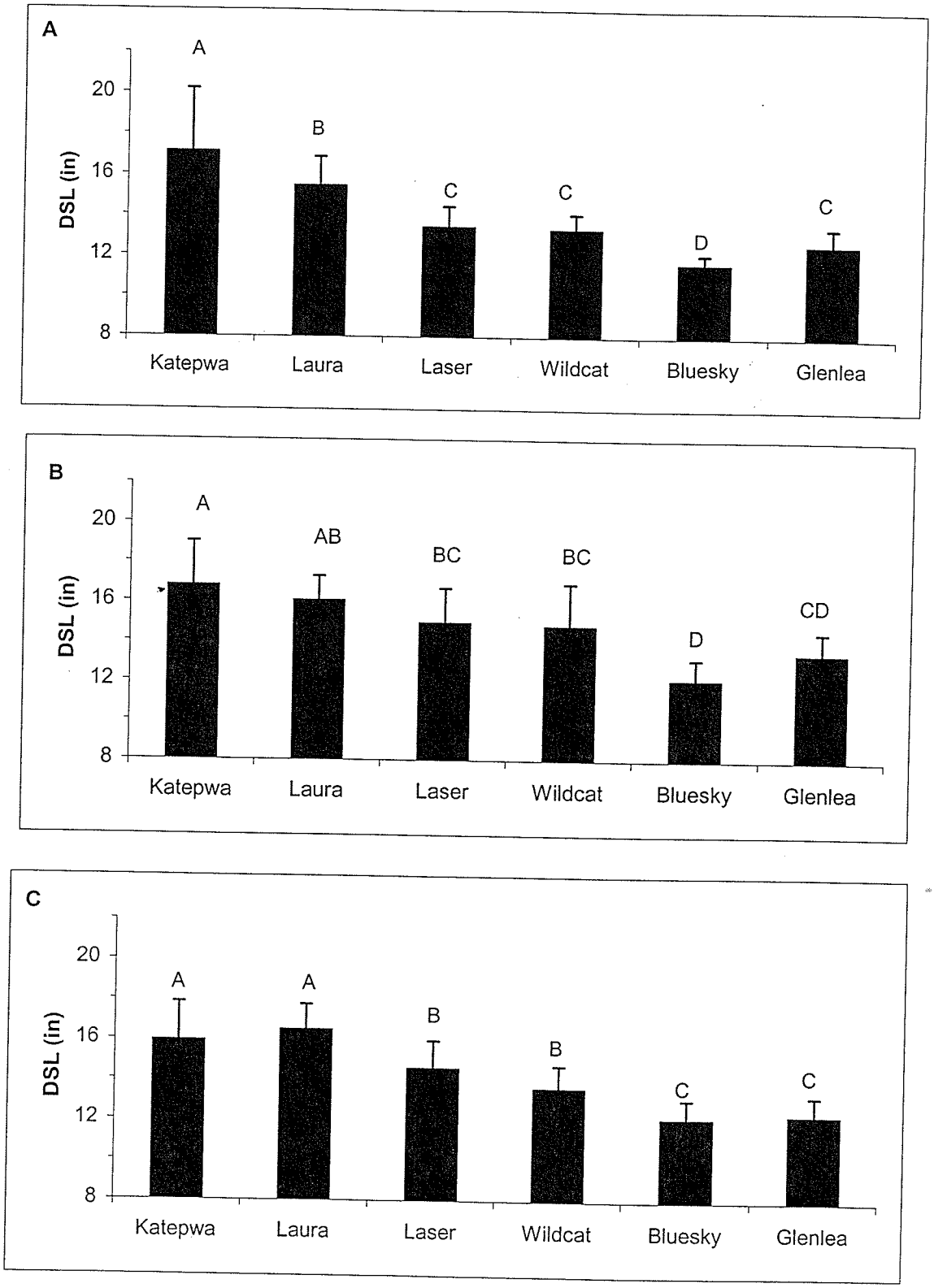


Figure 32. Average Dough Sheet Length (DSL) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

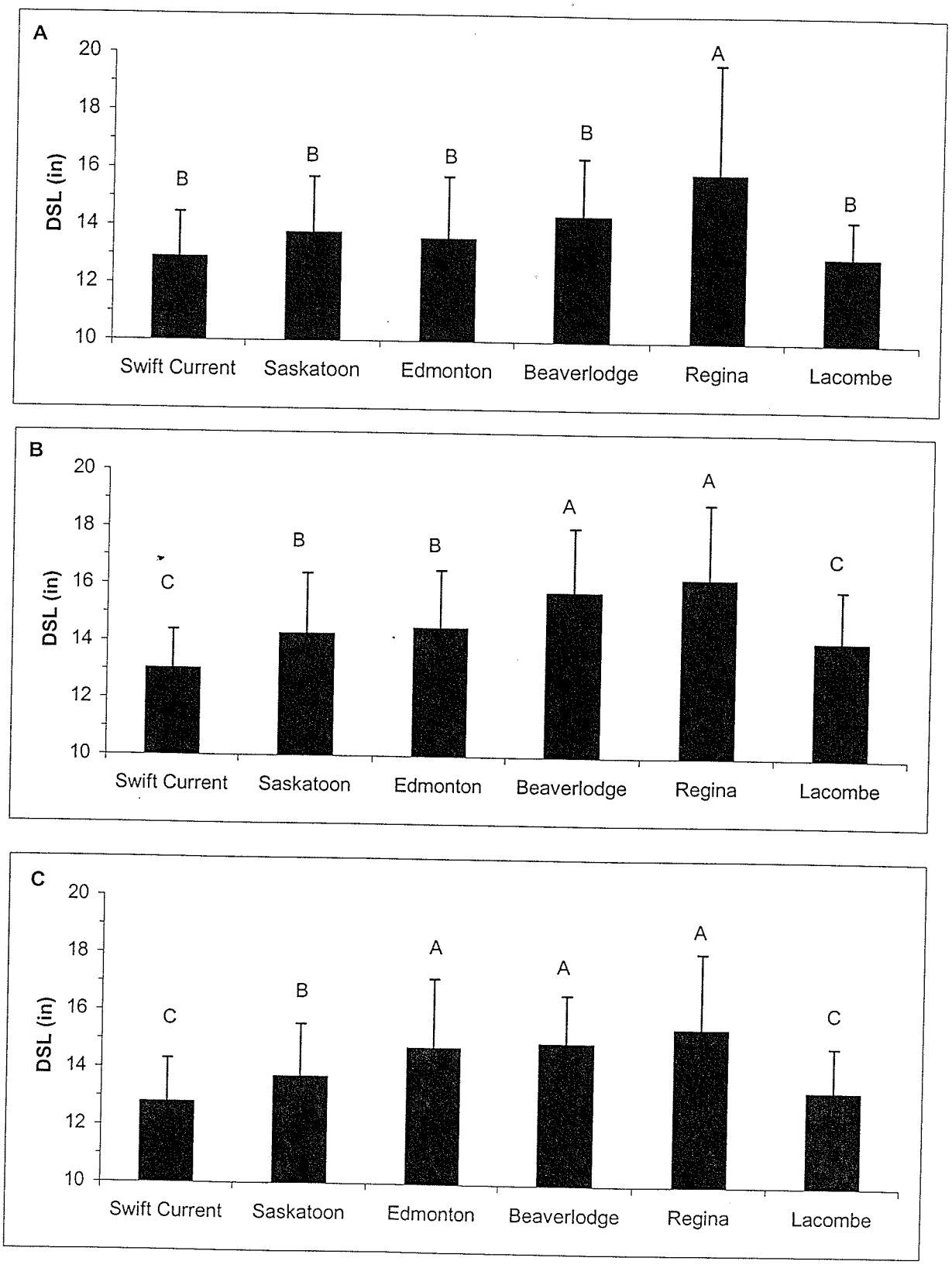


Figure 33. Average Dough Sheet Length (DSL) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

cultivars and locations making it more difficult to differentiate between the samples. The CV values decreased to 11.7, 8.1, and 15.2% for genotype, location, and the full sample set, respectively (Table 5). The strength of the genotypic effect decreased but remained larger than the location effect; the G/E variance ratio decreased from 4.0 to 2.1 (Figure 31). The addition of starch removed many differences between cultivars and locations, which was expected since the samples were given like protein content.

Removing shortening from the formulation caused a drop in DSL, but it was hardly substantial. Essentially, the values did not change much from the untreated samples with the addition of starch. This showed that shortening was important for maintaining the extensibility of the dough of a cultivar. The genotypic effect was dominant over locations, and there was clear separation among the cultivars (Figures 32C and 33C). The CV values were 12.9, 7.4, and 14.7% for genotype, location and the full sample set, respectively (Table 5). The G/E variance ratio showed that DSL was still highly influenced by genotype (Figure 31). Although this parameter was a simple measurement, DSL provided a useful method for significantly separating Glenlea and Bluesky, which was one of the objectives.

4.3.1.3 Loaf Volume

The range in loaf volume (LV) for the genotypes was 1148 to 1192 cc (Figure 34A), and the range for the locations was 1140 to 1193 cc (Figure 35A). There were no significant differences among the genotypes, but there were two levels of significance for the locations. Finding that the CWES cultivars were equal to the breadmaking cultivar

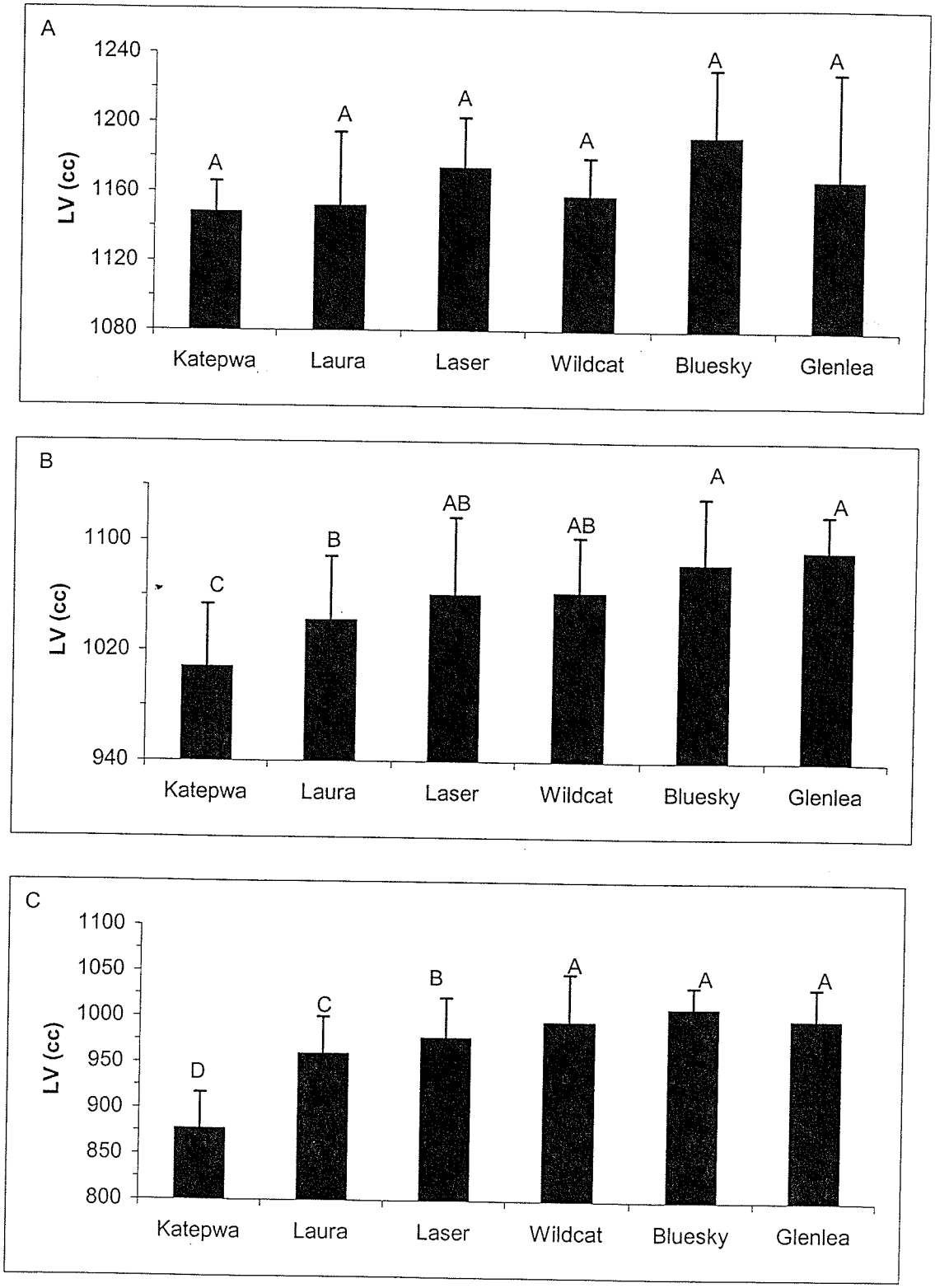
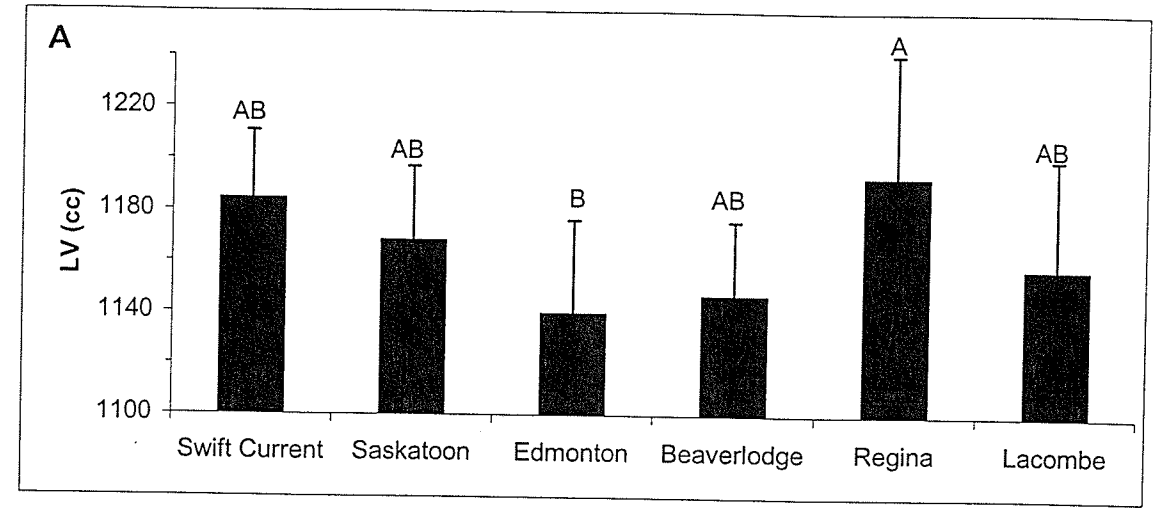
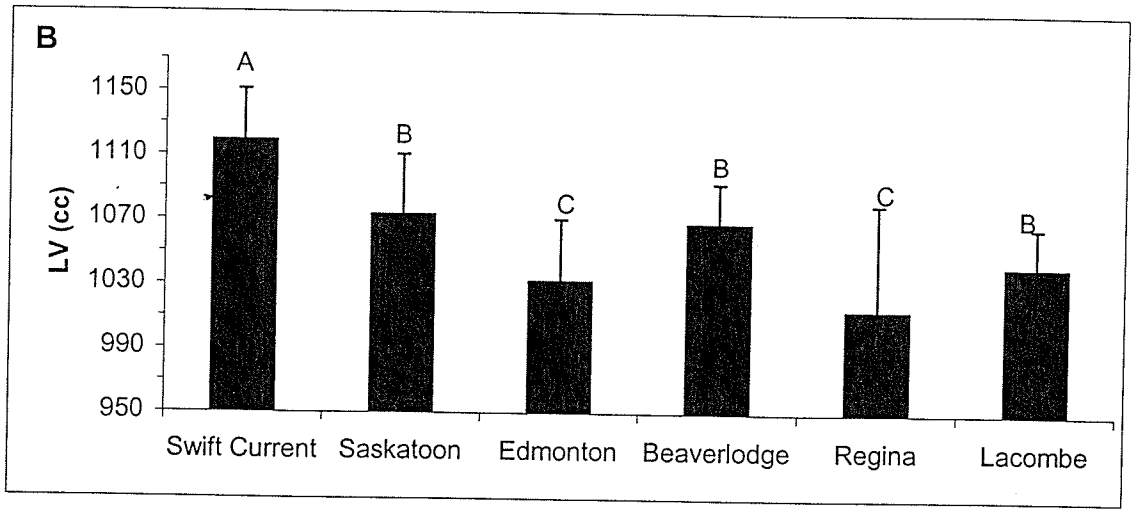


Figure 34. Average Loaf Volume (LV) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).





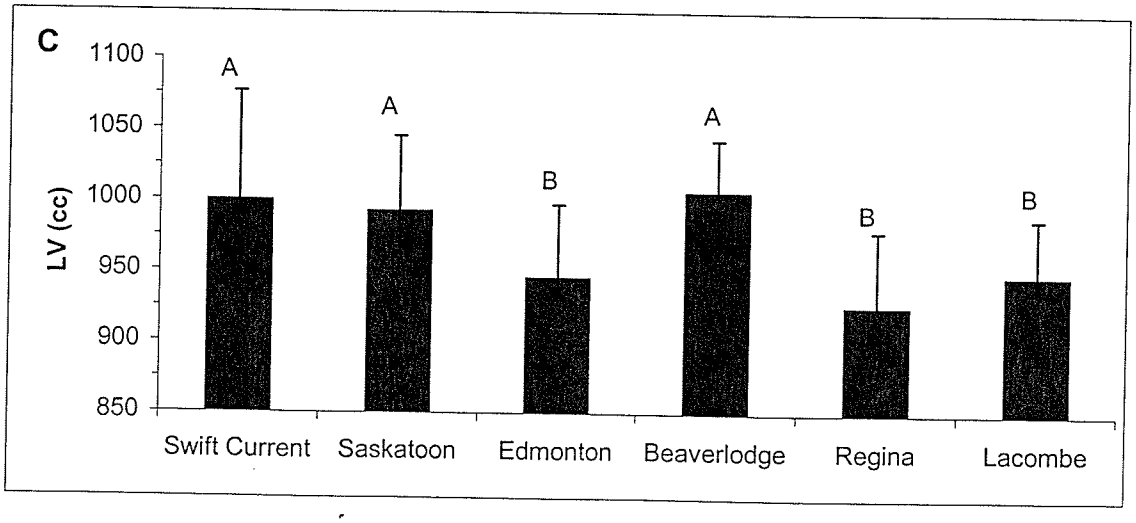


Figure 35. Average Loaf Volume (LV) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

Katepwa was important because CWES cultivars are traditionally not used for bread-making purposes, because of their overly strong characteristics. The CVs for genotype, location and the full sample set were 1.4, 1.8, and 3.3%, respectively (Table 5); these were the lowest CV values in the entire study. The G/E variance ratio was 0.61, which suggested a large influence by growing location (Figure 31). LV was the only baking parameter that was influenced more by the growing location than the inherent genetic characteristics. The lack of variation shown by the CV values suggested that there was not enough difference in any of the samples to warrant a significant conclusion.

Adding starch to the formulation was expected to extract a greater degree of variation because of the removal of FPC and a large environmental influence. The CPC resulted in a decrease in LV, and a greater degree of separation among the samples. The LVs decreased significantly, but had three levels of significance for cultivars (Figure 34B). The pattern remained essentially the same, with the CWES cultivars having the higher LV, but the CWRS cultivars decreased substantially. Interestingly enough, it was the cultivars with the highest FPC that had the lowest LVs. These cultivars also had the greatest amount of starch added before baking, which may be why they declined so much. However, the higher LVs were associated with the cultivars with the greatest amount of IGC. This suggested that the IGC enabled a cultivar to achieve a large LV. The anomaly was Laser, since it had a high FPC and IGC, and still had a LV that was statistically equal to Glenlea. Perhaps, the lower level of SPC was the reason for the higher LV. The locations were separated into three significant levels that were very similar to the untreated samples (Figure 35B). The biggest difference was that Regina went from the highest LV to the lowest; Regina had the highest average FPC. Again, it

was possible that the large amount of starch added to these samples caused the decline in LV. Nevertheless, Laser had both a high FPC and LV, while Glenlea had the lowest FPC and a high LV. There was definitely an issue with protein quality, because the FPC could not possibly explain these differences. The CV values nearly doubled to 2.9, 3.5, and 4.8% for genotype, location and the full sample set, respectively (Table 5). Starch was clearly beneficial for evaluating samples of different inherent dough strength for breadmaking purposes. The G/E variance ratio increased slightly to 0.67 (Figure 31), thus maintaining the environmental influence. This result was surprising, since for other parameters, if they were influenced by environment, they become genotype dependent after the FPC effect was removed. Added starch showed that the LV of a cultivar was influenced more by where it was grown than by its genotype.

The removal of shortening from the baking formulation resulted in a significant decrease in LV for all genotypes (Figure 34C) and locations (Figure 35C). Baking without shortening resulted in a shift from a location effect to significant genotypic effect; the G/E variance ratio was 2.1 (Figure 31). Katepwa had the lowest LV, followed by Laura, and then all the CWES cultivars that had the highest LVs and all were statistically equal to one another. The exception was Laser, which had the lowest LV of all the CWES and was not significantly equal to Glenlea or Bluesky. This made starch an attractive treatment for finding differences in the LV of different cultivars. This was essentially the same order found for the cultivars with the shortening present. The CV values were 5.0, 3.5 and 6.1% for genotype, location and the full sample set, respectively (Table 5). Shortening played a role in keeping variation down, because the increase in CV values when shortening was removed was significant. This suggested that shortening

had a coddling effect (i.e. the weaker cultivars performed better) on the weaker cultivars which was perhaps more apparent with the CWRS than the CWES cultivars. It could be argued that the effect seen here was directly related to the starch content in the sample (i.e. about 90%), but then that would allude to protein quality having no impact on LV, when it most definitely does. LV was important for detecting differences in genotypes and locations, but did not add much to the differentiation between CWES cultivars.

4.3.2 Textural Properties of Strong-Mixing Cultivars

4.3.2.1 Peak Force

Peak force (PF) was the amount of force required to fracture the bread crumb (see Figure 8). There was small but significant variation in PF associated with the cultivars and locations. The range in genotypes was 143.4 to 188.0 grams of force (g), and the locations ranged from 156.4 to 175.1 g (Figures 36A and 37A). There was significant separation of the genotypes, but there was no significant effect for the locations. The CV values for genotype, location and the full sample set were 9.7, 4.5 and 12.4% (Table 6). The G/E variance ratio was 4.63 (Figure 38), which was expected because there were no significant location effects.

When samples were diluted to a CPC of 10%, the PF decreased dramatically. Compared to the untreated samples, the starch diluted samples decreased by an average of 16%. The ranking of the cultivars changed considerably, and Glenlea and Bluesky

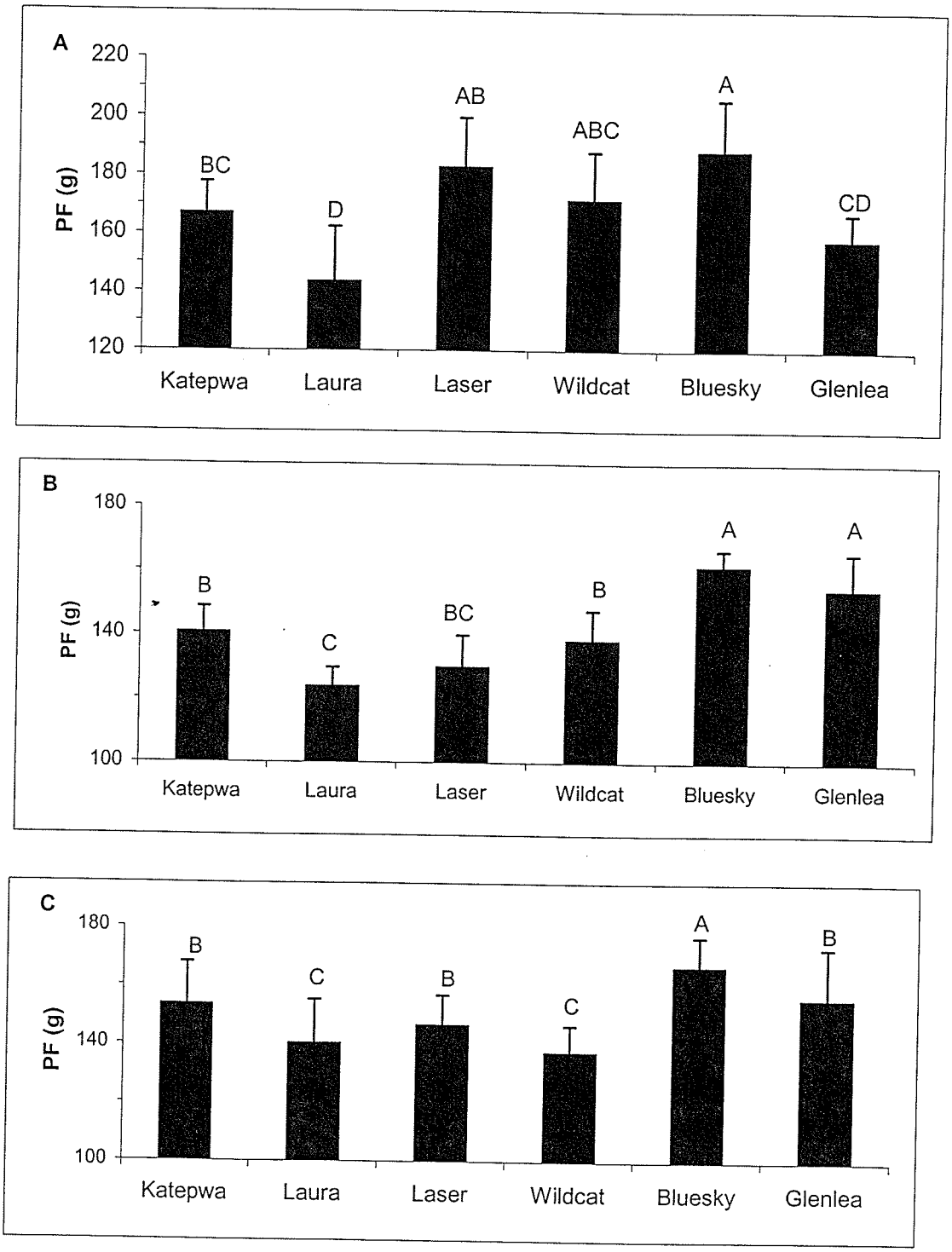


Figure 36. Average Peak Force (PF) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

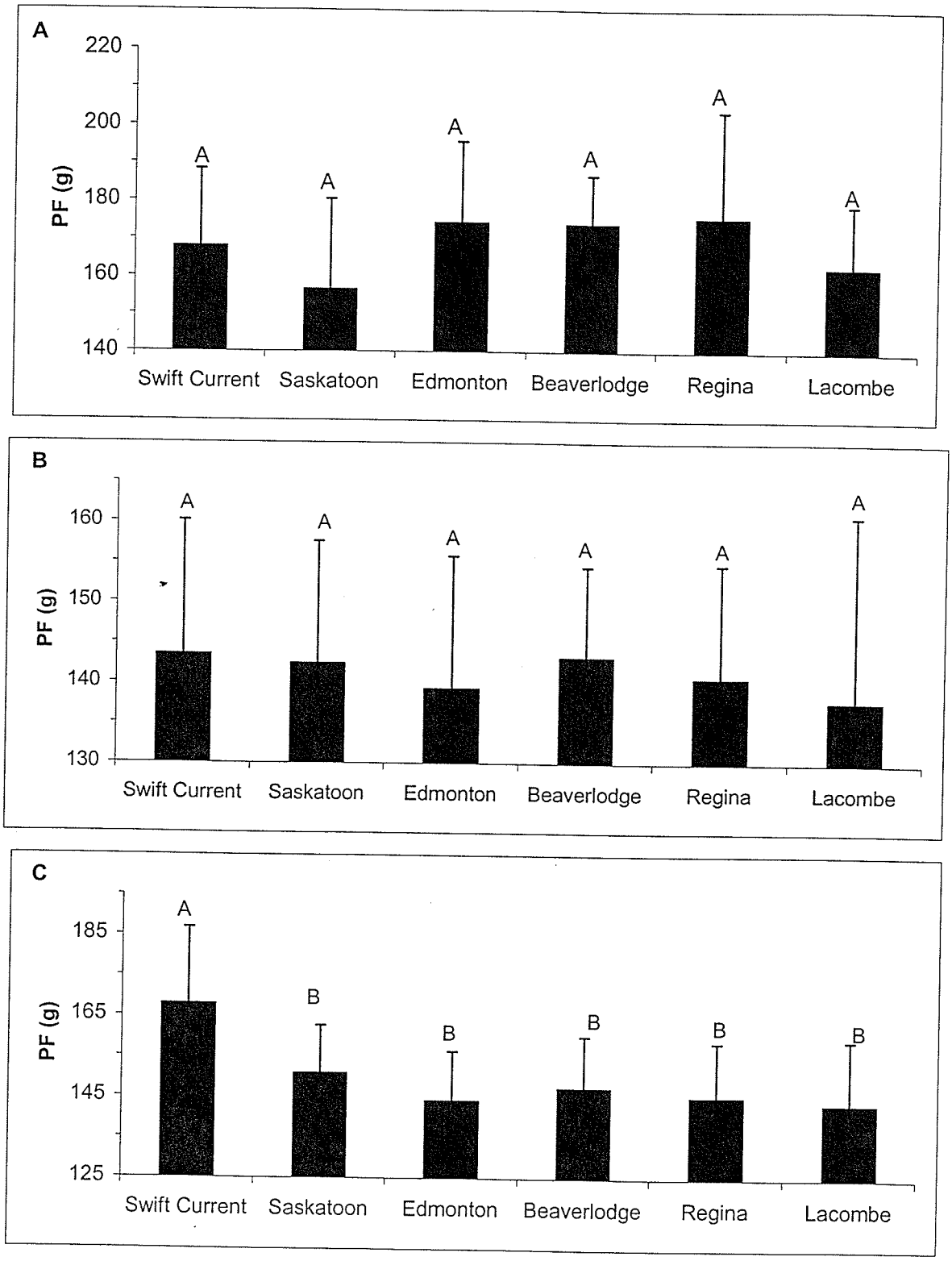


Figure 37. Average Peak Force (PF) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

Table 6

Coefficients of variation (CV) of texture parameters for effects of genotype, environment and full sample set.

		Coefficients of Variation (%)		
Treatment	**Parameter***	**Genotype**	**Location**	**Full Set**
Untreated	PF	9.73	4.52	12.39
	PT	5.27	5.63	9.02
	AS	3.19	9.84	12.83
	CAP	13.40	6.05	16.97
Starch	PF	10.12	1.65	10.95
	PT	6.98	5.59	9.48
	AS	5.01	8.60	11.56
	CAP	14.12	5.70	15.55
Starch without Shortening	PF	7.25	6.17	10.58
	PT	5.85	5.27	8.24
	AS	10.02	7.81	15.20
	CAP	11.09	9.58	15.68

*PF= peak force, PT= peak time, AS= ascending slope, CAP= curve area to peak.

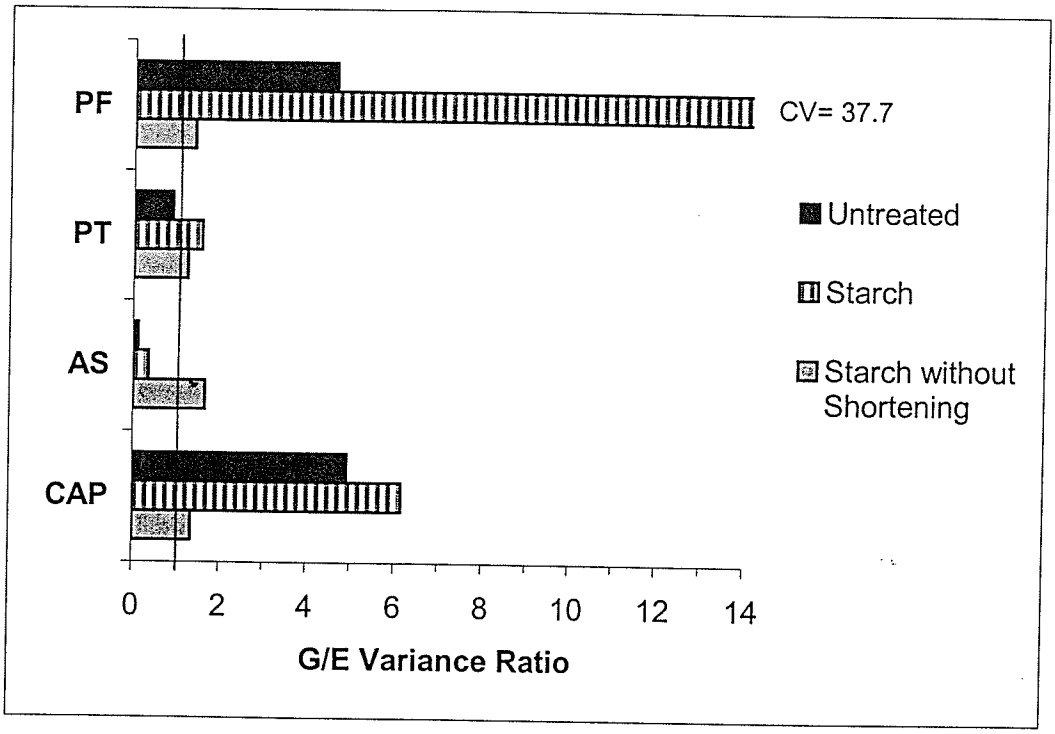


Figure 38. Ratio of Genotypic to Environmental (G/E) Variance for Textural Parameters. Equal Contributions of Genotype and Environmental Influence designated by Vertical Line.

now had the highest PF (Figure 36B). There was a similar distinct separation of the CWES class as seen with MT and WIP; Glenlea and Bluesky were significantly greater than all the other cultivars. Again, there was no significant location effect (Figure 37B). The CV values for genotype, location and the full sample set were 10.1, 1.7 and 11.0%, respectively (Table 6). The G/E variance ratio was the largest of the entire experiment at 37.7 (Figure 38) indicating a genotypic effect.

With the removal of shortening from the baking formulation, PF decreased. This result suggested that shortening increased the force required for breaking through the bread slice, and starch made it easier to fracture. It seems that starch causes the bread structure to become more brittle and not tougher. There were significant genotype and location effects. The separation of the cultivars was not as clear as when shortening was present, but there was still a good distinction between Bluesky and the rest of the cultivars (Figure 36C). This trend where Bluesky shows greater strength than Glenlea is consistent with other findings in this study. The locations consisted of two levels of significance (Figure 37C). The variation according to CV values for genotype, location and the full sample set were 7.3, 6.2 and 10.6%, respectively (Table 6). This showed a dramatic decline in the genotypic effect when starch was present, and was substantiated by the G/E variance ratio of only 1.4 (Figure 38).

4.3.2.2 Peak Time

Peak time (PT) was the amount of time required to reach PF (see Figure 8). PT showed significant variation for both genotypes and locations. The genotypic effect was significant, but smaller than the location effect. The range of values for genotypes was

20.9 to 24.3 seconds (Figure 39A), and for locations, the range was 21.2 to 25.0 (Figure 40A). The range for the full sample set was 18.4 to 28.1 seconds. The distinction between the cultivars was very difficult to determine; the genotypic effect was not large. The location effect was clearly stronger, but it too had overlapping levels of significance. The CV for genotype, location and the full sample set were 5.3, 5.6 and 9.0%, respectively (Table 6). There was little difference in variation, and the G/E variance ratio value of 0.88 was borderline environmental and genotypic influence (Figure 38).

The addition of starch caused a decrease in cultivar and location values by an average of 23%. The CPC increased the genotypic effect to a level greater than the location effect, and showed distinct separation among the cultivars. Glenlea and Bluesky had the longest PTs even in the presence of the weakening effect of starch, while the CWRS varieties had the shortest PT (Figure 39B). Location effects decreased slightly but remained significant (Figure 40B). The CV values increased for genotype and total range (7.0 and 9.5%, respectively), but remained the same for locations (Table 6). The increase in the genotypic effect also registered a similar increase in the G/E variance ratio of 1.56 (Figure 38). The addition of starch permitted a nice separation of the "strong" CWES cultivars of Glenlea and Bluesky from the "weak" CWES cultivars, Laser and Wildcat.

Removing shortening from the CPC treated samples resulted in a significant decline in PT. Genotype had a greater effect than environment (Figure 39C); Bluesky and Glenlea were still significantly greater, but all the other cultivars were statistically equal. There were still significant effects associated with the locations (Figure 40C).

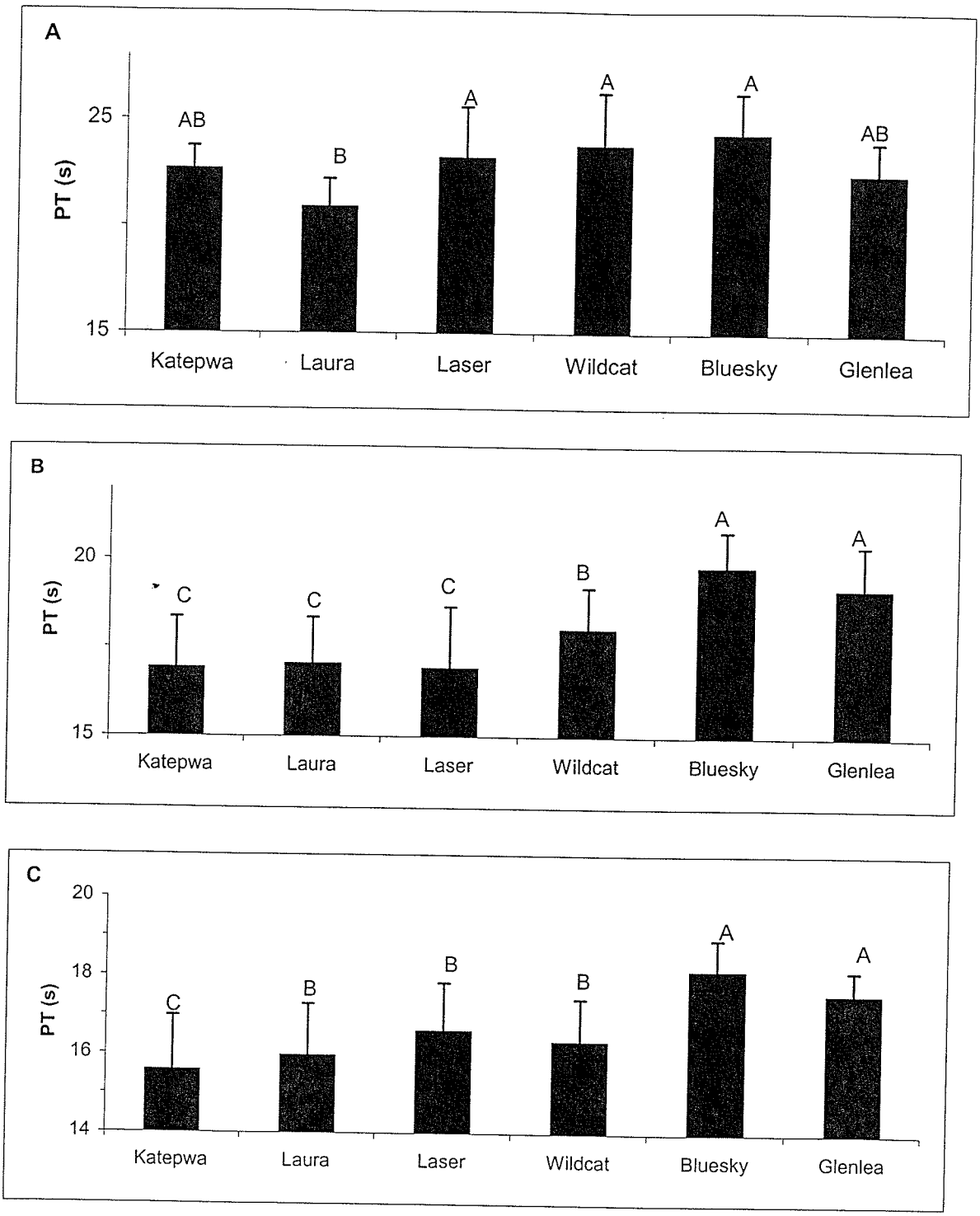


Figure 39. Average Peak Time (PT) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

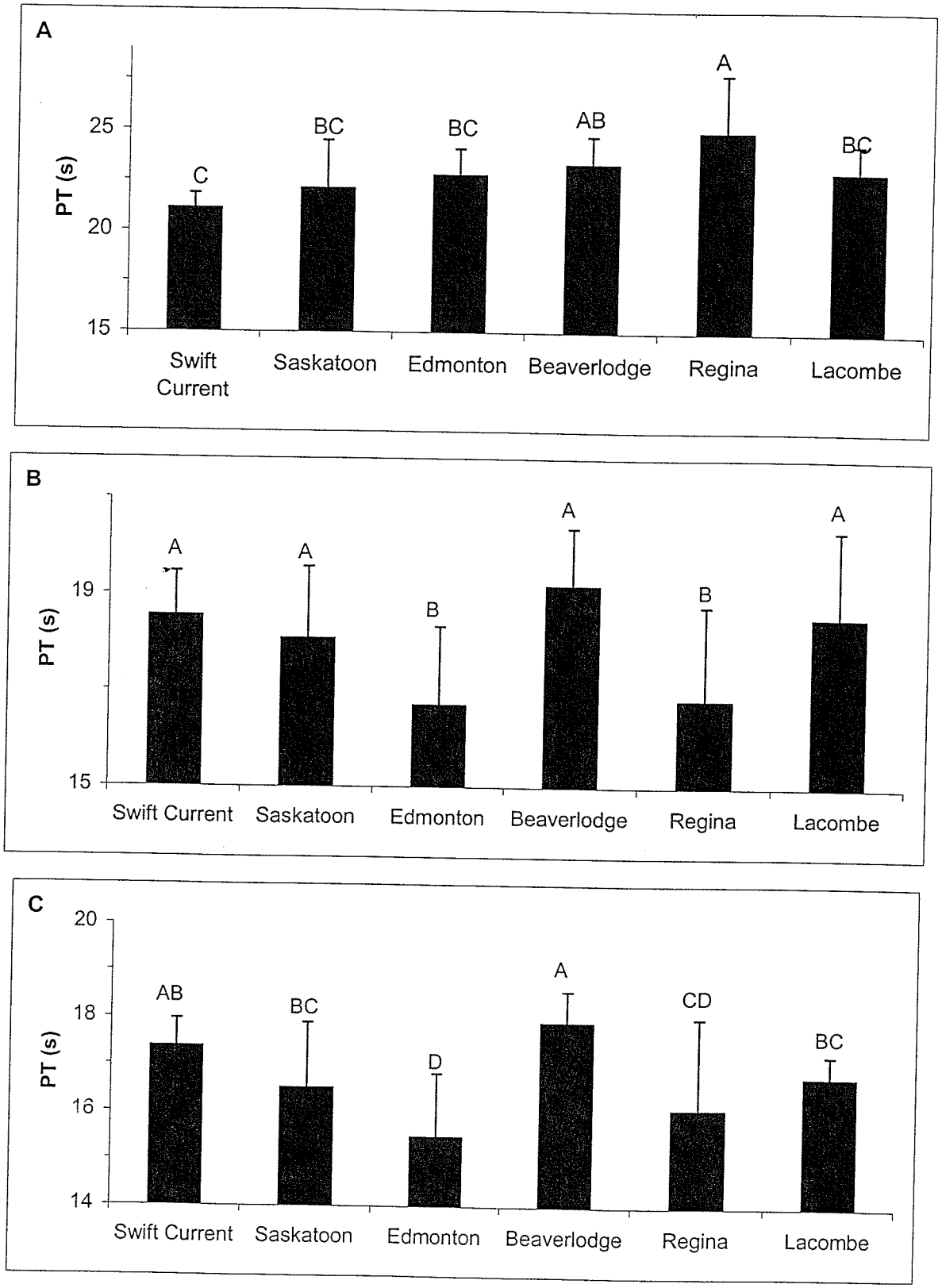


Figure 40. Average Peak Time (PT) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

The CV values for genotype, location and the full sample set all decreased compared to the untreated samples and were now 5.9, 5.3 and 8.2%, respectively (Table 6). There was also a subsequent decrease in the G/E variance ratio to 1.2, which was a slightly larger genotypic influence than environmental, although not significantly (Figure 38). PT provided significant separation in the CWES cultivars, but only when at a CPC.

4.3.2.3 Ascending Slope

Ascending slope (AS) was a measurement of the rate to fracture (see Figure 8), and showed large amounts of variation and low levels of significance. There was no genotypic effect for the untreated samples (Figure 41A); the location effects were significant (Figure 42A). The range of values for genotypes was 5.4 to 5.9 g/s, and the range for locations was 4.8 to 6.4 g/s. The full sample set ranged from 6.4 to 9.7 g/s. The CV values for location and the full sample set were 9.8 and 12.8% respectively, and that for genotype was 3.2% (Table 6). There was a very low G/E variance ratio of 0.10, which was expected with the lack of genotype effects (Figure 38).

The addition of starch to the formulation caused a significant increase in slope. This large increase in slope showed the fragility of the bread slices in the presence of starch. The genotypic effects were small but significant with only two levels of significance (Figure 41B). Again, the location effects were highly significant, and there were four overlapping but significant levels for the cultivars (Figure 42B). The CV values for genotype increased to 5.0%, while both the location and the full sample set declined to

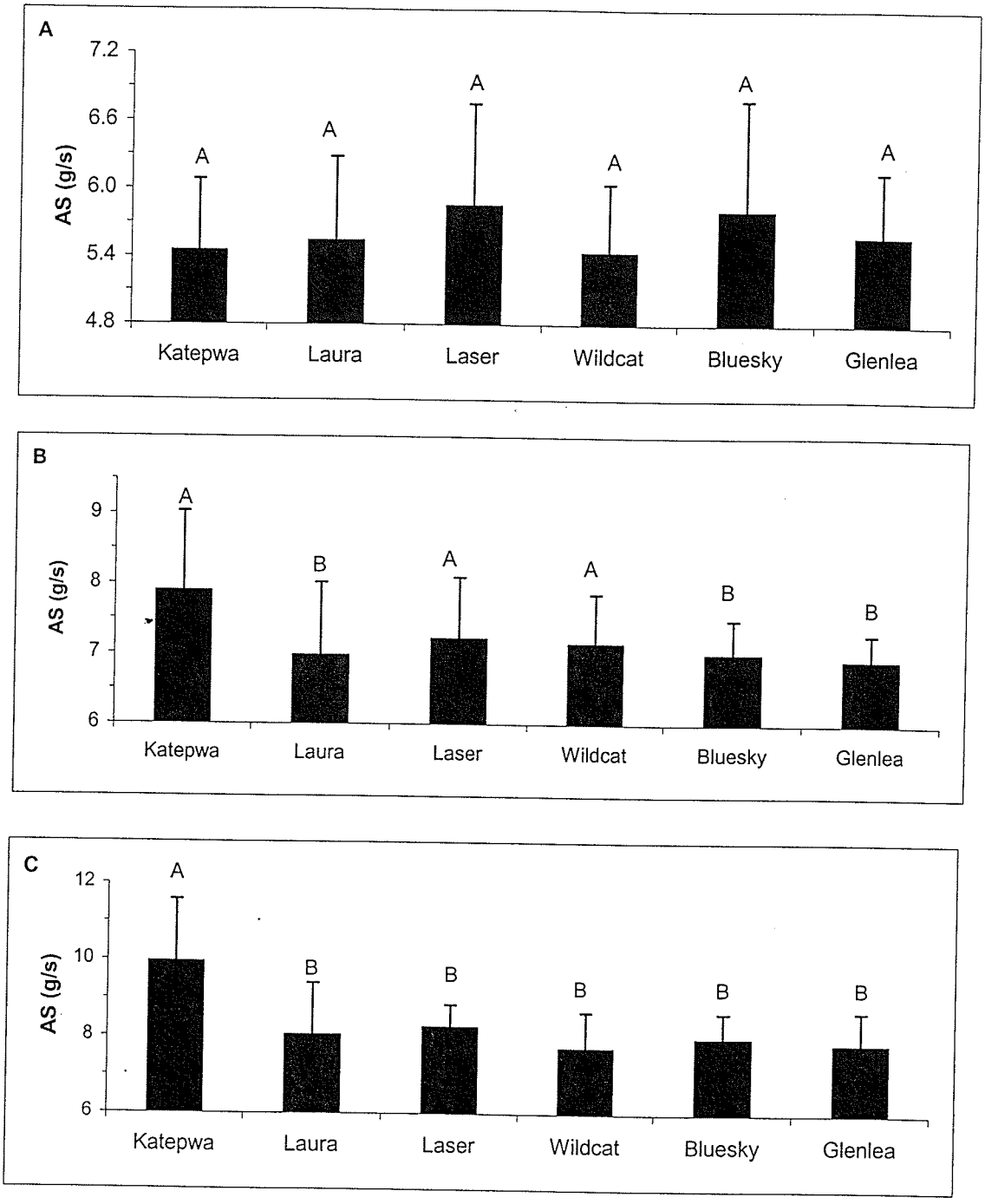


Figure 41. Average Ascending Slope (AS) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

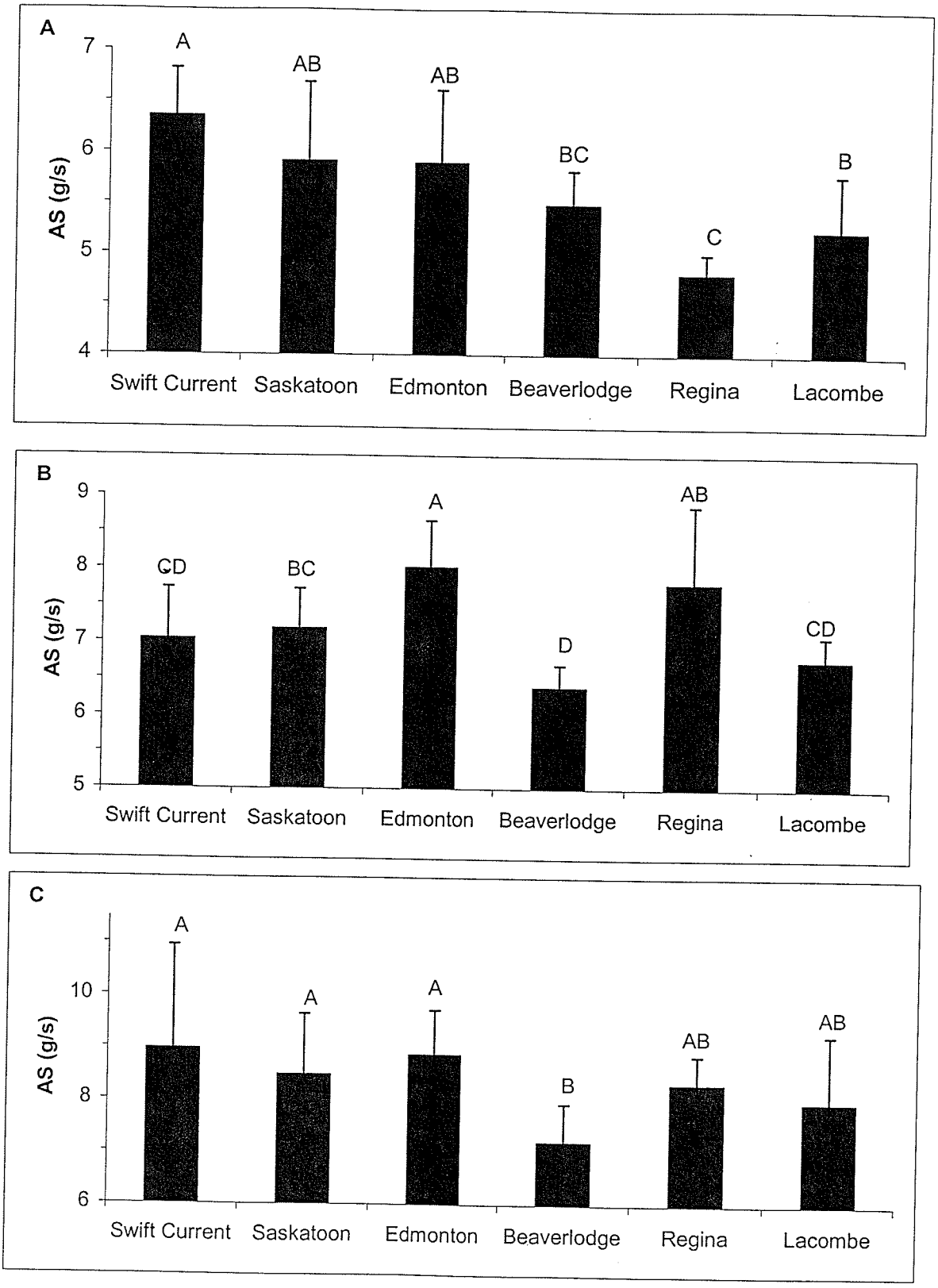


Figure 42. Average Ascending Slope (AS) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

8.6 and 11.6%, respectively (Table 6). The shift in variation was significant, because the G/E variance ratio was still in favour of the location effects, i.e. 0.34 (Figure 38).

Removing shortening from the treatment caused a significant increase in AS compared to the untreated samples. There were significant genotypic effects (Figure 41C), and location effects (Figure 42C) but there was no clear separation of CWES and CWRS, nor was the parameter useful for predicting the strength of the cultivars. The CV values for genotype, location and the full sample set were 10.0, 7.8 and 15.2%, respectively (Table 6). The variation in genotype doubled, while the location variation declined significantly, and resulted in a G/E variance ratio of 1.6 (Figure 38).

4.3.2.4 Curve Area to Peak

Curve area to peak (CAP) was the product of PF and PT, and gave an indication of the relative strength of the bread crumb (see Figure 8). The genotypic effect for CAP was significant for the untreated samples (Figure 43A), and showed significant separation among the cultivars. The range of values for genotype was 1501 to 2224 g*s. The CWES cultivars, with the exception of Glenlea, were significantly greater than the CWRS cultivars. Glenlea was statistically equal to Katepwa, and was significantly lower in CAP than Bluesky; this parameter differentiated between Bluesky and Glenlea quite well. There were no significant location effects (Figure 44A), and the values ranged from 1761 to 2060 g*s. There was no clear indication of bread strength; Bluesky had the highest value but Glenlea was statistically equal to Katepwa. The genotype, location and full sample set CV values were the highest of all texture parameters and were 13.4, 6.1

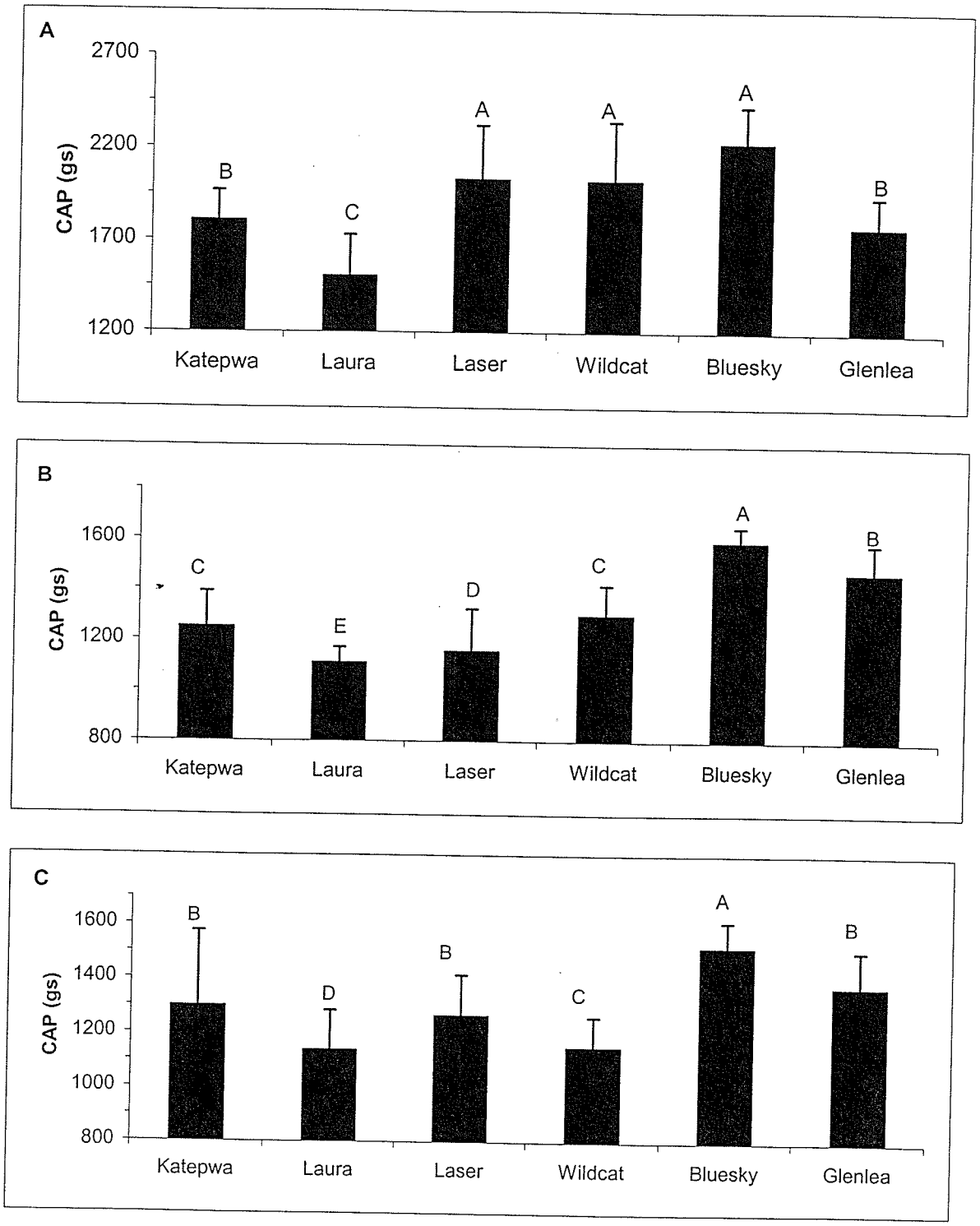


Figure 43. Average Curve Area to Peak (CAP) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

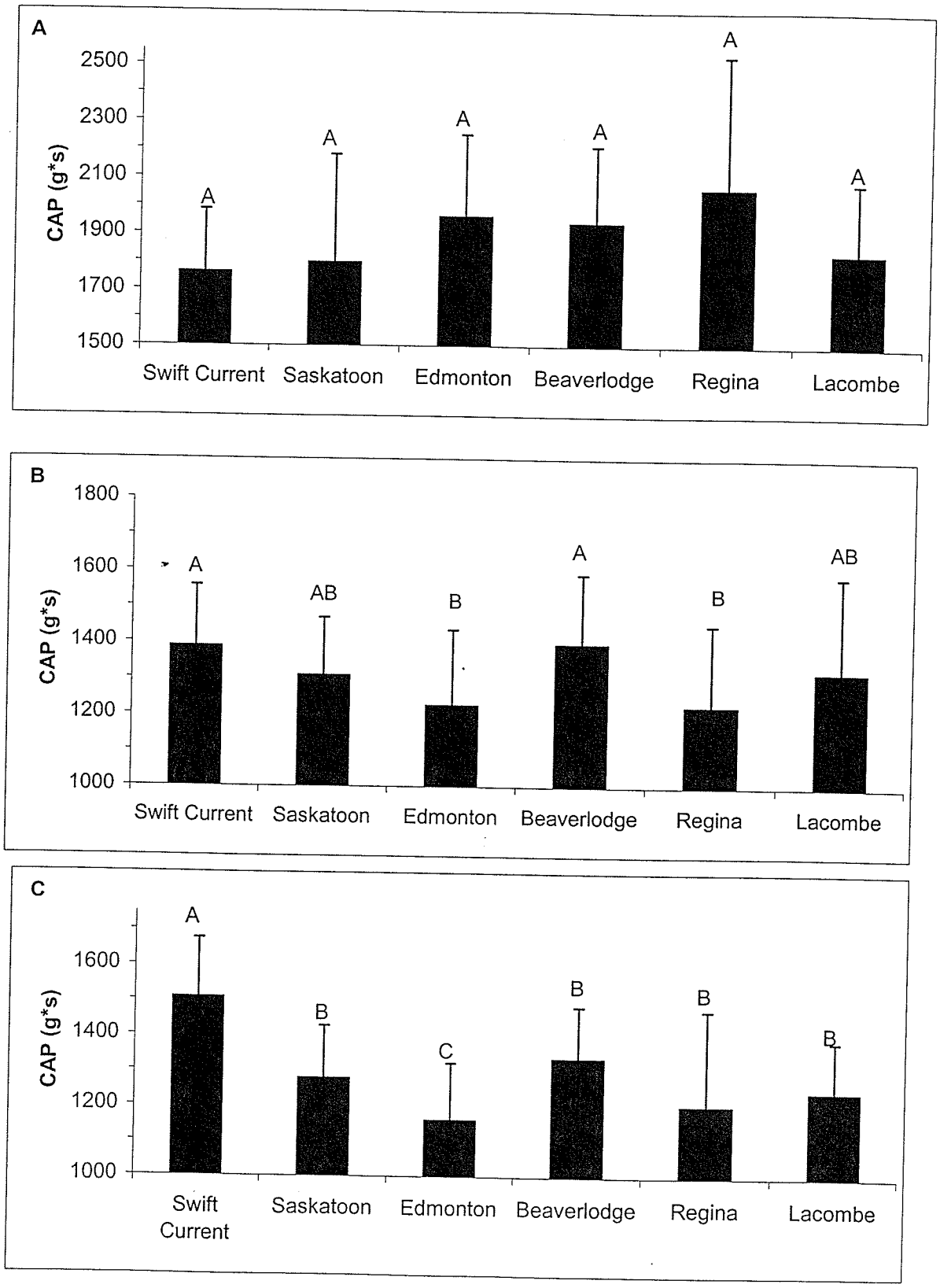


Figure 44. Average Curve Area to Peak (CAP) of Locations: Untreated (A), Starch Diluted (B and Starch Diluted without Shortening (C).

and 17.0%, respectively (Table 6). The G/E variance ratio was 4.9, which reflected the strong genotypic effect (Figure 38).

Treating the samples with starch to achieve CPC of 10% caused a substantial decline in CAP. This was expected due to the significant drop in PF and increase in AS. Genotype was significant, and showed a large division of cultivars loosely based on perceived crumb strength (Figure 43B). Bluesky and Glenlea were at the top of the cultivar list, but Bluesky was significantly greater than Glenlea. This time, Glenlea was significantly greater than Katepwa. Although the location effect was small, it was significant (Figure 44B). The CV values increased for genotypes to 5.0, but decreased for the locations and full sample set, 8.6 and 11.6%, respectively (Table 6). The increased genotypic CV resulted in an increased G/E variance ratio of 6.2 (Figure 38).

With the removal of shortening, the rankings remained the same, but the values decreased the same amount as with shortening present. Glenlea and Bluesky maintained the highest CAP values, but Glenlea was again equal to Katepwa (Figure 43C). This suggested that shortening had a great impact on maintaining the CAP of Glenlea, and the other cultivars. Location effects were also significant (Figure 44C). The CV values decreased for genotype, increased for locations, and did not significantly change for the full sample set (Table 6). The variation did change substantially as measured by the G/E variance ratio, which was reduced to 1.3 from 6.2 (Figure 38) clearly showing a decline in the genotypic effect due to the removal of shortening. The results showed that bread from starch-treated samples were significantly stiffer than untreated samples (Figure 43A). The starch treatment increased the fragility of the bread structure, and resulted in a decrease in PT and PF values.

4.3.3 Structural Properties of Strong-Mixing Cultivars

4.3.3.1 Cell Density

Cell density (CD) was a measurement of the quantity of cells, regardless of size, per unit area. It gave an indication of the relative cell size for the slice of bread. CD showed a significant effect for location, and a very minor genotypic effect. The ranges of values for genotype were 79.4 to 82.3 cells/ mm^2 (Figure 45A), and the range for the locations was 79.7 to 84.2 cells/ mm^2 (Figure 46A). There was a significant distinction among the cultivars, but no consistent trend according to dough strength. The impact of locations was due to the greater CD for the breads prepared from samples grown in Edmonton compared to other locations that were equal to one another. The CV values were 1.4, 2.1 and 3.0% for genotypes, locations and the full sample set, respectively (Table 7). The G/E variance ratio was 0.46 suggesting a significant environmental effect (Figure 47).

Starch addition resulted in very little statistical change, and very little difference in values. The genotypes lacked significant differences in CD, which suggested that starch had no affect on the genotypes (Figure 45B); the effect of a CPC on the locations was significant (Figure 46B). This was evident by the slight increase in CV for location (3.3%), and the decrease in the genotypic variation to 0.5% (Table 7). Although the G/E variance ratio was 0.03, it was not a significant value since there was no genotypic affect (Figure 47). Starch had no significant impact on the sample set for the density of the cell structure.

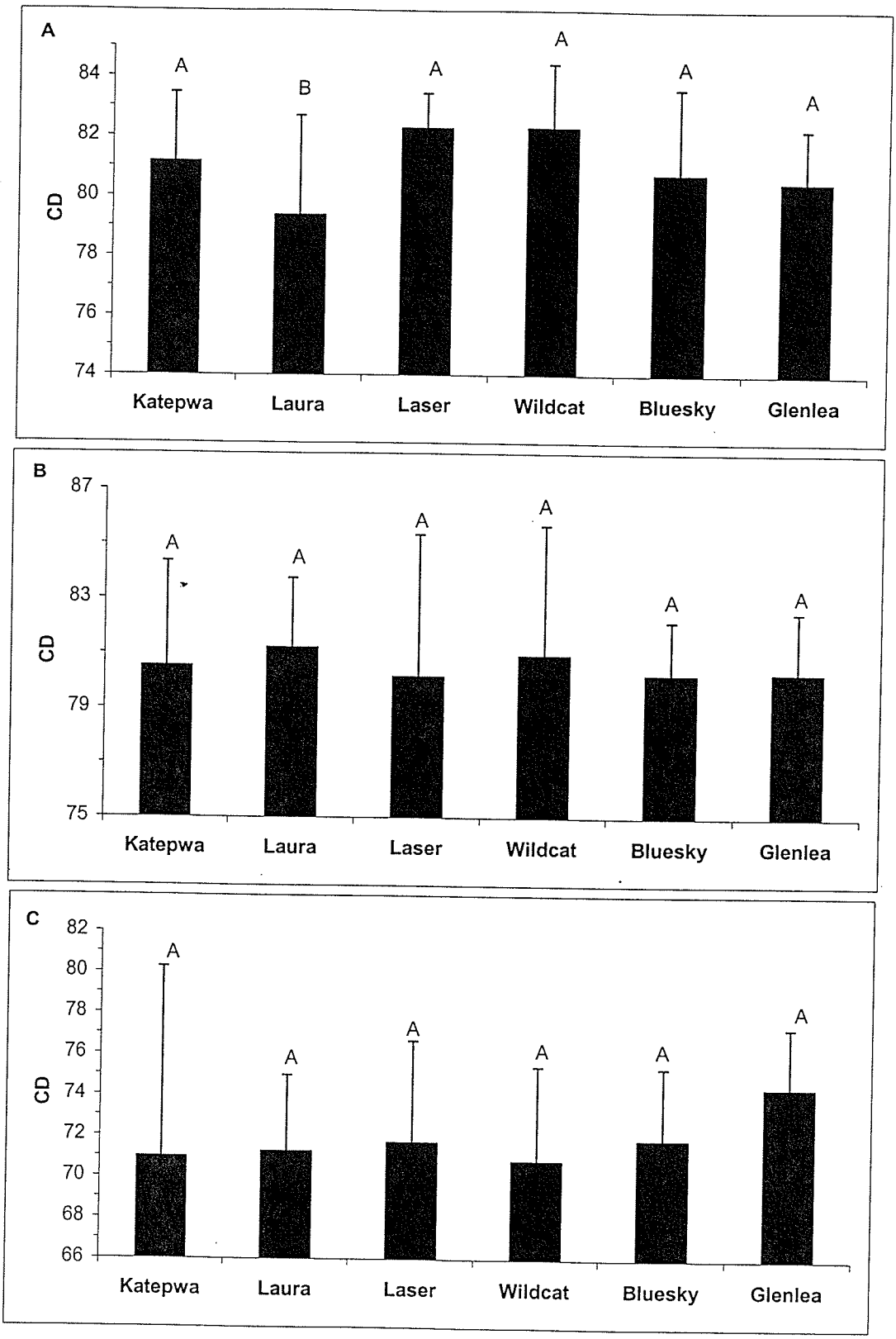


Figure 45. Average Cell Density (CD) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

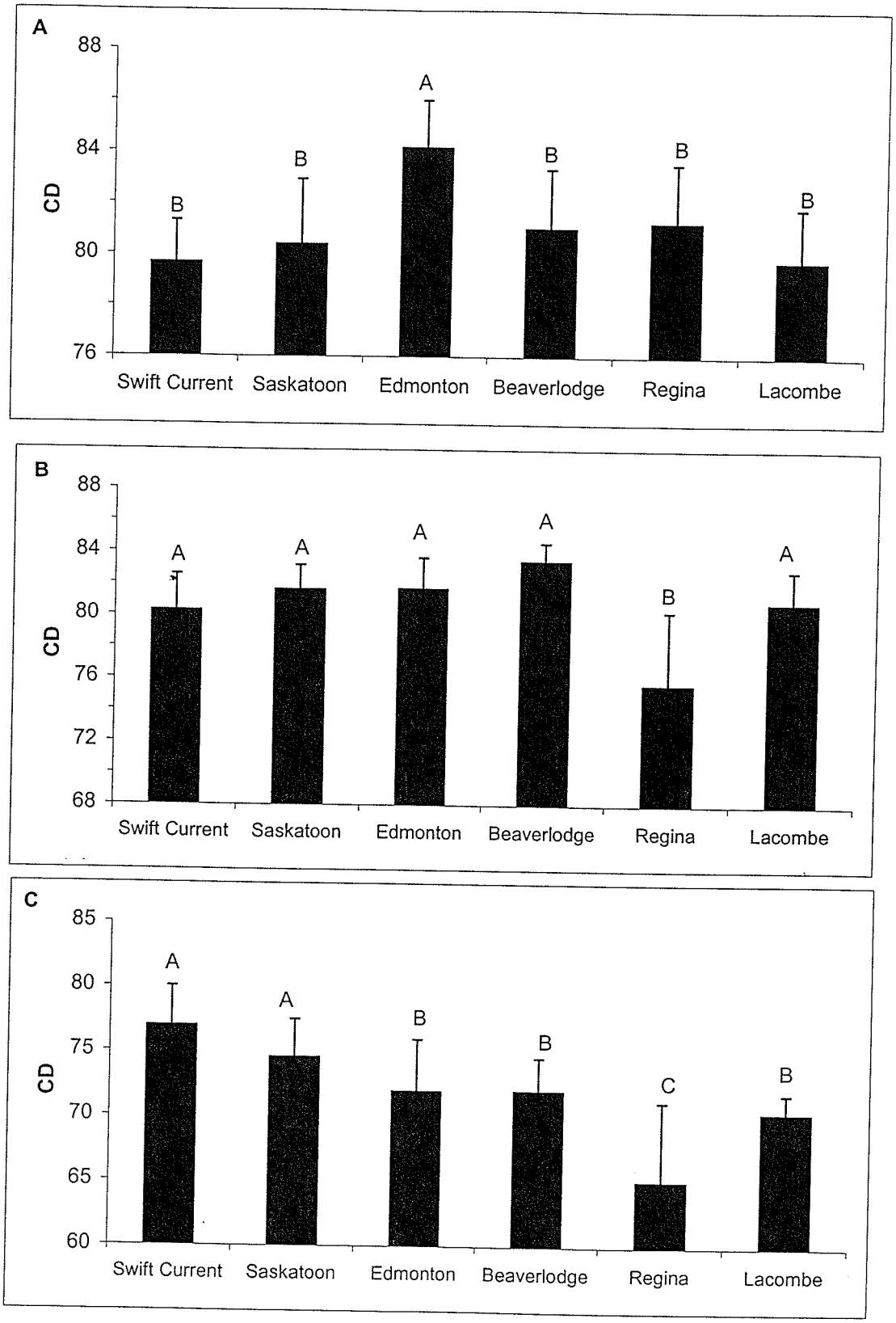


Figure 46. Average Cell Density (CD) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

Table 7

Coefficients of variation (CV) of structural parameters for effects of genotype, environment, and full sample set.

		Coefficients of Variation (%)		
Treatment	Parameter*	Genotype	Location	Full Set
Control	CD	1.39	2.05	3.00
	CWT	4.19	1.65	4.90
	CU	2.12	2.19	4.31
	GL	1.16	1.34	2.59
Starch	CD	0.53	3.28	4.22
	CWT	2.89	1.78	4.05
	CU	0.89	5.12	6.30
	GL	2.11	1.37	3.27
Starch without Shortening	CD	1.83	5.62	7.03
	CWT	6.22	3.35	6.87
	CU	4.70	7.57	10.27
	GL	3.78	4.40	6.04

*CD= cell density, CWT= cell wall thickness, CU= cell uniformity, GL= gray level

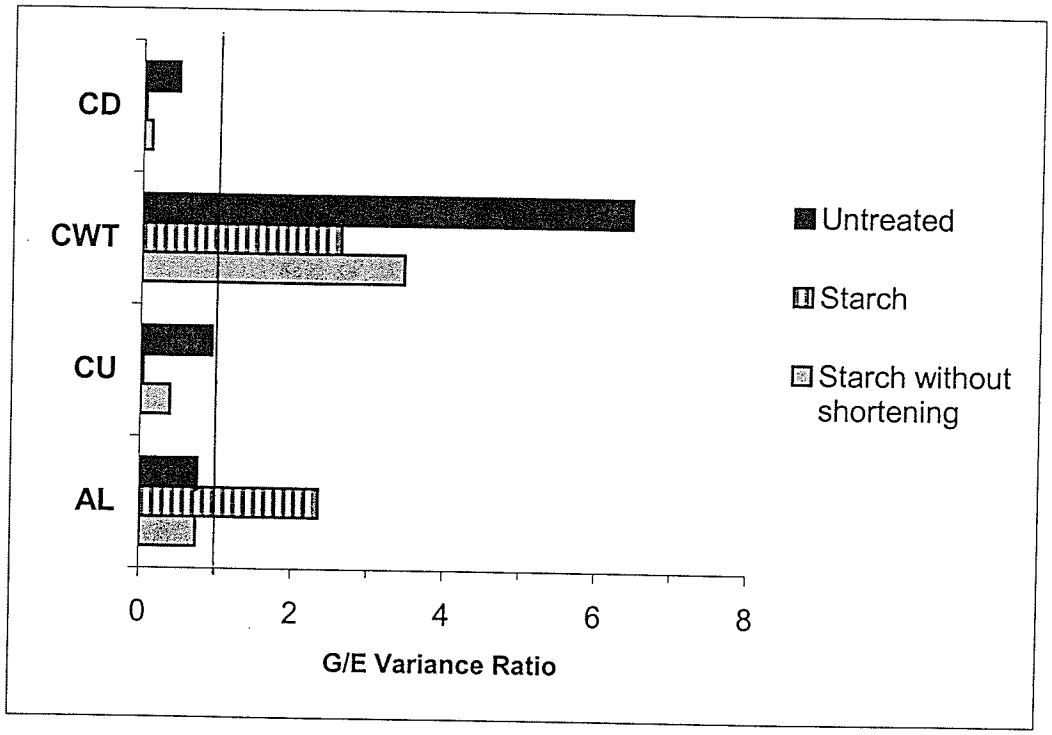


Figure 47. Ratio of Genotypic to Environmental (G/E) Variance for Structural Parameters. Equal Contributions of Genotype and Environmental Influence designated by Vertical Line.

Removing shortening caused a significant decline in CD for the samples. There was no significant genotypic effect (Figure 45C), but the location effect was significant (Figure 46C). The CV values for genotype, location and the full sample set were 1.8, 5.6 and 7.0%, respectively (Table 7). The G/E variance ratio (0.11) favoured the environmental effect (Figure 47).

4.3.3.2 Cell Wall Thickness

Cell wall thickness (CWT) was a measurement of the thickness of the walls between the bubbles in a slice of bread, and is an important parameter for indicating strength of cultivars. The genotype effect was significant and provided very clear separation of the CWES from the CWRS class (Figure 48A); the range of values for cultivars was 697 to 759 μm. This suggested that the gluten matrix of the stronger cultivars had the ability to withstand the stresses of breadmaking because of the quality of their protein. They have a greater quantity of the HMW-GS (insoluble glutenin) that asserts the strength effect in this parameter. The location effect was not highly significant (Figure 49A), and ranged from 708 to 739 μm. The CV values for genotype, location, and the full sample set were 4.2, 1.7, and 4.9%, respectively (Table 7). There was a substantial genotypic effect, which was evident by the high G/E variance ratio of 6.4 (Figure 47).

Diluting the samples to a CPC caused a 4% decrease in the CWT for cultivars and locations. This was not a large decrease, but it did cause differences in the samples. The genotypic effect was still significant and dominant over the location effect; it showed a greater degree of separation but with more overlapping (Figure 48B). The location effect

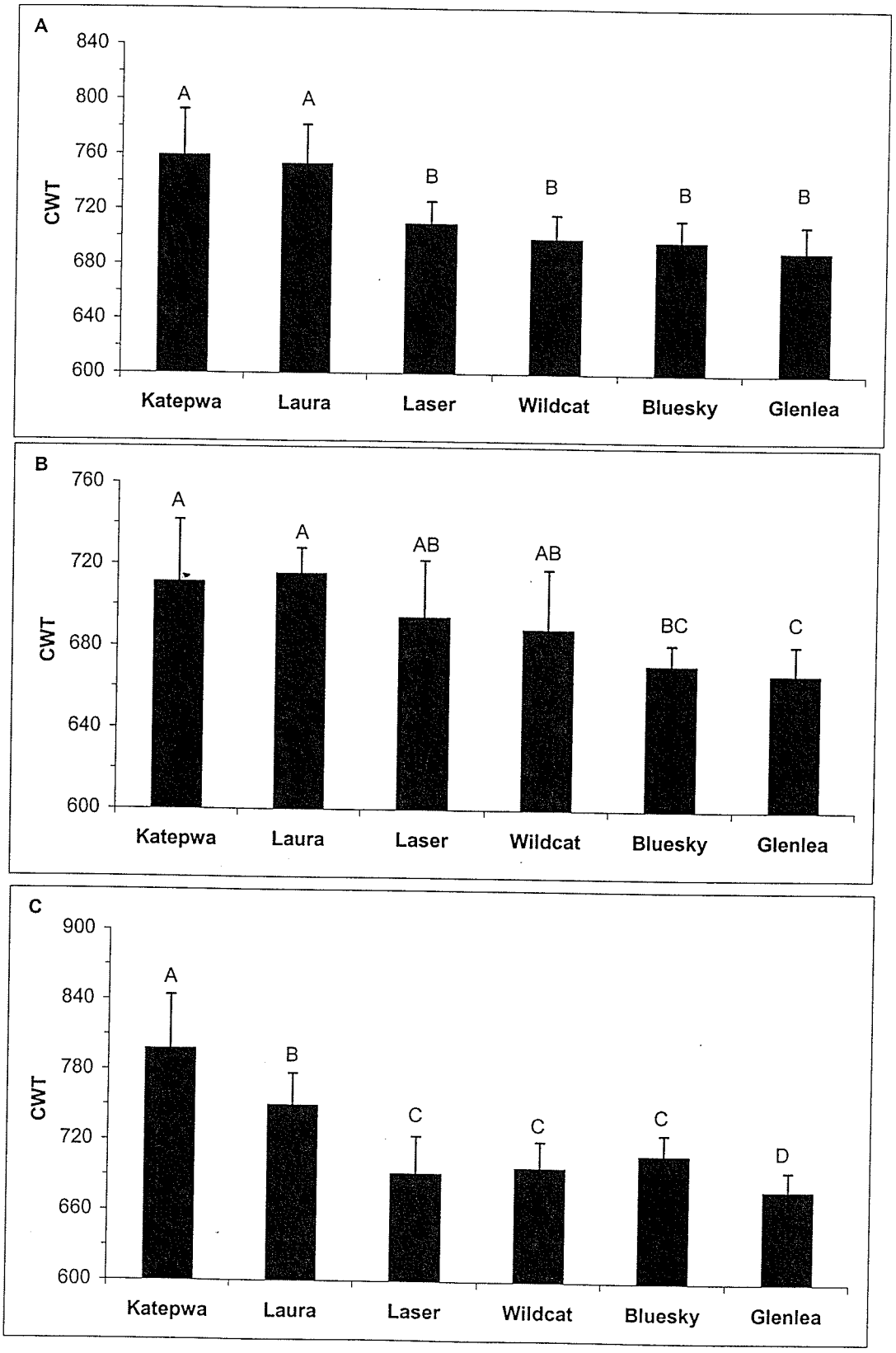


Figure 48. Average Cell Wall Thickness (CWT) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

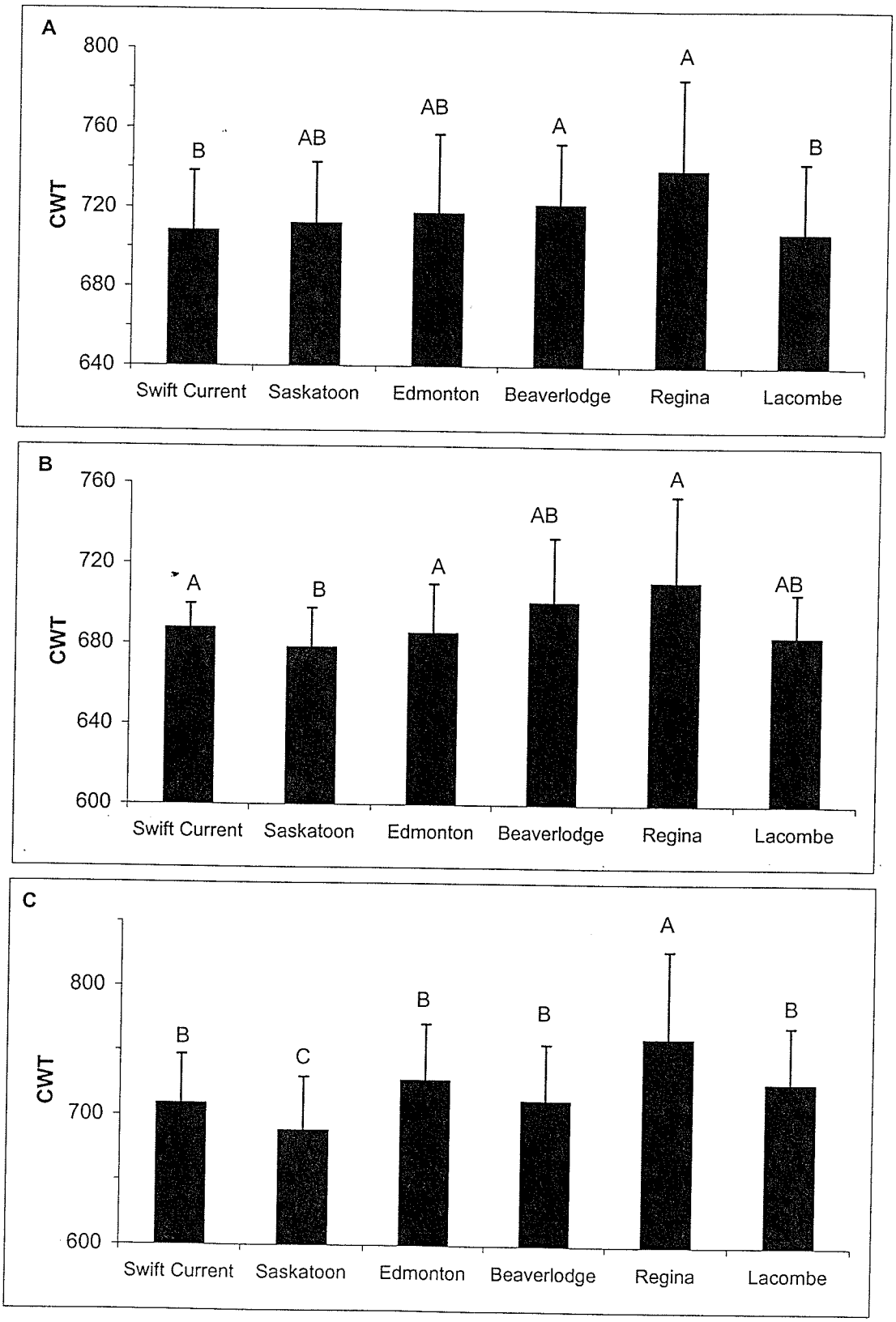


Figure 49. Average Cell Wall Thickness (CWT) of Locations: Untreated (A), Starch Dilutec (B), and Starch Diluted without Shortening (C).

was significant, but still had less variation than the genotypes (Figure 49B). The CV values for genotype decreased to 2.2%, locations increased to 1.8%, and the full sample set decreased to 4.1% (Table 7). The decrease in variation also led to the decline in the G/E variance ratio to 2.62 (Figure 47).

By removing shortening, virtually no change in CWT was found with the untreated samples. This suggests that shortening had a much stronger and more important effect than starch on CWT. The genotypic and location effects were both significant, but the genotype effect was still greater. There was good separation between the two classes and within each class for the cultivars examined, suggesting that CWT is an important parameter distinguishing differences among cultivars (Figure 48C). The CWRS cultivars were significantly greater than the CWES, which in turn were separated into two groups thus showing the gluten strength of the CWES class. Locations had a significant effect, but the effect was not as large as for genotypes (Figure 49C). The CV values increased for genotype, location and the full sample set to 6.2, 3.4 and 6.9%, respectively (Table 7). This suggested that shortening masked differences in CWT by helping the weaker cultivars to achieve the same potential as the CWES cultivars. The G/E variance ratio of 3.5 reflected the significant genotypic influence (Figure 47). Starch dilution without shortening played a significant role in separating the cultivars, because shortening had a tendency to push weaker cultivars to greater levels that could not be reached without shortening.

4.3.3.3 Cell Uniformity

Cell uniformity (CU) is a measure of the ratio of small to large cells within a slice of bread. CU showed significant differences for genotypes and locations. The range of CU for cultivars was 31.9 to 33.6 (Figures 50A), and the range for locations was 31.6 to 33.7 (Figure 51A). The CV values were 2.1 and 2.2% for genotypes and locations, respectively; the CV for the full sample set was 4.3% (Table 7). The G/E variance ratio was 0.9, which along with the low CV values made it difficult to conclude which effect was more influential on the CU (Figure 47).

With the addition of starch, the significant genotypic effect was lost, while the location effect remained significant (Figures 50B and 51B). The CV values for genotype, location, and the full sample set became 0.9, 5.1, and 6.3%, respectively (Table 7). This substantiated the large environmental variation, and the significance of the location effect. The G/E variance ratio also decreased significantly to 0.03 (Figure 47), suggesting a significant environmental effect on CU. The CPC treatment resulted in an increase in the magnitude of the location effect and a loss of the genotypic effect. This result was different from results achieved for other parameters where the CPC usually resulted in an increase in genotypic effect.

Removing shortening from the baking formulation decreased the CU values for all sample cultivars by about 10%. This treatment resulted in an increase in the genotypic effect (Figure 50C), but with overlapping levels of significance. The location effect remained essentially the same (Figure 51C). The CV values increased for both the genotypes and locations to 4.7 and 7.6%, respectively (Table 7). The G/E variance ratio

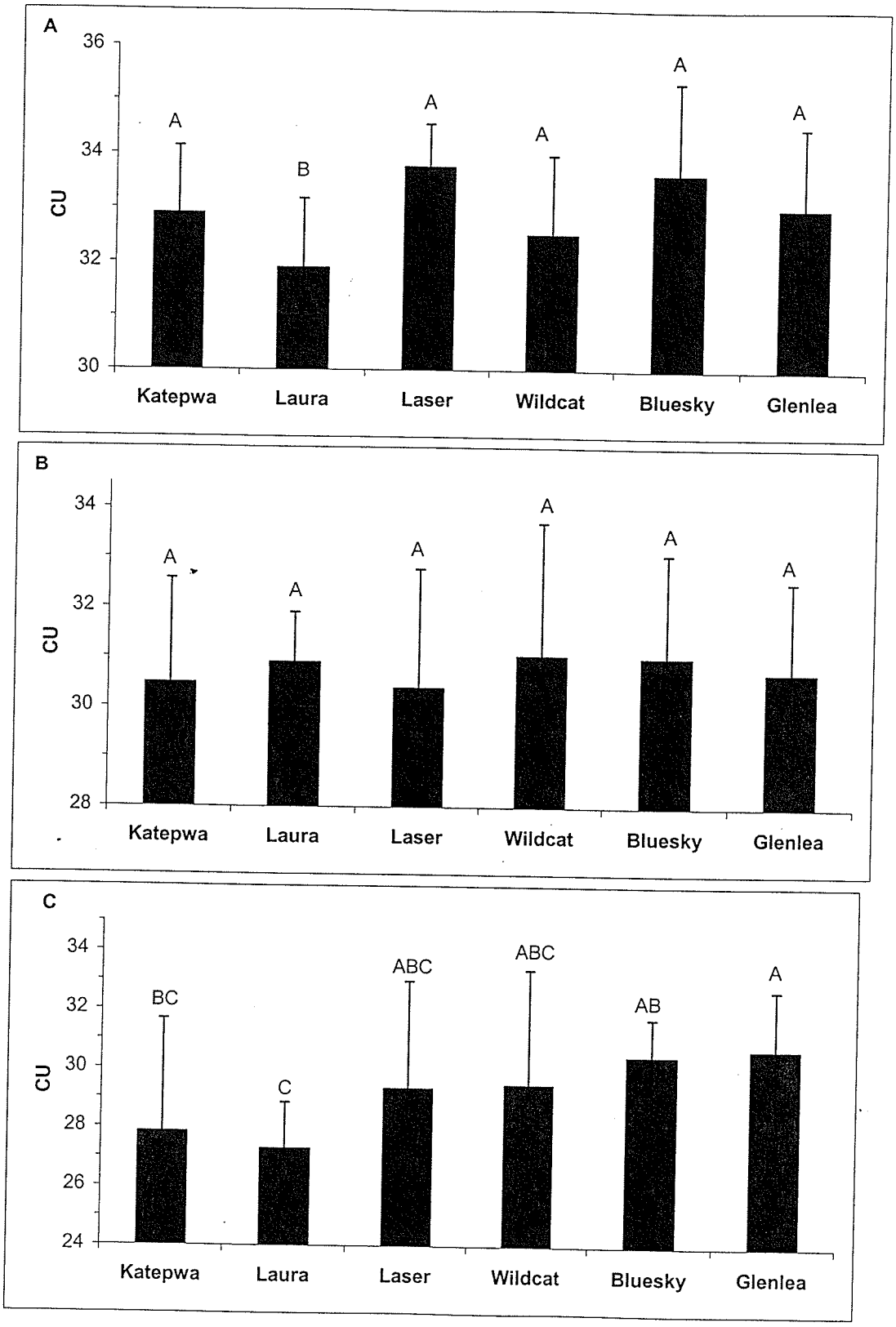


Figure 50. Average Cell Uniformity (CU) of Cultivars: Untreated (A), Starch Diluted (B), and starch Diluted without Shortening (C).

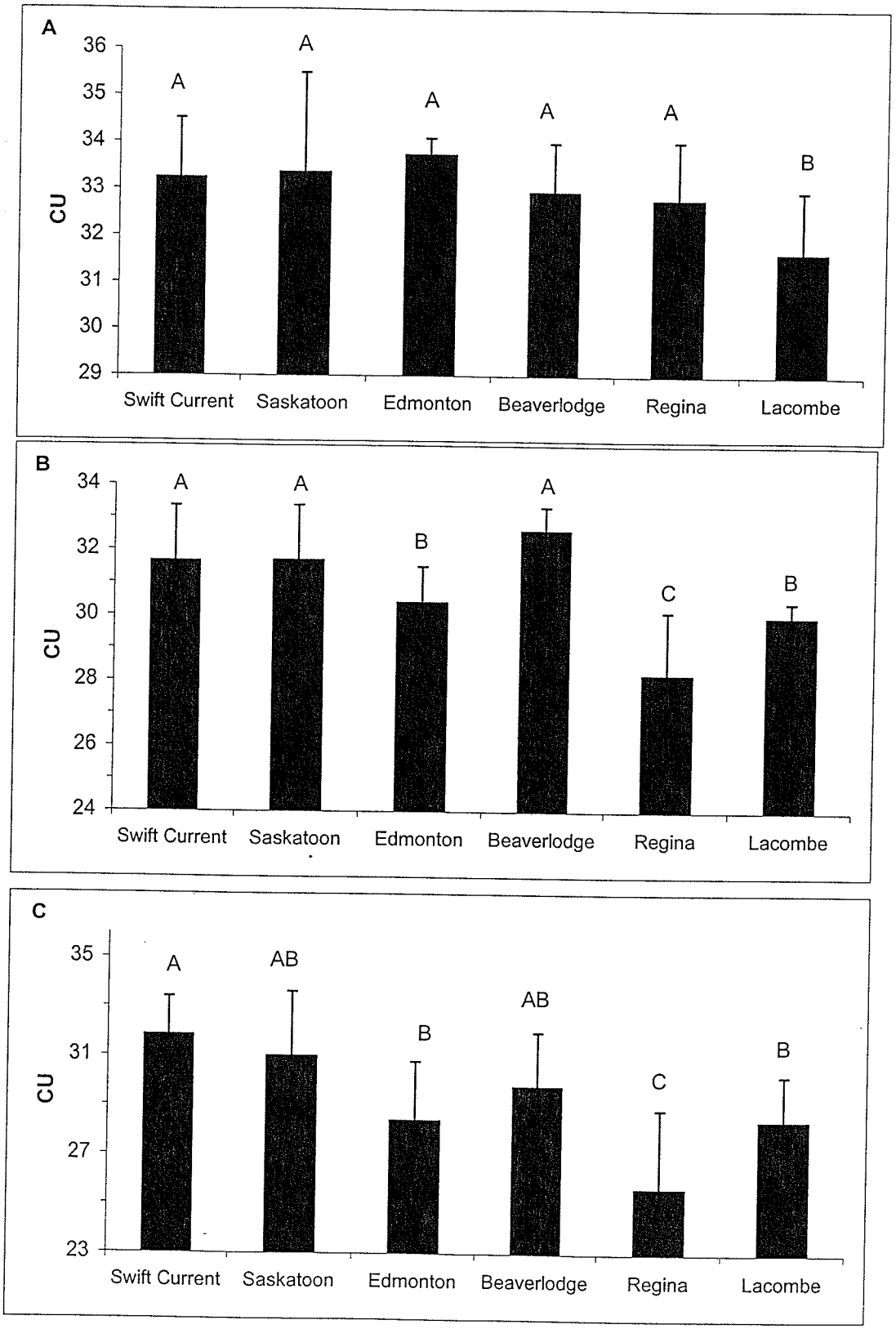


Figure 51. Average Cell Uniformity (CU) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

also increased from 0.03 to 0.4 (Figure 47), suggesting a significant location effect. CU was highly dependent on growing location and showed that inherent genetic material played a minor role in the uniformity of bread cell structure.

4.3.3.4 Gray Level

Gray level (GL) is the colour of a slice of bread based on a Kodak grey-scale. GL had no significant genotype or location effects (Figures 52A and 53A). The CV values in turn were small and not significant (Table 7), and the G/E variance ratio of 0.75 was not considered to be a useful number, since there were no significant effects associated with the samples (Figure 47).

Starch caused a slight increase in the GL of the samples of about 2%. The range of values for genotypes was 119.9 to 123.6 (Figure 52B), and showed significant separation of cultivars. The range of values for locations was 119.6 to 123.3 (Figure 53B), and showed significant separation of locations. The CV value for genotype increased to 2.1 and the CV for location remained the same (Table 7). The G/E variance ratio increased to 2.4 (Figure 47). The increase in the genotypic effect of GL was opposite to the CU trend, suggesting that GL was a more inherent trait than cell uniformity.

By removing shortening from the formulation, a number of changes were observed. The overall values of GL decreased by an average of 8%, and both genotypes and locations increased in significance. Glenlea and Bluesky were significantly greater in GL than all the other cultivars in the set suggesting a brighter slice (Figure 52C). The darkening of the slice can be attributed to the lack of shortening, which clearly made

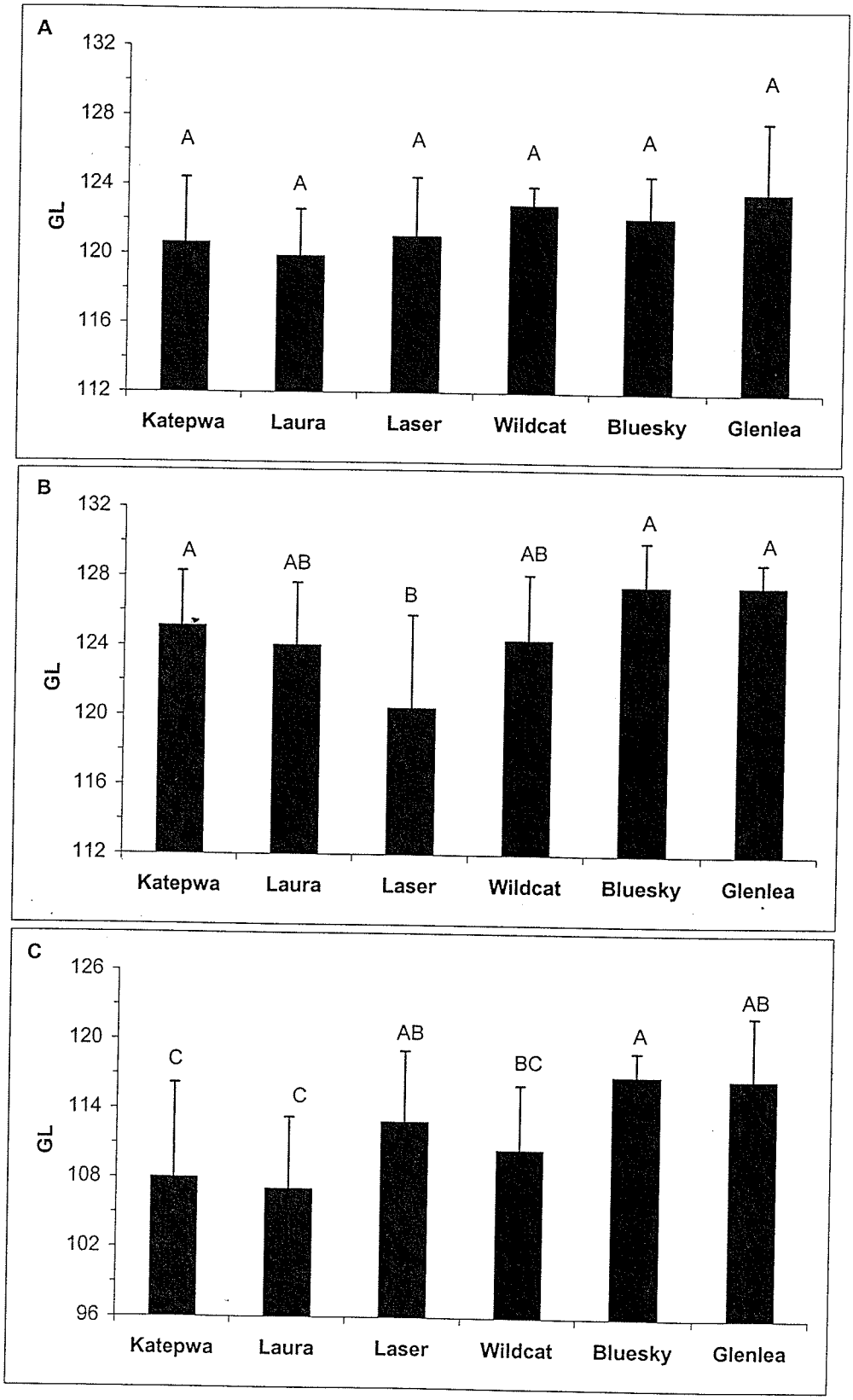


Figure 52. Average Gray Level (GL) of Cultivars: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

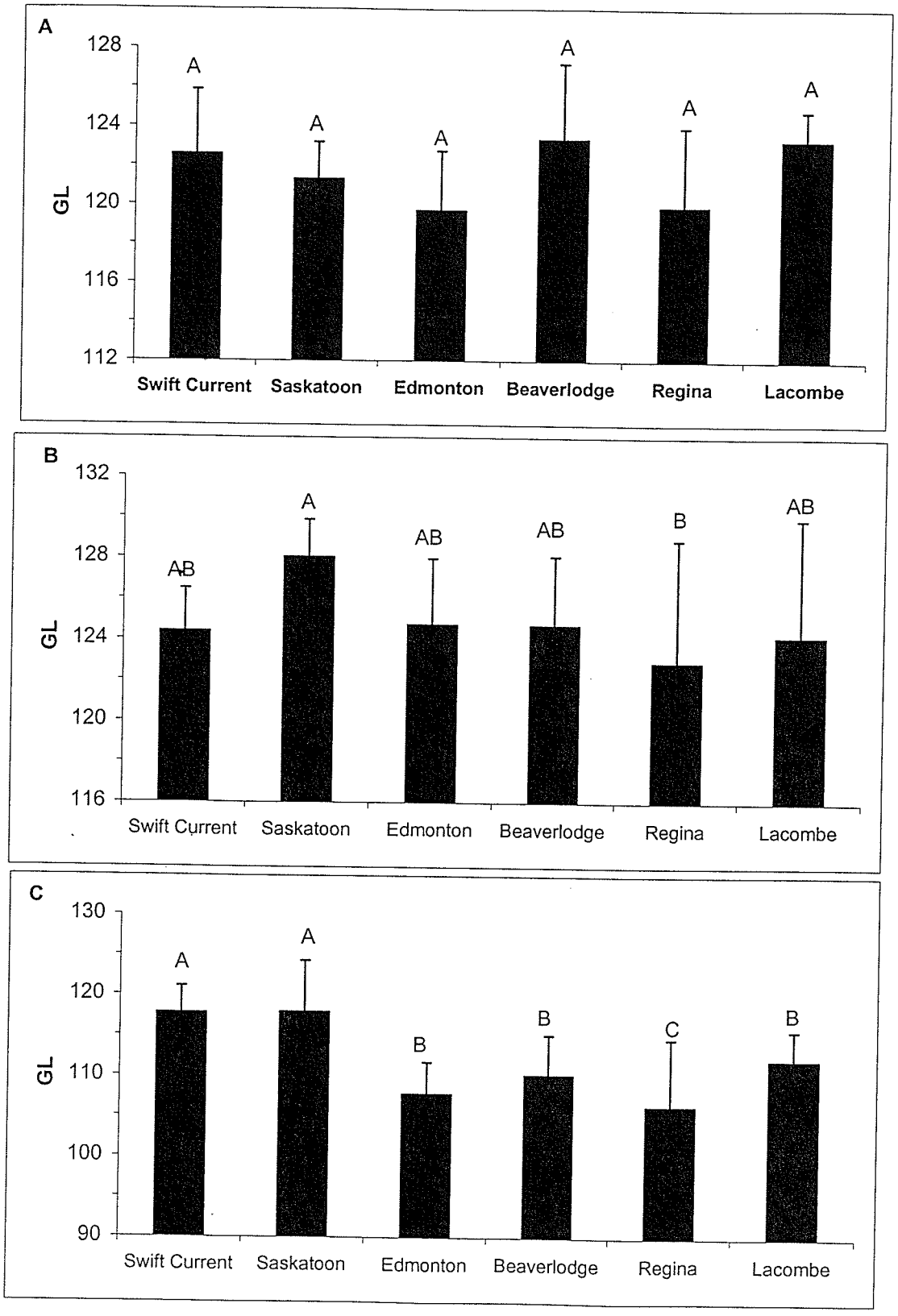


Figure 53. Average Gray Level (GL) of Locations: Untreated (A), Starch Diluted (B), and Starch Diluted without Shortening (C).

shortening important for the colour of bread. The locations also became more significant (Figure 53C). The CV values for genotype, location, and the full sample set were 3.8, 4.4, and 6.0%, respectively (Table 7). The increase in the effects was paralleled by an increase in significance of the G/E variance ratio of 0.7, which reflected the location effect (Figure 47). The colour of the bread slice only became apparent when baked at a CPC, and was more substantial with the removal of shortening.

4.4 Relationships of Protein Content and Protein Quality with Dough and Baking Quality Parameters

4.4.1 Relationships Between Protein Compositional Parameters

Relationships between the protein compositional parameters are shown in Table 8. There were significant relationships between FPC and SPC (% flour, r=0.85) and RPC (% flour, r=0.78), whereas, IGC (% flour) did not correlate well with FPC (r=0.42). FPC, SPC (% flour), and RPC (% flour) did not show any more large correlations with the HPLC parameters. As expected, IGC (% flour) correlated well with HMW-GS, LMW, GS, and TGS, because the fractions were essentially the same based on the extraction procedure. The correlations between the normalized values of the parameters increased (Table 8). SPC/ FPC was negatively correlated with every protein parameter. There were also significant correlations between IGC/FPC and IGC/SPC with the normalized values of the three HPLC parameters, but not with HMW-GS/LMW-GS. Normalisation increased the significance of the correlations.

An important relationship with SPC, IGC and RPC as a function of FPC is shown (Figure 54). The linear regression showed that as FPC increased, all protein components

increased, but SPC increased at a greater rate than RPC and IGC. The linear equations of each parameter were SPC (y = 0.5255x + 0.4982), IGC (y = 0.1189x + 1.289) and RPC (y = 0.3556x - 1.7873). Compared to SPC and RPC, the IGC did not change much along the entire range of FPC and was much more variable. RPC initially had a lower quantity than IGC, but as FPC increased, RPC surpassed IGC. Therefore, according to these findings, when diluting the samples to a CPC, the higher FPC samples were getting their SPC and RPC diluted more than IGC.

4.4.2 Relationships of Protein Composition and Mixing Parameters

PDR displayed highly variable results with no clear indication of cultivar quality according to dough strength. This was apparent when PDR showed significant separation between Glenlea and Bluesky, even though they were already shown to be statistically equal in mostl other parameters. PDR showed significant relationships with a select group of mixing parameters (Table 9). PDR achieved the highest relationship with FPC (r= 0.53) as was expected, but it was not a strong relationship. In addition, PDR correlated well with HMW-GS (r= 0.72) and IGC (r= 0.75), which was a surprise. PDR was traditionally well correlated with FPC and not usually with protein quality. Perhaps, the sample set of CWES cultivars caused this result, because there has never been a mixing study that focused on extra strong cultivars. With the addition of starch, the significance of correlations declined for all parameters (Table 10), except for IGC/ SPC (r=0.75), which maintained its high correlation. The removal of FPC allowed the protein quality parameters to show a relationship that was initially unable to determine because of the FPC. Without removing FPC, i.e. a dominant environmental effect, the

Table 8

Correlation Matrix (r) of Protein Compositional Parameters*.

	FPC	SPC (%flour)	SPC/ FPC	IGC (%flour)	IGC/ FPC	RPC (%flour)	RPC/ FPC	IGC/ SPC	HMW	HMW/ FPC	LMW	LMW/ FPC	TGS	TGS/ FPC	HMW/ LMW
FPC	1														
SPC(%flour)	0.85	1													
SPC/ FPC	-0.13	0.41	1												
IGC(%flour)	0.42	0.10	-0.54	1											
IGC/ FPC	-0.35	-0.56	-0.44	0.70	1										
RPC(%flour)	0.78	0.45	-0.51	0.19	-0.44	1									
RPC/ FPC	0.42	0.05	-0.65	-0.03	-0.39	0.89	1								
IGC/SPC	-0.21	-0.57	-0.71	0.75	0.94	-0.15	-0.06	1							
HMW GS	0.46	0.09	-0.61	0.94	0.60	0.31	0.12	0.70	1						
HMW GS/ FPC	-0.17	-0.46	-0.58	0.74	0.90	-0.19	-0.17	0.91	0.80	1					
LMW GS	0.51	0.20	-0.49	0.87	0.51	0.31	0.06	0.59	0.90	0.66	1				
LMW GS/ FPC	-0.23	-0.44	-0.42	0.63	0.85	-0.30	-0.29	0.82	0.64	0.87	0.71	1			
TGS	0.48	0.13	-0.57	0.92	0.58	0.31	0.09	0.67	0.96	0.74	0.98	0.71	1		
TGS/ FPC	-0.24	-0.50	-0.51	0.68	0.90	-0.27	-0.25	0.89	0.70	0.95	0.69	0.97	0.73	1	
HMW GS/ LMW GS	0.04	-0.21	-0.47	0.44	0.41	0.10	0.14	0.47	0.53	0.56	0.12	0.09	0.30	0.28	1

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits

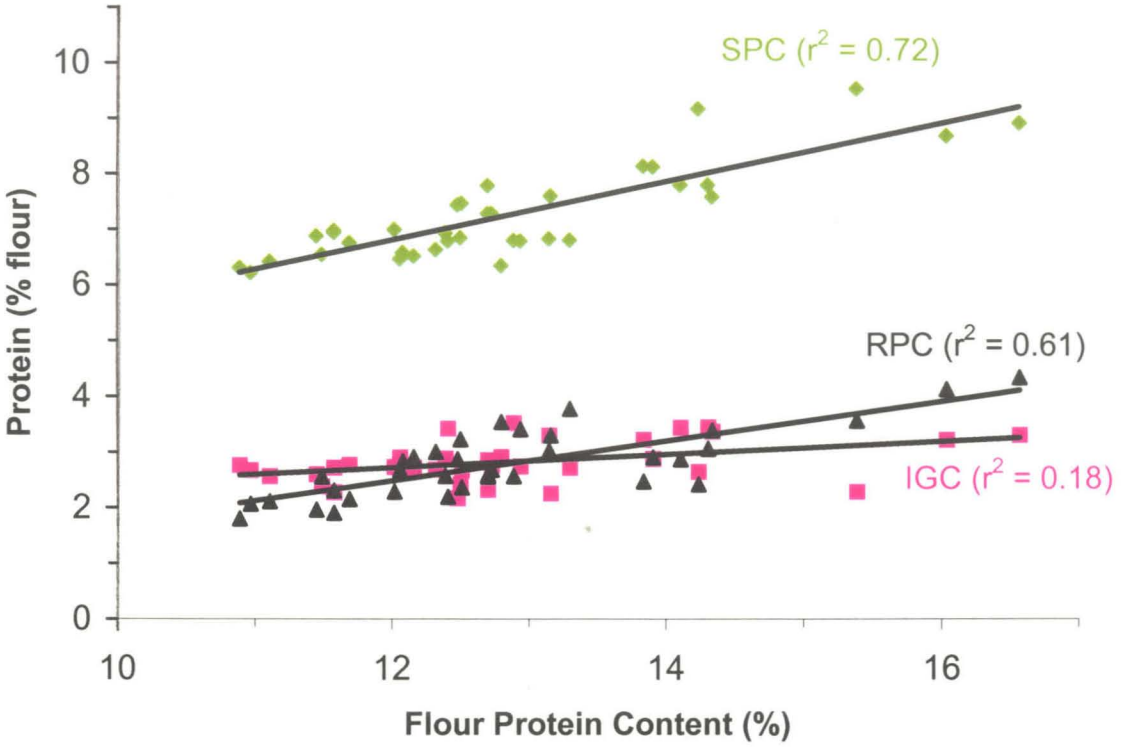


Figure 54. Scatterplot of Soluble Protein Content, Insoluble Glutenin Content, and Residue Protein Content as a function Flour Protein Content. The Linear Regression Equations were: SPC- y = 0.5255x + 0.4982, IGC- y = 0.1189x + 1.289 and RPC- y = 0.3556x - 1.7873 .

functionality of protein quality would not be realized for this sample set. The addition of salt did not change the relationships (Table 11), and the strength of correlations were similar in magnitude to the untreated samples. This was also shown with the starch and salt treated samples (Table 12), except that these results resembled the starch treated values. PDR correlated with protein quality parameters, but not to as large an extent as the remaining three mixing parameters.

BW correlated significantly with the protein quality parameters HMW-GS, TGS, IGC (% flour) and IGC/SPC (Table 9). BW correlated better with the quality parameters of protein than PDR did, thus showing a link with protein quality. In fact, the best relationship was with IGC (% flour, r= 0.83). The addition of starch (Table 10) caused significant increases for HMW-GS, TGS, IGC, and IGC/SPC. With the minimizing of the environmental effect (normalization for protein content), the protein quality parameters increased in their relationships. IGC/FPC was highly associated with BW, while SPC/FPC, LMW-GS/FPC and RPC/FPC were not. Salt treatment (Table 11) increased the BW of each cultivar, but did not change the correlations compared to the untreated samples. A similar result was shown when both salt and starch were added (Table 12). BW was more significant for predicting protein quality than PDR, but MT and WIP were more significant and useful.

MT and WIP were closely related to each other, as shown by the earlier analysis (Figures 20 and 27), therefore they will be discussed together. WIP was usually more strongly correlated than MT with all parameters for all treatments (Tables 9-12). Treating the samples with starch and the combination of starch and salt lead to an increase in strength of the correlations, while salt caused no change compared to the untreated samples. There

Table 9

Correlation coefficients (r) of average protein compositional parameters and untreated mixing parameters*.

	MT	BW	PDR	WIP
FPC	-0.52	0.23	0.53	-0.29
SPC (%flour)	-0.75	-0.08	0.33	-0.61
SPC/ FPC	-0.51	-0.53	-0.28	-0.63
IGC (%flour)	0.35	0.83	0.75	0.66
IGC/ FPC	0.79	0.66	0.34	0.91
RPC (%flour)	-0.35	0.11	0.28	-0.22
RPC/ FPC	-0.16	0.01	0.03	-0.12
IGC/ SPC	0.81	0.70	0.36	0.95
HMW GS	0.32	0.78	0.72	0.60
HMW GS/ FPC	0.73	0.68	0.40	0.86
LMW GS	0.33	0.64	0.58	0.55
LMW GS/ FPC	0.82	0.50	0.18	0.86
TGS	0.36	0.72	0.64	0.60
TGS/ FPC	0.81	0.58	0.26	0.89
HMW GS/ LMW GS	0.03	0.48	0.47	0.24

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, MT= mixing time, BW= band width, PDR= peak dough resistance, WIP= work input to peak.

Table 10

Correlation coefficients (r) of average protein compositional parameters and CPC mixing parameters*.

	MT	BW	PDR	WIP
FPC	NC	NC	NC	NC
SPC (%flour)	-0.65	-0.38	-0.20	-0.56
SPC/ FPC	-0.65	-0.38	-0.20	-0.56
IGC (%flour)	0.82	0.79	0.69	0.90
IGC/ FPC	0.82	0.79	0.69	0.90
RPC (%flour)	-0.03	-0.25	-0.35	-0.18
RPC/ FPC	-0.03	-0.25	-0.35	-0.18
IGC/ SPC	0.89	0.74	0.59	0.91
HMW GS	0.51	0.37	0.26	0.44
HMW GS/ FPC	0.80	0.74	0.63	0.84
LMW GS	0.51	0.18	0.04	0.38
LMW GS/ FPC	0.85	0.60	0.44	0.84
TGS	0.54	0.28	0.14	0.43
TGS/ FPC	0.86	0.68	0.54	0.87
HMW GS/ LMW GS	0.13	0.39	0.43	0.22

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, MT= mixing time, BW= band width, PDR= peak dough resistance, WIP= work input to peak, NC= no correlation.

Table 11

Correlation coefficients (r) of average protein compositional parameters and salt-treated mixing parameters*.

	MT	BW	PDR	WIP
FPC	-0.49	0.33	0.60	-0.28
SPC (%flour)	-0.73	0.05	0.41	-0.55
SPC/ FPC	-0.51	-0.47	-0.25	-0.54
IGC (%flour)	0.35	0.83	0.77	0.67
IGC/ FPC	0.77	0.57	0.31	0.92
RPC (%flour)	-0.32	0.18	0.31	-0.25
RPC/ FPC	-0.14	0.04	0.04	-0.18
IGC/ SPC	0.80	0.62	0.33	0.92
HMW GS	0.32	0.79	0.74	0.59
HMW GS/ FPC	0.71	0.61	0.38	0.83
LMW GS	0.34	0.66	0.61	0.55
LMW GS/ FPC	0.81	0.42	0.15	0.83
TGS	0.36	0.73	0.66	0.59
TGS/ FPC	0.80	0.50	0.24	0.86
HMW GS/ LMW GS	0.01	0.52	0.51	0.22

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, MT= mixing time, BW= band width, PDR= peak dough resistance, WIP= work input to peak.

Table 12

Correlation coefficients (r) of average protein compositional parameters with both CPC and salt-treated mixing parameters*.

	MT	BW	PDR	WIP
FPC	NC	NC	NC	NC
SPC (%flour)	-0.65	-0.23	-0.13	-0.48
SPC/ FPC	-0.65	-0.23	-0.13	-0.48
IGC (%flour)	0.79	0.75	0.69	0.85
IGC/ FPC	0.79	0.75	0.69	0.85
RPC (%flour)	-0.01	-0.38	-0.42	-0.22
RPC/ FPC	-0.01	-0.38	-0.42	-0.22
IGC/ SPC	0.87	0.66	0.57	0.84
HMW GS	0.47	0.21	0.19	0.31
HMW GS/ FPC	0.76	0.64	0.59	0.74
LMW GS	0.47	0.10	0.04	0.27
LMW GS/ FPC	0.83	0.59	0.47	0.78
TGS	0.50	0.17	0.12	0.31
TGS/ FPC	0.83	0.64	0.55	0.80
HMW GS/ LMW GS	0.10	0.20	0.29	0.13

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, MT= mixing time, BW= band width, PDR= peak dough resistance, WIP= work input to peak, NC= no correlation.

were significant negative correlations with FPC and SPC, and significant positive correlations were determined for IGC (%FPC), IGC/SPC and the HPLC GS (%FPC) for both MT and WIP. Normalisation of the parameters again proved to be an important correction factor that greatly increased the relationships between MT and WIP with the protein quality parameters. The relationships with the HPLC parameters remained essentially constant for all treatments, because added starch and salt had very little impact on these highly genotypically influenced parameters after the cultivar samples were normalised for FPC.

An interesting sequence of correlations was shown for the protein quality parameters. Beginning with FPC, relationships of MT and WIP were r= -0.52 and -0.29, respectively (Figure 55A and B). Continuing with the sequential fractionation, SPC (% flour) correlations with MT and WIP had values of r= -0.75 and -0.61, respectively (Figures 56A and B). Non-normalised IGC correlated with MT and WIP positively (r= 0.35, and 0.66, respectively; Figure 57A and B) and with a greater magnitude than SPC (% flour). With normalisation, the relationships between SPC/FPC and MT and WIP decreased (r= -0.51 and -0.63, respectively, Figures 58A and B), while they increased significantly for IGC to r= 0.79 and 0.91, respectively (Figures 59A and B). Normalisation with FPC was very beneficial for improving the relationships between protein quality and mixing strength. The final component was IGC/SPC, and it relayed the strongest relationship between mixing strength and protein quality for this sample set; MT and WIP correlated with IGC/SPC to the level of r= 0.81 and 0.95, respectively (Figures 60A and B). These findings clearly show that the mixing strength parameter WIP was better suited than MT for explaining the variation in the sample set. In all these

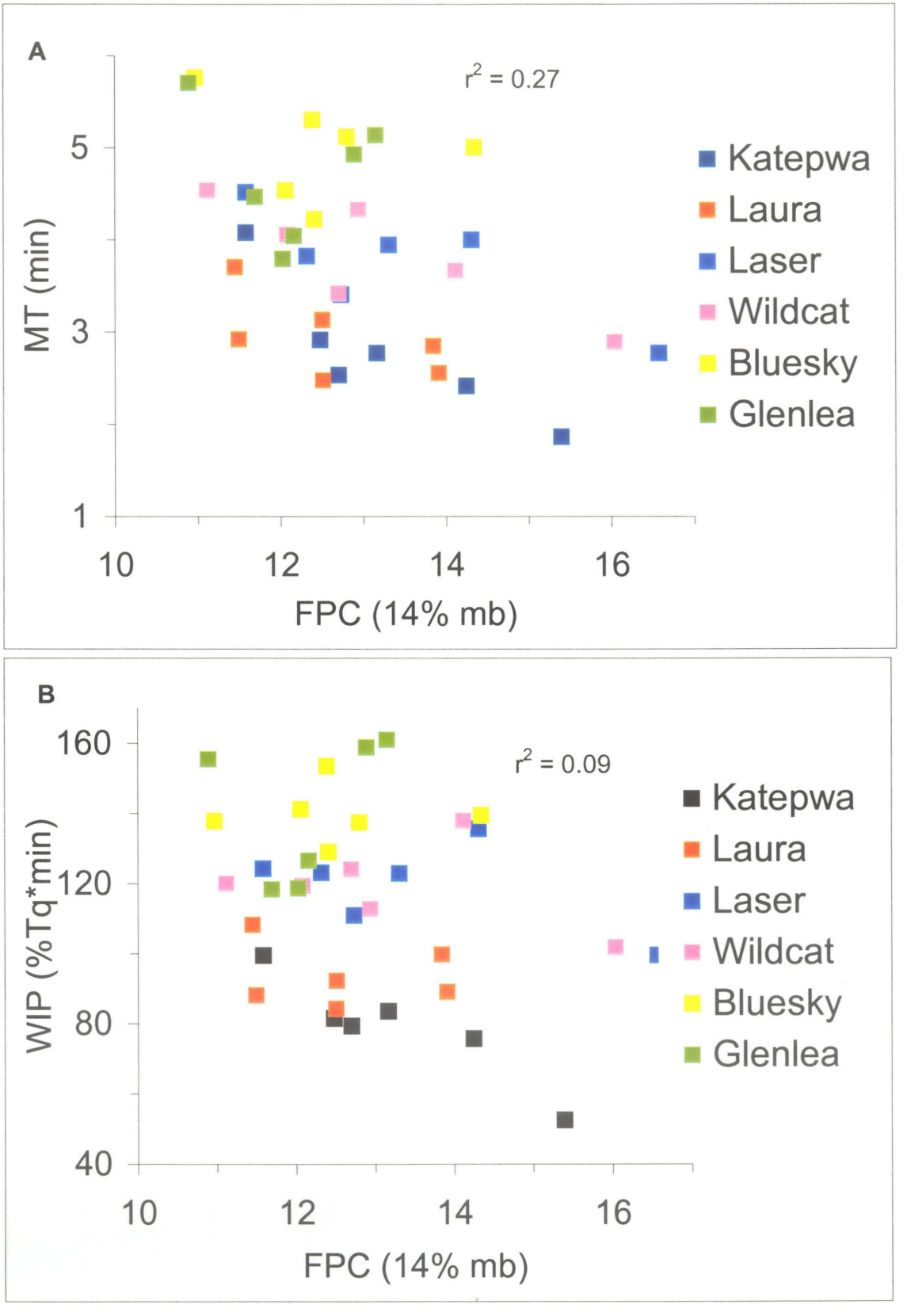


Figure 55. The Relationship of MT (A) and WIP (B) with FPC.

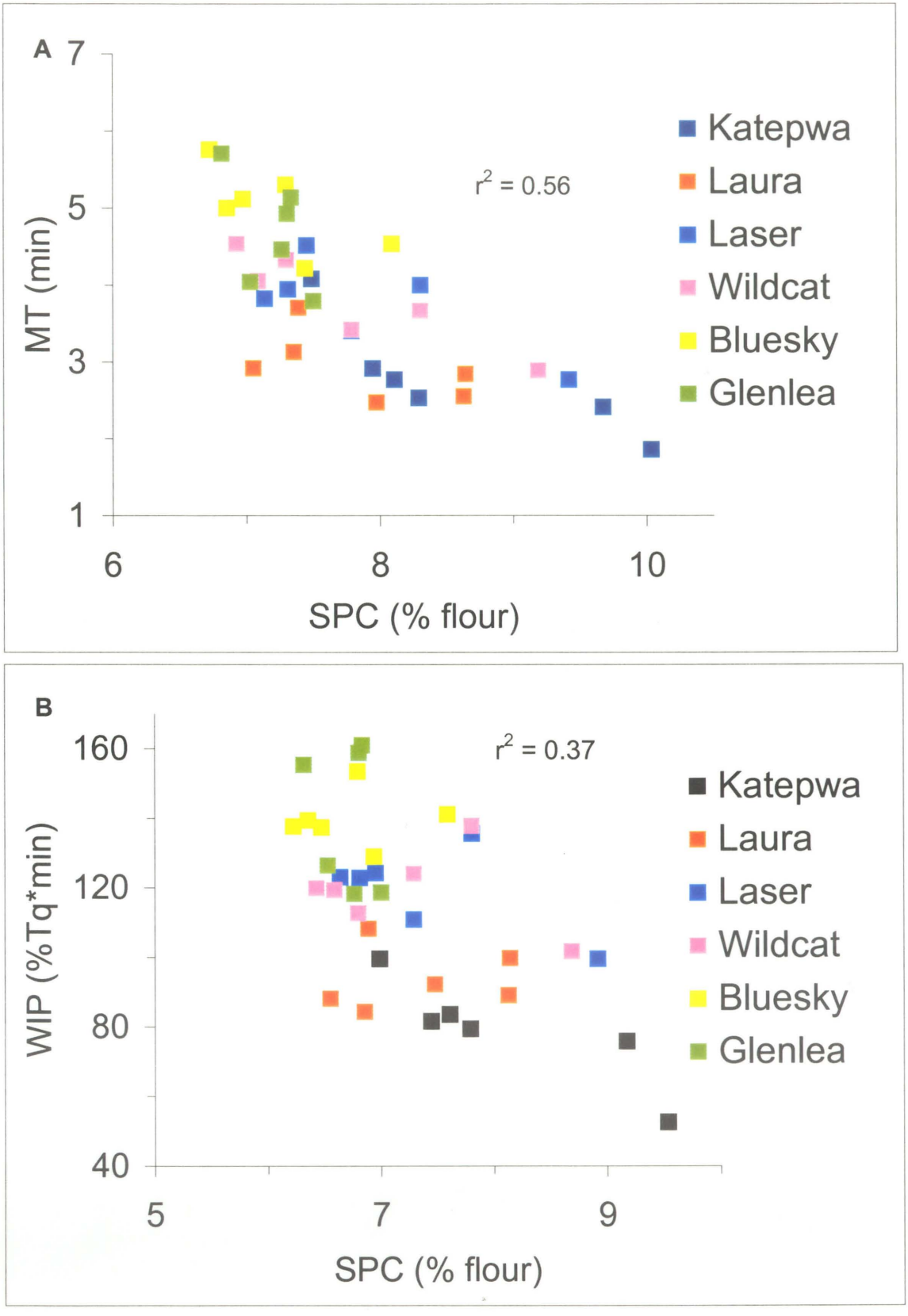


Figure 56. The Relationship of MT (A) and WIP (B) with SPC (% flour).

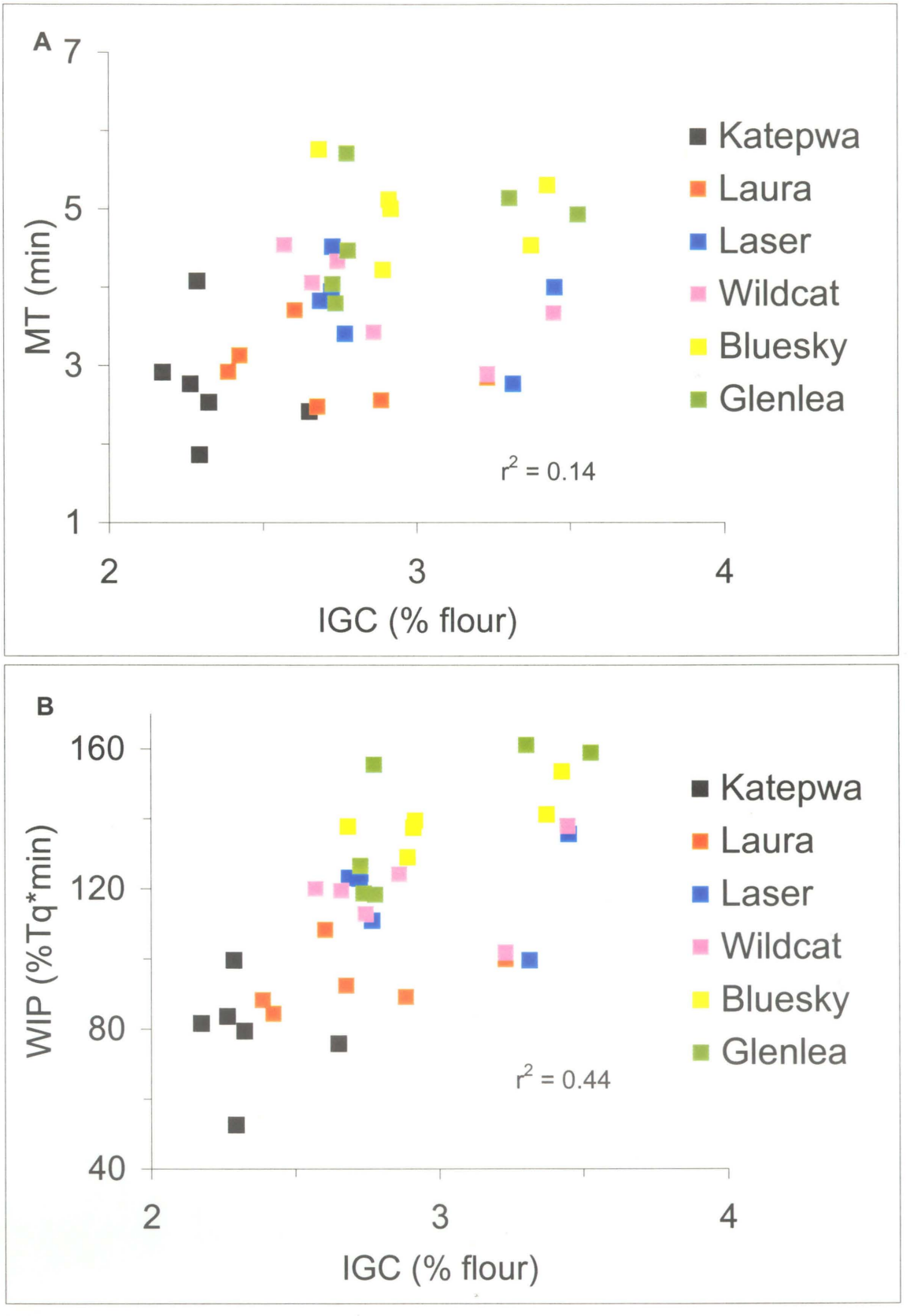


Figure 57. The Relationship of MT (A) and WIP (B) with IGC (% flour).

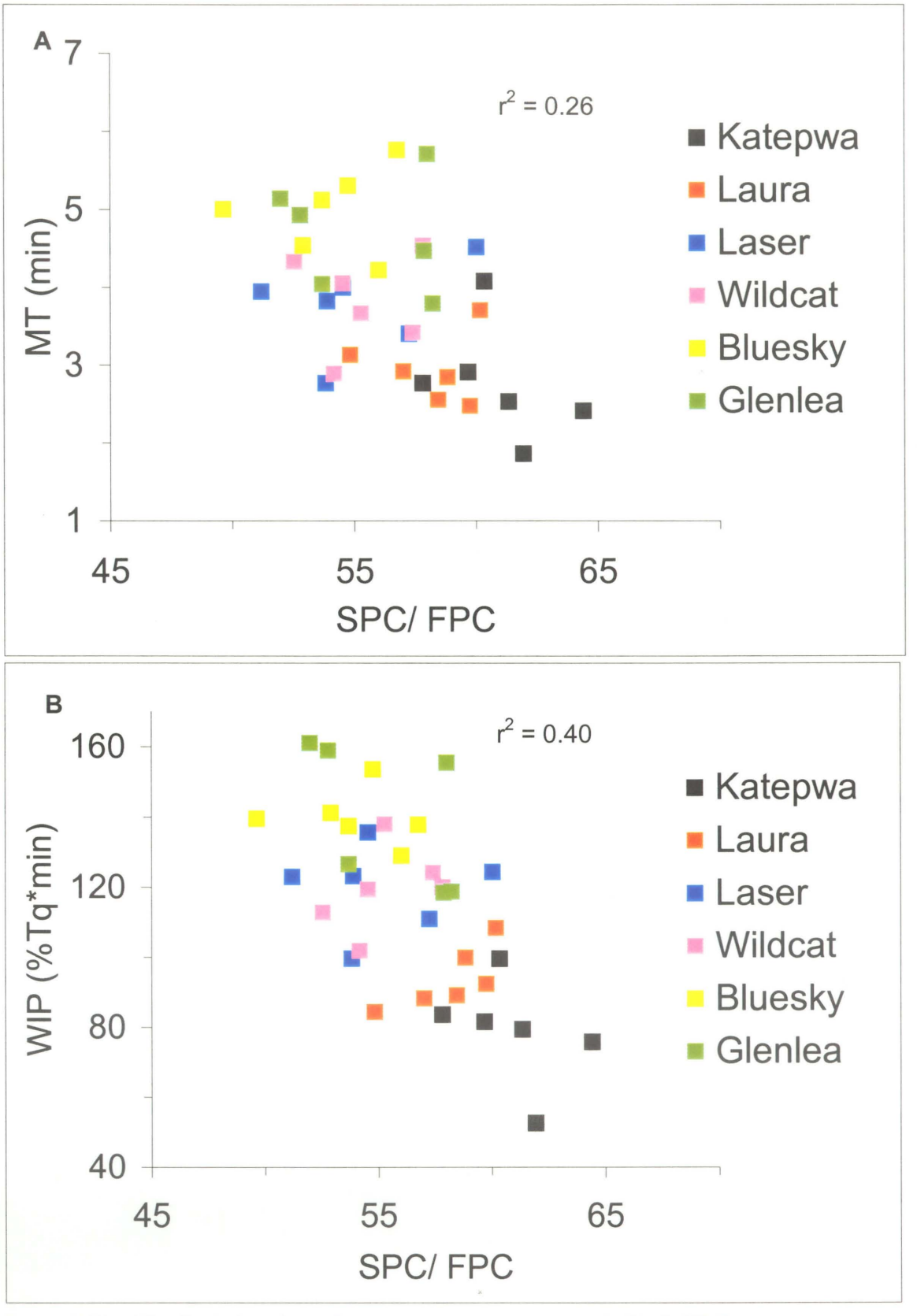


Figure 58. The Relationship of MT (A) and WIP (B) with SPC/ FPC.

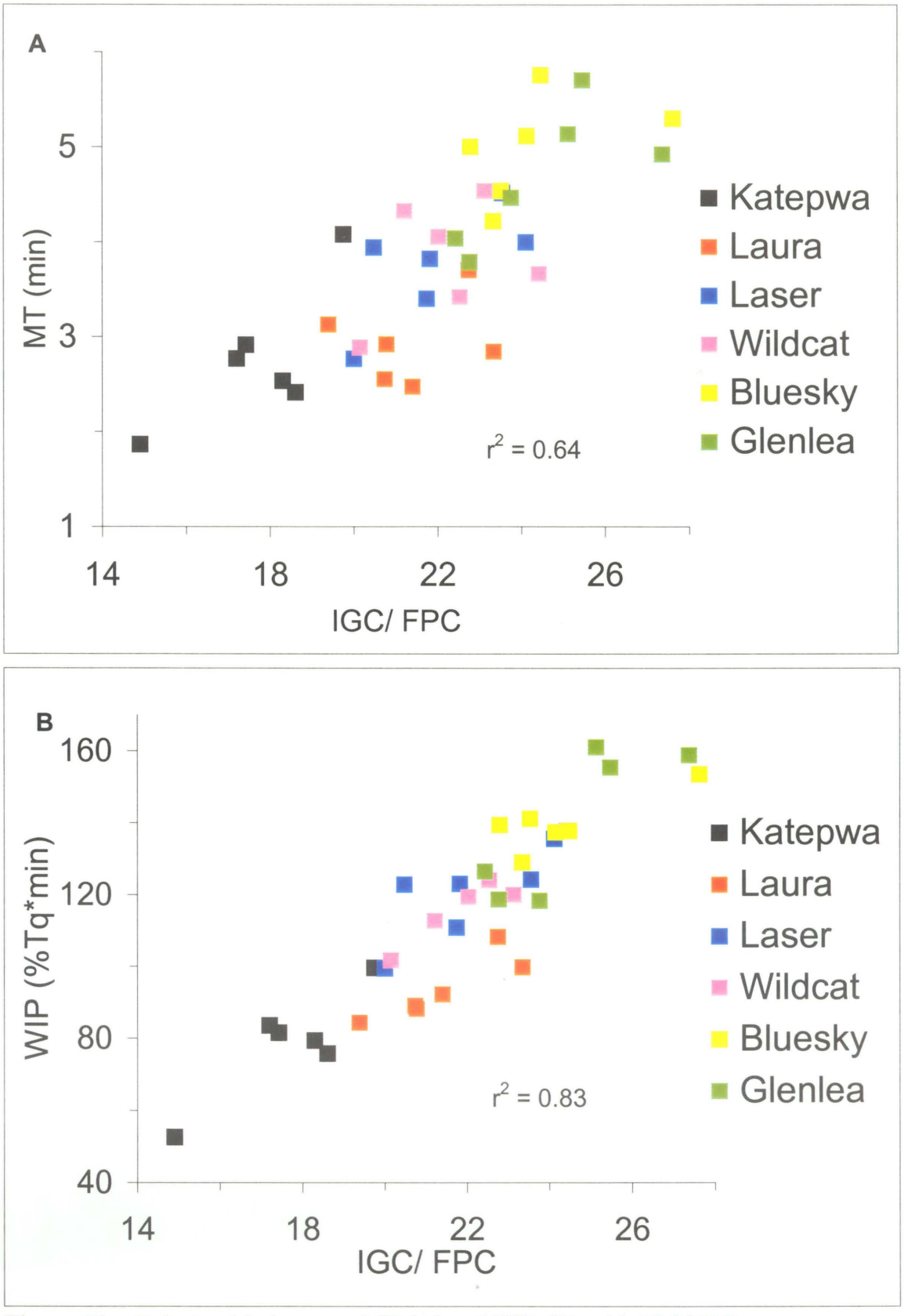


Figure 59. Relationship between MT (A) and WIP (B) with IGC/FPC.

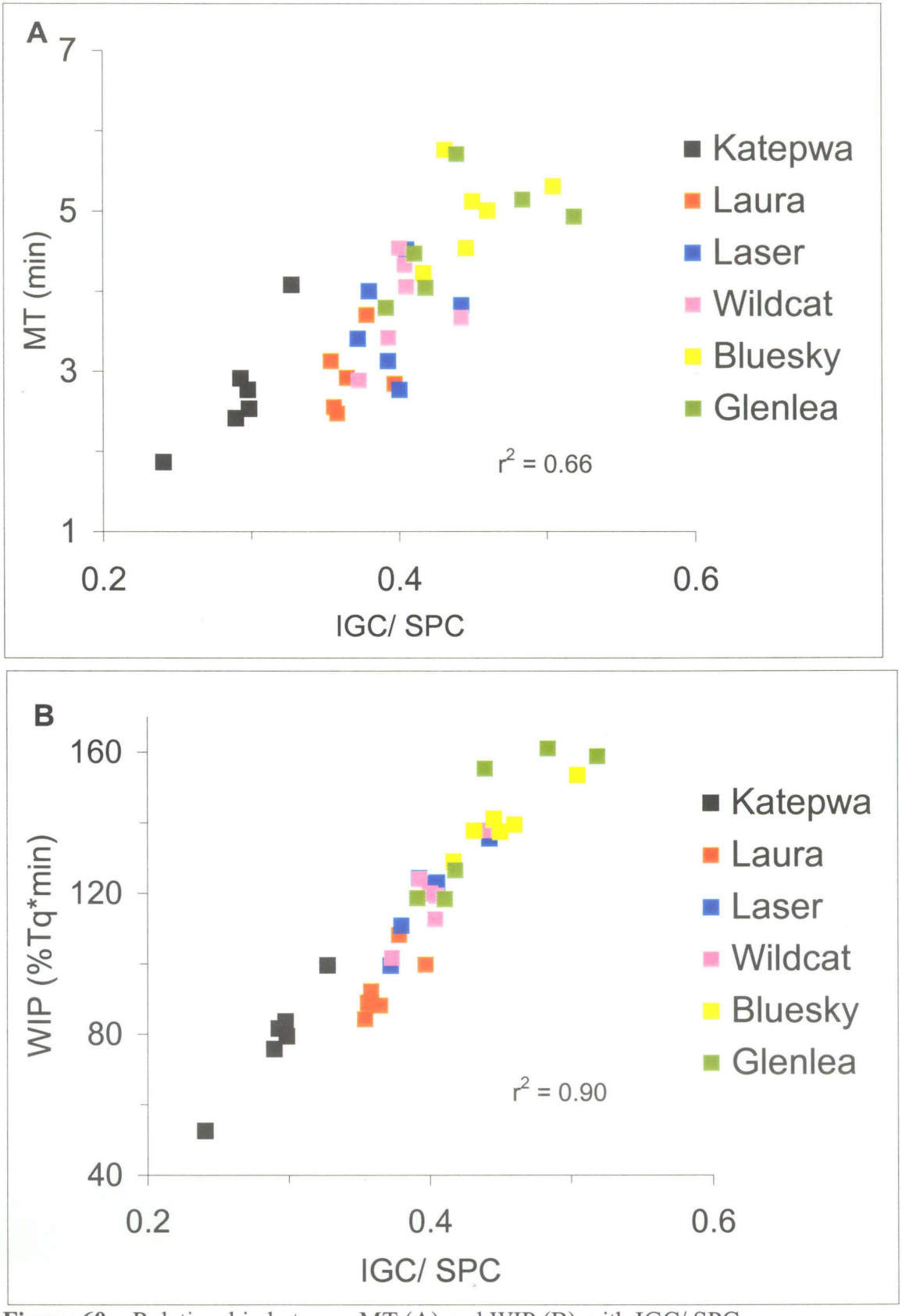


Figure 60. Relationship between MT (A) and WIP (B) with IGC/ SPC.

figures, the CWRS cultivars were always near the lower end of the linear relationship, and the CWES cultivars filled out the middle and top (see all above figures). Glenlea and Bluesky were found at the opposite end of the CWRS cultivars, which was further evidence of their dominance in strength, according to these parameters, in the CWES class and this sample set. An interesting result was found when three locations of Glenlea were located near the middle in these relationships, where Laser and Wildcat were found. This suggested that the CWES class was not only of varying strength, but that the standard Glenlea was not as strong as in the past.

These relationships showed that mixing parameters and protein quality were highly correlated with each other. One shortcoming was that the sample set was small and relatively specialised with only two classes of wheat a limited amount of cultivars. Nevertheless, such a specialised sample set made the relationships that were obtained that much stronger and believable.

4.4.3 Relationships of Protein Compositional Parameters with Baking Parameters

Significant relationships were found between protein compositional parameters and baking parameters, including baking process, textural and structural characteristics. All of the relationships between protein compositional parameters and baking parameters of untreated samples are shown in Table 13. ffMT was highly correlated with protein quality parameters, as shown by the significant correlations with IGC/FPC (r=0.72), IGC/SPC (r=0.82), the normalized HPLC parameters (r=0.69-0.73), and SPC/FPC (r=-0.67). There was a significant relationship between the development of dough and the strength and quality of protein; this result was very similar to the Mixograph MT

correlations. ffMT had virtually identical relationships to DSL and similar to CWT, only ffMT was positively correlated where DSL was negatively correlated. This suggested that DSL was also highly correlated with protein quality but inversely. LV did not correlate well with any parameters; the most significant relationship for LV was with LMW-GS/ FPC (r=0.37). There was no relationship between LV and FPC (r= -0.04), where traditionally there is. This could possibly be due to the tight sample set of cultivars that is mostly CWES with only two CWRS cultivar samples. This result requires more study, because it contradicts past research. Relationships between protein compositional parameters and LV are found in Appendix A. There was a lack of relationships for the untreated samples between the baking and protein compositional parameters.

The relationships for the samples treated at a CPC are shown in Table 14. A significant relationship (r= 0.63) was shown between IGC and LV (Figure 61) for starch-treated bread. This reflected the LV data, where the highest LVs were reported for the samples with the highest level of IGC and lowest SPC, i.e. Glenlea and Bluesky (see Figure 34). The LVs were concentrated near the area of lower SPC and higher LV suggesting that SPC had an inverse relationship with breadmaking quality. The high concentration of IGC was a result of the CWES biased sample set where the majority of the cultivars had lower SPC contents because of the genotypic effect. As can be seen, most of the relationships with the untreated parameters did not change in significance or magnitude with the addition of starch.

Table 13

Correlation coefficients (r) of protein compositional and untreated baking process parameters*.

	Abs	ffMT	DSL	LV	PF	PT	AS	CAP	CD	CWT	CU	GL
FPC	0.65	-0.34	0.40	-0.03	0.33	0.59	-0.54	0.40	0.45	0.31	-0.07	-0.38
SPC (%flour)	0.65	-0.66	0.70	-0.18	0.16	0.32	-0.44	0.13	0.41	0.61	-0.07	-0.45
SPC/ FPC	0.09	-0.68	0.60	-0.26	-0.25	-0.40	0.12	-0.41	-0.01	0.60	-0.01	-0.20
IGC (%flour)	0.12	0.42	-0.42	0.17	0.41	0.46	-0.07	0.50	0.44	-0.37	0.29	-0.10
IGC/ FPC	-0.41	0.72	-0.74	0.22	0.17	0.00	0.36	0.20	0.10	-0.63	0.35	0.17
RPC (%flour)	0.47	-0.10	0.19	0.06	0.26	0.59	-0.56	0.38	0.16	0.09	-0.24	-0.14
RPC/ FPC	0.26	0.09	0.01	0.08	0.09	0.41	-0.44	0.24	-0.09	-0.07	-0.28	0.07
IGC/ SPC	-0.35	0.82	-0.78	0.28	0.22	0.15	0.22	0.31	0.09	-0.70	0.29	0.18
HMW GS	0.10	0.40	-0.43	0.25	0.40	0.53	-0.14	0.55	0.37	-0.38	0.19	-0.18
HMW GS/ FPC	-0.34	0.68	-0.73	0.31	0.20	0.18	0.21	0.33	0.11	-0.62	0.25	0.03
LMW GS	0.01	0.36	-0.39	0.30	0.60	0.66	-0.12	0.72	0.48	-0.36	0.31	-0.16
LMW GS/ FPC	-0.54	0.69	-0.74	0.37	0.41	0.25	0.31	0.48	0.19	-0.65	0.40	0.11
TGS	0.04	0.41	-0.44	0.29	0.51	0.60	-0.12	0.66	0.44	-0.39	0.26	-0.14
TGS/ FPC	-0.49	0.73	-0.77	0.35	0.31	0.20	0.30	0.41	0.14	-0.67	0.34	0.11
HMW GS/ LMW GS	0.22	0.21	-0.24	-0.01	-0.30	-0.08	-0.10	-0.16	-0.11	-0.16	-0.20	-0.12

*FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, Abs= baking absorbance, ffMT= full formula mixing time, DSL= dough sheet length, LV= loaf volume, PF= peak force, PT= peak time, AS= ascending slope, CAP= curve area to peak, CD= cell density, CWT= cell wall thickness, CU= cell uniformity, GL= gray level.

Table 14

Correlation coefficients (r) of protein compositional and baking parameters for starch and shortening treated samples*.

	Abs	ffMT	DSL	LV	PF	PT	AS	CAP	CD	CWT	CU	GL
FPC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
SPC (%flour)	0.04	-0.71	0.44	-0.15	-0.15	-0.27	0.20	-0.23	0.15	0.42	0.22	0.01
SPC/ FPC	0.04	-0.71	0.44	-0.15	-0.15	-0.27	0.20	-0.23	0.15	0.42	0.22	0.01
IGC (%flour)	-0.36	0.75	-0.70	0.63	0.40	0.43	-0.23	0.46	0.18	-0.61	0.21	0.24
IGC/ FPC	-0.36	0.75	-0.70	0.63	0.40	0.43	-0.23	0.46	0.18	-0.61	0.21	0.24
RPC (%flour)	0.26	0.09	0.14	-0.38	-0.19	-0.08	-0.01	-0.15	-0.30	0.08	-0.40	-0.21
RPC/ FPC	0.26	0.09	0.14	-0.38	-0.19	-0.08	-0.01	-0.15	-0.30	0.08	-0.40	-0.21
IGC/ SPC	-0.30	0.86	-0.71	0.53	0.38	0.43	-0.23	0.46	0.08	-0.64	0.07	0.19
HMW GS	0.16	0.53	-0.25	0.00	0.06	-0.08	0.15	-0.02	-0.29	-0.22	-0.35	-0.13
HMW GS/ FPC	0.16	0.53	-0.25	0.00	0.06	-0.08	0.15	-0.02	-0.29	-0.22	-0.35	-0.13
LMW GS	0.03	0.47	-0.22	-0.02	0.22	-0.08	0.27	0.09	-0.31	-0.19	-0.33	-0.08
LMW GS/ FPC	0.03	0.47	-0.22	-0.02	0.22	-0.08	0.27	0.09	-0.31	-0.19	-0.33	-0.08
TGS	0.07	0.54	-0.26	0.02	0.16	-0.06	0.21	0.06	-0.28	-0.24	-0.33	-0.08
TGS/ FPC	0.07	0.54	-0.26	0.02	0.16	-0.06	0.21	0.06	-0.28	-0.24	-0.33	-0.08
HMW GS/ LMW GS	0.31	0.28	-0.16	0.06	-0.30	-0.01	-0.23	-0.21	-0.05	-0.13	-0.15	-0.13

*NC= no correlation, FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, Abs= baking absorbance, ffMT= full formula mixing time, DSL= dough sheet length, LV= loaf volume, PF= peak force, PT= peak time, AS= ascending slope, CAP= curve area to peak, CD= cell density, CWT= cell wall thickness, CU= cell uniformity, GL= gray level.

Table 15

Correlation coefficients (r) of protein compositional and baking parameters for starch treated samples without shortening*.

	Abs	ffMT	DSL	LV	PF	PT	AS	CAP	CD	CWT	CU	GL
FPC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
SPC (%flour)	0.06	-0.70	0.55	-0.30	0.05	-0.25	0.27	-0.06	0.09	0.50	-0.10	-0.29
SPC/ FPC	0.06	-0.70	0.55	-0.30	0.06	-0.25	0.27	-0.06	0.09	0.50	-0.10	-0.29
IGC (%flour)	-0.38	0.72	-0.65	0.69	0.31	0.47	-0.25	0.37	0.41	-0.70	0.49	0.47
IGC/ FPC	-0.38	0.72	-0.65	0.69	0.31	0.47	-0.25	0.37	0.41	-0.70	0.49	0.47
RPC (%flour)	0.26	0.10	-0.02	-0.28	-0.32	-0.15	-0.07	-0.26	-0.44	0.08	-0.32	-0.10
RPC/ FPC	0.26	0.10	-0.02	-0.28	-0.32	-0.15	-0.07	-0.26	-0.44	0.08	-0.32	-0.10
IGC/ SPC	-0.32	0.83	-0.71	0.63	0.23	0.45	-0.28	0.32	0.27	-0.71	0.41	0.46
HMW GS	0.15	0.45	-0.22	0.23	-0.13	0.01	-0.20	-0.13	-0.19	-0.35	-0.10	0.01
HMW GS/ FPC	0.15	0.45	-0.22	0.23	-0.13	0.01	-0.20	-0.13	-0.19	-0.35	-0.10	0.01
LMW GS	0.03	0.42	-0.24	0.13	0.04	0.03	-0.02	0.03	-0.15	-0.25	-0.04	0.05
LMW GS/ FPC	0.03	0.42	-0.24	0.13	0.04	0.03	-0.02	0.03	-0.15	-0.25	-0.04	0.05
TGS	0.06	0.48	-0.26	0.18	-0.04	0.04	-0.11	-0.03	-0.17	-0.31	-0.05	0.04
TGS/ FPC	0.06	0.48	-0.26	0.18	-0.04	0.04	-0.11	-0.03	-0.17	-0.31	-0.05	0.04
HMW GS/ LMW GS	0.28	0.21	-0.04	0.32	-0.39	0.00	-0.50	-0.37	-0.14	-0.34	-0.15	-0.04

*NC= no correlation, FPC= flour protein content, SPC= soluble protein content, IGC, insoluble glutenin content, RPC= residue protein content, HMW= high molecular weight glutenin subunits, LMW= low molecular weight glutenin subunits, TGS= total glutenin subunits, Abs= baking absorbance, ffMT= full formula mixing time, DSL= dough sheet length, LV= loaf volume, PF= peak force, PT= peak time, AS= ascending slope, CAP= curve area to peak, CD= cell density, CWT= cell wall thickness, CU= cell uniformity, GL= gray level.

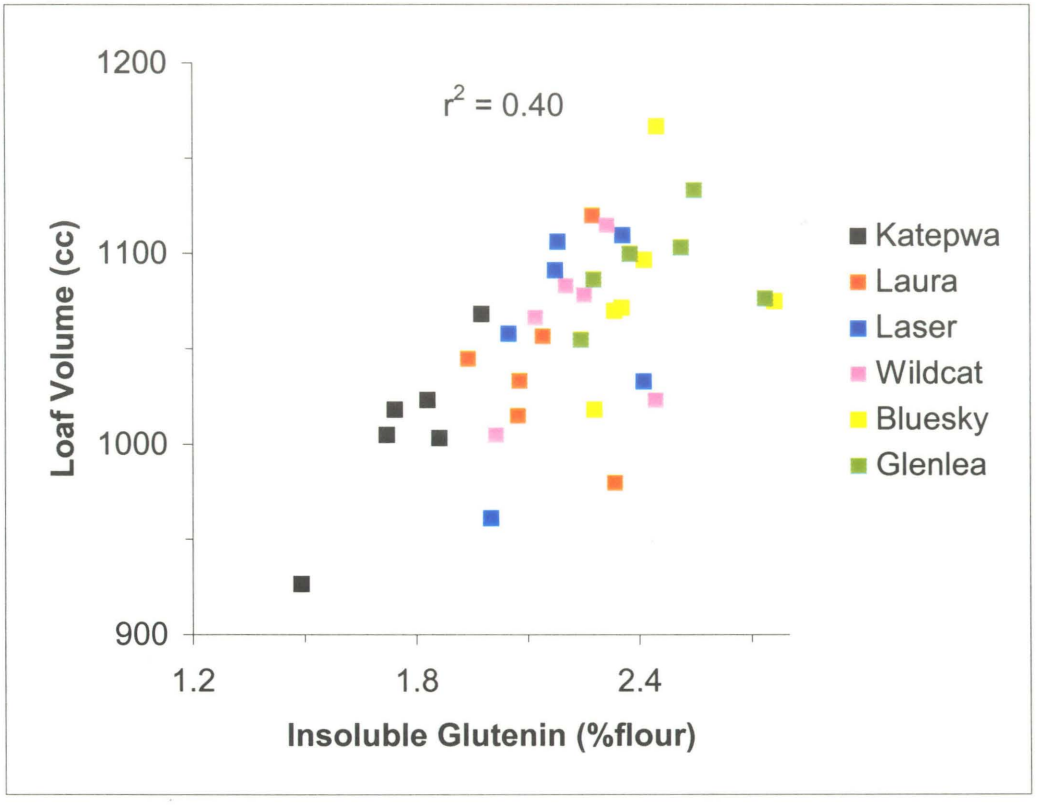


Figure 61. Relationship of Loaf Volume and Insoluble Glutenin Content (% flour) at a Constant Protein Content.

All of the relationships between the protein compositional parameters and baking parameters for starch treated samples without shortening are found in Table 15. Again, the relationships discussed previously were still present. The correlation between IGC and LV increased slightly, and was matched by LV as a function of IGC/SPC ($r = 0.63$).

5.0 GENERAL DISCUSSION

The Canada Western Extra Strong (CWES) wheat class was the focus of this study to determine how genotype and environment affected protein content, protein quality, mixing properties and breadmaking properties. Due to its extra strong gluten properties this class has likely the most functional protein quality (i.e. gluten composition) with respect to dough strength, and mixing tolerance among all Canadian classes of wheat. The objectives of this study were: to quantify genotypic differences in dough protein composition, mixing properties, and bread baking potential across locations; to similarly quantify the magnitude of location effects; and to establish which tests (protein composition, mixing and/ or baking) best characterize the unique properties of CWES wheat.

Protein content demonstrated an inverse relationship with dough strength and was a significantly environmentally influenced parameter. In other words, the lowest FPC was always associated with the strongest cultivars (i.e. Glenlea and Bluesky) and the locations of highest grade quality (i.e. 1CW grade for Swift Current and Saskatoon). All other cultivars had higher protein contents and lower grade levels of 2 and 3CW due to degrading factors (recall Table 1). The cultivars that had a higher protein content also had higher levels of SPC (i.e. CWRS cultivars), whereas the lower FPC cultivars had higher levels of IGC and a high ratio of IGC/SPC (i.e. CWES cultivars with the exception of Laser). Protein quality, defined as IGC, under normal growing conditions was a

highly inherited characteristic, whereas the quantity of protein correlated with protein composition parameters SPC and RPC depended primarily on growing location (Finney and Yamazaki 1967). These four protein parameters fulfilled the first and third objectives very well, because they proved very useful for showing significant differences in the CWES and CWRS classes of wheat. Wooding et al (1999) alluded to this protein quality issue in their mixing study involving multiple locations. They found that a higher level of applied nitrogen fertilizer resulted in higher FPC, but this did not necessarily lead the better relationship of mixing requirements and dough strength parameters as shown herein. Gupta et al. (1996) reported that because gliadins were synthesized at a higher rate than glutenins in the later stages of ripening, higher FPC would not necessarily confer stronger dough characteristics for a cultivar. A similar observation was observed with Figure 54 where SPC was present in a greater amount as protein content increased compared to IGC and RPC. FPC was highly susceptible to environmental effects, which explained why wheat breeders have such a challenge to increase wheat protein content.

FPC is well known to be highly influenced by environment (Graybosch et al. 1996; Peterson et al. 1998), whereas SPC/FPC, IGC/FPC, all RP-HPLC/FPC parameters and IGC/SPC were highly genotypic. This suggested that the magnitude of genotypic effects was hidden until the FPC was removed allowing for greater genotypic influence of protein quality parameters to surface. The use of the ratio of genotypic to environmental variance (G/E variance ratio) proved very useful for quantifying the magnitude of the effects for each parameter, and to better understand the performance of these cultivars across different location. Fowler and de la Roche (1975) also reported greater environmental variation in FPC for a number of cultivars in the Eastern

Cooperative Spring Test in the years under study (1968-1971). Similarly, Peterson et al. (1986) reported significant environmental variation of FPC in their study of 27 diverse cultivars of varying strength. With the sample set in this thesis, Laser and Wildcat had significantly higher FPC, similar SPC/FPC and significantly less IGC/FPC than that of Glenlea. Normalisation was an important calculation, because FPC was highly environmentally influenced. With the removal of FPC, large genotypic differences were found for many breadmaking quality parameters. FPC normalization caused the protein parameters to fulfill Objectives 1, 2, and 3 very well .

The RP-HPLC parameters also provided information on the importance of protein quality (i.e. IGC) and protein quantity (i.e. FPC). All of the CWES cultivars had the same HMW-GS composition (Hussain et al. 1998), and Glenlea and Laura had identical Glu-1 scores of 10, whereas Katepwa has a Glu-1 score of 9 (Khatkar et al. 1996). However, there were significant differences in dough mixing properties of these three cultivars and quantity of GS as the results showed. Of course, it may be that one or more individual components of GS have significant effects that were not discovered during the course of this thesis. For instance, the HMW-GS were similar for all CWES cultivars, but the LMW-GS were not analyzed. LMW-GS composition is a subject that has not received as much attention as HMW-GS, and may prove very useful for explaining why there is such a disparity within the CWES class.

From the dough mixing experiments, the parameters WIP and MT provided useful information on strength and quality of the CWES class, and was important- WIP> MT- for explaining variation in genotypes and locations. MT proved less useful for differentiating among cultivars and classes even though it had virtually identical

variation. WIP especially was very strongly related to protein quality and therefore protein strength; the correlation between IGC/SPC and WIP was r^2= 0.90. MT and WIP both clearly separated the two classes of wheat with the four CWES cultivars having characteristically and significantly greater mixing requirements than the two CWRS counterparts. In addition, within the CWES class there were two distinct levels of WIP and MT; Glenlea and Bluesky were significantly stronger than Wildcat and Laser. The mixing parameters MT and WIP fulfilled Objective 3 by quantifying the differences in the CWES class.

CWES shipments are/were mostly Glenlea. This cultivar, Glenlea, showed a difference among locations with its strength. Perhaps this was the reason for the complaints on the lower quality shipments of grain, although definitely not the only one. However, it was clear that the newer cultivars Wildcat and Laser do not have the same quality as Glenlea. As mentioned above, the reason is most probably protein quality related, but the reasons are beyond the experimental work within.

FPC normalization was a useful manipulation for evaluating the protein compositional parameters of the cultivar samples, because it removed the substantial environmental effect of FPC thus better revealing differences among the cultivars, which was an objective of this study. Adding starch to flour to achieve a constant protein content was beneficial for reveal intercultivar differences. Khatkar et al. (1996) reported an increase in MT in their study involving mixing fractionated and reconstituted flours at a CPC, of which Glenlea was one of the cultivar samples. Perhaps the increase in MT with added starch shown in this study was due to additional time being required for development of gluten in the presence of excess starch, i.e. it took longer for interactions

to occur between the gluten proteins and therefore longer time to dough development. The interesting aspect to this result was that the MT of CWRS cultivars decreased slightly (5%), but increased for the CWES cultivars with added starch (approximately 10%). Laser and Wildcat had very little increase in MT, but Bluesky and Glenlea increased more even when they had the lowest amounts of starch added. Perhaps, the additional starch interfered with the presumably larger glutenin (on average) in the CWES cultivars, which was greatest for Bluesky and Glenlea. This result suggested that mixing requirements were dependent not only on protein quality but also on starch quantity.

The other issue with the starch addition was a possibility that the relatively weaker cultivars with higher FPC were unfairly assessed. These cultivar samples required more starch dilution compared to the stronger cultivars with the lower FPC. Because IGC was relatively constant among all cultivar samples, greater starch dilution for high FPC samples would result in disproportionately lower IGC in the CPC treated samples. Clearly, protein composition does not change linearly with FPC changes (Figure 54). Research has shown that in later stages of protein synthesis an abundance of gliadin is synthesized while glutenin synthesis tapers off (Gupta et al. 1996). Thus, the addition of starch to a higher FPC sample would dilute the higher concentration of gliadins to a lesser percentage and the lower amount of glutenins to a greater percentage that would make the dough relatively weaker. In other words, the protein quality of the two higher FPC CWRS cultivars declined with the addition of starch; i.e. at a CPC the protein quality of the CWRS cultivars was lower than that for the CWES cultivars. The addition of starch provided a sound basis for evaluating FPC; starch addition helped

establish that FPC was largely environmental and protein quality was highly genotypic in nature. Although the effect of starch was variable and difficult to understand, it did show that MT and WIP were important parameters for quantifying the magnitude of location effects (Objective 2), and in significantly improving separation among the CWES cultivars into understandable groups based on perceived dough strength (Objective 3). The importance of this discovery directly affects breeders, because traditional breeding for FPC involves achieving high protein yielding cultivars. In the future, breeders of Canadian bread-wheat need to pay more attention to the importance of the quality of the protein. This result indicated that protein quality, especially content of insoluble glutenin, and not protein content, played a significant role in explaining variation in CWES strength and functionality and differences between CWES and CWRS (Objectives 1 and 3).

When treated with starch, the CWES flours achieved significantly higher LVs than the CWRS cultivars, which was opposite to traditional results showing strong cultivars had poorer breadmaking properties (MacRitchie 1984). The difference is that this thesis used a short-time test and traditional testing utilizes a long fermentation baking process. Since the environmental influence on FPC was dominant, it would seem likely that the higher FPC samples would give higher LV values, because they had greater amounts of gluten proteins. However, the CWES cultivars had similar LV to the CWRS cultivars with their native FPC, and greater when the quantity of protein was removed. This suggested again that the quality of the protein was much more important than the quantity.

A significant shortening effect was determined, which was strongly influenced by genotype. Shortening increased the LV by an average of 130 cc for the CWRS and by an average of 90 cc for the CWES (Figure 34B and C). There was clear separation between the CWES cultivars, which were significantly higher in LV than the CWRS cultivars. Perhaps the stronger cultivars did not require the improving effect that shortening provides as much as the weaker CWRS cultivars did. This would help explain why the CWES cultivars performed much better than the CWRS cultivars for LV with shortening removed.

6.0 CONCLUSIONS

The CWES wheat class varied in protein content, protein quality, dough mixing strength, and breadmaking quality depending on growing location and cultivar. Protein quality was proven more important than protein content for explaining the CWES cultivar quality. Large glutenin polymer, IGC, was strongly related to dough mixing requirements, and was highly correlated to dough mixing strength. The relationship that best explained variation in genotype and location was WIP and IGC/SPC ($r^2 = 0.90$). The spectrophotometric method for quantifying 50% 1-propanol soluble and insoluble protein was especially useful for evaluating the CWES cultivars. The method provided precise values of the protein components that were found significantly different between the two classes and within the CWES class. Further work with protein quality is required especially in the area of LMW-GS.

Evaluating the mixing properties of CWES wheats using the mixograph at constant absorption was very useful, and showed that MT and especially WIP explained much of the variation in dough strength across genotypes and locations. The CWES class required special settings (filters of 160, stages of 3, 10% peak fit window, and top-line for analysis) to obtain a precise peak that all dough mixing parameters are based on. In addition, there were two distinct levels of dough strength within the CWES class; Glenlea and Bluesky were significantly greater than Wildcat and Laser. It was difficult to differentiate between Glenlea and Bluesky. The baking study was not as useful as hoped, but further analysis with a more rigorous baking test, such as the remix-to-peak may

prove more useful for this sample set of CWES and CWRS cultivars. Because of this study, and other collaborative work at the various research centres in Winnipeg on CWES cultivars (Agriculture and Agri-Food Canada Cereal Research Centre and the Grain Research Laboratory), Wildcat and Laser are being phased out of the CWES class. Dough mixing was a better method for evaluating these samples compared to that of baking.

Starch dilution of the cultivar samples to a CPC was a useful treatment for determining the functionality of these cultivar samples grown in different environments and for evaluating this unique class. Starch addition was also very useful for increasing relationships between breadmaking quality and protein quality parameters. In fact, it was very difficult to differentiate the breadmaking quality parameters except at the CPC. Similar to this result, normalization of the key protein fractions per unit protein caused stronger relationships to be revealed with many parameters (e.g. IGC/FPC and WIP).

Breeders are challenged to develop varieties that contain both a high protein content and protein quality; the cultivars within the CWES class show two distinct levels of strength. In addition, there is a challenge to breed for high protein content because of the significant effect of the environment on FPC. The environmental effect was highly significant but will vary from year to year. Therefore, studying another crop year may provide more useful information on the effect of the environmental conditions. In fact, since the environmental influence is so prominent, a robust study of many different agricultural disciplines that includes weather data, whether hourly or daily, and stage of growth, as well as various other environmental factors, such as soil and water availability, is required. The environmental effect was a limitation of the study, because it was not

fully understood due to the lack of solid conclusions on environment that could be made with a one-year study. Attention needs to be paid to grain handling, because of the large environmental influence and significant decline in grain elevators within Western Canada. Protein quality, and not protein content, was a much more useful predictor of dough strength and sample quality.

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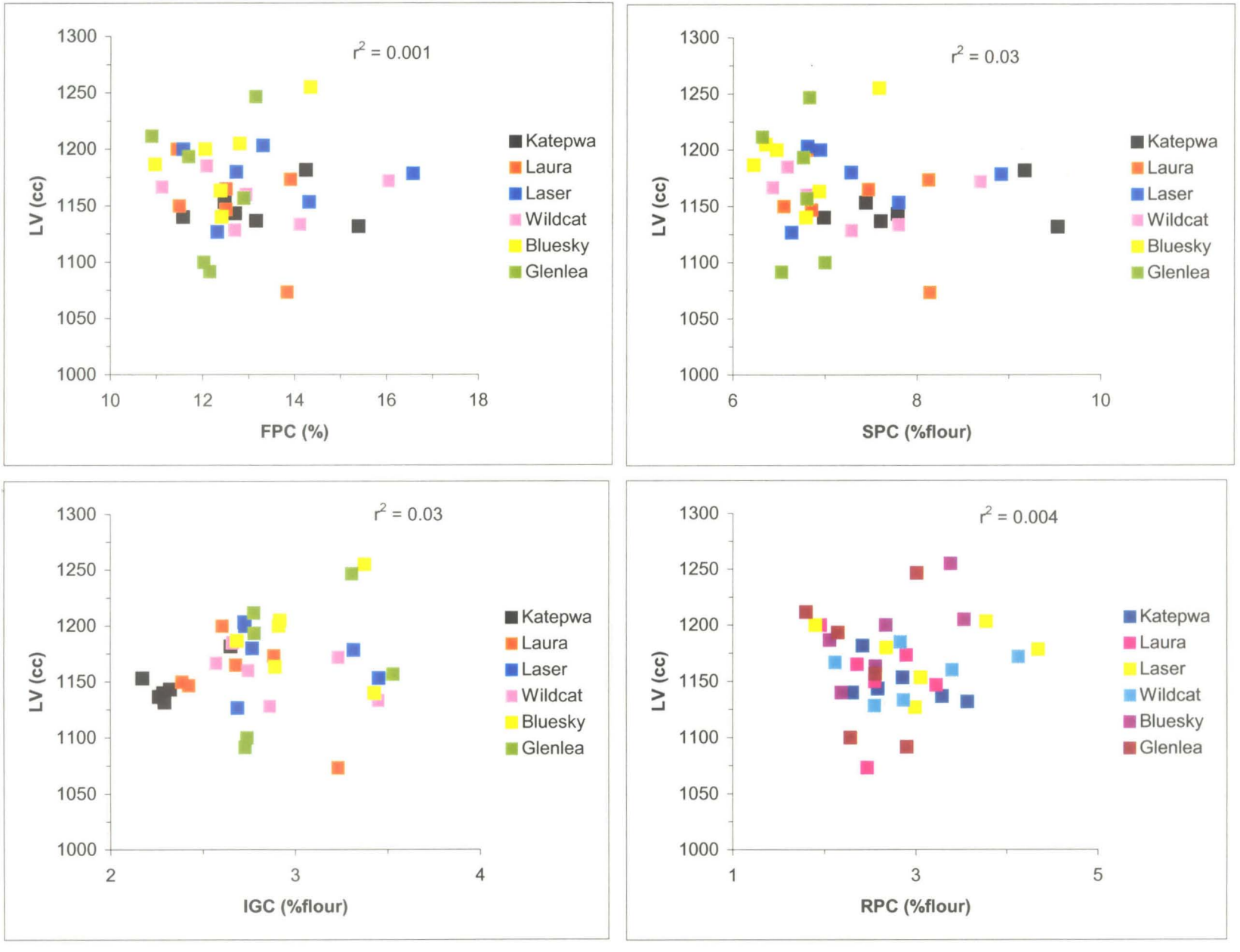
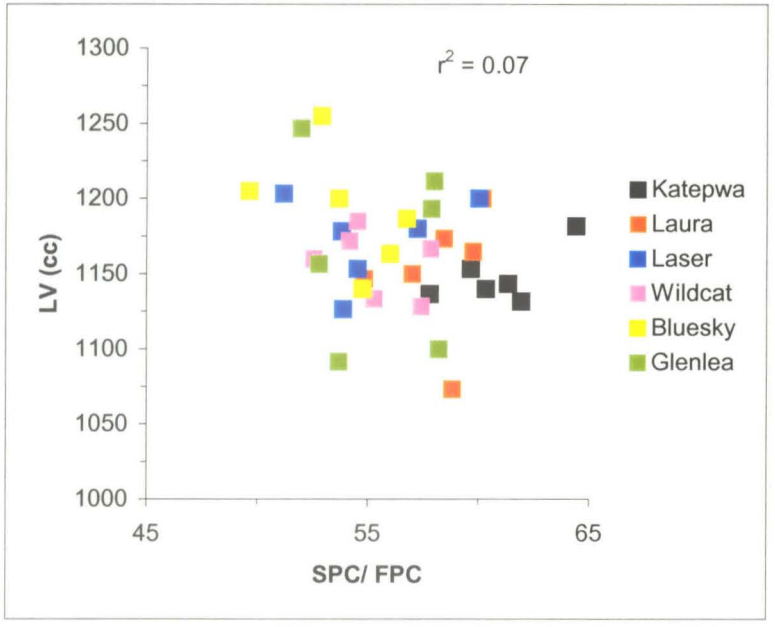
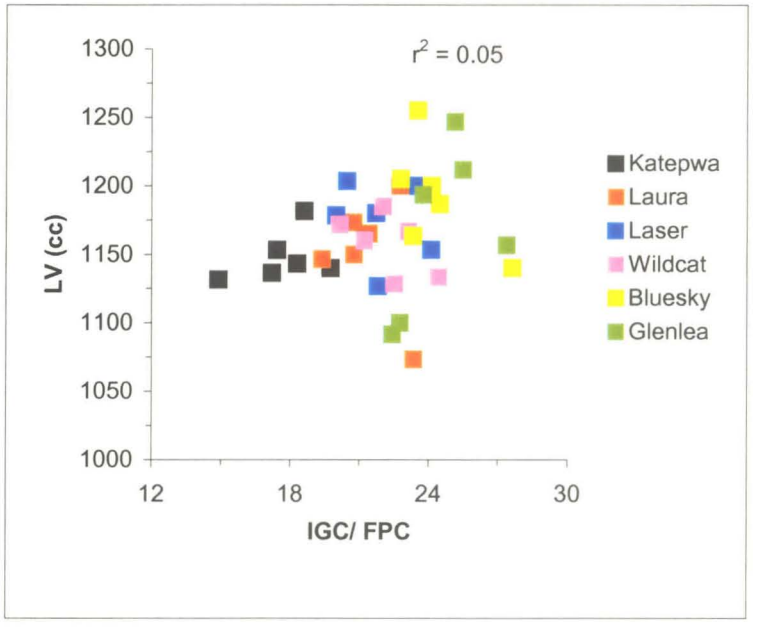
APPENDIX

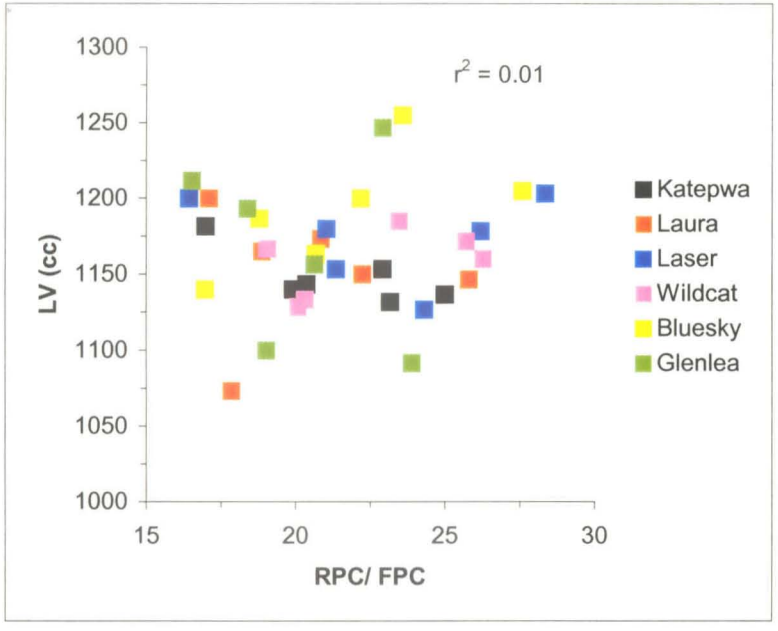
Appendix A Scatterplots of Loaf Volume as a Function of Protein Compositional Parameters

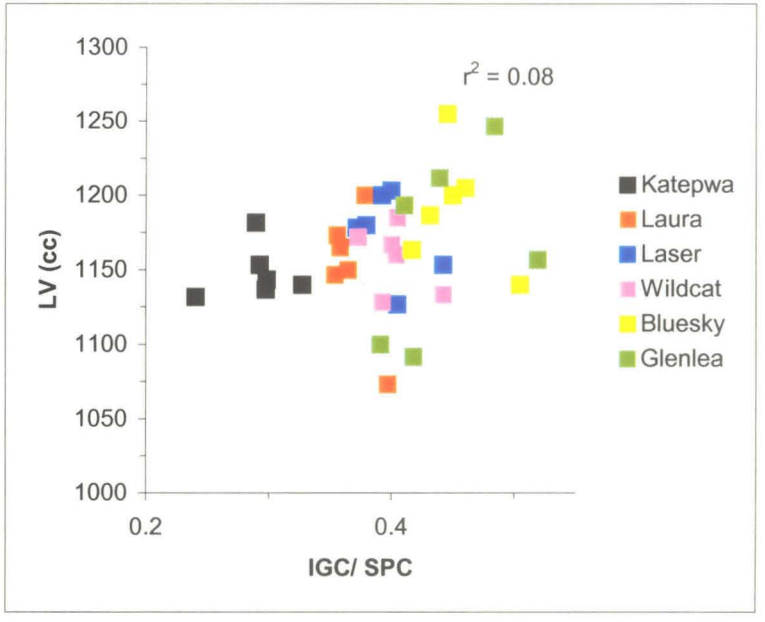


Loaf Volume as a Function of Protein Compositional Parameters for Untreated Samples

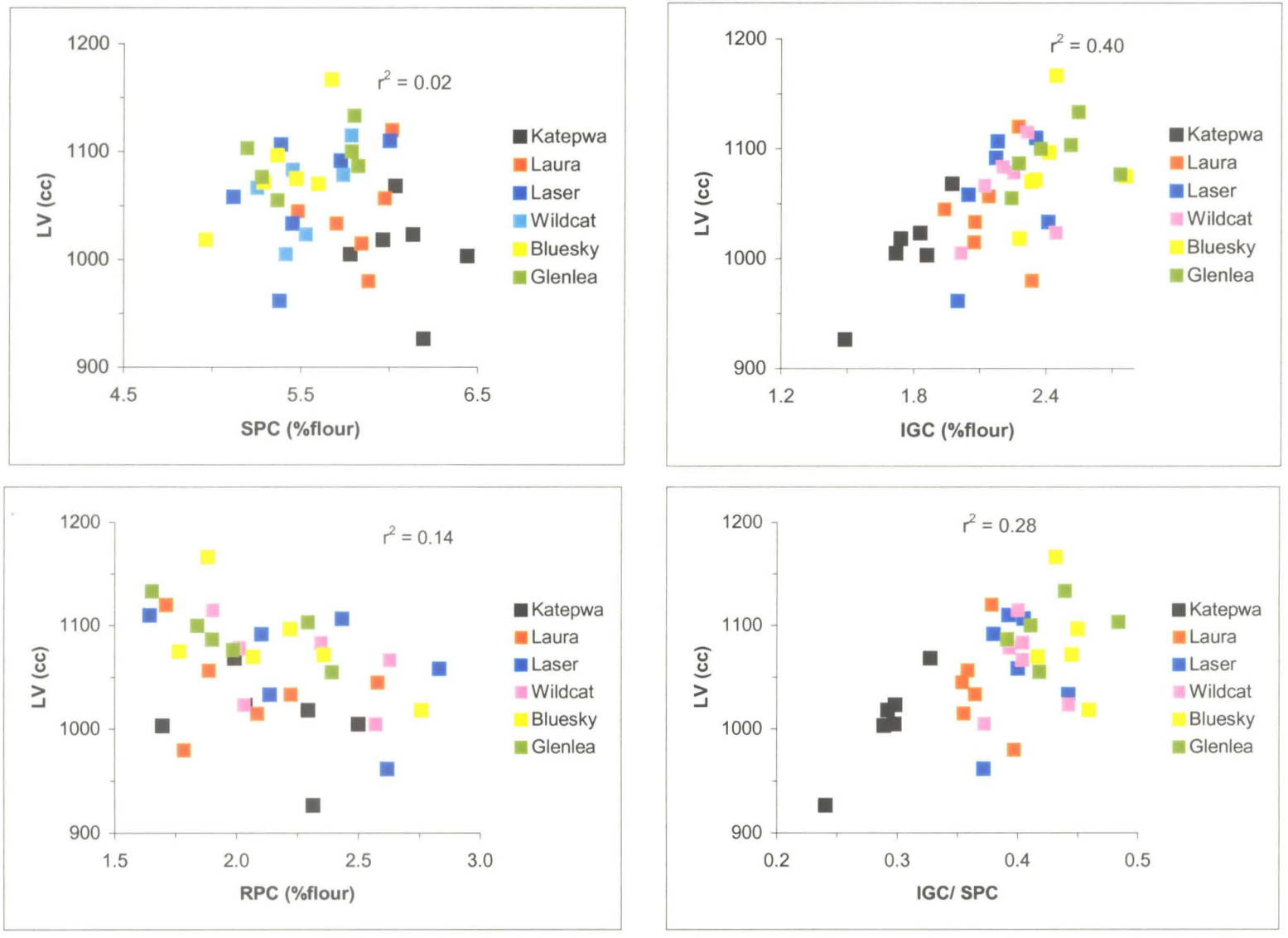




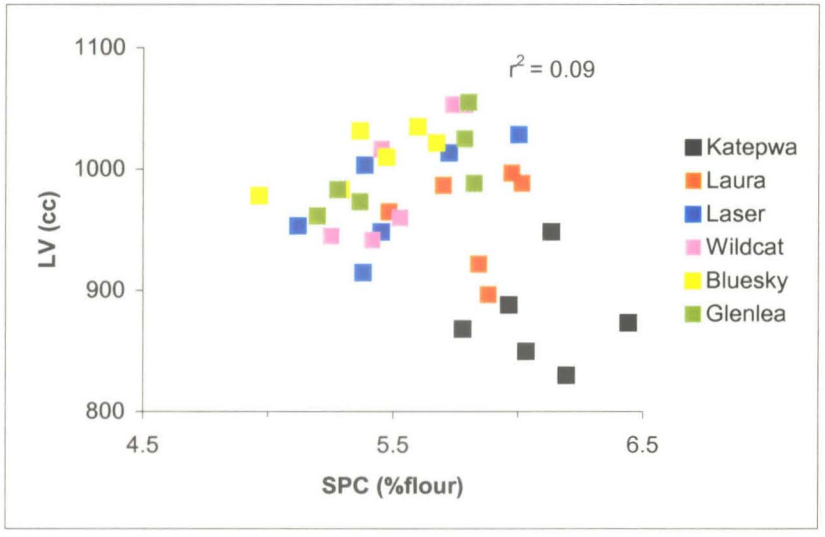


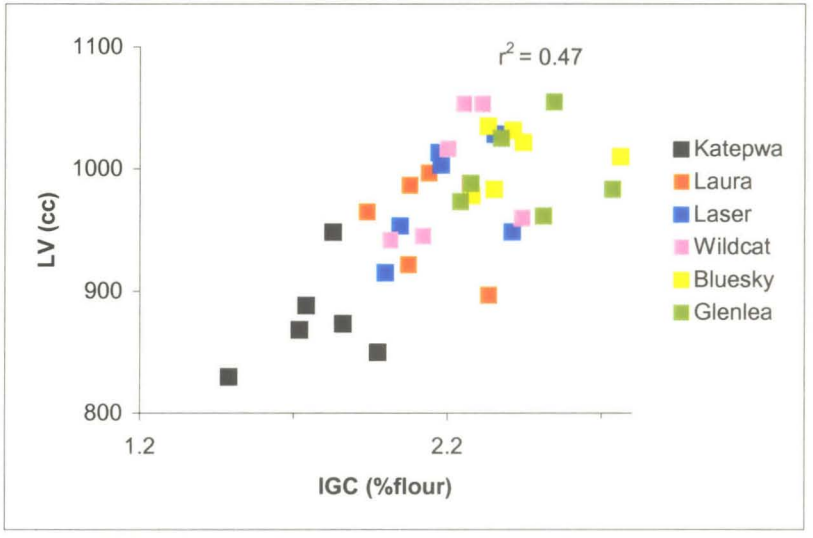


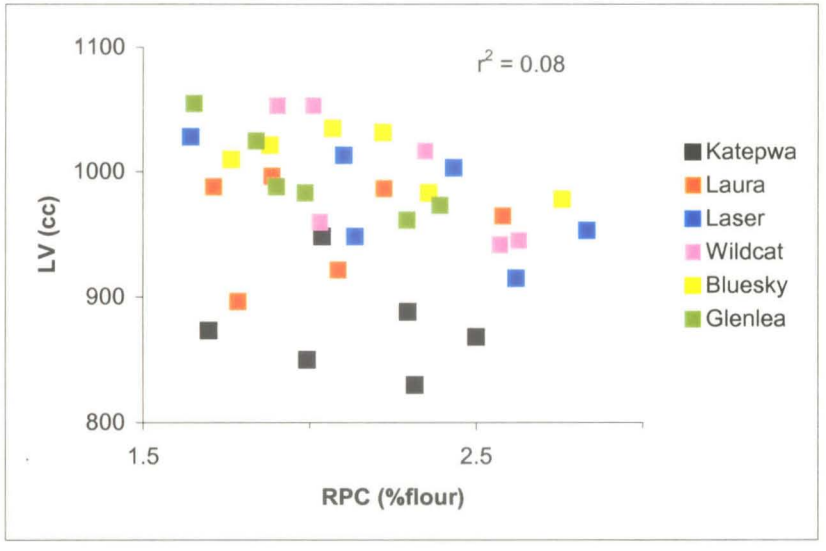
Loaf Volume as a Function of Protein Compositional Parameters for Untreated Samples

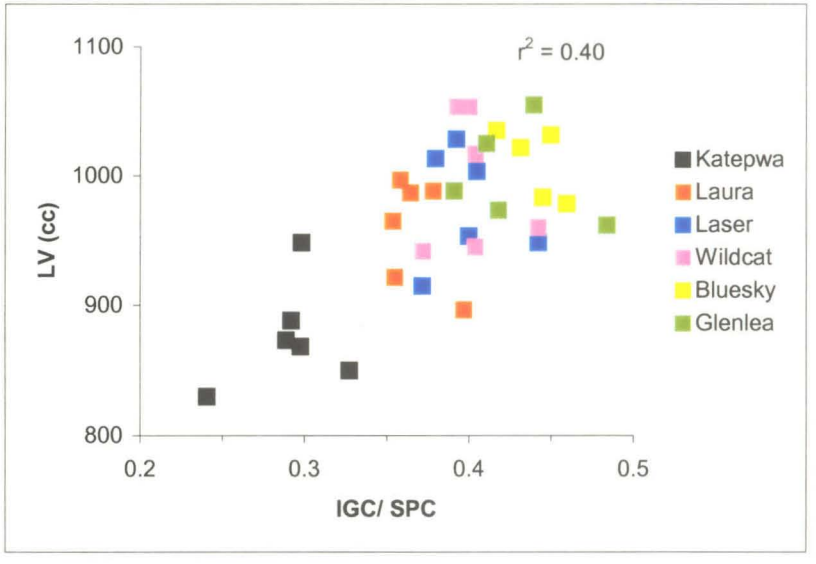


Loaf Volume as a Function of Protein Compositional Parameters for Starch Treated Samples









Loaf Volume as a Function of Protein Compositional Parameters for Starch Treated Samples without Shortening.