Effects of Wheat Millstream Refinement on Flour Colour, Dough Rheology, and Protein Composition

BY

Anne-Sophie Machet

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
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THE UNIVERSITY OF MANITOBA

FACULTY OF GRADUATE STUDIES

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Of

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LIST OF ABBREVIATIONS

Abbreviation	Description
BWPR	BandWidth at Peak Dough Resistance
CIE	Commission Internationale de l'Eclairage
CPS	Canada Prairie Spring
CWES	Canada Western Extra Strong
CWRS	Canada Western Red Spring
HWS	Hard White Spring
DDT	Dough Development Time
FER	Flour Extraction Rate
FP	Flour Protein
HMW-GS	High Molecular Weight Glutenin Subunits
IG	Insoluble Glutenin
LMW-GS	Low Molecular Weight Glutenin Subunits
M_r	Molecular Weight
MT	Mixing Time
MTI	Mixing Tolerance Index
PAGE	Polyacrylamide Gel Electrophoresis
PDR	Peak Dough Resistance
%R	Percentage Reflectance
RP	Residue Protein
RP-HPLC	Reverse Phase High Pressure Liquid Chromatography
SD	Starch Damage
SDS	Sodium Dodecyl Sulfate
SP	Soluble Protein
WIP	Work Input to peak

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ABSTRACT

The wheat kernel is highly heterogeneous in structure and composition. Wheat milling has adapted to this heterogeneity over time, and has evolved to optimize the transformation of wheat into different types of flour for bread and a very wide array of other foods. To accomplish this, wheat milling generates millstreams that can vary widely in refinement (bran content), protein and pentosan content, and overall functionality that can significantly impact the processing properties of dough or batter, along with the visual appeal of end products and their nutritional value. Knowledge and understanding of millstream composition is a very important consideration enabling millers to optimize the quality of flour blends for different products and customers. Good knowledge is available on millstream colour, protein content and general dough mixing properties as a function of flour refinement. However, very little is known about the protein composition of specific millstreams and how that protein composition relates to breadmaking quality. Also, while the relationship between instrumentally determined flour colour and flour refinement or ash content appears to be well understood, that knowledge is based on limited colour wavelength data provided by filter-based instruments.

This thesis research project investigated inter-relationships among spectrophotometrically determined colour, dough rheology and protein composition of millstreams of eight red and white hard spring wheat samples comprising six western

Canadian cultivars including two grown in two different locations. Wheat was milled to an extraction rate of 80% on a tandem Buhler laboratory mill which accommodated extensive stock separation. Thirteen flour streams, including four break streams (B1 to B4), six reduction streams (M1 to M6), a sizing stream (S1), a low quality stream (Q1), and a bran flour fraction (BF), as well as three millfeed fractions, including bran, fine bran, and shorts, were studied. All fractions were initially analyzed for yield, ash content, protein content, and the flour streams were analyzed for starch damage. The degree of flour refinement by colour measurement was evaluated using Agtron colour determination (reflectance at 546 nm), and a computerized diode array spectrophotometer with the capability to measure CIE colour coordinates (L* a* b*) and reflectance spectra from 400 to 700 nm. In addition to roller milling, wheat of four cultivar samples was pearled into six fractions, progressively removing 10% of kernel weight with each abrasion step. These fractions were analyzed for ash and protein contents. Millstreams were evaluated for their dough mixing properties at constant water absorption (65%, 14% mb) without and with 2% (flour basis) salt using a 2 g direct drive computerized mixograph. Mixograph parameters that were analyzed were peak dough development or mix time (MT, min), peak dough resistance (PDR, % torque), i.e. maximum dough consistency, bandwidth at PDR (BWPR) which measures the changing extensional viscosity of a dough as it is being mixed, and work input to peak dough development (WIP). Finally, millstreams and pearled wheat fractions were assessed for their protein composition, using a propanol-based protein fractionation procedure giving three fractions: soluble protein (SP) containing mainly gliadins, insoluble glutenin (IG), i.e. the high molecular weight (HMW) fraction of glutenin, and residue protein (RP). Protein composition results were evaluated for correlation with dough rheological

properties. The reduced IG fraction of several streams that were substantially different in protein content and dough mixing properties were analyzed by reversed-phase HPLC (RP-HPLC) for quantitative and qualitative variation in glutenin subunit (GS) composition.

The total yield of break flours was the lowest for AC Corinne (11.5%), and was highest for AC Barrie (18.3%). Conversely, flour yield of the first two reduction streams (M1+M2), which usually accounts for more than 50% of total yield, was highest for AC Corinne (55.6%), and lowest for AC Barrie (45.2%). These yield results were generally in accord with wheat hardness as evaluated by starch damage, weight-averaged over all flour streams. These starch damage results for AC Corinne and AC Barrie were 6.8 and 5.2%, respectively. Starch damage for reduction flours was more than double that of break flours (8.9 and 4.1%, respectively). Protein contents generally increased across millstreams representing fractions from inner to outer endosperm within the kernel, as reflected by variation from the first to last break (B1 to B4) and first to last reduction streams (M1 to M6). Ash and protein contents of the reduction streams in particular, increased in a systematic and linear fashion. On average from M1 to M6, ash content increased by over 700% (0.33-2.93%) and protein content increased by 55% (12.3-19.1%). For break flours B1 to B4 by comparison, a smaller protein increase of 30% (14.9-19.2%) was measured. A similar increase in ash and protein content from inner endosperm to the peripheral layers of the kernel was also found for the pearled wheat fractions.

Stepwise discriminant analysis showed that reflectance at 400 nm was the best wavelength to discriminate millstreams of different refinement according to ash content. Discrimination power decreased with increasing wavelength as shown by coefficient of

variation (CV) of reflectance across millstreams that progressively decreased from 27% to 8% from 400 to 700 nm, respectively. A particularly noteworthy result was that Agtron measurement of flour colour at 546 nm appeared to be significantly influenced by grain colour for millstreams with ash contents above about 0.50%. In contrast, measuring flour reflectance at 400 nm was independent of grain colour regardless of millstream refinement. As well, results at 400 nm were less influenced by co-variation of protein content compared to Agtron measurements; at 400 and 546 nm, protein content was responsible for 1.4% and 2.0% of the variation in flour colour, respectively. Results strongly point to the need in the milling industry to move away from determining flour reflectance at long wavelengths (e.g. 546 nm) to measurements based at 400 nm which was shown to be much more effective and accurate for assessing flour refinement. Either a computerized colour spectrophotometer could be used or a more economical filter-based instrument such as the Agtron could be adapted for this purpose.

The dough mixing characteristics of millstreams were very diverse. Break streams were considerably stronger than reduction streams. For example, the average PDR of break and reduction flours was 53 and 31%, respectively. Also, break streams had a different pattern of variation compared to reduction roll flours. PDR increased from the first to last break fraction by 56% on average. In contrast, early reduction streams were of similar strength, averaging a PDR value of 36%, but late reduction streams gave very weak doughs; the average PDR value of M6 was 18%. BF gave the strongest doughs, with an average PDR value of 64%. Among cultivar samples, AC Corinne millstreams produced the strongest doughs consistent with its classification as an extra strong wheat, while AC Barrie was the weakest sample.

Flour refinement as measured by ash content was not correlated with any of the mixing parameters although it seemed to play a role in the mixing properties of the last two reduction streams, M5 and M6. When considering dough mixing properties in relation to ash content, protein content and composition, results showed that MT was an unreliable measure of dough strength as no consistent patterns of correlation were evident. In contrast, PDR and BWPR, and WIP to a lesser extent, were very effective measures of dough strength. Ash content was negatively related to PDR and BWPR on average ($r = -0.20$). In contrast, protein content was significantly and positively correlated with these parameters ($r = 0.55$ and 0.52, respectively), but correlation coefficients were much greater when M5 and M6 flour streams were excluded ($r = 0.84$ and 0.79, respectively). For break flours, increasing protein content (i.e. increasing gluten protein fractions SP and IG) from B1 to B4 was accompanied by a corresponding increase in PDR and BWPR, suggesting a cause and effect relationship, although the apparent positive contribution of SP to dough strength appeared to be due to statistical co-variation ($r = 0.55$) with IG. For reduction streams for which protein content increased by an even greater amount from M1 to M6, the correspondence between PDR and BWPR and protein content was not clear. This was especially evident for the last two reduction streams, M5 and M6, which possessed very high ash contents (2.4% on average), high protein contents (17.7% on average) and produced very weak doughs. For these reduction flours, results indicated that their very high content of RP (400% greater than the average for break flours) combined with lower gluten protein content (SP plus IG, 33% lower than the average for break flours) was likely responsible for the observed trend in dough rheological properties.

The addition of salt increased all four mixograph parameters, with a greater effect on PDR and BWPR, however the extent of the effect of salt on specific millstreams was variable among samples. Salt had a greater strengthening effect on AC Corinne, the extra strong cultivar, compared to that of the other samples. For doughs mixed both without and with salt, very similar patterns of correlation were found between all protein fractions and dough mixing parameters, although correlations were slightly higher on average for the salted dough set.

Both millstreams and pearled fractions showed the same patterns of variation in protein composition. As flour became less refined, relative (to millstream protein content) levels of IG and SP decreased, and RP content increased. Accordingly, RP in millstreams most likely derives from contaminating bran residue. This protein fraction merits more attention than it has received to date in the literature, as it likely represents a negative factor in flour breadmaking quality.

Averaged across cultivar samples for roller-milled flours, the range of concentration of gluten protein fractions SP and IG (expressed as a percentage of millstream protein content) varied from 38 to 69% and 12 to 22%, respectively, and was positively correlated with flour refinement. In contrast, RP varied by about 330% across millstreams, from 11 to 47%, and was negatively correlated with flour refinement. Break streams, on average, had higher concentrations of SP/FP (65%) and IG/FP (21%) compared to reduction streams (55% SP/FP and 17% IG/FP). In contrast, break streams on average, had lower levels of RP (14%) compared to reduction streams (25% RP).

RP-HPLC revealed large differences in total IG subunit composition of selected millstreams of widely varying protein content and refinement that reflected quantitative differences in total IG contents. However, no differential expression of HMW or LMW

subunit amounts was found, i.e. relative glutenin subunit concentrations in M1 flour was identical to that of B3 and BF flours. Accordingly, glutenin subunit composition appeared to be identical regardless of the origin of the millstreams, whether from the center of the kernel or its periphery; only the concentration of glutenin varied.

Among protein composition parameters, SP and IG content and IG/FP of millstreams were correlated most highly with mixograph torque (PDR), bandwidth (BWPR) and work input (WIP) at peak dough development, indicating a close association with strong dough properties. It was noteworthy that protein content of millstreams had invariably lower correlations than either SP or IG content and IG/FP, indicating that the protein quality (composition) of millstreams was a more important factor than protein quantity in relation to dough mixing properties. This study confirmed that MT was not a reliable parameter to estimate the quality of millstreams, and could not be predicted from protein composition data.

Taking all the protein fractions into account, results indicated that millstreams with strong dough properties had high IG and SP content, high IG/FP values, and low levels of RP and RP/FP. Break streams on average, and B3 in particular, possessed these characteristics, whereas the latter reduction streams did not. These tail-end reduction streams (M5 and M6) had very weak dough mixing properties. Early reduction streams (M1 and M2) were intermediate in both technological quality and protein quality. The millstream with the strongest dough mixing characteristics for all cultivar samples was BF which had high levels of RP (similar to M4 and M5) but also distinctly high levels of IG that were ~ 25% higher on average than B3 millstreams. These results provide additional confirmation of the importance of HMW glutenin as the predominant wheat protein fraction associated with dough strength and breadmaking quality in general.

In conclusion, this thesis research has contributed considerable new knowledge on relationships between flour millstream refinement, colour, dough strength and protein composition. Knowledge of millstream protein composition, particularly IG and RP fractions, appears to be very beneficial to gain a more complete fundamental understanding of flour breadmaking quality. On the practical side, this information can provide millers with knowledge of properties of individual millstreams that will help to optimize blending for the production of flours with specific characteristics for different end-uses.

INTRODUCTION

Among the world's cereal grains for food use, wheat ranks second to corn in terms of production and first in human consumption. Its unique characteristics that make wheat the only cereal suitable for breadmaking have contributed to extensive research over the years. That uniqueness largely depends on the corresponding uniqueness of wheat endosperm proteins which were first fractionated in detail by Osborne (1907). Since then, extensive research has been done on aspects related to wheat quality improvement, including wheat genetics, wheat grain and flour composition, dough rheology, and bread characteristics. Considerable progress has been achieved through development of appropriate genotypes with optimal properties for a given class of wheat. For example, the CWRS wheat class is widely considered to be the foremost bread wheat class in the world in terms of absolute quality and uniformity of quality. However, variability in CWRS wheat utilization quality still exists. This variability arises from genotypic differences within the class combined with environmental effects. Another less often considered source of variation in utilization quality is that due to milling. Even though milling is the most important value-added processing step in the conversion of wheat into baked goods, knowledge is especially weak on protein composition of individual millstreams and inter-relationships with processing quality.

It is well established that wheat has a very heterogeneous physical and chemical composition, part of which is reflected by a considerable increase in protein and mineral contents from the inner to the outer layers of the kernel (Morris et al., 1945, 1946, Hinton, 1947, 1959, 1962, Stevens et al., 1963, Kent, 1966, Kent and Evers, 1969). Several studies have also shown that not only the content but also the protein

composition (in terms of ratios of the different types of protein) varies throughout the kernel (Hinton, 1947; McDermott and Pace, 1960; Stevens et al., 1963; Kent and Evers, 1969; Nelson and McDonald, 1977). Consequently, wheat milling generates millstreams of varying and distinct refinement, protein content and composition that will impact dough processing properties, bread loaf volume and texture, and nutritional value. Millstream refinement is widely used in the industry to facilitate blending of flours for different end-uses, and is generally based on the mineral content of the millstreams measured by ash determination. Since the minerals in the wheat kernel are concentrated in the outer endosperm and bran, as well as germ, and are present only at relatively low levels in starchy endosperm, the ash content of flour, or its degree of refinement, reflects its level of contamination by non-endosperm material, especially bran.

In this study, eight cultivar samples including six Canadian wheat genotypes (two grown in different locations) from five commercial classes were milled on a tandem Buhler Laboratory mill, leading to the production of 13 millstreams and 3 millfeed fractions differing greatly in composition and refinement. Wheat of four of these samples were pearled to provide fractions representing different layers of the kernel. This thesis is a comprehensive investigation of the physicochemical properties of the millstreams with focus in three areas: 1) colour measurement, 2) dough mixing properties, and 3) protein composition. The objectives of the study were as follows:

- To evaluate a new approach for flour refinement characterization, i.e. colour spectrophotometry. The wide refinement range of the millstreams accommodated an opportunity to achieve this objective. A computerized diode array colour spectrophotometer was used that was capable of simultaneous measurement of reflectance across the entire visible light spectrum as well as tristimulus colour

coordinates. Results were compared to those obtained with the conventional methods, i.e. ash content and colour measurement using the Agtron colour meter.

- To determine the dough mixing properties of the millstreams, using a 2 g direct drive computerized mixograph. Some researchers have previously determined some rheological properties of millstreams using relatively low intensity regimes provided e.g. by a Do-Corder (Endo et al., 1987), a farinograph (Holas and Tipples, 1978; Preston et al., 1982) and extensograph (Preston et al., 1982). These studies also usually focused on one generic wheat genotype or composite sample reflecting a class of wheat. Knowledge of millstream dough performance by high intensity experimental mixing as accommodated by the mixograph is very limited. Because of the small amount of some of the millstreams, the 2 g mixograph was convenient to conduct this study.
- To evaluate the variation in protein composition of the millstreams in terms of insoluble glutenin, soluble protein, and residue protein, using a propanol-based protein fractionation procedure. The quality or grade of different millstream flours is most often based on ash and protein content. However, protein composition likely varies widely as well. Since the protein composition of flour, most notably the glutenin component, is a key factor in breadmaking quality, it would be advantageous to gain a better understanding of the protein quality of different millstreams, particularly in relation to their dough mixing properties. Results were compared to the protein composition of pearled wheat fractions which provided material of varying refinement complementary to roller-milled flour. Several millstreams of very different refinement, protein content and quality were analyzed

using reversed-phase HPLC to evaluate whether quantitative and qualitative variation existed in glutenin subunit composition.

Chapter 1: Literature Review

1.1. Introduction

Among cereal crops, wheat is the second most grown and consumed worldwide only after corn. Its popularity stems mainly from its unique ability to produce leavened bread. After appropriate hydration and energy input, wheat flour is transformed into a viscoelastic dough in which gluten proteins form a continuous network. That protein network is capable of entrapping gas bubbles during mixing. Subsequent expansion of dough during fermentation and baking can result in bread of high volume.

The first step in processing wheat into bread is milling. The two main objectives in wheat milling are to separate as completely as possible the external covering of the wheat kernel (bran and germ) from the endosperm, and to reduce the endosperm to flour by grinding larger particles into fine particles. These two objectives are attained through several so-called break and reduction steps. At each step, flour is obtained. Because of the heterogeneous physical and chemical composition of the wheat kernel, the different millstreams vary in composition and quality. That quality is a function of ash content and colour, protein content and strength. The miller then combines the different flours streams together according to the type of flour needed for the end product. Accordingly, different qualities of finished flour can be produced from the same starting grist of wheat.

The milling process is therefore the most important value-added processing step in the conversion of wheat into baked goods. However, relatively little is known about the variation in composition and quality of the millstreams compared to what is known about these aspects in straight grade flour.

1.2. Wheat kernel composition

1.2.1. General composition

The wheat kernel is essentially composed of three histological sections: the endosperm, the germ, and the bran, each having a characteristic chemical composition. The germ (2.4-3.6% w/w of the kernel) comprises the embryonic axis and the scutellum, the endosperm (81-83%) includes the starchy endosperm and the aleurone layer, and the bran (14.7-15.0%) is comprised of at least six different tissue layers (Fig. 1.1). The main constituents and their typical chemical composition are presented in Table 1.1.

Table 1.1. Typical percentage composition of wheat germ, bran, aleurone, and endosperm.

	Grain	Germ	Endosperm	Bran	Aleurone
Protein (Nx5.7)	12.8	27.6	10.3	16.6	17.9
Lipid	2.5	10.6	1.1	5.4	7.9
Ash (mineral)	1.9	4.3	0.5	7.8	15.8
Carbohydrates	82.7	50.4	86.2	70.0	58.3
Starch		21.3	82.6	9.9	Nil
Sugars		17.0	1.3	5.3	12.9
Cellulose		8.5	0.2	24.6	4.6
Pentosans		7.7	2.1	30.2	24.6

Adapted from Pomeranz (1988).

1.2.2. Ash and protein gradients

Numerous studies have proven that both ash and protein contents have increasing gradients from the inner to the outer endosperm (Morris et al., 1945, 1946; Hinton, 1947, 1959, 1962; Stevens et al., 1963; Kent, 1966; Kent and Evers, 1969). The early studies have used hand-dissection or microdissection (done by securing and analyzing material from definite parts through drilling out layers from cross sectioned kernels). The small

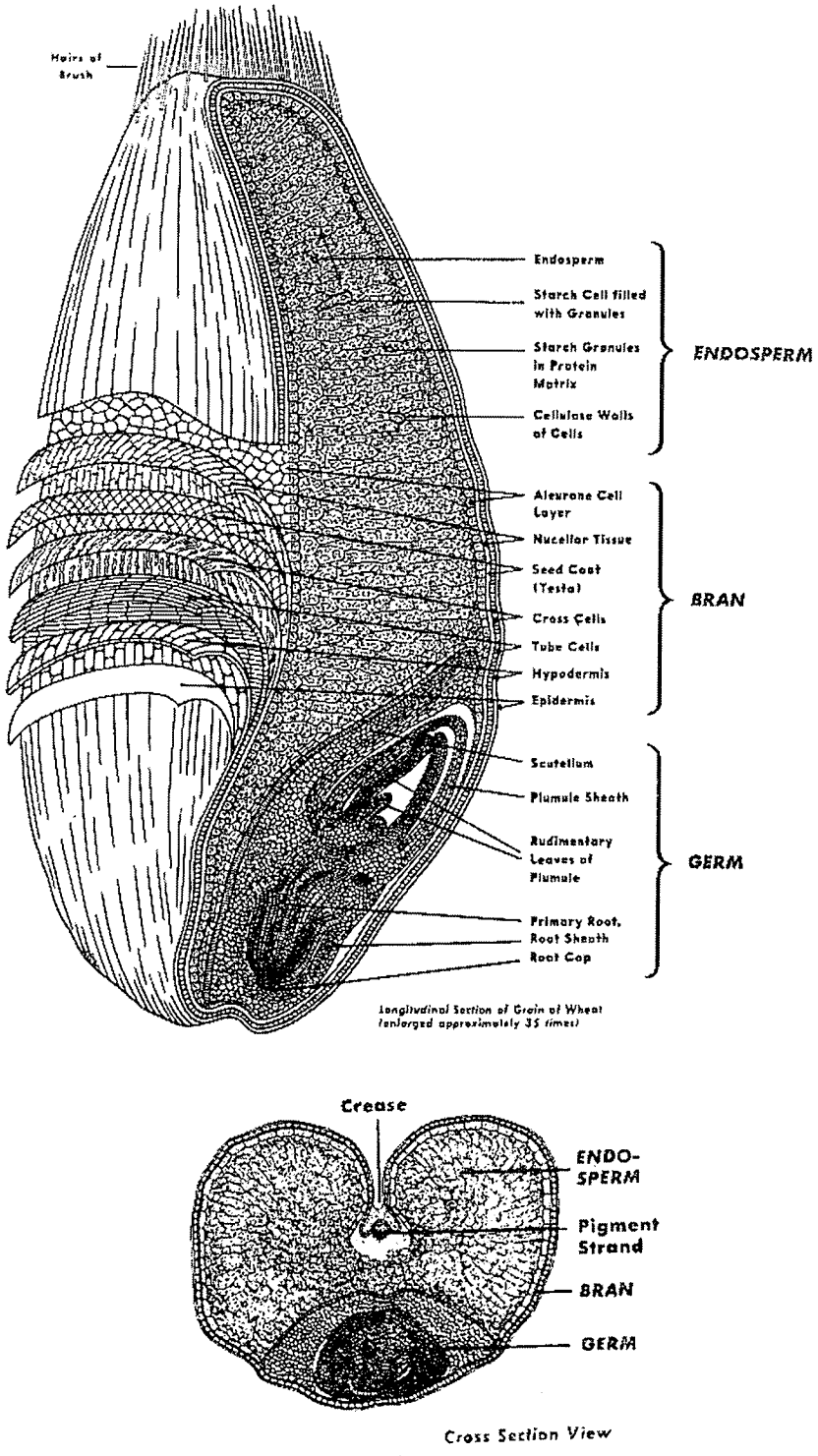


Figure 1.1. Longitudinal and cross sections of a wheat kernel (Source: Wheat Flour Institute, 1976, Washington, D.C.).

size of the wheat kernel and the presence of the crease render hand-dissection difficult and promote cross-contamination of the tissues. Although the data obtained might not be completely accurate, they give a good understanding of the variations in ash and protein contents within the kernel. By hand-dissecting wheat kernels and examining the composition of the different parts of endosperm, aleurone layer, pericarp and testa, Hinton (1959) found an increasing ash gradient from inner to outer endosperm (0.38% to 1.41%, mean of three wheat varieties). Ash content of the endosperm accounted for 20 to 26% of the total ash content of the kernel (Hinton, 1959; MacMasters et al., 1971). The aleurone layer contained 14.37 to 17.22% ash, and accounted for 56 to 60% of the total ash of the kernel (Hinton, 1959; MacMasters et al., 1971). The ash content in the bran layers was 13 to 18 times that of the endosperm (Morris et al., 1945).

Protein content has a similar increasing gradient from inner endosperm to the periphery of the kernel. The principal difference in protein distribution, compared to that for ash, is its concentration in the bran coat (Morris et al., 1945). In these tissues, the protein content was about 1.4 times that of the whole endosperm, in contrast to ash content which was 13 to 18 times that of the endosperm. Similar to ash, there is also a shallow increasing gradient of protein content within the inner endosperm (from the center to the outer cheek). The lowest concentrations of protein found by Morris et al. (1945) were in the center endosperm and ranged from 6.5 to 8.6%. Cobb (1905) and Hinton (1947) analyzed hand-dissected fraction of soft wheat and found a 2.2 fold gradient in protein content (7.4 to 16.5%, variety Purple Straw, and 5.7 to 12.5%, variety Vilmorin 27, respectively) from the center to the periphery of the endosperm. However, Kent (1966) reported a much steeper gradient in protein content, from 8% in inner endosperm to 43% in subaleurone endosperm. He separated the subaleurone endosperm

(outermost cellular layer of the starchy endosperm) from the inner endosperm using a sedimentation technique on coarse air-classified fractions of pin-milled hard red winter wheat. He confirmed that a difference existed between hard and soft wheat, due to differences in the composition of the subaleurone layer, which was much richer in protein content in hard wheat. He reported an increase in protein content in the subaleurone layer from 33 to 54%, whereas the increase in protein content in the inner endosperm was from 8 to 15%. Although a typical wheat grain contains about 11% of subaleurone endosperm by volume (Kent and Jones, 1952), this layer, because of its high protein content, accounts for almost 25% of the total protein of starchy endosperm in hard wheat.

Stevens et al. (1963) investigated the amino acid composition of endosperm protein and aleurone cell contents obtained by air-classification of milled wheat, and of the total protein complex in the parent flour. The amino acid composition of the aleurone cells was different than that of the endosperm cells, indicating a difference in protein composition between the aleurone layer and the endosperm. However, some segregation may have occurred during the air classification process. Several authors have reported a higher proportion of glutenin-to-gliadin ratio and a lower proportion of water-soluble proteins in the portion of endosperm of higher protein content than that of lower protein content (McDermott and Pace, 1960), and in high-protein flour fractions than in low protein fractions that were separated by air-classification (Jones and Dimler, 1962). Kent and Evers (1969) concluded that storage protein represents a larger proportion of the total protein in subaleurone endosperm than in inner endosperm.

1.3. Wheat flour proteins

1.3.1. Protein composition

Wheat proteins were first classified by Osborne (1907) into four groups according to their solubility: albumins, soluble in water; globulins, soluble in dilute salt solutions; gliadins, soluble in 70% ethanol; and glutenins, soluble in dilute acids or bases. Chen and Bushuk (1970) added a fifth fraction to the original four from Osborne's procedure, by dividing glutenin into two fractions: one soluble in dilute acetic acid (0.1 N) and the other insoluble in this solvent. Albumins and globulins represent about 9% and 5% of the total protein, respectively (Orth and Bushuk, 1972; Bushuk, 1993). From the perspective of flour functionality for breadmaking, albumins and globulins are considered as non-gluten proteins. They are mainly non-storage metabolic proteins, i.e. enzymes. Gliadins and glutenins are considered storage proteins (nitrogen-storage proteins) due to their very high glutamine content. According to the modified Osborne fractionation (Orth and Bushuk, 1972), they make up about 85% of the total proteins (40% each, respectively), and are also called gluten proteins. Gluten proteins are of outmost importance in wheat flour since they directly correlate to breadmaking properties. With gliadins providing the viscous component and glutenin the elastic component, these two types of proteins confer the well-known viscoelastic properties to the dough. When hydrated, the gluten forms a viscoelastic network capable of entraining air bubbles during dough mixing, and containing their subsequent growth during dough fermentation, processes that are necessary to obtain bread with good volume and fine crumb structure.

Gliadin proteins are extremely heterogeneous. They are single chain polypeptides covering a relatively wide range of molecular weight, and can be classified

into four groups, based on their electrophoretic mobilities on acid-polyacrylamide gel electrophoresis (PAGE): α-, β- , γ-, and ω-gliadins. ω-gliadins (M_r = 44,000 to 74,000) lack the sulfur-containing amino acids, cysteine and methionine (Field et al., 1983) but possess a relatively high proportion of residues of glutamine, proline and phenylalanine. α-, β- , and γ-gliadins (M_r = 30,000 to 44,000) have a more variable amino acid composition. Unlike the ω-gliadins, they contain about 2 to 3% cysteine plus methionine. All the cysteine residues are involved in intramolecular disulfide bonds.

Glutenins are very large polymeric proteins. These proteins remain in the insoluble residue after sequential extraction with 0.1*N* sodium chloride and 70% ethanol solutions. The true molecular weight of the glutenins is not known, but evidence suggests that it is above 1 million (Bushuk, 1993), and can be as much as 11 million (Wahlund et al., 1996). These proteins comprise a wide range of subunits of two main types, the high molecular weight glutenin subunits (HMW-GS, M_r = 95,000 to 140,000) and the low molecular weight glutenin subunits (LMW-GS, M_r = 30,000 to 51,000), joined by interpolypeptide disulfide bonds (Payne and Corfield, 1979). The HMW-GS form about 10 to 20% of the glutenin protein and have a major role in breadmaking quality of bread flour. A bread variety can have three to five HMW-GS and about 15 LMS-GS (Bushuk, 1993).

1.3.2. Protein quality as related to dough strength and breadmaking quality

Although the existence of a protein quality factor in wheat flour has long been known (Finney and Barmore, 1948), it is only in the early 1970's that a specific protein fraction was found to be related to breadmaking quality differences among different genotypes. Orth and Bushuk (1972) demonstrated that the breadmaking quality of a flour

was directly related to the amount of glutenin insoluble in dilute acetic acid. Since then, a major focus in cereal chemistry research has been the development of methods for extraction and fractionation of wheat flour proteins, especially glutenins, and investigation of their effects on the functional properties of doughs. Because of their polymeric structure and large size, glutenin molecules are highly aggregated. The degree of this aggregation has been associated with solubility properties. Flours of better quality have a greater proportion of aggregated glutenin molecules that leads to a greater proportion of protein insoluble in aqueous solutions of urea (Pomeranz, 1965), acetic acid (Orth and Bushuk, 1972), hydrochloric acid (MacRitchie, 1987), sodium dodecyl sulfate (Gupta et al., 1993), and propanol (Fu and Sapirstein, 1996).

Many researchers have now established a cause-effect relationship between glutenin fraction and rheological and baking properties of wheat flours. Reconstitution studies by Lee and MacRitchie (1971) and MacRitchie (1973) showed that the dough properties and thus the strength of the flour were highly influenced by the molecular weight distribution of gluten proteins. Adding high molecular weight protein fraction of the gluten to a weak flour rendered it strong. Another reconstitution study by MacRitchie et al. (1991) showed an increase in loaf volume and mixograph peak development time as the proportion of glutenins in the fractions increased. Huebner and Wall (1976), Zhu and Khan (2004), and others reported that flours containing a higher ratio of high molecular weight glutenin to low molecular weight glutenin were generally of better breadmaking performance. They also found that flours of weak mixing and baking characteristics usually contained lesser quantities of the high molecular weight fraction.

Payne et al. (1979) reported that the presence of a subunit of glutenin, termed glutenin subunit 1, whose molecular weight was about 145,000 (HMW-GS), correlated significantly ($r = 0.72$) with breadmaking quality, indicating that the HMW-GS composition as measured by SDS sedimentation volume was an important factor in breadmaking. Payne et al. (1981) and Ng and Bushuk (1988) also found a strong correlation between the presence of HMW-GS 5 and 10 and baking quality. Subsequently, Payne (1987) proposed a quantitative system, the Glu-1 Quality Score, in which glutenin subunits are named according to a numerical order based on their mobility on SDS-PAGE. This system ranks wheat varieties according to their HMW-GS composition, an indirect measure of breadmaking quality. Since then, a substantial body of work has demonstrated the relative importance of many different subunits (Branlard and Dardevet, 1985; Ng and Bushuk, 1988; Dong et al., 1992; Gupta et al., 1994, 1995; Huang and Khan, 1997; Beasley et al., 2002; Uthayakumaran et al., 2002; He et al., 2005)

Whether glutenin subunit composition alone is the main factor responsible for quality differences among wheat genotypes has been the subject of subsequent research. However, in the literature, discrepancies are found on interpretations of the functional properties of gluten proteins in breadmaking. Hoseney et al. (1969) dissolved gluten in 0.005 N lactic acid and obtained three fractions: an insoluble fraction, a gliadin-rich fraction, and a glutenin fraction. After reconstituting the gluten protein fraction with water-solubles and starch and then baking the flours, they found that the insoluble fraction had no effect on breadmaking, whereas the gliadin-rich protein was responsible for loaf volume, and the glutenin proteins for the dough mixing properties of the wheat flour. By fractionating gluten proteins from 26 wheat varieties using a modified Osborne

procedure, Orth and Bushuk (1972) and Orth et al. (1972) obtained high correlations between loaf volume and dough strength (as measured by the farinograph), and the proportion of insoluble glutenin proteins. MacRitchie (1978) used 0.1 M acetic acid to fractionate gluten proteins. After centrifugation, the supernatant was referred to as the gliadin fraction, and the residue as the glutenin fraction. The gluten fractions were reconstituted with their water-solubles and starch fractions, and then baked. The results showed that increasing levels of high molecular weight proteins strengthened the dough, suggesting that dough strength was a function of the molecular weight distribution of the gluten proteins. He suggested that differences in breadmaking quality were closely related to the properties of the more insoluble high molecular weight glutenin proteins. Preston and Tipples (1980) used 0.05 M acetic acid to fractionate gluten proteins. The acid-soluble fractions had a dough strengthening effect and increased loaf volume, whereas the acid-insoluble fraction decreased loaf volume. Marais and D'Appolonia (1981) found that loaf volume was positively correlated to protein content, but negatively correlated with the percentage of glutenin and residue proteins.

The two major variables responsible for these conflicting results were differences in isolation procedures, and differences due to different varieties. Chakraborty and Khan (1988b) have attempted to explain the different results by fractionating proteins of two hard red spring wheat varieties, one of poor quality and one of good breadmaking quality, using various isolation procedures (Chakraborty and Khan, 1988a). They then exchanged the water-solubles (albumins and globulins) and starch fractions, separately, between the two varieties, and also exchanged the gliadin and glutenin fractions. Their results showed that compositional differences in protein fractions (albumins and globulins, gliadins, glutenins, and residue) as obtained from various fractionation

procedures could lead to differences in loaf volume. However, the highest positive responses to loaf volume were associated with the fraction containing the larger amount of glutenin, independent of the fractionation procedure. Singh et al. (1990a,b) showed that sonication of flour suspensions in 2% SDS solution (pH 6.9) gave almost complete extraction of proteins, and therefore glutenin. A strong positive relationship was reported (Singh et al., 1990b) between the proportion of glutenin as measured by SE-HPLC and several flour quality parameters in a set of 15 cultivars. Notably, relative quantity of glutenin was highly positively correlated with loaf volume ($r = 0.72$).

Chakraborty and Khan (1988b) also found that optimum loaf volume was obtained when a certain portion of the glutenin fraction was in combination with gliadin proteins. These findings were further supported by Van Lonkhuijsen et al. (1992) and Sapirstein and Fu (2000). Van Lonkhuijsen et al. (1992) used RP-HPLC to correlate gliadin fractions and breadmaking quality for 32 wheat samples with the same HMW glutenin A subunit composition (null, 7, and 2+12) but different gliadin compositions. They found a good correlation between loaf volume and composition of γ-gliadins. Other peaks corresponding to ω-gliadins were negatively correlated to loaf volume. They demonstrated a strong statistical relation between breadmaking quality and the relative content of certain types of gliadins, for which only four gliadin peaks explained 82% of the variance in loaf volume. However, only one type of HMW-GS composition was used in this study and consequently these results might not be valid for other wheat genotype. Sapirstein and Fu (2000) have also proposed that it is the interaction of gliadin and glutenin which explains differences in dough strength of different wheats.

The effect of glutenin proteins on the rheological properties of dough has also been extensively studied. However, as previously noted, fractionation-reconstitution

studies are predominant and the fractionation methods used to isolate the gluten proteins are various. Lee and MacRitchie (1971) fractionated the protein component from four varieties of Australian wheats by a successive extraction procedure using urea solutions. When added to a base flour, early urea extracts decreased mixing stability as measured by a mixograph and gave weak doughs, as measured by an alveograph. On the contrary, late urea extract, containing higher levels of HMW protein, increase mixing stability and dough strength. Lawrence et al. (1988) used near-isogenic lines (three lines each null at one of the loci (*Glu-A1*, *Glu-B1*, or *Glu-D1*), three lines each null at two of the loci, and one line null at all three loci) and demonstrated that the amount of HMW glutenin related very strongly with dough properties. They reported that breadmaking quality declined dramatically with the absence of all HMW-GS. Absence of subunits 5+10 at the *Glu-D1* locus and 17+18 at the *Glu-B1* locus had a significantly greater effect on Mixograph peak time than loss of subunit 1 at the *Glu-A1* locus. Also, the lines deficient for both subunits 5+10 and 17+18 had significantly poorer quality than lines deficient for either 5+10 and 1, or 17+18 and 1. Singh et al. (1990a,b) extracted glutenins using sonication of flour suspensions in 2% SDS solution (pH 6.9), and reported strong correlations between the relative quantity of glutenin and extensograph dough resistance ($r = 0.84$), extensograph extensibility ($r = 0.84$), and mixograph peak development time ($r = 0.84$). Absolute quantity of glutenin was significantly correlated with farinograph dough development time ($r = 89$) and negatively correlated with dough extensibility ($r = -0.76$) and dough breakdown ($r = -0.65$) (Singh et al., 1990b). Sapirstein and Johnson (2001) developed a simple spectrophotometric method to measure the content of insoluble glutenin in flour. After removal of monomeric proteins with 50% 1-propanol, insoluble glutenins were solubilized using 50% 1-propanol and dithiothreitol (DTT). The

insoluble glutenin content, determined spectrophotometrically, was highly correlated (r^2 = 0.85) with mixing strength of diverse set of 88 Canadian wheat genotypes.

The use of the mixograph has been widely used in determining dough rheological properties. Martinant et al. (1998) found strong correlations between the amount of protein, the proportion of gliadin and that of glutenin, and mixograph parameters such as the peak dough resistance and the peak height for a set of 39 European bread wheat cultivars of medium breadmaking quality. The proportion of gliadin was negatively correlated with mixing time, whereas the residue protein (unextractable in 70% ethanol and 2% SDS) was positively correlated with mixing time. Khatkar et al. (1996) concluded from a correlation study with the mixograph involving 13 wheat cultivars from Canada, France, and the UK (selected on the basis of their wide range in breadmaking performance) that the protein quality appeared to primarily control the dough behaviour during mixing. The differences in the mixing characteristics among weak and extra strong cultivars were mainly due to differences in gluten protein quality (glutenin content) with protein content playing only a minor role. Dong et al. (1992) attempted to evaluate the effects of gliadin and glutenin proteins and their individual electrophoretic bands on dough mixing properties. Gliadin proteins, extracted with 70% aqueous ethanol, were analyzed by acid-PAGE and glutenin proteins, extracted with 0.125 M Tris buffer containing 1% SDS and 5% 2-mercaptoethanol, were analyzed by SDS-PAGE. The results provided evidence for the existence of direct associations between breadmaking quality and certain HMW-GS and gliadin subunits. HMW-GS 5+10 were associated with good dough mixing and baking quality and subunits 2+12 and 3+12 with poor dough mixing and baking quality. These results were consistent with previous studies (Branlard and Dardevet, 1985; Ng and Bushuk, 1988), providing that

some of the HMW-GS have consistently shown their effects on baking quality. However, Dong et al. (1992) did not include in their study the effect of LMW-GS. Roels et al. (1993) found the mixing characteristics of doughs to be well correlated with Glu-1 scores, supporting the data of Finney et al. (1982) that glutenin quality governs mixing requirements. Skerritt et al. (1996) extracted gliadin-rich and glutenin-rich fractions using a fractionation method based on solubilization in dilute hydrochloric acid after titration at different pH (5.3 for gliadin and 3.9 for glutenin). They found that the addition of glutenin fractions increased dough strength whereas gliadin fractions weakened the dough properties, as measured by a 2 g mixograph. This result was also found by Edwards et al. (2003) who isolated gluten from three durum wheat cultivars with a wide range of strength. Fido et al. (1997) extracted gliadins from wheat and investigated the effect of purified individual groups (α-, β-, γ-, ω -1 and ω-2). They found that addition of all goups of gliadin resulted in a decrease in dough strength as measured on a 2 g mixograph.

Although HMW-GS seem to have a fundamental role in dough rheological properties and breadmaking, the majority of the subunits in glutenins are LMW-GS. Allelic variation of LMW-GS can also affect quality. The co-migration of LMW-GS with gliadins in SDS-PAGE traditionally makes the study of these proteins difficult. However, Gupta and Shepherd (1987) developed a two-step SDS-PAGE method and were able to achieve the separation of these two types of proteins. They reported a significant variation of LMW-GS patterns among bread wheat varieties. The ratio of HMW-GS to LMW-GS has also been a subject of interest more recently. Gupta et al. (1991) found that the highest correlation coefficients between predicted and actual dough strength as measured by the extensograph (Rmax and Ext) were obtained when

predictions were based on both LMW and HMW glutenin subunits. Gupta et al. (1993) reported that protein unextractable in 0.5% SDS was very strongly and positively correlated with dough strength. Using size-exclusion HPLC they showed that the unextractable protein contained a greater proportion of larger polymers and a significantly higher ratio of HMW-GS to LMW-GS than the extractable fraction. Gupta et al. (1992) fractionated flour protein using sonication in 2% SDS-buffer solution. The HMW-GS and LMW-GS contained in the unreduced fraction obtained by sonication in 2% SDS buffer were separated by SDS-PAGE. Results showed that samples with a higher ratio of HMW-GS to LMW-GS had greater dough strength, i.e. higher dough maximum resistance and mixograph development time. Zhu and Khan (2004) fractionated gluten proteins form two cultivars of HRS wheat with good and poor breadmaking quality, using sequential extraction with dilute hydrochloric acid. They found that the cultivar of better breadmaking quality had a higher ratio of HMW to LMW glutenin subunits.

1.4. Wheat milling

1.4.1. Milling process

Aside from reducing the endosperm into very fine particles for flour production, the main objective of milling is to separate as completely as possible wheat bran and germ from the starchy endosperm. Roller milling is the traditional process wherein each mill is equipped with break (corrugated) rolls and reduction (smooth) rolls. Break rolls break open the grain, scraping off as much endosperm from the bran layers as possible (Bass, 1988). The reduction rolls gradually grind or reduce the endosperm into flour. After each roll passage, the stock is sifted, and the flour passing through the fine sieves

is collected. Depending on the stage in milling, the material remaining on the sieves is classified into three types of particles (all larger than flour): 1) pure or relatively pure endosperm, 2) composites of endosperm and bran varying in size, shape, and proportion of endosperm to bran, and 3) pure or relatively pure bran. Depending on the type of particles, they will be redirected to the appropriate stage for further purification or grinding. After each roll passage, the flour collected is called a millstream or flour stream, and is given the name and number of the corresponding roll passage, i.e. break flours are called B1, B2, etc, the middling flours coming from the reduction passages are called M1, M2, etc. A mill also includes one or more sizing passage (scratch process), and the resulting millstreams are called S1, S2, etc. In some cases, the mill also has a low quality clean up passage, and the resulting flour is given the name Q1. A commercial hard wheat mill can produce 30 or more millstreams. The by-products collected from the mill are bran and shorts. The bran is the coarse, flaky product that has been passed through the break system after adhering endosperm has been removed. After it emerges from the final break passage sifter, it is passed through a bran finisher (or bran duster) to recover the final vestiges of adhering endosperm. The collected flour is sieved and is called bran flour. Shorts are the finer branny material emerging from the final overtails of the later passages of purification and from the final reduction sifter. Shorts can also be passed through a shorts duster to strip the remaining endosperm. In a conventional milling system, some germ is recovered initially from the whole wheat by an impact machine, and later from the overtails of coarse reduction passages. Further details about the milling process are beyond the scope of this thesis, and are well explained in many publications and books (for review, see Bass, 1988).

By judiciously blending the different millstreams obtained, the miller can produce a wide range of flours of different qualities and refinement for specific end-uses. The miller can either combine all the streams together to produce a straight-grade flour, or blend together various machine flours in different combinations to produce split run flours to accommodate the various commercial grades of flours that are sold by the milling industry, e.g. patent flour, clear flour, etc.

The resulting flour should have good colour, be free of brown specks, and have improved digestibility and shelf life. The most common diagnostic tests for determining flour quality relate to the degree of flour refinement as measured by flour colour measurement (Patton and Dishaw, 1968; Murthy and Dietz, 1974) and ash content determination. These are discussed in the next section. The properties of flour can be defined in numerous ways including ash content and colour, protein content, as well as dough flour strength, enzyme activity, and ultimately a breadmaking test.

1.4.2. Flour refinement determination

Flour quality is defined as "the ability of the flour to produce a uniformly good end product under conditions agreed by the supplier and the customer" (Mailhot and Patton, 1988). The main flour components that contribute to quality are protein quantity and quality, mineral content, flour colour, flour water absorption, enzymatic activity, especially that of α-amylase, particle size, starch damage, and flour dough rheological properties in general. Among these components, mineral content and flour colour are the main criteria used in the industry as a measure of flour refinement. Mineral content is determined though the measure of ash content, i.e. the inorganic material left after incineration of flour (Approved AACC method 08-01, 2000).

Since mineral content increases from inner endosperm to the peripheral layers of the kernel, with the greatest amount being located in the aleurone layer, ash content is directly related with bran contamination in flour. While the relationship between flour ash and baking quality may not always be strong (Wichser and Shellenberger, 1948), it is a convenient quality testing procedure and millers and bakers have developed well established specifications corresponding to different levels of flour quality. However, different wheat cultivars vary in the amount of ash (mineral) natively found in the endosperm, and thus small variations in ash content between different wheat cultivars do not necessarily imply the presence of different amounts of bran in flours (Hoseney, 1998), although high ash content in flour is an indication of bran contamination.

An alternative test of flour refinement is flour colour. The Agtron colour meter and the Kent-Jones flour colour grader are widely used for this purpose. Both instruments are based on the photoelectric method, which measures the degree of reflectance at particular wavelengths using filters. Green filters, viz. 530 nm and 546 nm are currently used for the Kent-Jones and Agtron instruments, respectively. Selection of these filter wavelengths evolved from earlier research (Gillis 1963) which reported blue filter sensitivity at 436 nm to yellow endosperm carotenoid compounds, as bleaching of flour with benzoyl peroxide changed the blue filter-based Agtron readings. Agtron measurements using a green filter, measuring reflectance at 546 nm, were found to be insensitive to flour bleaching, and highly correlated to ash content (Patton and Dishaw, 1968; Murthy and Dietz, 1974), and were therefore recommended as a measure of bran content in flour. However, as for ash content, colour measurement to determine flour refinement has limitations. Li and Posner (1989), who evaluated hard white winter wheat milling properties, found differences in colour between red and white wheats for

highly bran contaminated millstreams that come from the light colour of the white wheat bran that makes its inclusion into flour less noticeable visually and instrumentally. Shuey and Skarsaune (1973) computed an equation to predict per cent flour ash from the flour colour reflectance values obtained by the green Agtron, and found that different regression equations were needed for wheats of different origins. Shuey (1975) also found an influence of variety and environment in flour colour reflectance values and flour ash content, and concluded that it is necessary to know the origin of the sample to be able to conclude that flours having the same colour values or ash contents are or not of the same grade or extraction. Moreover, none of these studies have taken into account the influence of protein content on flour reflectance. Barnes (1986) milled 13 UK wheats into straight-grade flours, and compared the flour colour grade (FCG) of these flours (measured with a Kent-Jones flour colour grader) with the reflectance at 540 nm of dissected endosperm. He found that the endosperm grayness could account for approximately three out of the four units of FCG by which the flours varied, leaving only one unit to be accounted for by differences in bran content. Moreover, he reported a negative correlation between endosperm paste reflectance at 540 nm and the protein content of the endosperm. Therefore, for refined flours, flour colour was not a valid measure of bran content, as the grayness of the gluten will be predominant in the variation in flour reflectance values.

Despite all the flaws existing in using colour and ash measurements for flour refinement determination, these two methods can be very effective in ranking flours from a single wheat cultivar according to the degree of refinement (Ziegler and Greer, 1971).

Relatively new approaches to quantify bran content in flour have been studied. Symons and Dexter (1992) tried to predict flour refinement using pericarp fluorescence on millstreams obtained from pilot-scale milling of hard wheat. This method appeared to be more accurate than ash or flour colour to predict bran contamination because it was based on direct quantization of biological tissue. The authors were able to discriminate between four divide flours, including first patent, second patent, straight grade, and clears, but could not measure small variations among the flours within each divide group. Symons and Dexter (1993) investigated the potential of estimating flour refinement by flour aleurone fluorescence, by using millstreams from pilot-scale milling of hard wheat. Like the previous method, measuring flour refinement using this method appeared to be more accurate that ash and colour measurements because of the direct quantization of biological tissue (Symons and Dexter, 1992). Ferulic acid is highly concentrated in aleurone tissue and is the compound responsible for fluorescence (Fulcher et al., 1972 as cited by Symons and Dexter, 1992). Aleurone fluorescence was highly correlated with ferulic acid content for every millstream except bran flour. However, break flours are higher in pericarp tissue than are prime quality reduction streams (Symons and Dexter, 1991, 1992) and pericarp is also high in ash content (Hinton, 1959; Morris et al, 1946) but low in ferulic acid (Fulcher et al, 1972). Therefore, the relatively high ash content of break flours was not related exclusively to aleurone contamination, accounting for the low aleurone fluorescence of break flours relative to reduction flours of comparable ash content. For both studies (Symons and Dexter 1992, 1993), the prediction of ash content or flour colour based on pericarp and aleurone fluorescence was not consistent among wheat classes.

Croes (1961) criticized the use of green filter for colour measurement and suggested that a preferred method for the measurement of a flour-water paste would include contributions from brightness, yellowness, and whiteness. These three components of reflected light are a measure of the tristimulus colour coordinates L*a* b*. The use of the tristimulus colour coordinates to predict flour refinement was later investigated by Symons and Dexter (1991), Allen et al. (1989), and Oliver et al. (1992). Symons and Dexter (1991) found a close relationship between L* value (brightness), and pericarp and aleurone fluorescence. They reported that L* was only sensitive to particle size, whereas a* and b* values were sensitive to both moisture changes and particle size of the flour. Oliver et al. (1992) found a linear relationship between L* values and Kent-Jones colour grade (r^2 = 0.72). Through multiple linear regression analysis they reported a high correlation between Kent-Jones colour grades and all three tristimulus values (r^2 = 0.81), and a higher correlation between ash content and L* (r^2 = 0.85).

More recently, researchers developed methods for bran measurement in flour by image analysis (Whitworth et al., 1997; Harrigan and Bussmann, 1999; Kim and Flores. 1999). Kim and Flores (1999) evaluated the determination of bran contamination in hard red wheat flour using a bran speck counting method based on image analysis. Bran speck count results showed that this new method was more accurate than conventional methods, i.e. colour measurement. However, some problems occurred, including the poor accuracy of the method. Even though flour ash was significantly correlated with the number of bran specks, a few large bran particles could contribute to high flour ash content. As well, for a single sample, the randomness of orientation and distribution of bran specks when the sample was placed in the holder produced a different image and a

different number of speck counts in each measurement. As a result, 17 out of the 21 flour samples studied showed significant differences in bran speck counts within triplicate measurements.

1.4.3. Effect of extraction rate on flour refinement

Flour extraction is defined as the proportion of the wheat recovered as flour by the process of milling. A typical sound wheat kernel contains about 83% of endosperm. Consequently, this percentage is a theoretical limit of white flour that can be produced from wheat. However, in practice, only 72 to 77% of white flour can be produced (D'Appolonia, 1993) depending on the milling equipment and wheat type. Two phenomena are responsible for the limitation in flour production. First, the shape of the kernel, with the presence of the crease that extends quite deeply in the kernel, represents an obstacle to the milling process. Second, bran adheres to the endosperm. Therefore, in order to avoid significant contamination of white flour by bran, bran must be removed intact and consequently some endosperm is inevitably left in the finished bran.

In the wheat kernel, the amount of ash is low in the center and increases to the outer layers (Morris et al., 1945, 1946; Hinton, 1947, 1959, 1962; Stevens et al., 1963; Kent, 1966; Kent and Evers, 1969). Hinton (1959) showed that for hand-dissected wheat kernels, the ash content increases slowly up to an extraction rate of about 77% and thereafter the ash content increases much more steeply as the outer endosperm is included. Therefore, the higher the flour extraction, the higher the ash content, and the greater will be the percentage of material closest to the bran portion of the kernel (D'Appolonia, 1993).

During milling, the increase in ash content in the flour is occurs normally in the latter stages of the reduction process, where flour yield is increased by regrinding and resieving the bran-rich streams. The flour gained from this process is from the outer endosperm layers of the kernel, and has a different composition than the flour obtained from the center of the kernel during the early stages of milling (Orth and Mander, 1975). Later reduction flours are notably higher in protein and ash contents than early reduction flours. Consequently, the flour that comes from the center is the whitest and the lowest in protein and ash contents. Conversely the flour that comes from the outer part of the kernel is the darkest and the highest in protein and ash contents. Incorporation of a relatively small quantity of bran particles, including aleurone cells, can account for a considerable increase in ash content, due to the high concentration of minerals in bran which is 10 to 20 times that of the inner endosperm (Morris et al., 1945). It is important to note that it is the aleurone layer that contains the majority of the minerals in bran, representing 56 to 60% of the total ash of the kernel (Hinton, 1959; MacMasters et al., 1971). Because of its direct relationship with bran contamination, ash content is widely used in the industry as a criterion of milling performance.

1.4.4. Effect of extraction rate on protein quantity and quality

Among flour quality criteria, flour protein quantity and quality are the most important parameters in breadmaking. Bushuk et al. (1969) defined protein quality as the property of flour proteins which gives rise to different baking performance with flours of the same protein content.

Like ash, protein concentration increases from the center of the endosperm to the outer layers of the wheat kernel. In addition to this protein gradient in the wheat kernel,

protein quality also varies within the kernel (D'Appolonia, 1993), as the concentration of protein and protein fractions will change relative to starch which is the other main constituent of endosperm.

The relationship between flour protein content and extraction rate is well known. According to Farrand (1974), at 90% extraction rate, flour protein is equal to the wheat protein. Consequently, the protein loss on milling is zero. When the extraction rate increases the flour protein increases. When the flour extraction rate is between 82 and 92%, the protein content in the flour increases due to the proteins obtained from the aleurone and outer endosperm layers of the kernel. At extraction rates above 92%, the outer pericarp tissue will be included. Because this layer contains a lower protein content, the flour protein decreases. Regardless of these details, the differences in wheat flour protein among different genotypes or wheat samples are mainly due to differences in endosperm protein content. Variation in protein content of the bran layers including the aleurone layer creates smaller changes (Farrand, 1974).

Protein content and composition are the major factors in determining breadmaking quality. Variation in these factors within the wheat kernel, and consequently their variation among flours of different extraction rates is very relevant to a complete understanding of wheat quality for bread or other products. Farrand (1974) studied the properties of two U.K. varieties and tried to elucidate the nature of the differences between wheat protein and flour protein over a range of extraction rates when milled on both a small commercial mill and a Buhler laboratory mill. He showed that gluten washed from a patent flour at 40 to 50% extraction has significantly different rheological properties compared with a straight-run flour milled at 70 to 75% extraction, when using the same wheat.

Orth and Mander (1975) studied the variation in protein quality of a series of flours covering different extraction rates (66 to 82%) for three wheat cultivars representing a range of hardness (hard, semi-hard, and soft) and milled on a Buhler laboratory mill. Protein quality was measured using rheological testing (farinograph and extensograph). The wheats were selected to produce flours at approximately the same protein content at a given extraction rate in order to minimize the influence of this variable when making intervarietal comparison of flour properties. They reported an increase in ash content, protein content, colour grade values, and farinograph absorption of the flours as the extraction rate increased from 66 to 82%. The gluten strength however decreased with increasing extraction rate to 82%, as shown by an increase in farinograph dough breakdown, and a decrease in maximum extensigram resistance. This decrease in strength occurred despite an increase in flour protein for the higher extraction flours. The extraction rate that gave the optimum loaf volume varied with the variety: 72% for the hard wheat variety, and 74% for the soft wheat variety. Beyond these extraction rates, loaf volume rapidly decreased. Because the high extraction flours produced doughs that broke down quickly during prolonged mixing and offered less resistance to extension than did those of lower extraction flours, Orth and Mander (1975) concluded that flours produced at different extraction rates had different flour protein quality and hence, composition. However, they did not elaborate the molecular basis for such a conclusion and also did not take into account the detrimental effect on loaf volume of increasing ash content in flour of increasing extraction rate. Gupta et al. (1992) reported the relationship between protein composition in terms of proportions of the three main groups of protein (polymeric, gliadins, and albumin-globulins) and various flour quality parameters for a set of 15 wheat cultivars grown at six different

nitrogen fertilizer levels, thus representing a wide range of protein content. As the flour protein increased, the proportion of glutenin did not vary systematically, the proportion of gliadin increased, and the proportion of albumin-globulin decreased. The total amount of each group increased. They also found that quality parameters such as extensograph resistance and mixograph dough development time appeared to depend on the balance between polymeric and monomeric proteins. Although the variation in protein composition in this study cannot be compared with the variation in protein composition in flours of different extraction rates proposed by Orth and Mander (1975), it is possible that the basis for the results of Orth and Mander (1975) lies in the variation in proportions of the three main groups of protein. In this case, higher extraction flours of higher protein content could possess a lower glutenin-to-gliadin ratio.

1.4.5. Effect of bran content in breadmaking

Bran is known to be detrimental to loaf volume. The effect of wheat bran in breadmaking has been studied by many researchers, although results are sometimes contradictory. Lai et al. (1989) replaced 14% of the flour in the baking formula with wheat bran. They observed that bran had a far greater detrimental effect on loaf volume than an inert ingredient that would have only contributed to a dilution of gluten protein. The effect of bran was linear, with a greater effect on flours with a higher loaf volume potential. β-glucosidase digestion as well as heat-treatment did not alter the effect of bran on loaf volume. Heat-treatment was also used by De Kock et al. (1999), who obtained contradictory results compared to Lai et al. (1989). The authors milled ten samples of wheat on a Buhler Miag roller mill. Untreated and heat-treated bran of

different particle size were added to a common base flour. The detrimental effect of bran on loaf volume was lessened with heat-treated bran than with untreated brans, indicating that heat-sensitive chemical components are in part responsible for reducing brown bread volume. The authors suggested that the heat-treatment inactivated lipase, and significantly reduced the level of reducing substances. Reducing bran particle size in general decreased loaf volumes, but a heat-treatment of the smaller particle size bran restored the original loaf volume.

Lai et al. (1989) found that doughs containing bran appeared dry and addition of 2% water had a beneficial effect. Doughs with up to 10% bran were manageable, whereas doughs with more than 10% bran were difficult to handle. When bran was presoaked in water, finely ground, and added to a base flour, loaf volume improved over the control loaf. The authors suggested that the reduction in loaf volume when bran was added in fiber-rich (brown) bread to the formula was due to competition for water between bran, starch, and gluten proteins; ultimately gluten was not sufficiently hydrated to develop optimally. Nelles et al. (1998) also studied the effect of bran pretreatments (hydration, wet heat, and wet oxidation) on breadmaking. All treatments significantly improved brown bread quality, with a greater effect for hydration and wet oxidation treatments. Three different hypotheses were proposed by the authors to explain these results: an increase in overall water absorption of the brown bread doughs resulting in improved hydration of all flour components, a decrease in potentially oxidizable substances in the bran through lipoxygenase activation, and a wash-out effect of free reduced glutathione, known to disrupt disulfide bonding (Schofield and Chen, 1995, Nelles et al. 1998), resulting in greater disulfide bonding and hence stronger dough.

Salmenkallio-Marttila et al. (2001) studied the effect of bran fermentation on breadmaking qualities of high-fiber bread, using commercial white flour and wheat bran. Prefermentation with added yeast and prefermentation with added yeast and lactic acid bacteria both improved loaf volume crumb structure, and shelf life, added flavor and good and homogeneous crumb structure, whereas spontaneous bran fermentation did not show the same positive effects. The effect of fermentation of bran on bread quality was evaluated using light microscopy, and the authors observed a well-developed protein network structure of the breads baked with fermented bran as opposed to the control bread made with unfermented bran. Moreover, the pretreatments of the bran had no detectable effect on the cell wall structure of the breadcrumb.

It has been reported that some cultivars have unexpected strengthening effects on dough characteristics and baking quality. Ozboy and Koksel (1997) milled two varieties of wheat, a hard red winter wheat (var. Bezostaya) and a soft white winter wheat (var. Gerek) on a Buhler laboratory mill. Addition of fine bran of both varieties to their respective straight grade flours had a weakening effect on doughs and was detrimental to bread volumes. Addition of Bezostava coarse bran to its corresponding straight grade flour also had a negative effect on dough strength and breadmaking properties. However, the addition of coarse bran of the Gerek variety to either of the straight grade flours strengthened dough and had reduced negative effect on loaf volume. Nevertheless, the authors did not explain their results. The different effects of bran prepared from different cultivars on breadmaking properties were also observed by Nelles et al. (1998). They found that brans of different cultivars had different water absorption, and that those with higher absorptions gave stronger doughs (longer farinograph development times and stabilities) and higher loaf volumes. They suggested that the different brans

have differing chemical composition, and their subsequent hydration kinetics influenced bread characteristics differently.

1.5. Flour millstreams composition and properties

As described previously, the milling process produces millstreams. Also, it has been shown that the wheat kernel has increasing gradients of ash and protein from inner to outer endosperm. It is therefore expected that depending upon the stage of the process the streams will have varying ash and protein contents, thus varying refinement and quality. Regular millstream analysis in the industry is essential in the control of milling operation and efficiency (Bass, 1988). Routine millstream analysis includes determination of moisture, ash content, and protein content. Therefore, the variation in composition of the different millstreams in terms of these parameters has been the subject of many studies. The composition in pentosans of the millstreams has also been subject of interest. Wang et al. (2005) found that pentosan content represents a more sensitive marker of flour refinement compared to ash content.

1.5.1. Yield

Total yields on the reduction side are always higher than that on the break side. However, the proportions depend on wheat hardness. Wheat hardness plays an important role in flour milling, influencing the pattern of endosperm fracture, ease of separation of bran from endosperm and consequently break flour release (Bass, 1988). Blakeney et al. (1979) as well as Kilborn et al. (1982) found a close relationship between grain hardness and break flour release. It is therefore expected that any study using different mills and different wheat varieties will obtain different results. Nelson and McDonald (1977)

milled four hard red spring wheat varieties on a pilot mill to a flour extraction rate (FER) of 76.2 to 77.8%, depending on the variety. For three of the varieties, the first middling stream (M1) gave the highest yield (12.7 to 14.7%), followed by M3 (11.0 to 12.9%). However, for the remaining sample, the yield of M3 was higher but close to that of M1 (12.8 and 12.7%, respectively). Holas and Tipples (1978) milled a No. 1 CWRS on a commercial scale Buhler mill to an extraction rate of 76.1 %. The break flour yields combined represented 22.2% of the total yield. M2 had the highest yield (16.5%) followed by M3 (14.0%) and M1 (11.5%). Consequently, the total yield of the first three middling fractions represented 55.2% of the total yield. Black et al. (1981) milled CWRS wheat on the Grain Research Laboratory Pilot Mill to a 74% FER which is typical for hard wheat roller milling. The highest proportion of flour was produced on the reduction rolls, with M1 and M2 accounting for about 30% of the 74% total yield. The four break flours and sizing flours accounted for 14% and 10%of total flour yield, respectively. Martin and Dexter (1991) milled a No. 2 CWRS, a No. 1 CWRW, and a No. 1 CPS (HY 320) wheat in a tandem Buhler laboratory mill also to a 74% extraction rate. The five break flours combined produced yields representing 15.5% of the FER for the red winter wheat, 16.1% for the CPS wheat, and 16.5% for the red spring wheat. M1 always gave the highest yield of 20% for the red spring wheat, 22.8% for the red winter wheat, and 23.3% for the CPS wheat. Depending on the type of wheat, M1 and M2 accounted for 42.7% of the FER for CWRS wheat to 49.2% of the FER for the CPS wheat.

For all studies described above, the yield of the tail end reduction streams was always low. Also, because of the different type of mills and wheat varieties of varying hardness used, the yield pattern is different among the different investigations.

1.5.2. Protein content

The protein gradient in the wheat kernel results in different qualities and quantities of flour protein in different millstreams (Wang and Flores, 1999). Black et al. (1981) reported a wide variation in protein content from 10.8% for M1 to 18.6% for bran flour (BF) for a CWRS wheat milled on the Grain Research Laboratory Pilot Mill. The four break flours, along with BF had high protein content. Wang and Flores (1999) found BF to have the highest protein content (15.8%, 14.9%, and 9.1% for a hard red winter, hard white winter, and soft red winter wheat, respectively), followed by B3 (14.2%, 13.8%, and 6.9% for the corresponding wheat samples. The different values they obtained for different wheats were a direct consequence of the wheat protein content. Most workers (Hinton, 1947, Kent; 1966; Nelson and McDonald, 1977; Endo et al., 1987; Prabhasankar et al., 2000) have also found that protein content increased from the first to the last break. The increasing protein content of the coarse fractions in later break and reduction flours was largely attributed to a concentration of subaleurone endosperm of high protein content in these fractions (Kent, 1966). However, Black et al. (1981) found the third break flour to be the highest in protein content. The authors explained this difference with the earlier studies by Hinton (1947) and Kent (1966) by considering that the fourth break does not produce a "true" break flour in the GRL Pilot Mill due to a different type of stock fed to the fourth break in this study as compared to the earlier ones.

Because they are derived from stocks consisting largely of lower-protein inner endosperm particles, the reduction flours possess lower protein content than the break flours (Black et al., 1981; Hinton, 1947; Kent, 1966; Nelson and McDonald, 1977; Orth et al., 1976; Endo et al., 1987; Prabhasankar et al., 2000). Nelson and McDonald (1977)

milled four hard red spring wheat varieties on a pilot mill and found that M1 and M4 streams had the lowest protein contents. Kent (1966) milled a hard red winter wheat using a laboratory mill and reported a slight increase in protein between M1 and M2 (13.9 and 15.5%, respectively). Black et al. (1981) milled a CWRS wheat milled on the Grain Research Laboratory Pilot Mill and found protein content increased from M1 to M3 (11.0 to 13.8%), then decreased for M5 (11.7%) and increased again for M6 flour (12.2%). The significantly higher protein content of M3 compared to that of the other reduction streams was due to the high protein content of the overs derived from the third break. According to Kent (1966), this result would occur due a higher percentage of aleurone cells present in the latter break flours. The protein content of the BF was usually the highest of all millstreams (Black et al., 1981; Preston and Dexter, 1994; Wang and Flores, 1999), except in the study of Nelson and McDonald (1977) in which, surprisingly, the protein content of BF was lower than that of the break streams, and only slightly higher than that of the reduction streams.

Thus, significant variation in protein concentration of millstreams is dependant on mill flow and wheat type, explaining some of the discrepancies reported in the literature.

1.5.3. Ash content

Morris et al. (1946) compared hand-dissected fractions with experimental millstreams milled on a Buhler laboratory mill (three break flours and three reduction flours) for three wheat varieties including a soft red winter, a hard red winter, and a hard red spring wheat. The lowest concentration of ash, which was found in the first break flour, was similar to that of the lowest ash hand-dissected fraction, located in the center

of the cheek. However, the highest ash flour streams, B3 and M3, were slightly lower in concentration than the highest ash hand-dissected fraction (endosperm close to the crease).

The ash content of a straight-grade flour is dependent on the flour extraction rate, and on how well the mill can achieve an optimum and efficient separation of endosperm and bran in the break section (Bass, 1988; Black et al., 1981). Sizing flours and first reduction flours are the most highly refined flour streams, i.e. those least contaminated by non-endosperm material (Izydorczyk et al., 2003). Black et al. (1981) and Preston and Dexter (1994), working with the same mill (GRL Pilot Mill) and a CWRS wheat, found M1, M2, S1, and S2 to have the lowest ash contents of all millstreams. M1 had the lowest ash content of 0.28% (Black et al., 1981) and 0.33% (Preston and Dexter, 1994), followed by M2, then either S1 or S2. Black et al. (1981) also obtained relatively low ash values for the reduction streams M3 (0.41%), M4 (0.39%), and M5 (0.48%), and for the break streams B1 (0.44%), B2 (0.40%), and B3 (0.49%), and much higher ash values for B4 (1.2%), M6 (0.79%) and BF (0.98%). On the contrary, Preston and Dexter (1994) reported higher ash values starting at M4 (0.82%), up to M6 (1.49%) and BF (2.61%). Wang and Flores (1999) also found the ash content to be the highest for the last reduction stream and bran flour. Because yields of these late reduction flour streams were low (below 11%, 8%, and 5% of the FER for the SRW, HRW, and HWW wheats, respectively), the ash content of the straight-grade flours was not greatly affected. In general, the streams having low ash also have low protein content. Nelson and McDonald (1977) found a correlation coefficient of 0.735 between protein and ash content.

1.5.4. Colour

Flour colour has been extensively studied (refer to section 1.4.2.). It is a general observation in wheat milling that flour colour measured by brightness is negatively correlated with ash content. Wang and Flores (1999) found that M1, M2 and M3 flours were brighter with higher Agtron reflectance values compared with flours of other streams. In contrast, BF had the darkest colour. Colour and ash content were highly correlated (r = -0.74). Flour brightness, measured as the tristimulus L* value, was highly correlated with Agtron colour values (r = 0.95). Holas and Tipples (1978) measured millstream colour with a Kent-Jones colour grader and obtained the most desirable values for the first reduction streams and the least desirable values for the low-grade streams. Similar results were found by Black et al. (1981) and Martin and Dexter (1991). Overall, the most highly refined streams have the brightest colour, while high ash streams have the darkest colour (Black et al., 1981).

1.5.5. Starch damage

Starch damage is influenced by the roller surface and the previous history of ground material. Corrugated rolls impart low starch damage as opposed to reduction rolls. Consequently, streams derived from the breaking process possess the lowest starch damage values, while those derived from stocks going through many reduction rolls tend to have higher starch damage content. Black et al. (1981) and Holas and Tipples (1978) found the break flours to have the lowest starch damage values. The highest starch damage values were associated with M5 and M6 flours. Similar results were found by Wang and Flores (1999), with higher value for hard wheats than for soft wheats.

1.5.6. Dough rheology

Differences in biochemical components present in flour streams will affect dough rheology and baking performance. Preston et al. (1982) investigated farinograph absorption, extensograph properties, baking absorption, and bread quality for each CWRS flour stream investigated by Black et al. (1981). Farinograph absorption (FA) increased from B1 to B3 (62.4 to 68.4%) then decreased for B4 (66%). This pattern of variation in FA was consistent with that of protein content (15.2 to 18.3% from B1 to B3, 15.7% for B4) and starch damage values (12 to 21 Farrand units from B1 to B3). Conversely, the millstreams with the lowest protein contents, S1 and S2, had the lowest farinograph absorptions. The reduction flours (M1 to M6) showed a clear trend in their relationship between starch damage (27-77 Farrand units) and FA (63.5%-77.9%). These results (Preston et al., 1982) were consistent with other studies (Holas and Tipples, 1978; Preston and Dexter, 1994). Generally flour water absorption increases with increasing protein content, starch damage, and fiber content and decreasing particle size (Holas and Tipples, 1978; Preston and Dexter, 1994).

Flour streams also possess a wide range of farinograph dough development times (DDT) and mixing tolerance index (MTI) values (Preston et al., 1982). DDT increased from B1 to B2 and B3 (6.5, 8.25, 8.0 min, respectively); MTI decreased with B3 (20 BU for B1 and B2, 5 BU for B3). B4 had the second shortest DDT (4.25 min) and the highest MTI (85 BU) values, indicating a low tolerance of B4 to overmixing. M1 had a long DDT (11.5 min) and an MTI of zero, consistent with strong flour properties. From M1 to M5, DDT decreased (from 11.5 to 3.75 min) and MTI increased (from 0 to 30 BU). Surprisingly, M6 had a longer DDT (5 min) than M5, although it had a much higher MTI than M5 (60 BU). On the contrary, Holas and Tipples (1978) reported

longer DDT and stabilities for break flours (DTT average 7 min) than for reduction flours (DDT average 5 min). These results indicate that wide variations exist in dough rheological properties for corresponding millstreams obtained from different mills.

The millstreams also had varying extensograph areas, lengths, and heights (Preston et al., 1982). The break streams had larger areas than the straight grade flour (from 140 to 180 cm^2, and 130 cm^2, respectively), except B4 which had a much lower area (30 cm^2) reflecting much weaker dough properties. The reduction streams had on average lower areas than the break streams.

Endo et al. (1987) obtained millstreams from a CWRS wheat milled on an experimental Buhler mill to a FER of 60%, and evaluated their rheological properties using a Brabender Do-Corder, without and with bromate, to accentuate the differences in rheological properties. Bromated break flour curves were characterized by three Do-Corder peaks at 75, 85, and 95°C, whereas the 95°C peak was absent for the non-bromated break flours. The reduction flours exhibited a predominant peak at 85°C and a slight rise at 75°C, but did not respond to bromate. It was thought that protein and starch are responsible for peaks obtained at 75 and 85°C. The authors also evaluated the changes in the properties of millstreams during dough mixing by measuring the rheological properties of flour streams using the Do-Corder on doughs pre-mixed in a mixograph at peak time and at peak time plus 5 min. The break streams underwent a dramatic change during mixing, unlike the reduction streams for which changes were slight. These flours also had a more rapid decrease in SH content from unmixed dough to dough mixed at peak time plus 5 min than the reduction flours, indicating that changes observed in Do-Corder curves during dough mixing appeared to be related to the removal of SH. Endo et al. (1987) concluded that the differences in the rheological

properties between break and reduction streams could be attributed to intrinsic differences in their components, such as changes in sulfhydryl (SH) content.

Surprisingly, there have been no reports investigating flour millstreams using the mixograph.

1.5.7. Breadmaking properties

Preston et al. (1982) investigated baking absorption for different millstreams of a CWRS wheat milled on a pilot mill. The first three break flours and BF had the highest baking absorption, due to higher protein content. The sizing flours had the lowest baking absorption due to their low protein content. From M1 to M3, baking absorption increased, then decreased to M5. Baking absorption was difficult to assess for M6 due to the lack of dough cohesion of this millstream. A bread score was also determined for each of the flour streams, baked using five different baking procedures. Depending upon the method used, the millstreams performed differently. On average, the best overall baking quality was obtained for the two sizing flours S1 and S2, followed by break-roll flours and early reduction flours. Significant correlations were obtained between loaf volumes and extensograph extensibility and area ($0.66 < r < 0.96$, depending on the baking process). Interestingly, no correlation was found between loaf volume and farinograph DDT, MTI, or extensograph maximum height, which normally is a good indicator of dough strength.

1.5.8. Protein quality

There are very few reports in the literature on the protein composition of different millstreams (Orth et al., 1976; Nelson and McDonald, 1977). Even though

many authors have indicated that the differences among millstreams in rheological and baking properties were related to differences in protein composition, few investigated the protein composition of the millstreams. This seems surprising since it is the protein composition of a flour that determines its rheological and baking properties. The few studies that are available are outdated, as more accurate protein extraction methods are now available.

Orth et al. (1976) milled a semi-hard bread wheat on a Buhler laboratory mill and fractionated the flours using the modified Osborne procedure described by Chen and Bushuk (1970) with minor alterations. They reported that approximately 83% of the protein break flour contributed to the gluten network, as opposed to 69% for the late reduction streams. Nelson and McDonald (1977) fractionated the protein of the streams of two varieties of hard red spring wheat (Waldron and Era) into glutenin, gliadin, albumin, and non-protein nitrogen fractions using the chromatographic filtration method of Bushuk and Wrigley (1971). Relative amounts of protein in each fraction were calculated from UV absorption at 280 nm. One variety (Waldron) contained more gliadin than glutenin in all the millstreams, whereas the other variety (Era) contained more gliadin than glutenin in only M1 and M4. They found that the small differences in protein fractions among the millstreams were not consistent from one variety to another. The gliadin fraction varied significantly between streams for both varieties studied, while the albumin and non-nitrogen protein fraction were significantly different for only one of the varieties. Analysis of the protein fractions in break and reduction streams showed no significant differences in the levels of glutenin, gliadin, and albumin.

Chapter 2. Colour Spectrophotometry of Wheat Millstreams for Estimation of Flour Refinement of Different Cultivars of Hard Spring Wheat

ABSTRACT

Colour spectrophotometry was evaluated as a new instrumental approach to measure degree of flour refinement. Eight hard spring wheat samples including white and red-grained cultivars were milled on a tandem Buhler laboratory mill to an extraction rate of 80%. Thirteen flour streams, including four break streams, one sizing stream, one low-quality stream, and six reduction streams, as well as three millfeed fractions were obtained and analyzed for yield, moisture, ash content, protein content, starch damage and colour (dry and wet slurry methods). Ash and protein content generally increased from first to last break and reduction streams as refinement decreased, reflecting their variation from inner to outer endosperm within the kernel. A very high correlation was obtained between protein content and ash for reduction flours across all samples ($r = 0.97$). Degree of flour refinement by colour measurement was evaluated in three ways: 1) Agtron colour determination (at 546 nm), 2) CIE colour coordinates L*, a*, b*, and 3) reflectance spectra from 400 to 700 nm. Methods 2) and 3) were accommodated simultaneously by a computerized colour spectrophotometer. Measuring flour reflectance at 400 nm provided the greatest discrimination of millstreams according to stepwise discriminant analysis. Discrimination of millstreams, as measured by the average squared canonical correlation statistic, increased systematically across all cultivars samples as wavelength decreased from 700 to 400 nm. Very high correlations were obtained between ash content and flour reflectance at 400 nm as well as at 546 nm ($r = -0.96$ for both). Correlations were similarly high between ash content and L* ($r = -$

0.96), a* (r = 0.92), and b* (r = 0.92). Results at 400 nm were less influenced by co-variation of protein content, which was responsible for 1.4% of the variation in colour at 400 nm, as opposed to 2.0% at 546 nm using the Agtron colour test. Significantly, the colour of wheat did not influence flour reflectance at 400 nm for any millstream regardless of ash content. In contrast, Agtron reflectance values began to diverge above about 0350% ash for millstreams deriving from red and white wheats, indicating an accuracy problem for this method. Since the computerized colour spectrophotometer can measure both reflectance and tristimulus colour coordinates at the same time, and gave very reproducible results, it appears to be an effective tool for rapid estimation of flour refinement.

2.1. INTRODUCTION

Flour refinement can be defined as the degree to which flour originating as pure starchy endosperm includes bran and germ constituents arising from the milling process. During wheat milling, individual flour streams are produced that derive from the inner to outer endosperm, and will be more or less contaminated by bran (pericarp, testa and aleurone) and germ (embryo and scutellum) depending on the stage of the process. As a result, each millstream will have a relatively distinct chemical composition and degree of refinement or flour grade that reflect the natural gradient of protein and minerals (Morris et al., 1945, 1946; Hinton, 1947, 1959, 1962; Stevens et al., 1963; Kent, 1966; Kent and Evers, 1969) as well as non-starch polysaccharides (Ciacco and D'Appolonia, 1982; Delcour et al., 1999; Wang et al., 2005) from the inner endosperm to the epidermis of the kernel.

The degree of refinement of a straight-grade flour is mainly affected by the flour extraction rate, but can also result from intrinsic differences in wheat quality, or environmental factors affecting the mineral content of bran. As the extraction rate increases above about 75%, the proportion of bran included in flour increases and thus the degree of refinement can deteriorate rapidly (Ziegler and Greer, 1971). Therefore, flour derived from higher extraction rates has increased bran content that brings nutritional benefits to the final product, but also processing difficulties, notably weaker doughs and lowering of breadmaking quality (Holas and Tipples, 1978). An increasing level of bran contamination is also detrimental to flour colour and brightness, making the flour and white baked good products less appealing. Accordingly, flour refinement is an important indicator of wheat milling quality and can significantly affect the price of flour.

The most widely used estimate of flour refinement is ash content, i.e. the inorganic material left after incineration of flour at 550-590°C (AACC, 2000). While the relationship between flour ash and baking quality may not always be strong (Wichser and Shellenberger, 1948), it is a convenient quality testing procedure and millers and bakers have developed well established ash specifications corresponding to different levels of flour quality.

An alternative test of flour refinement is flour colour, and the Agtron colour meter and the Kent-Jones flour colour grader are widely used for this purpose. Both instruments are based on the photoelectric method, which measures the degree of reflectance at particular wavelengths using filters. Green filters, viz. 530 nm and 546 nm are currently used for the Kent-Jones and Agtron instruments, respectively. Selection of these filter wavelengths evolved from earlier research (Gillis, 1963) which reported blue

filter sensitivity at 436 nm to yellow endosperm carotenoid compounds, as bleaching of flour with benzoyl peroxide changed the blue filter-based Agtron readings. Sims and Lepage (1968) showed that flour pigments from wheat had colour absorbance maxima at about 449 and 475 nm, with absorbances decreasing to essentially zero above 525 nm. Agtron measurements using a green filter, measuring reflectance at 546 nm, were found to be insensitive to flour bleaching, and highly correlated to ash content (Patton and Dishaw, 1968; Murthy and Dietz, 1974), and were therefore recommended as a measure of bran content in flour. However, despite advantages of ease and speed of flour colour determination and the possibility of continuous monitoring, longstanding questions exist concerning its accuracy to monitor flour grade (Shuey 1975). For example, Shuey and Skarsaune (1973) found that different wheat mixes had significantly different regression equations relating ash content and Agtron colour.

The challenge in measuring flour refinement in the milling and baking industry is to develop a method specific to bran content in flour that is routine to use and robust across diverse wheats. Relatively new approaches to quantify bran content in flour include measuring pericarp and aleurone fluorescence (Symons and Dexter, 1991, 1992, 1993), determining tristimulus colour coordinates L*, a* and b* (Croes, 1961; Symons and Dexter, 1991, 1992, 1993; Allen et al., 1989; Oliver et al., 1992) or counting bran specks by digital image analysis (Whitworth et al., 1997; Harrigan and Bussman, 1999; Kim and Flores, 1999). However, none of these methods appears to be a potential replacement of conventional flour ash or Agtron colour determination owing to the latters' convenience and simplicity, and widely accepted accuracy for estimating flour refinement. However, it should be noted that the standard AACC method 14-30 (2000)

for Agtron colour test for flour generates reflectance values on an arbitrary scale that is relative to calibration disks used, i.e. absolute reflectance values are not determined.

The objective of this study was to comprehensively evaluate the relationships between flour ash, colour and brightness using a new approach, i.e. computerized diode array colour spectrophotometry capable of simultaneous measurement of absolute reflectance across the entire visible light spectrum as well as tristimulus colour coordinates. The aim was to determine the relative accuracies of different wavelengths and tristimulus coordinates to predict ash content and flour refinement, and any dependencies of results on the colour of milled wheats which included both red and white grained cultivars. The ability of colour spectrophotometry to discriminate flour millstreams varying in ash and refinement was also evaluated.

2.2. MATERIALS AND METHODS

2.2.1. Wheats

Wheat was supplied by the Canadian Wheat Board and comprised eight cultivar samples comprising six genotypes of four commercial classes representing a wide range of intrinsic qualities for breadmaking. The samples are described in Table 2.1. All samples were grown in the 2001 crop year, and were of sound milling grade.

2.2.2. Milling

Approximately 45 kg of cleaned wheat were tempered for 18h to obtain a final moisture content of 16%. The wheat was then milled using a tandem Buhler pneumatic laboratory MLU 202 mill (Buhler Bros., Inc., Uzwil, Switzerland) (Martin and Dexter, 1991). The mill flow used (Fig. 2.1) has been modified from original. This mill was

Table 2.1. Description and origin of cultivar samples.

Cultivar	Class	Origin[5]	Protein Content [6]
Superb	CWRS[1]	Kelsey, AB	14.3
Superb 2	CWRS	Melfort, SK	12.6
AC Barrie	CWRS	Cudworth, SK	15.1
AC Snowbird	HWW[2]	BRS	14.5
AC Snowbird 2	HWW	BRS	13.6
AC Corinne	CWES[3]	Mayfair, SK	14.6
AC Crystal	CPS[4] Red	Valaraiso, SK	13.8
AC Vista	CPS White	Cudworth, SK	14.2

[1]Canada Western Red Spring; [2]Hard White Wheat; [3]Canada Western Extra Strong; [4]Canada Prairie Spring; [5]AB=Alberta, SK=Saskatchewan, BRS=blended representative sample; [6](%, 13.5% mb)

developed to produce flour streams with a diversity of quality similar to that produced by a commercial mill. The first mill is equipped with scratch rolls and provides four full break passages, a low quality cleanup passage and a sizing passage. The second mill is equipped with smooth rolls and accommodates six reduction passages. The following millstreams were obtained: 4 break fractions (B1-B4), one quality fraction (Q1), one sizing fraction (S1), and 6 middling fractions (M1-M6). Shorts, bran and fine bran were also collected. The bran was passed through a bran finisher and rebolted (183μm) to collect the bran flour (BF). The mill was set up to give an extraction rate of 80%. Millstreams were collected, weighed, and tested for moisture content, protein content, ash content and flour colour. After milling, the flour streams were double bagged to avoid oxidation and loss of moisture, left at room temperature for aging for 4 weeks, then stored at -30°C. Whenever flour was needed for analyses, a small amount of each flour stream was taken out of the freezer, placed in a sealed container in a plastic bag, and left in the fridge until further analysis.

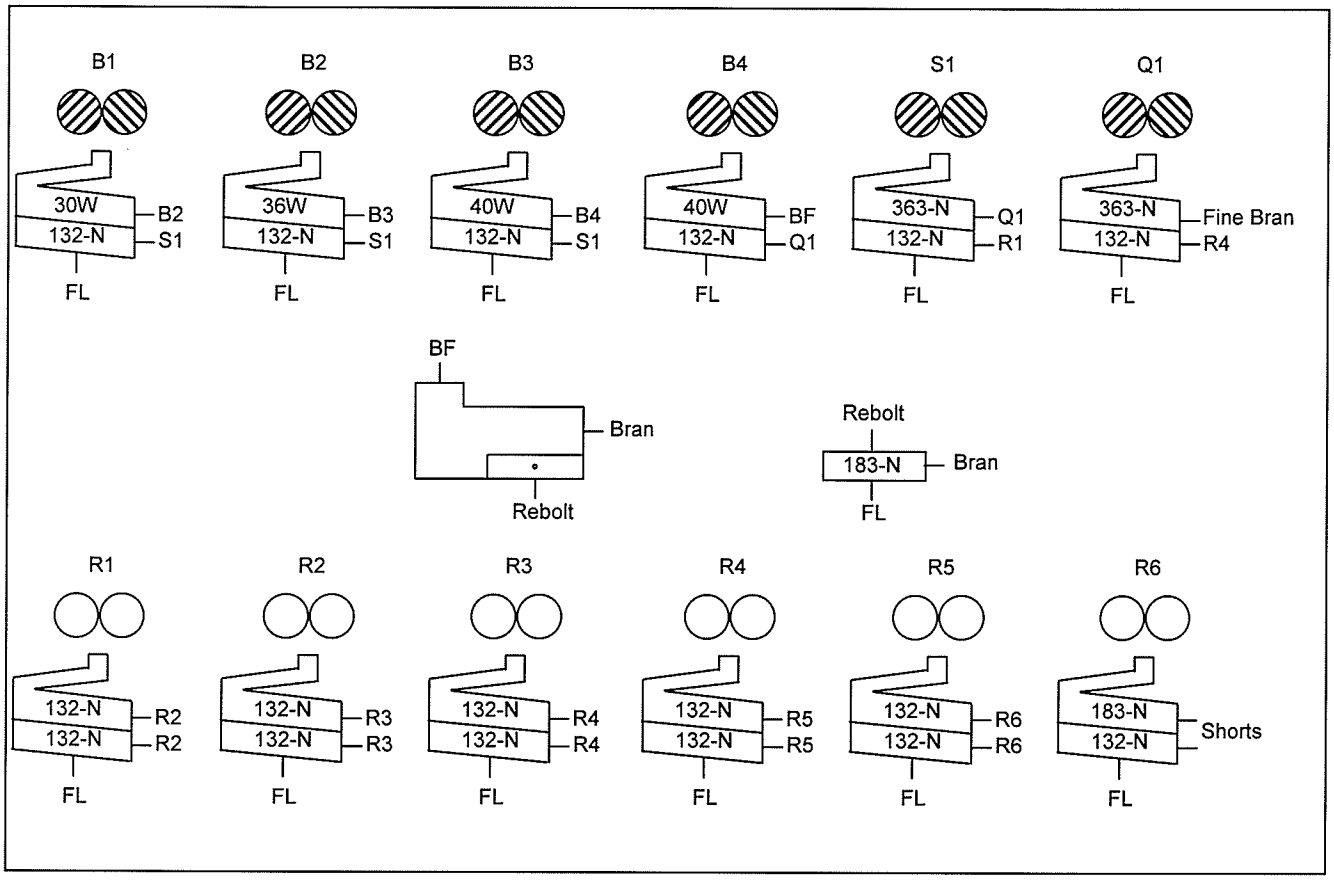


Figure 2.1. Wheat millflow for the tandem Buhler laboratory mill. FL = flour, BF = bran flour, B = break, R = reduction. Roll gaps (in mm): B1: 6.4 - B3: 1.0 – B4: 0.7 – Q1: 0.4 – R1: 0.5 – R3: 0.3 – R4: 0.3 – R6: 0.3; Feed rate: 300g/min; Break roll corrugations: B1: 4.7/cm – B2: 7.9/cm – B3: 11/cm – B4, S1, Q1: 11/cm; Sieves: N = nitex – W = wire (all sizes in micron); Roll velocity: Back rolls: 522 rpm; Front rolls: 264 rpm (differential 2:1).

2.2.3. Quality tests

All wheat and flour quality tests were performed at least in duplicate and the results are expressed on a 14% moisture basis. Wheat moisture was determined using the Halross 919 moisture meter (Labtronics, Winnipeg, MB) according to Approved AACC method 44-11 (2000). Flour moisture was determined using a Brabender Rapid Moisture Tester (C.W. Brabender, South Hackensack, NJ) in which 10 g is heated for 1 h at

130°C. Flour colour was determined according to Approved AACC method 14-30 (2000) using an Agtron colour meter model M-45 digital colour meter (Agtron Inc., Reno, Nevada, USA). The instrument was operated with a green filter (546 nm) and was calibrated with disks #63 and #85 for 0 and 100% relative reflectance, respectively. Ash content of the flour was determined using Approved AACC standard method 08-01 (2000). Protein content (Nx5.7) was determined by combustion nitrogen analysis (CAN) on a Leco Model FP-428CNA instrument calibrated with EDTA. Starch damage expressed as percent of flour weight was determined using AACC method 76-31, i.e. MegaZyme method. This last test was performed on flour from only four of the eight samples (AC Barrie, Superb, AC Corinne, and AC Snowbird), which was a sufficient number to demonstrate variation across millstreams.

2.2.4. Minolta colour measurements

In addition to Agtron colour testing, the colour of flour millstreams (dry and flour-water slurries) was evaluated using a computerized Minolta spectrophotometer (Model CM-3500d, Minolta Co., Ltd. Osaka, Japan). Data acquisition and analysis of the colour measurements was performed using the computer software program Spectramagic. The instrument settings for use with the supplied optical glass Petri dish were downloaded from the disk provided with the instrument. The 10° standard observer as defined by the CIE (1964) and the D65 (noon daylight) illuminant were used. The colour characteristics measured were CIE L*, a*, b*, as well as percentage reflectance in the visible spectrum (400 to 700 nm). The instrument was calibrated using a reference white tile and a black cylinder for 100% and 0% reflectance, respectively. As described

in the CM-3500d Communication Manual, the basic flow of operations for a reflectance measurement is as follows:

1) Light produced by a pulsed xenon arc lamp is diffused inside the integrating sphere and then evenly illuminates the specimen surface.

2) Light reflected from the specimen surface at an angle of 8° to the normal is reflected by a mirror and passes out of the integrating sphere. That light is collected by a lens system and enters a spectral sensor for measurement. At the same time, a second beam of light (that which illuminates the specimen surface) enters the optical fiber cable for monitoring illumination and is transmitted to the spectral sensor for monitoring illumination. By utilizing two spectral sensors in this way, the effects of slight variations in the spectral characteristics or intensity of the illumination can be eliminated by calculation.

3) The light entering each spectral sensor is divided by wavelength from 400 to 700nm at 20 nm intervals by a continuous interference filter and strikes the corresponding segments of the silicon photodiode array. The segments convert the received light into electrical currents proportional to the intensity of the light, and these electrical currents are subsequently digitized and computed as spectral reflectance values for each wavelength range. The results are passed to the computer connected to the spectrophotometer.

For colour measurement of dry flour, the Petri dish containing 4 g of flour (14% moisture basis) was placed on the recessed illumination port of the instrument which formed part of the surface of an integrating sphere inside the instrument. Preparation of samples for colour measurement of flour-water slurries was done according to the Agtron test procedure with slight modifications: 4 g of flour (14% moisture basis) were

used instead of 20 g, and 5 mL of water were added instead of 25 mL, thus the same ratio of flour to water was used. When the water was added to the flour in the Petri dish, the mixture was blended using a stirring rod with a rubber policeman, with a smooth circular motion for 2 min. The slurry was set aside to stand for 5 min after the mixing, in order to allow escape of air bubbles entrained during mixing and time for oxidative enzymes to work. The Petri dish containing the flour-water slurry was then placed on the instrument for measurement. Duplicate measurements were averaged. Reflectance values at 546 nm (%R 546) were obtained by extrapolation between the 540 and 550 nm values.

Bleached flours were prepared by adding 50 ppm or 100 ppm of a 32% benzoyl peroxide powder (American Ingredients Company, Kansas, USA) to the straight grade flours from Superb, AC Barrie, AC Corinne, and AC Snowbird. After adding the reagent, the flours were well mixed, then left at room temperature for 48 hours before analyses.

2.2.5. Statistical analyses

All statistical analyses were performed on duplicate measurements using the procedures of the SAS (1988) software system version 8.2. Correlation analyses of the data were performed using Pearson's correlation coefficient analysis. Stepwise discriminant analysis was performed using the StepDisc procedure.

2.3. RESULTS AND DISCUSSION

2.3.1. Flour yield

Total or almost total recovery of mill stock from wheat milling is a good initial indicator of the precision in yields of the different streams obtained. Cumulative millstream yields ranged from 99.3 (Superb) to 100.4% (AC Snowbird 2). The extraction rate for all flours ranged from 76.2% (AC Snowbird 2) to 81.4% (AC Corinne), with an average of 79.1%. For all samples, the highest production of flour was obtained on the reduction side of the mill. The first and second reduction flours accounted for at least 50% (average 54.3%) of the total yield and the sizing stream added another 10.7 to12.8%, depending on the cultivar sample. The first reduction stream M1 gave the highest yield followed by the sizing flour S1 and the second reduction stream M2. The lowest yields obtained were from B4, M5 and M6. This trend was reported in previous studies (Black et al., 1981; Martin and Dexter, 1991).

The amount of break flour and the amount of reduction flour produced for all samples exhibited a range of yields consistent with that of commercial, pilot-scale, and other laboratory millings (Martin and Dexter, 1991; Black et al., 1981). The yields of the break flours and of the reduction flours were moderately variable among the samples. For example, for AC Corinne, B1 to B4 and M1 plus M2 accounted for 11.5% and 55.6%, respectively, of the total yield. In contrast, for AC Barrie, the four break flours accounted for 18.3% of the total yield, and M1 plus M2 for 50.4% of the total yield. Lesser amounts of break flour and greater amounts of reduction flour usually reflect the influence of harder kernels during milling (Bass, 1988; Blakeney et al., 1979; Kilborn et al., 1982). These flour yield results obtained therefore suggested that AC Corinne was the hardest wheat and AC Barrie and AC Crystal were the softest, with 72.2 and 62.1%

Table 2.2. Yield and analytical data of millstreams.

	Yld[1]	Mst[2]	Ash[3]	Pro[4]	Agt[5]	SD[6]	Yld	Mst	Ash	Pro	Agt	SD
	Superb						**AC Barrie**					
B1	1.72	14.1	0.54	14.89	47.5	4.00	2.54	14.3	0.49	16.57	59.9	3.31
B2	6.20	14.6	0.45	16.25	65.6	4.53	7.58	14.5	0.42	17.63	70.5	3.72
B3	1.96	13.8	0.55	18.65	50.6	4.39	2.76	14.1	0.49	20.04	55.4	3.60
B4	1.41	14.2	0.61	19.43	37.8	4.51	1.76	14.3	0.49	21.66	40.8	3.91
S1	11.66	14.9	0.41	13.96	80.9	5.25	12.54	14.8	0.38	14.67	81.5	4.64
Q1	1.52	14.3	0.66	14.97	40.8	5.22	1.47	14.3	0.69	16.24	37.1	5.02
M1	32.50	14.6	0.36	12.68	86.7	8.06	28.06	14.3	0.32	13.09	92.6	7.12
M2	11.00	13.4	0.43	12.85	62.2	9.93	12.14	14.0	0.38	12.83	79.2	8.80
M3	3.31	13.2	0.64	13.45	42.2	11.20	3.70	13.2	0.65	13.54	46.5	10.11
M4	4.17	13.8	0.85	14.02	28.5	8.88	3.71	13.7	1.00	14.70	18.8	8.37
M5	1.12	13.0	1.74	15.69	-24.2	9.79	1.22	13.0	1.98	16.58	-32.7	9.39
M6	0.57	12.5	2.65	17.99	-52.6	9.53	0.65	12.3	3.11	19.61	-59.0	8.63
BF	1.21	14.1	2.40	23.56	-46.3	5.01	1.49	14.1	2.31	26.95	-45.8	4.20
CBr	14.55	14.3	5.87	16.99			14.77	14.5	6.17	16.07		
FBr	3.58	13.5	4.82	16.58			2.85	13.4	4.71	17.97		
Shts	2.85	11.7	3.66	17.55			2.53	12.2	3.65	18.50		
Cum	*99.3*		*1.54*				*99.8*		*1.54*			
	AC Corinne						**AC Snowbird**					
B1	1.35	13.9	0.76	15.95	32.4	4.89	1.90	14.3	0.50	15.28	60.0	3.11
B2	5.12	14.3	0.57	17.18	55.4	5.32	8.11	14.9	0.43	16.59	71.3	3.54
B3	1.63	13.9	0.70	19.57	41.3	4.99	2.10	14.1	0.51	18.87	48.6	3.40
B4	1.24	14.1	0.71	19.86	33.6	5.01	1.52	14.4	0.57	18.86	72.7	3.62
S1	10.73	14.6	0.50	14.33	77.1	6.04	11.99	14.8	0.38	14.1	84.5	4.14
Q1	1.38	14.2	0.76	15.57	46.0	5.81	1.33	14.4	0.66	14.84	54.2	4.22
M1	30.40	14.2	0.38	13.28	87.7	8.65	30.79	14.6	0.32	12.74	91.8	6.14
M2	14.84	13.5	0.42	13.29	76.8	10.60	11.09	14.2	0.37	12.78	84.4	7.32
M3	5.09	13.2	0.59	13.78	57.9	11.76	3.13	13.7	0.64	13.36	60.8	8.27
M4	4.79	13.7	0.91	14.68	36.6	9.46	3.62	13.8	0.94	14.22	44.4	6.88
M5	1.55	12.7	1.77	16.32	-8.5	10.27	1.06	13.1	1.96	16.94	-4.7	7.62
M6	0.76	12.1	2.81	18.95	-45.5	10.25	0.52	12.2	3.01	20.06	-39.9	7.15
BF	1.07	13.6	2.74	25.1	-57.3	4.30	1.29	14.2	2.39	23.34	-40.2	4.31
CBr	14.08	14.3	6.43	15.21			16.77	14.6	5.89	14.89		
FBr	3.25	13.2	4.83	17.17			2.62	13.5	4.21	18.59		
Shts	3.04	11.7	3.36	17.50			2.13	12.1	3.42	19.98		
Cum	*100.3*		*1.61*				*99.9*		*1.55*			

[1]Yield (%); [2]Moisture (%); [3]Ash (%); [4]Protein (%); [5]Agtron colour value (Agtron units); [6]Starch Damage (MegaZyme Unit). CBr = Coarse bran, FBr = fine bran, Shts = Shorts, Cum = cumulative value. All values corrected to 14% mb.

Table 2.2. (Cont'd).

	Yld	Mst	Ash	Pro	Agt	Yld	Mst	Ash	Pro	Agt
	AC Vista					**AC Crystal**				
B1	1.50	14.0	0.46	14.91	54.4	2.54	14.0	0.50	14.51	60.8
B2	7.09	14.2	0.38	15.88	76.6	7.57	14.3	0.43	15.38	73.3
B3	2.62	13.7	0.43	18.49	66.4	2.27	13.9	0.50	18.07	59.7
B4	1.94	14.2	0.47	20.07	54.2	1.94	14.0	0.54	19.28	43.8
S1	12.25	14.5	0.34	13.45	89.2	12.80	14.5	0.40	12.94	83.7
Q1	1.45	14.0	0.60	15.62	54.9	1.47	14.0	0.71	14.53	38.8
M1	29.42	14.3	0.28	11.91	95.9	31.81	14.1	0.34	11.51	91.2
M2	11.05	14.0	0.36	11.99	81.4	10.26	13.8	0.42	12.09	71.4
M3	3.49	13.2	0.57	13.05	56.5	2.28	13.1	0.89	13.59	32.4
M4	4.07	13.5	0.84	14.50	40.7	3.72	13.5	1.09	14.01	13.4
M5	1.18	13.0	1.65	16.30	-7.3	1.16	12.9	2.10	16.39	-37.7
M6	0.61	12.3	2.46	19.39	-38.8	0.59	12.3	2.85	18.55	-57.5
BF	1.60	13.8	1.53	24.90	-25.4	1.77	13.8	1.73	24.38	-30.4
CBr	14.52	14.3	4.70	16.13		13.64	14.0	6.55	16.52	
FBr	4.05	13.3	3.94	18.55		3.24	12.9	4.84	17.05	
Shts	3.17	12.0	3.11	18.20		2.61	11.7	3.50	16.45	
Cum	*100.0*		*1.28*			*99.7*		*1.56*		
	AC Snowbird 2					**Superb 2**				
B1	1.73	14.0	0.53	14.53	61.7	1.98	14.1	0.53	12.45	54.5
B2	7.70	14.4	0.47	15.78	73.6	6.40	14.5	0.45	13.86	71.3
B3	1.92	13.9	0.57	17.88	60.1	1.75	14.0	0.52	16.04	57.4
B4	1.39	14.3	0.62	17.75	51.5	1.52	14.5	0.57	16.36	47.1
S1	11.67	14.6	0.41	13.56	88.2	12.69	14.9	0.38	12.09	85.8
Q1	1.22	14.2	0.73	14.29	58.0	1.55	14.3	0.62	12.97	52.3
M1	31.66	14.5	0.31	12.25	95.0	34.67	14.4	0.33	11.16	94.0
M2	12.63	14.0	0.38	12.39	86.3	10.94	13.8	0.40	11.43	81.1
M3	2.93	13.3	0.80	13.13	56.8	2.43	13.3	0.82	12.67	40.6
M4	3.51	13.4	1.09	13.88	42.1	3.62	13.6	0.99	13.01	30.0
M5	1.08	12.8	2.19	16.49	-3.0	0.83	13.1	2.24	15.91	-30.4
M6	0.55	12.1	3.21	19.64	-37.3	0.40	12.0	3.36	18.88	-55.7
BF	1.17	13.9	2.86	21.70	-40.8	1.36	14.0	2.26	20.04	-46.0
CBr	16.30	13.8	6.08	14.89		14.66	14.4	6.09	15.65	
FBr	2.72	13.1	4.47	17.73		2.89	13.2	4.73	16.02	
Shts	2.21	12.0	3.61	18.51		2.04	11.9	3.69	16.72	
Cum	*100.4*		*1.59*			*99.7*		*1.50*		

of the total flour yield obtained on the reduction side, respectively. However, starch damage results indicate that AC Snowbird was softer than AC Barrie.

Direct comparison of millstream yield results with previous studies is difficult since different studies use different mills, mill flows, and extraction rates. However, a major difference was obtained for the yield of M1 flour, which was much higher in this study (up to 150%) than those reported elsewhere (Nelson and McDonald, 1977; Holas and Tipples, 1978; Black et al., 1981; Martin and Dexter, 1991; Preston and Dexter, 1994). The high yield of M1 flour originated from the overs of S1 and B1 to B3 resulting from the small gap (0.5 mm) in reduction rolls that was set to achieve the high extraction rate of 80% used in this study. Consequently, the yields of M5 and M6 were lower than has been reported in other studies. A higher yield of B2 and a lower yield of B4 in this study compared to others were also obtained. In order to achieve a high extraction rate, break rolls were likely set tighter than that used in other studies, resulting in higher yields for the early break flours, and thus lower yields for the last break stream, B4.

2.3.2. Moisture

The mill room is temperature- and relative humidity- controlled to give reproducible flour moisture. Drying of stocks would affect yield, ash and colour. The decrease in moisture from the early break streams (over 14%) to the latter reduction streams (12%) can be explained by the number of wheat milling passages. As the wheat or stock passes through an increasing number of roll passages, friction heat of roller milling will increase, resulting in moisture loss by evaporation. These results are in accordance with previous studies (Black et al., 1981).

2.3.3. Ash content

Ash content of a flour is a measure of its mineral content. Since the minerals in the wheat kernel are concentrated in the outer endosperm, especially the aleurone layer (Hinton, 1959; MacMasters, 1971) and are present in lower levels in the starchy endosperm, the ash content of a flour reflects the degree of contamination by non-endosperm material, especially bran, and hence provides a good estimate of flour refinement. The first and second middling streams, M1 and M2, as well as the sizing stream S1, were the most highly refined streams as indicated by their low ash content (Fig. 2.2). Accordingly, these millstreams originate from the inner part of the endosperm. These results are similar to other reports (Izydorczyk et al., 2003; Black et al., 1981; Preston and Dexter, 1994). The ash content rose in the latter middling streams (from M3 to M6) as the incorporation of the subaleurone, aleurone, and germ increased in these streams.

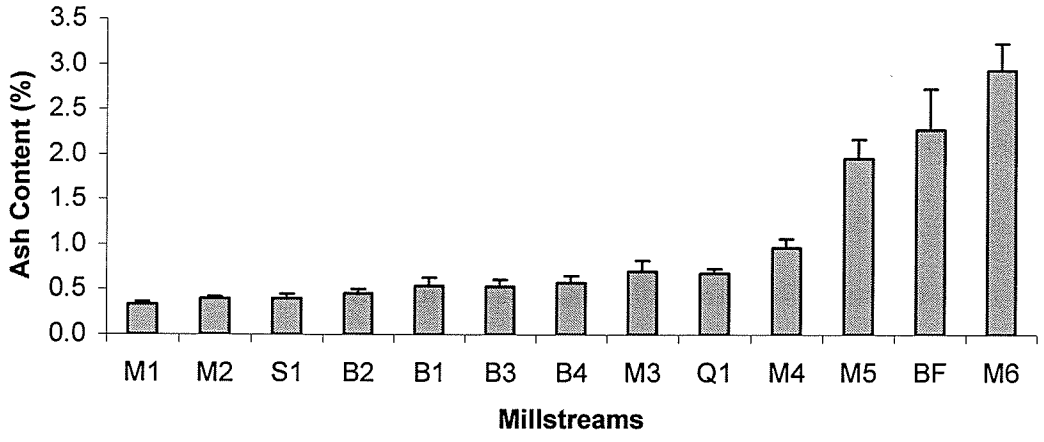


Figure 2.2. Averaged ash content of the millstreams of the eight cultivar samples. Standard deviation bars represent the variation in ash content between the cultivars.

For all samples, B1 had a higher ash content than B2 (Fig. 2.2), which is due to bran powdering as the seed coat is opened. On average, the difference was about 0.10%. Thereafter the ash content increased from B2 to B4. Ash values varied with sample; for the break streams, AC Corinne had the highest value (0.69% on average) while AC Vista had the lowest values (0.44% on average). The reduction streams had progressively increasing ash contents from M1 to M6 (average values of 0.33 and 2.93%, respectively). From M1 to M3, ash values are acceptable for commercial breadmaking flour. However, the M4 to M6 streams possessed ash values that were very high (over 0.80%), indicating substantial contamination of these streams by bran and germ. The high bran content of these streams is normal and can be explained by the high extraction rate used, which reflects a less efficient separation of bran from endosperm in the break section had a lower extraction been used. This increase of ash content from B2 to B4 and from M1 to M6 reflects the tendency of the milling process to mill from the inner to the outer layers of the kernel. The relationship between the ash gradient of the kernel from the inner to outer endosperm, and typical outcomes of roller milling as obtained in this study are well documented (Morris et al., 1946; Hinton, 1947; Hinton, 1959; Ziegler and Greer, 1971).

Bran flour stream had a slightly lower ash content compared to M6 (on average values of 2.28 and 2.93%, respectively), and for some samples (AC Vista and AC Crystal), bran flour ash was lower than that of M5. Bran flour is obtained from the bran flakes collected after the fourth break and is produced by impacting rather than by grinding. M5 and M6 flours are produced from re-grinding middlings that are highly contaminated with bran. Accordingly, bran flour contained a very different composition

of protein and had very different functionality compared to the last reduction streams M5 and M6 (refer to following sections).

Whole wheat ash normally varies from one genotype to another, and is dependent on both genotype and the growing environment. In evaluating flour quality in terms of ash content, it is necessary to also consider wheat ash. Usually, a greater ash content in all flour streams of one cultivar sample compared to another, is a reflection of higher wheat ash content. AC Corinne possessed the highest wheat ash content (1.61%), followed by AC Snowbird 2 (1.59%), while AC Vista had the lowest wheat ash content (1.28%). The average wheat ash of the eight samples was 1.52%, and the majority of results were in the range of 1.54 to 1.56%. The variation of wheat ash content was reflected in the ash content of the millstreams. AC Corinne had the highest flour ash values ranging from 0.38% for M1, to 2.81% for M6. In contrast, AC Vista had the lowest values ranging from 0.28% for M1, to 2.46% for M6.

2.3.4. Protein content

The protein content of the millstreams was highly variable from one flour stream to another and from one cultivar sample to another. Millstream protein contents reflected in general differences in protein contents of whole wheat itself: the lowest and highest protein samples, AC Corinne and Superb 2 had the highest and lowest M1 protein, respectively. As was observed for ash values, the gradient in protein content within the wheat kernel was reflected in the millstream results. M1 and M2 possessed the lowest protein content, which ranged from 11.2% for Superb 2, M1, to 13.3% for AC Corinne, M2 (Fig. 2.3).

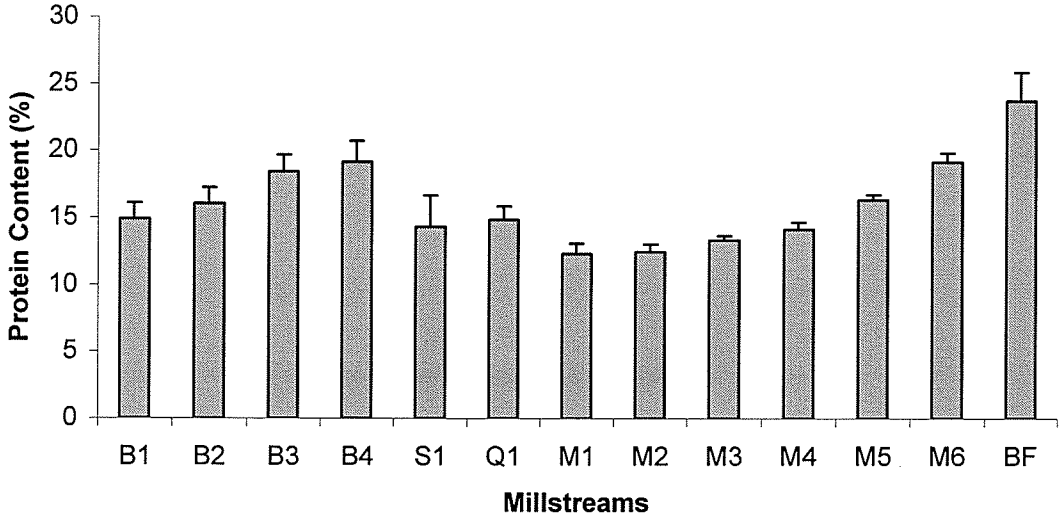


Figure 2.3. Averaged protein content of the millstreams of the eight cultivar samples. Standard deviation bars represent the variation in ash content between the cultivars.

Protein content for all samples increased progressively from B1 to B4 (14.9 to 19.2% on average, respectively). These results are consistent with findings of other workers (Hinton, 1947, Kent, 1966, Nelson and McDonald, 1977, Endo et al., 1987). Similarly protein content increased from M1 to M6 for all samples (12.3 to 19.1% on average, respectively). For AC Barrie, M2 flour had slightly less protein than M1 (12.8 and 13.1%, respectively). Trends in protein content variation within reduction flour streams vary widely in the literature. Nelson and McDonald (1977) reported M4 to have a protein content as low as M1 for four hard red spring wheat samples that were pilot milled. Black et al. (1981) found protein content to decrease between M4 and M5 for one sample of CWRS milled on the GRL pilot mill. Generally, variation from one study to another is directly related to the type wheat, mill and mill flow.

BF had the highest protein content of all millstreams, with values ranging between 20.0% for Superb 2, and 26.9% for AC Barrie. Similar results have been reported (Wang and Flores, 1999; Black et al., 1981). BF was obtained from the bran

flakes collected after the fourth break. Kent (1966) found that hard wheat varieties possessed a high protein subaleurone layer underneath the bran layers. This layer is the outermost cellular layer of the starchy endosperm but derives at a later stage of development from aleurone cells (Gordon, 1922 as cited by Kent, 1966). The author showed that in a HRW wheat of 13.7% protein, the subaleurone layer possessed 33 to 54% protein in comparison with 8 to 15% for the remainder of the inner endosperm. Protein contents of corresponding subaleurone and endosperm regions of a HRS wheat (14.7% protein) were estimated to range from 31 to 35% and 9 to 18%, respectively. Although the wheat grain contains about 11% of subaleurone endosperm by volume, this layer, because of its high protein content, accounts for almost 25% of the total protein of the starchy endosperm in these wheats. As well, the subaleurone content of the last two break flours was reported to be 39 and 54%, respectively. Accordingly, it appears plausible that BF contains significant quantities of subaleurone tissue. The protein composition results described in Chapter 4 indicate that BF contains a high content of glutenin and residue protein (protein of bran origin) content similar to M3 on average. Accordingly, subaleurone protein appears to comprise, in part, protein of very high quality for breadmaking, and is likely different from protein of the aleurone layer itself.

For all samples, the average protein content of reduction flours (14.6%) was lower than that of the break flours (17.1%). This result is explained by the nature and yield of the stocks from which the reduction flours are obtained. M1 and M2, which consist largely of lower protein inner endosperm particles (Black et al., 1981; Hinton, 1947; Kent, 1966; Nelson and McDonald, 1977; Orth and Mander, 1975; Endo et al., 1987), comprised more than 80% of the total reduction flours (M1 to M6).

2.3.5. Starch damage

The pattern of variation of starch damage across millstreams is shown in Fig. 2.4. For all cultivar samples, the third reduction stream, M3, had the highest starch damage (SD), followed by either M2 or M5 depending on the sample. M4 had lower starch damage compared to M3 because the fourth reduction rolls are mainly fed by overs from the Q1 rolls, which are low in starch damage due to the corrugation rolls of Q1. However, starch damage in M5 and M6 is lower than that in M2 and M3 presumably because the latter reduction streams have significantly lower starch content, derived in relation to their higher protein content and pentosan content (Wang et al., 2005). Lower starch content translates into lower total starch damage, other factors being equal. BF had low starch damage, due not only to the way it is obtained (impacting rather than grinding), but also to its high protein content and low starch content, as a result.

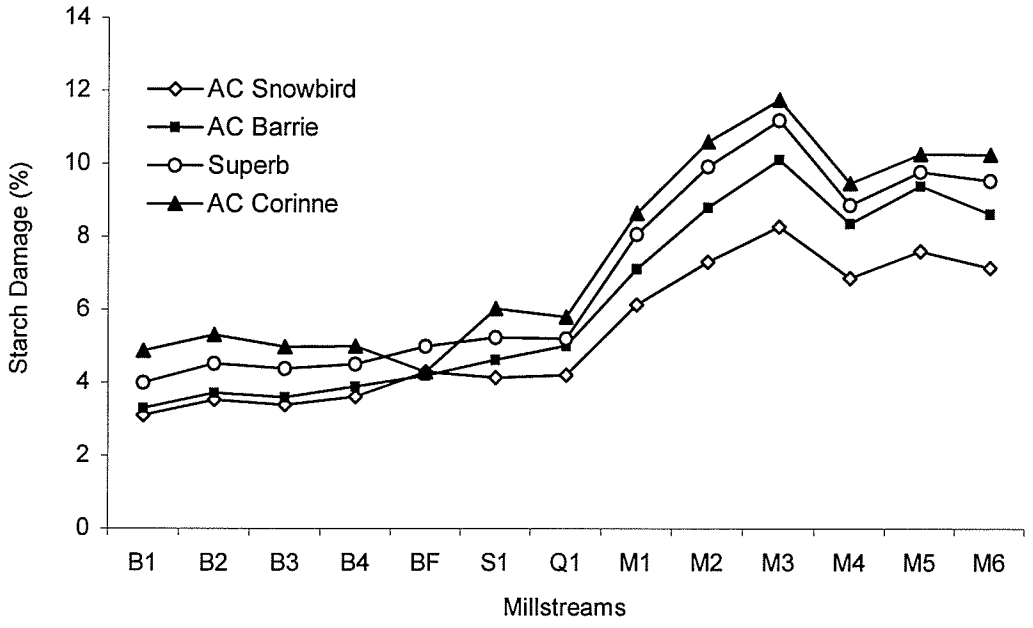


Figure 2.4. Variation in starch damage across millstreams for four samples.

The highest starch damage values were observed for AC Corinne. This was expected, since AC Corinne like Glenlea, which is a close relative, is known to be a relatively hard-textured wheat, and therefore, the higher the starch damage values will be generated in the flour. AC Snowbird possessed the lowest SD values, followed by AC Barrie and Superb.

A certain proportion of damaged starch is a necessary requirement in flour for breadmaking to furnish sufficient substrate of fermentable carbohydrate for yeast fermentation, as essentially only damaged starch can be readily hydrolyzed by α-amylase. As well, the damaged granules will absorb proportionately more water than undamaged starch (Greer and Stewart, 1959) and will swell accordingly, thereby increasing their size and thus the surface area to be covered by the gluten (Farrand, 1972). If the protein or gluten content is relatively low and the proportion of damaged starch is too high, the increase in surface area may be such that there is insufficient gluten to form a continuous matrix, ultimately resulting in bread of low volume. In addition, flour with a high level of starch damage could compete with the gluten for water, thereby preventing gluten from complete hydration and thus optimum development during mixing. Fig. 2.5 shows how the ratio of SD to protein content varied in the different millstreams. With their low starch damage and relatively high protein content, the break fractions in this study should be of good quality for breadmaking. On the other hand, early reduction flours may have too high a ratio of damaged starch to protein. There appears to be no information in the literature to confirm or dispute these speculations.

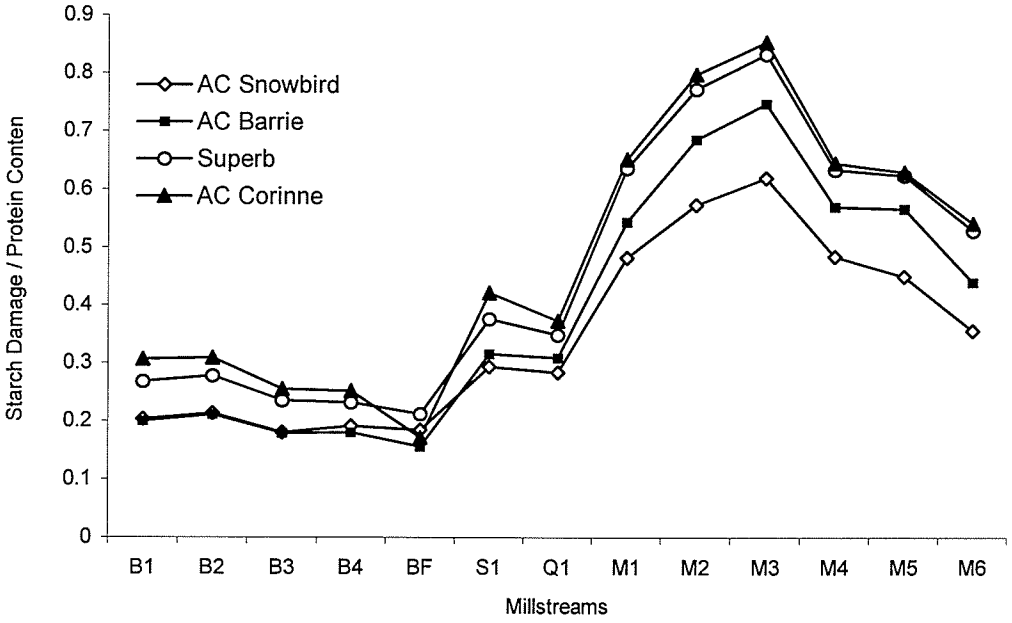


Figure 2.5. Variation in the ratio of SD to protein content in the different millstreams.

2.3.6. Colour

Several types of measurements were done to evaluate the colour of the different millstreams, leading to different types of values: colour grade as measured by the Agtron colorimeter (546 nm), L* a* b* values and % reflectance at different wavelengths as measured by colour spectrophotometry.

2.3.6.1. Agtron colour measurements

It is widely considered to be true within the milling industry that the higher the Agtron value, the higher the flour refinement. The highest colour values obtained for all samples were associated with the low ash flour streams M1 and S1 (91.8 and 83.8% on average, respectively), as expected. On the other hand, the high ash flour streams M5, M6 and BF gave negative values. These negative values arise from lower light

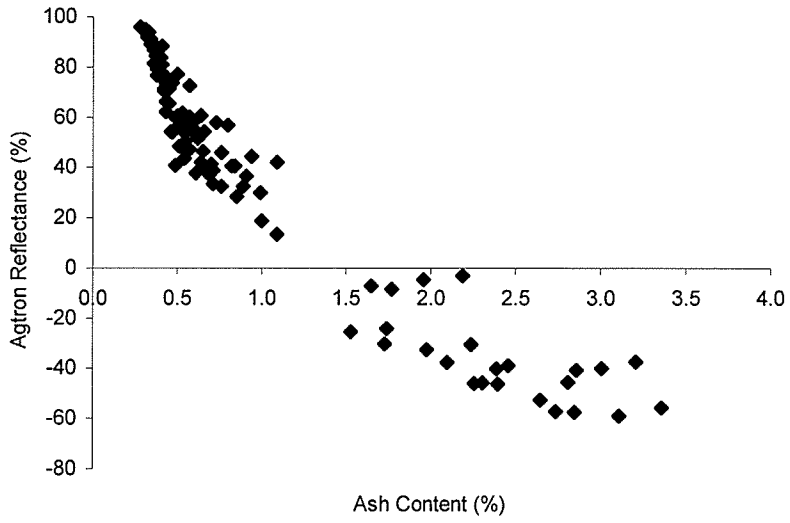


Figure 2.6. Relationship between Agtron reflectance values and ash content of the combined millstreams.

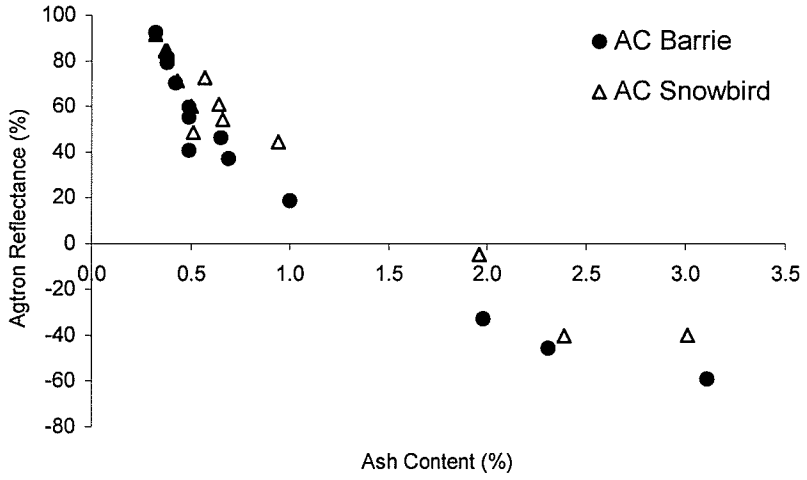


Figure 2.7. Agtron reflectance values as a function of ash content between a white wheat (AC Snowbird) and a red wheat (AC Barrie).

reflectance of these samples than the #63 calibration disk used to zero the instrument. Despite this inaccuracy, the Agtron colour values were highly correlated to the ash content ($r^2 = 0.902$) (Fig. 2.6). When comparing the wheat flour samples to one another, the white wheats AC Snowbird, AC Snowbird 2 and AC Vista, tended to have higher reflectance values than the red wheats for some flour streams (Fig. 2.7, Table 2.2). This was especially true for millstreams of relatively high bran contamination. For example, for M4 flours, the red wheat reflectance values ranged from 40.7 to 44.4%, while that of the white wheats ranged from 13.4 to 36.6%. However, their respective ash contents did not discriminate the same way. AC Snowbird, which had the highest Agtron reflectance value of 44.4% for M4 flour, had an ash content of 0.94%, whereas the lowest ash content for M4 (0.84%) was associated with AC Vista, which had a reflectance value of 40.7%. The M4 stream of AC Crystal had the lowest Agtron reflectance value of 13.4%, and the highest ash content of 1.09%. However, the M4 stream of AC Snowbird 2 also had an ash content of 1.09% but had a much higher reflectance of 42.1%. This indicates that although ash content and Agtron reflectance values were highly correlated across all millstreams, considerable discrepancies exist for millstreams of lower refinement when wheats of different bran colour are compared. Li and Posner (1989) evaluated the difference in colour of flours produced at increasing extraction rates (74 to 82%) between red and white wheats. They found that the inclusion of white wheat bran was less noticeable visually and instrumentally compared to red wheat bran.

Differences between red and white wheat is a problem encountered when using flour colour as a measure of ash content by the Agtron 546 nm approach, which is why wheat of diverse origin may not be comparable (Shuey, 1975; Barnes, 1986; Symons and Dexter, 1996) as each wheat can possess its own relatively distinct relationship. This

was also found by Shuey and Skarsaune (1973) who computed an equation to predict percent flour ash from flour colour reflectance values obtained by the green Agtron method, and found that different regression equations were needed for different mill mixes (in this case, two genotypes grown at two different locations). Shuey (1975) concluded that because both genotype and environment influence flour colour reflectance values and flour ash content, it is necessary to know the origin of the sample to be able to conclude that flours having the same colour values or ash contents are or not of the same grade or extraction. Evaluating colour, as well as ash content, can however be very effective in ranking flours from a single wheat sample according to the degree of refinement (Ziegler and Greer, 1971).

Moreover, although all previous studies have reported a linear correlation or regression between ash content and Agtron values, the current study clearly shows that the scattered points vary according to a logarithmic curve or equation. Table 2.3 shows the linear and logarithmic relationship.

Table 2.3. Correlation coefficients between Agtron reflectance values and ash content.

	Linear r^2	Logarithmic r^2
AC Barrie	0.918	0.983
Superb	0.937	0.988
AC Corinne	0.934	0.975
AC Snowbird	0.941	0.953
AC Vista	0.912	0.966
AC Crystal	0.926	0.986
Superb 2	0.919	0.983
AC Snowbird 2	0.957	0.965

2.3.6.2. Colour spectrophotometry

The objective of using colour spectrophotometry for flour colour measurement was to evaluate its performance as an alternative and more comprehensive approach for measuring flour refinement other than by ash content and Agtron colour measurement. The spectrophotometer used in this study can evaluate in one measurement both the tristimulus colour coordinates L* a* b*, and the percentage reflectance (%R) of flour obtained as spectra from 400 to 700 nm. All correlations between ash and reflectance values were always greater for slurries than for dry flour (Fig. 2.8). When analyzing dry flour, the error associated with particle size has to be considered. In general, softer wheat gives flour of finer granularity which leads to higher reflectance values, and vice-versa.

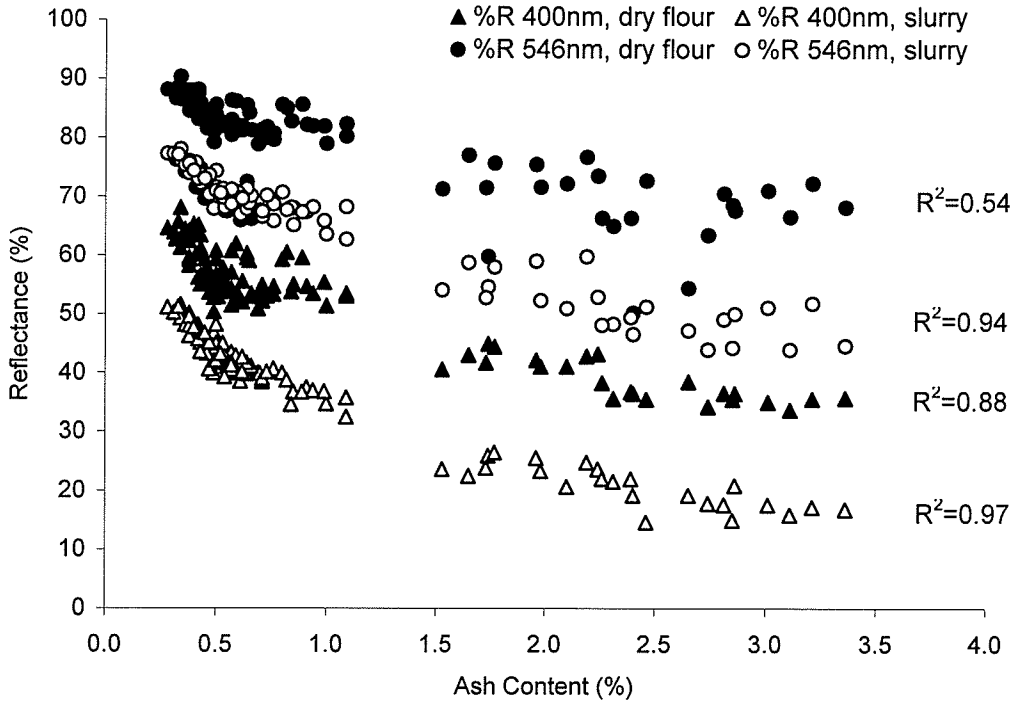


Figure 2.8. Logarithmic correlations between % reflectance at 400 and 546 nm and ash content for dry flour and slurries, as measured by spectrophotometry.

To eliminate this error, flour-water slurries were used instead of dry flour, as was proposed by Patton and Dishaw (1968). Therefore, only results obtained from flour-water slurries are discussed below.

L*

L* measures the brightness, and therefore the whiteness of the flour. L* reflectance properties of the flour streams were highly correlated with ash content (r^2 = 0.929) (Fig. 2.9). As ash content increased, the slurries became darker. Clearly, bran inclusion in the flour decreased its brightness. Although values of L* and ash content were highly correlated, the ranking of the millstreams according to each parameter was different, indicating that L* and ash do not measure the same properties among millstreams. According to Table 2.4, M1 always ranked first according to ash content and L* value. M2 always ranked second according to ash, but either second or third according to L*. According to ash content, M3 ranked towards the end of the series, just before Q1 and M4, which are millstreams quite contaminated by bran. However, according to L* values, M3 was ranked earlier on the series, depending on the type of wheat. Similar discrepancies were observed for B4. BF and tail-end reduction streams (M5 and M6) were expected to rank last by both ash and L*, although M6 was most often placed last as determined by ash content and sometimes before BF as determined by L* values depending on the type of wheat. Table 2.4 also ranks wheat according to Agtron reflectance values, and similar discrepancies were observed. L* values have been found to be correlated to pericarp fluorescence (r^2 = 0.90) (Symons and Dexter, 1991), to Kent-Jones colour values (Oliver et al., 1992) (r^2 = 0.72) and to ash content (Oliver et al., 1992) (r^2 = 0.85). This indicates that L* could be a satisfactory indicator of flour

refinement. In this study, L* values did not change when the flour was bleached with benzoyl peroxide.

Table 2.4. Ranking of millstreams according to ash content, Agtron reflectance, and L*, for a red wheat (AC Barrie), a white wheat (AC Snowbird), and the overall average.

AC Barrie			AC Snowbird			Average		
Ash	**Agtron**	**L***	**Ash**	**Agtron**	**L***	**Ash**	**Agtron**	**L***
M1	M1	M1	M1	M1	M1	M1	M1	M1
M2	S1	M2	M2	S1	M2	M2	S1	S1
S1	M2	S1	S1	M2	S1	S1	M2	M2
B2	B2	B2	B2	B4	B2	B2	B2	B2
B1	B1	B1	B1	B2	M3	B1	B3	B3
B3	B3	B3	B3	M3	B1	B3	B1	B1
B4	M3	M3	B4	B1	B3	B4	M3	M3
M3	B4	B4	M3	Q1	Q1	Q1	B4	Q1
Q1	Q1	Q1	Q1	B3	B4	M3	Q1	B4
M4	M4	M4	M4	M4	M4	M4	M4	M4
M5	M5	M5	M5	M5	M5	M5	M5	M5
BF	BF	BF	BF	M6	M6	BF	BF	BF
M6	M6	M6	M6	BF	BF	M6	M6	M6

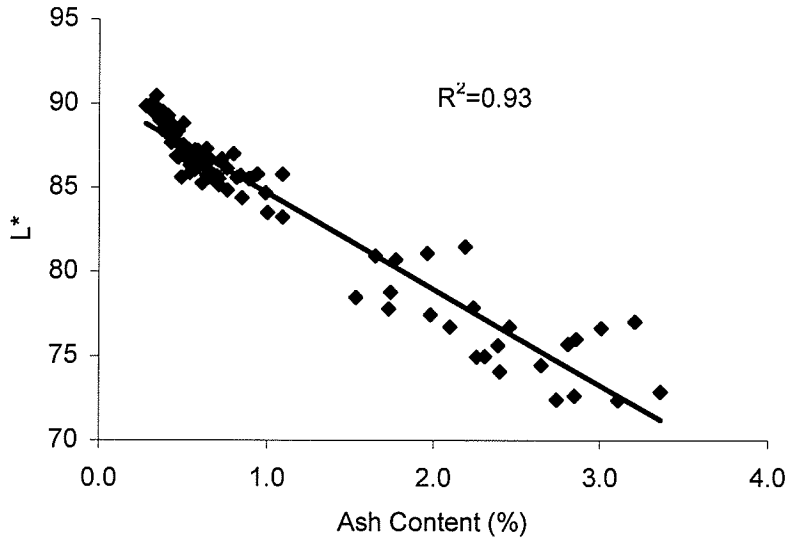
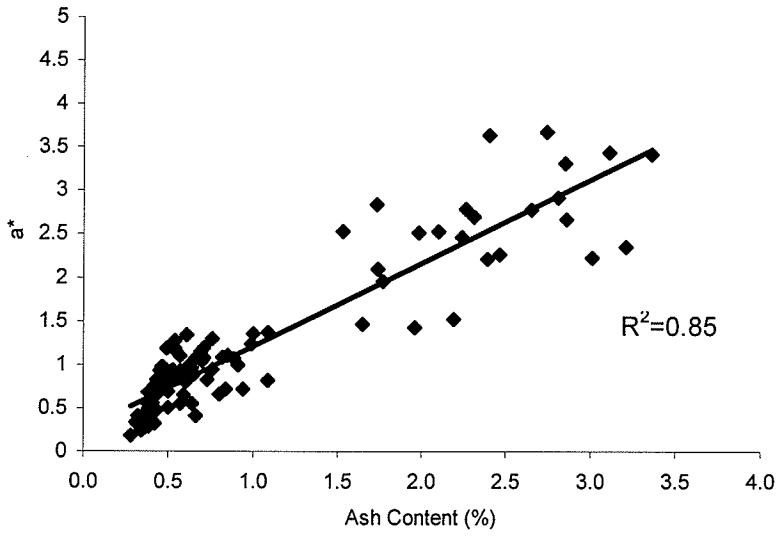


Figure 2.9. Relationship between ash content and L* values, all samples combined.

a* and b*

The CIE colour coordinates a* and b* (red/green axis and yellow/blue axis on the colour coordinates, respectively) also had relatively high correlations with ash content (r^2 = 0.85 and 0.86 respectively, Fig. 2.10) but slightly lower than that of the Agtron and L* values. As the ash content increased, the a* values increased. All a* values were positive, indicating an increased redness of the flour streams as flour refinement decreased, even for white grained wheats. As the ash content increased, the b* values also increased. All b* values were also positive, indicating an increase in yellowness of the flour streams as flour refinement decreased. Although a* and b* parameters are used to measure flour colour, they were found to be sensitive to flour moisture changes and flour particle size (Symons and Dexter, 1991). By using flour-water slurries this problem can be easily overcome. The b* values are related to the yellowness of the flour, which is more likely due to the carotenoid pigments that can be bleached by benzoyl peroxide (Gillis, 1963; Patton and Dishaw, 1968; Murthy and Dietz, 1974). Accordingly, the b* values did change (decreased) when the flour was bleached, and thus cannot always be related to flour refinement. However, a* values, although also sensitive to flour moisture changes and particle size (Symons and Dexter, 1991), could be used as a bran contamination indicator. On the other hand, scattered points did not fit the regression line well, especially for the less refined streams, indicating that discrepancies exist for these streams among samples. These results indicate that a* and b* are less suitable than L* to measure flour refinement.

A.



B.

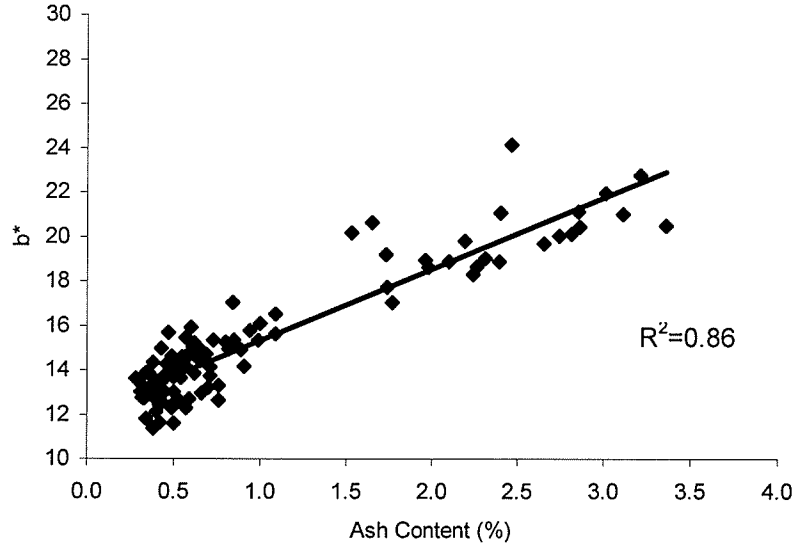


Figure 2.10. Relationship between ash content and a* (A) and b* (B) values, all samples combined.

Percentage Reflectance (%R)

Using the Minolta CM-3500d spectrophotometer, reflectance spectra between 400 and 700 nm were obtained for flour-water slurries of all millstreams of all eight varieties. An example of the spectra obtained for AC Barrie is shown in Fig. 2.11.

To evaluate the spectrophotometer for flour colour measurements in comparison with the Agtron, the percentage reflectance at 546 nm (wavelength used by the Agtron colour meter) obtained for each millstream was calculated by extrapolation. This was done because the diode array sensors of the spectrophotometer were calibrated at 10 nm intervals. Furthermore, the scale and range of reflectance generated by the spectrophotometer and Agtron instrument were not the same. The former was calibrated using a black cylinder and a white tile for reflectances of 0 and 100%, respectively. In

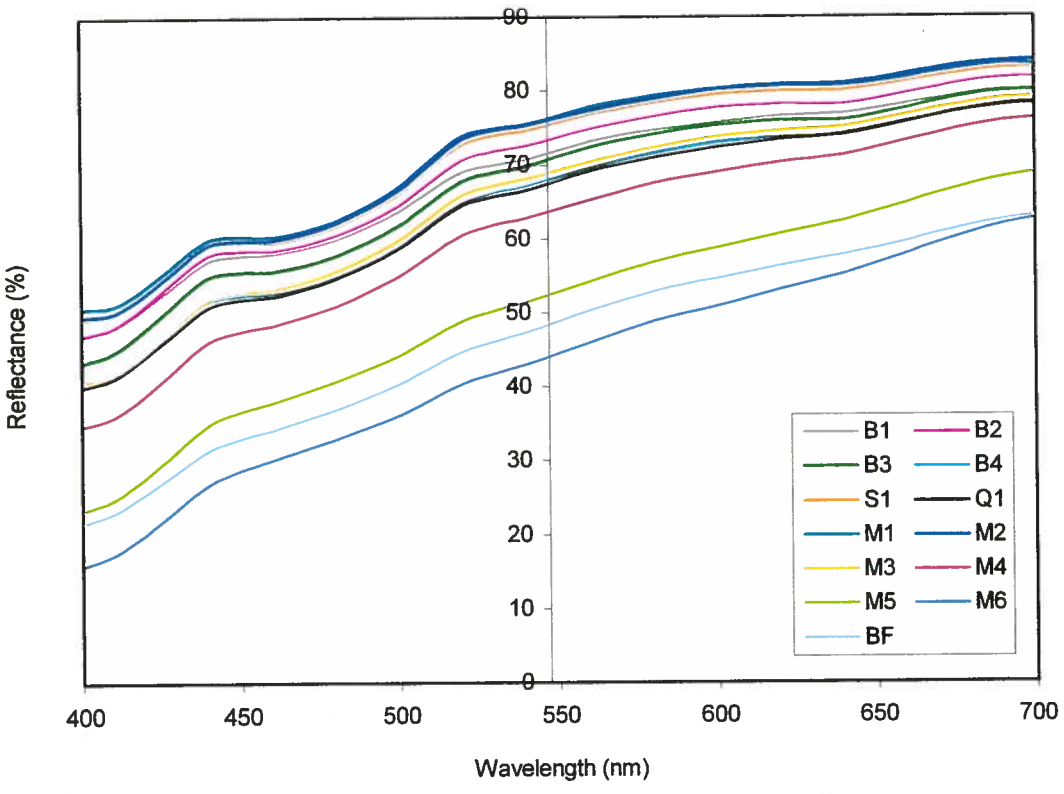


Figure 2.11. Colour reflectance spectra obtained for the millstreams of AC Barrie. The y-axis is located at the intersection with the 546 nm wavelength.

contrast, the Agtron method uses disks #63 and #85 for 0 and 100% relative reflectance, respectively, which leads to inaccurate results (negative values) for samples of low refinement. Accordingly, the range of reflectance values for the spectrophotometer was substantially narrower than that of the Agtron instrument. However, values obtained with both instruments were highly correlated ($r^2 = 0.99$), and the percentage reflectance at 546 nm as determined by the Minolta spectrophotometer was closely related to ash content ($r^2 = 0.92$). Figure 2.12 shows the slightly curvilinear relationship between Agtron and Minolta spectrophotometer reflectance values at 546 nm (%R 546). Predicted Agtron values were calculated from the %R 546 values, and correlated to the observed Agtron values. The relationship (Fig. 2.13) was close to perfect.

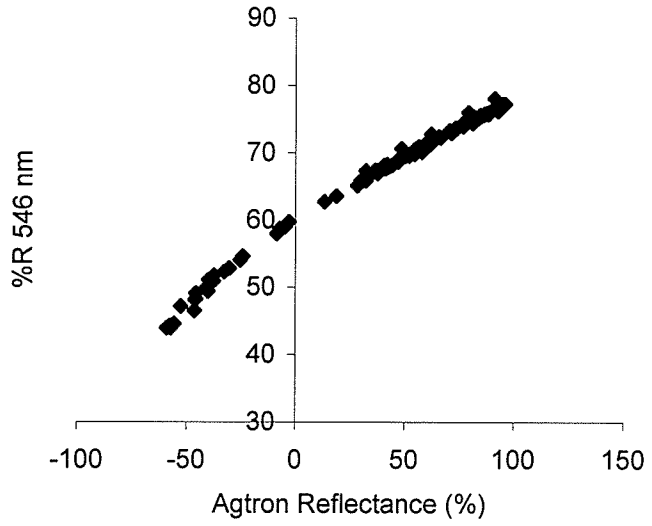


Figure 2.12. Relationship between Agtron reflectance values and %R 546 nm values determined using the Minolta spectrophotometer. The regression equation was y = 58.654 + 0.208x. All millstreams from all samples are included.

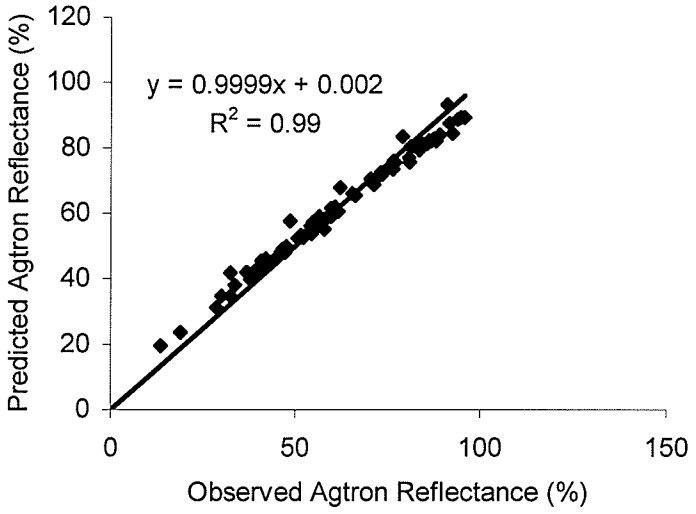


Figure 2.13. Relationship between observed and predicted Agtron values.

The more highly refined the stream, the higher the reflectance values from 400 to 700 nm. According to the reflectance spectra of all varieties, the lowest values were obtained for the last three reduction streams M4 to M6 and the BF. The reflectance spectra values ranked BF before M6, in line with ash contents. For the break streams, reflectance spectra values increased from B1 to B2, then decreased from B2 to B4. For the reduction streams, reflectance spectra values decreased progressively from M1 to M6, again in accordance with ash. The curves of all millstreams of all samples showed two broad maxima in the lower wavelength range, near 440 and 530 nm. The concavity of reflectance between these two maxima can be explained by the absorption of light due to the carotenoid pigments of the endosperm. Barnes (1986) investigated the spectra of flour unbleached and bleached by benzoyl peroxide. He obtained peaks between 400 and 500 nm that corresponded to carotenoid absorbances. When the flour was bleached, the carotenoid absorption bands disappeared from the spectra. In this study, four straight

grade flours (Superb, AC Barrie, AC Corinne, AC Snowbird) were bleached with benzoyl peroxide. Figure 2.14 shows the reflectance spectra of AC Snowbird straight grade flour that was unbleached, bleached with 50ppm of benzoyl peroxide, and bleached with 100ppm of benzoyl peroxide. Results for the other samples are shown in Appendix I. Although there was a small difference in reflectance for the flours bleached with the two concentrations of benzoyl peroxide (not true for all the genotypes), the concavity of the non-bleached flour due to the carotenoid pigments disappeared, confirming Barnes' results.

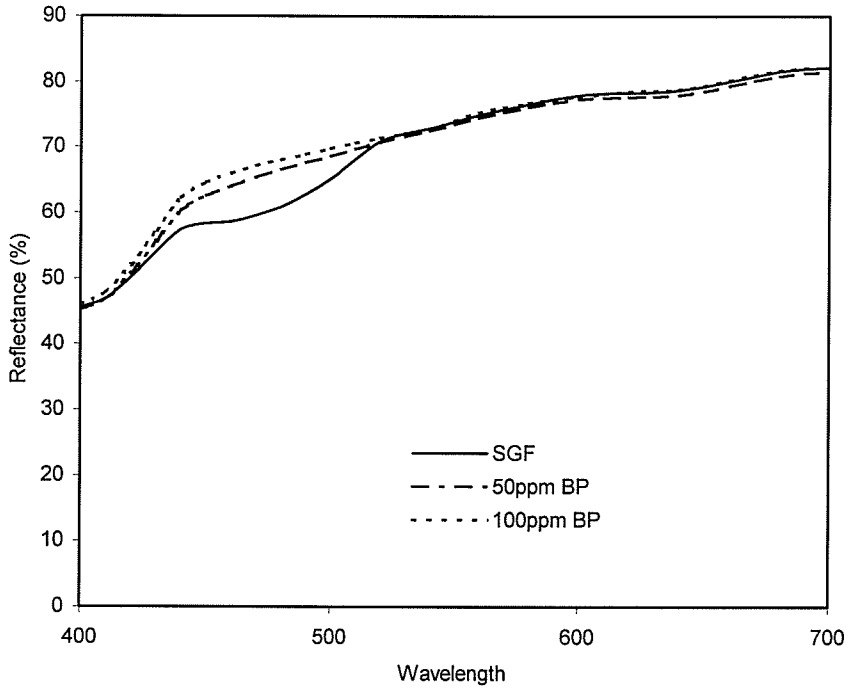


Figure 2.14. Colour reflectance spectra of AC Snowbird straight grade flour (SGF) unbleached and bleached with 50 and 100 ppm of benzoyl peroxide.

To provide another test of colour spectrophotometry to gauge flour refinement, stepwise discriminant analysis was applied to the spectra to determine the relative power of different wavelength to discriminate the millstreams. Results (Table 2.5.) show the ranking of the wavelengths for each sample according to their discrimination power, which is indicated by the ASCC (average squared canonical correlation statistic). The wavelengths at which carotenoid pigments absorb (440 to 530 nm) cannot be considered, as explained earlier. For four samples (Superb, AC Snowbird, AC Vista, and AC Snowbird2), 400 nm was the first selected wavelength, indicating that 400 nm was the

Table 2.5. Stepwise discriminant analysis selection of wavelengths by Average Squared Canonical Correlations (ASCC).

λ (nm)	AC Barrie		Superb		AC Corinne		AC Snowbird	
ranking	λ (nm)	ASCC	λ (nm)	ASCC	λ (nm)	ASCC	λ (nm)	ASCC
1	560	0.083	400	0.0833	520	0.083	400	0.083
2	400	0.166	460	0.163	410	0.166	580	0.166
3	430	0.239	410	0.243	490	0.237	470	0.231
4	470	0.321	420	0.322	580	0.318	430	0.306
5	680	0.393	570	0.385	440	0.371	680	0.373
6	620	0.428	630	0.410	680	0.443	640	0.419
7	570	0.501	680	0.471	620	0.488	410	0.484
8	520	0.556	560	0.541	460	0.517	530	0.544
9	700	0.589	520	0.575	590	0.533	610	0.570
10	410	0.643	510	0.641	430	0.587	690	0.631

λ (nm)	AC Vista		AC Crystal		AC Snowbird 2		Superb 2	
ranking	λ (nm)	ASCC	λ (nm)	ASCC	λ (nm)	ASCC	λ (nm)	ASCC
1	400	0.083	550	0.083	400	0.083	560	0.083
2	470	0.165	410	0.165	510	0.166	400	0.166
3	430	0.244	480	0.242	490	0.238	500	0.245
4	520	0.315	430	0.315	420	0.313	410	0.322
5	690	0.369	640	0.356	470	0.385	450	0.382
6	650	0.438	400	0.425	440	0.439	470	0.451
7	480	0.484	530	0.452	700	0.485	680	0.524
8	670	0.547	590	0.526	610	0.543	630	0.558
9	580	0.586	680	0.588	580	0.585	470	0.581
10			610	0.636	640	0.601	620	0.622

most discriminatory wavelength for these samples. For AC Barrie and Superb2, 400 nm was the second selected wavelength and for AC Corinne and AC Crystal, 410 nm was selected as the second beast wavelength for discrimination. As 400 nm was the most frequently selected wavelength among the top two selections, it is likely the optimal wavelength for discriminating among millstreams.

The second method used to assess millstream discrimination was by determining the coefficient of variation of reflectance values among millstreams calculated at each wavelength (Fig. 2.15). The result indicates that reflectance variation among millstreams increases with decreasing reflectance. Therefore, the wavelength at which the millstreams varied most was 400 nm, which confirms the results found by the statistical method, stepwise discriminant analysis.

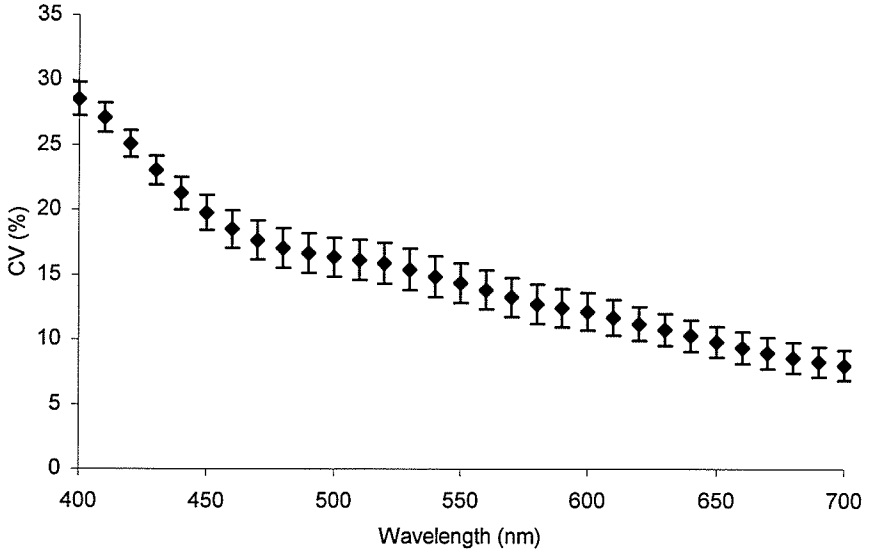


Figure 2.15. Averaged coefficient of variation (CV %) of reflectance values of each millstream calculated at each wavelength. The standard deviation bars indicate the variation among samples.

Correlations between reflectance at 400 nm (%R 400) and other parameters of flour refinement and colour were analyzed. Results indicated that %R 400 was highly correlated to ash content (Table 2.6. and Fig. 2.16) and to many other parameters. %R 400 was most strongly correlated with Agtron reflectance. Figure 2.17 shows the relationship between ash content and colour reflectance for a number of selected wavelengths such as 400, 500, 600, and 700 nm. For these wavelengths, the highest r^2 was achieved using 400 nm. The result confirms 400 nm as the best wavelength for measuring millstream refinement.

Table 2.6. Correlation coefficients between the flour refinement determination methods, ash content, and protein. All coefficients are significant ($p<0.001$).

	Ash	Protein	Agtron	L*	a*	b*	%R546
Ash							
Protein	0.598						
Agtron	-0.950	-0.687					
L*	-0.964	-0.694	0.989				
a*	0.921	0.733	-0.962	-0.978			
b*	0.925	0.621	-0.910	-0.910	0.864		
%R546	-0.959	-0.685	0.994	0.999	-0.976	-0.908	
%R400	-0.958	-0.667	0.985	0.975	-0.934	-0.953	0.980

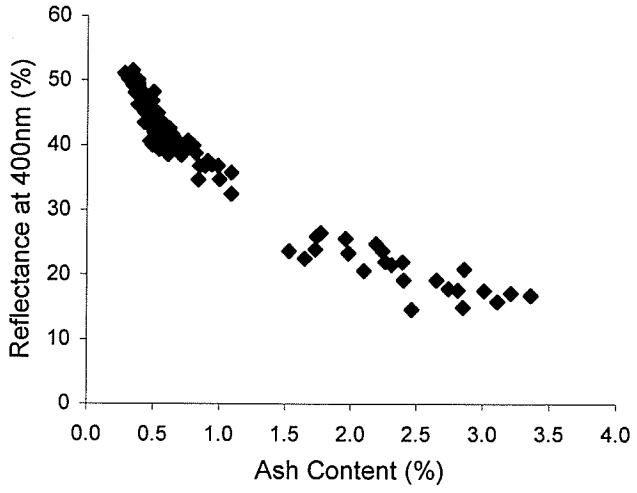


Figure 2.16. Relationship between reflectance at 400 nm as measured by spectrophotometry and ash content of the millstreams.

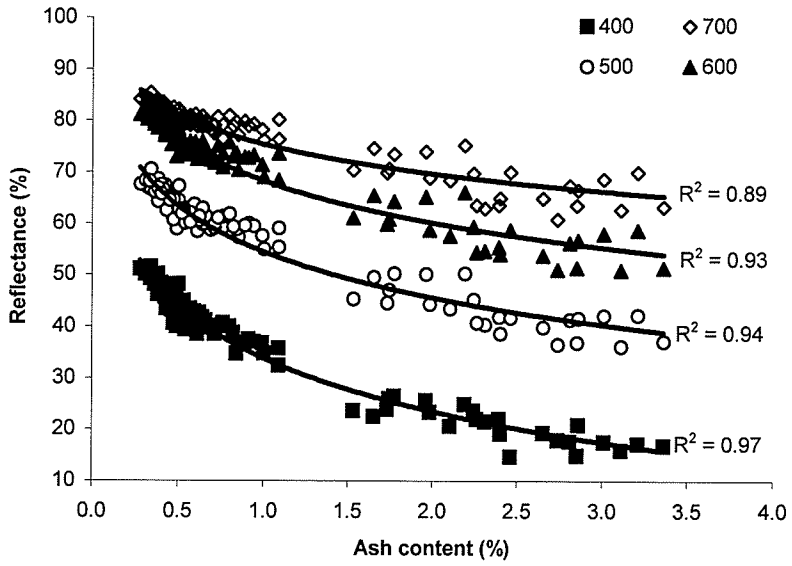


Figure 2.17. Relationship between reflectance of millstreams and ash content at different wavelengths.

2.3.6.3. Effect of grain colour

The effect of wheat grain colour on millstream reflectance was determined by separating the white wheats from the red wheats for data analyses. Figure 2.18 shows the relationship between ash content and percentage reflectance measured by spectrophotometry at two different wavelengths, 546 and 400 nm, and by Agtron reflectance. Using the 546 nm wavelength, the red and white wheat correlation curves begin to diverge from each other from about 0.50% ash (Fig. 2.18.A), indicating that bran colour had an effect on millstream reflectance values. Agtron reflectance results (Fig. 2.18.B) were similar. However, millstream reflectance measurements at 400 nm did not differentiate between red and white grained samples regardless of ash content. These results provide further evidence that %R at 400 nm would be a more accurate basis to determine flour refinement, and flour colour in general.

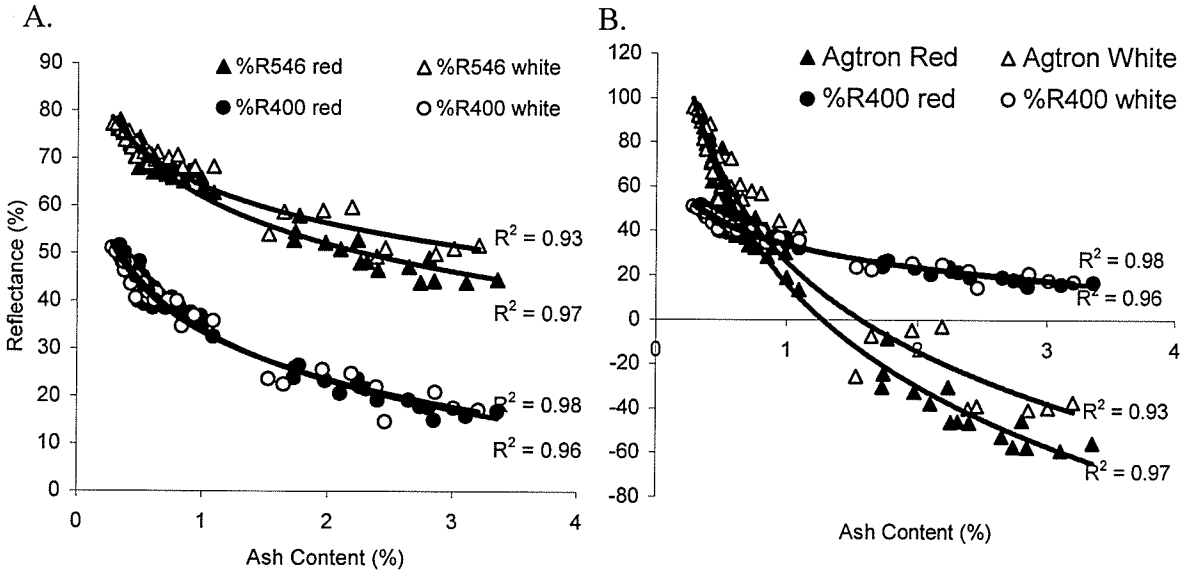


Figure 2.18. Relationship between ash content and the percentage reflectance at 546 and 400 nm (A) and for the Agtron reflectance values (B) for white wheats and red wheats. R^2 values are for logarithmic-based relationships.

2.3.6.4. Comparison of the various methods of flour refinement determination

The correlation coefficients among the various methods used in this study to measure flour refinement (Ash content, Agtron, tristimulus L*, a* and b*, percentage reflectance at 546 nm and at 400 nm) are presented in Table.2.6. High correlations suggest that all methods can be potentially used as a measure of flour refinement.

For further understanding of the various methods and parameters, millstreams were separated into two groups, the highly refined streams including B1 to B4, S1, M1 and M2, and the low grade streams, Q1, BF, M3 to M6. Correlations were re-calculated and results are presented in Table 2.7.

Table 2.7. Linear correlation coefficients between the flour refinement determination methods, ash content, and protein content for the high and the low refinement streams separated.

	Streams of high refinement B1 to B4, S1, M1 and M2							
	Ash	Protein	Agtron	L*	a*	b*	%R546	%R400
Ash								
Protein	0.681							
Agtron	-0.898	-0.767						
L*	-0.915	-0.791	0.970					
a*	0.858	0.794	-0.935	-0.948				
b*	0.269	0.589	-0.420	-0.477	0.509			
%R546	-0.924	-0.782	0.972	0.999	-0.948	-0.443		
%R400	-0.878	-0.854	0.929	0.962	-0.916	-0.602	0.958	
	Streams of low refinement Q1, M3 to M6, BF							
	Ash	Protein	Agtron	L*	a*	b*	%R546	%R400
Ash								
Protein	0.713							
Agtron	-0.948	-0.790						
L*	-0.948	-0.802	0.991					
a*	0.897	0.801	-0.960	-0.976				
b*	0.905	0.714	-0.884	-0.866	0.811			
%R546	-0.947	-0.800	0.995	0.999	-0.975	-0.868		
%R400	-0.963	-0.752	0.976	0.962	-0.913	-0.951	0.965	

For the highly refined streams, ash was most highly correlated to %R 546, followed by L*, Agtron reflectance and %R 400, however, the range of correlation variation was small, from r = -0.92 (%R 546) to r = -0.88 (%R 400). For the streams of low refinement, ash was most highly correlated with %R 400 (r = -0.96), followed by Agtron, L*, and %R 546 (r = -0.95). These rankings according to correlation coefficients were slightly different when all millstreams were combined (Table 2.6). The largest differences in correlation coefficients between high and low refinement flour streams were those concerning b*. Correlations between b* and other reflectance parameters and ash were relatively high (> 0.81) for the low grade streams. However, b* was poorly correlated to the other parameters for streams of high refinement, indicating that b* was

not a satisfactory parameters for flour colour assessment in general. As mentioned earlier, b* values were affected by flour bleaching.

2.3.6.5. Influence of protein content on colour measurements

The correlation coefficients between protein and the various methods used in this study to measure flour refinement (Ash content, Agtron, tristimulus L*, a* and b*, percentage reflectance at 546 nm and at 400 nm) are presented in Table.2.6.

It is important to consider the effect of protein content when measuring flour colour. Barnes (1986) showed that flour colour was strongly influenced by a contribution from the endosperm, apart from any bran contamination. He found that endosperm paste reflectance at 540 nm was negatively correlated with the protein content of the wheat and flour, and that was partly influenced by genotype. This variation in the endosperm caused differences in the grayness of flour-water pastes.

Table 2.6 provides correlation coefficients between protein content and flour refinement methods. Protein content was negatively correlated to Agtron, L*, %R 546, and %R 400, and positively correlated with a* and b*. Although protein content was not as highly correlated to any test of flour refinement as was ash content, the correlation coefficients are significant ($p < 0.001$). This indicates that protein content had an effect on flour colour.

Colour induced by protein content is not related to bran contamination of the millstream. Although, since both ash content and protein content increase from inner to outer endosperm, millstreams having high protein content can also have high bran content. Thus, the effect of protein on flour colour can be greater for low grade millstreams. Correlation coefficients between protein content and flour refinement

measures were on average slightly higher for low grade millstreams than for high grade counterparts (Table 2.7). However, %R 400 was an exception as it had a lower correlation coefficient with protein content for millstreams of lower refinement (r = -0.75) than that for millstreams of higher refinement (r = -0.85). This further indicates that %R 400 is a better test to measure flour refinement as opposed to Agtron and tristimulus colour coordinates L*a*b*, especially for flours of lower refinement.

Table 2.8 shows the contribution of protein content to flour colour values using partial r^2 statistics.. Although its contribution was low compared to that of ash content, protein content was responsible for approximately 2% of the variation in colour as measured by Agtron, L*, and %R 546 nm. By comparison, protein content had less influence on %R 400 nm values (partial r^2 = 1.4%). L* values were influenced least by protein content (partial r^2 = 0.7%). Analogous results were shown in Table 2.6.

Table 2.8. Contribution of protein content to colour values.

	Agtron	L*	a*	b*	%R 546	%R 400
Partial R^2 ash	0.902	0.929	0.848	0.856	0.920	0.917
Partial R^2 protein	0.022	0.022	0.052	0.007	0.023	0.014
Total R^2	0.924	0.951	0.900	0.863	0.943	0.931

2.4. CONCLUSIONS

Analytical properties of millstreams were consistent with previous studies. The most refined streams (M1, M2, and S1) possessed low ash and low protein contents, and the least refined ones (M5 and M6) had high ash and high protein contents. The head reduction streams originating on average from inner endosperm particles had lower protein contents than the break streams. The increasing trends in ash and protein

contents from first to last break and reduction flours reflected the well-known increasing gradient of minerals and protein from inner to outer endosperm. The BF fraction, in which subaleurone endosperm is concentrated contained the highest protein content of all millstreams, a characteristic typical of hard wheats. Results indicated that break flours and reduction flours were different in composition in terms of mineral and protein content.

The millstream flours covered a wide range of ash and protein content and colour, and therefore provided an excellent opportunity for a flour refinement study, using a computerized diode array colour spectrophotometer. In industry, flour refinement is commonly measured by ash content and/or colour instruments (Agtron) using a single wavelength (546 nm). In this study, it was shown that although Agtron results as well as L* (brightness) values were highly correlated with ash content, there were discrepancies in millstream discrimination and deficiencies in accuracy for determining refinement. The spectrophotometer provided a reflectance spectra from 400 to 700 nm, and it was found that the most discriminating wavelength was 400 nm. This wavelength was clearly superior to the green Agtron wavelength (546 nm) in terms of discrimination power among millstreams. The 400 nm wavelength was also outside the range of absorption of flour carotenoid pigments (440-475 nm), indicating that flour refinement measurements at 400 nm could also be accomplished on bleached flour. Reflectance values at 400 nm were highly correlated to ash content and L* values. The principal limitation of using flour colour to determine bran contamination arises from differences in light reflectance between bran from red and white wheats. By using a 546 nm wavelength, wheats of diverse origin cannot be compared. However, the present research has shown that reflectance at 400 nm was insensitive to wheat colour even for

flour streams containing high levels of bran. Furthermore, 400 nm was less sensitive than the Agtron-based 546 nm to the negative contribution of colour by flour protein. Results showed that using a 400 nm wavelength in flour refinement determination was clearly more accurate than using the Agtron 546 nm wavelength.

The computerized diode array colour spectrophotometer was a very effective tool for evaluating flour colour and refinement. Its capability of measuring simultaneously the CIE colour coordinates and reflectance over a scale from 400 to 700 nm presents extra advantages over filtered-based instruments.

Chapter 3. Dough Mixing Characteristics of Wheat Millstreams of Different Cultivars of Hard Spring Wheat

ABSTRACT

Rheological properties of individual millstreams of four wheat cultivars were investigated. Wheat comprised two cultivar samples of Canada Western Red Spring wheat (Superb and AC Barrie), one representative sample of Canada Western Hard White wheat (AC Snowbird), and one sample of Canada Western Extra Strong wheat (AC Corinne). Wheat was milled on a tandem Buhler pneumatic laboratory mill to produce 13 flour streams; 4 break flours, 1 bran flour, 1 sizing flour, 1 low quality flour, and 6 reduction flours. These streams were evaluated using a computerized 2 g mixograph without and with 2% salt (NaCl) at constant absorption (65%, 14% mb). The mixograph parameters that were evaluated were mixing time (MT), peak development resistance (PDR), bandwidth at peak resistance (BWPR), and work input to peak (WIP). MT values showed no trend among streams for individual cultivars and were not correlated with protein content. PDR and BWPR values were significantly correlated with protein content ($r = 0.55$ and 0.52, respectively), but correlation coefficients were greater when M5 and M6 were excluded ($r = 0.84$ and 0.79, respectively). For the flour break streams, the 64.8% and 69.5% increase in PDR and BWPR respectively from B1 to B4 was accompanied by a corresponding 78.6% increase in protein content, on average. On the other hand, for the reduction streams (M1-M6), the correspondence between PDR and BWPR and protein content was not that clear. This was especially evident for the last two reduction streams, M5 and M6, which possessed high protein content but produced weak doughs, indicating that the protein quality or composition of

these millstreams was different from that of the break roll flours. Flour refinement as measured by ash content was not correlated with any of the mixing parameters although seemed to play an indirect role in the mixing properties of the last reduction streams, M5 and M6, which had high ash contents. The results indicated that protein composition, and most importantly glutenin content, varied widely among millstreams, and was the main factor responsible for the different dough mixing characteristics of the millstreams.

3.1. INTRODUCTION

Wheat milling is a key source of variation in flour quality for breadmaking, mainly because the wheat kernel is heterogeneous in physical and chemical composition. Of major importance is the increasing protein content which mirrors the mineral (ash) gradient from inner endosperm to the outer layers of the kernel (Morris et al., 1945, 1946; Hinton, 1947, 1959, 1962; Stevens et al., 1963; Kent, 1966;, Kent and Evers, 1969). Pentosan content has also been reported to change from inner to the outer layers of the kernel with the aleurone layer of bran having the highest concentration (Ciacco and D'Appolonia, 1982; Delcour et al., 1999). Thus the milling process will provide millstreams whose variable composition, notably protein and pentosans, can affect breadmaking performance of the flours.

Although flour stream quality in terms of protein content has been subject to numerous research studies (Morris et al., 1945, 1946; Hinton, 1947, 1959, 1962; Stevens et al., 1963; Kent, 1966; Kent and Evers, 1969), and pentosans to a lesser extent (Stephen et al., 1949; Ciacco and D'Appolonia, 1982; Delcour et al., 1999; Wang et al., 2005), the rheological properties of millstreams have not been comprehensively studied.

The few studies regarding dough mixing properties of flour millstreams include

work with the Do-Corder (Endo et al., 1987), the farinograph (Holas and Tipples, 1978, Preston et al., 1982), and the extensograph (Preston et al., 1982). Endo et al. (1987) obtained millstreams from a CWRS wheat milled on an experimental Buhler mill to a flour extraction rate (FER) of 60%, and evaluated their rheological properties using a Brabender Do-Corder, without premixing, and on pre-mixed dough samples mixed to peak and peak + 5 min using a mixograph at 70% absorption. The break streams underwent a dramatic change during mixing as measured by the Do-Corder, unlike the reduction streams for which changes were slight. Endo et al. (1987) concluded that the differences in the rheological properties between break and reduction streams could be attributed to intrinsic differences in their components, such as changes in sulfhydryl (SH) content, which decreased dramatically during mixing for the break streams. Preston et al. (1982) measured the dough development time (DDT) and mixing tolerance index (MTI), as well as extensograph parameters, for CWRS millstreams. The authors found that while the break streams had greater extensograph areas than the reduction streams, indicating greater dough strength, they had shorter DDT and lower tolerance to overmixing than the reduction streams. Among the reduction streams, M1 had a long tolerance to overmixing, while DDT decreased and MTI increased from M1 to M5, indicating a progressive decrease in dough strength. On the contrary, Holas and Tipples (1978) found longer DDT and stabilities for break streams than for reduction streams.

The mixograph has been widely used in cereal science research since being initially introduced (Swanson and Working, 1933) and later with the development of a 2 g direct drive computerized mixograph (Rath et al., 1990) for automated recording and easy interpretation of the results. It has proven to be a convenient tool in research and in flour quality evaluation, especially when the sample size is limited. An important

drawback of the mixograph is that it cannot measure water absorption (Finney and Shogren, 1972), although many researchers have attempted to do so (Baig and Hoseney, 1977; Hazelton et al., 1997; Ingelin and Lukow, 1999). Dupuis (1999) has shown that using an optimum absorption as measured by the mixograph is likely not possible, as different cultivar samples had water absorptions varying in 30% range based on one peak height, and subsequently would likely not produce optimum dough consistency for breadmaking. Preston and Dexter (1994) also found farinograph absorption to range from about 59% to 79% depending on the millstream of a CWRS wheat bland. Also, the mixograph is still mainly used at constant water absorption, usually from 60 to 66%. The mixograph has been useful in determining quality of wheat, dough (Johnson et al., 1943; Finney and Shogren, 1972; Bruinsma et al., 1978; Roels et al., 1993; Khatkar et al., 1996; Primard et al., 1991; Martinant et al., 1998; Weegels et al., 1995b) or gluten (Neufeld and Walker, 1990) and in the evaluation of the effect of a single or several parameters in reconstitution studies (Tsen, 1969; Danno and Hoseney, 1982; Lang et al., 1992; MacRitchie et al., 1991; Pena and Balance, 1987; Bekes and Gras, 1992; Bekes et al., 1994; Skerritt et al., 1996).

Despite the fact that the mixograph is one of the most popular instruments to evaluate dough mixing properties, especially in cultivar development activities, there have been no reports on its use for millstream analysis. Accordingly, this study was undertaken to determine the dough rheological properties of flour millstreams using a 2 g computerized mixograph.

3.2. MATERIALS AND METHODS

3.2.1. Materials

Four cultivar samples representing three commercial wheat classes were used in this study: AC Barrie (Canada Western Red Spring wheat), Superb (Canada Western Red Spring wheat), AC Corinne (Canada Western Extra Strong wheat) and AC Snowbird (Canada Hard White Spring wheat). All samples were from the 2001 crop year. The origin of the samples is specified in Chapter 2, Table 2.1.

3.2.2. Milling and quality testing

A description of the milling procedure as well as quality tests performed on the wheat and flour streams is presented in Chapter 2. A flour extraction rate of 80% was used. The mill produced four break flours (B1-B4), one sizing flour (S1), one low quality flour (Q1), one bran flour (BF), six reduction flours (M5-M6), and three by-products, bran, fine bran, and shorts.

3.2.3. Preparation of straight-grade flours

Straight grade flour was prepared (about 9 kg) by combining flour streams in proportion to their milling yield (Table 2.2, Chapter 2). An extraction rate of 77% was used for the preparation of the straight-grade flours. To accomplish this, it was necessary to exclude the M6 flour, which had very low yield (< 1%), and add varying amounts of the M5 fraction. For AC Barrie and AC Corinne it was necessary to exclude M5 flour as well and add appropriate amounts of the BF fraction.

3.2.4. Dough Mixing Experiments

Dough mixing experiments were performed using a 2 g direct-drive Mixograph (National Manufacturing Division, TMCO, Lincoln, NE, USA). Mixing conditions for each sample were as follows: 1.5 g of flour (14% mb), mixer speed of 88 rpm, constant temperature (25°C), and constant water absorption (65%). Constant water absorption provided dough mixing based on constant dough mass for all samples. This allowed direct comparison of the dough mixing properties from the different millstreams and wheat cultivar samples. Constant water absorption of 65% was determined such that all mixograms fit below the 100% torque scale of the mixograph. All mixing experiments were performed at least in duplicate. Data acquisition and analysis of the mixograms was performed using the computer software program, Mixsmart, version 3.73 (Walker and Walker, 1990). All mixing parameters were measured from the mid-line of the mixograms. Four different mixogram parameters were evaluated: mixing time to peak dough resistance (MT, min.), peak dough resistance (PDR, %), bandwidth at peak dough resistance (BWPR, %), and work input to peak dough resistance (WIP, %torque.min). WIP was calculated by integrating the area under the mid-line curve from zero time to PDR. The measure of torque transferred of the mixing bowl was determined by the power consumption of the mixograph. Two mixing treatments were performed: the traditional flour-water experiments, and addition of NaCl at a 2% level (flour weight basis). The latter treatment was included as it accommodated measuring the dough mixing properties using an ingredient commonly used in dough formulations for breadmaking.

3.2.5. Statistical analyses

All statistical analyses were performed on duplicate measurements using the procedures of the SAS (1988) software system version 8.2. Treatment means (among millstreams, and for each millstream without and with 2% salt) were compared using Sheffe's LSD test to determine significant differences. Correlation analyses of the data were performed using Pearson's correlation coefficient analysis.

3.3. RESULTS AND DISCUSSION

3.3.1. Analytical properties of flour millstreams

Detailed analytical results of the millstreams of the eight cultivar samples used in the thesis research are presented and discussed in Chapter 2. Table 3.1 presents the ash and protein contents of the flour streams of the four samples used in this study. The increasing gradient of mineral and protein content from the inner endosperm to the outer

Table 3.1. Ash and protein contents of the millstreams and straight-grade flours (SGF) of the four varieties.

	Superb		AC Barrie		AC Corinne		AC Snowbird	
	Ash[1]	Pro[2]	Ash	Pro	Ash	Pro	Ash	Pro
B1	0.54	14.89	0.49	16.57	0.76	15.95	0.50	15.28
B2	0.45	16.25	0.42	17.63	0.57	17.18	0.43	16.59
B3	0.55	18.65	0.49	20.04	0.70	19.57	0.51	18.87
B4	0.61	19.43	0.49	21.66	0.71	19.86	0.57	18.86
S1	0.41	13.96	0.38	14.67	0.50	14.33	0.38	14.1
Q1	0.66	14.97	0.69	16.24	0.76	15.57	0.66	14.84
BF	2.40	23.56	2.31	26.95	2.74	25.1	2.39	23.34
M1	0.36	12.68	0.32	13.09	0.38	13.28	0.32	12.74
M2	0.43	12.85	0.38	12.83	0.42	13.29	0.37	12.78
M3	0.64	13.45	0.65	13.54	0.59	13.78	0.64	13.36
M4	0.85	14.02	1.00	14.70	0.91	14.68	0.94	14.22
M5	1.74	15.69	1.98	16.58	1.77	16.32	1.96	16.94
M6	2.65	17.99	3.11	19.61	2.81	18.95	3.01	20.06
SGF	0.48	13.76	0.44	14.60	0.50	14.15	0.45	13.93

[1]Ash content (%); [2]Protein content (%). All values corrected to 14% mb.

layers of the kernel was reflected in the millstream results; there was a general increase in ash and protein contents among the break flours, from B1 to B4, and among the reduction flours, from M1 to M6. Ash and protein contents varied depending on the wheat sample, i.e. the total protein and mineral content and their distribution in the whole kernel.

3.3.2. Flour-water dough mixograph parameters of millstreams

Representative mixograms of millstreams of AC Barrie are presented in Fig. 3.1. Mixograph results are summarized in Table 3.2 and shown in Fig. 3.2. The mixograms illustrate a very wide range of dough strength and mixing properties in general among the different flour streams.

There was no apparent trend in the MT values among the flour streams for individual cultivar samples. For example, the average MT between break and reduction flours was similar (5.6 and 6.0 min, respectively). However the different genotypes showed very different MT ranges (Fig. 3.2.A). AC Corinne, the extra strong wheat, had the highest MT values ranging between 7.11 (M5) and 11.41 min (S1). In contrast, AC Barrie had the lowest dough mixing requirements with MTs ranging from 2.92 min (B3) to 6.27 min (M6). Superb and AC Snowbird had intermediate dough mixing requirements. Superb had MTs ranging from 4.08 (B1 and S1) to 5.65 min (M6) and AC Snowbird had MTs ranging from 4.63 (M4) to 9.52 min (BF).

A progressive increase in PDR from B1 to B4 for AC Barrie is evident in Fig. 3.1 and is similar to the trend in results for the other cultivar samples (Table 3.2, Fig. 3.2.B). This increase in PDR from B1 to B4 flour streams averaged 23% among all the samples. The sizing stream S1 and the low quality stream Q1 had lower PDR values

than those of the break flours. Reduction streams on average had considerably lower PDR values than break streams (53.5 and 31.0%, respectively). The first three reduction streams, M1, M2 and M3, showed little variation in PDR values (Fig. 3.1, Table 3.2, Fig. 3.2.B). However, PDR decreased progressively from M3 to M6 for all samples by 48% for AC Barrie, 58% for Superb, 40% for AC Snowbird, and 53% for AC Corinne.

Table 3.2. Mixing parameter values obtained for the four samples.

	Superb				AC Barrie			
	MT[1]	PDR[2]	BWPR[3]	WIP[4]	MT	PDR	BWPR	WIP
B1	4.08	40.8	25.4	81	3.16	39.0	22.0	70
B2	4.44	46.8	28.0	103	3.27	47.7	26.4	88
B3	4.32	56.9	34.7	118	2.92	52.0	29.0	88
B4	4.67	66.5	37.1	153	2.74	65.2	34.7	110
S1	4.08	40.9	23.8	90	3.41	33.9	18.0	68
Q1	4.16	38.9	24.3	81	3.19	33.7	18.9	61
BF	5.05	58.1	38.1	143	3.26	68.3	38.5	124
M1	4.46	40.9	24.2	107	3.99	35.3	18.5	79
M2	4.81	37.9	20.6	104	3.83	30.1	15.9	71
M3	4.80	38.7	22.6	107	3.82	31.6	15.6	78
M4	4.15	37.5	17.9	100	3.74	31.1	14.3	72
M5	5.10	27.1	14.9	99	5.01	22.9	12.0	81
M6	5.65	20.4	10.6	88	6.27	15.3	7.4	76
SGF	4.11	39.3	23.6	91	3.15	36.0	19.7	70
LSD[4]	0.440	3.48	3.09	16.92	0.400	4.27	3.08	14.34
	AC Corinne				**AC Snowbird**			
	MT	**PDR**	**BWPR**	**WIP**	**MT**	**PDR**	**BWPR**	**WIP**
B1	9.17	53.2	34.6	216	5.85	36.2	22.3	123
B2	9.44	58.6	39.8	233	5.89	43.3	27.3	123
B3	9.97	71.7	43.8	267	5.44	50.0	31.8	131
B4	9.46	70.1	42.2	261	5.20	58.3	34.9	156
S1	11.49	46.8	32.2	217	6.00	32.9	19.7	101
Q1	7.78	50.0	31.0	163	4.90	34.9	21.0	94
BF	9.67	75.8	45.8	279	9.52	55.3	39.2	230
M1	10.28	38.4	26.4	198	6.90	31.0	19.3	103
M2	10.56	39.9	26.1	192	6.85	28.7	15.7	104
M3	8.02	43.0	26.8	174	6.64	33.8	16.8	108
M4	6.45	39.5	24.8	133	4.63	29.5	14.4	85
M5	7.11	34.3	19.6	146	6.17	21.2	11.7	95
M6	9.17	22.8	10.5	146	7.20	13.6	6.2	82
SGF	8.58	50.9	32.0	202	5.32	30.3	18.1	93
LSD	1.45	5.16	4.06	29.32	0.92	2.86	3.03	17.65

[1] Mixing Time (min), [2] Peak Dough Development (%), [3] BandWidth at Peak Dough Resistance (%), [4] Work Input to Peak (%torque.min), [4]Least Significant Difference

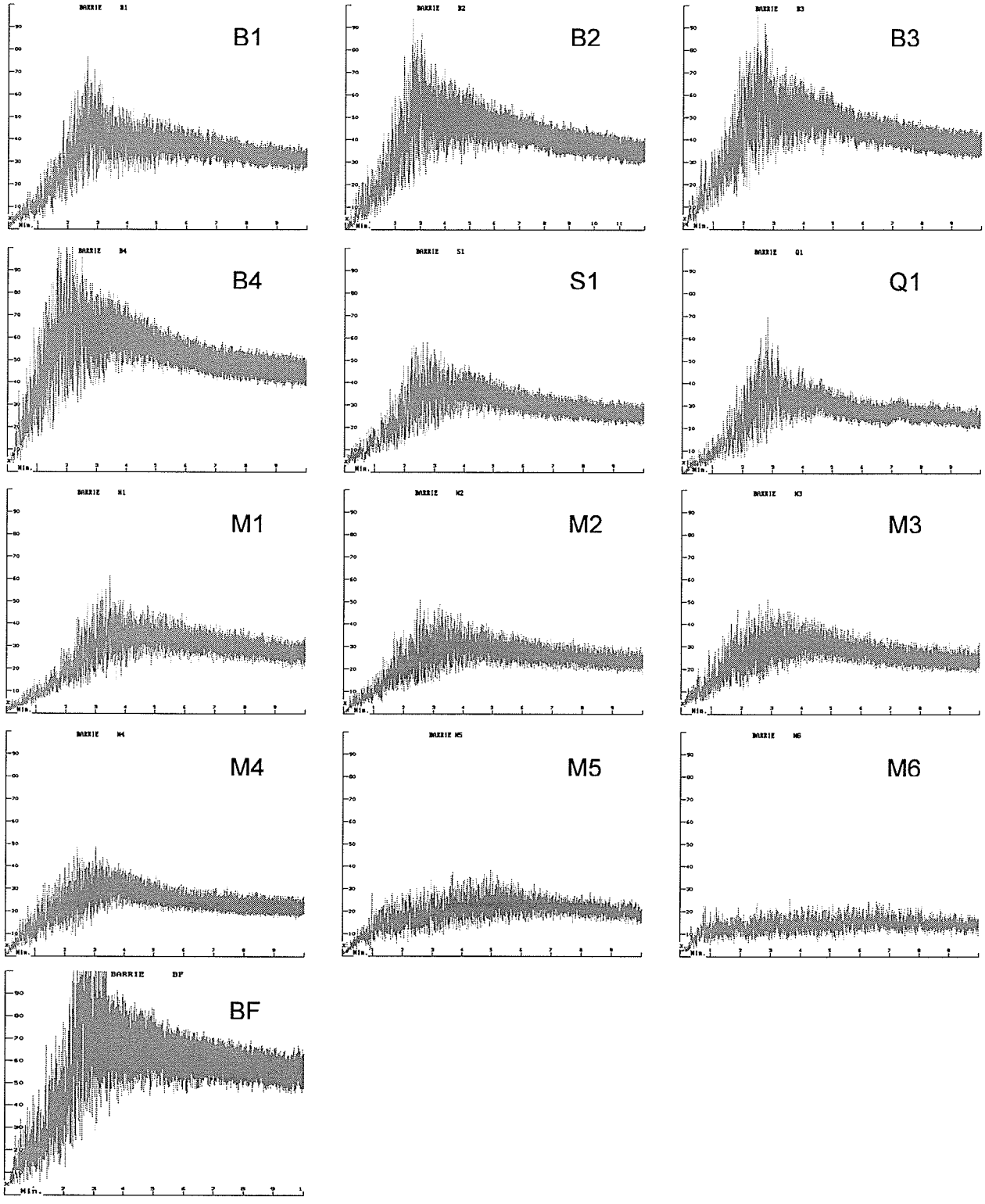
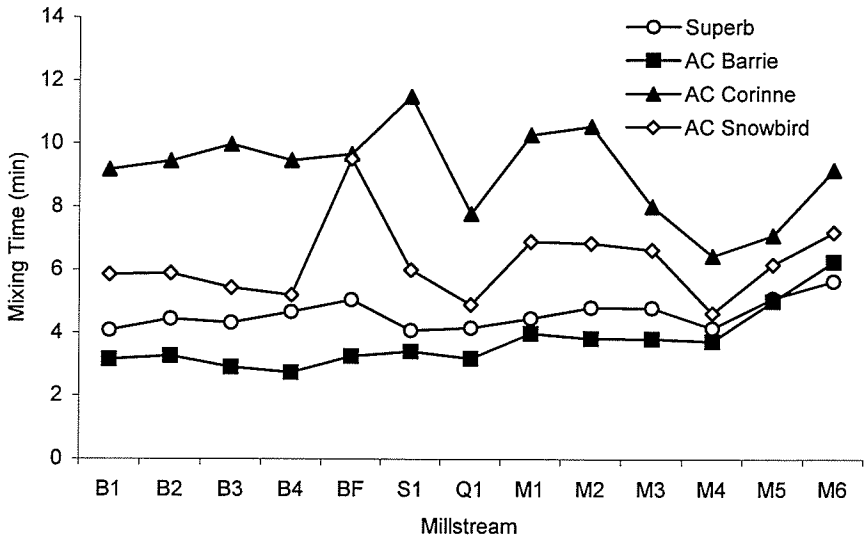
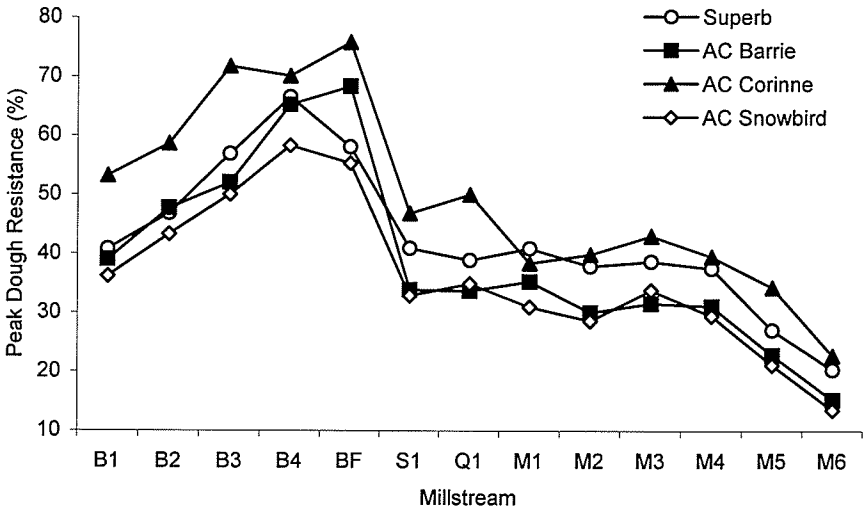


Figure 3.1. Representative mixograms obtained for AC Barrie millstreams.

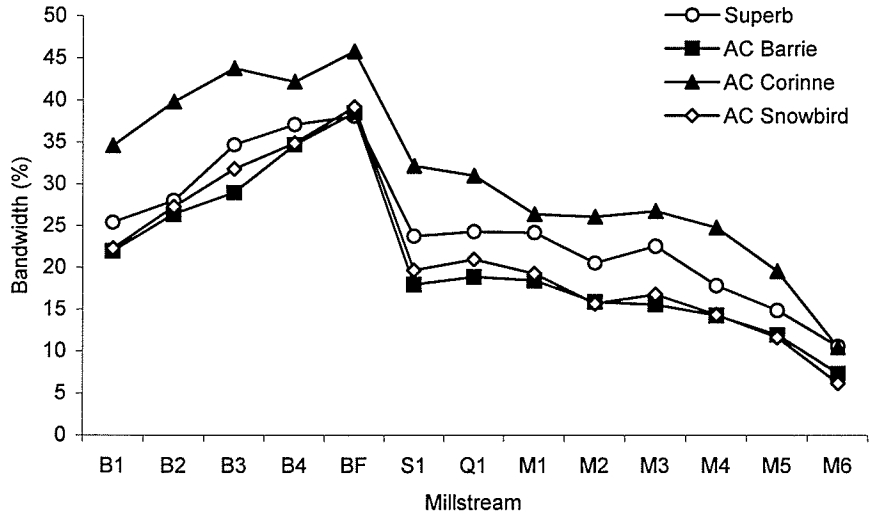
A.


Superb
AC Barrie
AC Corinne
AC Snowbird
Mixing Time (min)
0
2
4
6
8
10
12
14
B1
B2
B3
B4
BF
S1
Q1
M1
M2
M3
M4
M5
M6
Millstream

B.


Superb
AC Barrie
AC Corinne
AC Snowbird
Peak Dough Resistance (%)
10
20
30
40
50
60
70
80
B1
B2
B3
B4
BF
S1
Q1
M1
M2
M3
M4
M5
M6
Millstream

C.



D.

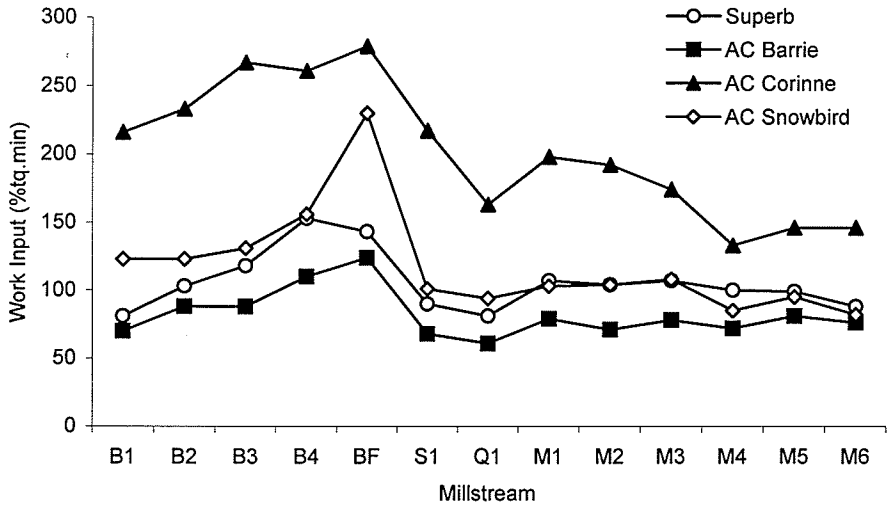


Figure 3.2. Variation in mixograph mixing time (A), peak dough resistance (B), bandwidth at peak dough resistance (C), and work input to peak dough resistance (D) across millstreams of the four samples.

Most noteworthy were the dough mixing results for the last two middling flours, M5 and M6, which gave mixograms that were very flat, showing little dough development, despite higher protein content compare to M1-M3 flours. As will be shown later, these millstreams (M4-M6) contained protein of very different composition compared to early reduction streams and break streams, and the pentosan contents of these M4-M6 millstreams were substantially higher (Wang et al., 2005) as well as their germ content.

For all genotypes, doughs made from BF had high PDR values among the millstreams, ranging from 55.3% for AC Snowbird to 75.8% for AC Corinne. The protein composition of BF, as will be discussed in Chapter 4, was different than that of all other streams. Finally, PDR values of the straight-grade flours were the highest for AC Corinne, followed by Superb, AC Barrie and AC Snowbird. Again, this variation in PDR values appears to reflect correlated variation in the concentration of gluten protein in the millstreams.

BWPR represents the bandwidth at peak dough resistance, and is easily interpreted from the mixograms. BWPR values followed precisely the same trend as for PDR (Table 3.2, Fig. 3.2.C). High PDR and high BWPR values have been associated with good dough strength and good breadmaking quality (Khatkar et al., 1996). Accordingly, among all the millstreams, BF possessed the strongest dough rheological properties, followed by the break flours and B4 flours in particular, and then by the reduction flours, which were the weakest on average. As expected, AC Corinne, the extra strong wheat possessed flour streams with the strongest dough properties among the four cultivar samples.

WIP values followed a similar trend to PDR and BWPR results (Table 3.2, Fig. 3.2.D). In general, WIP increased from B1 to B4. WIP for S1 and Q1 were lower on average than those for the break streams. The reduction streams had the lowest WIP values (on average) among the millstreams; however, there was no increasing or decreasing trend for WIP among the reduction flours. AC Barrie and AC Corinne had the lowest and highest WIP values, respectively.

From a subjective perspective, millstreams of different cultivar samples varied widely in dough appearance and feel. The break flours typically gave stronger and more elastic doughs than the reduction flours, while BF possessed superior dough physical properties. For M5 and especially M6 flours, it is noteworthy that after mixing, the "doughs" appeared wet, were not cohesive and appeared not to have been developed by the mixing action of the pins. Because of the flat mixograms obtained for these two millstreams, it was difficult to assess the behaviour of the dough during mixing.

3.3.3. Role of ash and protein contents in mixograph parameters

A correlation matrix of mixograph parameters, ash, and protein contents for all millstreams, with and without M5 and M6 data, is presented in Table 3.3. Among the mixograph parameters, PDR and BWPR were well correlated with WIP ($r = 0.83$ for both, averaged over all samples); as well, PDR was highly correlated with BWPR ($r = 0.98$, averaged over all samples). MT was not correlated with the other mixing parameters except when all samples are combined. In general, ash content was not significantly correlated with any of the mixing parameters. A negative relationship between ash content and PDR and BWPR was evident when M5 and M6 were included

Table 3.3. Correlation coefficients among mixograph parameters and flour quality parameters.

	All						All (without M5 and M6)					
	Ash[1]	**Pro[2]**	**MT**	**PDR**	**BW[3]**	**WIP**	**Ash**	**Pro**	**MT**	**PDR**	**BW**	**WIP**
Ash	1.0						1.0					
Pro	0.62**	1.0					0.74**	1.0				
MT	0.20	0.06	1.0				0.16	0.28	1.0			
PDR	-0.20	0.55**	0.17	1.0			0.49**	0.84**	0.29	1.0		
BW	-0.19	0.52**	0.30	0.97**	1.0		0.49**	0.79**	0.46*	0.96**	1.0	
WIP	0.08	0.35*	0.83**	0.66**	0.74**	1.0	0.38	0.42*	0.88**	0.69**	0.79**	1.0
	Superb						**Superb (without M5 and M6)**					
	Ash	**Pro**	**MT**	**PDR**	**BW**	**WIP**	**Ash**	**Pro**	**MT**	**PDR**	**BW**	**WIP**
Ash	1.0						1.0					
Pro	0.62	1.0					0.76*	1.0				
MT	0.80**	0.40	1.0				0.54	0.44	1.0			
PDR	-0.30	0.53	-0.32	1.0			0.39	0.86**	0.39	1.0		
BW	-0.23	0.59	-0.30	0.97**	1.0		0.50	0.92**	0.41	0.95**	1.0	
WIP	0.13	0.66	0.24	0.79*	0.73*	1.0	0.51	0.75*	0.71	0.87**	0.78*	1.0
	AC Barrie						**AC Barrie (without M5 and M6)**					
	Ash	**Pro**	**MT**	**PDR**	**BW**	**WIP**	**Ash**	**Pro**	**MT**	**PDR**	**BW**	**WIP**
Ash	1.0						1.0					
Pro	0.52	1.0					0.71	1.0				
MT	0.73*	-0.14	1.0				-0.08	-0.69	1.0			
PDR	-0.22	0.69*	-0.75*	1.0			0.52	0.95**	-0.70	1.0		
BW	-0.21	0.71*	-0.75*	0.99**	1.0		0.52	0.96**	-0.73	0.99**	1.0	
WIP	0.28	0.84**	-0.25	0.81**	0.80**	1.0	0.63	0.87**	-0.42	0.94**	0.91**	1.0
	AC Corinne						**AC Corinne (without M5 and M6)**					
	Ash	**Pro**	**MT**	**PDR**	**BW**	**WIP**	**Ash**	**Pro**	**MT**	**PDR**	**BW**	**WIP**
Ash	1.0						1.0					
Pro	0.69*	1.0					0.92*	1.0				
MT	-0.49	0.08	1.0				-0.10	0.08	1.0			
PDR	-0.09	0.64	0.27	1.0			0.59	0.94**	0.18	1.0		
BW	-0.24	0.51	0.34	0.98**	1.0		0.53	0.90**	0.29	0.98**	1.0	
WIP	-0.07	0.61	0.62	0.90**	0.90**	1.0	0.43	0.79*	0.60	0.87**	0.91**	1.0
	AC Snowbird						**AC Snowbird (without M5 and M6)**					
	Ash	**Pro**	**MT**	**PDR**	**BW**	**WIP**	**Ash**	**Pro**	**MT**	**PDR**	**BW**	**WIP**
Ash	1.0						1.0					
Pro	0.69*	1.0					0.74*	1.0				
MT	0.55	0.46	1.0				0.67	0.42	1.0			
PDR	-0.31	0.42	0.05	1.0			0.46	0.91**	0.23	1.0		
BW	-0.21	0.50	0.18	0.98**	1.0		0.54	0.95**	0.37	0.97**	1.0	
WIP	0.18	0.69*	0.58	0.81**	0.87**	1.0	0.79*	0.92**	0.71	0.82*	0.88**	1.0

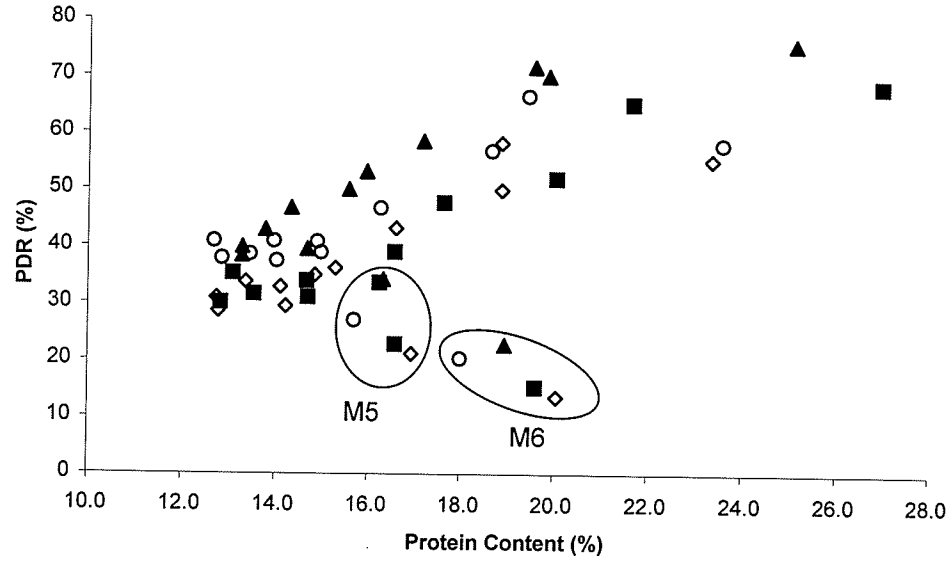
[1]Ash (%), [2]Protein (%), [3]BWPR. Ash and protein values corrected to 14% mb.
**$p < 0.001$, *$p < 0.01$

in the correlation matrices, but it became positive when M5 and M6 were excluded, although the relationship was not significant for individual samples.

In contrast, protein content was less well correlated with PDR (r = 0.57) and BWPR (r = 0.58). The correlation increased considerably when M5 and M6 streams were excluded. Protein content was not correlated with MT and it was unclear which chemical factor in flour could be related to dough development time in the mixograph. These findings are in agreement with those of Khan et al. (1989), who found that protein content had no relationship to mixing time. The direct relationship between protein content and PDR has also been demonstrated in previous studies (Finney and Shogren, 1972; Lang et al., 1992). Figures 3.3.A and 3.3.B show the relationship between PDR and protein content, and between BWPR and protein content, respectively. These charts show several outliers. These outliers correspond to M5 and M6 millstreams. It is noteworthy that each cultivar sample possessed a distinct relationship. Thus, except for M5 and M6, dough strength increased as protein content increased. This relationship was most evident for the break streams (Table 3.2 and 3.3); the increase in protein content was accompanied by a corresponding increase in PDR and BWPR. Next to BF, the break flours on average produced the strongest doughs. Break flours also had higher protein contents than the reduction streams. However, the relationship between dough strength and protein content was less clear for the reduction flours. Although protein content increased from M1 to M4, the PDR and BWPR values showed little variation.

Previous work has shown that although protein content is highly correlated with loaf volume (Finney and Barmore, 1948; Bushuk et al., 1969; Khan et al., 1989), it is the protein composition that is responsible for the dough mixing properties of the flour (Orth

A.



B.

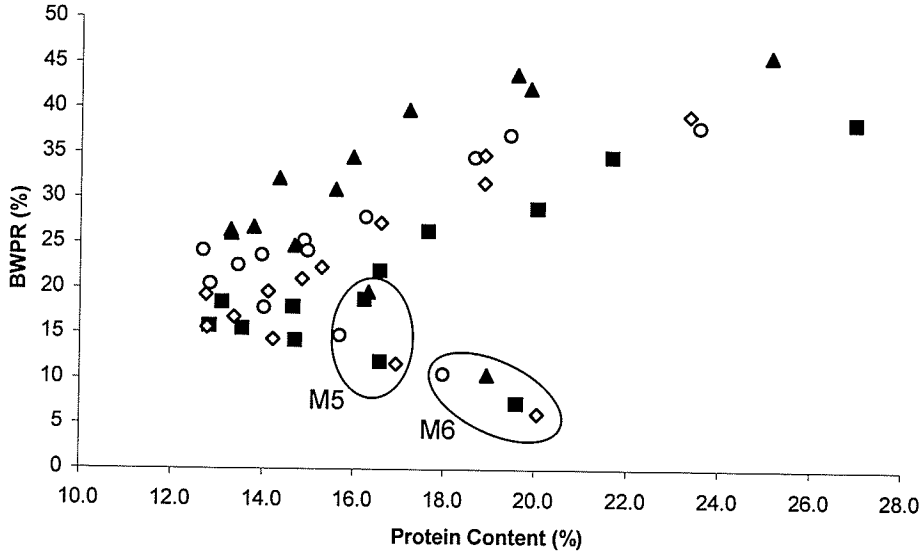


Figure 3.3. Relationship between millstream protein content and peak dough resistance (PDR) (A) and between millstream protein content and bandwidth at peak dough resistance (BWPR) (B) for Superb (o), AC Barrie (■), AC Corinne (▲), and AC Snowbird (◊). Outliers (circled) correspond to M5 and M6 flours.

and Bushuk, 1972; Khan et al., 1989; Dong et al., 1992; Roels et al., 1993; Weegels et al., 1995a; Skerritt et al., 1996); glutenin protein fractions strengthen the doughs (Orth and Bushuk, 1972; Gupta et al., 1992; Roels et al., 1993; Sapirstein and Fu, 1998; Uthayakumaran et al., 1999; Edwards et al., 2003), while gliadin protein fraction (Skerritt et al., 1996; Uthayakumaran et al., 2001; Edwards et al., 2003) and low Mr non-gluten proteins (Weegels et al., 1995b) weaken the rheological properties of the dough. Accordingly, protein composition of the reduction streams likely had a much greater impact on the dough rheological properties than protein content, and it appeared that the millstreams varied quite widely in protein composition (as will be shown later in Chapter 4). This was particularly evident for the last two reduction streams, M5 and M6, which had very poor dough properties despite a high protein content. In addition, these two streams possessed high ash content reflecting a high level of bran contamination, and especially high levels of pentosans, as was shown by Wang et al. (2005). They studied pentosan content and composition of millstreams using the same samples as in the present study and found that M5 and M6 streams possessed high levels of total pentosans (5.0 and 6.4%, respectively), water extractable pentosans (WEP) (1.0 and 0.9%, respectively), and low levels of WEP/WUP (water unextractable pentosans) (0.26 and 0.17%, respectively), hence high levels of WUP, compared to that of other streams. Pentosans can absorb up to 10 times their weight of water (Jelaca and Hlynka, 1971), and both WSP (Jelaca and Hlynka, 1971) and WUP (Jelaca and Hlynka, 1971; Michniewicz et al., 1990) contribute to the water absorption of wheat flour. On the contrary, proteins can absorb water only about twice their weight. It has been shown that the high water-binding capacity of pentosans can have large effects on dough properties. Jelaca and Hlynka (1971) added pentosans to dough and found an increase in

farinograph DDT from 2 to 3.5 min compared to the control. However, WIP requirements, as measured by the GRL mixer, decreased. Effect of added pentosans was shown clearly by the marked increase in dough consistency. Courtin and Delcour (2002) found that WEP increase the stability of the dough foam structure whereas WUP, which are present in discrete cell wall fragments, can form physical barriers for the gluten network during dough development, thus lowering the stability of the dough structure. However, Wang et al. (2002) reported that WEP interfered with gluten formation both in a direct and an indirect manner. Due to their nature, WEP interfere indirectly by competing for water and thus changing conditions for gluten development. Also, arabinoxylan-bound ferulic acid may be involved in the direct effect of WEP on gluten formation, by linking to gluten proteins. In the present study, the dough consistency of M5 and M6 was almost non-existent. For these millstreams, competition for water binding probably occurred between pentosans and gluten proteins, restraining the proteins from having access to the water and thus restricting the development of the gluten network. However, all of this indicates that while the pentosan effect is likely significant, the main reason for the inferior mixing properties of M5 and M6 is most likely protein based, with lower percentages of insoluble glutenin and much higher proportion of non-gluten forming RP, as will be shown in Chapter 4.

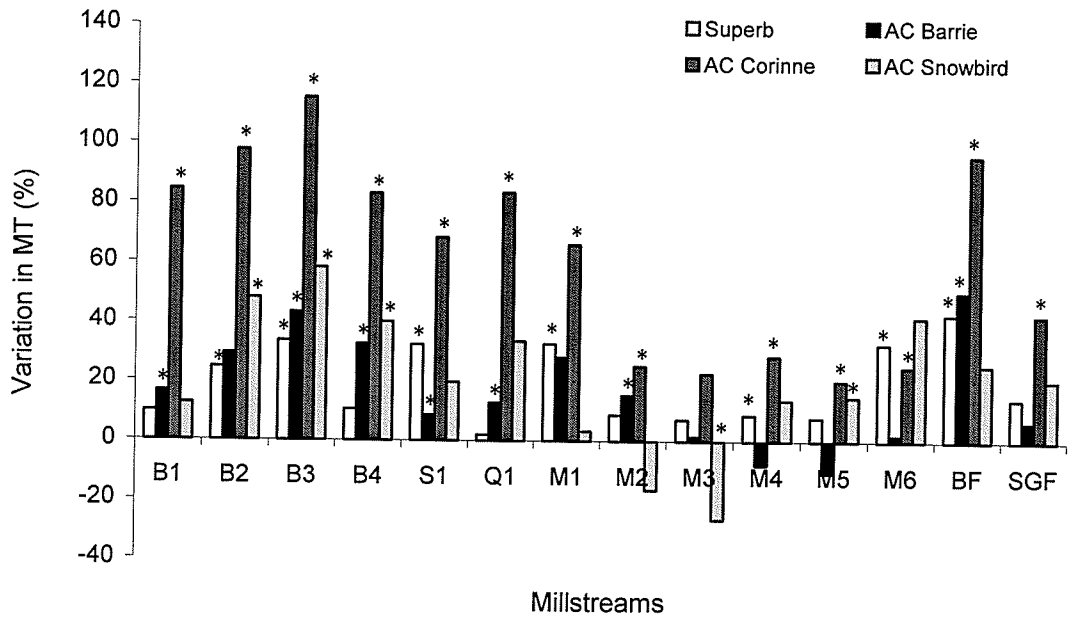
Contrary to M5 and M6, BF exhibited very strong dough properties, with high PDR and high BWPR, despite a similar ash content and similar total pentosan content and water extractable pentosan to water unextractable pentosan ratio (4.8 and 0.16%, respectively) (Wang et al., 2005). This confirms that protein composition most likely has the key role in dough mixing properties, and unlike that of M5 and M6 flours, the protein composition of BF was high in insoluble glutenin content (Chapter 4).

3.3.4. Effect of salt on dough mixing properties of the millstreams

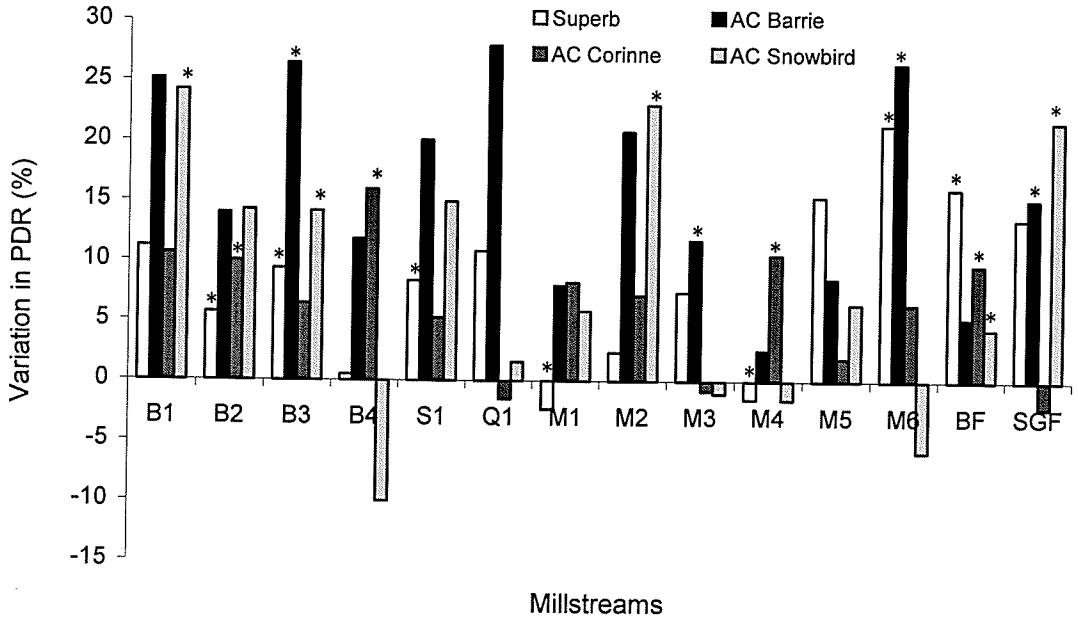
Dough rheological properties are sensitive to ionic interaction of salts with gluten proteins (Hlynka, 1962; Tanaka and Tipples, 1969; Danno and Hoseney, 1982; Kim and Bushuk, 1995). The tendency of salts to strengthen dough properties is attributed to electrostatic shielding of ionic amino acids on the surface of the gluten proteins. This shielding effect reduces electrostatic repulsion between individual gluten proteins, which are positively charged (Yoshino and Matsumoto, 1966). This results in stronger inter-protein hydrophobic and hydrophilic interactions and consequently increased aggregation (Bernardin, 1978; Preston, 1981).

In this study, salt addition significantly affected all mixing parameters for essentially all the millstreams. Except for a few millstreams, salt addition caused a significant increase in MT (Figure 3.4.A). However, the effect was very variable depending on millstream and cultivar sample. For the break streams, the strengthening effect of salt on MT was progressively greater from B1 to B3, and then decreased for B4. Similar to the break streams, salt had a large positive effect on MT for BF. For the reduction streams, the effect of salt was less clear; in general, MT decreased from M1 to M3. It was interesting that for AC Snowbird and AC Barrie, millstreams M2 and M3, and M4 and M5, respectively, responded negatively to the addition of salt. No explanation can be offered for this result, however, the difference in MT between doughs without and with 2% salt for these streams was not significant. The increased dough mixing time to peak with the presence of salt is likely a consequence of the increased protein aggregation that occurs, as explained previously. The most noteworthy effect of salt on dough MT was observed for AC Corinne, for which the effect of salt was significant for all streams but M4. Huebner

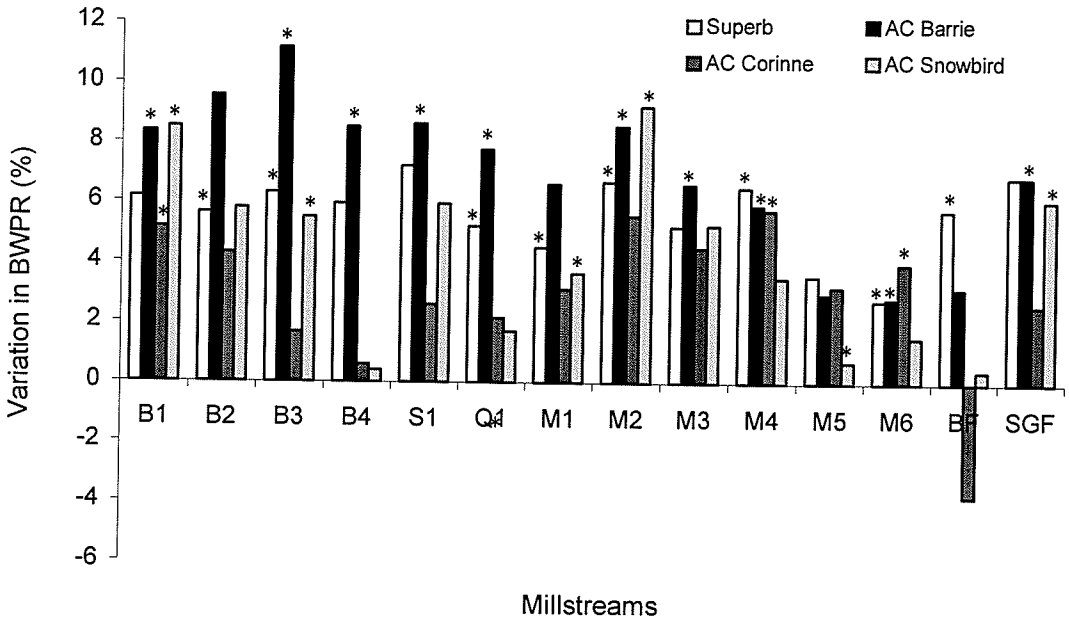
A.


Superb
AC Barrie
AC Corinne
AC Snowbird
Variation in MT (%)
140
120
100
80
60
40
20
0
-20
-40
B1
B2
B3
B4
S1
Q1
M1
M2
M3
M4
M5
M6
BF
SGF
Millstreams

B.


Superb
AC Barrie
AC Corinne
AC Snowbird
Variation in PDR (%)
30
25
20
15
10
5
0
-5
-10
-15
B1
B2
B3
B4
S1
Q1
M1
M2
M3
M4
M5
M6
BF
SGF
Millstreams

C.



D.

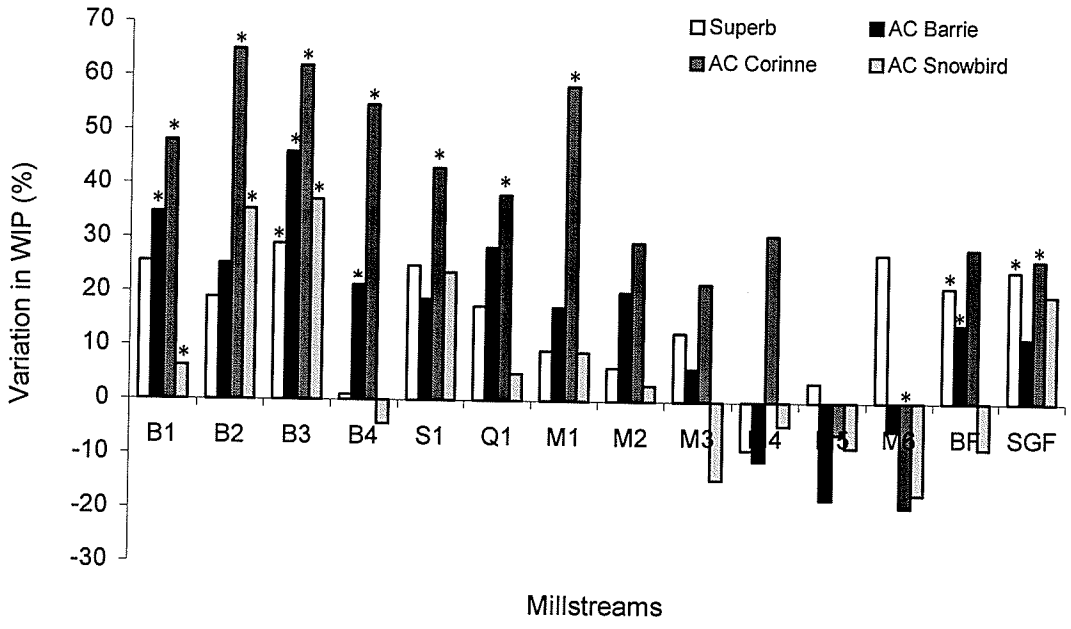


Figure 3.4. Effect of 2% salt on mixograph dough mix time (MT) (A), on mixograph peak dough resistance (PDR) (B), on mixograph bandwidth to peak resistance (BWPR) (C), and on mixograph work input to peak (WIP), as expressed as the percentage difference compared to control doughs without salt. Bars marked with (*) indicate a significant difference between no salt and 2% salt doughs.

(1970) observed that within wheat proteins, glutenin is more affected by salt than gliadins and that glutenins of wheat cultivars of higher breadmaking potential (hard wheats) were more sensitive to salt than those of lesser quality for breadmaking (soft wheats). Kim and Bushuk (1995) further studied salt sensitivity of glutenins by comparing the effects of salt on two cultivars, Glenlea, a CWES wheat, and Katepwa, a CWRS wheat substantially weaker than Glenlea. They found that the combination of molecular weight (M_r) and subunit composition of glutenin and the relative proportion of HMW glutenin subunits were the main factors influencing NaCl sensitivity. Also, protein of Glenlea was more sensitive to salt than Katepwa because the former contained polymeric glutenin molecules with higher structural features than the latter. In the present study, AC Corinne, a CWES similar to Glenlea (Kim and Bushuk, 1995), was greater than that of the other cultivar samples, due to its substantially higher concentration of HMW glutenin subunits, as will be shown in Chapter 4.

Addition of salt also increased PDR and BWPR values (Figures 4.3.B and 4.3.C), indicating increased dough strength compared to untreated samples. The extent of the effect of salt on PDR and on BWPR, however, showed no pattern among samples, but seemed to have a greater effect on AC Barrie. Interestingly, PDR and BWPR for millstreams of AC Corinne were less affected by salt than MT.

WIP was affected by salt addition similarly to MT (Figure 4.3.D). Nevertheless, in some cases, i.e. the tail-end reduction streams for some samples, WIP had lower values with salt than for the untreated samples. A greater effect was observed for AC Corinne, similar to MT. This was likely due to the higher concentration of HMW glutenin subunits in AC Corinne, as opposed to the other samples, as explained previously.

Overall, the addition of salt increased the magnitude of all the mixograph parameter values, reflecting an overall increase in dough strength, in general agreement with many earlier findings (Hlynka, 1962; Bakhoum and Ponte Jr., 1982; Lang et al., 1992; Butow et al., 2002). Yet, the effect of salt on specific millstreams was very variable. As mentioned earlier, the gluten sensitivity to salt is thought to be due to conformational differences in tertiary protein structure (Galal et al., 1978; Kim and Bushuk, 1995). Kim and Bushuk (1995) found that the combination of M_r and subunit composition of glutenin and the relative proportion of HMW glutenin subunits were the main factors influencing NaCl sensitivity. Also, amino acid composition of HMW and LMW glutenin subunits differ among cultivars, and polymeric glutenin molecules with different M_r also differ in their conformations and charge densities, and consequently sensitivities to salt. These factors can explain differences in salt sensitivity not only among cultivar samples, but also among millstreams, suggesting that millstreams have different protein composition, most notably in the proportions of HMW glutenin.

3.4. CONCLUSIONS

Part of the rationale for using the mixograph is that there have been no reports on its use for millstream analysis, despite its popularity to evaluate dough mixing properties of straight grade flours. The 2 g mixograph was a very useful tool as it can accommodate small sample size.

The wide range of protein content and refinement provided by the millstreams resulted in large differences in dough mixing properties. Flour refinement as measured by ash content was not correlated with any of the mixing parameters. MT was not correlated to protein content, and showed no consistent patterns of correlation among

millstreams of individual samples, but MT values were in different ranges for the different genotypes. The CWES sample, AC Corinne, had the longest MT across millstreams, while AC Barrie had the shortest. This indicated that although MT should be related to glutenin molecular size, it is not a reliable parameter in dough strength evaluation of millstreams, when the mixograph is used at constant water absorption. In contrast, PDR, BWPR and WIP to a lesser extent were found to be effective measures of dough strength. High PDR and BWPR values have been associated with good dough strength and good breadmaking quality. For break flours, increasing protein content from B1 to B4 was accompanied by a corresponding increase in PDR, BWPR, and WIP. For reduction streams for which protein content content increased by an even greater amount form M1 to M6, the correspondence between PDR and BWPR and protein content was not clear. This was especially evident for the last two reduction streams M5 and M6, which possessed very high ash (and pentosan) content and high protein content, and produced very weak doughs. It seemed that gluten in M5 and M6 did not develop more likely because of their poor protein quality, rather than because of the detrimental effect of their high pentosan content on gluten development. On the contrary, BF had strong dough properties despite their high pentosan content, indicating that dough mixing properties of BF are likely due to its good protein quality.

Addition of salt generally increased all four mixograph parameters compared to untreated doughs, although the intensity of the effect on specific millstreams was variable among samples. Compared to other cultivar samples, salt had a greater strengthening effect on AC Corinne, the CWES sample. The sensitivity of dough rheological properties to salt appears to be due to ionic interaction of salts with gluten proteins, which result in stronger inter-protein hydrophobic and hydrophilic interactions

and consequently increased aggregation. Also, among the various protein fractions of wheat, glutenin was most sensitive to salt effects. The distinct strengthening effect of salt on AC Corinne doughs could therefore be attributed to a higher concentration of glutenin.

Chapter 4. Variation in Gliadin, Glutenin, and Protein of Bran Origin in Wheat Millstreams

ABSTRACT

The heterogeneous composition of the wheat kernel results in flour millstreams of varying refinement, chemical composition and hence functional quality for breadmaking. Some aspects of millstream variation sometimes in relation to breadmaking functionality such as colour, mineral content (ash), protein content, and pentosan content and composition, are relatively well studied. However, surprisingly, little is known how protein composition varies, and in particular gluten protein fractions, gliadin and glutenin. Eight Canadian hard spring wheat samples of six cultivars were milled on a tandem Buhler laboratory mill to 80% extraction. Thirteen flour streams and three millfeed fractions (coarse bran, fine bran, and shorts) were collected. Additionally, four cultivar samples were processed by abrasion into six fractions that represent different layers of the kernel. Protein was fractionated using a propanol-based method, without and with reducing agent, into soluble protein (SP, mainly gliadins), insoluble glutenin (IG, mainly HMW glutenin), and residue protein (RP). The quantity of the latter was found to vary inversely with the level of flour refinement and so mainly contains protein of bran origin. Both millstreams and pearled wheat fractions showed the same pattern of variation in protein composition corresponding to levels of refinement. Averaged across cultivar samples, the range of concentration of gluten protein fractions (expressed as a percentage of millstream protein fraction) SP and IG varied approximately by 85% from 38 to 69% and 12 to 22%, respectively, and was positively correlated with flour refinement. In contrast, RP varied by about 330% across

millstreams, from 11 to 47%, and was negatively correlated with flour refinement. Break streams, on average, had higher concentration of SP (65%) and IG (21%) compared to reduction streams (55% SP and 17% IG). In contrast, break streams had lower levels of RP (14%) compared to reduction streams (25% RP). Among break streams, there was no clear differentiation of any of the three protein fractions across cultivar samples. In contrast, a clear pattern of variation was evident for reduction streams; the latter reduction streams (M4 to M6) had progressively increasing concentration of gluten protein and decreasing concentration of residue protein. Reversed-phase HPLC of reduced glutenin subunits of selected millstreams of widely varying protein content and refinement indicated no differential expression of subunit concentration, i.e. glutenin composition of M1 flour was identical to that of B3 and BF flours. Knowledge of millstream protein composition, particularly IG and RP fractions, appears to be very beneficial to gain a more complete understanding of flour breadmaking quality, as well as to guide stream selection in blending for different products.

4.1. INTRODUCTION

Variation in the end-use quality of different samples or parcels of wheat for breadmaking is commonly explained by differences in genotype and/or growing environment, both of which can affect the concentration or composition of several functionally important constituents of wheat, most notably the protein component. A third source of quality variation derives from the processing of wheat into flour, i.e. milling, which is the key value-added step in the conversion of wheat into baked goods. The nature of this variation derives from the heterogeneous structural and chemical composition of wheat kernels (MacMasters et al., 1971). An often cited reflection of this

heterogeneity is the increasing gradient of protein and mineral (ash) content in wheat from the inner endosperm to the outer bran layers that has been well established from analysis of hand-dissected kernel tissues (Morris et al., 1945, 1946; Hinton, 1947, 1959, 1962; Stevens et al., 1963; Kent, 1966; Kent and Evers, 1969). The milling industry over time, has adapted well to this heterogeneity by adjusting the degree of flour extraction and the extent to which different millstreams of the same grist can be combined to produce different flour blends with different qualities to accommodate requirements of the baking industry.

Many workers have studied the technological quality characteristics of different millstreams (Nelson and McDonald, 1977; Holas and Tipples, 1978; Preston et al., 1982; Endo et al., 1987; Preston and Dexter, 1994; Prabhasankar et al., 2000) or flours of different extraction rates (Orth and Mander, 1975). Taken together, these studies point to several important findings: viz. flour protein content increases with successive break roll passages, and is higher in break flours than in reduction flours; farinograph absorption increases while development time decreases with successive break and reduction roll passages; break flours had larger extensograph areas than reduction flours; as flour extraction increases from 66-82% (Orth and Mander 1975), farinograph absorption increases and dough strength progressively decreases, while loaf volume can be optimized at an intermediate extraction rate.

By comparison, the chemical composition of wheat millstreams has been investigated to a much lesser extent but those studies include comprehensive reports dealing with lipids (Morrison et al., 1982; Morrison and Hargin, 1981), α-amylase (Kruger, 1981), phenolic acids (Beta et al., 2005), and pentosans (Delcour et al., 1999; Wang et al., 2005). In contrast, few reports have been published detailing how protein

composition varies in different millstreams, or how that variation relates to end-use quality. What little information is available is contradictory. Orth et al. (1976) milled a hard red spring bread wheat on a Buhler laboratory mill and found a significant difference in Osborne protein fractions between break and reduction roll flours; break flours contributed substantially more (83%) to gluten formation compared to reduction roll flours (69%). In contrast, Nelson and McDonald (1977) found little or no significant difference (depending on genotype) in quantity of gliadin and glutenin protein within and among selected break and reduction roll flours for two HRS wheats whose protein was fractionated by size-exclusion chromatography of total protein extracts.

In light of the available literature and common breadmaking practice, it is generally understood that significant if not substantial differences in flour millstream quality exist. The goal of this study was to determine whether these differences have their nature in either protein content and/or protein composition differences of millstreams. Accordingly, this study had two main objectives: to determine the protein composition of flour millstreams obtained by extensive stock separation on an experimental mill; to evaluate the relationships between the protein composition of millstreams and dough mixing properties. As well, protein composition of roller milling streams was compared to the protein composition of pearled wheat fractions.

4.2. MATERIALS AND METHODS

4.2.1. Materials

Wheat comprised eight cultivar samples comprising six genotypes of four commercial classes representing a wide range of intrinsic qualities for breadmaking: AC Barrie (Canada Western Red Spring wheat), 2 samples of Superb (Canada Western Red

Spring wheat), AC Corinne (Canada Western Extra Strong wheat), 2 samples of AC Snowbird (Canada Hard White Spring wheat), AC Vista (Canada Prairie Spring White), and AC Crystal (Canada Prairie Spring Red). All samples were grown in the 2001 crop year, and were of sound milling grade. The origin of the samples is specified in Chapter 2, Table 2.1.

4.2.2. Milling

A description of the milling procedure as well as quality tests performed on the wheat and flour streams is presented in Chapter 2. A flour extraction rate of 80% was used. The mill produced four break flours (B1-B4), one sizing flour (S1), one low quality flour (Q1), one bran flour (BF), six reduction flours (M5-M6), and three by-products, bran, fine bran, and shorts.

4.2.3. Pearling

Four cultivar samples were selected for further investigation of protein composition of wheat fractions: Superb, AC Barrie, AC Corinne, and AC Snowbird. For each sample, 3 kg of wheat were pearled on a barley pearler (Type TM, Satake, Hiroshima, Japan) in order to obtain fractions of progressively increasing refinement. Each consecutive passage removed 10% of the wheat kernels by weight. As a result, 6 separate fractions were obtained and named thereafter 10%, 20%, 30%, 40%, 50%, and 60%.

4.2.4. Protein fractionation procedure

Protein composition of the millstreams was determined according to the propanol-based protein fractionation procedure described by Sapirstein and Johnson (2001). This method was developed on the basis that the traditional Osborne (1907) or modified Osborne (Chen and Bushuk, 1970) approach using water and salt and salt and water, respectively, as the initial solvents for non-gluten protein extraction causes a substantial proportion of gliadins to become unextractable by 70% ethanol. The gliadin is then extracted along with glutenin protein fraction. This was confirmed by Dupuis et al. (1996) and Sapirstein and Fu (2000).

The protein fractionation method of Sapirstein and Johnson (2000) allows the quantification of soluble protein (SP, mainly gliadins), insoluble glutenin (IG), and residue protein (RP) by difference.

4.2.4.1. Soluble protein extraction

To extract the soluble (monomeric) protein (SP), 50 mg of flour was suspended in 1 mL of 50% (v/v) 1-propanol in a 1.5 mL centrifuge tube. The suspension was allowed to rest for 30 min at room temperature (23°C) with intermittent vortexing (every 10 min for 5 sec). The mixture was then centrifuged for 3 min at 2,200 *g* in a table top centrifuge (Biofuge A, Heraeus-Christ). The supernatant was then decanted in a 2 mL microfuge tube. A second extraction was performed by adding 1 mL of 50% 1-propanol to the pellet. The dense starch-concentrated pellet was suspended with a microspatula, and the suspension was allowed to rest for 30 min at room temperature with intermittent vortexing, and then centrifuged for 3 min at 15,000 *g*. The supernatant was combined with that from the first extraction. Any liquid remaining in the centrifuge tube was

removed using a Pasteur pipette. The 2 mL microcentrifuge tube containing the pooled supernatants was vortexed and inverted for 10 sec to assure homogenization. The SP extract was then diluted 100-fold with 50% 1-propanol (i.e. 10 μL SP, 990 μL 50% 1-propanol) for analysis by spectrophotometry. The SP fraction was previously (Fu and Sapirstein, 1996) shown to contain total monomeric protein (mainly gliadins) and a small proportion (about 12%) of soluble LMW glutenin.

4.2.4.2. Insoluble glutenin extraction

The insoluble residue was reduced with 1 mL of a solution containing 0.1% (w/v) dithiothreitol (DTT) in 50% 1-propanol. This concentration of DTT was found to be sufficient to solubilize all the "insoluble" or HMW glutenin unextractable by 50% 1-propanol alone (Sapirstein and Johnson, 2000). The pellet was suspended in the reducing solution with a microspatula. The tubes were placed in a heating block at 55°C, vortexed after 5 min to homogenize the suspension, and returned to the heater for 30 min. The tubes were vortexed intermittently every 10 min. Subsequently, the mixture was centrifuged for 3 min at 15,000 *g*. The microcentrifuge tubes were then inverted once to obtain a homogeneous supernatant, and placed in a rack at room temperature. Dilutions were done within 20 to 30 min after extraction, because the partially reduced glutenin tended to re-aggregate and precipitate if left for longer time. An aliquot of the supernatant was diluted 100-fold in a 1.5 mL microcentrifuge tube for spectrophotometric analysis.

4.2.4.3. Spectrophotometric analysis

UV absorbance measurements were done at a wavelength of 214 nm. This shorter wavelength, as opposed to 280 nm which is often used for proteins absorbance measurements has two advantages: 1) considerably greater sensitivity arising from peptide bond absorbtivity, and 2) smaller variation in absorbtivity across protein samples stemming from composition differences in gluten proteins (e.g. gliadin to glutenin ratio) affecting amino acid composition (Wetlaufer, 1962). An aliquot of 50% 1-propanol solution and an aliquot of the 0.1% DTT in 50% 1-propanol solution were used as the blanks for absorbance measurements of the SP and IG fractions, respectively. RP concentration was calculated from the difference between flour protein and the sum of the concentrations of SP and IG.

4.2.5. Reversed-phase HPLC of reduced 50% 1-propanol-insoluble glutenin

Reversed-phase HPLC was conducted in order to determine whether there was a change in the subunit composition of the IG fraction among millstreams, specifically the ratio of HMW to LMW-GS, and/or differential expression of subunits was examined.

The method used to prepare samples of reduced glutenin subunits of millstreams for RP-HPLC analysis followed the procedure described by Fu and Sapirstein (1996). Samples were analyzed by RP-HPLC using an Agilent model 1100M liquid chromatograph incorporating a binary solvent delivery system, autosampler, vacuum degasser, heated column compartment maintained at 60°C for analyses, and diode array detector incorporating a 6-mm path length, and 1.7 μL microflow cell. A Zorbax 300 SB-C8 Narrow Bore RR column (Agilent Technologies, Inc.) was used (300 Å pore size, 3.5 μm particle size, 10 cm × 2.1 mm i.d.) in conjunction with a Zorbax 300 SB-C8

Narrow Bore guard column (1.25 cm × 2.1 mm i.d.). Solvents for RP-HPLC were A) filtered (0.2 μm) deionized-distilled water using a Milli-Q system, and B) acetonitrile (ACN, HPLC grade, Fisher Scientific). Both solvents contained 0.1% (v/v) trifluoroacetic acid (HPLC grade, Sigma), and were filtered using 0.45 μm filters in respective reservoirs. Solvent flow rate was maintained at 0.2 mL/min. Sample injection volume was 2.0 μL. After an initial 3 min isocratic condition at 23% solvent B, proteins were eluted in a 51 min linear gradient from 23-44% solvent B. At 54 min (total run time), the gradient was adjusted back to 23% solvent B, and data was acquired for 6 more minutes. The column was equilibrated at 23% solvent B for 10 min between runs. Column eluent was monitored at 206 nm. Control of the chromatograph and data quantitation was provided by Agilent HPLC Chemstation software version A.10 implemented on a Pentium III 500 MHz personal computer.

4.2.6. Statistical analyses

All statistical analyses were performed on duplicate measurements using the procedures of the SAS (1988) software system version 8.2. Significant differences were calculated using Sheffe's Least Significant Difference. Correlation analyses of the data were performed using Pearson's correlation coefficient analysis.

4.3. RESULTS AND DISCUSSION

4.3.1. Analytical properties of flour millstreams and pearled wheat fractions

Results for colour, ash, protein content, and dough mixing of the millstreams were presented previously in Chapters 2 and 3. Ash and protein contents of the

millstreams are shown later in Table 4.2 along with corresponding protein fractionation results. Ash and protein content generally increased from inner to outer endosperm within the kernel, as reflected by variation in the results from the first to last break and reduction streams.

The pearling fractions all showed a progressive decrease in ash content from the 10% fraction (4.94%, on average) to the 60% fraction (1.08%, on average) (Table 4.1). The relationship between ash and protein content for the pearled fractions (Fig. 4.1) shows a maximum protein content for the 20% fraction. This finding is in accord with that of Normand et al. (1965) who indicated that the first pearled fraction (or fractions, depending on the weight removed) contained the entire pericarp and aleurone layer. Once this layer was removed, the next fraction contained the high-protein-bearing material in the kernel, i.e. the subaleurone layer. This high protein layer, detected also in hand-dissected hard wheat kernels (Kent, 1966), was reported to contain 33 to 54% protein. Normand et al. (1965) also concluded that it was necessary to remove 12 to 15% by weight of the original kernel to ensure fairly complete removal of the bran layer. This explains why the 20% pearled fraction in the present study had the highest protein content. It is important to note that because of the crease and the oval to ovate shape of wheat kernels, pearling cannot produce fractions that are composed of specific tissue layers. Results of this study reach a similar conclusion. The final pearling removed 60% of original kernel weight. Accordingly, the pearled wheat from that fraction should represent the inner endosperm. However, its ash content varied from 0.99% for AC Corinne to 1.22% for Superb. Evidently considerable bran tissue remained in this fraction, partly because of the crease and the shape of the kernel.

Table 4.1. Analytical properties of pearled fractions

	Superb		AC Barrie		AC Corinne		AC Snowbird		Average	
	Ash[1]	Prot[2]	Ash	Prot	Ash	Prot	Ash	Prot	Ash	Prot
10%	5.13	19.39	5.15	21.03	4.93	19.63	4.55	19.32	4.94	19.84
20%	3.52	21.21	3.54	22.76	3.68	22.08	3.36	21.73	3.53	21.95
30%	2.33	19.86	2.44	21.40	2.43	21.16	2.42	20.69	2.41	20.78
40%	1.88	18.64	1.92	19.76	1.83	19.12	1.86	18.56	1.87	19.02
50%	1.52	17.17	1.44	17.98	1.36	17.44	1.36	16.85	1.42	17.36
60%	1.22	15.62	1.07	16.34	0.99	15.79	1.03	15.32	1.08	15.77

[1]Ash content (%),[2]Protein content (%). All values corrected to 14%mb.

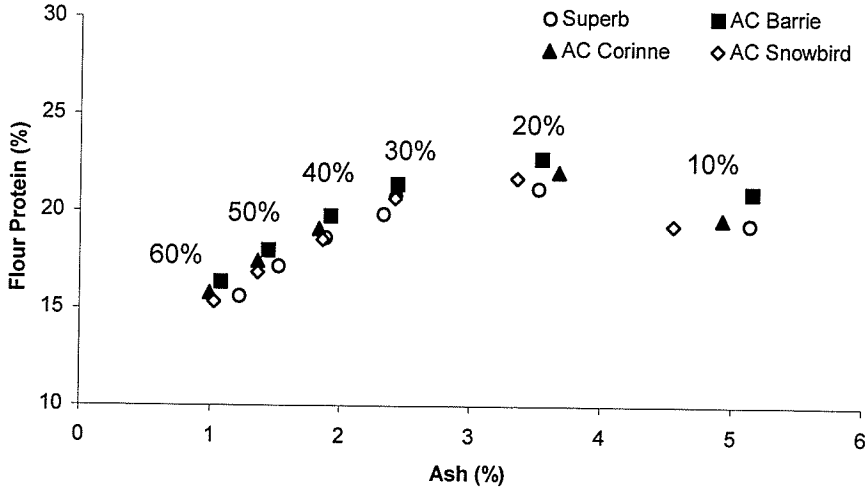


Figure 4.1. Relationship between flour protein content and ash content in pearled fractions (10 to 60% by weight)

4.3.2. Protein composition of millstreams

Protein composition results of millstreams relative to flour and flour protein (to eliminate the effect of flour protein) are presented in Table 4.2 and 4.3, respectively.

Table 4.2. Ash and protein contents, soluble protein (SP), insoluble glutenin (IG), and residue protein (RP) in millstreams of each cultivar sample.

	Superb					AC Barrie				
	Ash[1]	FP[1]	SP[1]	IG[1]	RP[1]	Ash	FP	SP	IG	RP
B1	0.54	14.89	10.66	2.75	1.49	0.49	16.57	12.06	2.66	1.84
B2	0.45	16.25	11.13	3.45	1.67	0.42	17.63	12.46	3.02	2.16
B3	0.55	18.65	12.09	3.86	2.70	0.49	20.04	14.86	3.44	1.75
B4	0.61	19.43	12.12	4.19	3.13	0.49	21.66	17.28	3.51	0.87
S1	0.41	13.96	9.59	2.93	1.44	0.38	14.67	10.52	2.32	1.83
Q1	0.66	14.97	9.43	2.89	2.65	0.69	16.24	10.58	2.65	3.01
M1	0.36	12.68	8.16	2.28	2.24	0.32	13.09	9.58	2.04	1.48
M2	0.43	12.85	8.37	2.36	2.13	0.38	12.83	9.17	1.89	1.77
M3	0.64	13.45	7.86	2.20	3.39	0.65	13.54	8.78	1.93	2.84
M4	0.85	14.02	7.74	2.44	3.84	1.00	14.70	9.50	2.20	3.00
M5	1.74	15.69	7.54	2.45	5.70	1.98	16.58	9.29	1.86	5.43
M6	2.65	17.99	7.61	2.50	7.88	3.11	19.61	8.46	2.16	8.98
BF	2.40	23.56	13.49	5.13	4.94	2.31	26.95	17.52	5.08	4.35
LSD[2]			1.15	0.30	1.19			1.13	0.30	1.25
	AC Corinne					AC Snowbird				
	Ash	FP	SP	IG	RP	Ash	FP	SP	IG	RP
B1	0.76	15.95	9.18	3.95	2.82	0.50	15.28	10.39	2.94	1.96
B2	0.57	17.18	10.09	4.67	2.42	0.43	16.59	11.41	3.36	1.81
B3	0.70	19.57	10.53	5.35	3.69	0.51	18.87	12.58	3.85	2.44
B4	0.71	19.86	11.14	5.34	3.38	0.57	18.86	12.79	3.96	2.11
S1	0.50	14.33	8.32	3.76	2.26	0.38	14.10	9.91	2.58	1.61
Q1	0.76	15.57	8.62	3.91	3.04	0.66	14.84	9.26	2.77	2.81
M1	0.38	13.28	7.78	3.39	2.11	0.32	12.74	9.23	2.25	1.26
M2	0.42	13.29	7.36	3.29	2.64	0.37	12.78	8.19	2.26	2.32
M3	0.59	13.78	7.59	3.20	2.99	0.64	13.36	8.14	2.32	2.89
M4	0.91	14.68	7.58	3.26	3.84	0.94	14.22	8.37	2.28	3.57
M5	1.77	16.32	7.13	3.34	5.85	1.96	16.94	7.27	2.21	7.46
M6	2.81	18.95	6.90	3.28	8.77	3.01	20.06	7.23	1.85	10.98
BF	3.36	25.10	12.57	6.72	5.81	3.42	23.34	13.15	4.67	5.52
LSD			0.66	0.38	0.72			1.04	0.21	1.21
	AC Vista					AC Crystal				
	Ash	FP	SP	IG	RP	Ash	FP	SP	IG	RP
B1	0.46	14.91	9.49	3.15	2.26	0.50	14.51	8.44	3.06	3.01
B2	0.38	15.88	10.63	3.18	2.07	0.43	15.38	9.5	3.53	2.35
B3	0.43	18.49	11.96	4.04	2.50	0.50	18.07	10.38	4.16	3.53
B4	0.47	20.07	12.51	4.43	3.12	0.54	19.28	11.24	4.49	3.55
S1	0.34	13.45	8.82	2.77	1.86	0.40	12.94	7.99	2.83	2.12
Q1	0.60	15.62	9.31	3.21	3.10	0.71	14.53	8.68	2.96	2.89
M1	0.28	11.91	7.82	2.31	1.77	0.34	11.51	7.44	2.48	1.59
M2	0.36	11.99	7.50	2.31	2.17	0.42	12.09	7.72	2.37	1.99
M3	0.57	13.05	8.43	2.38	2.24	0.89	13.59	7.75	2.44	3.40
M4	0.84	14.50	8.69	2.65	3.15	1.09	14.01	7.84	2.53	3.64
M5	1.65	16.30	7.93	2.44	5.93	2.10	16.39	7.09	2.38	6.93
M6	2.46	19.39	7.47	2.19	9.73	2.85	18.55	7.13	2.32	9.10
BF	1.53	24.90	13.87	5.81	5.22	1.73	24.38	13.53	5.95	4.90
LSD			0.83	0.32	0.96			0.61	0.25	3.31

[1]In %, all values corrected to 14% mb; [2]Least Significant Difference

Table 4.2. Cont'd.

	Superb 2					AC Snowbird 2				
	Ash[1]	FP[1]	SP[1]	IG[1]	RP[1]	Ash	FP	SP	IG	RP
B1	0.53	12.45	8.34	2.39	1.72	0.53	14.53	9.76	2.54	2.22
B2	0.45	13.86	9.13	2.86	1.87	0.47	15.78	10.26	3.07	2.45
B3	0.52	16.04	10.81	3.34	1.88	0.57	17.88	12.23	3.65	2.00
B4	0.57	16.36	10.94	3.62	1.80	0.62	17.75	11.7	3.45	2.60
S1	0.38	12.09	8.14	2.33	1.62	0.41	13.56	8.40	2.52	2.64
Q1	0.62	12.97	8.4	2.62	1.95	0.73	14.29	8.66	2.56	3.07
M1	0.33	11.16	7.59	2.19	1.38	0.31	12.25	8.15	2.05	2.06
M2	0.40	11.43	7.61	2.20	1.62	0.38	12.39	8.15	2.03	2.21
M3	0.82	12.67	7.73	2.37	2.57	0.80	13.13	7.73	2.11	3.29
M4	0.99	13.01	7.70	2.30	3.01	1.09	13.88	7.73	2.14	4.01
M5	2.24	15.91	7.19	2.21	6.51	2.19	16.49	6.87	2.03	7.58
M6	3.36	18.88	6.97	2.06	9.85	3.21	19.64	6.46	1.78	11.40
BF	2.26	20.04	11.37	4.34	4.33	2.86	21.70	11.72	4.27	5.71
LSD[2]			0.57	0.23	0.68			1.20	0.24	1.23

Table 4.3. Insoluble glutenin (IG), soluble protein (SP), and residue protein (RP), as a percentage of total flour protein content (FP) and ratio of IG to SP in millstreams of each cultivar sample.

	Superb				AC Barrie			
	SP/FP	IG/FP	IG/SP	RP/FP	SP/FP	IG/FP	IG/SP	RP/FP
B1	71.58	18.44	0.26	9.98	72.82	16.07	0.22	11.12
B2	68.50	21.21	0.31	10.30	70.66	17.10	0.24	12.23
B3	64.84	20.68	0.32	14.48	74.14	17.15	0.23	8.71
B4	62.35	21.56	0.35	16.08	79.76	16.20	0.20	4.03
S1	68.71	20.96	0.31	10.33	71.71	15.83	0.22	12.46
Q1	62.97	19.34	0.31	17.69	65.12	16.34	0.25	18.55
M1	64.36	17.97	0.28	17.67	73.17	15.55	0.21	11.28
M2	65.10	18.32	0.28	16.58	71.48	14.72	0.21	13.80
M3	58.41	16.39	0.28	25.20	64.83	14.22	0.22	20.95
M4	55.19	17.40	0.32	27.41	64.60	14.97	0.23	20.44
M5	48.03	15.62	0.33	36.35	56.01	11.22	0.20	32.77
M6	42.29	13.90	0.33	43.81	43.16	11.04	0.26	45.81
BF	57.27	21.77	0.38	20.96	65.01	18.84	0.29	16.15
LSD	7.54	1.89	0.05	7.48	6.29	1.78	0.03	6.91
	AC Corinne				**AC Snowbird**			
	SP/FP	IG/FP	IG/SP	RP/FP	SP/FP	IG/FP	IG/SP	RP/FP
B1	57.55	24.75	0.43	17.70	67.95	19.21	0.28	12.84
B2	58.72	27.18	0.46	14.10	68.80	20.27	0.29	10.93
B3	53.81	27.31	0.51	18.88	66.67	20.42	0.31	12.91
B4	56.10	26.90	0.48	17.00	67.83	21.00	0.31	11.18
S1	58.03	26.20	0.45	15.76	70.30	18.31	0.26	11.39
Q1	55.38	25.13	0.45	19.49	62.38	18.71	0.30	18.91
M1	58.60	25.54	0.44	15.86	72.45	17.65	0.24	9.91
M2	55.40	24.78	0.45	19.83	64.13	17.70	0.28	18.17
M3	55.04	23.22	0.42	21.73	60.95	17.40	0.29	21.65
M4	51.66	22.17	0.43	26.16	58.88	16.05	0.27	25.07
M5	43.71	20.48	0.47	35.81	42.92	13.05	0.30	44.03
M6	36.42	17.30	0.47	46.28	36.06	9.22	0.26	54.72
BF	50.08	26.76	0.53	23.16	56.33	20.01	0.36	23.66
LSD	3.50	2.32	0.05	4.20	6.10	1.25	0.03	7.74

Table 4.3. Cont'd.

	AC Vista				AC Crystal			
	SP/FP	**IG/FP**	**IG/SP**	**RP/FP**	**SP/FP**	**IG/FP**	**IG/SP**	**RP/FP**
B1	63.67	21.15	0.33	15.18	58.15	21.10	0.36	20.75
B2	66.93	20.05	0.30	13.02	61.79	22.95	0.37	15.27
B3	64.67	21.83	0.34	13.50	57.45	23.01	0.40	19.54
B4	62.34	22.09	0.35	15.57	58.31	23.28	0.40	18.41
S1	65.56	20.58	0.31	13.86	61.78	21.85	0.35	16.37
Q1	59.62	20.55	0.34	19.83	59.74	20.39	0.34	19.87
M1	65.69	19.40	0.30	14.90	64.65	21.56	0.33	13.79
M2	62.56	19.30	0.31	18.14	63.87	19.64	0.31	16.48
M3	64.56	18.25	0.28	17.20	56.99	17.98	0.32	25.03
M4	59.96	18.30	0.31	21.74	47.43	18.03	0.32	25.99
M5	48.63	14.96	0.31	36.41	43.23	14.50	0.34	42.27
M6	38.54	11.27	0.29	50.19	38.46	12.51	0.33	49.04
BF	55.70	23.34	0.42	20.96	55.51	24.39	0.44	20.09
LSD	4.76	2.16	0.03	5.96	6.10	1.25	0.03	7.74
	Superb 2				**AC Snowbird 2**			
	SP/FP	**IG/FP**	**IG/SP**	**RP/FP**	**SP/FP**	**IG/FP**	**IG/SP**	**RP/FP**
B1	66.98	19.19	0.29	13.83	67.19	17.51	0.26	15.31
B2	65.84	20.64	0.31	13.51	65.02	19.47	0.30	15.51
B3	67.43	20.84	0.31	11.74	68.41	20.41	0.30	11.19
B4	66.86	22.11	0.33	11.02	65.92	19.41	0.29	14.67
S1	67.37	19.25	0.29	13.38	61.95	18.61	0.30	19.44
Q1	64.74	20.23	0.31	15.03	60.57	17.94	0.30	21.49
M1	68.00	19.64	0.29	12.36	66.51	16.71	0.25	16.78
M2	66.59	19.23	0.29	14.18	65.79	16.39	0.25	17.82
M3	60.97	18.71	0.31	20.32	58.85	16.11	0.27	25.04
M4	59.17	17.67	0.30	23.16	55.71	15.43	0.28	28.86
M5	45.20	13.90	0.31	40.91	41.68	12.33	0.30	45.99
M6	36.91	10.93	0.30	52.16	32.88	9.09	0.28	58.03
BF	56.75	21.66	0.38	21.59	54.02	19.66	0.36	26.31
LSD	3.55	1.49	0.03	3.96	7.75	1.59	0.05	7.76

The relationship between RP and RP/FP levels in millstreams was very high (R^2 = 0.92) (Fig. 4.2.A), whereas that between IG and IG/FP was lower (R^2 = 0.55) (Fig. 4.2.B). In contrast, SP and SP/FP were poorly correlated (R^2 = 0.23) (Fig. 4.2.C). For the RP fraction, increasing millstream protein concentration translates into corresponding increases in RP in a predictable way, i.e. as millstream protein content increases, both absolute and normalized levels of RP increase. In contrast, the relationship between absolute and normalized protein composition results for SP and IG are complex. For break streams, Table 4.2 indicates that increasing protein content (from B1 to B4)

corresponds to increasing absolute levels of both SP and IG. However, for reduction streams, M1 through M6, while the trend in increasing total protein content is clear, SP and IG levels, respectively, do not change appreciably. In fact, the ratios of SP/FP and IG/FP decline with increasing FP (Table 4.3). This explains the absence of a clear relationship between absolute and normalized levels of SP and IG.

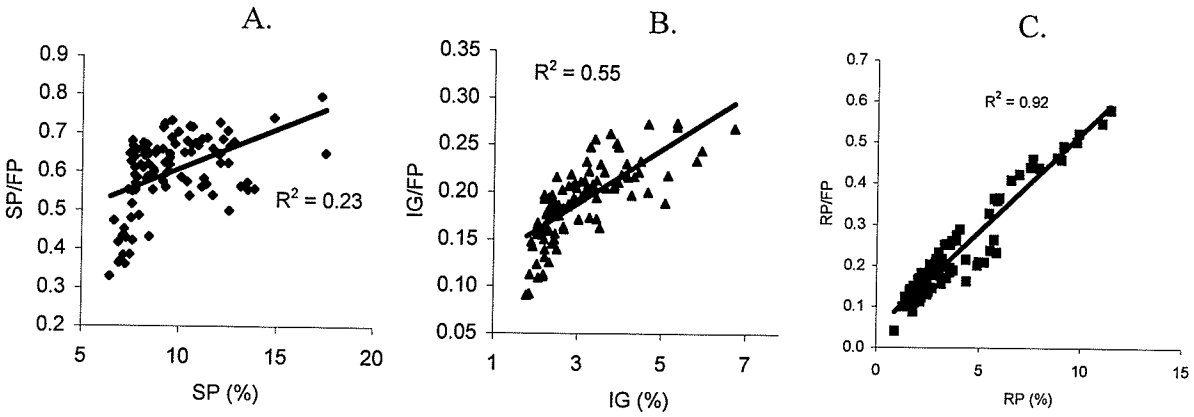


Figure 4.2. Relationship between SP and SP/FP (A), IG and IG/FP (B), and RP and RP/FP (C).

Cumulative protein content of total protein and of each of the fractions i.e. soluble protein (SP), insoluble glutenin (IG), and residue protein (RP) are shown in Fig. 4.3. The result indicates a much higher rate of (330%) accumulation of gliadins in high grade flour streams (< 0.50% ash) compared to glutenin which reflects the higher concentration of SP compared to IG in the wheat itself; 9.5% versus 3.0% (flour basis), respectively, averaged over all samples and millstreams. The lower rate of accumulation after about 0.50% is likely occurring as the gluten protein fractions in the millstreams are becoming increasingly diluted with bran residue which contains no gluten protein.

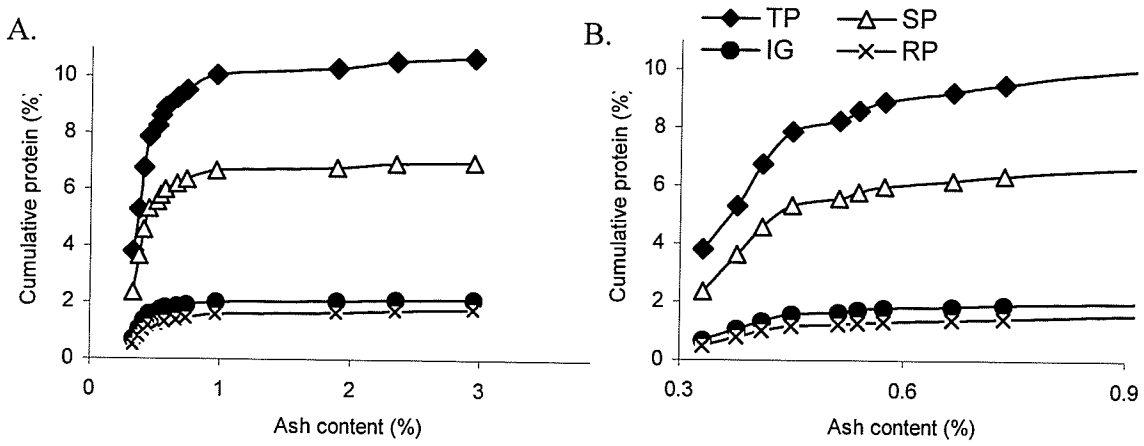


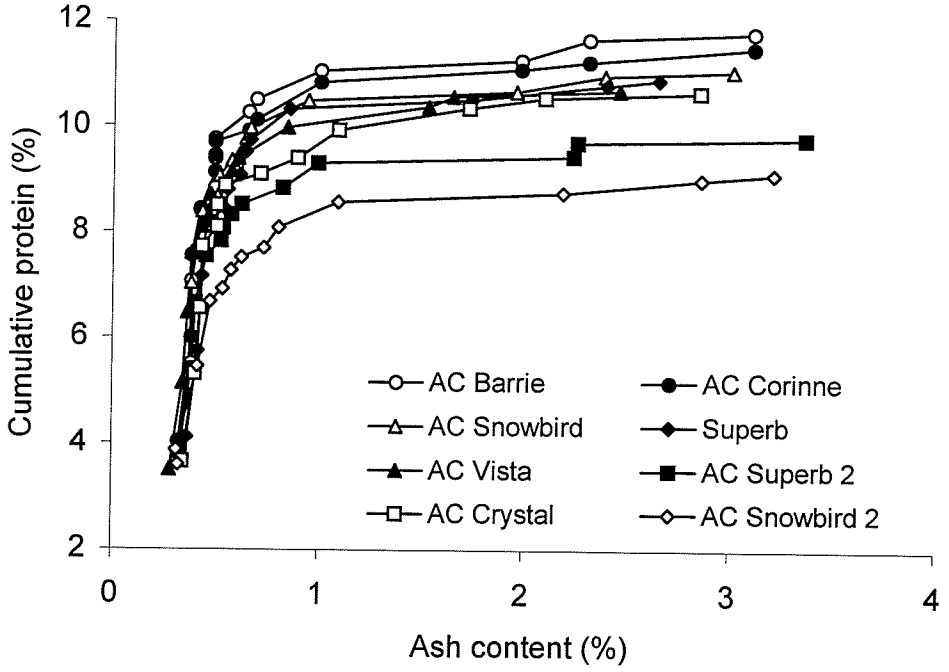
Figure 4.3. Cumulative protein content of different fractions as a function of ash, averaged over all cultivar samples; total protein (TP), soluble protein (SP), insoluble glutenin (IG), and residue protein (RP). Figure 4.3.B: details of the relationship for higher grade flour streams up to 1.0% ash content.

The trend of cumulative millstream protein content and composition as a function of increasing ash for the eight cultivar samples is shown in Fig. 4.4. Regardless of cultivar sample, approximately 50% of flour protein (excluding milfeeds) is contributed by the flour fractions of highest refinement, either M1 and M2 or M1 and S1. AC Barrie despite having the highest protein content, and the highest SP content, also has the lowest IG content, which explains its weak mixing properties.

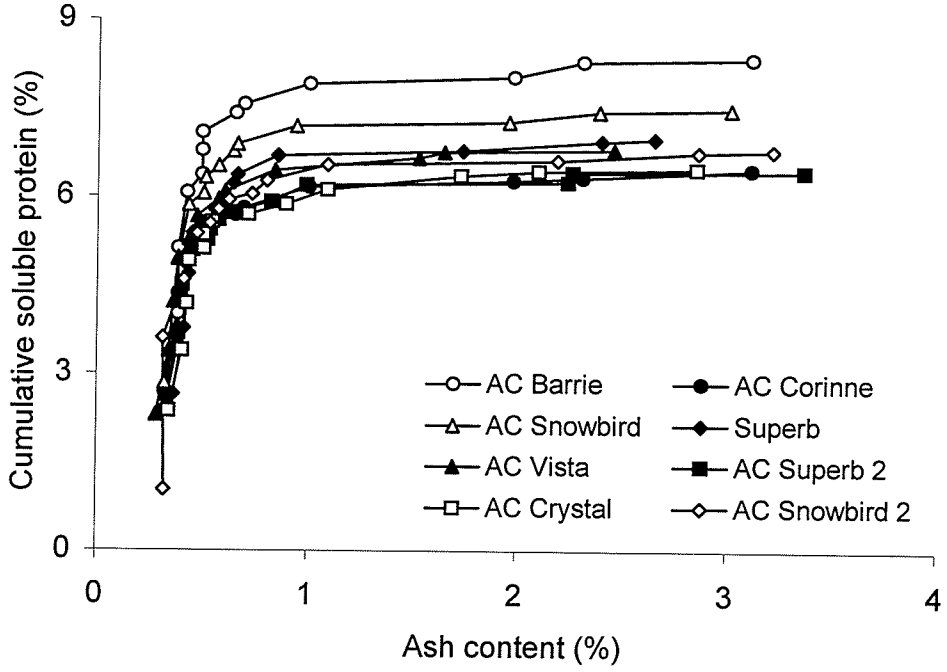
The variation in protein composition relative to flour protein (in order to eliminate the effect of protein content) in relation to flour refinement is illustrated in Fig. 4.5, 4.6, and 4.7.

Figure 4.5.A-C depicts the variation in SP/FP (proportion of SP in flour protein) as a function of stream refinement, i.e. ash content. On Fig. 4.5.A the BF data points appear as outliers of the trend. There was a highly significant (r = -0.75, on average)

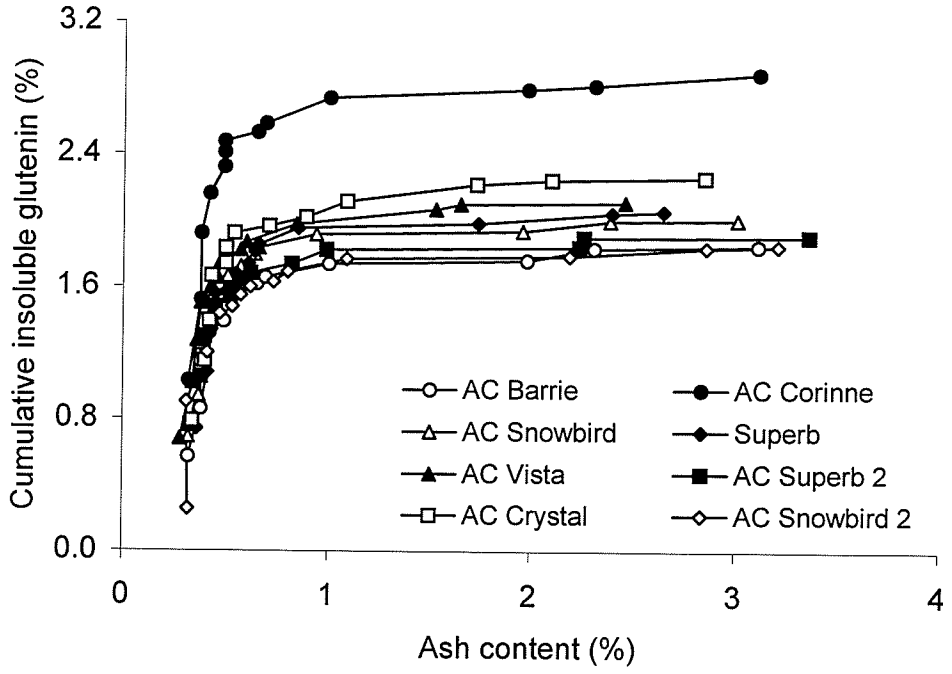
A.


Cumulative protein (%)
12
10
8
6
4
2
0
1
2
3
4
Ash content (%)
AC Barrie
AC Corinne
AC Snowbird
Superb
AC Vista
AC Superb 2
AC Crystal
AC Snowbird 2

B.


Cumulative soluble protein (%)
9
6
3
0
0
1
2
3
4
Ash content (%)
AC Barrie
AC Corinne
AC Snowbird
Superb
AC Vista
AC Superb 2
AC Crystal
AC Snowbird 2

C.



D.

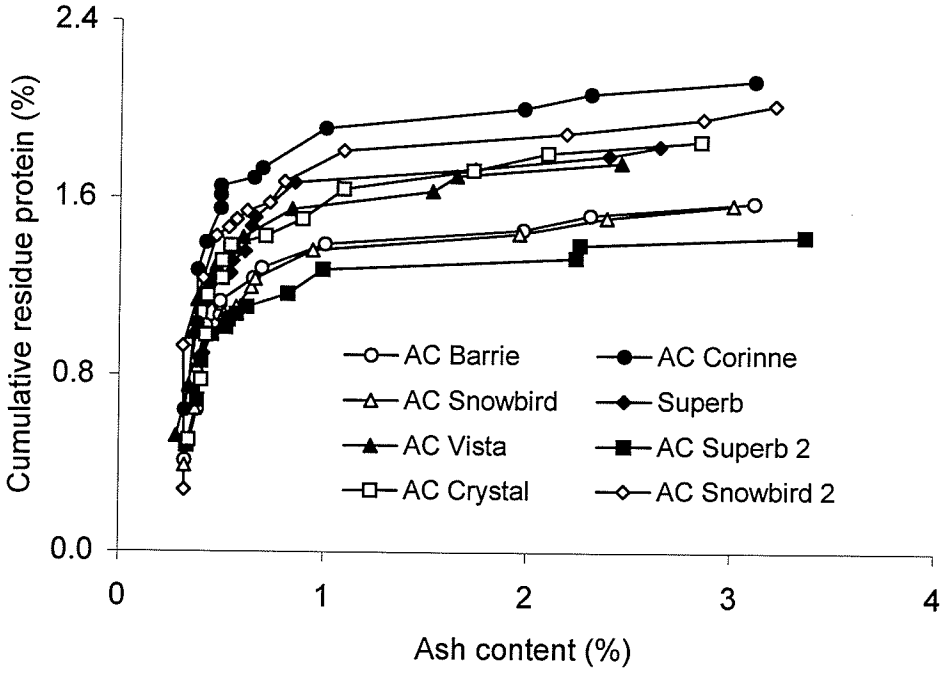


Figure 4.4. Cumulative protein content of different fractions as a function of ash for different cultivar samples; A, total protein; B, soluble protein; C, insoluble glutenin; D, residue protein.

negative correlation between SP/FP and ash content for each genotype. When BF was excluded, the correlation coefficient increased to r = -0.83, on average). This result indicates that the gliadin concentration per unit protein of flour varies inversely with flour refinement, i.e. bran contamination. The levels of SP in flour protein are high, and usually represent more than 50% of the total flour protein. SP/FP levels of M1 flours (representative of a highly refined stream) ranged from 58.6% (AC Corinne) to 73.2% (AC Barrie). In contrast, for M6 flours (representative of a highly bran contaminated stream), SP/FP levels ranged from 32.9% (AC Snowbird 2) to 43.2% (AC Barrie). When these results are presented by separating break streams B1-B4 and reduction streams M1-M6 (Fig. 4.5.B and 4.5.C, respectively), these trends become even clearer for the reduction flours. Each genotype also follows a relatively distinct trend (Fig. 4.5) with AC Corinne having the lowest levels of SP/FP, and AC Barrie possessing the highest levels for all millstreams.

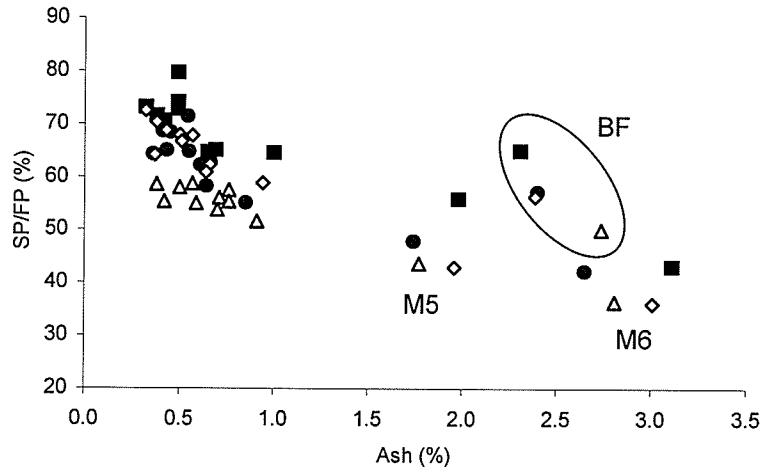
The corresponding variation of IG/FP as a function of ash content (Fig 4.6.A) also reveals BF streams as outliers. In the absence of BF results, a decrease in IG/FP levels as ash content increases is more clearly evident, and like the relationship for SP/FP, each cultivar sample has a distinct pattern of variation of IG in flour streams, particularly for the extra strong cultivar AC Corinne, which has significantly higher levels of IG/FP for all millstreams. As will be shown later, this variation in IG/FP was strongly correlated to dough mixing characteristics. Like for SP/FP results, while IG/FP in break streams did not vary as ash content increased (Fig. 4.6.B), that in reduction streams clearly decreased as ash content increased (Fig. 4.6.C).

The proportion of insoluble glutenin in flour has been positively correlated with breadmaking performance (Pomeranz, 1965; Orth and Bushuk, 1972; Khan and Bushuk,

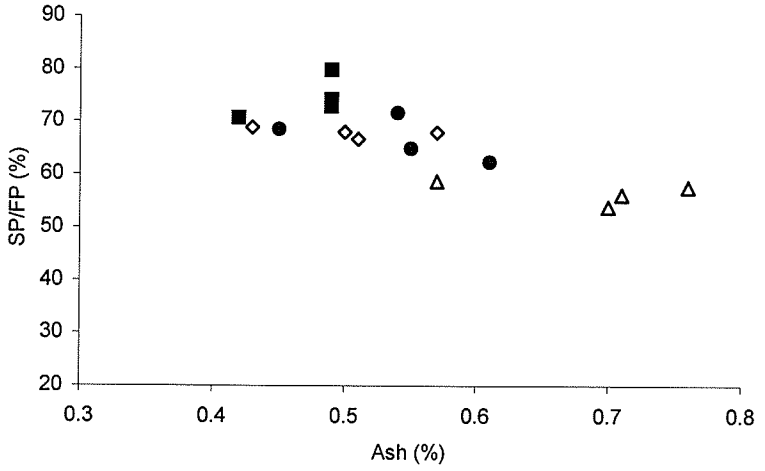
1978; Axford et al., 1979; Moonen et al., 1983; Sapirstein and Fu, 1998). For all samples, the break streams clearly had significantly high levels of IG/FP compared to reduction streams. Averaged over all cultivar samples, levels of SP/FP for the break (B1-B4) and reduction streams (M1-M6) were 65 and 21%, respectively, while levels of IG/FP were 55 and 17%, respectively. Flour streams M5 and M6, despite their high protein content, possess a significantly lower proportion of SP/FP and IG/FP, i.e. total gluten protein, compared to all other streams. On the other hand, BF, which appears to be a low quality stream according to its very high ash content (Table 4.1), typically possessed among the highest levels of IG/FP. Interestingly, SP/FP levels of BF were generally quite low and consistent with levels in M3 and M4 flour streams. The ratio of IG/SP further distinguishes BF (Table 4.3) as a very high protein quality millstream. Accordingly, as bran flour by its nature likely contains the highest level of subaleurone endosperm among all flour streams, it is the first report indicating that the subaleurone layer in wheat likely contains the highest concentration of HMW glutenin in wheat. These results partly explain the strong dough mixing properties of BF shown in Chapter 3.

Compared to the decreasing levels of both SP/FP and IG/FP with decreasing flour refinement, RP/FP had a completely opposite trend; it increased with increasing ash (Fig. 4.7). This result was highly consistent for all cultivar samples. As a proportion of flour protein, RP was the most variable fraction varying by as much as 330% from lowest levels in break flours (11% on average) to highest levels in M6 flours (47% on average) (Table 4.3). Sapirstein and Fu (1998) previously investigated the nature of RP in straight grade flour by SDS-PAGE, and found it contained a relatively low amount of essentially only Glu-D1 subunits of glutenin and lower M_r subunits of unknown identity.

A.



B.



C.

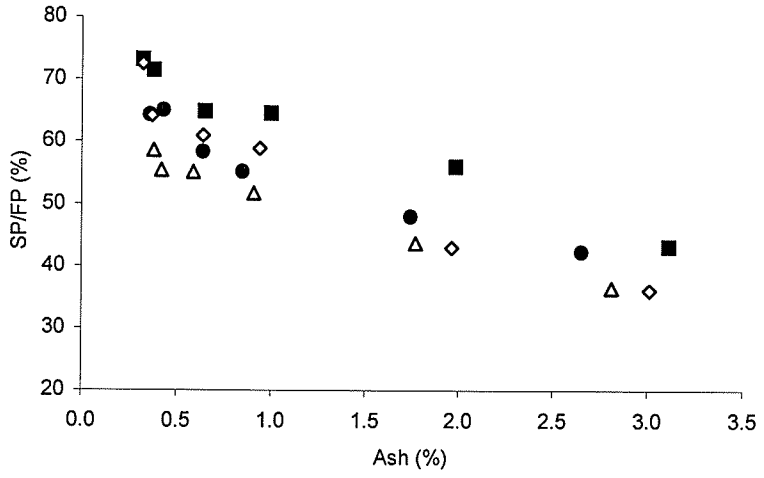
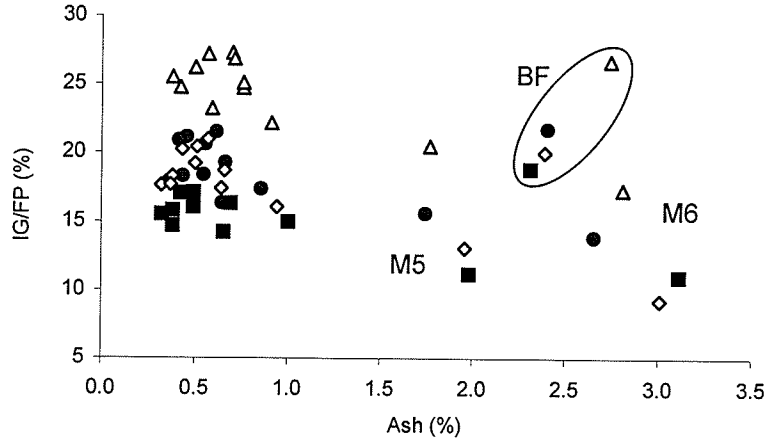
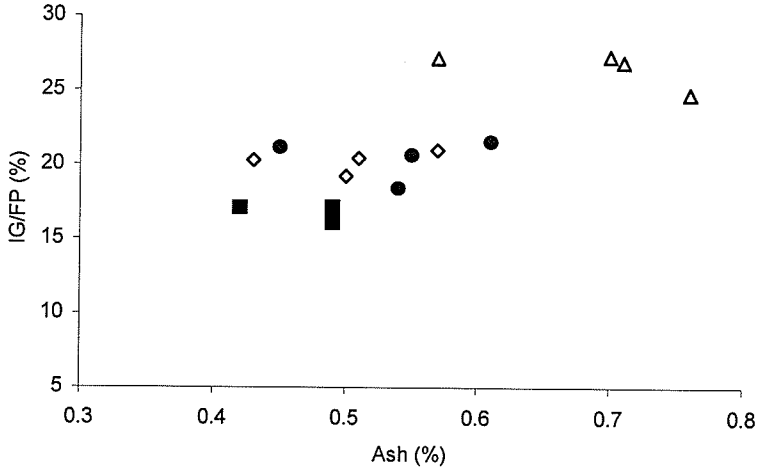


Figure 4.5. Variation in levels of soluble protein in flour protein (SP/FP) (%) as a function of ash content (%) in wheat flour millstreams of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇); in all millstreams (A), in break streams only (B), and in reduction streams only (C).

A.



B.



C.

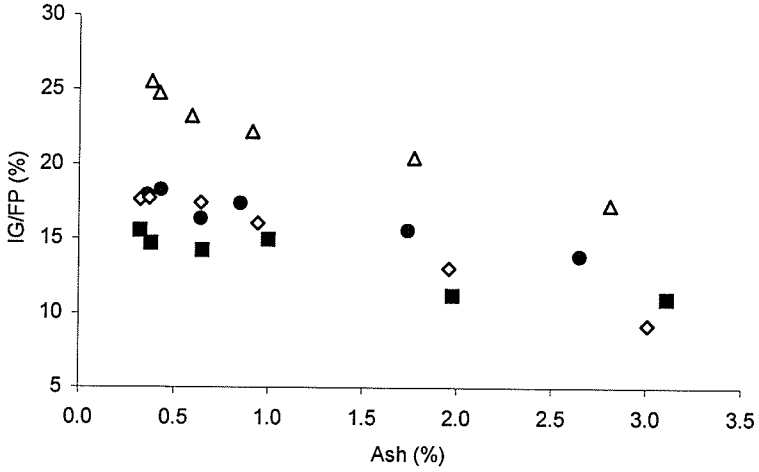


Figure 4.6. Variation in levels of insoluble glutenin in flour protein (IG/FP) (%) as a function of ash content (%) in wheat flour millstreams of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇); in all millstreams (A), in break streams only (B), and in reduction streams only (C).

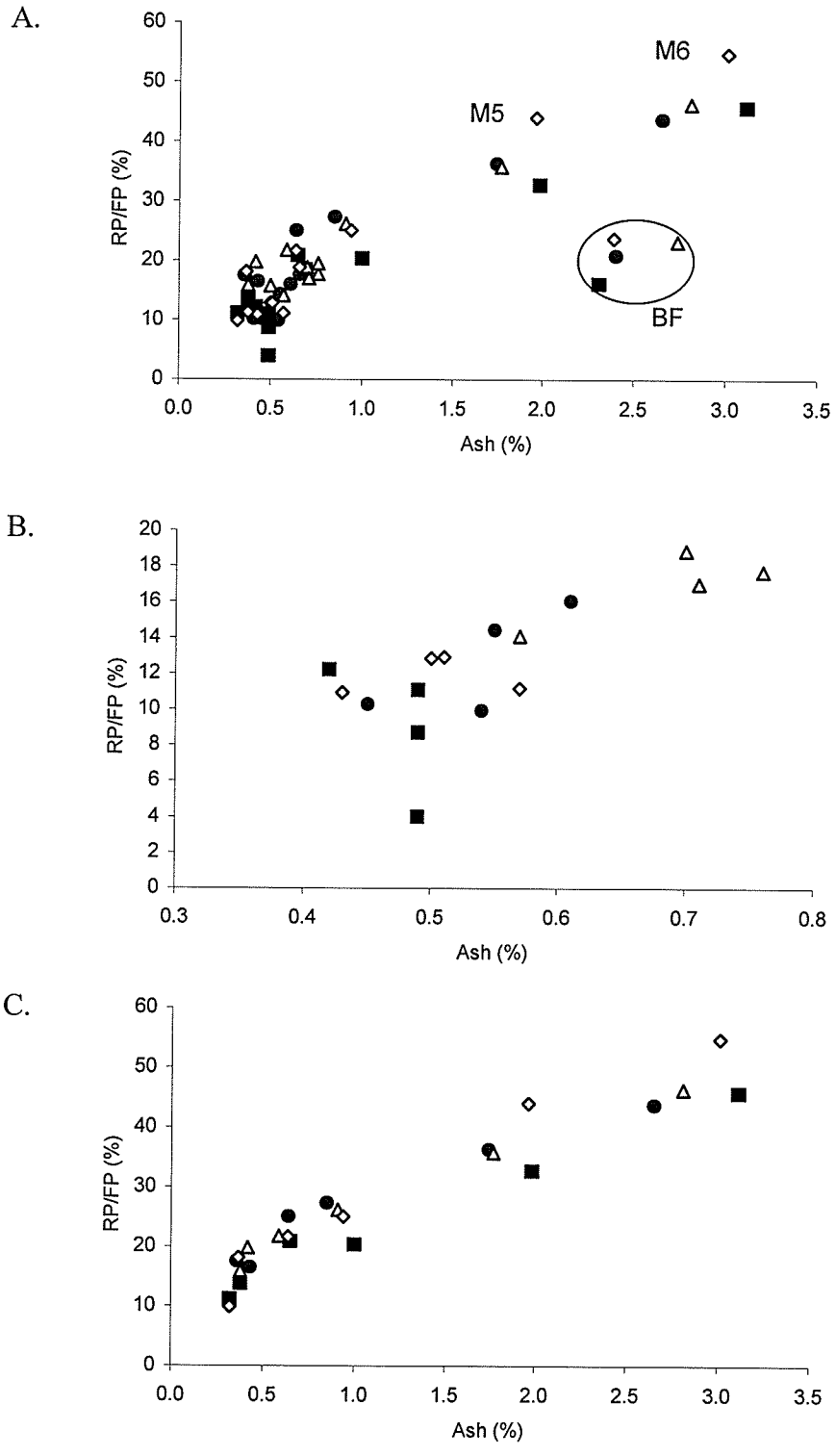


Figure 4.7. Variation in levels of residue protein in flour protein (RP/FP) (%) as a function of ash content (%) in wheat flour millstreams of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇); in all millstreams (A), in break streams only (B), and in reduction streams only (C).

They concluded that the RP fraction contained a large proportion of non-gluten protein consistent with structural protein as described previously (Byers et al., 1983; Kruger et al., 1988). The results shown in Table 4.3 and Fig. 4.7 provide evidence for the first time that RP in flour likely derives from contaminating bran residue. This is a protein fraction that merits more attention than it has received to date, as it likely represents a negative factor in flour breadmaking quality; interestingly, among all the protein fractions investigated by Sapirstein and Fu (1998), the RP protein was the only one that was significantly (negatively) correlated with dough extensibility, whose biochemical nature has been very elusive in the cereal chemistry literature. These results also point to the importance of separating residue protein from IG in protein fractionation work. Beginning even with the original Osborne (1907) fractionation, many methods have been reported which do not distinguish IG and RP (Chen and Bushuk, 1970; Bean et al., 1998).

In summary, millstreams of different genotypes have different and sometimes very distinct protein solubility properties. Flour that is less refined has decreased proportions of gliadin and glutenin protein and higher proportions of residue protein. Overall, break streams possessed high protein contents and good protein quality (i.e. high percentages of IG and SP and low percentage of RP in flour protein). For the reduction streams, it was very interesting to find that the most highly refined streams had a good protein quality despite low flour protein content, whereas the low grade streams of high ash content had poor protein quality despite a high protein content. It was noteworthy that the most highly refined reduction streams (M1, M2) had a poorer protein quality than the break flours, on a total flour protein basis.

The cultivar sample with the highest concentrations of IG and lowest concentrations of SP and RP in flour protein for all millstreams was AC Corinne, an extra strong wheat, which characteristically possesses strong dough mixing properties (Sapirstein and Fu, 1998).

Pearled wheat results shown later indicate that the variation in protein composition of the millstreams reflect corresponding variation within the wheat kernel structures, from inner to outer endosperm. It is also shown below that this variation in millstream protein composition is very closely related to the dough mixing properties.

4.3.3. RP-HPLC of reduced subunits of insoluble glutenin

Results presented in the previous section indicated large quantitative differences in the levels of IG among different millstreams. The question remains whether there exists a qualitative difference in composition of this HMW glutenin fraction. To answer this question, different millstreams were selected representing different parts of the wheat kernel with very different levels of IG, viz. M1, B3, and BF, whose flours have correspondingly very different dough mixing properties (Fig. 3.2, Chapter 3).

Reverse-phase HPLC was conducted on the reduced IG fractions. Representative results for Superb are shown in Fig. 4.8. The IG concentrations of M1, B3 and BF for this cultivar sample were 2.28, 3.86, and 5.13%, respectively. These differences in quantitative difference exists in relative amounts of any glutenin subunit, nor in the ratio absolute amounts of IG are reflected in Fig. 4.8.A for total HMW- and LMW-GS, and in Fig. 4.8.B, which depicts the same result for HMW-GS only. However, when the data are normalized relative to the highest peak (Fig. 4.8.C), there was essentially no difference in the chromatograms. This result indicates that in all three millstreams, no

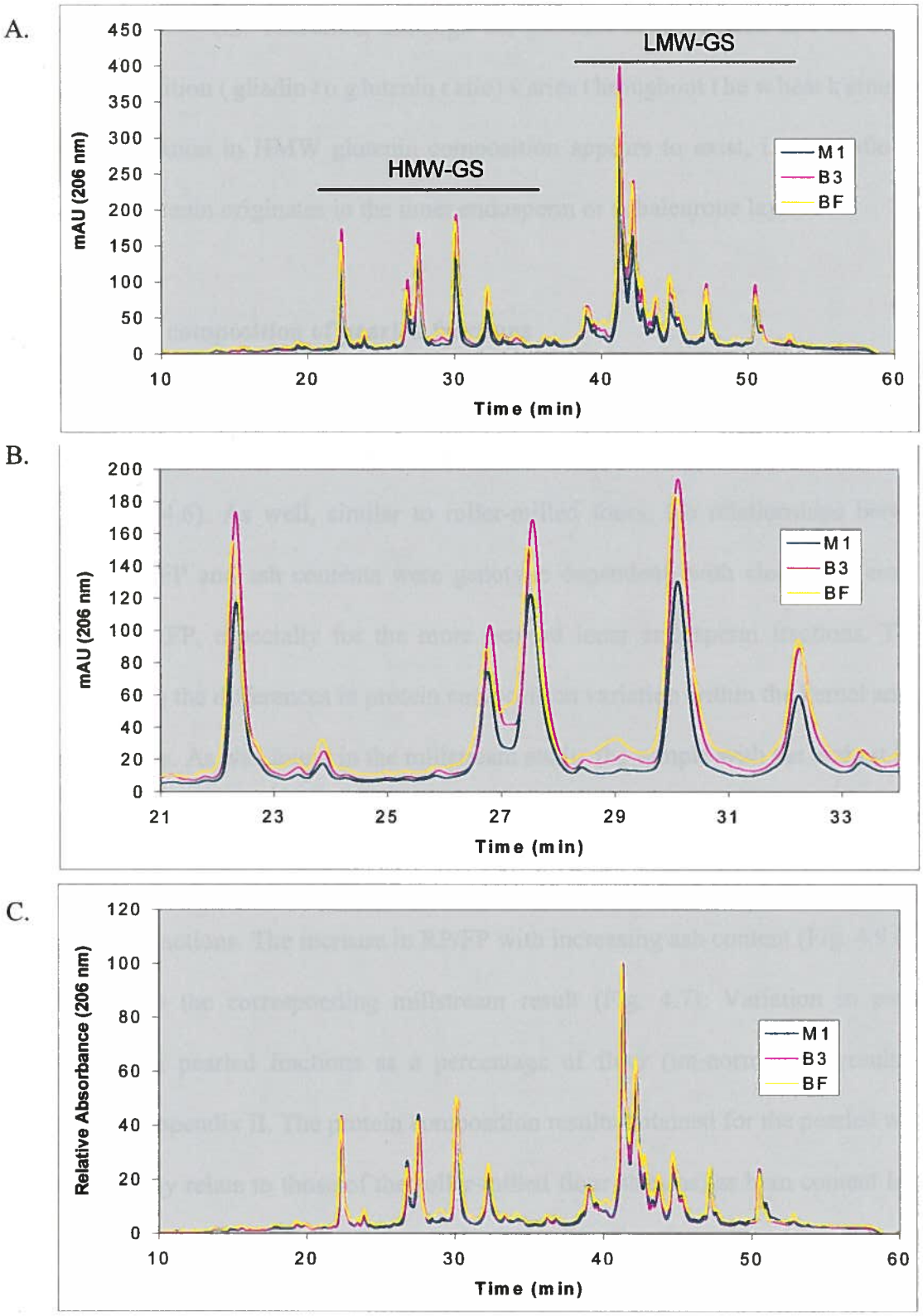


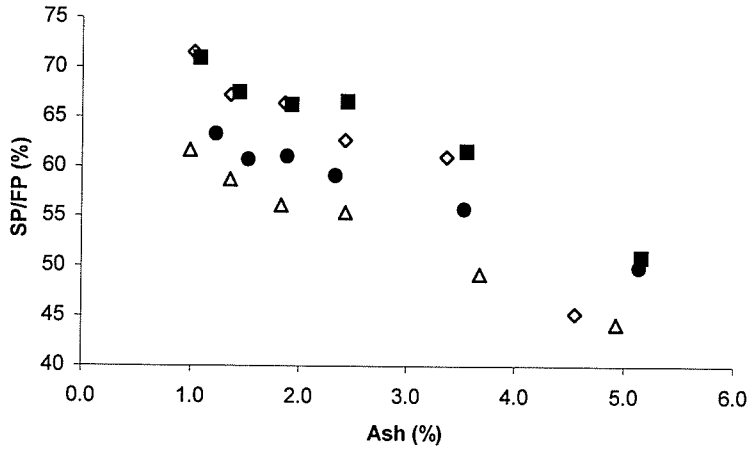
Figure 4.8. RP-HPLC of reduced insoluble glutenin subunits (A), of HMW subunits of reduced insoluble glutenin (B), and of reduced subunits of insoluble glutenin normalized relative to LMW-GS peak of largest magnitude at about 41 min (C), of selected millstreams of Superb M1 (first reduction), B3 (third break), and BF (bran flour).

of HMW to LMW-GS. Therefore, although the glutenin concentration and the overall protein composition (gliadin to glutenin ratio) varies throughout the wheat kernel, no qualitative variation in HMW glutenin composition appears to exist, i.e. regardless of whether the glutenin originates in the inner endosperm or subaleurone layer.

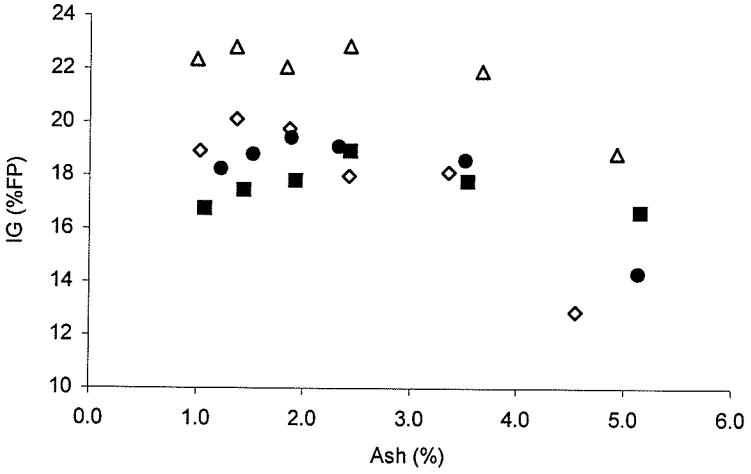
4.3.4. Protein composition of pearled fractions

The relationships between SP/FP and IG/FP and ash content in pearled wheat fractions (Fig. 4.9. A and B) are similar to corresponding results of roller milled flour streams (Fig. 4.5. and 4.6). As well, similar to roller-milled fours, the relationships between SP/FP and IG/FP and ash contents were genotype dependent, with clearer differences evident for IG/FP, especially for the more pearled inner endosperm fractions. These results illustrate the differences in protein composition variation within the kernel among cultivar samples. As was found in the millstream study, the sample with the highest level of IG/FP (AC Corinne) also had the lowest amount of SP/FP for all pearled fractions. Conversely, the sample with the lowest IG/FP (AC Barrie) had the highest SP/FP for most pearled fractions. The increase in RP/FP with increasing ash content (Fig. 4.9.C) is also similar to the corresponding millstream result (Fig. 4.7). Variation in protein composition in pearled fractions as a percentage of flour (un-normalized results) is presented in Appendix II. The protein composition results obtained for the pearled wheat fractions closely relate to those of the roller-milled flour streams; as bran content levels increase, SP/FP and IG/FP decrease and RP/FP increases. This result strongly supports the premise that the roller-milled flour stream results are a close reflection of variation in protein composition from inner to outer endosperm and that within the bran layers.

A.



B.



C.

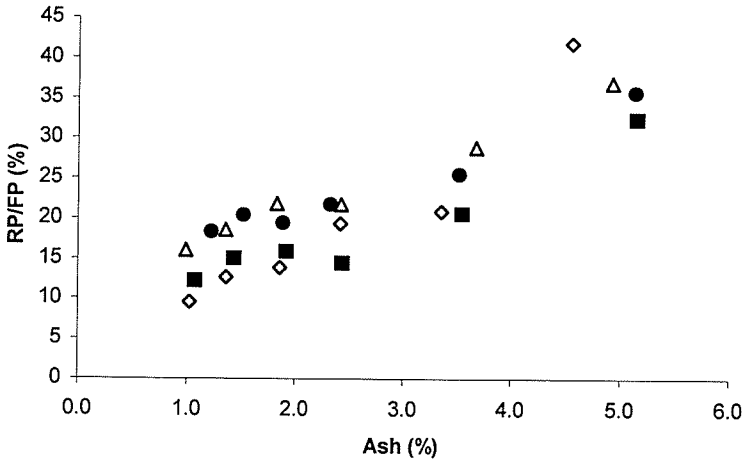


Figure 4.9. Relationship between soluble protein in flour protein (SP/FP) (A), insoluble glutenin in flour protein (IG/FP) (B), and residue protein in flour protein (RP/FP) (C) and ash content in wheat pearled fractions of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇).

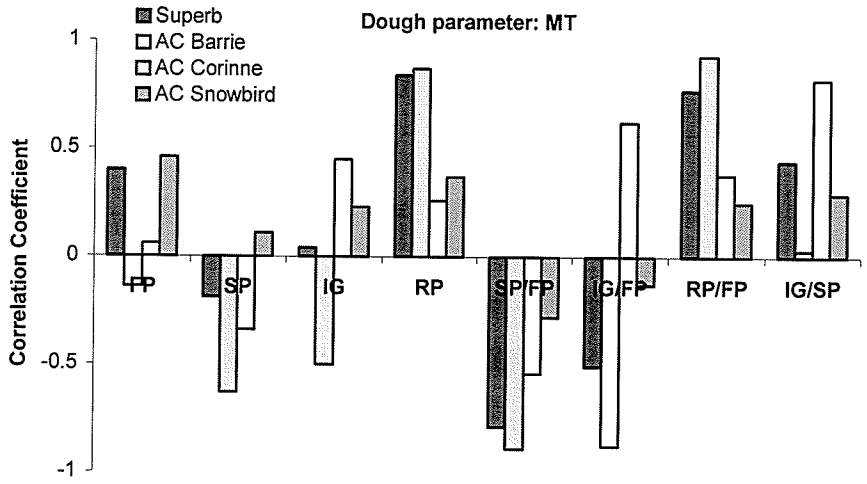
4.3.5. Correlations between protein composition and dough mixing properties

Figures 4.10 and 4.11 present correlation results between dough mixing properties and protein composition of millstreams for unsalted and 2% salted doughs, respectively. The underlying data for these plots along with statistical significance of the correlations are provided in Appendix III (Table 1).

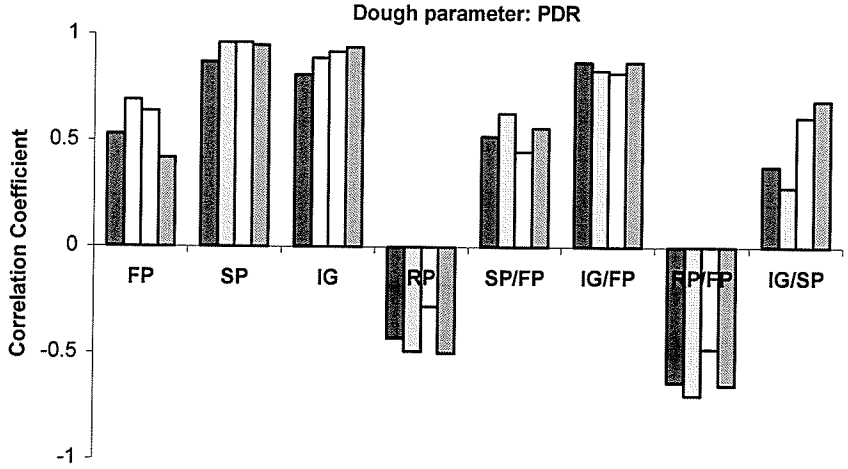
For unsalted and salted doughs, results indicate clear patterns of correlation for all cultivar samples between millstream protein fractions and the majority of dough mixing properties. Because of the large variation of protein content among millstreams (Table 4.2), relationships were compiled for both the absolute concentrations of SP, IG and RP in millstreams and corresponding levels normalized per unit protein, i.e. (SP/FP, IG/FP and RP/FP). As previously explained, the SP, IG, and RP fractions represent mainly gliadins, glutenin and non-gluten protein, respectively. The need to evaluate normalized protein composition results is justified because of the non-uniform relationships between levels of absolute and normalized protein composition in millstream fractions varying widely in refinement. As previously shown (Fig. 4.2), RP and RP/FP levels in millstreams were very closely related ($R^2 = 0.92$), whereas for IG and IG/FP a lower correlation was found ($R^2 = 0.55$). In contrast, SP and SP/FP were poorly correlated ($R^2 = 0.23$).

For doughs mixed both without (Fig. 4.10) and with salt (Fig. 4.11), very similar patterns of correlation were found between all protein fractions and dough mixing parameters, although correlations were slightly higher on average for the salted dough set, particularly between RP fractions and WIP (discussed below). Discussion of results will mainly focus on correlation results for the unsalted doughs.

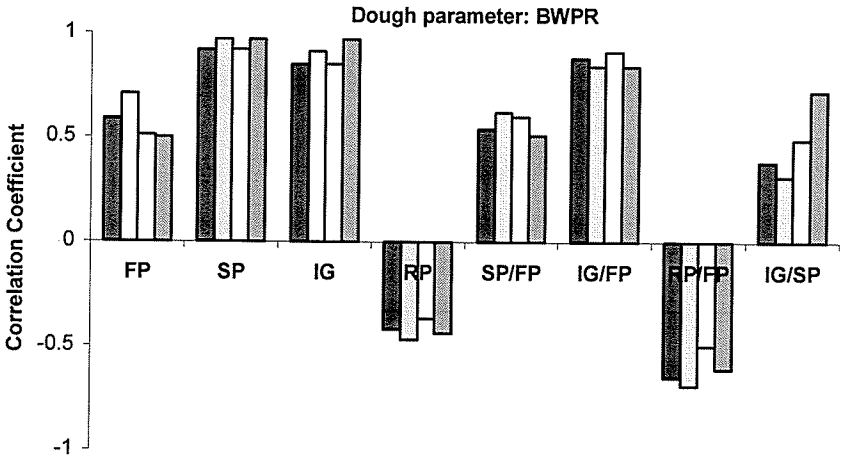
A.


Superb
AC Barrie
AC Corinne
AC Snowbird
Dough parameter: MT
Correlation Coefficient
1
0.5
0
-0.5
-1
FP
SP
IG
RP
SP/FP
IG/FP
RP/FP
IG/SP

B.


Dough parameter: PDR
Correlation Coefficient
1
0.5
0
-0.5
-1
FP
SP
IG
RP
SP/FP
IG/FP
RP/FP
IG/SP

C.


Dough parameter: BWPR
Correlation Coefficient
1
0.5
0
-0.5
-1
FP
SP
IG
RP
SP/FP
IG/FP
RP/FP
IG/SP

D.

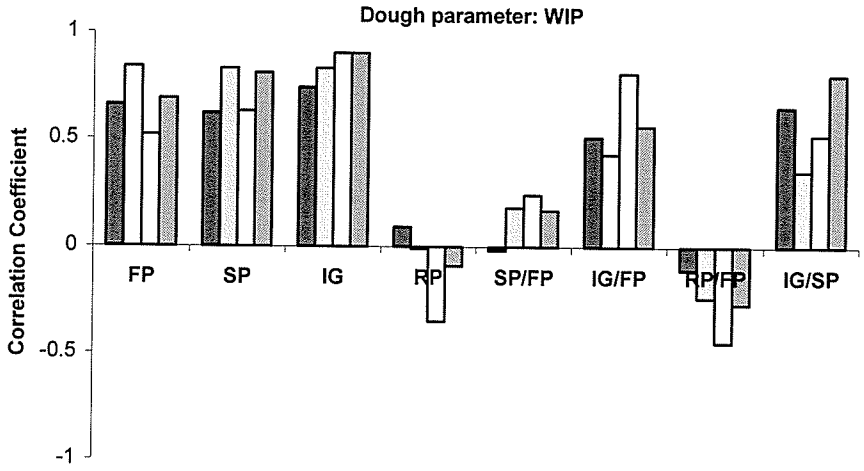
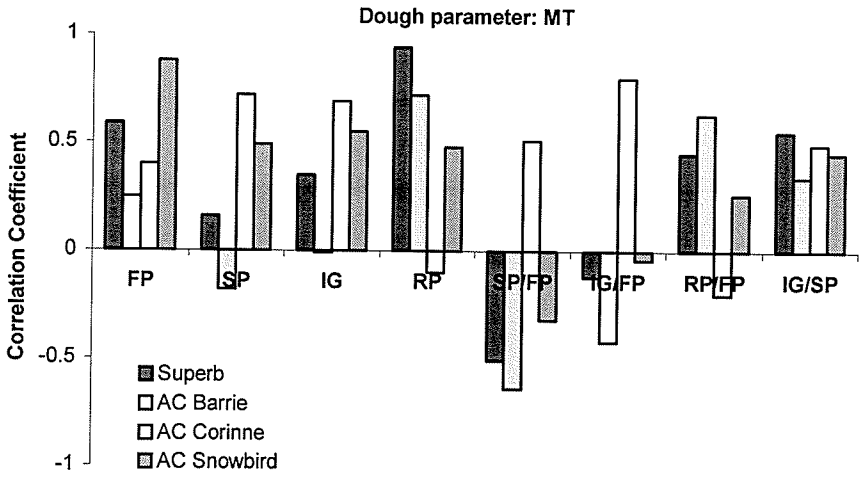


Figure 4.10. Correlations between protein composition fractions (flour protein FP, soluble protein SP, insoluble glutenin IG, residue protein RP, soluble protein in flour protein SP/FP, insoluble glutenin in flour protein IG/FP, residue protein in flour protein RP/FP, and ratio insoluble glutenin-soluble protein) and mixing time (MT) (A), peak dough resistance (PDR) (B), bandwidth at peak dough resistance (BWPR) (C), and work input to peak (WIP) (D) of the four cultivar samples.

A.



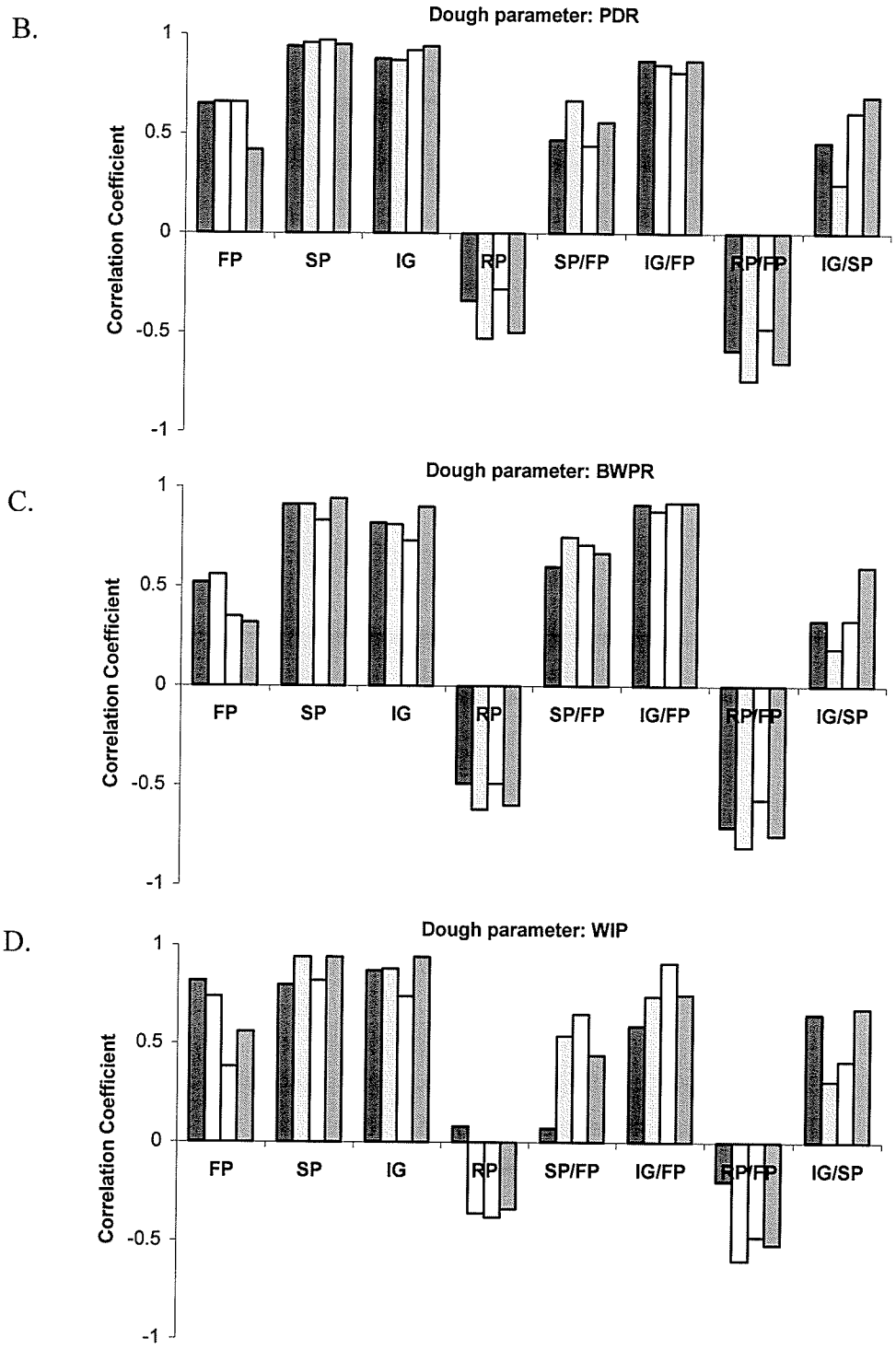


Figure 4.11. Correlations between protein composition fractions (flour protein FP, soluble protein SP, insoluble glutenin IG, residue protein RP, soluble protein in flour protein SP/FP, insoluble glutenin in flour protein IG/FP, residue protein in flour protein RP/FP, and ratio insoluble glutenin-soluble protein) and mixing time (MT) (A), peak dough resistance (PDR) (B), bandwidth at peak dough resistance (BWPR) (C), and work input to peak (WIP) (D) of the four cultivar samples for doughs with 2% salt.

MT was distinct among all four mixograph parameters, as no consistent correlation trend emerged across cultivar samples for any protein fraction including protein content (Fig. 4.10 and 4.11). As well correlation between MT and other dough mixing parameters were very low (refer to Table 3.3, Chapter 3). Other workers have reported similar results in attempting to establish a link between dough mix time and protein content or composition for straight grade flour samples of different genotypes (Khaktar et al., 1996). In controlled dough mixing experiments where base flours were enriched with gliadin or glutenin protein (Sapirstein and Fu, 1998; Uthayakumaran et al., 1999), adding gliadin or glutenin protein reduced and increased, respectively, mixograph mixing times. Flour millstreams, originating from relatively distinct parts of the wheat kernel, represent a much more complex type of flour material compared to straight grade flour (even of different genotypes) in terms of constituent composition. This complexity includes varying protein content and composition as shown in this study, varying pentosan content and composition (Delcour et al., 1999; Wang et al., 2005), and varying starch content, and possibly starch composition and granule size distribution about which nothing is known. Accordingly, understanding the molecular nature of peak dough development time, which is already problematic for conventional flour samples, poses many research challenges for flour millstreams.

In contrast to MT, other mixograph properties had much clearer and similar relationships to protein fractions. Two distinctly different relationships were observed: 1) millstream protein content along with SP, IG and SP/FP and IG/FP were all positively and often highly correlated with dough strength parameters WIP, PDR and BWPR, and

2) RP and RP/FP were negatively correlated with the same parameters, and accordingly contributed to dough weakness.

Among protein composition parameters, SP and IG content and IG/FP of millstreams were correlated most highly with mixograph torque (PDR), bandwidth (BWPR) and work input (WIP) at peak dough development, indicating a close association with strong dough properties. It is important to note that protein content of millstreams had invariably lower correlations than either SP or IG content and IG/FP, indicating that the protein quality of millstreams was a more important factor than protein quantity in relation to dough mixing properties.

Despite very high correlations between dough mixing properties PDR and BWPR (and WIP to a lesser extent) and protein fractions SP, IG and IG/FP, cultivar effects were evident in the millstream results. Three of the four cultivar samples used in

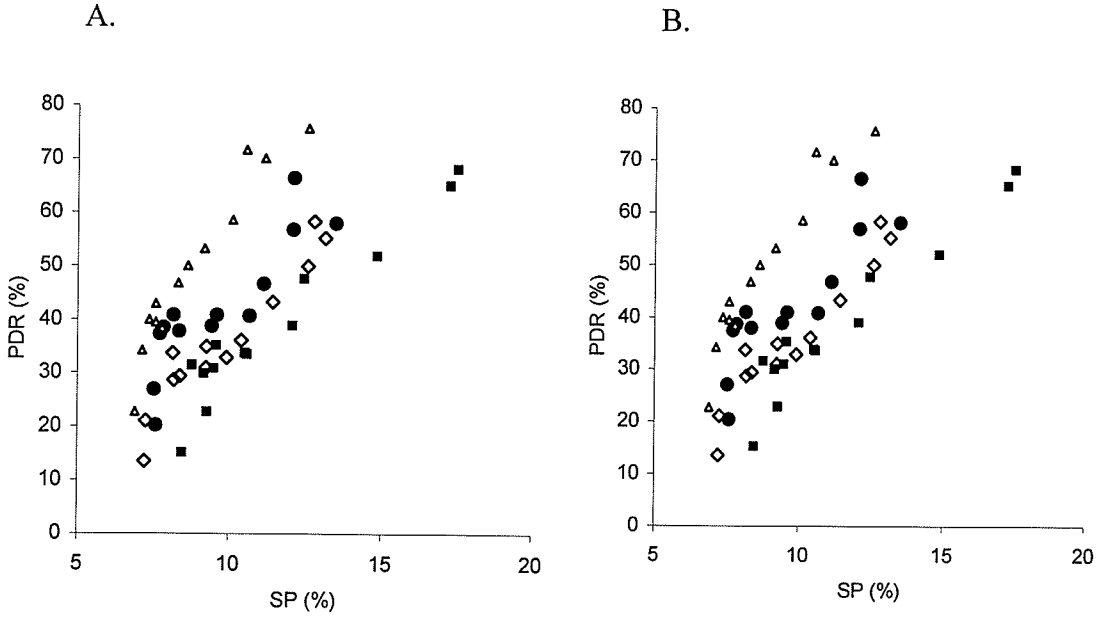


Figure 4.12. Peak Dough Resistance (PDR, %) in relation to (A) soluble protein (SP, %) and (B) insoluble glutenin (IG, %) content in millstreams of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇).

this study (Superb, AC Barrie and AC Snowbird) responded similarly to variation in dough mixing properties in relation to SP, IG and IG/FP, although each cultivar sample tended to follow a more or less separate trend depending on the protein fraction and mixing parameter under evaluation (e.g. Fig. 4.12.A, B, C). However, the CWES wheat sample AC Corinne could be readily distinguished in relationships involving the SP fraction in particular (Fig. 4.12.A and Appendix III Fig. 2); for all mixing parameters, the millstreams appeared to be much stronger than the SP fraction could account for. This result appears to stem from a statistical covariation between SP and IG ($r = 0.55$). Combining all samples, the correlation between millstream PDR values and SP content was $r = 0.70$. By comparison, the corresponding relationship between PDR and IG content of millstreams (Fig. 4.12.B) was much closer (overall correlation $r = 0.88$) as cultivar samples were relatively indistinct in IG content of millstreams. Accordingly, compared to the SP fraction, IG content of millstreams was a more accurate predictor of dough PDR as well as BWPR (refer to Appendix III Fig. 3) regardless of the cultivar sample; i.e. the higher the insoluble glutenin content of a millstream, the higher the dough torque and mixogram bandwidth at peak mix time.

Another problematic aspect of the strong positive association between SP content and PDR and BWPR, is that increasing levels of gliadins protein (i.e. SP) are well known to contribute to weaker dough properties (MacRitchie 1987; Skerritt et al., 1996; Weegels et al., 1995b). On the other hand, increasing levels of glutenin protein increase dough strength (Orth and Bushuk, 1972; MacRitchie, 1987; Gupta et al., 1992; Roels et al., 1993). Accordingly, results involving SP as a predictor of millstream dough strength were not considered to be reliable.

A more accurate comparison of the relative influence of SP and IG protein fractions in relation to dough mixing properties becomes evident when normalized results (SP/FP and IG/FP) are considered (Fig. 4.10); when averaged across cultivar samples, substantially higher correlations were obtained for IG/FP (Fig. 4.13). The proportion of insoluble glutenin in total protein explained considerably more of the variation in dough mixing properties of millstreams compared to the counterpart gliadins fraction (SP/FP).

While correlations were relatively high between IG/FP and the majority of mixograph dough mixing properties, a plot of PDR versus IG/FP (Fig. 4.14) reveals considerable scatter in the data for millstreams of higher dough strength (above about 40% PDR) reflecting sample or genotype differences in IG/FP. It is important to note as well, that millstreams of different genotypes having similar levels of IG/FP can be very

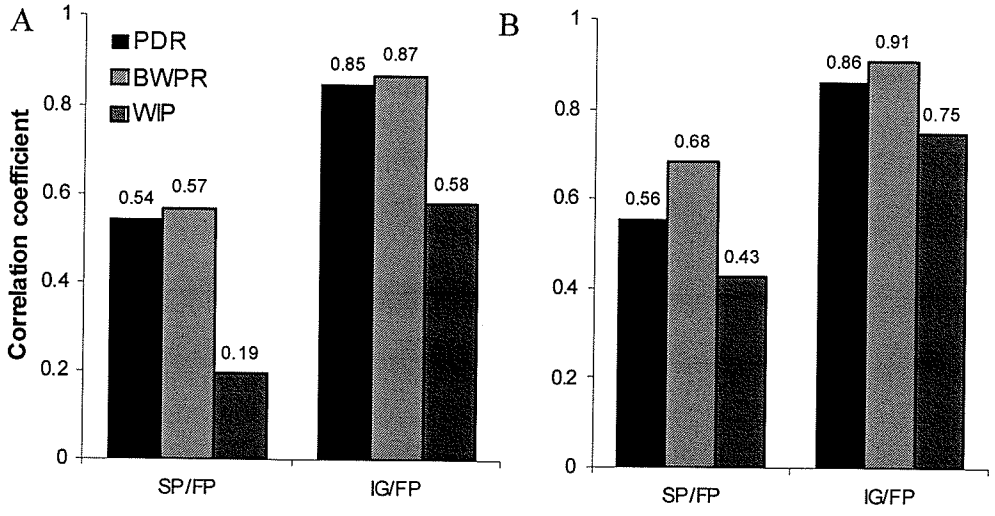


Figure 4.13. Correlations between normalized soluble protein (SP/FP) and insoluble glutenin (IG/FP) and indicated dough mixing properties for unsalted (A) and salted doughs (B).

different. For example, comparing AC Corinne and AC Barrie millstreams with similar IG/FP values of 17.3% and 17.2%, respectively, the corresponding PDR values were 22.8% and 52%, i.e. the pertinent millstream of AC Corinne is weaker than that for AC Barrie despite having similar levels of IG/FP. The corresponding millstreams of AC Corinne and AC Barrie that were the basis of this result were M6 and B3, respectively, i.e. the weakest millstream of AC Corinne, and close to the strongest millstream of AC Barrie. Whereas these millstreams had comparable levels of IG/FP, other protein fractions as well as detrimental components such as germ, were very different in respective quantities.

These results underscore the complexities that exist when comparing breadmaking properties and protein composition results for different millstreams of different genotypes. While it is important to understand that it is the balance of gliadin, glutenin and residue protein in any given millstream that largely determines breadmaking quality, differences in general can be ascribed to the glutenin component,

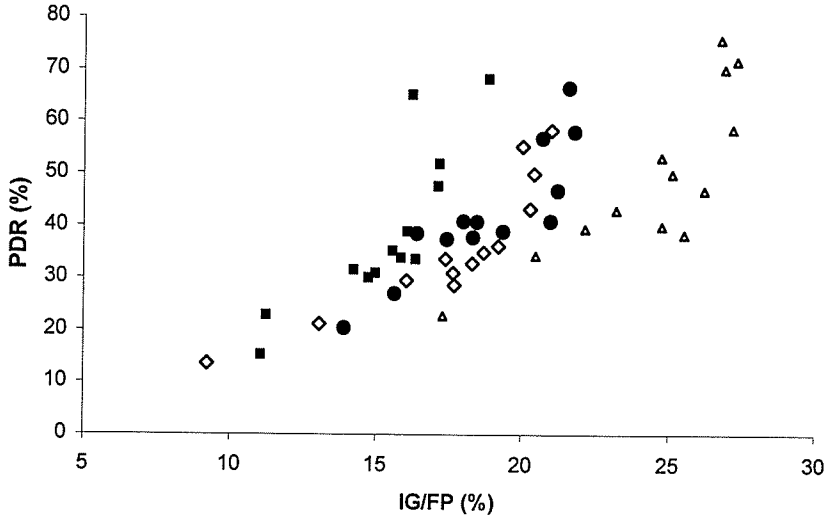


Figure 4.14. Relationship between peak dough resistance (PDR, %) and proportion of insoluble glutenin in flour protein (IG/FP) for millstreams of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇).

i.e. a strong dough must possess not only a high amount of IG, but also a high proportion of IG in the protein fraction.

Figure 4.13 indicates that among the mixograph parameters PDR, BWPR and WIP, the latter had the weakest relationships to IG/FP based on correlations (r = 0.58 on average across samples compared to r = 0.86). However, when WIP and IG/FP data are plotted (Fig. 4.15.A), it can be seen that the relationship is curvilinear and very tight for the salted doughs (Fig. 4.15.B). This explains the lower correlations that were generated between WIP and IG/FP which was based on a linear statistical analysis.

As has already been presented (Fig. 4.7), the RP fraction varied in an opposite manner to that of either SP and IG. Whereas the content of SP and IG protein decreased with decreasing millstream refinement, RP levels increased. As was previously observed (Fig. 3.2 Chapter 3), millstreams with the highest levels of RP, particularly tail-end reduction streams, had very poor dough mixing properties. Correlation results (Fig. 4.10)

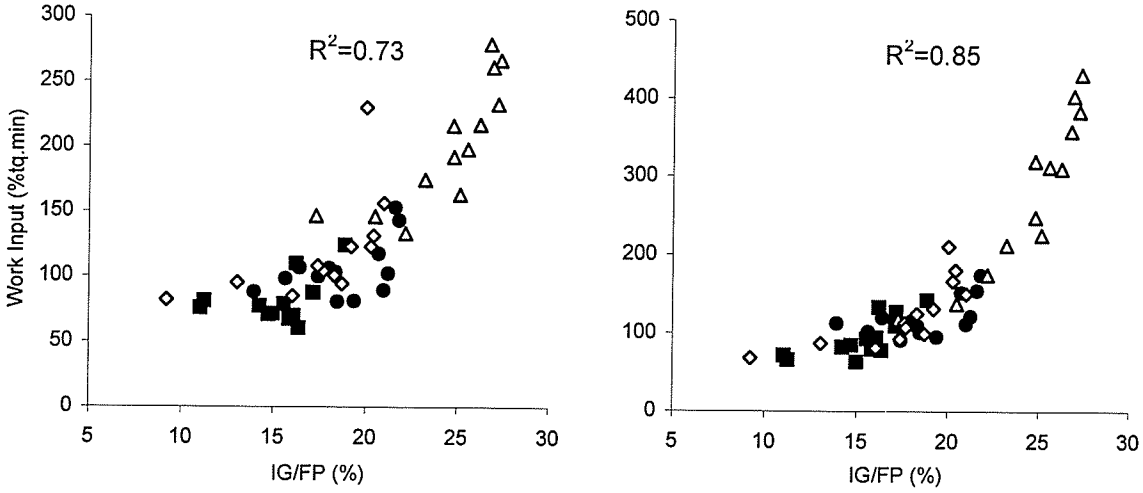


Figure 4.15. Relationship between work input to peak (WIP, %) and proportion of insoluble glutenin in flour protein (IG/FP) for millstreams of Superb (●), AC Barrie (■), AC Corinne (△), and AC Snowbird (◇).

clearly confirmed that RP and RP/FP, for all cultivar samples, had negative relationships to PDR, BWPR and WIP. This is in agreement with enrichment studies of a base flour of medium strength (MacRitchie 1987), where the residue proteins (after prior extraction of gluten proteins using HCl) caused dough weakening and depression of loaf volume. Sapirstein and Fu (1998), using the same protein fractionation as was used in this thesis research, also found by correlation analysis that the amount of final residue in total flour protein was negatively related to many breadmaking quality parameters.

4.4. CONCLUSIONS

Both millstreams and pearled wheat fractions displayed the same trends in protein composition variation. With decreasing flour refinement (increasing levels of bran contamination), both SP/FP and IG/FP decreased, while RP/FP increased. RP therefore has its origin in bran tissue. Furthermore, as a proportion of flour protein, RP content varied much more extensively across millstreams compared to SP and IG protein (381% vs. 83%). These results, and distinctly opposite trends of correlation between RP and dough mixing properties (invariably negative correlations) compared to those for SP and IG (positive correlations) clearly underscore the value of distinguishing and quantifying RP in wheat protein fractionation research.

With regard to the SP fraction, strongly positive correlations to millstream dough strength properties (PDR, BWPR and WIP) were problematic. In particular, the CWES cultivar AC Corinne could be readily distinguished in relationships involving the SP fraction; millstreams appeared to be much stronger than the SP fraction could account for. This result appeared to stem from covariation between SP and IG. Because the SP fraction is comprised mainly of gliadins protein, and the latter is generally associated

with dough weakness properties, results involving SP as a predictor of millstream dough strength were not considered reliable. Furthermore, for millstreams and pearled wheat fractions that contain significant amounts of bran, and hence bran constituents, it is plausible that the SP fraction contains increasing amounts of non-gluten proteins shown to be soluble in 50% propanol (Fu and Sapirstein, 1996), and other compounds such as thiols (glutathione), known to have significant effects on dough rheology (Villegas et al., 1963; Chen and Schofield, 1995). Also, this aspect deserves further attention.

Taking all the protein fractions into account, results indicated that millstreams with strong dough properties had high IG and SP content, high IG/FP values, and low levels of RP and RP/FP. Break streams on average, and B3 in particular, possessed these characteristics, whereas the latter reduction streams did not. These tail-end reduction streams (M5 and M6) had very weak dough mixing properties. Early reduction streams (M1 and M2) were intermediate in both technological quality and protein quality. The millstream with the strongest dough mixing characteristics for all cultivar samples was BF which had high levels of RP (similar to M4 and M5) but also distinctly high levels of IG that were ~ 25% higher on average than B3 millstreams. These results provide additional confirmation of the importance of HMW glutenin as the predominant wheat protein fraction associated with dough strength and breadmaking quality in general.

The distinct protein composition and dough mixing behaviour of late reduction streams as well as BF, while scientifically interesting, are not practically significant as the contribution of these millstreams to the overall yield of flour is very small, about 3% on average. As well, the strong dough mixing properties of BF flour is very likely offset by the weak mixing late reduction streams. Consequently, when these streams are blended together into straight grade flour, no effect is likely to be seen.

Particularly noteworthy were RP-HPLC results of reduced insoluble glutenin showing no proportional changes in individual HMW- and LMW-GS content even for millstreams of widely different dough mixing properties (M1, B3, BF). This result indicates that glutenin only varies in concentration, but not composition, from inner to outer endosperm. The qualities of wheat millstreams previously ranked by dough mixing properties alone using the farinograph and extensograph (Holas and Tipples, 1978; Preston et al., 1982) were similar to those obtained in the present study. Accordingly, results of this study provide for the first time, an explanation of dough mixing properties of millstreams based on protein composition or quality (in terms of proportions of IG, SP and RP). Compared to the latter, protein content was a much less important factor in explaining dough mixing properties of millstreams. For straight grade flour samples, Khatkar et al. (1996) also concluded that differences in the mixing characteristics among weak and extra strong wheat cultivars are due mainly to differences in gluten protein quality with protein content playing only a minor role.

Among the dough mixing properties that were studied, results indicated that PDR and BWPR were most strongly related to all protein composition variables, particularly IG and IG/FP. WIP was somewhat less consistent in this regard, while MT showed no consistent pattern of relationship to protein fractions and could not be predicted. MT continues to be widely used in reporting dough rheological properties of normal straight grade flours, but its association with protein composition and bread making quality is not strong (Dong et al., 1992; Roels et al., 1993; Khatkar et al., 1996; Martinant et al. 1998; Sapirstein and Fu, 1998). This study confirmed that when the mixograph is used at constant water absorption, MT was not a reliable parameter to estimate the quality of millstreams, and could not be predicted from protein composition data.

In conclusion, the present investigation has contributed considerable new knowledge on relationships between flour millstreams and protein composition. On the practical side, this information provides millers with knowledge of properties of individual millstream that will help optimize the blending of different millstreams for the production of flours of specified characteristics for different end-uses.

GENERAL DISCUSSION AND CONCLUSIONS

The main focus of this thesis research project was to investigate inter-relationships among measures of dough rheology and protein composition of wheat millstreams. The material for this study comprised eight red and white hard spring wheat samples comprising six western Canadian cultivars in five commercial classes including two grown in two different locations. Wheat was milled on a tandem Buhler MLU-202 laboratory mill which was equipped with six corrugated rolls on the first mill, and six smooth reduction rolls on the second, providing extensive stock separation. A total of 16 mill products were generated: four break roll flours (B1-B4), one so-called quality fraction (Q1), one sizing roll flour (S1), six middling or reduction roll flours (M1-M6), and three by-products: bran, fine bran, and shorts. The bran was passed through a bran finisher and rebolted to provide bran flour (BF). The extraction rate was set at 80% for a check wheat control, but ranged from 76.2% (AC Snowbird 2) to 81.4% (AC Corinne), depending on the wheat sample. Also, four of the wheat samples (Superb, AC Barrie, AC Corinne, and AC Snowbird) were pearled in six steps, each generating product representing 10% by weight of the original wheat sample or previously pearled residue. These fractions were used for comparison with millstreams protein composition results (Chapter 4).

The degree of mill product refinement was measured by ash content. Based on ash results, S1 flour (0.40%) and M1 and M2 reduction roll flours (0.36% average) were the most highly refined streams which is consistent with their typical commercial use in patent flour blends. Refinement decreased for the balance of break roll flours (ash contents are indicated in parentheses): B2 (0.45%), B1 and B3 (combined ash of 0.53%),

B4 (0.52%), and Q1 (0.68%). The ash content of M3 flour was 0.70%. Flour streams of lowest refinement were BF (2.28% ash) and the final two reduction streams M5 and M6 with ash contents of 1.95 and 2.93%, respectively. Protein content increased progressively from B1 (14.9%) to B4 (19.2%) and M1 (12.3%) to M6 (19.1%). These trends reflect the well-known gradient of minerals and protein, which increase from the center of the endosperm to the peripheral layers of the wheat kernel (Morris et al., 1945, 1946, Hinton, 1947, 1959, 1962, Stevens et al., 1963, Kent, 1966, Kent and Evers, 1969). The BF fraction, in which subaleurone cells are likely concentrated (Kent, 1966) contained the highest protein content of all millstreams (23.8%), a characteristic typical of hard wheats (Kent, 1966). The reduction streams originating on average from inner endosperm particles (Hinton, 1947; Kent, 1966; Orth and Mander, 1975; Nelson and McDonald, 1977; Black et al., 1981; Endo et al., 1987) had lower protein contents than the break streams. Overall, ash and protein contents of millstreams were consistent with previous studies (Izydorczyk et al., 2003; Black et al., 1981; Preston and Dexter, 1994), and indicated that break and reduction flours were different in composition. In subsequent experiments, it was found that ash content results, while closely related to flour colour, was not related to dough mixing properties. And while mixograph measures of dough strength and protein content were moderately correlated ($r = 0.57$), protein composition was a much more effective predictor in that regard.

The ash content of pearled wheat increased by about 500% from the inner to the outermost fractions (1.08 to 4.94%). These ash contents were on average much higher than those of the millstreams, in-line with the nature of pearling as a pearling process, i.e. pearling fractions maximize bran as opposed to maximizing endosperm product which is the aim in roller-milling. However, similar to roller-milling, the protein

contents of the pearled wheat fractions reflected an increasing gradient from inner endosperm to the outer layers of the kernels, up to the 20% fraction presumably containing the high protein subaleurone layer. Protein content increased on average from about 16 to 22% from the 60% to the 20% pearled wheat fractions. The 10% pearled fraction (outermost layer) was lower in protein content (19.8%) than the 20% fraction (22.0% protein), analogous to roller milling bran fractions in which the protein content (15.8% on average) was lower than that of BF (23.7%) and M6 flour (19.1%).

Because the millstream flours covered such a wide range of ash and protein content, as well as colour, this material provided an excellent opportunity for a study on measuring flour refinement. The objective was to comprehensively evaluate the relationship between flour ash, colour and brightness using a new instrumental approach based on a computerized diode array colour spectrophotometer. This instrument is capable of simultaneous measurement of reflectance across the entire visible spectrum as well as tristimulus colour coordinate values. In industry, flour refinement is commonly measured by ash content data and/or single wavelength colour (546 nm) provided e.g. by a filter-based Agtron instrument (per AACC Official Method 14-30). In this study, it was shown that although Agtron results as well as L* (brightness) values, were highly correlated with ash content (r^2 = 0.90 and r^2 = 0.93, respectively), there were discrepancies in millstream discrimination and deficiencies in accuracy for determining refinement. Colour spectrophotometry results clearly indicated the efficacy of using 400 nm as the basis to measure flour colour in general and refinement in particular. From the standpoint of discrimination among millstreams, 400 nm was clearly superior to green Agtron measurements at 546 nm. Also, flour bleaching results showed that 400 nm was effectively outside the range of the absorption of flour carotenoid pigments

which have absorbance maxima at about 440 and 475 nm (Sims and Lepage, 1968). Accordingly determining flour refinement at 400 nm could also be accomplished even with bleached flour. Reflectance values at 400 nm were highly correlated to ash content (r^2 = 0.93) and to CIE colour system parameters L*, a* and b*. As reported in the literature, the principal limitation of using flour colour to determine bran contamination arises from differences in light reflectance between bran from red and white wheats (Li and Posner, 1989). It was previously found that wheats of diverse origins cannot be compared (Shuey and Skarsaune, 1973; Shuey, 1975; Barnes, 1986). However, the present thesis research has shown that reflectance at 400 nm was insensitive to wheat colour (red and white wheats were compared) even for flour streams (e.g. M6) containing the highest levels of (ash) bran contamination (ash > 3.0%). Furthermore, 400 nm was less sensitive than the Agtron-based 546 nm to the negative contribution of colour by flour protein. These results strongly suggest that measuring flour colour reflectance at 400 nm has its basis in the absorbance of a chemical biomarker of bran. This aspect merits further research. As well, it would be useful to know the extraction rate above which Agtron flour colour begins to be influenced by wheat colour.

It should be noted as well, that because the standard AACC Method 14-30 is optimized for straight grade flour, it determines relative reflectance on an arbitrary expanded scale between 0 and 100% created by using calibration disks of 63 and 85% reflectance, respectively. As a result, negative reflectance values are generated for low grade flour streams such as M5, M6 and BF.

The computerized diode array colour spectrophotometer was clearly a very effective instrument for evaluating flour colour and refinement. Because of its capability to simultaneously measure both CIE colour coordinates as well as reflectance on a

continuous scale from 400 to 700 nm, it presents extra advantages over filter based instruments. If the latter could be adapted for use with an appropriate 400 nm filter, such an instrument would represent a very satisfactory low cost solution for measuring flour refinement with greater accuracy and precision than current methods accommodate.

The principal goal of this thesis research was to establish the extent and nature of relationships between dough mixing properties assessed using the mixograph and the protein component of different millstreams. Part of the rationale for using the mixograph is that there have been no reports on its use for millstream analysis, despite its popularity to evaluate dough mixing properties of straight grade flours, especially in cultivar development activities. Also, compared to the farinograph, the mixograph mixes doughs at higher intensity, so it represents a more appropriate instrument to study the mixing behaviour of stronger bread wheat cultivars, especially CWES wheats, one of which was included in this study.

Given the wide range of protein content and refinement of millstreams, large differences in dough mixing properties were expected and found. Interestingly, flour refinement as measured by ash content was not correlated with any of the mixing parameters although it seemed to play a role in the mixing properties of the last two reduction streams, M5 and M6 (see below). When considering dough mixing properties within cultivar samples in relation to ash content, protein content and composition, results showed that dough development time in the mixograph was an unreliable measure of dough strength as no consistent patterns of correlation were evident. However, among the different genotypes or cultivar samples, some differences were evident; the CWES sample, AC Corinne, had the longest MT, and AC Barrie, had the shortest. MT, whether from the mixograph or farinograph, continues to be widely used

in reporting dough rheological properties of normal straight grade flours. However, its association with breadmaking quality and protein composition is, on the whole, not strong (Roels et al., 1993; Khatkar et al., 1996; Dong et al., 1992; Martinant et al. 1998). The present research indicates that a factor other than protein content or composition is responsible for differences in dough development time among millstreams of varying refinement.

In contrast to MT, PDR and BWPR, and WIP to a lesser extent, were found to be very effective measures of dough strength. High PDR and BWPR values have been previously associated with good dough strength and good breadmaking quality (Khatkar et al., 1996). For break flours, increasing protein content and increasing gluten protein fractions SP and IG, from B1 to B4 was accompanied by a corresponding increase in PDR and BWPR, suggesting a cause and effect relationship, although the apparent positive contribution of SP to dough strength appeared to be due to statistical co-variation ($r = 0.55$) with IG (see below). For reduction streams for which protein content increased by an even greater amount from M1 to M6, the correspondence between PDR and BWPR and protein content was not clear. This was especially evident for the last two reduction streams, M5 and M6, which possessed very high ash contents (2.4% on average), high protein contents (17.7% on average) and produced very weak doughs. Protein composition appeared to be a key factor in the dough mixing properties of these low grade flours; RP content was very high (400% greater than the average for break flours), gluten protein content was considerably lower (SP plus IG, 33% lower than the average for break flours).

The addition of salt increased all four mixograph parameters, with a greater effect on PDR and BWPR, however the extent of the effect of salt on specific

millstreams was variable among samples. For doughs mixed both without and with salt, very similar patterns of correlation were found between all protein fractions and dough mixing parameters, although correlations were slightly higher on average for the salted dough set. Compared to other cultivar samples, salt had a greater strengthening effect on millstreams of the CWES sample. The sensitivity of dough rheological properties to salt appears to be due to ionic interaction of salts with gluten proteins (Hlynka, 1962; Tanaka and Tipples, 1969; Danno and Hoseney, 1982), which result in stronger inter-protein hydrophobic and hydrophilic interactions and consequently increased aggregation (Bernardin, 1978; Preston, 1981). The distinct strengthening effect of salt on AC Corinne doughs can be attributed to its substantially higher concentration of IG protein, i.e. HMW glutenin. This is in line with previous reports indicating that among the various protein fractions of wheat, glutenin was most sensitive to salt effects (Huebner, 1970; Kim and Bushuk, 1995).

The protein composition results of millstreams were very compelling, in part because the knowledge base on this subject is largely non-existant, and also because the protein composition variation of millstreams provided very good explanation of their dough strength properties. Both millstreams and pearled wheat fractions showed the same patterns of variation in protein composition, and results were very consistent across c ultivar samples. A veraged across c ultivar samples for roller-milled flours, the range of concentration of gluten protein fractions SP and IG (expressed as a percentage of millstream protein content) varied from 38 to 69% and 12 to 22%, respectively, and was positively correlated with flour refinement. In contrast, RP was much more variable, ranging by about 330% from lowest levels in break flours (11% on average) to highest levels in M6 flours (47% on average), and was negatively correlated with flour

refinement. Break streams, on average, had higher concentrations of SP/FP (65%) and IG/FP (21%) compared to reduction streams (55% SP/FP and 17% IG/FP). In contrast, break streams on average, had lower levels of RP (14%) compared to reduction streams (25% RP).

These results for the first time provide evidence that RP in millstreams, and most likely straight grade flour in general, derives from contaminating bran residue. Sapirstein and Fu (1998) previously investigated the nature of RP in straight grade flour by SDS-PAGE, and found it contained relatively low amounts of glutenin comprising mainly Glu-D1 subunits, with the balance being lower M_r subunits of unknown identity. They concluded that the RP fraction contained a large proportion of non-gluten protein consistent with structural protein as described previously (Byers et al., 1983; Kruger et al., 1988). The RP fraction merits more attention than it has received to date, as it likely represents a significant negative factor in flour breadmaking quality. Interestingly, among all the protein fractions investigated by Sapirstein and Fu (1988), the RP protein was the only one that was significantly (negatively) correlated with dough extensibility, whose biochemical basis has been very elusive in the cereal chemistry literature. These results also point to the importance of separating residue protein from IG in protein fractionation work, or glutenin-related tests to predict dough mixing properties.

Another noteworthy result was that provided by RP-HPLC analysis of reduced IG of different millstreams. There were large differences in total IG subunit composition of selected millstreams of widely varying protein content and refinement that closely reflected quantitative differences in total IG contents. However, no differential amounts of HMW or LMW subunit amounts were found, i.e. relative glutenin subunit concentrations in M1 flour was identical to that of B3 and BF flours. Accordingly,

glutenin subunit composition appeared to be identical regardless of the origin of the millstreams, whether from the center of the kernel or its periphery; only the concentration of glutenin varied.

Among protein composition parameters, SP and IG content and IG/FP of millstreams were correlated most highly with mixograph torque (PDR), bandwidth (BWPR) and work input (WIP) at peak dough development, indicating a close association with strong dough properties. This study also showed that MT could not be predicted from protein composition data. It was noteworthy that protein content of millstreams had invariably lower correlations than either SP or IG content and IG/FP, indicating that the protein quality (composition) of millstreams was a more important factor than protein quantity in relation to dough mixing properties. Many other workers have previously shown that protein composition was more important than protein content in determining dough mixing properties of flour (Orth and Bushuk, 1972; Khan et al., 1989; Dong et al., 1992; Roels et al., 1993; Weegels et al., 1995; Skerritt et al., 1996).

Taking all the protein fractions into account, results indicated that millstreams with strong dough properties had high IG and SP content, high IG/FP values, and low levels of RP and RP/FP. Break streams on average, and B3 in particular, possessed these characteristics, whereas the latter reduction streams did not. These tail-end reduction streams (M5 and M6) had very weak dough mixing properties. Early reduction streams (M1 and M2) were intermediate in both technological quality and protein quality. BF, based on its very high ash content, poor colour and high levels of RP (similar to M4 and M5) appeared to be a low quality stream. However, this millstream had the strongest dough mixing characteristics for all cultivar samples. A likely explanation for this result

was that BF had a distinctly high level of IG/FP, ~ 25% higher on average than the next strongest millstream, B3. As bran flour likely contains the highest level of subaleurone endosperm among millstreams, results also indicate that the subaleurone layer in wheat likely contains the highest concentration of HMW glutenin subunits. These results provide additional confirmation of the importance of HMW glutenin or IG as the predominant wheat protein fraction associated with dough strength and breadmaking quality in general.

In conclusion, this thesis research has resulted in a promising new approach for measuring wheat flour refinement, and has contributed considerable new knowledge on wheat flour millstreams, their physical properties, and inter-relationships between flour millstream refinement, dough strength and protein composition. Knowledge of millstream protein composition, particularly IG and RP fractions, appears to be very beneficial to gain a more complete fundamental understanding of flour breadmaking quality. On the practical side, this information can provide millers with knowledge of properties of individual millstreams that will help to optimize blending for the production of flours with specific characteristics for different end-uses.

CONTRIBUTION TO KNOWLEDGE

- A new approach to measuring flour refinement was investigated. Diode array colour spectrophotometry showed that measuring flour reflectance at 400 nm was much more effective than the conventional Agtron green wavelength (546 nm) for measuring flour refinement which is the basis for the official AACC Method 14-30 for measuring flour colour. Assessing flour colour at the shorter wavelength resulted in much greater discrimination of millstreams according to ash contents. In contrast to the Agtron method, measuring flour reflectance at 400 nm, produced results that were independent of grain colour, regardless of millstream refinement, and were also less sensitive to the influence of protein content on flour colour.
- This thesis research has characterized the dough mixing properties of wheat millstreams for the first time using the mixograph, at constant water absorption. Millstream dough rheology has been previously studied using the farinograph and Do-Corder only. The mixograph, which mixes doughs with greater intensity compared to the farinograph, has seen widespread use in North America and Australia for evaluating dough mixing properties of straight grade flours, especially in cultivar development activities. Results showed that flour millstreams had very diverse mixing properties, that could be very well measured and distinguished using the mixograph.
- Wheat flour millstreams of six wheat genotypes were characterized for the first time in terms of protein composition by solubility fractionation. There has been only one previous report (> 25 years ago) investigating the protein composition of

millstreams of two U.S. cultivar samples by a size-exclusion chromatography technique. Results provided considerable new information regarding the molecular basis for the functionality of flours of varying refinement. Whereas predicting the dough mixing quality of millstreams according to ash and protein contents was very poor and marginal, respectively, protein composition parameters were often very highly correlated suggesting cause and effect relationships, particularly for the IG fraction. Results provide additional evidence supporting the importance of HMW glutenin or IG as the predominant wheat protein fraction associated with dough strength and breadmaking quality in general.

- Because the variation of RP in millstreams varied in an opposite fashion to that of gluten protein fractions SP and IG, and was highly positively correlated with ash content, RP most likely derives from protein of bran origin, which has not been reported previously. RP was also the most variable protein fraction among millstreams, varying from 13 to 50% of total protein depending on millstream source. Results point to the importance of distinguishing RP from IG in wheat protein fractionation procedures to ensure that insoluble glutenin is not confounded with non-gluten (insoluble) protein, as the RP fraction likely represents a significant negative factor in flour breadmaking quality.
- MT of millstreams was an unreliable measure of millstream dough strength and could not be predicted from protein composition data. A factor other than protein content or composition appears to be responsible for differences in mixograph dough development times. In contrast, mixograph torque (PDR), bandwidth (BWPR) and work input (WIP) at peak dough resistance were highly correlated

with SP and IG content and IG/FP. Results indicated that these mixing parameters best described dough properties. However, high positive correlations between SP (mainly gliadins) and dough mixing strength parameters such as PDR, BWPR, and WIP appear to result from statistical covariation with IG protein content, and likely do not represent a true cause and effect relationship.

- The subaleurone layer of wheat appears to be the source of the highest concentration of HMW glutenin in the wheat kernel. This conclusion was reached based on the nature of bran flour (it contains the highest level of subaleurone endosperm among millstreams) combined with protein composition data showing BF having distinctly high levels of insoluble glutenin, ~ 25% higher on average than the next strongest millstream (B3), and on the protein composition of pearled fractions with the 20% fraction having the highest levels if IG. As well, BF had the strongest dough mixing characteristics consistent across all cultivar samples.
- Different millstreams of the same genotype, regardless of their level of refinement, contain identical composition of glutenin subunits in identical proportions. For example, the ratio of HMW-to-LMW glutenin subunits is the same regardless of their origin in wheat endosperm. Only the concentration of subunits as a whole varied, corresponding to the protein concentration of the parent IG fraction.

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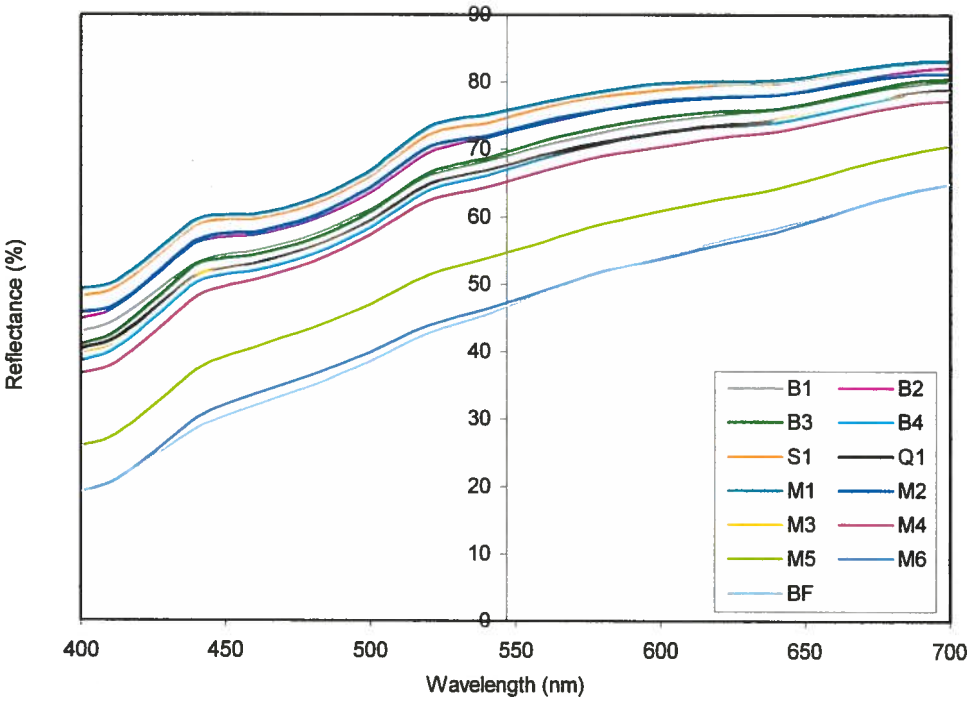
APPENDIX I



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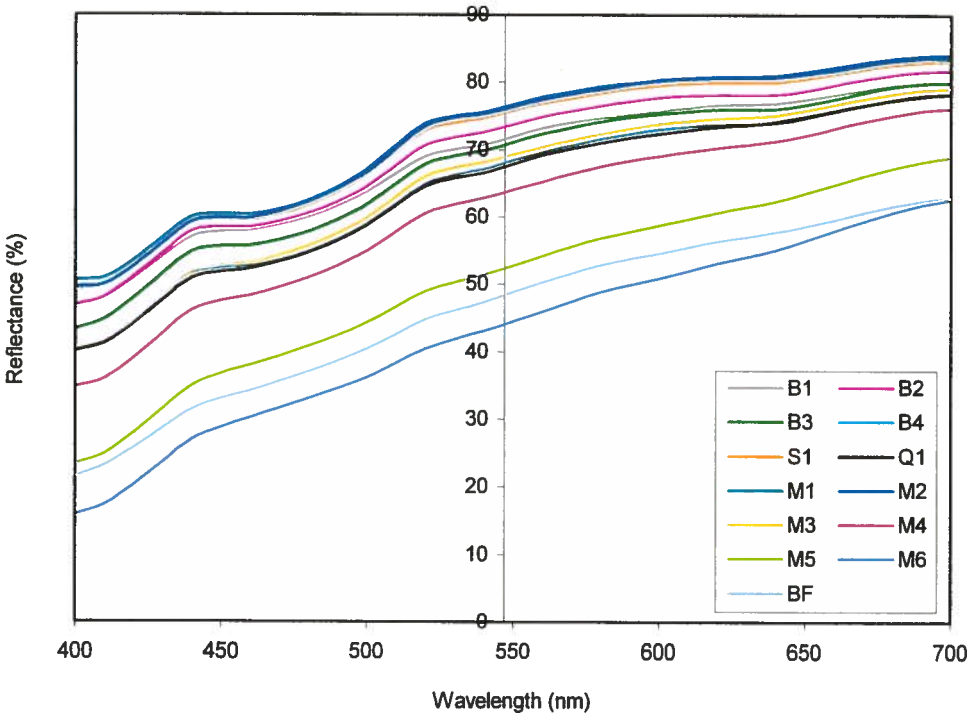

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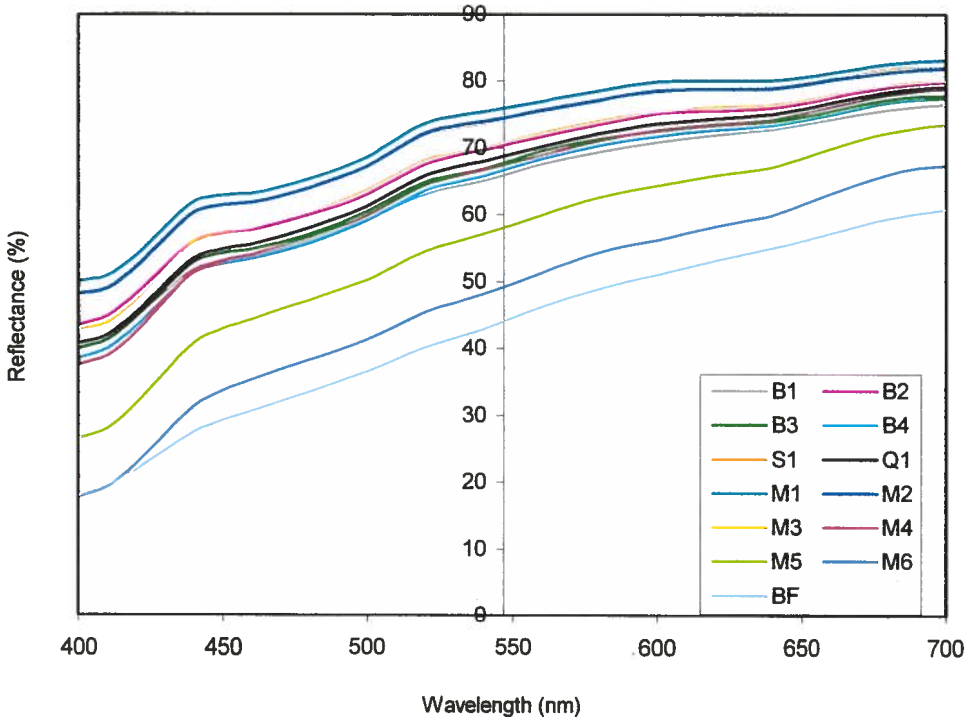

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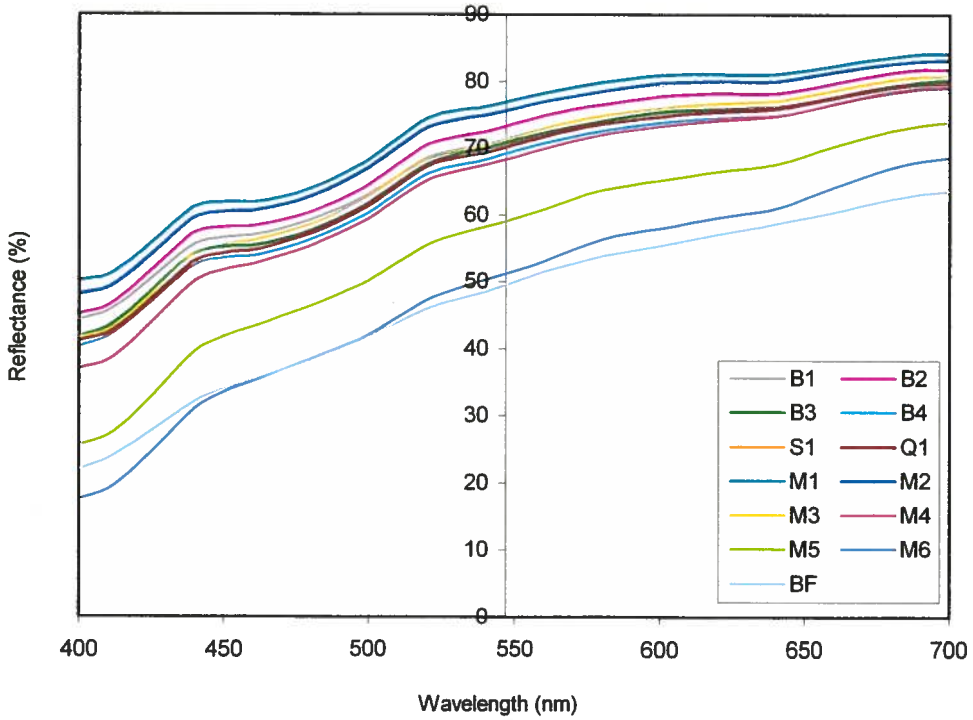

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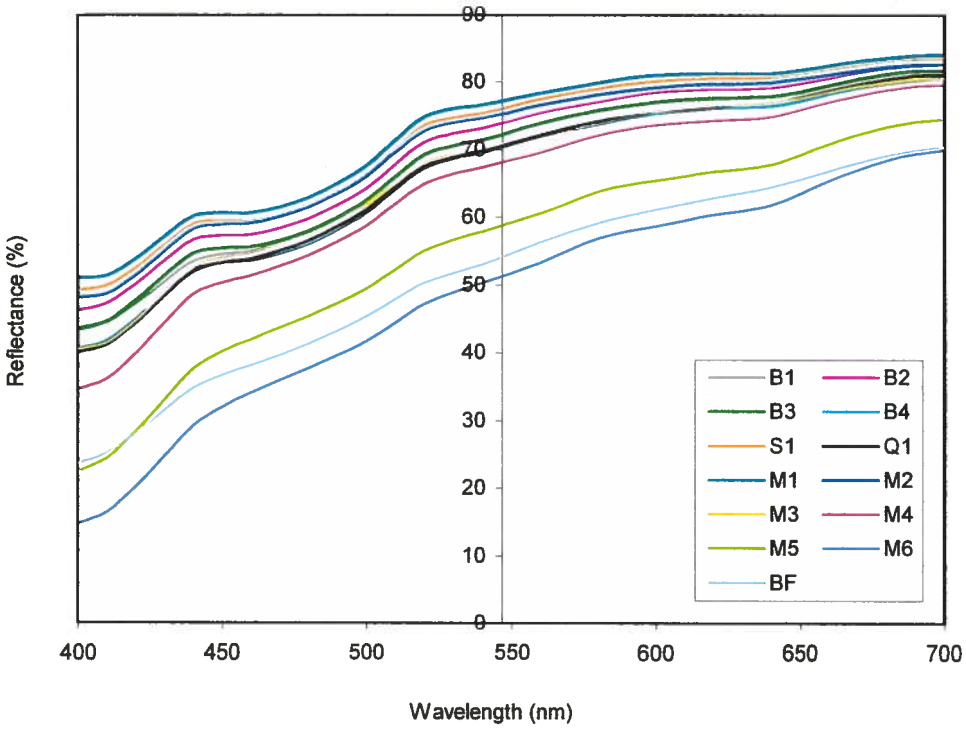

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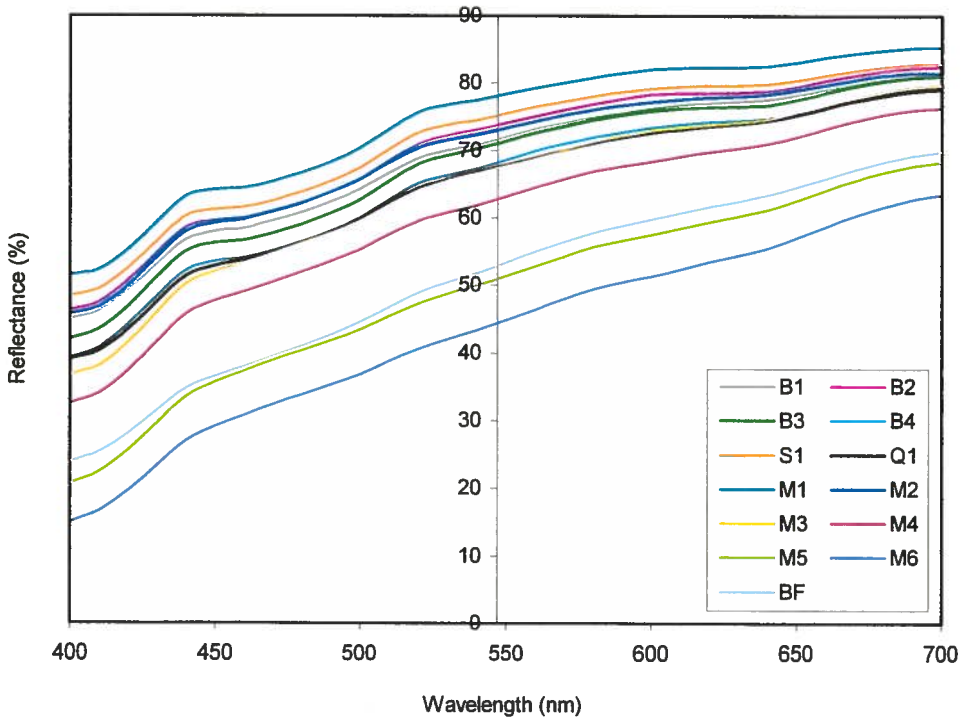

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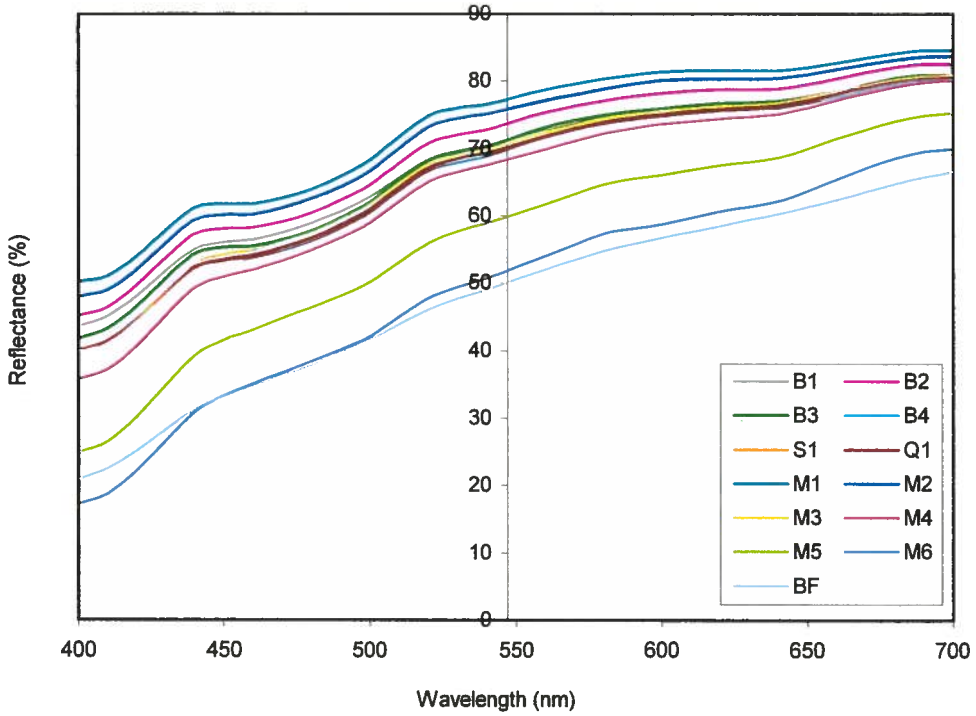
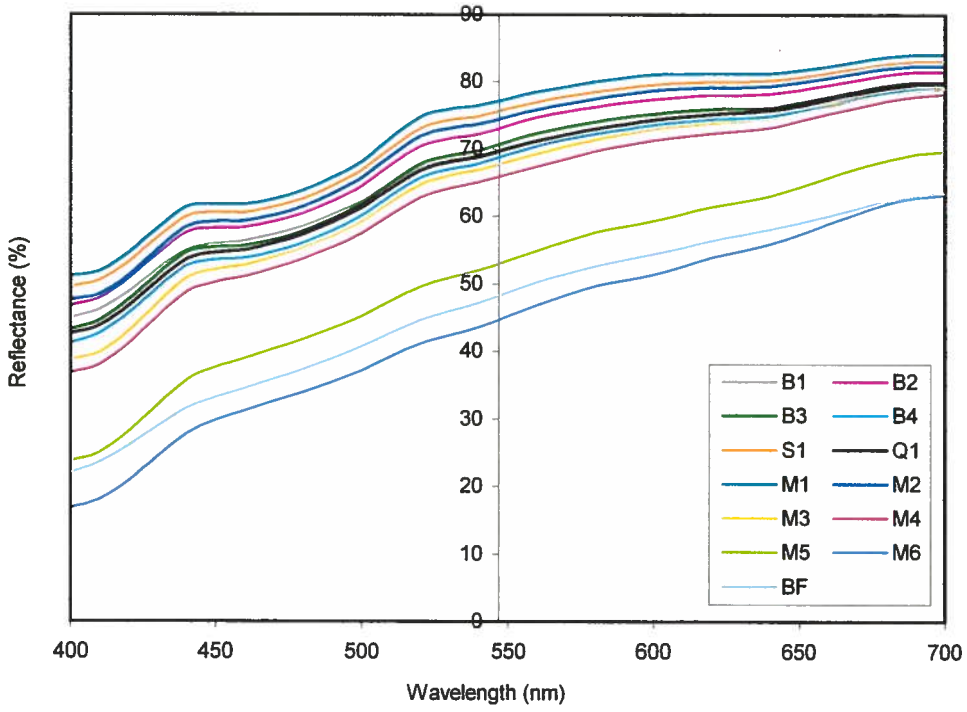


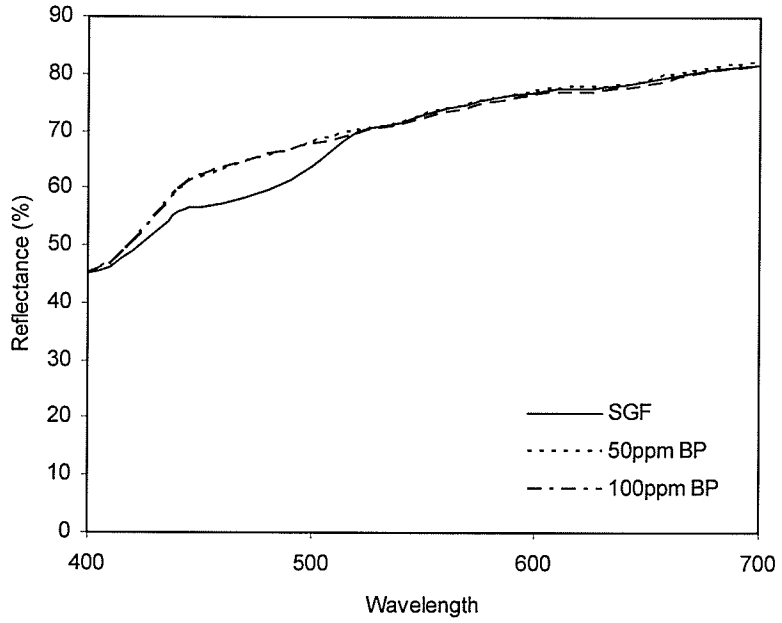
G. AC Snowbird 2



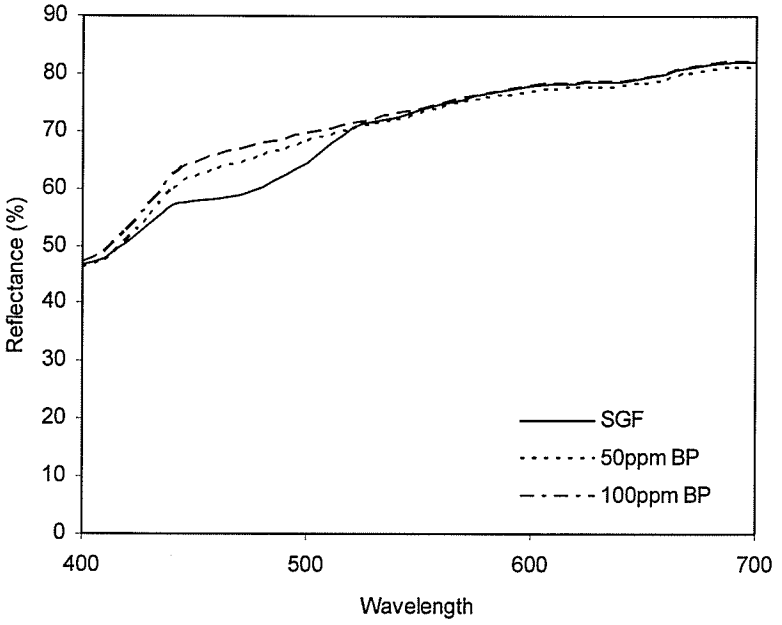
H. Superb 2

Figure 1. Reflectance spectra obtained from the Minolta spectrophotometer for millstreams of each cultivar sample.

A.


90
80
70
60
50
40
30
20
10
0
Reflectance (%)
400
500
600
700
Wavelength
SGF
50ppm BP
100ppm BP

B.


90
80
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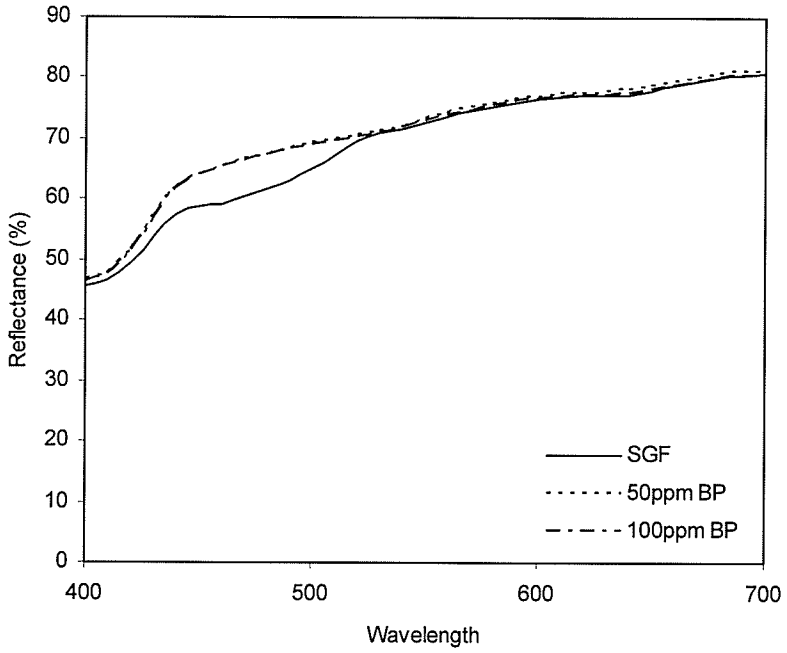
0
Reflectance (%)
400
500
600
700
Wavelength
SGF
50ppm BP
100ppm BP

C.



D.

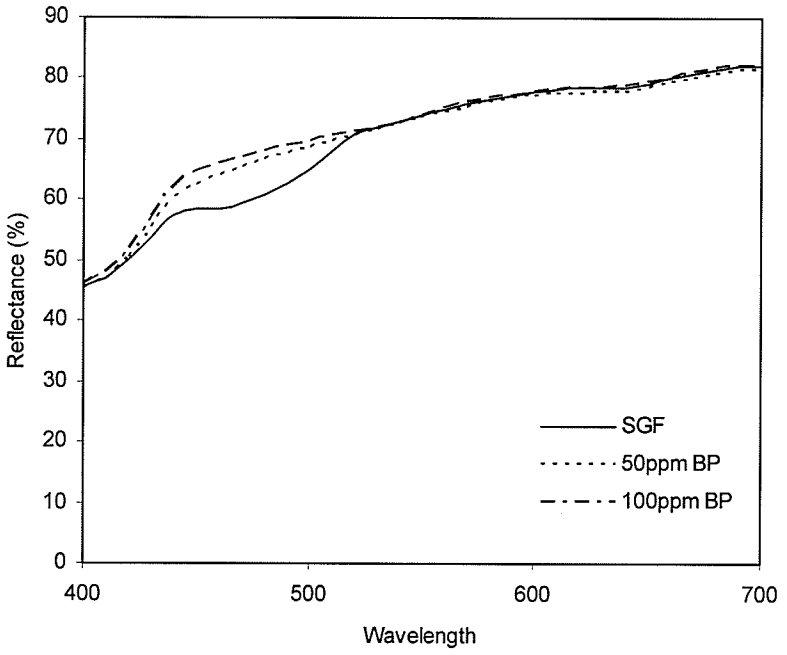


Figure 2. Reflectance spectra of unbleached and bleached straight-grade flours (SGF) from Superb (A), AC Barrie (B), AC Corinne, (C), and AC Snowbird (D).

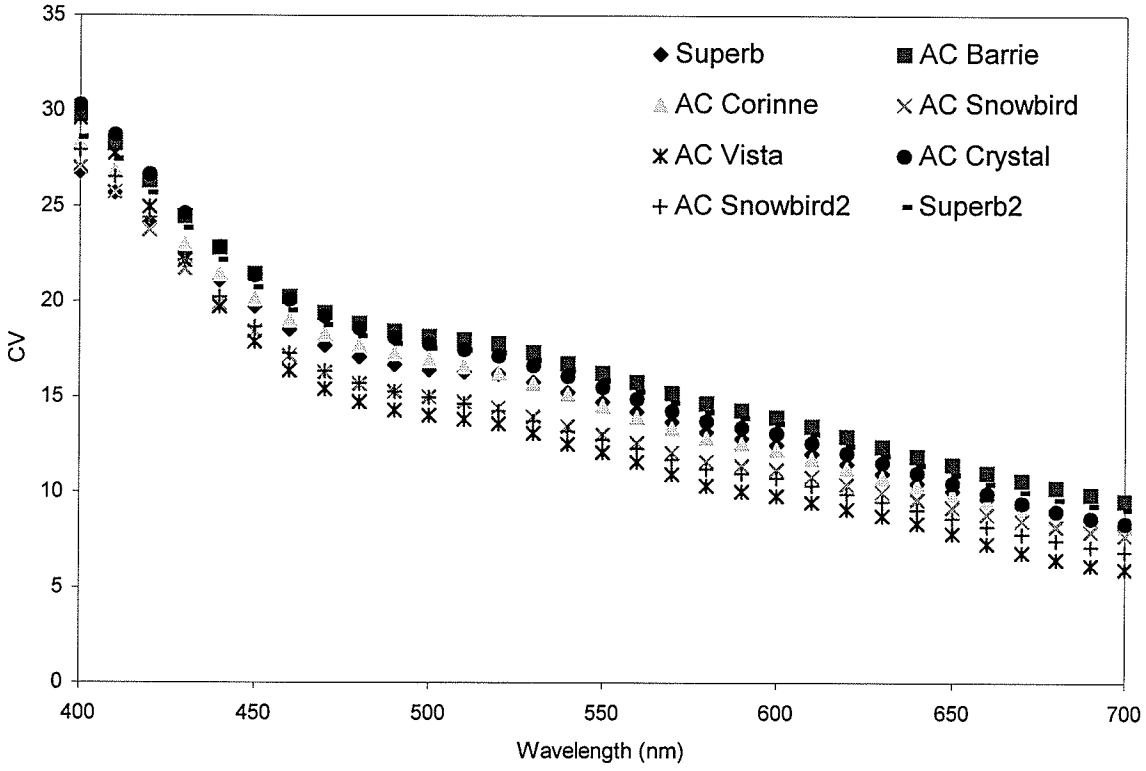


Figure 3. Coefficients of variation calculated among millstreams for each variety and for each wavelength, used for the determination of the most discriminant wavelength in colour measurement.

APPENDIX II

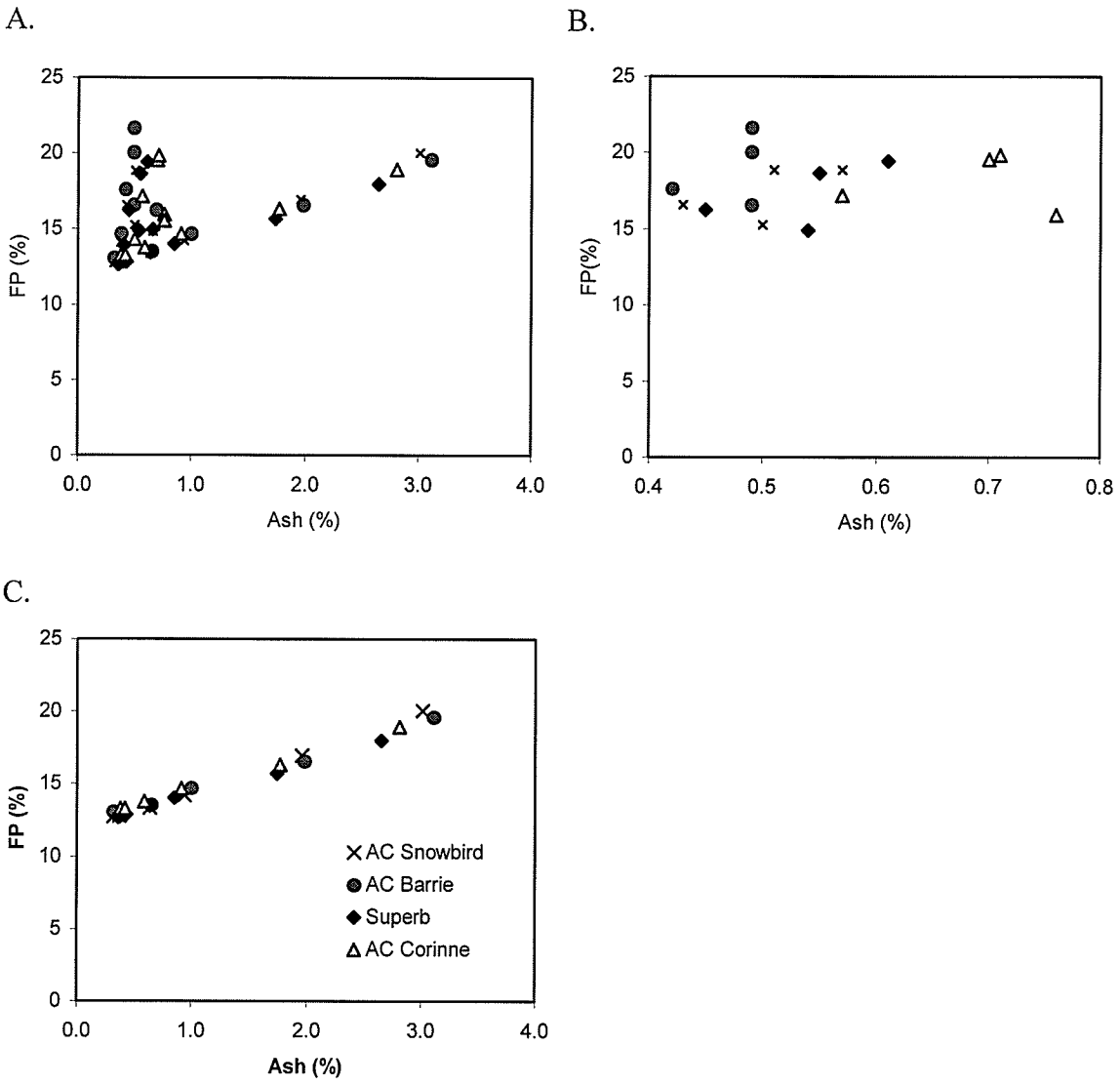


Figure 1. Relationships between flour protein and ash content (A) of all millstreams, (B) of break streams, and (C) of reduction streams, of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

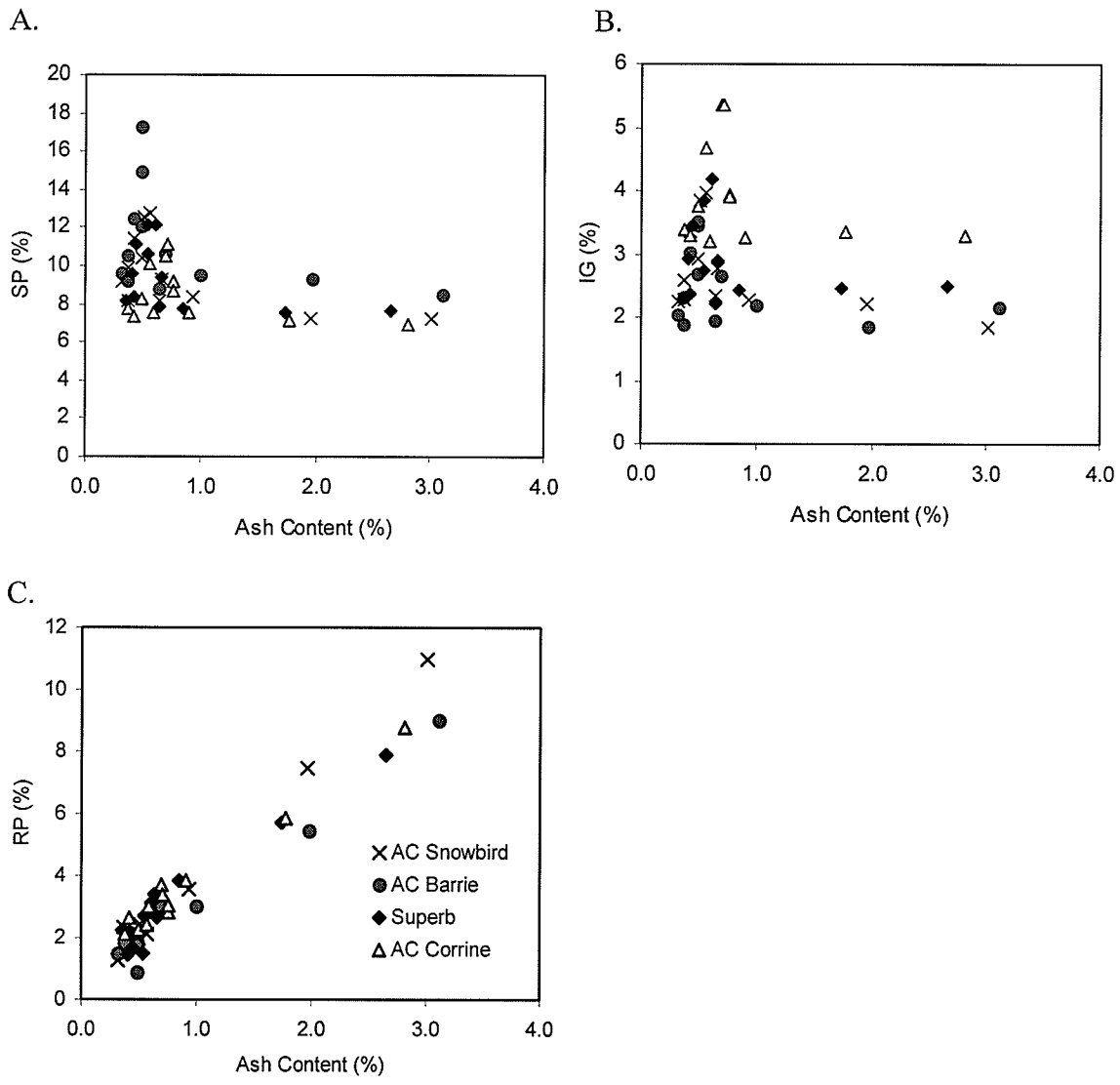


Figure 2. Variation in (A) soluble protein (SP), (B) insoluble glutenin (IG), and (C) residue protein (RP) in flour as a function of flour refinement of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

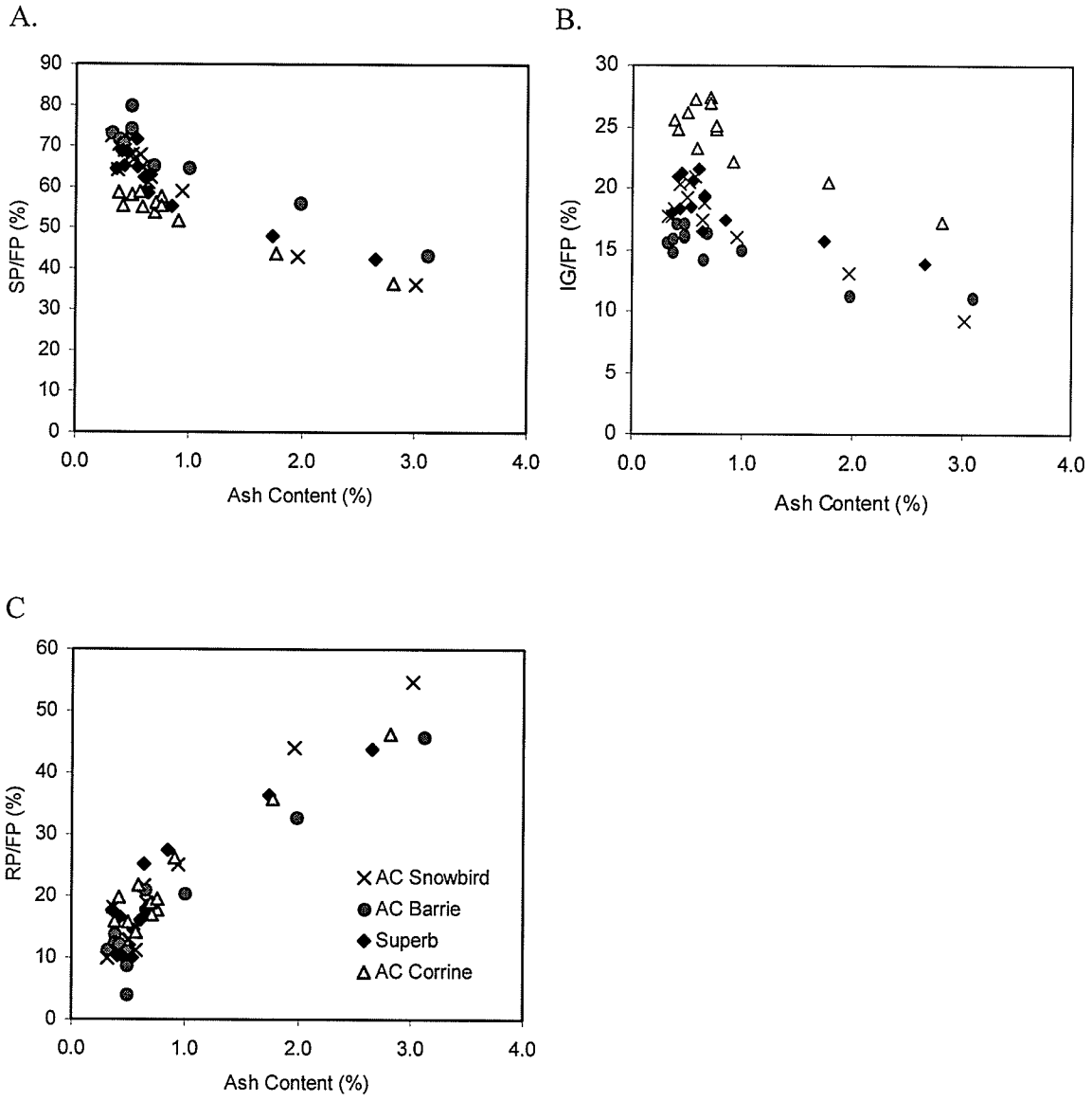


Figure 3. Variation in (A) soluble protein (SP), (B) insoluble glutenin (IG), and (C) residue protein (RP) as a percentage of flour protein as a function of flour refinement for millstreams of samples AC Snowbird, AC Barrie, AC Corinne, and Superb.

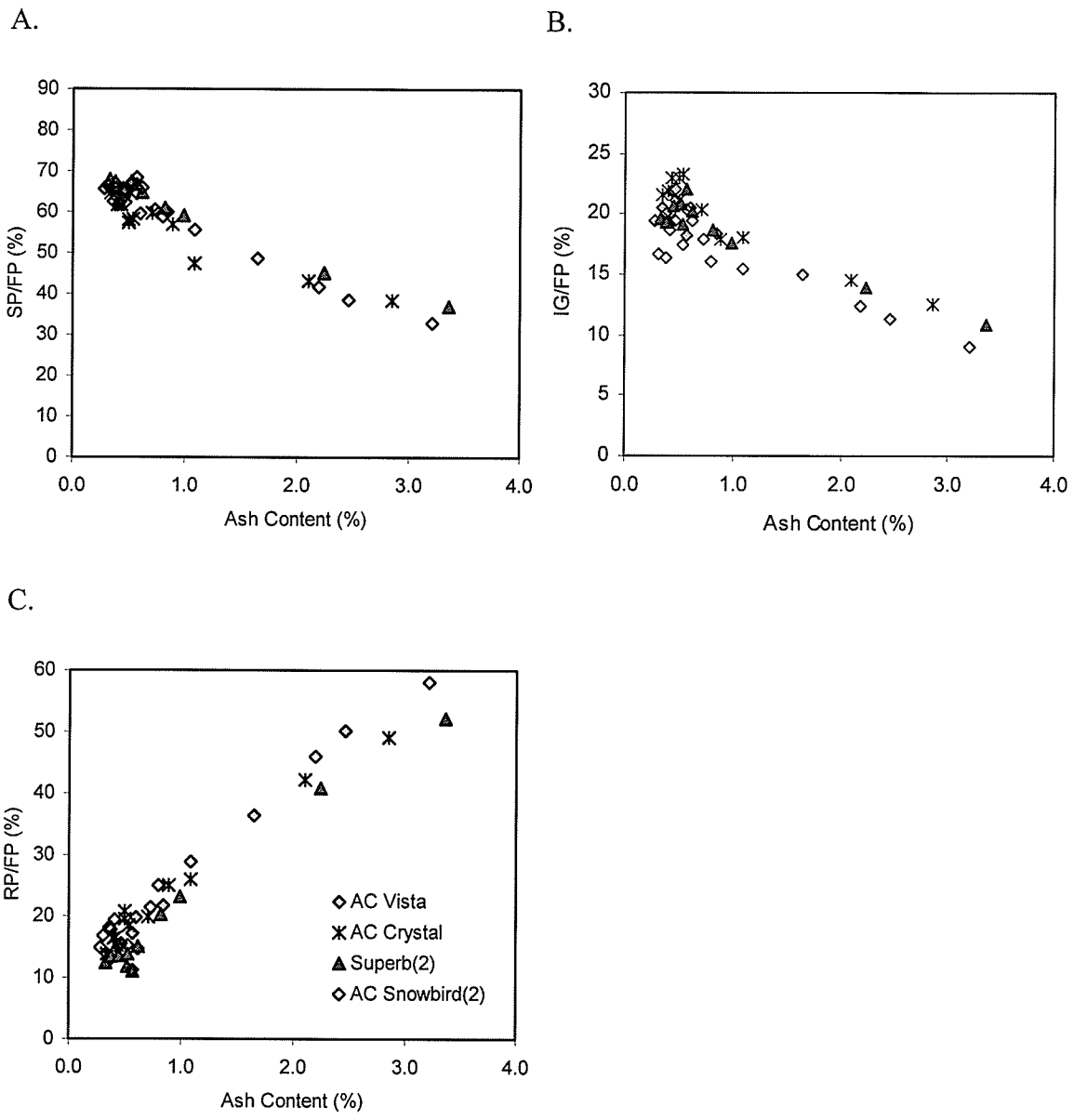


Figure 4. Variation in (A) soluble protein (SP), (B) insoluble glutenin (IG), and (C) residue protein (RP) as a percentage of flour protein as a function of flour refinement for millstreams of samples AC Vista, AC Crystal, Superb2, and AC Snowbird2.

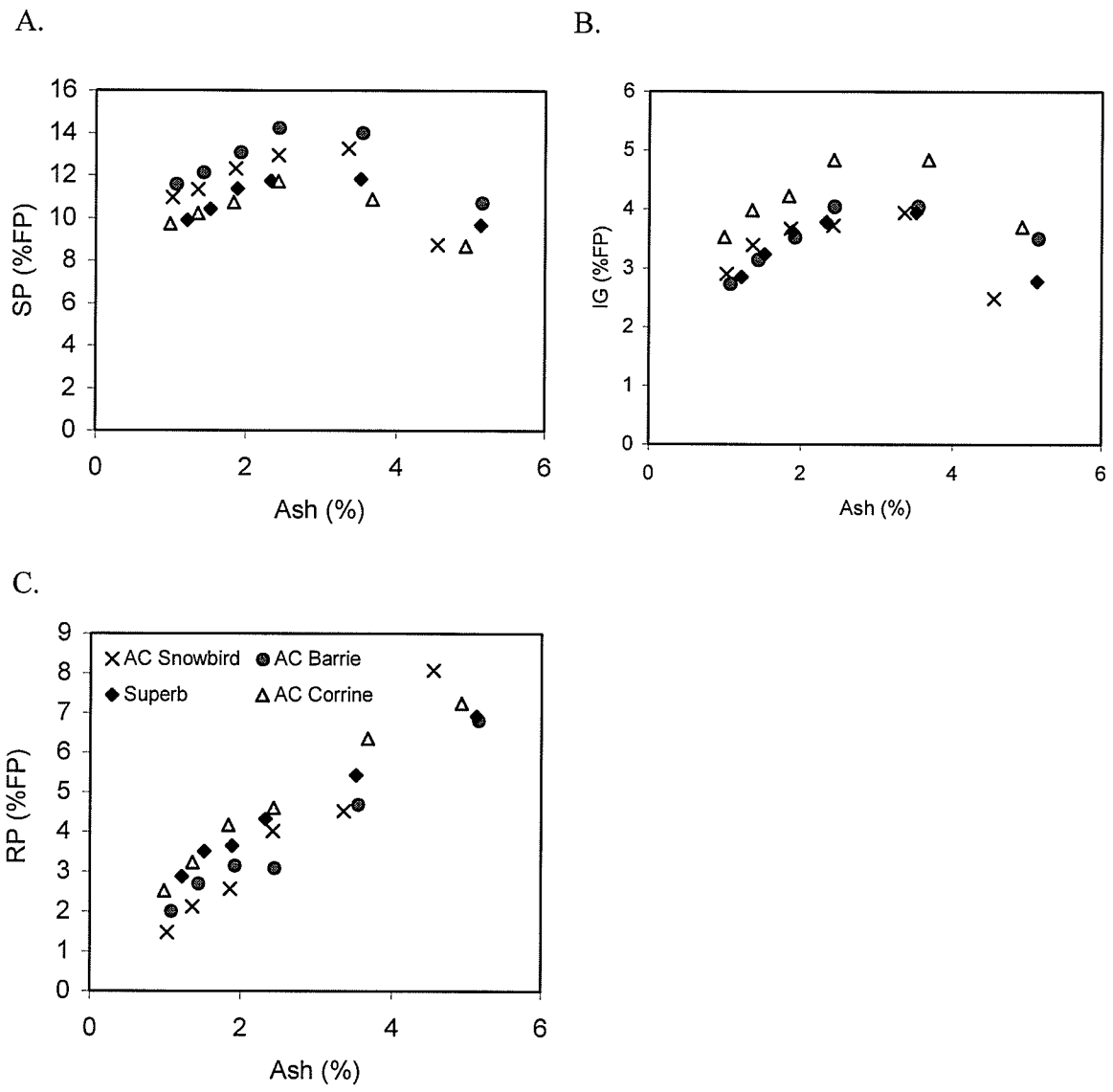


Figure 5. Variation in (A) soluble protein (SP), (B) insoluble glutenin (IG), and (C) residue protein (RP) in flour as a function of flour refinement in pearled fractions of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

APPENDIX III

Table 1. Correlation coefficients (r) between the mixograph parameters and the protein composition of the millstreams for each genotype, without and with salt. ***p < 0.001; **p < 0.01; *p < 0.05

	Superb (w/o salt)				Superb (2% salt)			
	MT	**PDR**	**BWPR**	**WI**	**MT**	**PDR**	**BWPR**	**WI**
FP	0.40	0.53	0.59*	0.66*	0.59*	0.65*	0.52	0.82***
SP	-0.19	0.87***	0.92***	0.62*	0.16	0.94***	0.91***	0.80***
IG	0.04	0.81***	0.85***	0.74**	0.35	0.88***	0.82***	0.87***
RP	0.84***	-0.43	-0.42	0.09	0.94*	-0.34	-0.49	0.08
SP/FP	-0.79**	0.52	0.54	-0.02	-0.51	0.47	0.60*	0.07
IG/FP	-0.51	0.87***	0.88***	0.51	-0.12	0.87***	0.91***	0.59*
RP/FP	0.77**	-0.64*	-0.65*	-0.11	0.45	-0.59*	-0.71**	-0.20
IG/SP	0.44	0.38	0.38	0.65*	0.55*	0.46	0.33	0.65*
	AC Barrie (w/o salt)				**AC Barrie (2% salt)**			
	MT	**PDR**	**BWPR**	**WI**	**MT**	**PDR**	**BWPR**	**WI**
FP	-0.14	0.69**	0.71**	0.84***	0.25	0.66*	0.56*	0.74**
SP	-0.63*	0.96***	0.97***	0.83***	-0.18	0.96***	0.91***	0.94***
IG	-0.50	0.89***	0.91***	0.83***	-0.01	0.87***	0.81***	0.88***
RP	0.87***	-0.49	-0.47	-0.01	0.72**	-0.53	-0.62*	-0.36
SP/FP	-0.89***	0.63*	0.62*	0.18	-0.64*	0.67*	0.75**	0.54
IG/FP	-0.88***	0.83***	0.84***	0.43	-0.42	0.85***	0.88***	0.74**
RP/FP	0.93***	-0.70**	-0.69**	-0.24	0.63*	-0.74**	-0.81***	-0.60*
IG/SP	0.03	0.28	0.31	0.35	0.34	0.25	0.19	0.31
	AC Corinne (w/o salt)				**AC Corinne (2% salt)**			
	MT	**PDR**	**BWPR**	**WI**	**MT**	**PDR**	**BWPR**	**WI**
FP	0.08	0.64*	0.51	0.61*	0.40	0.66*	0.35	0.38
SP	0.28	0.96***	0.92***	0.90***	0.72**	0.97***	0.83***	0.82***
IG	0.28	0.92***	0.85***	0.87***	0.69**	0.92***	0.73**	0.74**
RP	0.11	-0.28	-0.37	-0.16	-0.10	-0.28	-0.49	-0.38
SP/FP	0.36	0.45	0.60*	0.44	0.51	0.44	0.71**	0.65*
IG/FP	0.50	0.82***	0.91***	0.81***	0.80***	0.81***	0.92***	0.91***
RP/FP	0.12	-0.48	-0.50	-0.34	-0.20	-0.48	-0.57*	-0.48
IG/SP	0.25	0.61*	0.49	0.63*	0.49	0.61*	0.33	0.41
	AC Snowbird (w/o salt)				**AC Snowbird (2% salt)**			
	MT	**PDR**	**BWPR**	**WI**	**MT**	**PDR**	**BWPR**	**WI**
FP	0.46	0.42	0.50	0.69**	0.88***	0.35	0.32	0.56*
SP	0.11	0.95***	0.97***	0.81***	0.49	0.95***	0.94***	0.94***
IG	0.23	0.94***	0.97***	0.90***	0.55*	0.92***	0.90***	0.94***
RP	0.37	-0.50	-0.44	-0.09	0.48	-0.58*	-0.60*	-0.34
SP/FP	-0.28	0.56*	0.51	0.17	-0.32	0.64*	0.67*	0.44
IG/FP	-0.13	0.87***	0.84***	0.56	-0.04	0.92***	0.92***	0.75**
RP/FP	0.25	-0.65*	-0.61*	-0.27	0.26	-0.72**	-0.75**	-0.52
IG/SP	0.29	0.69**	0.72**	0.80***	0.45	0.64*	0.6*	0.68*

Cont'd

	All (w/o salt)				All (2% salt)			
	MT	**PDR**	**BWPR**	**WI**	**MT**	**PDR**	**BWPR**	**WI**
FP	0.06	0.55***	0.52***	0.35**	0.21	0.57***	0.42**	0.27
SP	-0.34*	0.70***	0.63***	0.09	-0.13	0.74***	0.69***	0.11
IG	0.45***	0.88***	0.90***	0.82***	0.64***	0.86***	0.80***	0.78***
RP	0.26	-0.36**	-0.35*	0.04	0.26	-0.36**	-0.47***	-0.01
SP/FP	-0.54***	0.29*	0.24	-0.29*	-0.41**	0.34*	0.43**	-0.17
IG/FP	0.62***	0.73***	0.81***	0.84***	0.75***	0.69***	0.75***	0.87***
RP/FP	0.38**	-0.49***	-0.45***	0.04	0.22	-0.51***	-0.59***	-0.06
IG/SP	0.82***	0.44**	0.52***	0.87***	0.85***	0.38**	0.37**	0.82***

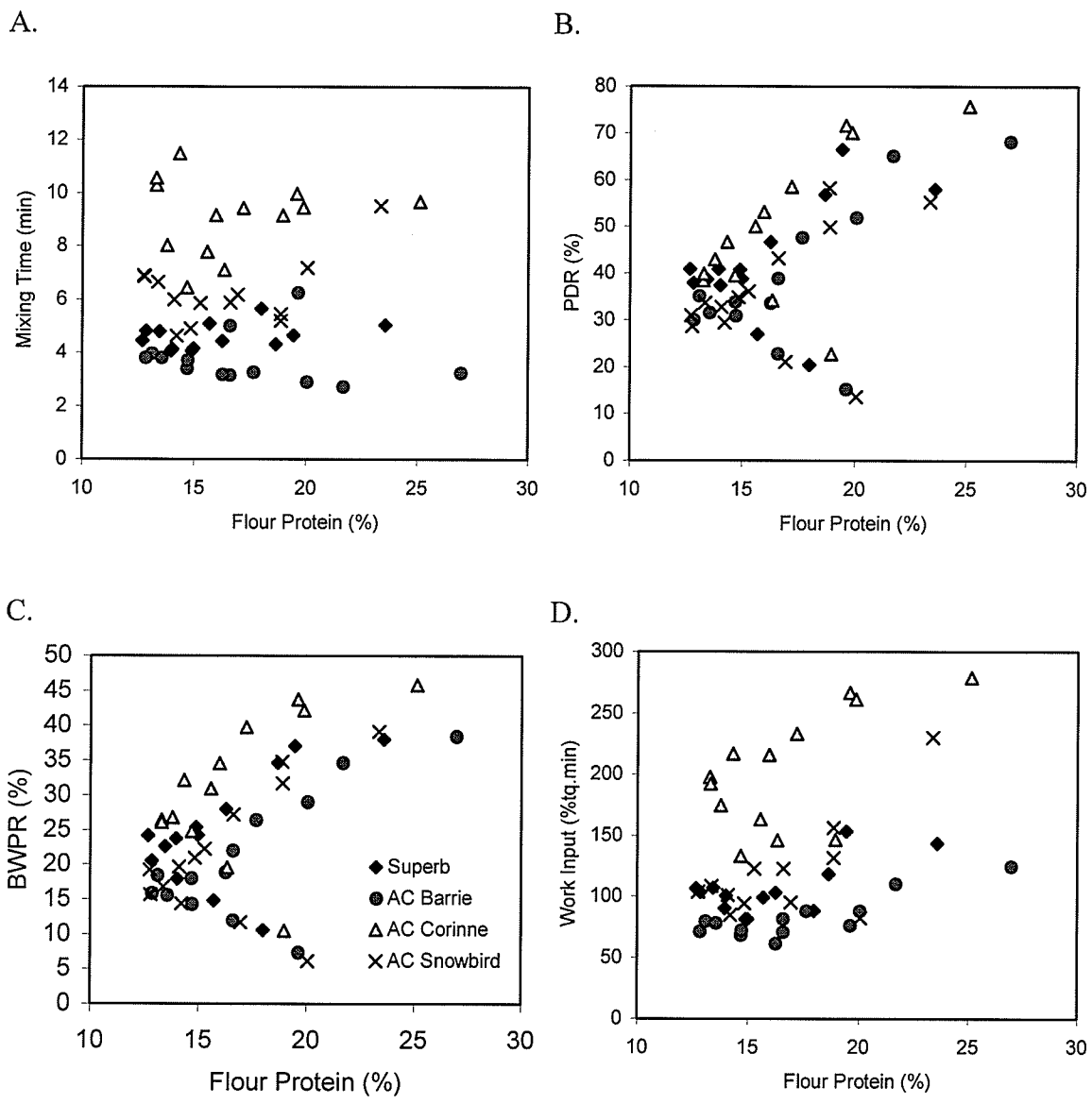


Figure 1. Relationships between flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

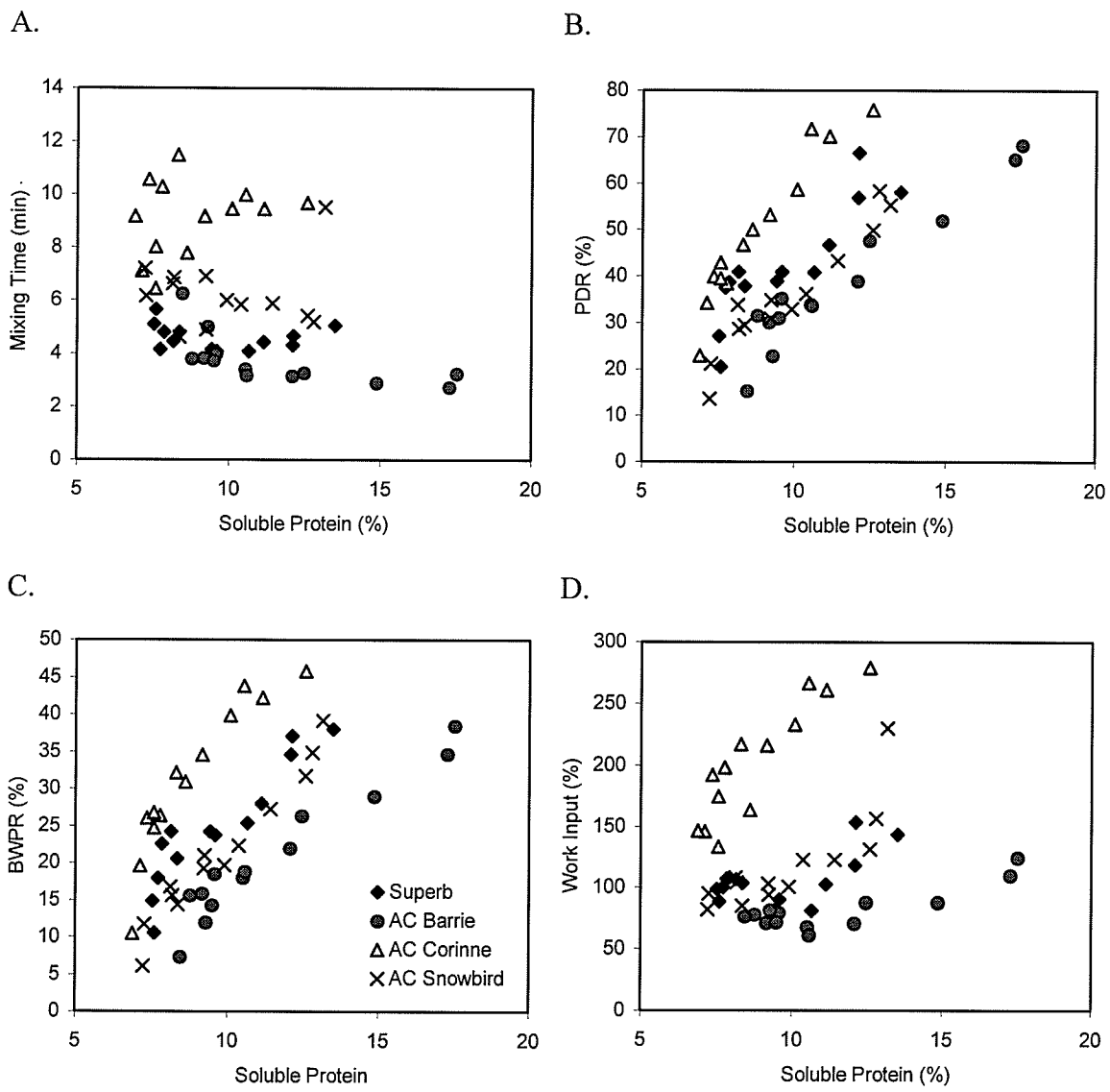


Figure 2. Relationships between soluble protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

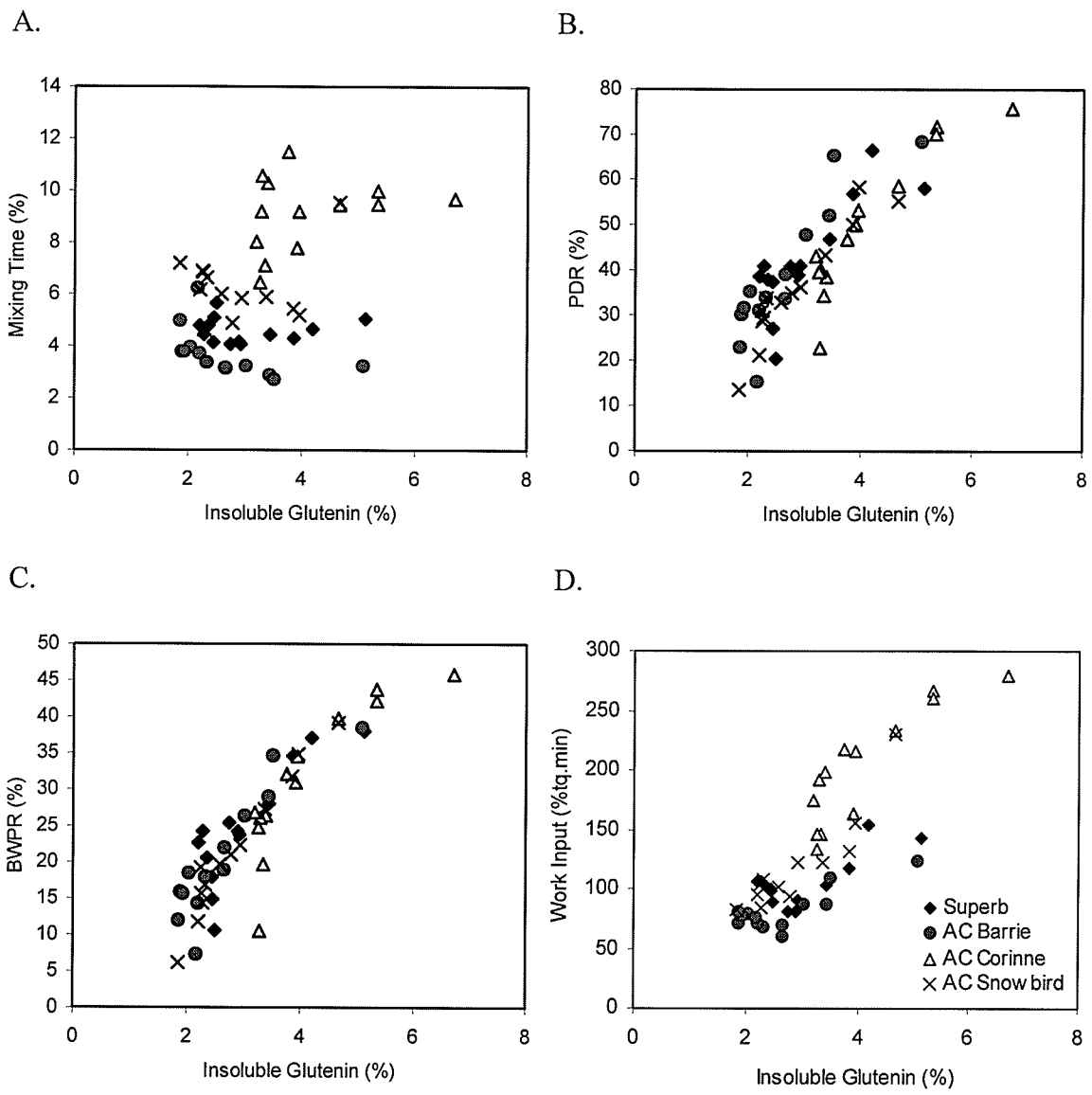


Figure 3. Relationships between insoluble glutenin and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

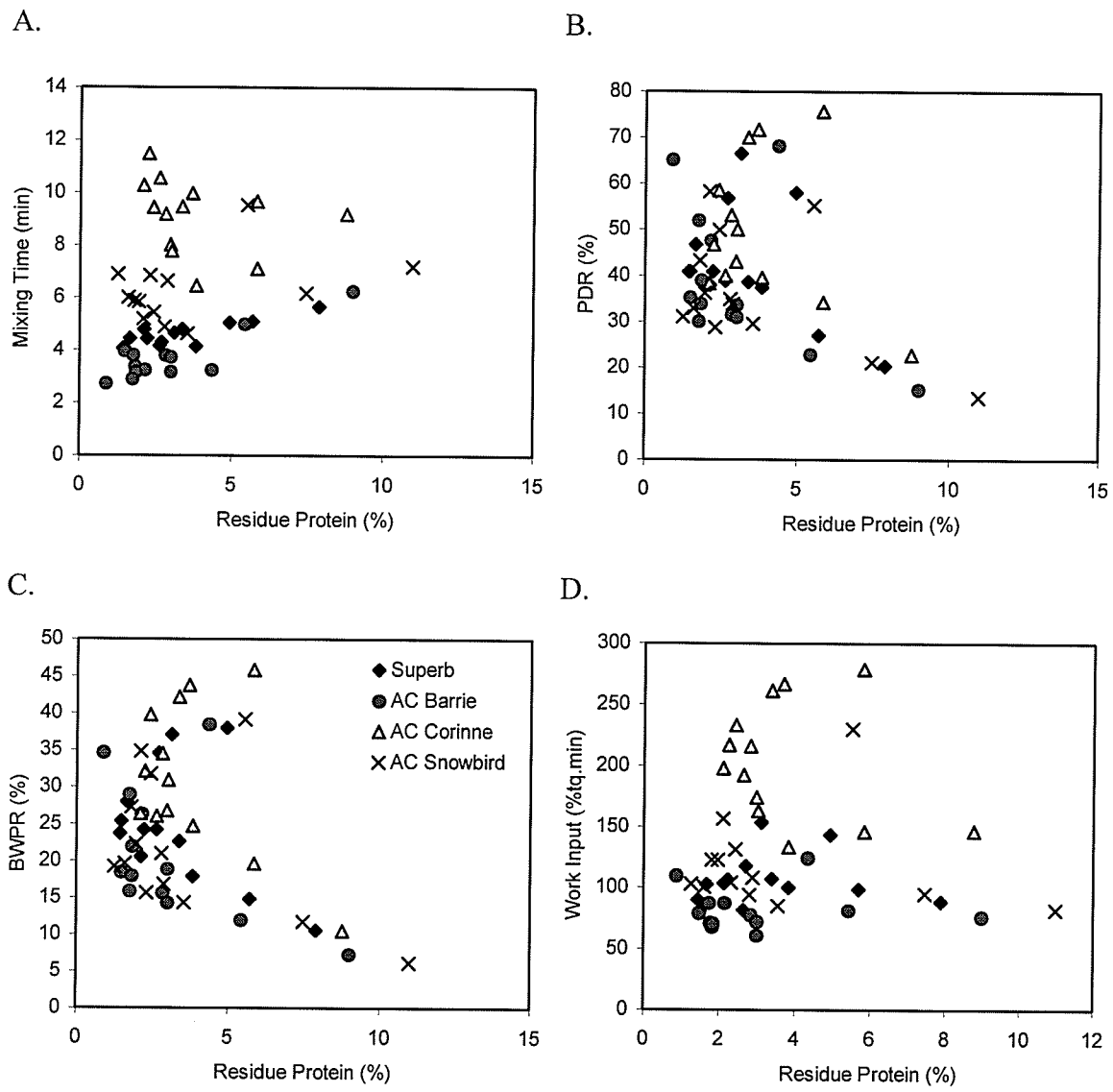


Figure 4. Relationships between residue protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

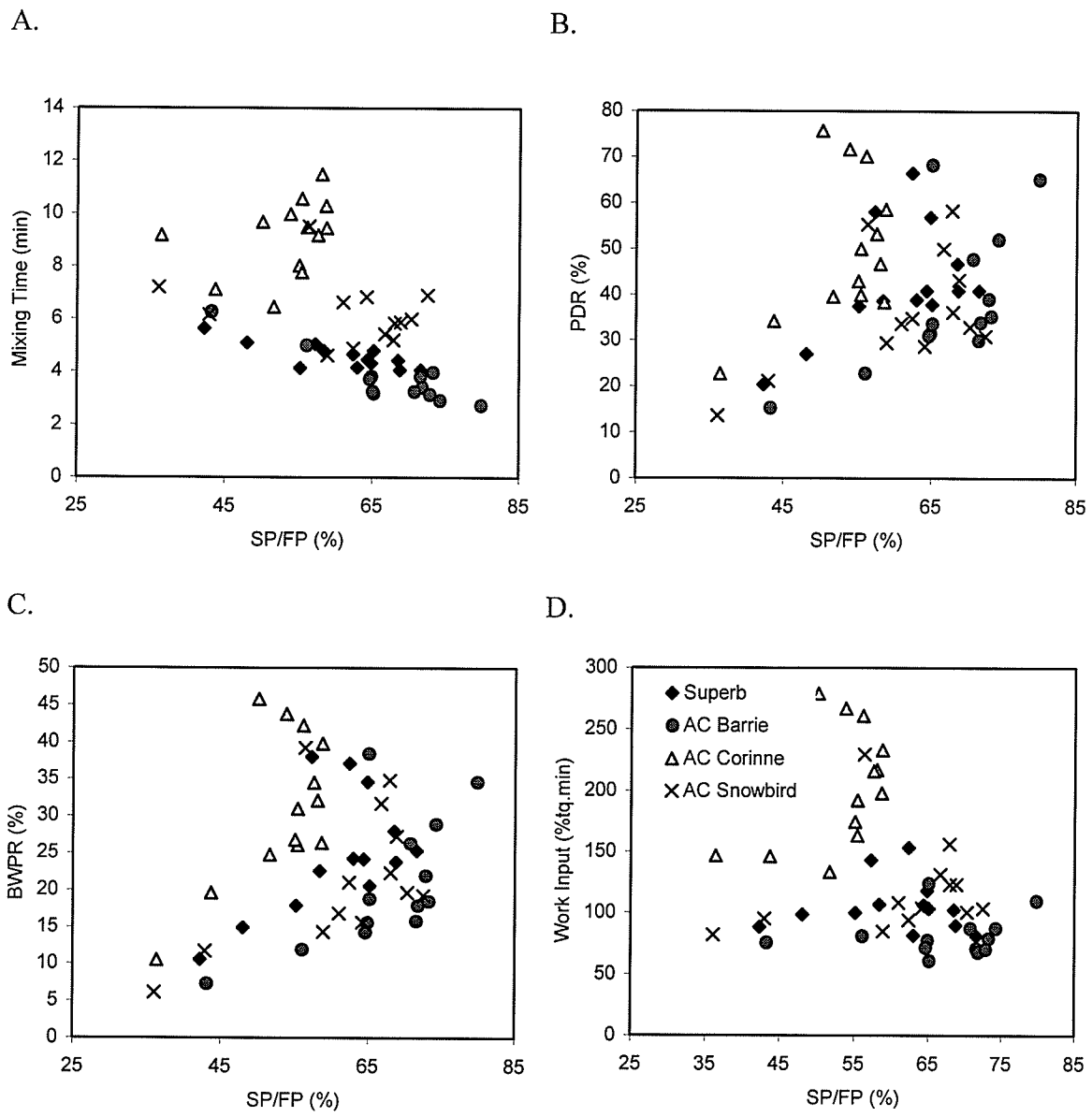


Figure 5. Relationships between percentage of soluble protein in flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

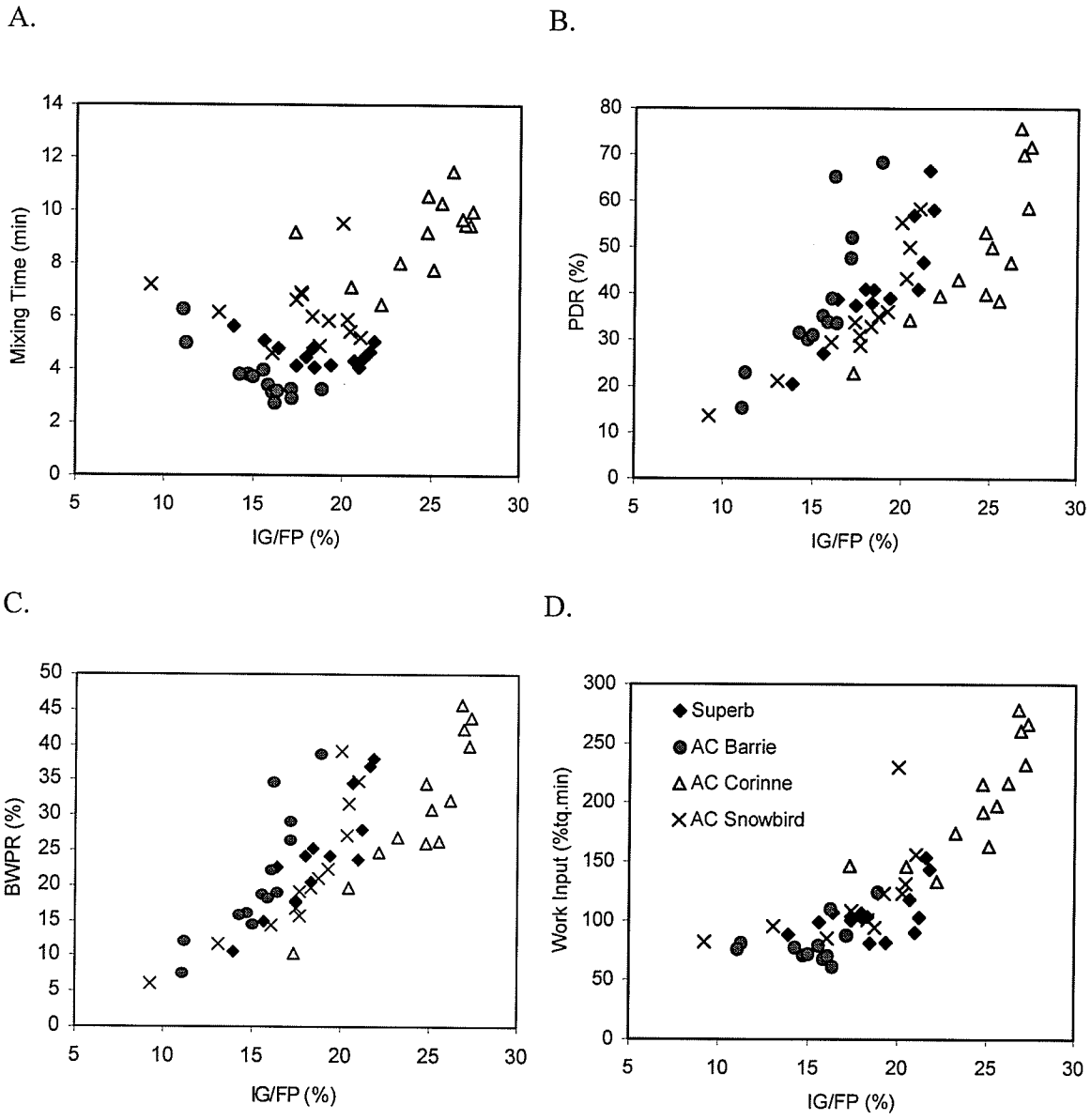


Figure 6. Relationships between percentage of insoluble glutenin in flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

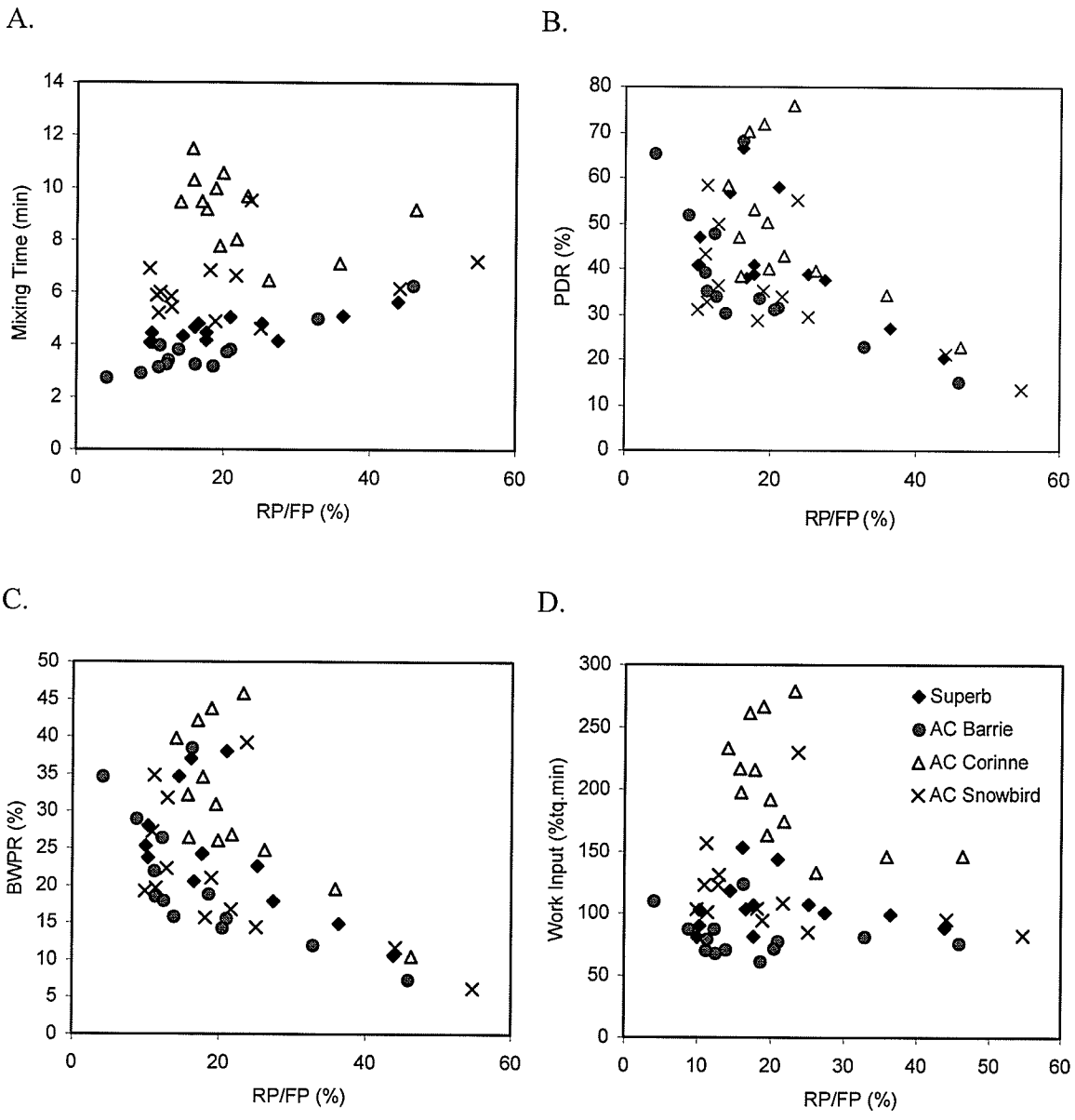


Figure 7. Relationships between percentage of residue protein in flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

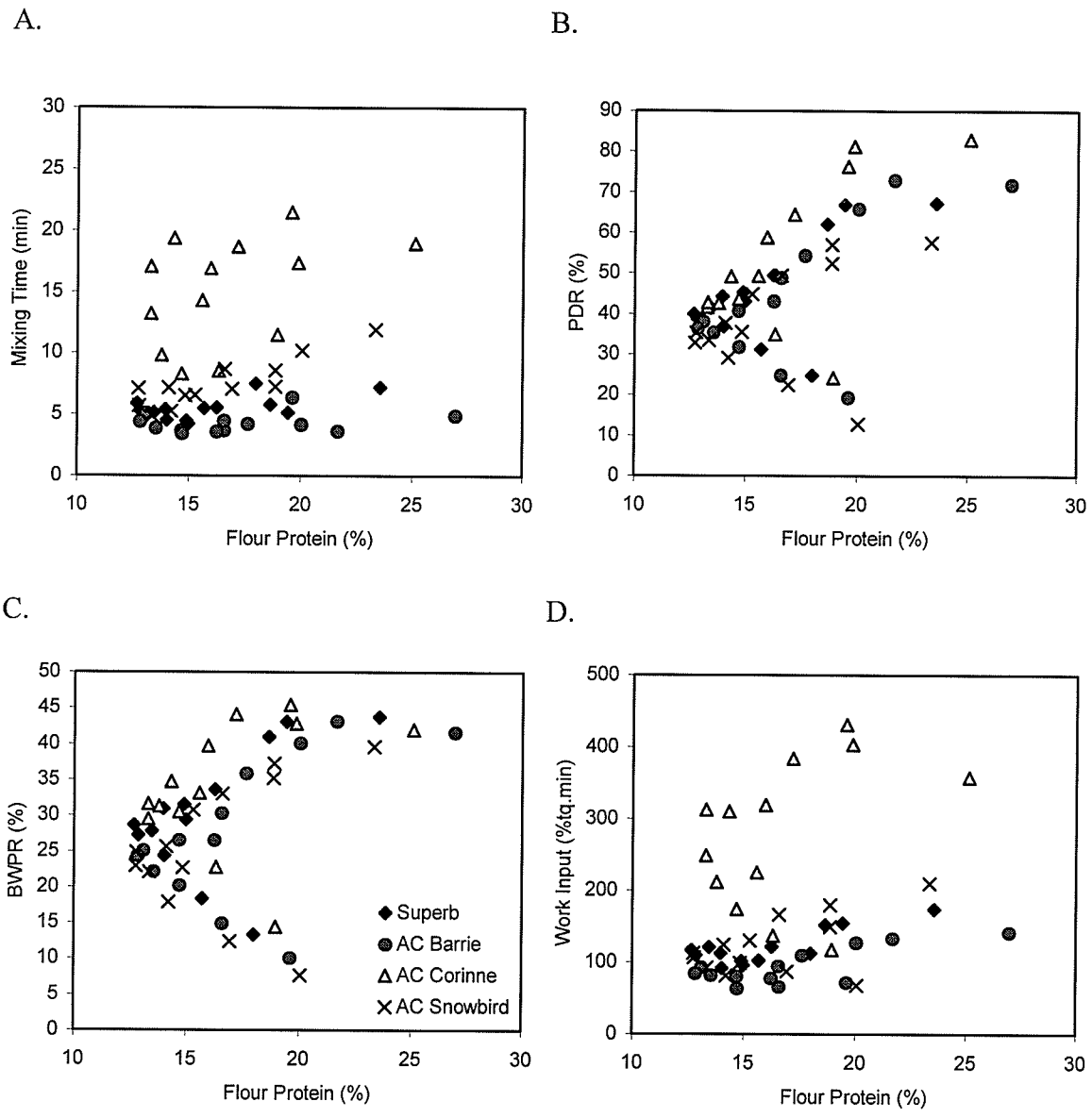


Figure 8. Relationships between flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

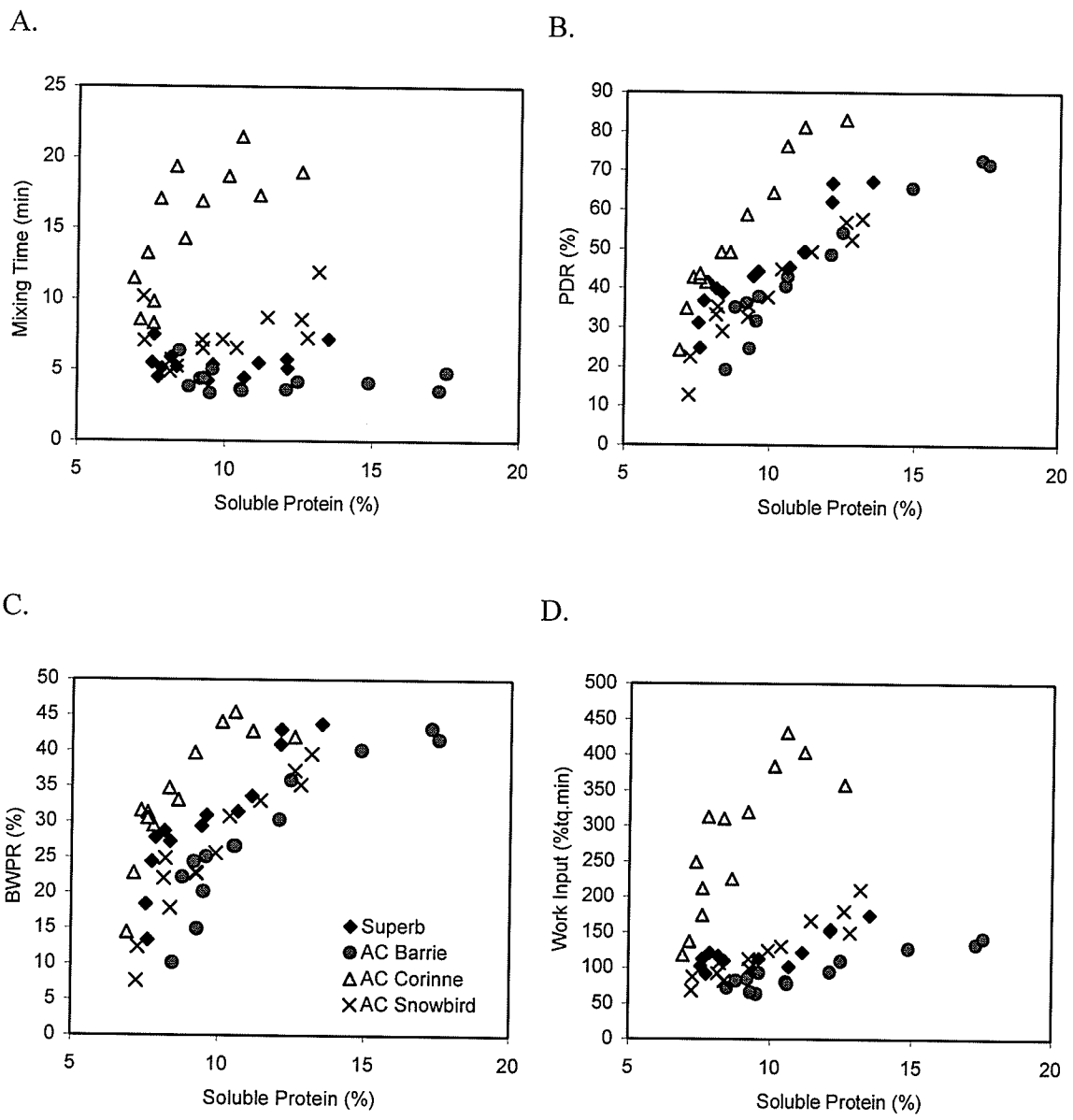


Figure 9. Relationships between soluble protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

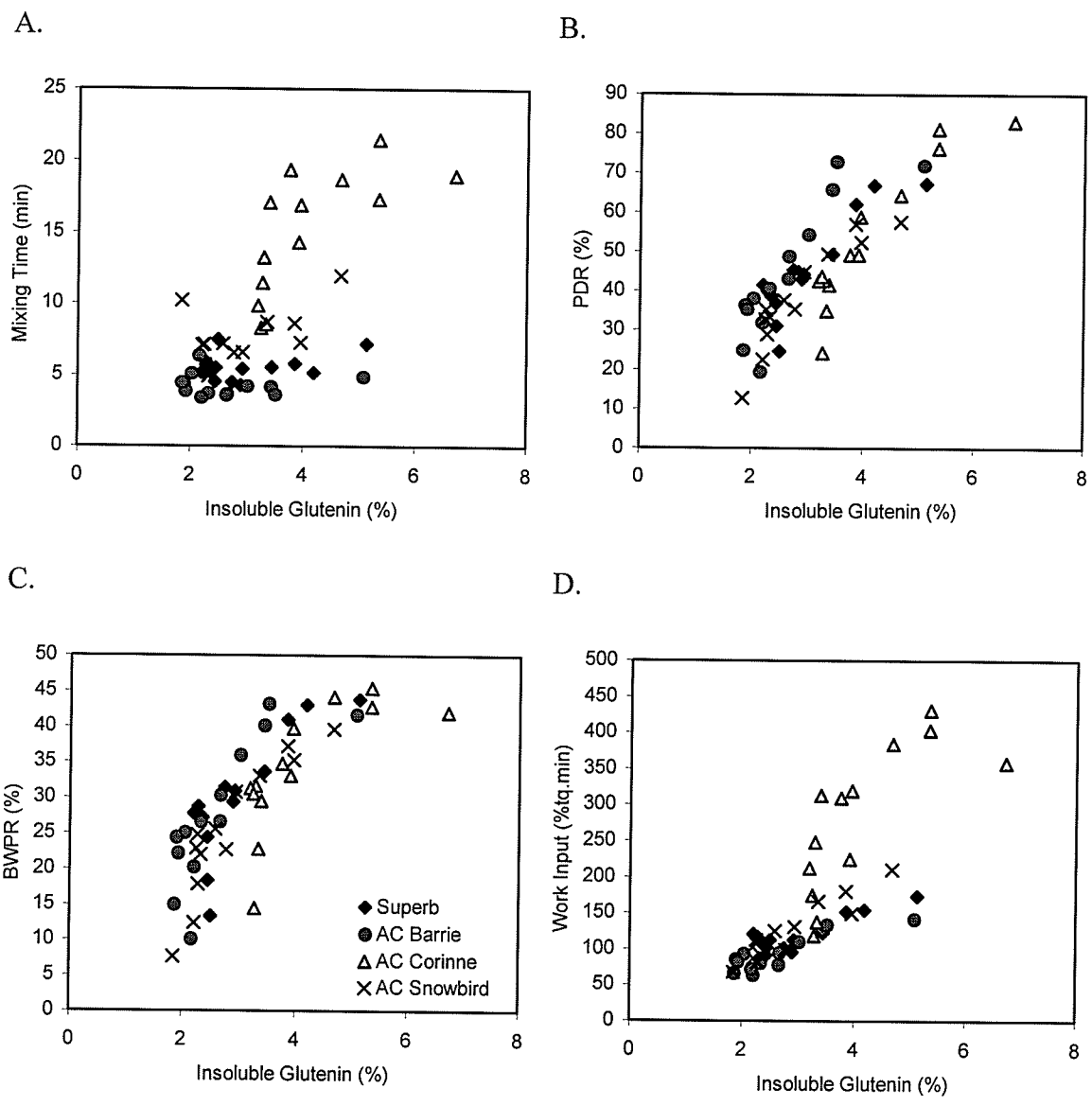


Figure 10. Relationships between insoluble glutenin and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

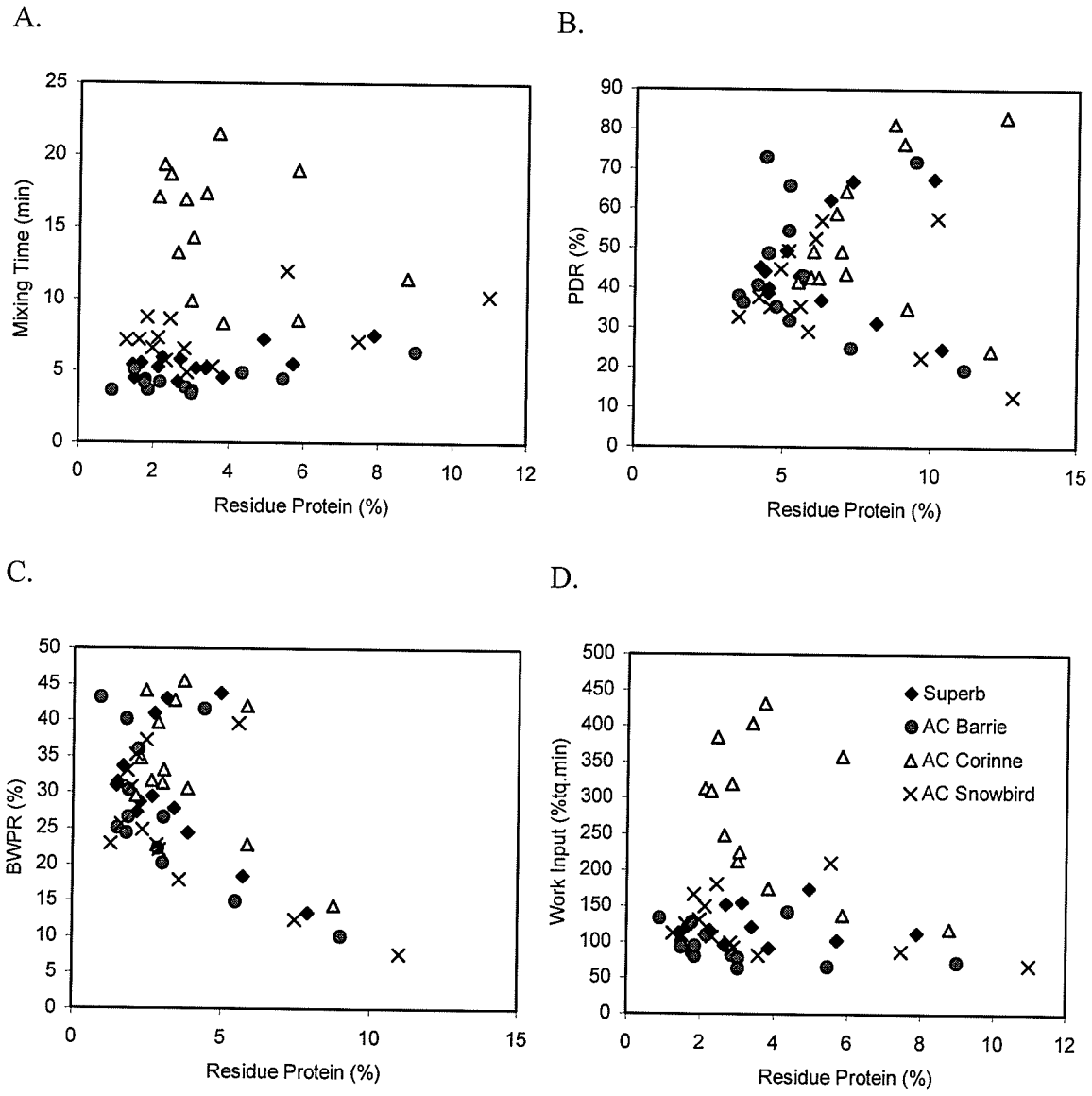


Figure 11. Relationships between residue protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

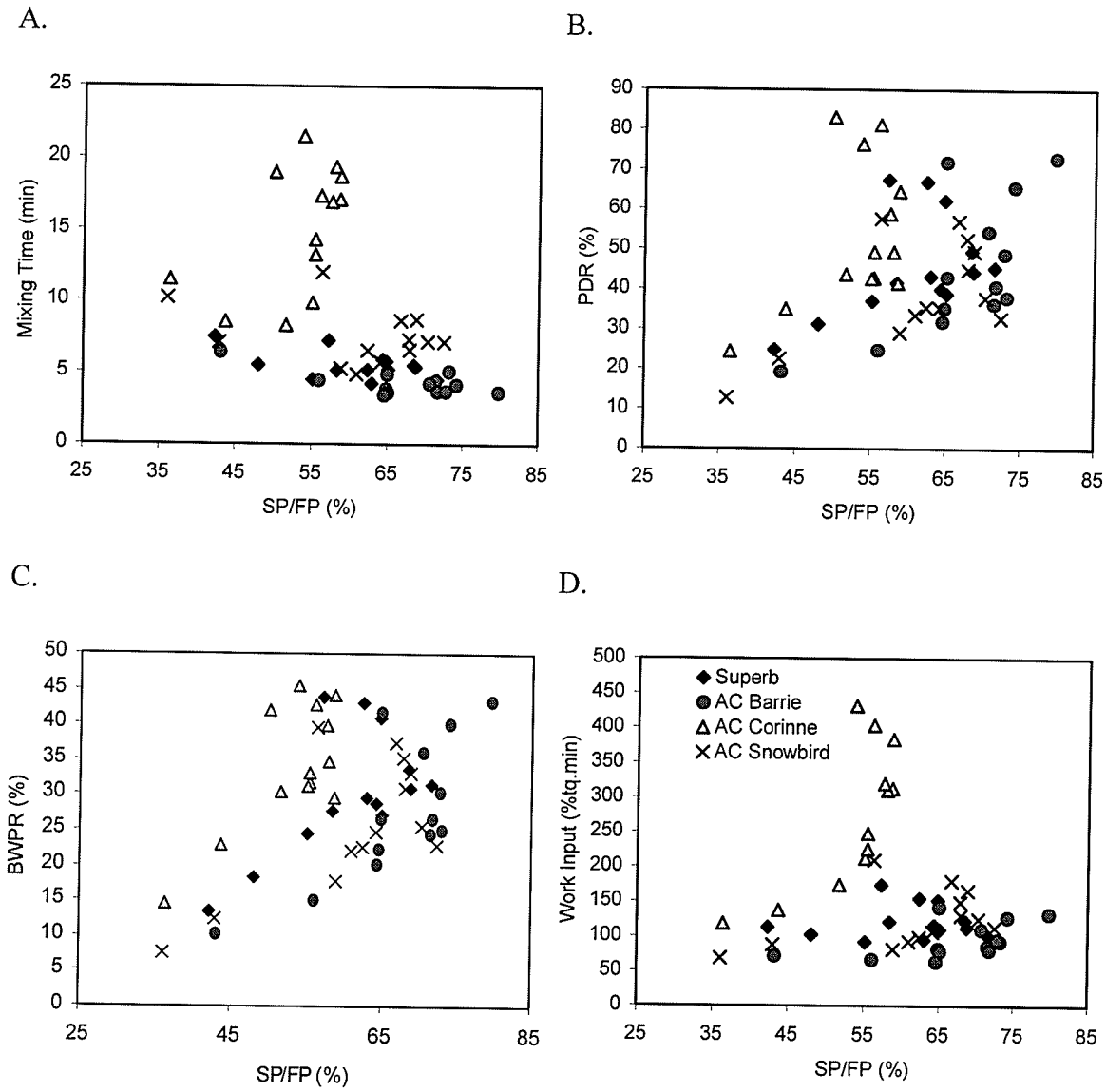


Figure 12. Relationships between percentage of soluble protein in flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

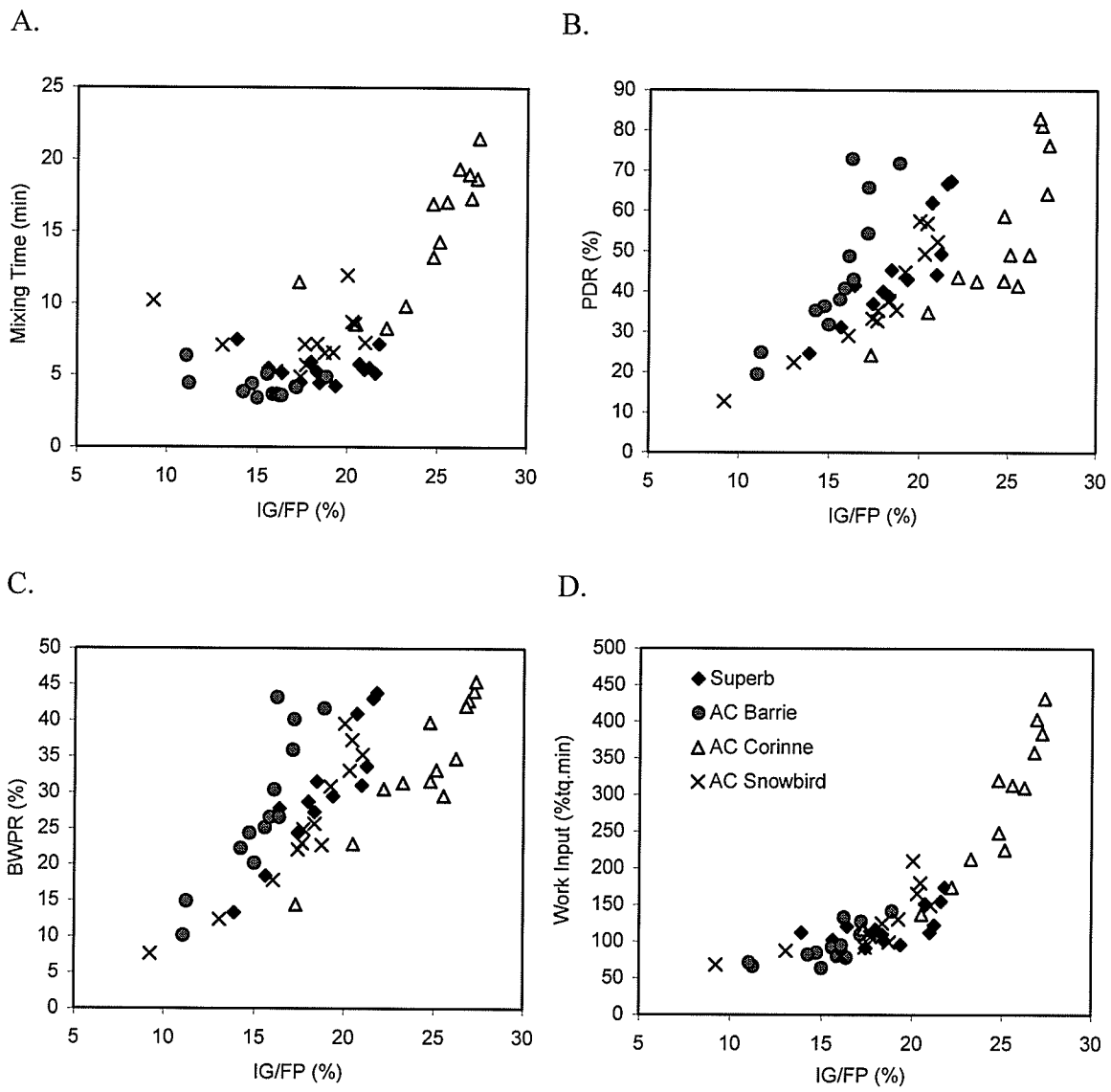


Figure 13. Relationships between percentage of insoluble glutenin in flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.

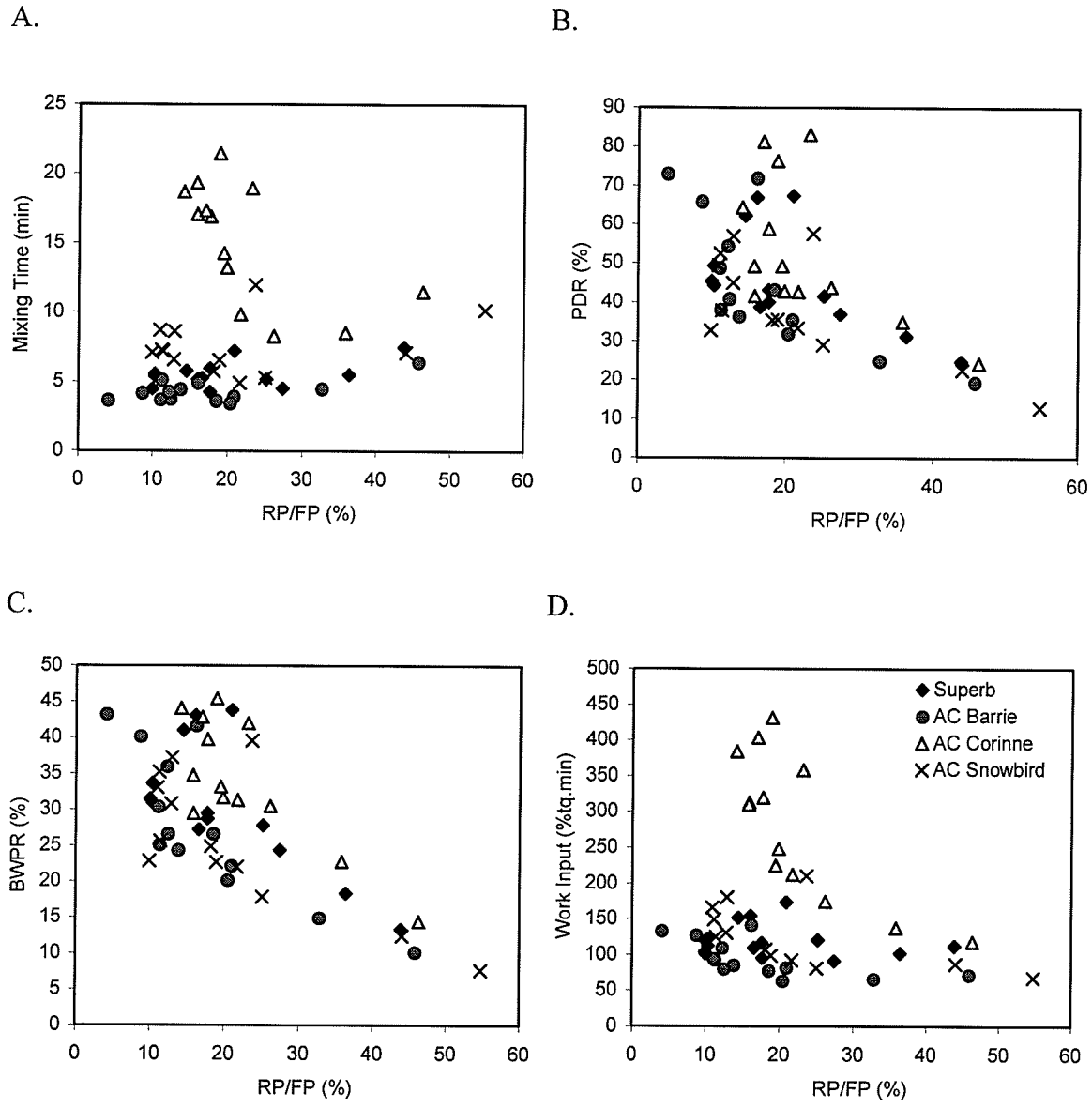


Figure 14. Relationships between percentage of residue protein in flour protein and (A) mixing time, (B) peak dough resistance, (C) bandwidth at peak dough resistance, and (D) work input to peak for doughs with 2% salt for millstreams of samples AC Snowbird, AC Barrie, Superb, and AC Corinne.