

**EFFECTS OF MICRONIZATION ON THE PHYSICOCHEMICAL  
AND RHEOLOGICAL PROPERTIES  
OF WHEAT VARIETIES**

by

**SHOUCHEN SUN**

A Thesis

Submitted to the Faculty of Graduate Studies

in Partial Fulfillment of the Requirements

for the Degree of

**MASTER OF SCIENCE**

Department of Human Nutritional Sciences

University of Manitoba

Winnipeg, Manitoba

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## ABSTRACT

A more comprehensive understanding of micronization (infrared treatment) has become necessary due to the potential use of this technique for processing grains and seeds. The main objective of this research was to investigate the effects of micronization, at different moisture levels, on physical, chemical, and rheological properties of wheats. Four wheat varieties (AC Karma, AC Barrie, Glenlea, and AC Ivory) were subjected to infrared radiation, at three moisture levels (as is, 16%, and 22%), to reach an average layer temperature of  $100 \pm 5^\circ\text{C}$ . The wheat samples were then milled and analyzed for single kernel characteristics, flour yield, ash and total protein content, protein fraction characteristics, alpha-amylase activity, and rheological behaviors. The protein fractionation test revealed significant decreases ( $P < 0.01$ ) in both monomeric proteins (from 54% of total protein in the control to 37% in the tempered micronized sample) and soluble glutenins (from 9.4 to 2.5%). There was a strong negative correlation ( $r = -0.98$ ) between the percentages of monomeric proteins and insoluble glutenins. Total extractable proteins of micronized samples tempered to 22% moisture decreased 43.5% compared with non-micronized control samples using SE-HPLC. Micronization had a remarkable effect on the gluten properties as seen from the significant decreases of water absorption ( $P < 0.01$ ) and dough development time ( $P < 0.01$ ). Results suggest that micronization to high temperature level has detrimental effects on gluten

functionality by decreasing protein solubility and impairing physicochemical and rheological properties of wheat flour. This behavior is largely independent of wheat varieties.

## ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to my advisor, Dr. Beverley M. Watts, for her insight, guidance, patience, and unfailing encouragement during this study. I also wish to thank Dr. Odean M. Lukow and Dr. Susan D. Arntfield who participated enthusiastically in the project from the outset and gave generously their advice and time.

Thanks are due to the members of the wheat quality research team – Kathy Adams, Jerry Suchy, Tatiana Kowalchuk, Margaret Prochownik, Kim Kuzminski, Sumathi Ambalamaatil, Kulwinder Kaur, and Surjani Uthayakumaran for their invaluable assistance, technical support, and friendship which made my work in the group a pleasure.

Special recognition must also be given to those specialists in various fields, to Ms. Aniko Bernatsky for her kindness and help in operating the micronizer equipment, to Mr. Chun Wang for his support in SE-HPLC test, and to Dr. Sheila Woods for the assistance in statistical analysis.

Warm appreciation is extended to Dr. Miklos Kovacs, Dr. John Noll, and Dr. Nancy Ames for permitting the use of the facilities.

Above all, I wish to thank my husband, Kai, my parents, and all my other relatives for their love, support, and encouragement at every step of this research.

Finally, the financial assistance from Agriculture and Agri-Food Canada is gratefully acknowledged.

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## ABBREVIATIONS

BDT	Time to Breakdown.
BF	Break Flour
CPSR	Canada Prairie Spring Red
CPSW	Canada Prairie Spring White
CWAD	Canada Western Amber Durum
CWES	Canada Western Extra Strong
CWRS	Canada Western Red Spring
CWRW	Canada Western Red Winter
CWSWS	Canada Western Soft White Spring
CWWS	Canada Western White Spring
DDT	Dough Development Time
DTT	Dithiothreitol
EXTENS	Extensibility from start until rupture
FAB_14	Farinograph water absorption on 14% m.b.
HI	Hardness Index
HMW-GS	High Molecular Weight Glutenin Subunits
IG	Insoluble Glutenin
LMW-GS	Low Molecular Weight Glutenin Subunits
m.b.	Moisture Basis
MCE	Mercaptoethanol
MP	Monomeric Protein
MPH	Mixograph Peak Height
MPT	Mixograph Peak Time
MWD	Molecular Weight Distribution
M_NT	Micronized, Non-tempered
M_T16	Micronized, Tempered to 16% Moisture
M_T22	Micronized, Tempered to 22% Moisture
NA	Not Available

NM_NT	Non-micronized, Non-tempered
NM_T22	Non-micronized, Tempered to 22% Moisture
PBW	Peak Band Width
RF	Reduction Flour
R <sub>max</sub>	Maximum Resistance to Extension
RP	Residue Protein
SDS-PAGE	Sodium-Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis
SE-HPLC	Size-Exclusion High-Performance Liquid Chromatography
SG	Soluble Glutenin
SH	Sulphydryl
SKCS	Single Kernel Characteristics System
STAB	Stability
WIP	Work Input to Peak

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## INTRODUCTION

Micronization is a short-time, high-temperature food processing technology. By using humidity and temperature to treat cereals or legumes in bulk, micronization improves their utilization for human food and animal feed (Micronizing Co. Ltd. 2002). The basic mechanism of micronization technology is that an infrared lamp or gas fired radiation equipment emits infrared waves, which are absorbed by the processed material causing the constituent molecules to vibrate. The inter-molecular friction brings about rapid heating that results in some precooking of the products. Micronization is becoming an important heat treatment in food industry. The major advantage of micronization over traditional convection heating is its high efficiency in both energy and time. Micronization was originally applied for treating animal feed to achieve starch gelatinization, eliminate antinutrient factors, and therefore increase starch digestibility and improve nutritional benefits. More applications of this technology in agricultural products were developed in the past twenty years, such as in the productions of breakfast cereals, whole grain breads, and brewery cereal adjuncts (Blenford 1980).

Wheat is the leading crop grown in Western Canada, and plays an important role in the economy of the Prairie Provinces. In 2000, total wheat production for Western Canada was approximately 24.1 million tonnes, and wheat export contributed about 2.3 billion US\$ to the Canadian economy (Canadian Wheat Board 2001). The development of new technologies, such as

micronization, will widen the potential applications and nutritional advantages of wheat for human society.

During the twentieth century, seven wheat classes were developed, each of them with specific characteristics and suitable for a particular range of end products. Wheat, such as Canada Western Red Spring (CWRS), is excellent for breadmaking, whereas, Canada Western Soft White Spring (CWSWS) wheat is used mainly for the production of biscuits and cookies. End use of wheat is determined mainly by its gluten strength, which ranges from very strong to weak. This characteristic of wheat flour is the main factor in the determination of flour quality, which can be determined by analyzing the physicochemical and rheological properties of flour. In this study, four wheat varieties (AC Karma, AC Barrie, Glenlea, and AC Ivory), representing different wheat classes, were chosen to characterize the micronized wheat with respect to the physical, chemical, and rheological properties.

Micronization is still a relatively new technology, and most of the research that has been conducted on micronization of food ingredients related to legumes. Although micronized wheat has been used commercially in many healthy whole grain products, few studies have been reported in terms of the physical, chemical, and rheological properties of micronized wheat. The effects of micronization on wheat protein fractions and related wheat quality factors also have not been reported. Therefore, the general goal of this research was to investigate the effects of micronization on the physical and rheological properties, and protein compositions of wheat. Typical micronizing conditions for

cereal processing were chosen in this study. The physical characteristics were determined using single kernel characteristics system. The protein analyses included total protein content, protein solubility, protein SDS-PAGE, and  $\alpha$ -amylase activity testing. And the rheological behavior was examined using Farinograph, Mixograph and TA.XT2 Texture Analyzer results.

The specific objectives of this project were to compare micronization effects among wheat varieties, to elucidate the micronizing effects on proteins, to determine the contribution of moisture to the effects of the micronization treatment, and to explore the potential of micronization as a viable method to decrease alpha-amylase activity in sprouted wheat.

## REVIEW OF LITERATURE

### 2.1 MICRONIZATION (INFRARED HEAT PROCESSING)

Micronization, or infrared heat processing, is an efficient food processing technology that employs short time and high temperature to process grains or seeds before their final applications in human food, animal feed, or brewing industry, in order to decrease the moisture content, affect the cooking ability, and improve nutritive value of the products (Zheng et al 1998, Botero Uribe 1997).

#### 2.1.1 Micronization versus Microwave Heating

“Micronization” is a term used to describe infrared heat processing, during which, materials are exposed to infrared wavelengths for a very short period of time. Although, micronization and microwave heating can both be used as a heat source in food processing, they have a couple of major differences. First, they have different electromagnetic spectrum wavelength. Micronization (infrared heating) uses infrared rays, which is in the range of 0.76  $\mu\text{m}$  to 1000 $\mu\text{m}$  approximately (Orfeuil 1987). Microwave heating uses microwaves, which is in the range 1000  $\mu\text{m}$  to 100mm (Vanzetti 1972). Second, micronization is characterized by superficial heating and the lack of coherent wavelengths, whereas, microwave heating is to penetrate deeply inside the processed materials (Copson 1975, Datta 2001).

## 2.1.2 The Fundamentals of Infrared Radiation

### 2.1.2.1 Infrared Radiation Region

The electromagnetic spectrum is the distribution of electromagnetic radiation according to wavelength, frequency, or energy content of the photon (Vanzetti 1972). It includes radio, microwaves, infrared, visible, ultraviolet, X-rays, and gamma rays. As shown in Fig. 2.1, infrared rays are part of the electromagnetic spectrum, between the visible and microwaves. According to wavelength, the infrared region is further divided into near, middle, or far wave band. Near infrared rays ( $0.76 \mu\text{m} - 1.4 \mu\text{m}$ ) are the closest to visible light. Therefore, they are easily reflected. In contrast, middle infrared ( $1.4\mu\text{m} - 3 \mu\text{m}$ ) and far infrared waves ( $>3 \mu\text{m}$ ) are more readily absorbed by materials as heat sources (Zhao 2000, Fasina and Tyler 2001).

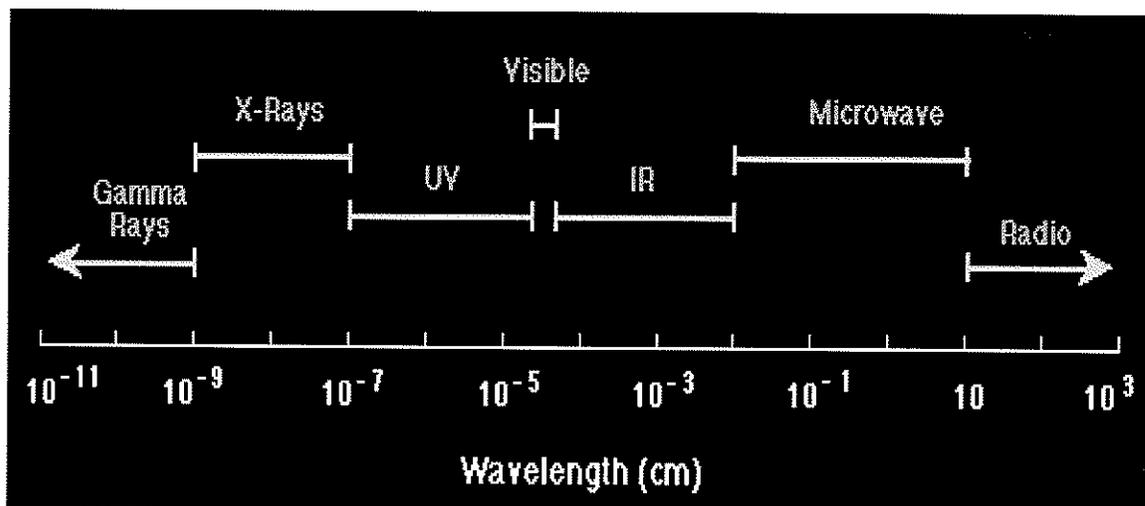


Fig. 2.1 The electromagnetic spectrum (modified from Vanzetti 1972)

### 2.1.2.2 Infrared Radiation Heating

All objects, with a temperature above absolute zero, radiate in a wide range of wavelengths of varying intensities. Meanwhile, radiation energies have been transferred from one to the other (Orfeuill 1987). The most obvious example is the sun transmits energy to our body, which makes us feel warm.

Infrared heating is a form of radiation heating. Like the other forms of electromagnetic radiation, infrared radiation can be described as a stream of photons, which travel in a wave-like pattern, move at the speed of light and carry some energy (Vanzetti 1972). When materials are exposed to infrared radiation, the infrared energy may cause atomic and molecular vibrations, which leads to a heating phenomenon (Toews 2001, Rusnak et al 1980, Sarantinos and Black 1996). Infrared radiation heating obeys three basic laws: Planck's Equation, Wien's Displacement Law, and Stefan-Boltzmann Law (Vanzetti 1972, Zhao 2000). These laws provide the means to determine the spectral distribution and intensity of the emitted infrared energy from a radiator. In general, only the radiation temperature controls the heating power produced by a radiator. The hotter the emitter, the shorter the wavelength of infrared radiation will be (Orfeuill 1987, Zhao 2000).

Either electrically heated or gas fired generators of infrared energy are available in various types. The electrical generators of infrared radiation include incandescent lamps, quartz tubes, and resistance elements. Since, the maximum wavelength of first two groups is normally less than  $1.3 \mu\text{m}$  and part of the energy is in the visible region, they are referred to as light (short-wave)

radiators, which emit at temperature from 1773°K to 2073 °K (Ginzburg 1969). In contrast, the gas generators and resistance elements are dark (long-wave) radiators with the predominant radiations rays larger than 1.3  $\mu\text{m}$ , and reach a temperature much lower than light radiators (Fasina and Tyler 2001), which is used in this study.

Since the usual emitters vary from 600°K to 2,500°K, almost all the energy is actually located in the infrared area. For instance, a stove burner and a space heater are two common uses of infrared heating in our daily life.

### **2.1.3 Advantages and Disadvantages of Micronization (Infrared Heating)**

#### **Process**

Infrared heating mainly uses radiation as its heat transfer mechanism. Compared with the other two heat transfer mechanisms (conduction and convection), high-energy efficiency is the most significant advantage of infrared radiation heating method. This is due to infrared transfers energy from the emitting source to the heated body without heating the intervening space, and the transmitted power is proportional to the fourth power of temperature. However, traditional convection heat transmission is dependent on the surrounding environment, and the power is only proportional to the first power of temperature (Orfeuill 1987, Abe and Afzal 1997). Thereby, infrared heating can provide higher heat density, which is much greater (20 to 100 times) than that of convection heating (Botero Uribe 1997). By improving heat transfer efficiencies, micronization may reduce processing time and save energy.

Infrared radiation heating is different with the other electromagnetic radiation heating with regard to its effect. Unlike the other radiations, such as ultraviolet, which may produce photochemical effect, infrared radiation is purely a thermal effect. Therefore, infrared heating is very safe for use in food industry (Orfeuil 1987).

From an industry processing point of view, there are many more advantages of micronization. For instance, micronizer equipment is easy to construct and operate, and often has a large consistent processing capacity. Micronizers are quite flexible in various aspects of products processing with respect to the amount of energy supplied, the distance between radiator and the processed materials, and the dwell time of the materials exposed to the infrared heating process (Blenford 1980). Both granule and powder materials can be processed under micronization, and uniform heating of product can be easily achieved (Botero Uribe 1997, Fasina and Tyler 2001).

The major disadvantage of infrared heating is that a micronizer is best suited for thin materials. Therefore, it is difficult to treat complex shaped product uniformly (Orfeuil 1987). However, using a vibrating conveyor can alleviate this weakness.

#### **2.1.4 Effects of Micronization on Agricultural Products**

Due to the combined effects of electromagnetic radiation and intense heat, micronization has complicated effects on processed products. Infrared heating not only dries materials, but may also decrease the cooking time, increase starch gelatinization, reduce the activity of antinutritional substances of the micronized products, and destroy insects in the products. These properties of micronization have a wide range of applications in agricultural products and food industries, and comprehensive studies on micronization effects have been undertaken.

##### **2.1.4.1 Drying Biological Materials**

One major application of micronization is as a source of dry heat to reduce the moisture content of materials. This application has been explored in various agriculture products, from grains, flour, legumes, to fruit, vegetables, and meats (Abe and Afzal 1997, McCurdy 1992, Blenford 1980). Abe and Afzal (1997) investigated the drying effect of thin-layer infrared radiation on rough rice at three initial moisture contents using four levels of radiation intensity. They found the typical drying curves were a function of radiation intensity. A higher moisture removed rate was observed at higher radiation intensity. The initial moisture content did not influence the drying behavior.

The major reason to dry grains by micronization is to partially dry freshly raw or washed grain with 25-30% moisture before storage or milling to avoid germination and mould. Zheng et al. (1998) reported on moisture losses in

various micronized cereals and legumes. After micronization treatment at 115°C, moisture content was reduced by 6% (rye) to 40% (barley) in the cereals. The differences of moisture loss among cereals were primarily due to the deviations in their original moisture content.

Ginzburg (1969) summarized the studies and applications of micronization done by Russian researchers. He suggested that wheat be considered as a thermo-labile material. Within the permissible temperature limits, micronized wheat can achieve higher yield of flour, without the breadmaking qualities and germination properties of the grain being affected. Schofield et al. (1983) indicated that the properties of wheat proteins were affected at temperatures above 50°C. Ginzburg also suggested it is necessary to lower the energy illumination for certain foods, such as fruit, vegetable, grain, or milk, to preserve their biological value.

#### 2.1.4.2 Reducing Cooking Time of Legumes

Reducing cooking time of legumes is a new application of micronization. Cooking of untreated legumes may require a long period of time. By softening the texture of seeds, allowing for easier water penetration, and increasing starch gelatinization, the micronization process will shorten the cooking time of legumes (Scanlon et al 1998). A study conducted by Cenkowski and Sosulski (1997) showed that the cooking time was reduced from 30 minutes in the control lentils to 15 minutes in lentils subjected to micronization for 55 second. Arntfield et al. (1997) reported a similar result in their study on the effects of tempering

conditions and moisture content of micronized lentils. They found that shorter cooking times could be achieved with lentils micronized after tempering to higher moisture conditions.

#### 2.1.4.3 Gelatinization of Starch

Micronization increases the content of gelatinized starch, and enhances the starch availability and digestibility (Blenford 1979). This approach has been used extensively in the production of cereal flakes, animal feed, or for cereal adjuncts in the brewing industry (Micronizing Co. Ltd. 2002, Rusnak et al 1980). Arntfield et al. (1997) indicated the importance of moisture content in the lentils with respect to gelatinizing starch during micronization. Higher moisture content resulted in a higher degree of starch gelatinization. With 33% tempering level, about 70% starch was gelatinized. A similar result was reported by Fasina et al. (1999) in a barley study. They suggested that both temperature and moisture were indispensable to gelatinization of starch. With higher micronization temperature or initial greater moisture level, the degree of starch gelatinization was increased.

#### 2.1.4.4 Eliminating Antinutritional Factors and Disinfecting Insects

Another change that can take place, in micronized products, is the reduction in protein solubility. Fasina et al. (1999) studied the effect of micronization on protein solubility in hullless and pearled barley samples at pH 2.0 to 12.0. A reduction of protein solubility at all pH levels was found. In other

research, the classic Osborne extraction procedure was conducted to prepare soluble protein fractions by consecutive extraction from ground cereal meals with deionized water, 0.5M NaCl, and 70% ethanol. The results indicated the solubilities of all three protein groups were reduced after micronizing treatment (Zheng et al 1998). It was suggested that protein denaturation might take place during micronization.

An advantage of this denaturation process could be the destruction of antinutritional or inhibiting substances, such as trypsin inhibitors and lectins. As a result, both protein digestibility and stability of the products are enhanced (Igbasan and Guenter 1996, Shiau and Yang 1982, Micronizing Co. Ltd. 2002). However, Savage and Clark (1988) found a negative effect of micronization on the biological value of sorghum protein. Micronization has also proved effective in lowering tannin content of winged bean by 12%, compared with untreated seed (Kadam et al 1987).

The micronizing operation can largely reduce the amount of enterobacteria, moulds, and insects, and without loss of cocoa butter. This property of micronization has been applied in the cocoa industry, which has obtained good results in using this technique as a pretreatment to process cocoa beans. The result is the improvement both in the bacteriology and productivity (Micronizing Co. Ltd. 2002, Blenford 1979).

Optimizing micronization conditions are crucial to achieve the desired end product. Several parameters should be taken into account in micronizing process, which include: the initial moisture content, shape and size of the

processed materials, the surface temperature of emitter, the distance from emitter to heated body, and the residence time of the material under infrared radiator (Zhao 2000).

## **2.2 WHEAT CHARACTERISTICS AND QUALITY**

Wheat is the most suitable grain for the production of leavened bread (Cornell and Hoveling 1998). Wheat flour has a unique ability to form visco-elastic dough, which retains gas produced by yeast fermentation. This results in leavened, light baked bread products. This peculiar characteristic of wheat is primarily due to its proteins, and especially the storage proteins, which form gluten when hydrated and mixed with water. It has been generally accepted that wheat proteins are crucial in determining wheat enduse properties and baking quality (Janssen et al 1996, MacRitchie 1992).

### **2.2.1 Classification and End Use of Wheat**

Wheat varieties exhibit different dough handling properties, generally referred to as flour strength. On the basis of flour strength, wheats are usually classified as either hard (strong) wheat or soft (weak) wheat. Hard wheat flour normally has relatively high protein content, high water absorption, good gas-retaining properties, and needs a long development time to optimize the visco-elastic properties of the dough. These characteristics make the flour suitable for breadmaking. In contrast, soft wheat flour has low protein content, poor gas-

retaining properties, and requires a short development time. Such flour is more suited for cookies and pastries (Canadian Wheat Board 2001).

Based on flour strength, kernel color, planting season, and end use purposes, Canadian Wheat Board has developed seven wheat classes. Canada Western Red Spring (CWRS) wheat is noted for its superior milling and baking quality, which is ideal not only for the production of high-volume pan breads, but also commonly used to blend with weaker wheats for different purposes. Canada Western Amber Durum (CWAD) wheat is specially used to produce semolina for making high quality pasta. Canada Western Extra Strong (CWES) wheat is best known for its extra strong gluten strength. It is particularly used as a blending wheat in pan breads and frozen dough applications. Canada Prairie Spring Red (CPSR) and Canada Prairie Spring White (CPSW) have medium-strong gluten suitable for many types of flat breads, noodles, and related products. Canada Western Red Winter (CWRW) wheat is also a hard wheat of medium gluten strength suitable for the production of various noodles and French-style breads. Canada Western Soft White Spring (CWSWS) wheat typically has a low protein content and weak gluten strength, which makes it appropriate for making cookies, crackers, and biscuits (CWB 2001).

Two hard white spring wheat varieties (AC Ivory and AC Snowbird) have been registered recently. They deliver comparable breadmaking quality to CWRS wheat with some color and aftertaste advantages (Agritel Grain Ltd. 2002). One of these varieties (AC Ivory) was included in this study. Because a

separate class has not yet been formed for these varieties, AC Ivory is referred to as a Canada Western White Spring (CWWS) wheat in this thesis.

## 2.2.2 General Characterization of Wheat Proteins

### 2.2.2.1 Major Groups of Wheat Proteins Based on Solubility

Proteins play a dominant role in governing breadmaking quality (MacRitchie 1992, Weegels et al 1996). A number of research studies have been conducted in optimizing quantitative fractionation of wheat protein components. Based on the differential solubility of the proteins extracted by sequential solvents, wheat proteins were originally classified by Osborne into four major groups: albumins, globulins, gliadins, and glutenins (MacRitchie 1992). The main characteristics of these four fractionation groups were shown in Fig. 2.2.

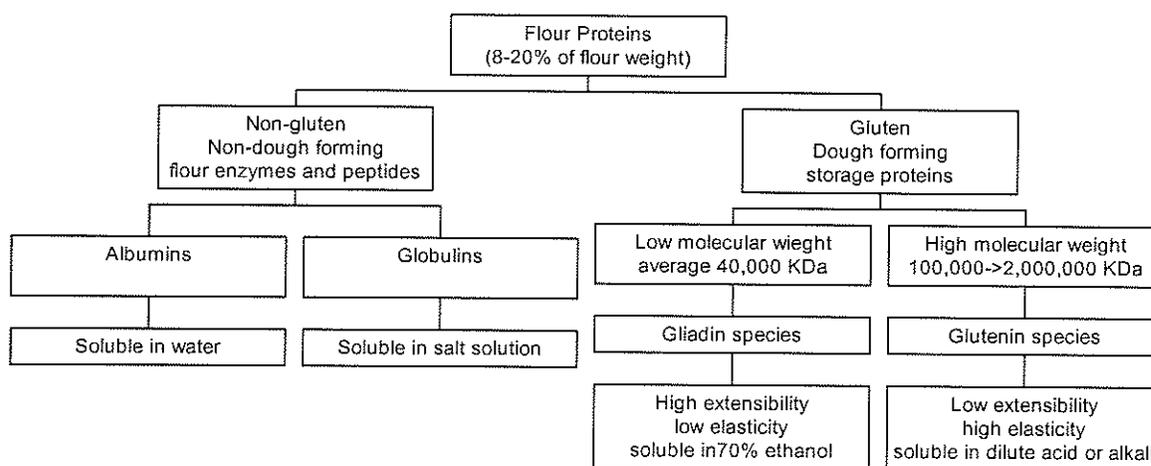


Fig. 2.2 Major components of wheat proteins on the basis of the Osborne procedure (Modified from Daniels and Frazier 1976)

The Osborne procedure is still a sound basis for the separation of the wheat protein groups (Fu 1996). However, due to the great complexity of wheat proteins and the overlapping between the different fractions, this solubility-based method does not result in complete separation of molecular species. Numerous modified fractionation procedures have been developed in the past ninety years.

Chen and Bushuk (1970) sequentially extracted proteins using water, 0.5M NaCl, 70% ethanol, and 0.05M acetic acid solution to further divide the glutenin group into two sub-fractionations, one soluble in 0.05M acetic acid (HAc), and the other insoluble in acetic acid. They discovered the major difference between the hard red spring wheat and the other three grains (rye, triticale and durum) was its lower water-soluble protein content, and higher gluten protein content. Their study provided a new approach to investigating the correlation between the quantity of protein components and wheat quality.

Lee and MacRitchie (1971) fractionated soluble protein groups by a successive extraction procedure using water, urea of various molarities, and 0.1M sodium hydroxide (NaOH) solution. They reported that the early urea extracts decreased mixing stability, and gave weaker doughs, whereas, the late extracts (urea and NaOH) increased mixing stability, and gave stronger doughs.

Kobrehel and Bushuk (1977) found that unreduced glutenin could be solubilized in solvents comprising of distilled water and sodium salts of fatty acids. The solubility of glutenin in solutions was improved with the increasing of carbon chain length. This result suggested that hydrophobic interactions exist between the soaps molecules and the polypeptide chains.

Graveland et al. (1982) combined fractionation and centrifugation methods, and with the aid of a 1.5% sodium dodecyl sulfate (SDS) solution, separated gluten further to gain more insight into gluten behavior and composition.

To date, separation of polymeric proteins from monomeric proteins is still a big challenge. Cross-contamination of glutenin with gliadin during sequential extraction is the main problem in preparing relatively pure protein fractions (Fu 1996). Fu and Sapirstein (1996) separated polymeric proteins from monomeric proteins using 70% 1-propanol. Flour proteins were first divided into 50% 1-propanol soluble and insoluble groups. The 50% 1-propanol insoluble group was mainly comprised of glutenin, whereas, the 50% 1-propanol soluble group included monomeric proteins and contaminated polymeric proteins. Polymeric proteins were then separated from the monomeric protein group by precipitating them with 70% 1-propanol.

Fu and Kovacs (1999) developed a new extraction procedure to obtain relatively purified protein fractions by applying the solvent of 0.3M NaI-7.5% 1-propanol because NaI could selectively remove the contaminating  $\omega$ -gliadins from glutenin. Aussenac and Carceller (2000) reported that the NaI/1-propanol insoluble fraction contained mainly polymeric proteins. Thus, the monomeric and polymeric proteins were completely separated.

Based on Fu and Kovacs' extraction procedure, Lukow et al. (2000) elaborated a rapid, reproducible protein fractionation method, and using the

Dumas (Combustion Nitrogen Analysis) test, to quantify relatively homogeneous protein groups and efficiently screen for wheat protein quality.

#### 2.2.2.2 Applications of SE-HPLC in Wheat Protein Studies

Wheat proteins are heterogeneous, and have a tendency to aggregate (Fu 1996). Many of them interact non-covalently with components, such as lipids and starch, and they may also associate with each other through non-covalent or covalent bonds. Therefore, separation and analysis of wheat proteins become extremely difficult. Size-exclusion High-performance Liquid Chromatography (SE-HPLC) technique is stable, uniform, fast, and reproducible in separating protein groups. As a consequence, SE-HPLC, combined with other means, such as Sodium-Dodecyl-Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and sonication (ultrasound), can be a very useful approach in wheat protein studies. Good results were obtained in qualifying and quantifying polymeric and monomeric protein fractions and in investigating the molecular weight distribution of glutenin in relation to breadmaking characteristics of wheat flour (Ciaffi et al 1996). SE-HPLC separates proteins on the basis of molecular size differences. The support pores retard proteins in inverse relation to their molecular size (Bietz 1985). The larger the proteins, the faster they elute from the column.

Dachkevitch and Autran (1989), using SE-HPLC, with sodium phosphate buffer containing 2% SDS, obtained four chromatographic fractions corresponding to different sizes of unreduced proteins. They reported that this

chromatographic method could be applied to assess the relationship between protein fractions and the baking quality. The distribution between the excluded peak (F1, MW>650 KDa) and the intermediate aggregates peak (F2, MW 115-650 KDa) was a good indicator of the potential baking strength of various genotypes.

Singh et al. (1990a, 1990b) achieved close to complete extraction of proteins, without chemical reduction of disulfide bonds, by applying a sonication technique. The extracts were analyzed by SE-HPLC, yielded into three distinct peaks, glutenin, gliadin, and albumin-globulin. They observed the relative quantity of glutenin (relative area of peak 1) was highly positively correlated with baking performance. Ciaffi et al. (1996) also reported the positive correlation of both absolute and relative amounts of soluble polymeric proteins with dough extensibility.

Batey et al. (1991) described an alternative elution condition that, by removing SDS from the buffer, extended column life to at least 2,000 injections. More recently, Larroque et al. (2000) investigated the procedures to obtain stable extracts for SE-HPLC. They suggested that endogenous proteases were the sources of the instability of SE-HPLC extracts. They concluded that heating protein extracts for 2 minutes at 80°C in a water bath, immediately after extraction, was a viable solution to avoid sample instability, especially in whole meal. These evolutions of SE-HPLC make this technique more powerful, sensitive, and reliable for wheat protein studies.

### 2.2.2.3 Composition and Structural Models of Wheat Gluten Proteins

Wheat gluten can be defined as proteinaceous, visco-elastic material that remains after starch and other soluble substances (including soluble proteins) are removed from flour dough by washing and kneading in a stream of water (Tatham et al 1990). Gluten contains approximately 80% protein, with gliadins and glutenins accounting for about 40% each (Fu 1996).

Gliadins are a group of single-chained proteins, with intramolecular disulfide bond linkages. They contribute to the cohesiveness and extensibility of dough (MacRitchie 1992). Based on the mobilities in electrophoresis at low pH, they are divided into  $\alpha$ -,  $\beta$ -,  $\gamma$ -, and  $\omega$ - types. The  $\omega$ -gliadins are distinguished from the others by their much higher molecular weight and complete deficiency in cysteine and methionine (Lasztity 1996). Therefore, Shewry et al. (1986) proposed a new classification of the gliadins into sulfur-rich ( $\alpha$ -,  $\beta$ -, and  $\gamma$ -) and sulfur-poor ( $\omega$ -) components. Another difference between these two fractions is the molecular structures. S-rich gliadins appear to be compact, tightly folded molecules. In contrast, S-poor ( $\omega$ -) gliadins are rod-shaped molecules with an unusual secondary structure based on repetitive  $\beta$ -turns, which are made up almost entirely (98%) of similar repeating amino acids sequences (Tatham et al 1990 and Kasarda 1989).

On the other hand, glutenins are multi-chained polymers, with a wide distribution of molecular weights from 100,000 to several million (Daniels and Frazier 1976), and have an attribute of resistance to extension and elasticity. Glutenins consist of individual subunits that form polymers stabilized by disulfide

bonds (Wrigley and Bekes 1999). The subunits can be separated by breaking intermolecular disulfide bonds with reducing agents such as dithiothreitol (DTT) or mercaptoethanol (MCE) (MacRitchie 1992). The reduced subunits can be subdivided into two types: the high-molecular-weight-glutenin subunits (HMW-GS), with molecular weights of 95,000-140,000, and the low-molecular-weight-glutenin subunits (LMW-GS), with molecular weights of 30,000-55,000 (Fu 1996).

A number of models have been proposed in an attempt to explain the structure and functional properties of glutenins. The basis of these models relies on the most common view of the structure of the glutenin, as a mixture of polypeptide subunits cross-linked by either intermolecular, or intramolecular, or both, disulfide bonds (Ewart 1972, Graveland et al 1985, and Karsarda 1989). A series of disulfide-linked gliadins and glutenins structures are shown in Fig. 2.3.

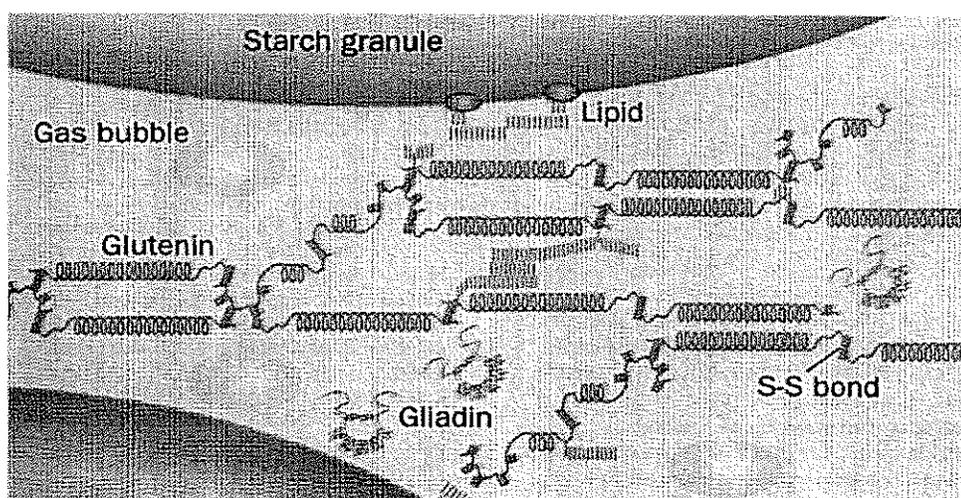


Fig. 2.3 A model for the disulfide-bonded structure of gluten network

(Wrigley and Bekes 1999)

To recapitulate the knowledge about the gluten network, it can be stated that gliadins and glutenins interact with each other by covalent disulfide bonds and non-covalent forces (hydrogen bonds and hydrophobic interactions). It is speculated that this combination may determine the unique properties of the whole gluten (Lasztity 1996).

#### 2.2.2.4 Correlation between Proteins and Wheat Quality

The “ quality” of wheat flour is a term used to describe the performance of that flour in breadmaking, including mixing tolerance, dough development time, and baking performance (Cornell and Hoveling 1998). It is universally accepted that protein is the predominant factor governing wheat end use quality. Normally, for a single wheat variety or a class of wheat varieties, increased loaf volume is directly related to increased protein content (Weegels et al 1996). However, for different varieties, the quality of protein becomes much more important than its quantity.

Protein quality is a very complicated trait influenced by many factors. These include growing conditions and genetics, amount and subunit composition of glutenin, molecular weight distribution (MWD) of proteins, and the ratio of gliadin/glutenin polymers (Kasarda 1989).

Gluten proteins (gliadins and glutenins) are largely responsible for the functionality of wheat flour in breadmaking. The unique gas retention property of bread dough is derived almost entirely from these storage proteins. Studies done so far showed that the albumins and globulins have little effect on gas

retention and dough formation. Gliadins and glutenins combine to form a complex gluten network. Wrigley et al. (1984) found the reduction in gliadin content was associated with an increase in the toughness and a decrease in the extensibility of the dough. Reconstitution studies have indicated that glutenins (especially HMW-GS) are capable of undergoing large deformations under stretching without breaking, i.e., high elasticity properties. Reconstitution flour with high proportions of glutenin increased the resistance to extension, the dough development time, and loaf volume (MacRitchie et al 1991, Gupta et al 1992). Therefore, to obtain a preferred breadmaking quality, an optimum ratio of gliadins and glutenins is indispensable.

Molecular weight distribution of wheat proteins can be a good indicator to show the linkage between protein composition and dough properties (MacRitchie 1999). Huebner and Wall (1976) separated glutenin protein into two fractions: I) a very high molecular weight fraction, and II) some lower molecular weight proteins. They found the ratio of the glutenin I to glutenin II was generally higher in dough from good quality varieties than from poor quality varieties, which means that a greater proportion of high molecular weight glutenin in flour dough would result in stronger dough and higher loaf volume. Dachkevitch and Autran (1989) reported a similar result using SE-HPLC.

Genetic studies (Payne et al 1981, Lukow et al 1989) established that some specific alleles controlling peculiar HMW-GS were positively associated with baking behavior whereas others were negatively associated. For example, subunits 5+10 generally contribute greater dough strength and better quality

than the allelic pair 2+12. The number and distribution of cysteine residues could be an explanation for this. The additional cysteine residue present in subunit 1Dx5 compared with subunit 1Dx2 could permit branching of the glutenin polymers and lead to a higher cross-linked (more elastic) network. Therefore, the cultivars with subunits 5+10 are stronger and have better baking quality compared to the cultivars with subunits 2+12 (Kasarda 1989, Shewry et al 1992). Environment also has effects on protein composition and functionality. The variability of nitrogen and/or sulphur input may influence protein content and protein composition (Wrigley et al 1984).

### **2.3 RHEOLOGICAL STUDIES OF DOUGH**

Rheology is the science dealing with deformation of matter. It involves two basic properties: viscosity and elasticity (Bushuk 1985). Dough rheological properties are important to bakers for two reasons. Firstly, these properties determine the behavior of dough during mechanical handling, such as rounding and molding, and secondly, they affect the baking performance of wheat flour (Bloksma and Bushuk 1988, Botero Uride 1997). The physical dough tests, such as Farinograph and Mixograph, can be used as rapid substitutes, as well as useful complements, for baking tests (Bloksma and Bushuk 1988).

### **2.3.1 Correlation between Rheological Characteristics and Protein Composition**

The rheological properties of dough mainly depend on gluten proteins, gliadin and glutenin (Bushuk 1985). During the formation of dough, with the addition of water, the gliadin fraction becomes highly viscous, whereas the glutenin part is very elastic, and for this reason, it is postulated gliadins contribute to the viscosity, and glutenins contribute to the elasticity of dough. Stathopoulos et al. (2000) confirmed this speculation by performing rheological tests on a number of gluten sub-fractions with distinct molecular weight distribution ranging from HMW to LMW.

In spite of the fact that the HMW-GS accounts for only 20% of the total glutenins, they are the major contributor to the elasticity of gluten and dough strength related parameters (Shewry et al 1989). A number of research studies have been carried out on HMW-GS at molecular and genetic level. Gupta et al. (1995) stated that both HMW and LMW GS are crucial components to maintain wheat flour as a dough. With a complete loss of either HMW-GS or LMW-GS, gluten elasticity diminishes. They also found that the polymers of Glu-1 (HMW-GS) had greater positive effects to the functionality of dough development and maximum dough resistance than the polymers of Glu-3 (LMW-GS). Two remarkable characteristics of HMW-GS have been concluded to contribute to dough's functionality. First, HMW subunits have efficient cysteine residues present allowing reactions between themselves and other polypeptides, thus forming disulfide bond networks of glutenins. This cross-linking structure of the

glutenin polymers may have notable effects on elasticity property of dough. Second, the protein conformation, and in particular the  $\beta$ -turn rich repetitive domain, could also contribute to gluten properties. The  $\beta$ -turn rich conformation represents an energetically favored state. Stretching would probably disrupt this state, deform the peptide backbone and hydrogen bonds, and expose hydrophobic groups to the aqueous environment of the wet dough. This is an energetically unstable state. So when removing the stress, the stable structure would reform, resulting in elastic recoil and the recovery of the dough (Shewry et al 1992, Shewry et al 1989).

Proteins dominate the performance of the dough. Meanwhile, the other components in dough also play important roles in dough rheological behavior. For example, the carbohydrate components may be directly involved in the aggregation tendency of specific glutenin subunits and thereby contribute to the functionality of the protein in gluten (Lasztity 1996, Shewry et al 1989). In general, during dough formation, glutenins would interact with gliadins, residue starch, and lipids by hydrogen, hydrophobic, ionic forces, and disulphide bonds to construct gluten network.

### **2.3.2 Measuring Methods**

The rheological properties of dough are usually determined by using empirical methods rather than fundamental tests. Equipments used to conduct these tests include Farinograph, Mixograph, Extensograph, and Alveograph. Oliver and Allen (1992) stated that these apparatus are very useful to monitor

and control wheat flour quality and to predict the behavior of dough in baking performance.

### 2.3.2.1 Farinograph

The Farinograph is one of the two most widely used instruments for testing the rheological behaviors of dough (Kunerth and D'Appolonia 1985). The Farinograph curve (farinogram), obtained when a dough is kneaded between two broad sigma-shaped paddles, records the resistance that the dough offers to the mixing blades during a prolonged and gentle mixing action at a controlled temperature (Shuey 1975). This methodology requires a certain amount of water be added to the flour to produce a flour-water system. During the mixing process, the Farinograph curve height increases as the dough system becomes more resistant to stretching, until it reaches to a high peak point. Time to reach this certain point is termed as dough development time (DDT). After this peak point, the dough becomes less resistant to the mixing, and starts to breakdown. The 500 Brabender Units (BU) line is generally used to define optimum dough consistency. On the basis of a set of parameters of which dough development time (DDT), stability, mixing tolerance index (MTI), time to breakdown are the most important, the Farinograph provides information for evaluating flour quality. The most important information obtained is the Farinograph water absorption and the tolerance of the dough to mechanical mixing.

The shape of the farinogram can vary considerably depending on the variety, class of wheat and growing environment, as well as the grade and

storage conditions (Kunerth and D'Appolonia 1985). With regard to the correlation with protein, both protein content and protein quality may affect the farinogram markedly.

#### 2.3.2.2 Mixograph

The Mixograph is another widely used recording dough mixer that is used to measure dough handling properties. Two pairs of moving vertical pins, attached to the mixing head, provide the mixing action of the Mixograph. At the bottom of the bowl, there are three fixed pins opposing the rotating pins. During mixing, initially, with the folding and stretching action of the pins, water is absorbed by flour, and the dough starts to develop. The force resisting the movement of the pins gradually increases to the maximum point, which corresponds to the top of the curve. Beyond this point, the mobility of dough increases resulting in the downward slope of the curve (Kunerth and D'Appolonia 1985).

Like the Farinograph, various parameters of Mixograph curves are correlated with the quality factors of wheat. Lukow (1997) indicated that mixogram peak height, which includes three important quality attributes: protein content, water absorption, and dough strength, is a critical parameter to monitor breadmaking quality. Compared to the Farinograph, the Mixograph is much smaller, generally requires less wheat flour to carry out an analysis, and uses a higher speed. As a result, the Mixograph is suited for early generation wheat quality screening. Suchy et al. (2000) successfully developed a small-scale

method using a 2-g Mixograph combined with the TA.XT2 texture analyzer for Canadian wheat breeding programs.

## **2.4 SUMMARY**

Micronization (infrared heat processing) is a relatively new technology with much potential. Interest in the application of micronization to agricultural products has increased in the past twenty years due to its high efficiency and many other advantages. Micronization has been used in cereals, legumes, and oil seeds to reduce cooking time, achieve starch gelatinization, and enhance nutritional value extensively. Wheat is the most important grain grown in the Prairie Provinces. Wheat proteins dominate breadmaking quality of wheat. Because of the importance and complexity of wheat proteins, cereal chemists are always vigorously involved in qualifying and quantifying individual protein components. Separating wheat proteins based on solubility and molecular size are two important and effective approaches in protein study. The relationship between wheat quality and wheat protein composition and rheological behaviors has been investigated extensively.

## MATERIALS AND METHODS

### 3.1 MATERIALS

#### 3.1.1 Wheat Samples

Two sets of wheat samples representing varieties from three different bread wheat classes and a newly registered hard white bread variety were chosen in the primary study to reflect a wide range of physicochemical properties and dough mixing behaviors, as listed in Table 3.1.

Table 3.1 Sources and Characteristics of Wheat Samples in the Primary Study

Variety	Class	Characteristics	Location	Year
AC Karma (1)	CPSW	Medium strong	Manitoba	1997
AC Karma (2)			Saskatchewan	1998
AC Barrie (1)	CWRS	Strong	Manitoba	1999
AC Barrie (2)			Manitoba	1999
Glenlea (1)	CWES	Extra strong	Manitoba	1999
Glenlea (2)			Manitoba	1997
AC Ivory (1)	<sup>1</sup>	Strong, similar to	Saskatchewan	1998
AC Ivory (2)		AC Barrie	Saskatchewan	2000

<sup>1</sup> Referred as Canada Western White Spring (CWWS) wheat in this thesis.

The grades of all the wheat samples for the primary study were Canada No.1. The same AC Barrie wheat sample was used to study the contribution of tempering to micronization effects. Sprouted AC Karma wheat, grown in

Saskatchewan in 2000, was used in the research on the effects of micronization on sprouted wheat. Wheat samples were stored in a walk-in freezer at  $-10^{\circ}\text{C}$ , before the micronizing treatment.

### 3.1.2 Experimental Equipments

The experimental equipments are listed in Table 3.2.

Table 3.2 Major Experimental Equipment Used in the Study

MANUFACTURER	EQUIPMENT
Biochrom Ltd., UK	Ultrospec 1000 UV/Visible spectrophotometer
Brabender Instruments Inc., USA	Do-corder 2200-3
Buhler Brothers, Switzerland	Buhler Automatic Laboratory Mill, Type: MLU-202
Dickey-john Corp., USA	Instalab 800 (NIR product analyzer)
LECO Corp., USA	LECO FP-528
Micronizing Co., UK	MR2 Micronizer
National Manufacturing, USA	2-g micromixograph with computerized analysis
LKB-Bromma, USA	2150 HPLC pump
LKB-Bromma, USA	2152 HPLC controller
LKB-Bromma, USA	2141 chromatography system
Perten Instruments NA Inc., USA	Single Kernel Characterization System (SKCS) 4100
Phenomenex, Torrance, USA	Phenomenex Biosep SEC-4000 column
Texture Technologies Corp., UK	TA.XT2 Texture Analyzer

## 3.2 METHODS

### 3.2.1 Sample Tempering

Wheats were tempered to specific moisture contents by adding water to the samples for a specific time before micronization. The water required was calculated as follows (Arntfield et al 1997):

$$\text{Weight of H}_2\text{O (kg)} = \frac{\text{Weight of wheat (kg)} \times [\% \text{ H}_2\text{O (target)} - \% \text{ H}_2\text{O (initial)}]}{100 - \% \text{ H}_2\text{O (initial)}}$$

For each treatment, five kilograms of wheat were divided into two equal portions and placed in closed 11.4 L (40.6 × 27.9 × 15.2 cm) Rubbermaid® containers. Wheats were tempered with distilled water to 16%, or 22% moisture content, and allowed to stand overnight at room temperature (25°C). During the tempering period, the wheats were shaken occasionally to equilibrate the moisture level throughout the tempering container.

### 3.2.2 Micronizing Treatment

The pilot scale, gas-fired MR2 micronizer (Micronizing Co., UK), shown in Figure 3.1, was used to process non-tempered and tempered grain seeds. During operation, the seeds were fed, in a single layer, to the conveyor via a hopper and a vibratory feeder. In order to achieve uniform surface exposure to the radiator, the conveyor vibrated seeds constantly during the radiation process.

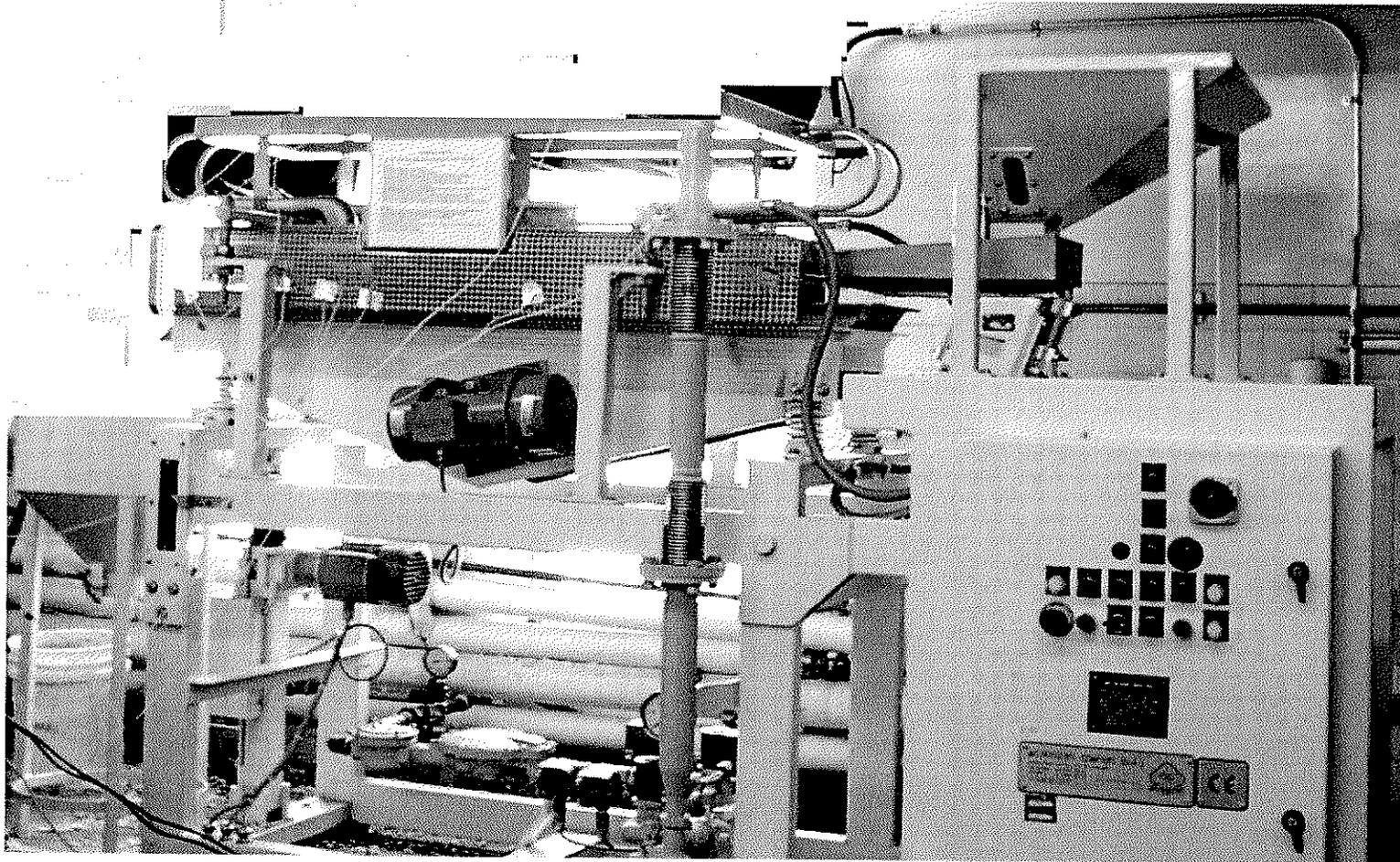


Fig. 3.1 Photograph of MR2 Micronizer used in the study

To conduct an operation, the natural gas-fired burners were lit, and then a warm-up pea sample was fed through the micronizer to preheat the equipment to stabilize processing conditions. Thereafter the samples were mixed and poured into the hopper. The gas input to the radiator, which was set at level 7 in the whole process, controlled the surface temperature of the infrared sources. The feeding speed was 60-65, adjusted by changing the setting of the rheostat. The slope of the vibratory conveyor bed was between 0 and  $-1$ . An infrared thermometer (Cole-Palmer Instrument Co., Illinois) was applied to measure surface temperature of the micronized seeds (Toews 2001). Based on a preliminary study, in which no significant rheological difference was found between samples which surface temperatures heated to  $100^{\circ}\text{C}$  and the ones that had reached higher surface temperatures, the final target temperature for the micronized seeds was set at  $100 \pm 5^{\circ}\text{C}$ . The treated samples were collected, cooled to room temperature, and then sealed in plastic bags and stored in a walk-in freezer at  $-10^{\circ}\text{C}$  for future analyses.

### **3.2.3 Physical Properties**

#### **3.2.3.1 Single Kernel Characteristics**

The Single Kernel Characteristics System (SKCS) 4100 (Perten Instruments NA Inc., USA) was used to conduct kernel characteristics testing. Three hundred grains were measured in each test using the computerized system. The distributions of kernel weights, diameters, hardness indices, and

moisture contents for the 300 grains were shown graphically, and the data were summarized as mean values and standard deviations for each characteristic.

#### 3.2.3.2 Milling Performance

After the various micronizing treatments, the wheat samples (except the ones with 22% moisture conditions) were tempered overnight to 16.5% moisture level before milling. The samples with 22% moisture were milled at as is. A Buhler Automatic Laboratory Mill (Type: MLU-202) (Buhler Brothers, Switzerland) was used to mill the wheat to produce straight-grade flour. The amounts of brans, shorts, break flours, reduction flours, and the total flour yields of all the samples were reported. The break flour and reduction flour were mixed together for further study.

#### 3.2.3.3 Ash Content

The ash content was determined using AACC method 08-01 (American Association of Cereal Chemists 1997).

### 3.2.4 Chemical Analyses of Protein Components

#### 3.2.4.1 Total Protein Content

For each test, 100 mg flour (14% moisture basis (m.b.)) was added to a tin foil cup, wrapped and tightly compressed into a pellet. Then the flour was analyzed for total flour nitrogen by applying the modified Dumas (Combustion Nitrogen Analysis) method using LECO FP-528 (LECO Corp.). The results were

corrected to protein content using a 5.7 factor (nitrogen  $\times$  5.7 = protein) (Lukow et al 2000).

### 3.2.4.2 Protein Fractionation Analysis

The protein fractionation was performed using a small-scale protein fractionation method (Lukow et al 2000). Three sequential extractions of flour were carried out. The first one was with 7.5% 1-propanol and 0.3M NaI, the second with 50% 1-propanol, and the third with 40% 1-propanol and 0.2% DTT. The proteins were separated into four fractions: monomeric protein (MP), soluble glutenin (SG), insoluble glutenin (IG), and residue protein (RP) as shown in Fig.3.2. The protein contents ( $N \times 5.7$ ) of these four groups were quantified by

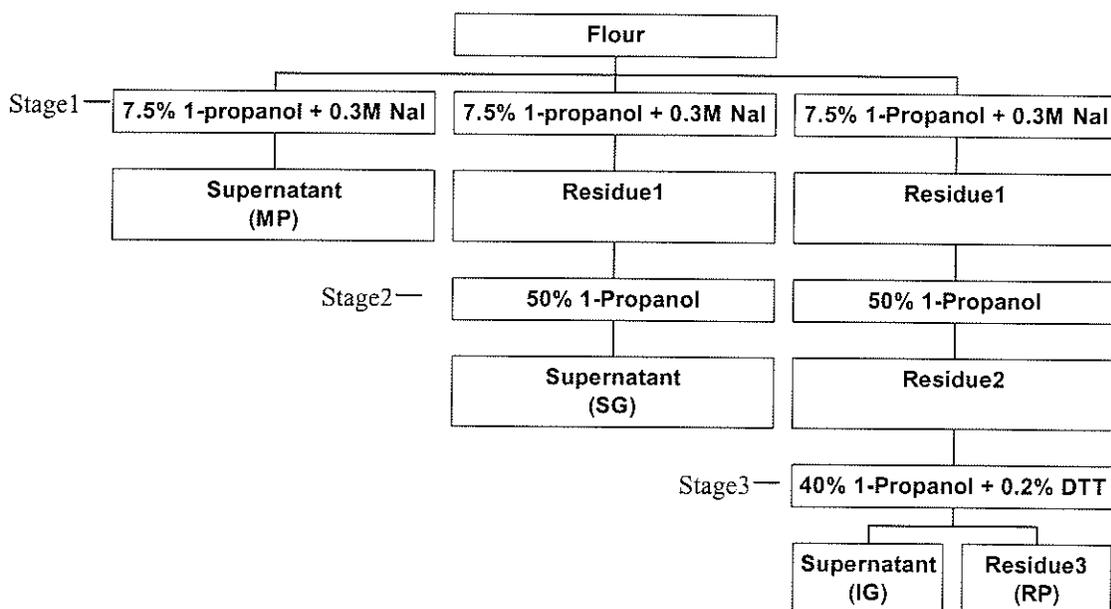


Fig.3.2 Simplified protein fractionation scheme (modified from Lukow et al 2000)

first analyzing the dried insoluble residues at each stage using the modified Dumas method (FP528, LECO Corp.), and then by mathematical calculation. Monomeric protein equaled the difference of protein content between flour and residue 1, SG equaled the difference of protein content between residue 1 and residue 2, IG equaled the difference of protein content between residue 2 and residue 3, and RP equaled the amount of protein left in residue 3.

#### 3.2.4.3 Size-Exclusion HPLC Procedure

SE-HPLC was carried out using typical running conditions (Larroque et al 2000). Flour (50 mg, 14% m.b.) was stirred for 50 min at 25°C in the presence of 1 ml 50% 1-propanol. The supernatant, obtained by centrifuging the sample twice (15 min. each at 15,000 × g), was filtered through a 0.45 µm filter (0.45 micron, Cameo 3N Syringe Filter Nylon, Osmonics Inc.). Ten microliter protein extract was then injected into a Phenomenex Biosep SEC-4000 column (Phenomenex, Torrance, USA), and run for 30min on isocratic gradient of 50% water (+0.05% TFA) and 50% acetonitrile (+0.05% TFA) using an HPLC system (LKB-Bromma, USA), which comprised a model 2150 pump, a model 2152 controller, and a model 2141 chromatography system at 214 nm. The valleys were considered as cutoff points between peaks for all the control samples (Singh et al 1990a), and the peaks of the micronized samples were determined accordingly. A fixed time of 22 minutes was applied as the cutoff point for the last peak in all samples.

#### 3.2.4.4 Electrophoretic Analyses

Electrophoretic analyses included SDS-PAGE for high molecular weight (HMW) gels (Lukow et al 1989), low molecular weight (LMW) gels (Lukow et al 1994), and  $\omega$ -gliadin gels (Nieto-Taladriz et al 1994).

#### 3.2.4.5 Alpha-amylase Activity

Alpha-amylase activity testing was performed according to an alpha-amylase assay for flour produced from pre-harvest sprouted grains and food products containing trace levels of  $\alpha$ -amylase, with some modifications (procedure 2, AMZ 8/96, Megazyme International Ireland Ltd., Wicklow, Ireland). Flour samples ( $0.5 \pm 0.01$  g) were weighed into test tubes. Sodium maleate buffer (5 ml) was added to each tube. The tubes were placed in the incubation bath at  $60.0^{\circ}\text{C}$  for 5 min., and briefly vortexed at intervals. An amylazyme tablet was then added to each tube, and the tubes were vortexed 5 sec. every 2 min. (at the beginning, and at the end of 2 and 4 min.). Trizma base (6 ml) was added at exactly 5 min. to terminate the reaction. The tubes were left at room temperature for 5 min., and the flour slurry was filtered through Whatman GF/A glass fiber filter paper. The absorbance of the filtrate was measured at 590nm using Ultrospec 1000 UV/Visible spectrophotometer (Biochrom Ltd., UK).

### 3.2.5 Rheological Measurements

#### 3.2.5.1 Farinograph

The Do-corder 2200-3 (Brabender Instruments Inc.) was used to conduct Farinograph tests. Water, based on preliminary test, to produce a Farinograph curve with maximum consistency centered on the 500 BU line, was added to 50 gram of flour (14% m.b.) (American Association of Cereal Chemists 1997).

#### 3.2.5.2 Two-gram Mixograph

Mixograph analysis was performed using the computerized 2-g Mixograph (National Manufacturing, Lincoln, NE, USA). A 2 g of flour sample (14% m.b.) and 0.29 g of 2% salt (w/w) were used at water absorption level FAB+6% (Suchy et al 2000).

#### 3.2.5.3 Microscale Extension Test

Micro-extension tests were performed using a TA.XT2 Texture Analyzer (Texture Technologies Corp., Stable Microsystems, Surrey, UK). Dough was made under the same mixing conditions as in 3.2.5.2, and the mixing was stopped at peak development. The dough was taken out gently from the mixing bowl and positioned over three to four channels of the Teflon-coated block (Suchy et al 2000). After a resting time of 40 min. at 25°C in an incubator, the individual dough strips were measured on the texture analyzer at a hook speed of 3.3 mm/s and a trigger force of 0.02 N.

### 3.3 Experimental Design and Statistical Analyses

This research was divided into three studies, which were designed to investigate I) the effects of micronization on the physicochemical and rheological properties of four varieties (primary study), II) the contribution of tempering to micronization effects (tempering study), and III) the effects of micronization on sprouted wheat (sprouting study).

The primary study included two sets of all four wheat varieties, which made a total of eight samples. The study was designed as a factorial experiment with two factors and performed within blocks. Each set of samples was considered as a block, and the two factors were variety and treatment. There were four levels (AC Karma, AC Barrie, Glenlea, and AC Ivory) in factor one, variety. In factor two, treatment, there were also four levels (non-micronized control, i.e., the samples were neither micronized nor tempered (NM\_NT), micronized but not tempered (M\_NT), tempered to 16% moisture level then micronized (M\_T16%), and tempered to 22% moisture level then micronized (M\_T22%)). These two factors resulted in a total of sixteen variety  $\times$  treatment combinations (Table 3.3).

Table 3.3 Experimental Design of the Blocks for the Primary Study

VARIETY	TREATMENT			
	NM_NT	M_NT	M_T16%	M_T22%
AC Karma	√	√	√	√
AC Barrie	√	√	√	√
Glenlea	√	√	√	√
AC Ivory	√	√	√	√

The treated and untreated samples were evaluated for physical, chemical and rheological parameters. Except for milling performance, two replications were conducted for each test, and means of replications were analyzed. After finishing the first set of samples, the second set was processed and tested in the same way. For the analysis of variance (ANOVA), the sources of variation (with degrees of freedom (df)) are: block (1), variety (3), treatment (3), variety x treatment interaction (9), error (15), making a total of 31 df.

Factorial experimental designs were also applied in the studies to determine the contribution of tempering to micronization effects of AC Barrie wheat and the effects of micronization on sprouted AC Karma wheat. For the contribution of tempering to micronization effects study, there were two levels (non-micronized (NM) and micronized (M)) in factor one, micronizing condition, and in factor two, tempering condition, there were also two levels (non-tempered (NT) and tempered to 22% moisture level (T22%)). For the effects of micronization on sprouted wheat study, there were two levels (sound and sprouted) in factor one, and in factor two, treatment, there were three levels (NM\_NT, M\_NT, and M\_T22%).

All statistics calculations were conducted using general linear models (GLM) or correlation analysis procedure of the SAS system V8.1 (SAS Institute Inc, Cary, NC). Whenever there was a significant ( $\alpha = 0.05$ ) main effect, mean values were compared by Ryan-Einot-Gabriel-Welsch Multiple Range Test (REGWQ) at  $\alpha = 0.05$  level.

## RESULTS AND DISCUSSION

The study focused on the physicochemical and rheological properties, which are important indices for wheat quality and end-use purposes, and can be affected by many factors, including genotype, growing environment, and protein quality and quantity. Two sets of wheat samples from different sources were chosen for this study. Some variations in basic characteristics among duplicate wheat control samples were observed (Table 4.1). The hardness data for two

Table 4.1 Basic Physical and Chemical Properties of Two Sets of Wheat Control Samples<sup>1</sup>

Variety	Weight (mg)	HI <sup>2</sup>	Moisture (%)	Protein <sup>3</sup> (%)	Ash (%)
Karma (1)	35.46±0.06	93.79±0.29	12.94±0.06	11.40±0.00	0.59±0.01
Karma (2)	37.79±0.21	87.88±0.70	10.95±0.02	11.85±0.07	0.51±0.00
Barrie (1)	32.62±0.09	107.29±0.11	13.91±0.06	12.95±0.07	0.48±0.00
Barrie (2)	31.68±0.01	83.29±1.22	12.03±0.01	13.10±0.00	0.49±0.02
Glenlea (1)	44.16±0.15	96.73±0.23	9.99±0.06	10.80±0.00	0.59±0.00
Glenlea (2)	41.09±1.25	90.71±2.27	12.68±0.01	12.75±0.07	0.55±0.00
Ivory (1)	30.69±0.08	101.84±1.54	14.15±0.01	14.70±0.00	0.42±0.00
Ivory (2)	28.60±0.34	93.56±0.42	11.72±0.04	15.50±0.14	0.45±0.00

<sup>1</sup> Means of two determinations ±standard deviation.

<sup>2</sup> HI: Hardness Index.

<sup>3</sup> Data are reported on a 14.0% moisture basis.

AC Barrie samples were quite different, ranging from 83.29 to 107.29. This could be due to various factors, such as growing environment, moisture level, and storage condition. There was an obvious variation in protein content between the two Glenlea samples (10.8% and 12.8%). The difference in protein content could affect the rheological behaviors and other properties of dough made of these flours.

#### **4.1 PHYSICAL PROPERTIES**

##### **4.1.1 Single Kernel Characteristics**

Wheat physical characteristics are important factors for distinguishing wheat class and predicting flour milling and breadmaking performance (Ohm et al 1998). Some of the characteristics are evaluated by Single Kernel Characteristics System (SKCS), which include single kernel weight, single kernel diameter, single kernel hardness index, and single kernel moisture content (Appendix I). Additional data, such as the distribution of moisture contents of individual kernels and the uniformity of hardness, could provide information about storage stability and the consistency of the milling performance (Osborne et al 1997). The overall means of these physical parameters for each of the four wheat varieties are summarized (Table 4.2). No significant interactions were found between variety and treatment for these parameters.

Table 4.2 Mean Values over all the Treatments for Single Kernel Characteristics of Four Wheat Varieties <sup>1</sup>

Variety	Single Kernel Characteristics <sup>2</sup>			
	Weight (mg)	Diameter (mm)	HI <sup>3</sup>	Moisture (%)
AC Karma	37.48 <sup>b</sup>	3.30 <sup>b</sup>	87.43 <sup>b</sup>	13.75 <sup>a</sup>
AC Barrie	32.68 <sup>c</sup>	3.24 <sup>b</sup>	96.04 <sup>ab</sup>	13.64 <sup>a</sup>
Glenlea	43.31 <sup>a</sup>	3.54 <sup>a</sup>	92.22 <sup>b</sup>	13.11 <sup>a</sup>
AC Ivory	29.77 <sup>d</sup>	3.10 <sup>c</sup>	103.80 <sup>a</sup>	13.85 <sup>a</sup>

<sup>1</sup> Each value in the table is the overall mean value for each variety, n=16.

<sup>2</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

<sup>3</sup> HI: Hardness Index.

Kernel weight is an indication of the density and soundness of the wheat (Ohm et al 1998). The results obtained for single kernel weight were close to those of comparable wheat reported (Canadian Wheat Board 2000). There was a significant difference in kernel weight for the four wheat varieties (Table 4.2). Glenlea, as an extra strong variety, had the highest kernel weight (43.31 mg), followed by AC Karma prairie spring wheat (37.48 mg).

Diameter was a function of weight. A larger kernel size was observed at higher kernel weight (Table 4.2). Recently developed AC Ivory wheat had the smallest kernel size (3.10 mm) and weight (29.77 mg).

Hardness is an important parameter for the differentiation of wheat classes, and is closely related to the end-use properties of wheat (Ohm et al

1998). The SKCS 4100 system classifies wheat into hard or soft based on HI values, which segregate wheat on a numeric scale. Hard wheat is assigned a value of 75, whereas, 25 for soft wheat (Gaines et al 1996). All four wheat varieties chosen for this project were hard wheats, and suitable for bread making. In general, Glenlea (CWES) has a harder kernel than AC Barrie (CWRS), which results in a longer period of time for tempering Glenlea to optimize milling performance (CWB 2000). In this study, the HI values of AC Barrie and Glenlea were very close (Table 4.2), which is probably due to environmental variations and relatively small sample size of the study.

There were some variations in kernel properties for wheat samples after the treatments. The average results for single kernel characteristics for each of the four processing conditions are given in Table 4.3. There was no significant difference between non-micronized control samples and the micronized that had been not tempered for all parameters. However, softer kernels were observed for all varieties at the highest tempering level, when compared to the M\_NT and M\_T16% samples. Meanwhile, increased moisture contents in the samples were also detected after the tempering process. The effects were consistent across all varieties.

Table 4.3 Mean Values over all the Varieties for Single Kernel Characteristics of Four Treatments <sup>1</sup>

Treatment <sup>3</sup>	Single Kernel Characteristics <sup>2</sup>			
	Weight (mg)	Diameter (mm)	HI <sup>4</sup>	Moisture (%)
NM_NT	35.26 <sup>ab</sup>	3.26 <sup>a</sup>	94.38 <sup>ab</sup>	12.29 <sup>c</sup>
M_NT	34.68 <sup>b</sup>	3.28 <sup>a</sup>	101.36 <sup>a</sup>	10.89 <sup>c</sup>
M_T16%	35.79 <sup>ab</sup>	3.27 <sup>a</sup>	96.41 <sup>a</sup>	14.39 <sup>b</sup>
<b>M_T22%</b>	<b>37.51 <sup>a</sup></b>	<b>3.37 <sup>a</sup></b>	<b>87.33 <sup>b</sup></b>	<b>16.77 <sup>a</sup></b>

<sup>1</sup> Each value in the table is the overall mean value for each treatment, n=16.

<sup>2</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

<sup>3</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

<sup>4</sup> HI: Hardness Index.

The increase in moisture content would likely increase kernel weight and size. For hard wheat, like the samples investigated in this study, moisture content has a pronounced impact on hardness. It has been reported that wheat became progressively softer with increasing moisture level (Pomeranz and Williams 1990). This raised the question of whether moisture alone rather than in combination with the micronizing treatment changed the other three parameters.

Table 4.4 Mean Value for Single Kernel Characteristics on the Effect of Micronizing/Tempering Treatments from AC Barrie Wheat <sup>1</sup>

Treatment <sup>3</sup>	Single Kernel Characteristics <sup>2</sup>			
	Weight (mg)	Diameter (mm)	HI <sup>4</sup>	Moisture (%)
NM_NT	32.15 <sup>b</sup>	3.20 <sup>b</sup>	95.29 <sup>a</sup>	12.97 <sup>b</sup>
M_NT	31.36 <sup>b</sup>	3.20 <sup>b</sup>	102.89 <sup>a</sup>	10.62 <sup>b</sup>
M_T22%	34.83 <sup>a</sup>	3.37 <sup>a</sup>	87.97 <sup>a</sup>	16.98 <sup>a</sup>
NM_T22%	34.88 <sup>a</sup>	3.34 <sup>a</sup>	77.40 <sup>a</sup>	17.47 <sup>a</sup>

<sup>1</sup> Each value in the table is the average of four determinations.

<sup>2</sup> Means in the same column under the same factor (micronizing/tempering) with different letters are significantly different ( $P \leq 0.05$ ).

<sup>3</sup> NM: non-micronized; M: micronized; NT: non-tempered; T22%: tempered to 22% moisture.

<sup>4</sup> HI: Hardness Index.

In order to determine whether the alterations of single kernel characteristics were due to the tempering or the micronizing-tempering interaction, two AC Barrie samples as used in the previous study were tempered to 22% moisture level without micronization. After that, they were air-dried to similar moisture content as the M\_T22% samples. There was no interaction between tempering and micronizing condition. No significant differences were found for parameters with or without micronization at two tempering levels (Table 4.4). This result suggests that micronization had no obvious influence on kernel characteristics. On the contrary, tempering level had significant impacts on single kernel weight and diameter. Considering the great variation in hardness index value between two AC Barrie control samples, no significant

difference was found in hardness parameter for all samples. Moisture content had a strong positive correlation with single kernel weight (Fig. 4.1). There was a relatively weak negative correlation between single kernel moisture and HI (Fig. 4.2). It was concluded that moisture content was a factor in the differences for single kernel characteristics within varieties.

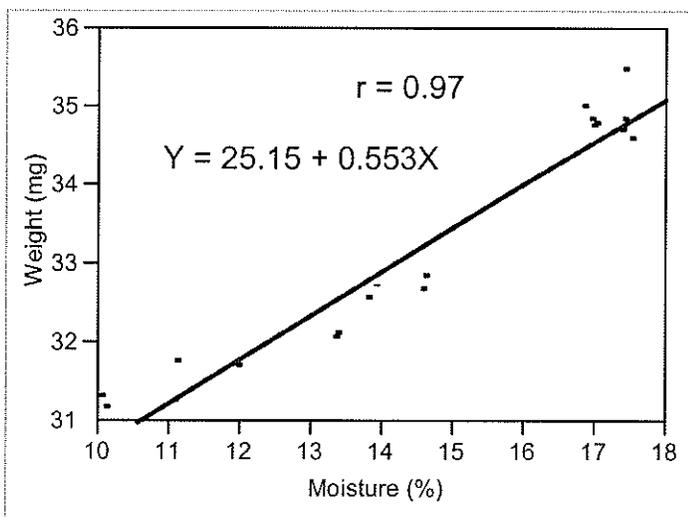


Fig. 4.1 Correlation of weight (mg) with moisture content (%) of AC Barrie

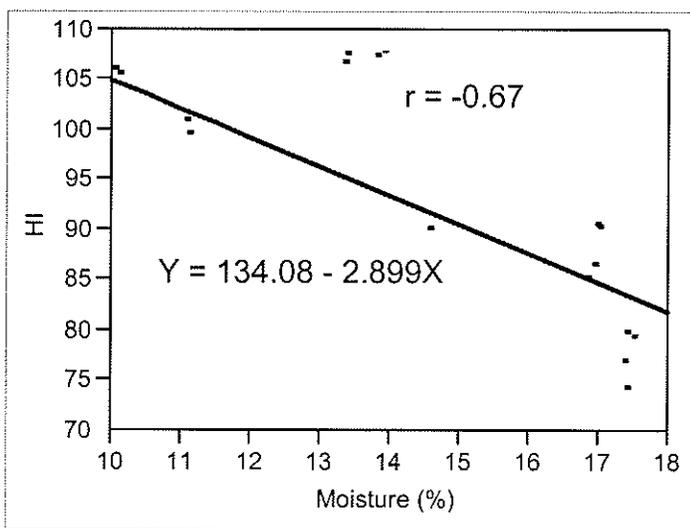


Fig. 4.2 Correlation of hardness index with moisture (%) of AC Barrie

### 4.1.2 Milling Performance

No obvious differences for milling parameters were found between control samples and the samples micronized at low moisture levels (Appendix II). However, there were some significant differences for milling parameters between control samples and the samples subjected to micronizing at 22% moisture level. Flour yield is one of the most important indices for milling performance. M\_T22% treated samples had the lowest average flour yield (67.96%) among the four treatments (Table 4.5). Compared to the control samples, the overall drop was 6.7%. This indicated that the endosperm was

Table 4.5 Mean Values over all the Varieties for Wheat Milling Properties of Four Treatments <sup>1</sup>

Treatment <sup>3</sup>	Milling Parameters <sup>2</sup>			
	Yield (%)	Bran (%)	BF (%) <sup>4</sup>	RF (%) <sup>5</sup>
NM_NT	72.85 <sup>a</sup>	15.04 <sup>b</sup>	23.45 <sup>a</sup>	49.72 <sup>a</sup>
M_NT	73.44 <sup>a</sup>	15.91 <sup>b</sup>	22.41 <sup>a</sup>	51.39 <sup>a</sup>
M_T16%	72.94 <sup>a</sup>	15.69 <sup>b</sup>	22.09 <sup>a</sup>	51.57 <sup>a</sup>
<b>M_T22%</b>	<b>67.96 <sup>b</sup></b>	<b>19.09 <sup>a</sup></b>	<b>20.88 <sup>a</sup></b>	<b>47.66 <sup>b</sup></b>

<sup>1</sup> Each value in the table is the mean value for each treatment, n=8.

<sup>2</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

<sup>3</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

<sup>4</sup> BF: break flour.

<sup>5</sup> RF: reduction flour.

hard to separate from the bran, which resulted in the loss of flour yield. This corresponded to the increase of bran content, from 15.04% to 19.09%. However, no obvious changes were found among NM\_NT, M\_NT, and M\_T16% samples. In the tempering study, there were also no differences in milling results between NM\_NT and NM\_T22% samples of AC Barrie. These results suggested that either micronizing without tempering or tempering alone had no influence on milling performance. Only micronization at high moisture level produced a negative impact on milling performance.

#### **4.1.3 Ash Content**

Ash content is a good indicator for flour milling quality. In general, the lower the flour ash, the more desirable is that wheat for milling. There was a significant difference in ash content among the wheat samples (Table 4.6, Appendix III). AC Ivory wheat had the lowest ash content (0.45%) among the four wheat varieties, followed by AC Barrie (0.49%). Glenlea had a higher ash level (0.57%) compared to the first two samples. This is due to the higher natural mineral content in the endosperm of Glenlea (CWB 2000). The average ash content of AC Karma control samples (0.55%) was lower than that of Glenlea control. However, the ash content of AC Karma was higher after the micronization, probably due to the physical and structural variations between these two varieties after the treatments. The ash contents were progressively increased after the micronization with the increase of moisture level. The ash

Table 4.6 Ash Content (%) from Four Varieties of Four Treatments <sup>1</sup>

Variety	Treatment <sup>2</sup>				Mean <sup>3</sup>
	NM_NT	M_NT	M_T16%	M_T22%	
AC Karma	0.546	0.586	0.614	0.661	0.6015 <sup>a</sup>
AC Barrie	0.483	0.457	0.507	0.505	0.4878 <sup>c</sup>
Glenlea	0.570	0.522	0.559	0.600	0.5624 <sup>b</sup>
AC Ivory	0.433	0.450	0.455	0.478	0.4540 <sup>c</sup>
Mean <sup>3</sup>	0.5078 <sup>b</sup>	0.5034 <sup>b</sup>	0.5336 <sup>a b</sup>	0.5609 <sup>a</sup>	

<sup>1</sup> Each value in the table is the average of four determinations.

<sup>2</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

<sup>3</sup> Means in the same column/row with different letters are significantly different ( $P \leq 0.05$ ).

content of M\_T22% treated samples was significantly higher than that of control samples overall the varieties, 0.56% and 0.51%, respectively. This substantiated the results from the milling performance. Because of the same reason as the decrease of flour yield, M\_T22% treated flours may contain some ground bran materials. Therefore, they had a higher ash content than the others.

## 4.2 CHEMICAL ANALYSES OF PROTEIN COMPONENTS

### 4.2.1 Total Protein Content

The protein contents (14% m.b.) for the wheat flours are summarized (Table 4.7), and a significant correlation between protein content and wheat variety was found ( $P < 0.01$ ). Protein content of AC Barrie (12.9%) was typical for flour from the CWRS class (CWB 2000). The lower protein contents of AC Karma and Glenlea were also typical values for these types of wheats. Furthermore, AC Ivory had the highest protein content (14.9%). As expected, there were no differences in protein content among various processing conditions except a slight drop in the samples micronized at 22% moisture level (Appendix IV).

Table 4.7 Protein Content (%) from Four Varieties of Four Treatments <sup>1</sup>

Variety	Treatment <sup>2</sup>				Mean <sup>3</sup>
	NM_NT	M_NT	M_T16%	M_T22%	
AC Karma	11.7	11.7	11.6	11.4	11.6 <sup>c</sup>
AC Barrie	13.1	13.0	13.1	12.4	12.9 <sup>b</sup>
Glenlea	11.8	11.6	11.6	11.3	11.6 <sup>c</sup>
AC Ivory	15.1	15.0	14.9	14.6	14.9 <sup>a</sup>
Mean <sup>3</sup>	12.9 <sup>a</sup>	12.8 <sup>a</sup>	12.8 <sup>a</sup>	12.4 <sup>a</sup>	

<sup>1</sup> Each value in the table is the average of four determinations, 14% m.b.

<sup>2</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

<sup>3</sup> Means in the same column/row with different letters are significantly different ( $P \leq 0.05$ ).

#### 4.2.2 Protein Fractionation Analysis

Based on solubility, proteins of wheat flour samples were separated into four fractions. Monomeric protein (MP) and soluble glutenin (SG) groups were soluble without reduction. Whereas, insoluble glutenin (IG) can only be soluble by adding the reducing agents, and residue protein (RP) fraction was not soluble even after reduction. For the control samples, there was no significant difference in the percentage of RP (6.56 - 8.19%). In contrast, there were significant variations in MP, SG, and IG content among the four wheat varieties (Table 4.8). Glenlea, usually considered as the strongest wheat among these four varieties, had the highest percentage of IG (32.56%), whereas, AC Karma, which belongs to the CPSW class, and considered to have medium dough strength properties (CWB 2000), had the lowest percentage of IG (25.79%). The percentages of SG for Glenlea and AC Karma were also notably different, which were 8.41 and

Table 4.8 Percentage of Protein Fractions of Wheat Control Samples <sup>1</sup>

VARIETY	Percentage of Protein Fractions <sup>2,3</sup>				
	MP	SG	IG	RP	IG/SG
AC KARMA	54.05 <sup>ab</sup>	12.21 <sup>a</sup>	25.79 <sup>c</sup>	7.95 <sup>a</sup>	2.16 <sup>b</sup>
AC BARRIE	55.32 <sup>a</sup>	7.47 <sup>b</sup>	29.03 <sup>b</sup>	8.19 <sup>a</sup>	4.08 <sup>a</sup>
GLENLEA	52.46 <sup>b</sup>	8.41 <sup>b</sup>	32.56 <sup>a</sup>	6.56 <sup>a</sup>	3.95 <sup>a</sup>
AC IVORY	52.85 <sup>b</sup>	9.36 <sup>ab</sup>	30.70 <sup>ab</sup>	7.09 <sup>a</sup>	3.45 <sup>ab</sup>

<sup>1</sup> Each value in the table is the average of four determinations.

<sup>2</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

<sup>3</sup> MP: monomeric protein; SG: soluble glutenin; IG: insoluble glutenin; RP: residue protein.

12.21%, respectively. In absolute terms, Glenlea had approximately 26% more IG and more than 31% less SG when compared to AC Karma. Sapirstein and Fu (1998) studied the protein fractions of seven Canadian cultivars with diverse breadmaking quality. They reported that soluble and insoluble glutenin were highly correlated with dough properties and bread loaf volume, whereas, MP and RP were not.

The results indicated that the main difference in flour protein was the relative quantities of SG and IG. Accordingly, the ratio of IG to SG was evaluated (Table 4.8). The ratio for AC Barrie and Glenlea was significantly higher than that for AC Karma ( $P < 0.05$ ), which was 4.08, 3.95 and 2.16, respectively. This result suggested that there was a close correlation between gluten strength and the relative amount of IG and SG. The distribution between IG and SG could be used as a potential indicator for screening wheat functionality.

Micronizing treatments significantly and progressively reduced the amounts of MP and SG proteins in all wheat samples at all moisture levels. On the contrary, the percentages of IG and RP fractions consistently increased after the micronizing treatments (Table 4.9, Appendix V). No interaction was found between variety and treatment among all the protein groups (Table 4.10- 4.13).

Table 4.9 Mean Values of Protein Fractions (%) from Varieties of Four Treatments<sup>1</sup>

SAMPLE	PERCENTAGE OF PROTEIN FRACTIONS			
	MP	SG	IG	RP
AC KARMA NM_NT	54.05±0.75	12.21±0.95	25.79±1.46	7.95±0.23
AC KARMA M_NT	46.48±4.58	6.54±1.06	34.715±4.86	12.27±0.79
AC KARMA M_T16%	43.36±3.49	3.37±1.52	39.96±4.55	13.33±0.47
AC KARMA M_T22%	32.61±3.63	2.70±0.57	51.96±1.85	12.74±1.22
AC BARRIE NM_NT	55.32±0.45	7.47±0.19	29.03±0.62	8.19±0.88
AC BARRIE M_NT	51.45±1.20	4.94±2.27	32.30±2.48	11.33±0.98
AC BARRIE M_T16%	46.56±3.85	3.79±1.87	36.69±4.65	12.97±1.05
AC BARRIE M_T22%	39.71±2.13	2.48±1.24	43.76±2.57	14.06±0.79
GLENLEA NM_NT	52.46±1.88	8.415±1.36	32.56±0.03	6.56±0.48
GLENLEA M_NT	46.78±6.12	4.50±1.16	37.55±6.87	11.18±0.41
GLENLEA M_T16%	42.03±0.79	3.57±0.85	42.51±1.65	11.89±0.01
GLENLEA M_T22%	39.70±3.44	2.31±1.66	45.33±4.58	12.67±0.52
AC IVORY NM_NT	52.85±0.70	9.36±2.11	30.70±2.33	7.10±0.46
AC IVORY M_NT	44.31±0.22	4.41±0.70	38.00±0.34	13.30±1.27
AC IVORY M_T16%	43.64±4.89	3.48±1.91	40.21±4.91	12.67±1.90
AC IVORY M_T22%	37.43±0.16	2.33±0.78	46.36±2.49	13.88±1.57

<sup>1</sup> Means of four determinations ± standard deviation.

Table 4.10 The ANOVA for Monomeric Protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	19.4546	19.4546	2.31	0.149
Variety	3	83.5190	27.8397	3.31	0.0492
Treatment	3	1109.0314	369.6771	43.91	<0.0001
VarietyxTreatment	9	69.5975	7.7331	0.92	0.5354
Error	15	126.2876	8.4192		
Corrected Total	31	1407.8900			

Table 4.11 The ANOVA for Soluble Glutenin

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	3.0403	3.0403	1.65	0.2189
Variety	3	12.8589	4.2863	2.32	0.1166
Treatment	3	220.5724	73.5241	39.82	<0.0001
VarietyxTreatment	9	18.5990	2.0666	1.12	0.4067
Error	15	27.6940			
Corrected Total	31	282.7647			

Table 4.12 The ANOVA for Insoluble Glutenin

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	20.3207	20.3207	1.78	0.2018
Variety	3	75.3346	25.1115	2.20	0.1301
Treatment	3	1273.8006	424.6002	37.24	<0.0001
VarietyxTreatment	9	127.5734	14.1748	1.24	0.3407
Error	15	171.0213	11.4014		
Corrected Total	31	1668.0506			

Table 4.13 The ANOVA for Residue Protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	2.7117	2.7117	3.48	0.0816
Variety	3	7.0266	2.3422	3.01	0.0633
Treatment	3	171.7227	57.2409	73.55	<0.0001
VarietyxTreatment	9	7.6496	0.8500	1.09	0.4226
Error	15	11.6745			
Corrected Total	31	200.7850			

Table 4.14 Mean Percentage Values over all the Varieties for Protein Fractions of Four Treatments <sup>1</sup>

Treatment <sup>4</sup>	Percentage of Protein Fractions <sup>2,3</sup>				Total of MP and IG
	MP	SG	IG	RP	
NM_NT	53.67 <sup>a</sup>	9.36 <sup>a</sup>	29.52 <sup>d</sup>	7.45 <sup>c</sup>	83.19
M_NT	47.25 <sup>b</sup>	5.09 <sup>b</sup>	35.64 <sup>c</sup>	12.02 <sup>b</sup>	82.89
M_T16%	43.90 <sup>b</sup>	3.55 <sup>bc</sup>	39.84 <sup>b</sup>	12.71 <sup>ab</sup>	83.74
M_T22%	37.36 <sup>c</sup>	2.45 <sup>c</sup>	46.85 <sup>a</sup>	13.34 <sup>a</sup>	84.21

<sup>1</sup> Each value in the table is the mean value for each treatment, n=16.

<sup>2</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

<sup>3</sup> MP: Monomeric protein; SG: Soluble glutenin; IG: Insoluble glutenin; RP: Residue protein.

<sup>4</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

The influence of micronization on the distribution of flour proteins was studied. The overall means of the percentage of protein fractions for each treatment are summarized (Table 4.14). The percentage of soluble MP, fell from 53.67 for non-treated samples to 37.36 for those treated at the highest moisture level. These represented a decrease in MP of 30.4%. Even for micronization alone without changing moisture level, there was a significant drop in MP. The same trend was observed in the soluble fraction extracted with 50% 1-propanol (SG). The mean value fell from 9.36 for the control samples to 2.45 for those treated at the highest moisture (Table 4.14). This represented an overall decrease in SG of 73.9%. The proportions of MP and SG gradually decreased

as tempering level increased. This loss in soluble proteins could be due to protein denaturation under micronizing (Arntfield et al 1997).

The decrease in extractable proteins in MP and SG fractions for treated samples corresponded to an increase in the amounts of IG and RP fractions. Compared to non-treated samples, IG fraction was increased by 21-59%, and RP group was increased by 61-79% under the various micronizing treatments. The extent of the alteration was greater if the initial moisture content of the micronized samples was higher (Table 4.14).

It has been suggested that heat-induced denaturation might involve aggregation of polypeptide chains through disulfide bonding (Zheng et al 1998). Such heat-denatured proteins should be solubilized by reducing agent DTT or MCE. In this study, when the reducing agent DTT was added, a higher proportion of IG became extractable. This suggested that the aggregated MP and SG proteins produced during the micronization could still be broken down by a reducing agent, and became soluble after reduction, which was a convincing evidence to support the theory that micronizing heat denatured proteins were aggregated through disulfide bonding.

The combined total amounts of MP and IG fractions for each treatment were approximately the same, ranging from 82.89 to 84.21% (Table 4.14). There was a strong negative correlation between MP and IG fractions ( $r = -0.98$ ) (Fig. 4.3). These results substantiated the previous observations in terms of the marked changes induced by micronization heating for all protein fractions.

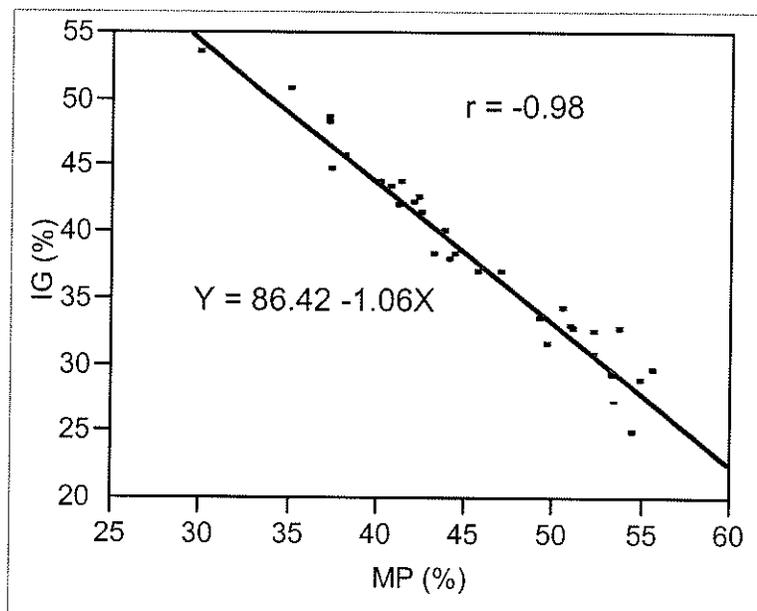


Fig. 4.3 Correlation of insoluble glutenin with monomeric protein  
of all the wheat samples

In order to examine the effects of tempering alone without micronization on protein fractions, fractionation analysis was conducted for NM\_T22% AC Barrie samples. The distribution of protein fractions for tempered samples was similar to that for the control samples (Appendix Vb). This result indicated that only tempering did not affect protein solubility.

### 4.2.3 Size-Exclusion HPLC Procedure

Size-exclusion chromatography separated flour protein fractions into three distinct peaks based on molecular size. These peaks represented polymeric proteins, gliadins, and albumins-globulins (Fig 4.4). Polymeric proteins (>100 KDa), mostly soluble glutenins, were eluted mainly in peak 1. All classes of gliadins (80-25 KDa) were eluted in peak 2. And albumins-globulins (25-5 KDa) corresponded to peak 3 (Singh et al 1990a). For the control samples, AC Ivory had the highest absolute values for the areas of peak 1, peak 2, and the total. This was attributed to the highest protein content of AC Ivory (15.1%). It has been reported that total protein content was highly correlated with absolute areas of individual peaks and total chromatographic area (Ciaffi et al 1996). As the protein contents of the wheats in this study, ranged from 11.7 to 15.1%, the relative amounts of protein were easier to compare than the absolute peak area values for the control samples (Table 4.15).

Table 4.15 Mean Values for Unreduced Extractable Proteins of Control Samples <sup>1</sup>

VARIETY	$10^{-5} \times$ Absolute HPLC Area <sup>2</sup>				Percentage of Total Area <sup>2</sup>		
	Peak 1	Peak 2	Peak 3	Total	Peak 1	Peak 2	Peak 3
AC KARMA	21.2 <sup>a</sup>	40.4 <sup>c</sup>	16.1 <sup>a</sup>	77.8 <sup>bc</sup>	27.2 <sup>a</sup>	52.0 <sup>c</sup>	20.7 <sup>a</sup>
AC BARRIE	17.6 <sup>b</sup>	56.4 <sup>b</sup>	13.6 <sup>a</sup>	87.6 <sup>b</sup>	20.1 <sup>b</sup>	64.4 <sup>a</sup>	15.5 <sup>bc</sup>
GLENLEA	15.0 <sup>b</sup>	43.2 <sup>c</sup>	12.7 <sup>a</sup>	70.9 <sup>c</sup>	21.1 <sup>b</sup>	61.0 <sup>b</sup>	17.9 <sup>b</sup>
AC IVORY	23.2 <sup>a</sup>	66.5 <sup>a</sup>	14.7 <sup>a</sup>	104.5 <sup>a</sup>	22.2 <sup>b</sup>	63.7 <sup>a</sup>	14.1 <sup>c</sup>

<sup>1</sup> Each value in the table is the average of four determinations.

<sup>2</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

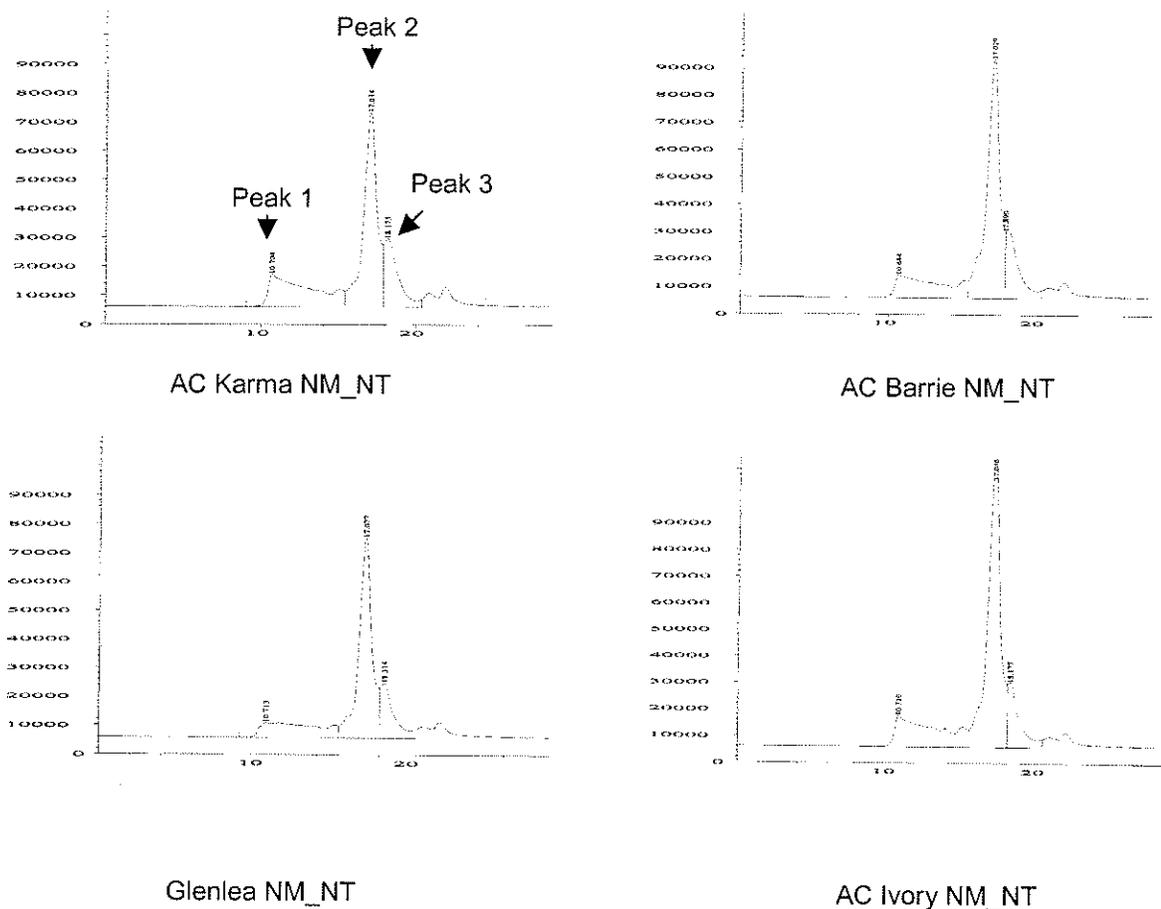


Fig. 4.4 SE-HPLC separation of unreduced protein fractions  
from untreated control wheat varieties

The percentage of peak 1 (SG) for AC Karma control (27.2) was significantly higher than that for the other three control samples ( $P < 0.05$ ). This result was consistent with the observation in the protein fractionation analysis, since the highest proportion of SG was also found in the AC Karma in that study. AC Karma had the smallest percentage of protein in peak 2 and the largest

percentage of protein in peak 3 among four wheat varieties, which indicated that although there was no obvious variation in total soluble MP among wheat control samples, the actual compositions of MP could be quite different. AC Karma had less gliadin and more albumin-globulin proteins than the other three varieties. This might be used as an index to explain the relatively weak dough properties of AC Karma wheat. In general, Glenlea, as an extra strong wheat, has the lowest percentage of extractable polymeric proteins. In this study, the percentage of polymeric proteins (peak1) for Glenlea, AC Barrie and AC Karma was not significantly different. This is probably due to environment variations and relatively small size of the study. There was no difference between AC Barrie and AC Ivory in percentage areas for these three peaks. This result suggested that these two varieties had similar protein compositions and might have similar dough properties as well.

Micronizing treatments significantly and progressively reduced SE-HPLC extractable protein fractions for all treated samples (Table 4.16, Appendix VI). There were no significant interaction effects between variety and treatment for the absolute peak areas (Table 4.17 - 4.20). The impact of micronization on the three main fractions was investigated (Table 4.21). The amounts of both total extractable protein and individual protein fractions were reduced after processing. Therefore the alteration of absolute peak areas was more noteworthy than the changes of percentage areas for studying the effect of micronization on protein fractions. The following discussion is based on the absolute areas of protein groups.

Table 4.16 Mean Values for Unreduced Soluble Protein Fractions Using SE-HPLC<sup>1</sup>

SAMPLE	10 <sup>-5</sup> x Absolute HPLC Area <sup>2</sup>			
	Peak 1	Peak 2	Peak 3	Total
AC KARMA NM_NT	21.25±1.20	40.45±3.18	16.15±1.06	77.85±5.44
AC KARMA M_NT	12.40±6.08	39.45±5.73	12.10±2.69	63.95±14.35
AC KARMA M_T16%	7.05±2.62	35.35±1.91	10.85±1.63	53.30±6.22
AC KARMA M_T22%	2.10±0.57	28.45±5.30	6.65±1.63	37.15±7.42
AC BARRIE NM_NT	17.60±0.00	56.40±2.83	13.55±0.07	87.60±2.69
AC BARRIE M_NT	11.15±2.05	57.3±4.38	11.6±0.99	80.05±7.28
AC BARRIE M_T16%	7.15±3.32	52.80±1.84	9.70±0.71	69.65±5.87
AC BARRIE M_T22%	3.10±1.27	42.15±0.92	7.30±0.99	52.50±3.25
GLENLEA NM_NT	15.00±1.98	43.25±3.18	12.75±1.63	70.90±6.79
GLENLEA M_NT	9.85±3.32	42.70±3.11	9.40±1.27	61.95±1.48
GLENLEA M_T16%	4.90±0.28	38.20±4.95	6.70±0.99	49.80±6.22
GLENLEA M_T22%	3.30±0.99	34.10±2.69	6.30±0.14	43.70±1.56
AC IVORY NM_NT	23.20±0.14	66.50±2.26	14.75±1.63	104.45±4.03
AC IVORY M_NT	9.35±0.49	57.00±3.25	9.10±0.57	75.40±4.24
AC IVORY M_T16%	8.35±4.17	55.35±3.18	9.15±0.78	72.75±8.13
AC IVORY M_T22%	3.95±0.07	48.50±6.36	6.90±1.41	59.25±7.85

<sup>1</sup> Means of four determinations ± standard deviation.

<sup>2</sup> Arbitrary unit for HPLC peak area was millivolts x centiminutes.

Table 4.17 The ANOVA for Peak 1

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	1.8201E10	1.8201E10	0.29	0.5968
Variety	3	3.9450E11	1.3150E11	2.11	0.1417
Treatment	3	1.1481E13	3.8271E12	61.43	<0.0001
VarietyxTreatment	9	6.8906E11	7.6562E10	1.23	0.3478
Error	15	9.3444E11			
Corrected Total	31	1.3517E13			

Table 4.18 The ANOVA for Peak 2

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	4.6434E11	4.6434E11	3.94	0.0656
Variety	3	2.3824E13	7.9414E12	67.46	<0.0001
Treatment	3	8.0859E12	2.6953E12	22.90	<0.0001
VarietyxTreatment	9	9.8405E11	1.0934E11	0.93	0.5282
Error	15	1.7658E12			
Corrected Total	31	3.5124E13			

Table 4.19 The ANOVA for Peak 3

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	1.4691E10	1.4691E10	0.87	0.3658
Variety	3	2.9624E11	9.8746E10	5.85	0.0075
Treatment	3	2.3891E12	7.9637E11	47.15	<0.0001
VarietyxTreatment	9	1.7271E11	1.9190E10	1.14	0.3971
Error	15	2.5334E11			
Corrected Total	31	3.1261E12			

Table 4.20 The ANOVA for Total Area

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	4.4585E11	4.4585E11	1.04	0.3233
Variety	3	2.6872E13	8.9572E12	20.95	<0.0001
Treatment	3	5.8067E13	1.9356E13	45.28	<0.0001
VarietyxTreatment	9	4.0391E12	4.4878E11	1.05	0.4482
Error	15	6.4120E12			
Corrected Total	31	9.5835E13			

Table 4.21 Mean Values over all the Varieties for Unreduced Extractable Proteins of Four Treatments by SE-HPLC <sup>1</sup>

Treatment <sup>2</sup>	10 <sup>-5</sup> ×Absolute HPLC Area <sup>3</sup>			
	Peak 1	Peak 2	Peak 3	Total
NM_NT	19.3 <sup>a</sup>	51.6 <sup>a</sup>	14.3 <sup>a</sup>	85.2 <sup>a</sup>
M_NT	10.7 <sup>b</sup>	49.1 <sup>ab</sup>	10.5 <sup>b</sup>	70.3 <sup>b</sup>
M_T16%	6.9 <sup>c</sup>	45.4 <sup>b</sup>	9.1 <sup>b</sup>	61.4 <sup>c</sup>
M_T22%	3.1 <sup>d</sup>	38.3 <sup>c</sup>	6.8 <sup>c</sup>	48.2 <sup>d</sup>

<sup>1</sup> Each value in the table is the mean value for each treatment, n=16.

<sup>2</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

<sup>3</sup> Means in the same column with different letters are significantly different ( $P \leq 0.05$ ).

The absolute areas represented the amount of extractable proteins. The total protein extractability was significantly affected by micronization ( $P < 0.01$ ). Compared to the control samples, the average protein solubility was reduced by 17% for M\_NT samples (Table 4.21). The analysis of total protein solubility indicated that tempering level of the wheat before micronization also affected protein solubility. The samples micronized at higher tempering level (M\_T22%) were significantly less soluble than the samples micronized at as is moisture level (M\_NT) ( $P < 0.05$ ) (Fig. 4.5). Compared to control samples, a further 20% decrease of solubility was observed. It has been suggested that higher moisture contents during micronization made proteins more susceptible to denaturation and aggregation, therefore lead to lowering solubility (Arntfield et al 1997).

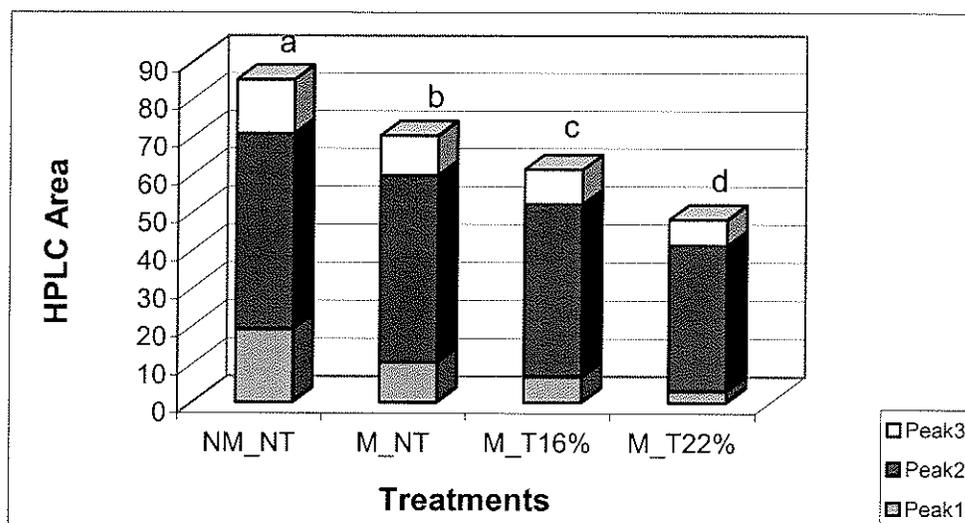


Fig. 4.5 Total extractable proteins by SE-HPLC for four micronizing/tempering treatments

In terms of the changes of extractable amount for individual protein fraction after micronization, it was observed that increasing grain moisture level during micronization progressively reduced the extractability of all wheat protein groups (Fig. 4.6). The average amount of polymeric protein fraction had a pronounced drop after micronization, varying from 19.3 for non-treated flours to 10.7 for non-tempered micronized ones. Increasing moisture to 22% resulted in an additional 39% reduction of extractable polymeric proteins, compared to non-treated samples (Table 4.21). The amounts of gliadins and albumins-globulins groups were also decreased after treatments, which were clearly demonstrated in Fig 4.6. Polymeric protein (peak 1) was especially affected by micronization. Under the most severe treatment (M\_T22%), only 18% extractable polymeric

protein was left compared to that of the control samples (Table 4.16). Similar patterns were observed in all the other three wheat varieties. All these results confirmed that micronizing heat treatments aggregated and denatured wheat proteins therefore decreased protein solubility. A similar result was reported by Zheng et al. (1998).

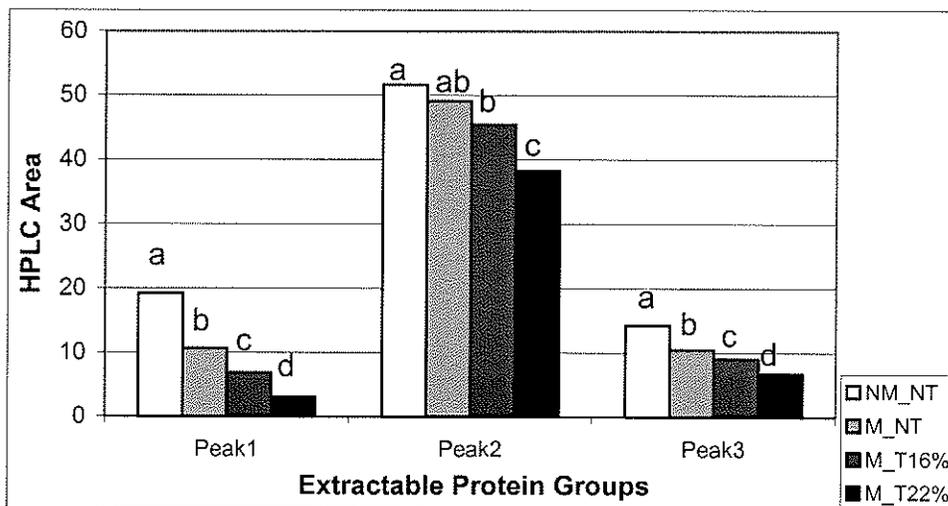


Fig. 4.6 Three peaks of protein fractions by SE-HPLC for four micronizing/tempering treatments

A strong positive correlation between the absolute areas of peak 1 by SE-HPLC and the percentages of SG by protein fractionation analysis for all the samples was found ( $r = 0.92$ ), which further supported the above conclusions (Fig 4.7).

Although micronization treatments influenced all the protein fractions, the reducing rates of extractable amounts for individual protein groups were not exactly the same. Polymeric proteins (peak 1) had the fastest decreasing rate

compared to the other two protein groups (Fig 4.6). Gliadins (peak 2) appeared to withstand more micronizing heat damage, especially when not tempered. It has been reported that glutenin fraction was more susceptible to heat than the gliadin fraction (Schofield et al 1983).

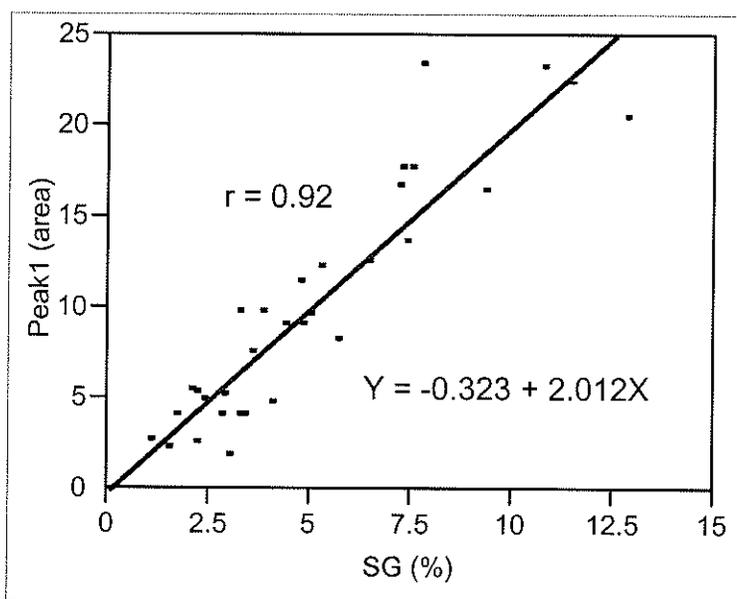
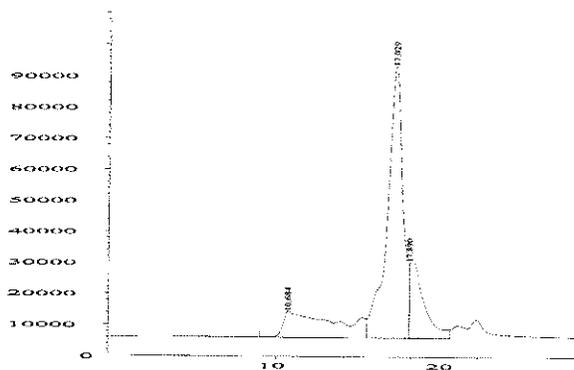
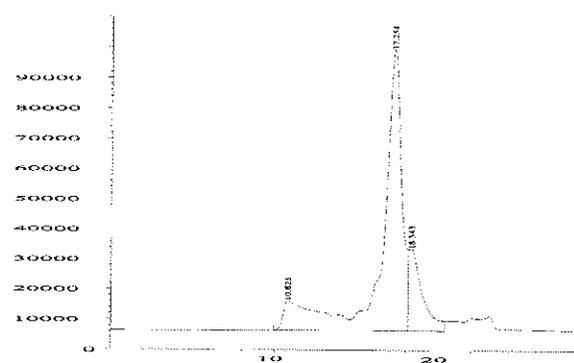


Fig. 4.7 Correlation of peak 1 ( $10^{-5} \times$  Absolute HPLC Area) with SG of all the wheat samples

Typical elution profiles were obtained in the two related studies. No difference of protein solubility was found either between NM\_NT and NM\_T22% AC Barrie samples or between sound and sprouted AC Karma samples (Fig 4.8, Fig. 4.9). These observations suggested that neither tempering alone, nor mild germination impacted wheat protein solubility.

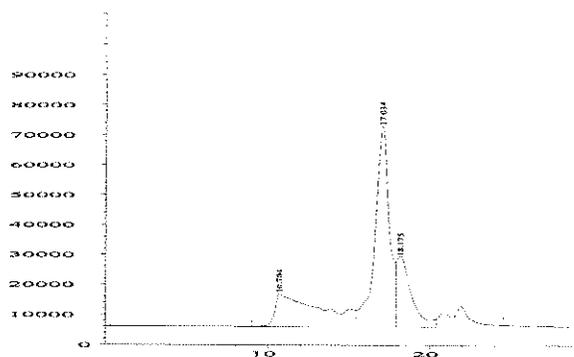


AC Barrie NM\_NT

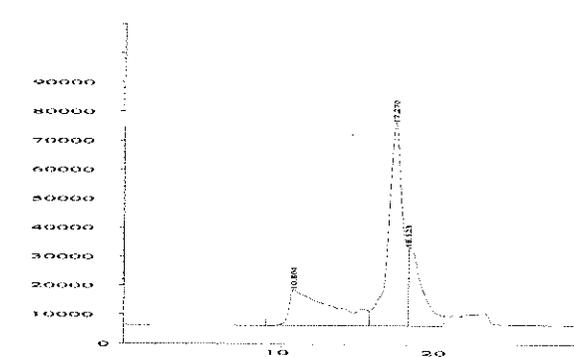


AC Barrie NM\_T22%

Fig 4.8 SE-HPLC separation of proteins from AC Barrie before and after tempering to 22% moisture level without micronization



AC Karma sound NM\_NT



AC Karma Sprouted NM\_NT

Fig 4.9 SE-HPLC separation of proteins from sound or sprouted AC Karma without micronization

#### 4.2.4 Electrophoretic Analyses

Typical electrophoretic patterns were obtained for the wheat proteins of each variety. High molecular weight glutenin subunits (HMW-GS), as a quantitatively minor but functionally important group, have been intensely studied for more than two decades because of their relationship to breadmaking quality (Shewry et al 1992). The HMW-GS compositions of the varieties are as follows: Glenlea and AC Barrie, 2\*, 7+8, 5+10; AC Karma, 1, 7+9, 2+12; AC Ivory, 2\*, 7+9, 5+10. The positions of these bands are shown in Fig. 4.10-4.13.

For all varieties, micronized and control flour samples had essentially the same composition of both glutenin subunits and gliadins. However, the quantities of protein components were progressively changed after micronization. In the  $\omega$ -gliadin gel, because only the 50% 1-propanol soluble protein components were fractionated, there was a quantitative decrease of protein components. This decrease in intensity of protein bands corresponded to the reduction in MP observed in the protein fractionation analysis. In contrast, in the LMW gel, a quantitative increase of reduced protein subunits was observed after micronization. This increase in intensity of protein bands corresponded to the increase of IG observed in the protein fractionation analysis as well. In the HMW gel, total flour, reduced by MCE, was separated by SDS-PAGE. There was no apparent effect for treatments of each variety. Electrophoretic patterns strongly supported the results from protein fractionation tests, and were convincing evidence to support the theory that micronizing heated proteins were aggregated by disulphide bonding.

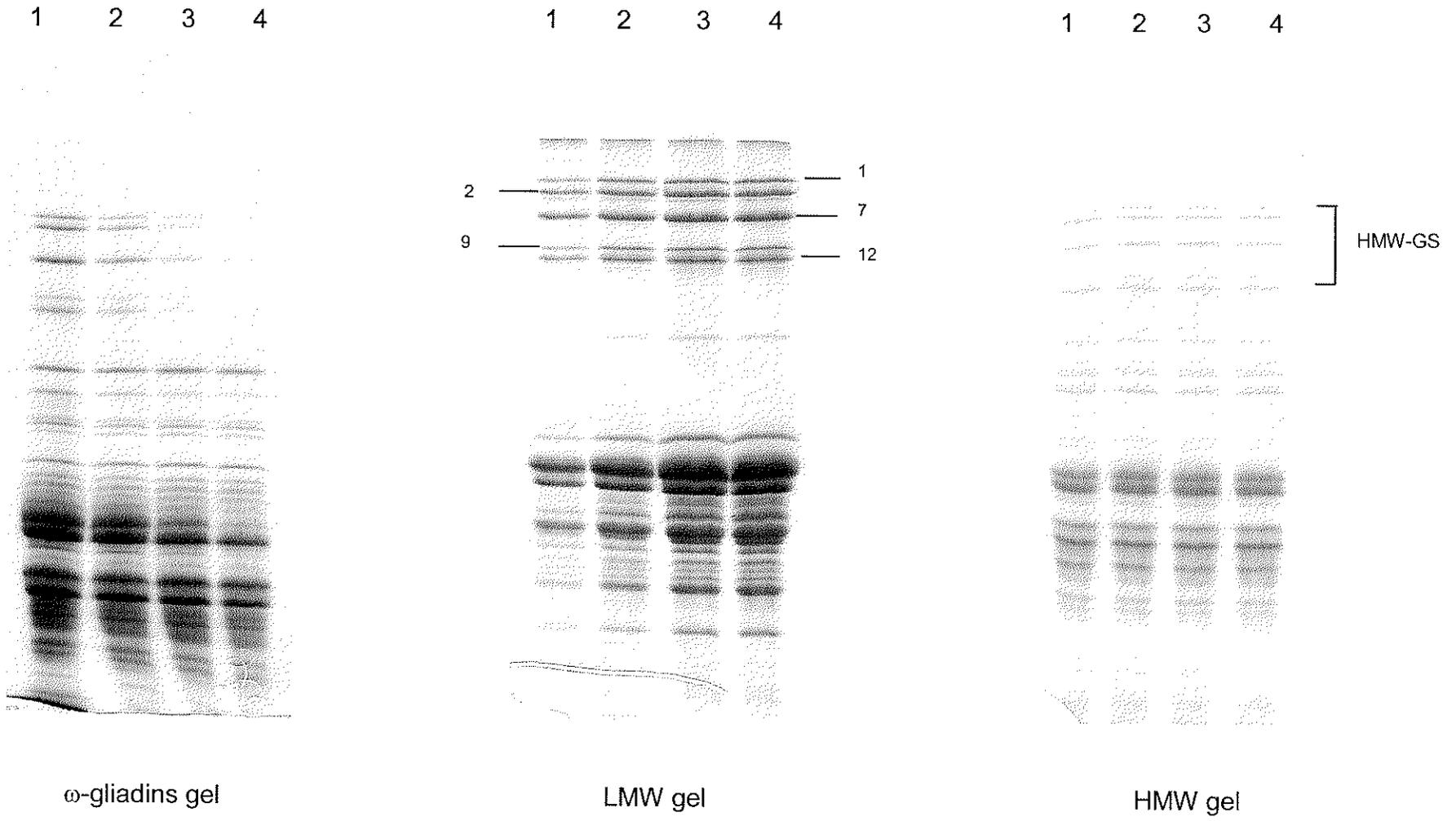


Fig. 4.10 SDS-PAGE separation of the HMW and LMW glutenins and the  $\omega$ -gliadins of AC Karma for four micronizing/tempering treatments: Lane\_1, NM\_NT; Lane\_2, M\_NT; Lane\_3, M\_T16%; Lane\_4, M\_T22%

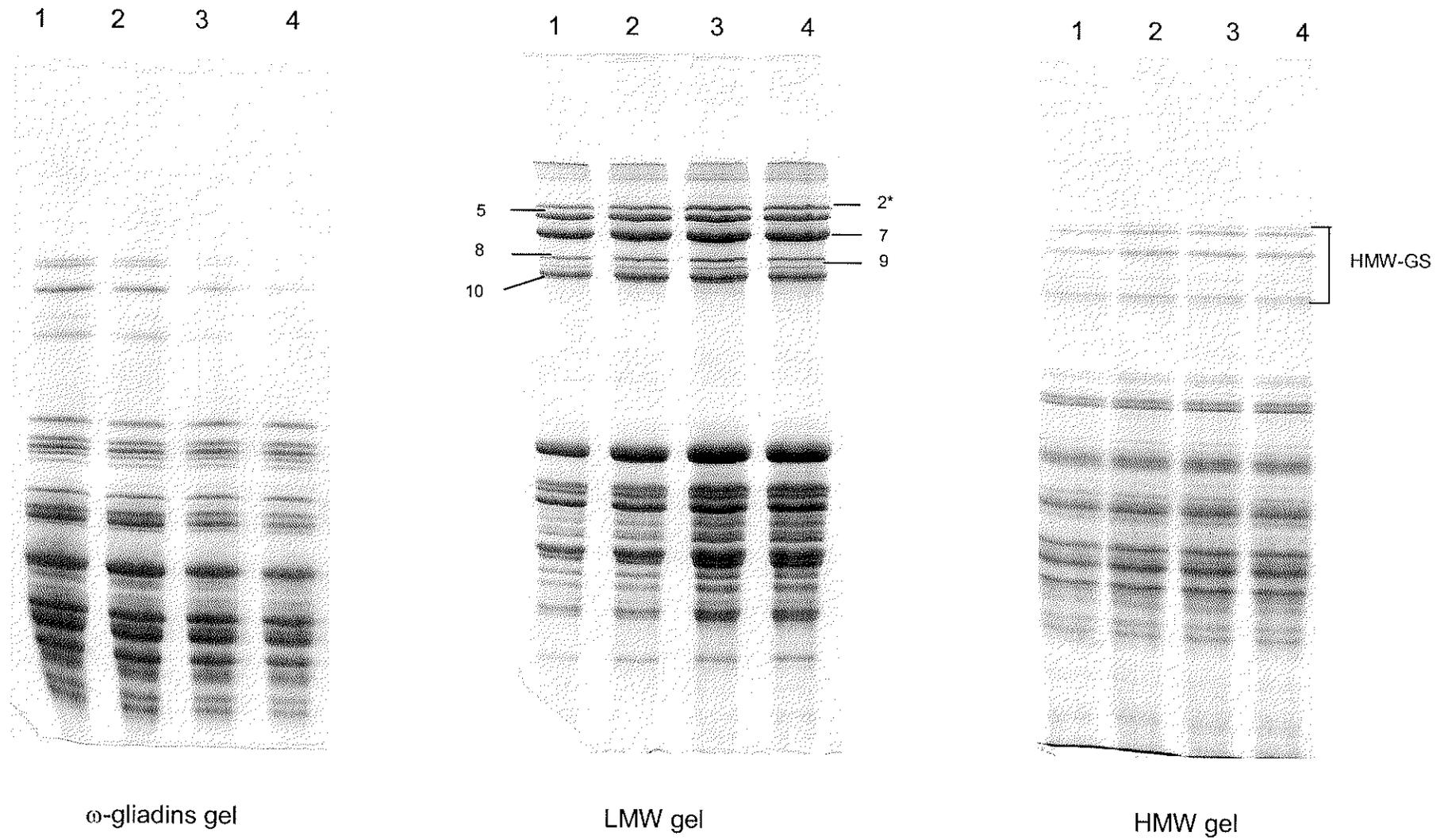


Fig. 4.11 SDS-PAGE separation of the HMW and LMW glutenins and the  $\omega$ -gliadins of AC Barrie for four micronizing/tempering treatments: Lane\_1, NM\_NT; Lane\_2, M\_NT; Lane\_3, M\_T16%; Lane\_4, M\_T22%

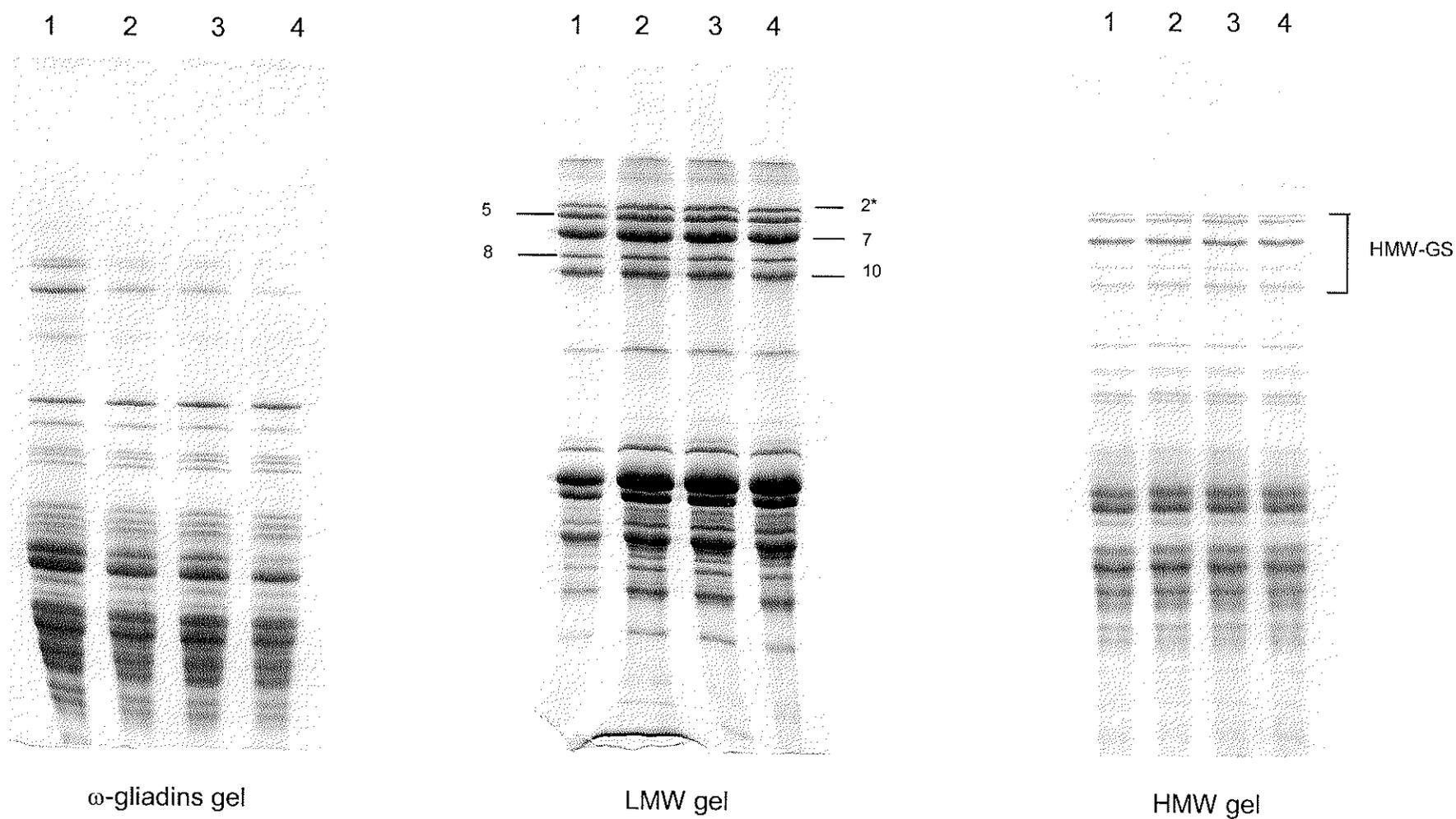


Fig. 4.12 SDS-PAGE separation of the HMW and LMW glutenins and the  $\omega$ -gliadins of Glenlea for four micronizing/tempering treatments: Lane\_1, NM\_NT; Lane\_2, M\_NT; Lane\_3, M\_T16%; Lane\_4, M\_T22%

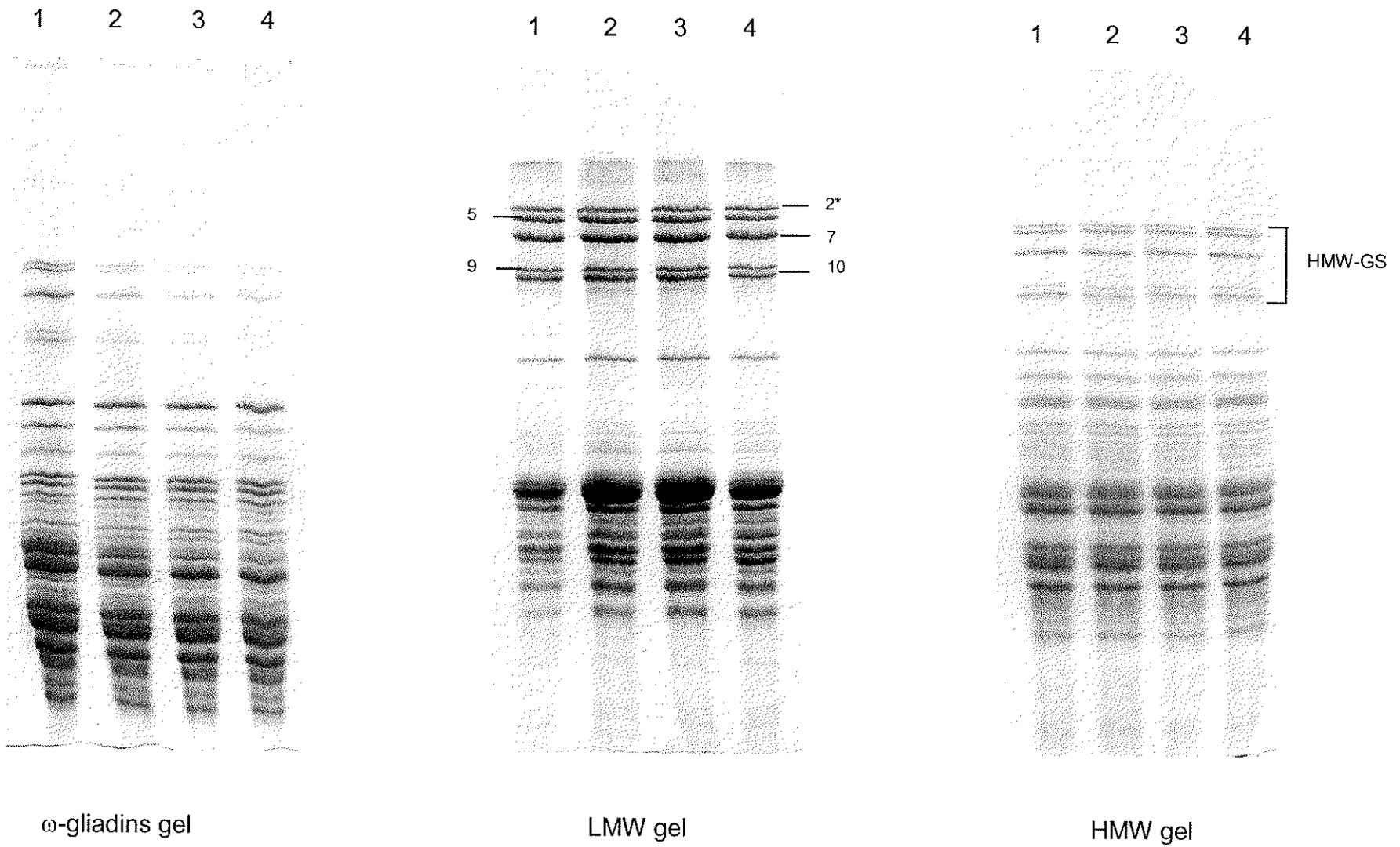


Fig. 4.13 SDS-PAGE separation of the HMW and LMW glutenins and the  $\omega$ -gliadins of AC Ivory for four micronizing/tempering treatments: Lane\_1, NM\_NT; Lane\_2, M\_NT; Lane\_3, M\_T16%; Lane\_4, M\_T22%

#### 4.2.5 Alpha-amylase Activity

Alpha-amylase level in wheat is one of the key quality parameters. Elevated levels of  $\alpha$ -amylase are used as an indicator of sprouting (Megazyme 1996). In this study,  $\alpha$ -amylase activity of AC Karma flours was determined on the Azurine-crosslinked amylose substrate (Appendix VII). All activities were converted to Ceralpha units through standard curves. The results are shown in Table 4.22. Sound AC Karma control samples contained an average of 0.025 unit/g alpha-amylase activity. The activity of sprouted AC Karma wheat increased pronouncedly to 3.634 unit/g, i.e., activity increased 145 times, in agreement with Edwards et al (1989). It has been reported that the thermal inactivation of  $\alpha$ -amylase can be achieved by raising the temperature above 80°C (Zawistowska 1989). In this study, there was a significant decrease of  $\alpha$ -amylase activity for sprouted wheat after micronizing ( $P < 0.01$ ). The activity was reduced by approximately 50% after micronizing at 22% moisture. However, the  $\alpha$ -amylase activity of the sprouted wheat after micronization was still much higher compared to that of sound wheat (Table 4.22). The result indicated that

Table 4.22 Alpha-amylase Activity (unit/g) for Sound/Sprouted AC Karma Wheat<sup>1</sup>

Condition	Treatment		
	NM_NT	M_NT	M_T22%
Sound	0.025±0.012	0.014±0.004	0.008±0.000
Sprouted	3.634±0.045	3.191±0.118	1.837±0.074

<sup>1</sup> Means of four determinations ± standard deviation.

micronization might have some effect on decreasing  $\alpha$ -amylase activity for very mildly germinated wheat. Micronization was not a viable method to treat severely sprouted wheat. This is probably due to the thermostable properties of  $\alpha$ -amylase, and very short processing time for the sprouted wheat under micronization.

### 4.3 RHEOLOGICAL MEASUREMENTS

#### 4.3.1 Farinograph

The Farinograph is widely used for wheat quality evaluation studies. Farinograph curves of wheat flours were generated to determine changes in water absorption, dough development time (DDT), and stability (STAB), due to the difference in wheat varieties and micronization treatments. The shape of Farinograph curves varies according to wheat class and variety (Kunerth and D'Appolonia 1985). The curves shown in Fig. 4.14 were typical for samples used. AC Karma control (a medium strong wheat) and Glenlea control (an extra strong wheat) had similar protein content, yet extremely different farinograms. For the control samples, the differences between varieties were evident.

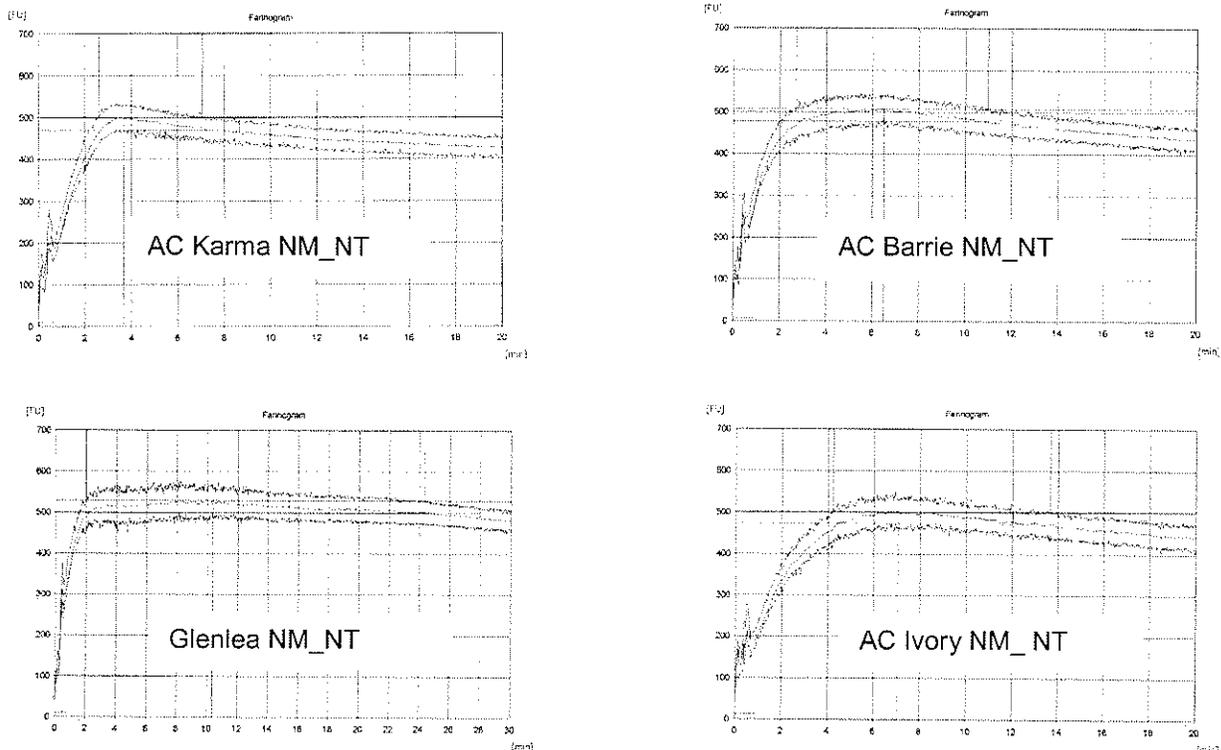


Fig. 4.14 Farinograms for non-treated wheat samples

Farinograph DDT and STAB were significantly higher for Glenlea than for the other three varieties ( $P < 0.05$ ). Similar curves were obtained for AC Barrie and AC Ivory, although AC Ivory had slightly higher values in water absorption and DDT (Table 4.23), likely due to the higher protein content of AC Ivory (15.1% vs. 13.1%).

The farinograms for the AC Barrie control and treated flours are shown in Fig.4.18. Micronization treatments significantly decreased DDT and STAB values ( $P < 0.01$ ). The average DDT of AC Barrie dropped from 6.5 min. for untreated samples to 1.9, 1.5 and 1.2 min. for the flour micronized at as is, 16%, and 22% moisture levels, respectively (Table 4.23). This result indicated a loss of gluten functionality due to micronization treatments.

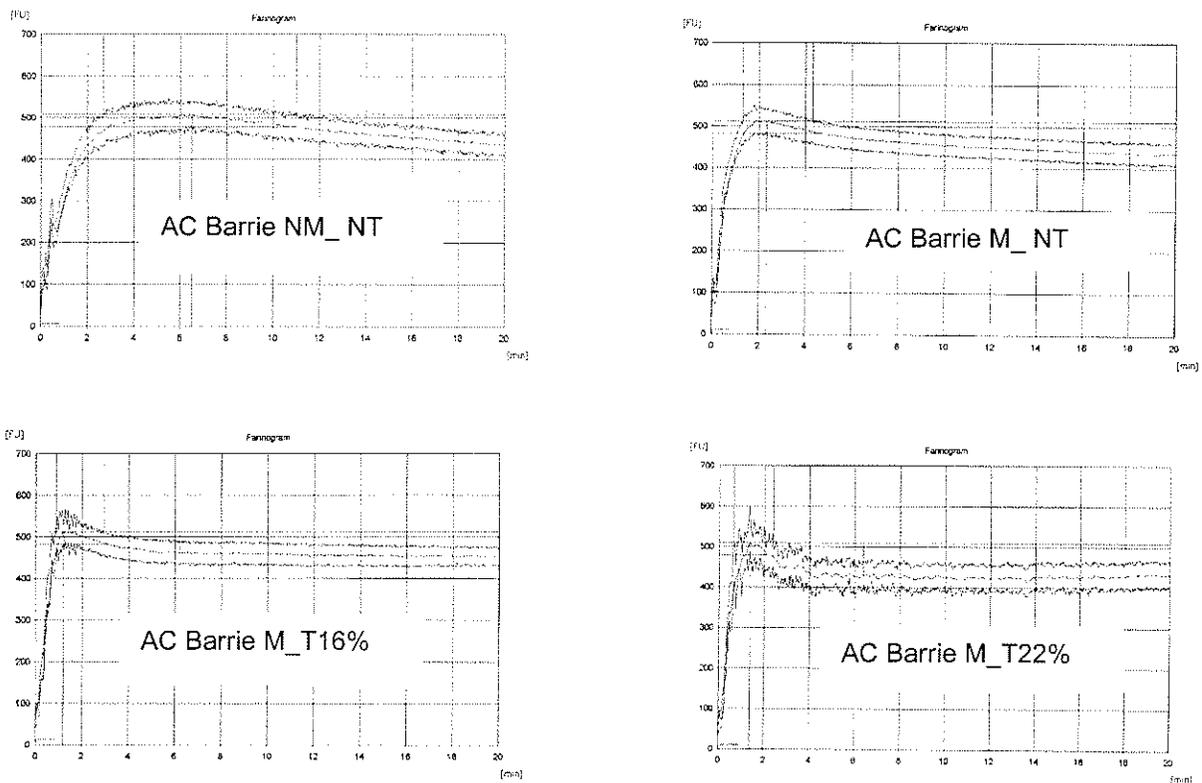


Fig. 4.15 Farinograms of AC Barrie for four micronizing/tempering treatments

Table 4.23 The Farinograph Data of Wheat Varieties for Four Micronizing/Tempering Treatments

SAMPLE <sup>1</sup>	Farinograph Parameters		
	FAB_14 <sup>2</sup> (%)	DDT <sup>3</sup> (min)	STAB <sup>4</sup> (min)
AC KARMA NM_NT (1)	57.6	4.7	4.7
AC KARMA NM_NT (2)	58.1	3.7	4.5
AC KARMA M_NT (1)	56.4	1.2	1.6
AC KARMA M_NT (2)	56.0	2.0	NA <sup>5</sup>
AC KARMA M_T22% (1)	56.1	1.9	13.4 <sup>6</sup>
AC KARMA M_T22% (2)	54.7	1.9	3.7
AC BARRIE NM_NT (1)	59.2	6.5	12.6
AC BARRIE NM_NT (2)	58.3	6.5	8.3
AC BARRIE M_NT (1)	59.2	1.5	1.9
AC BARRIE M_NT (2)	56.6	2.3	3.0
AC BARRIE M_T22% (1)	58.8	0.9	1.1
AC BARRIE M_T22% (2)	57.6	1.4	1.8
GLENLEA NM_NT (1)	57.9	20.4	39.0
GLENLEA NM_NT (2)	58.5	10.4	22.3
GLENLEA M_NT (1)	57.8	33.9 <sup>6</sup>	NA <sup>5</sup>
GLENLEA M_NT (2)	57.1	1.4	1.3
GLENLEA M_T22% (1)	57.9	1.7	2.0
GLENLEA M_T22% (2)	57.6	2.2	2.5
AC IVORY NM_NT (1)	60.6	8.3	11.3
AC IVORY NM_NT (2)	61.1	7.0	9.4
AC IVORY M_NT (1)	57.2	1.9	19.1 <sup>6</sup>
AC IVORY M_NT (2)	57.6	1.9	3.3
AC IVORY M_T22% (1)	57.2	2.4	22.3 <sup>6</sup>
AC IVORY M_T22% (2)	57.7	1.9	19.1 <sup>6</sup>

<sup>1</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T22%: micronized, tempered to 22% moisture.

<sup>2</sup> FAB\_14: Farinograph water absorption on 14%mb.

<sup>3</sup> DDT: dough development time.

<sup>4</sup> STAB: stability.

<sup>5</sup> NA: not available.

<sup>6</sup> because the patterns for micronized samples were so unusual, the Farinograph can't read the data properly in some cases.

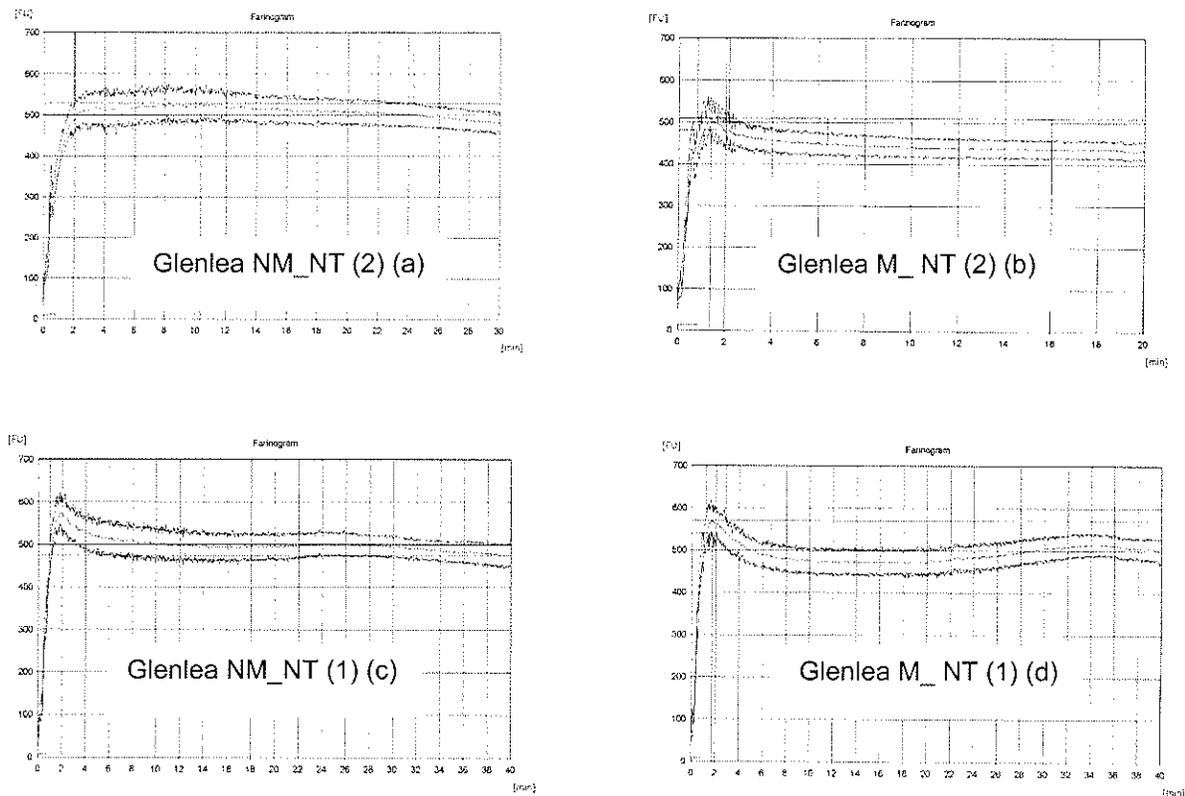


Fig. 4. 16 Farinograms of Glenlea samples with or without micronizing treatments

The M\_T16% and M\_T22% treatments produced similar effects on the Farinograph properties (Appendix VIII). However, M\_NT Glenlea samples had two extremely different Farinograph curves (Fig 4.16 b, d). Glenlea M\_NT (2) sample had a curve similar to the curves for the other varieties micronized without tempering. Glenlea M\_NT (1) sample appeared to need higher DDT than its control, 33.9 and 20.4 min., respectively. The STAB value was also very high, which was out of the measuring range of the equipment. We also noticed the dough properties of two Glenlea control samples were quite different (Fig. 4.16 a, c). The cause of this big difference within varieties might be environmental and storage conditions. The farinogram for Glenlea control (1) was not typical. It might have some property changes for this sample during storage period.

In order to examine the effects of tempering alone without micronization on Farinograph parameters, farinograms for NM\_T22% AC Barrie were obtained. Tempering alone did not have any noticeable effect on the Farinograph parameters. The farinograms were identical between control and tempered samples (Fig. 4.17). This result indicated that micronization alone was the major contributor to the Farinograph changes of micronized samples.

In order to study the effect of germination on Farinograph parameters, sprouted AC Karma samples were tested. Sprouted sample had lower water absorption, DDT, and STAB values compared to untreated sound wheat (Fig. 4.18, Appendix VIIIc). This indicated that wheat quality deteriorated during sprouting (Singh et al 1987).

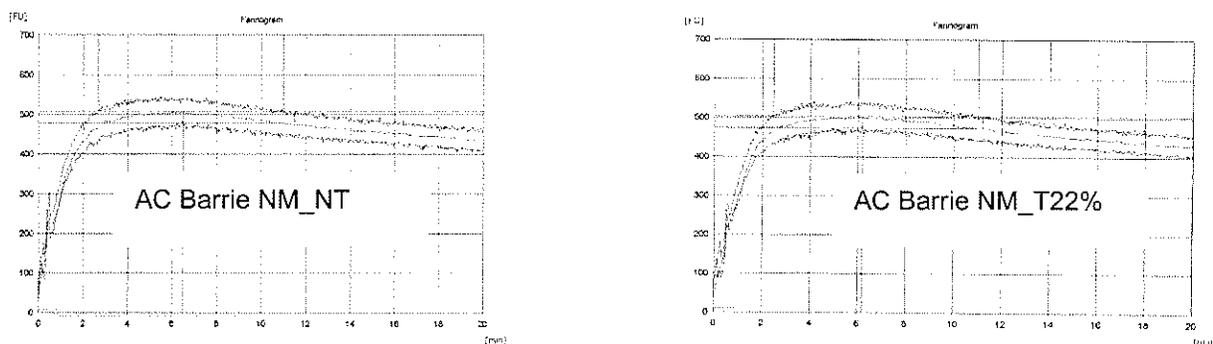


Fig. 4. 17 Farinograms of AC Barrie before and after tempering to 22% moisture level without micronization

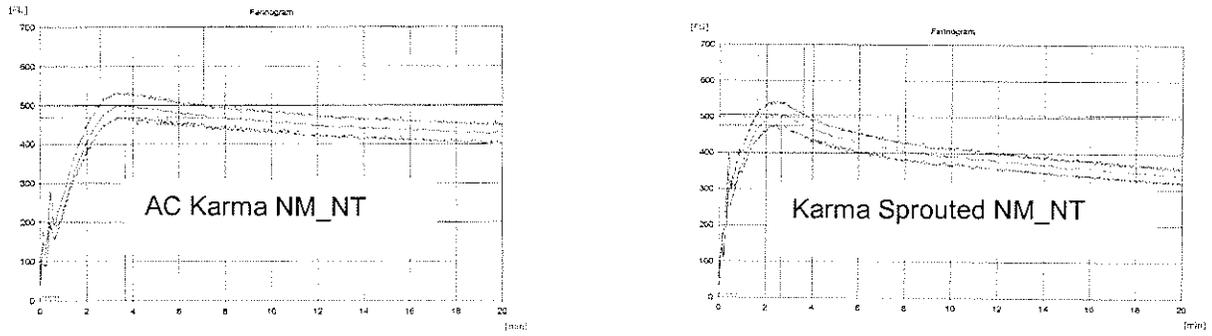


Fig. 4. 18 Farinograms of sound or sprouted AC Karma without micronization

### 4.3.2 Two-gram Mixograph

The Mixograph is used for measuring gluten structure related wheat quality (Kunerth and D'Appolonia 1985, Martinan et al 1998). Mixograph curves of wheat flours were produced to determine changes in Mixograph peak time (MPT), Mixograph peak height (MPH), and work input to peak (WIP), due to the difference of wheat varieties and micronization treatments. The values of MPT and WIP for the Glenlea control samples were significantly higher than the values for other three controls ( $P < 0.05$ ). The types of curves depended on varieties, and were typical for the classes that the varieties belong to (Fig. 4.19).

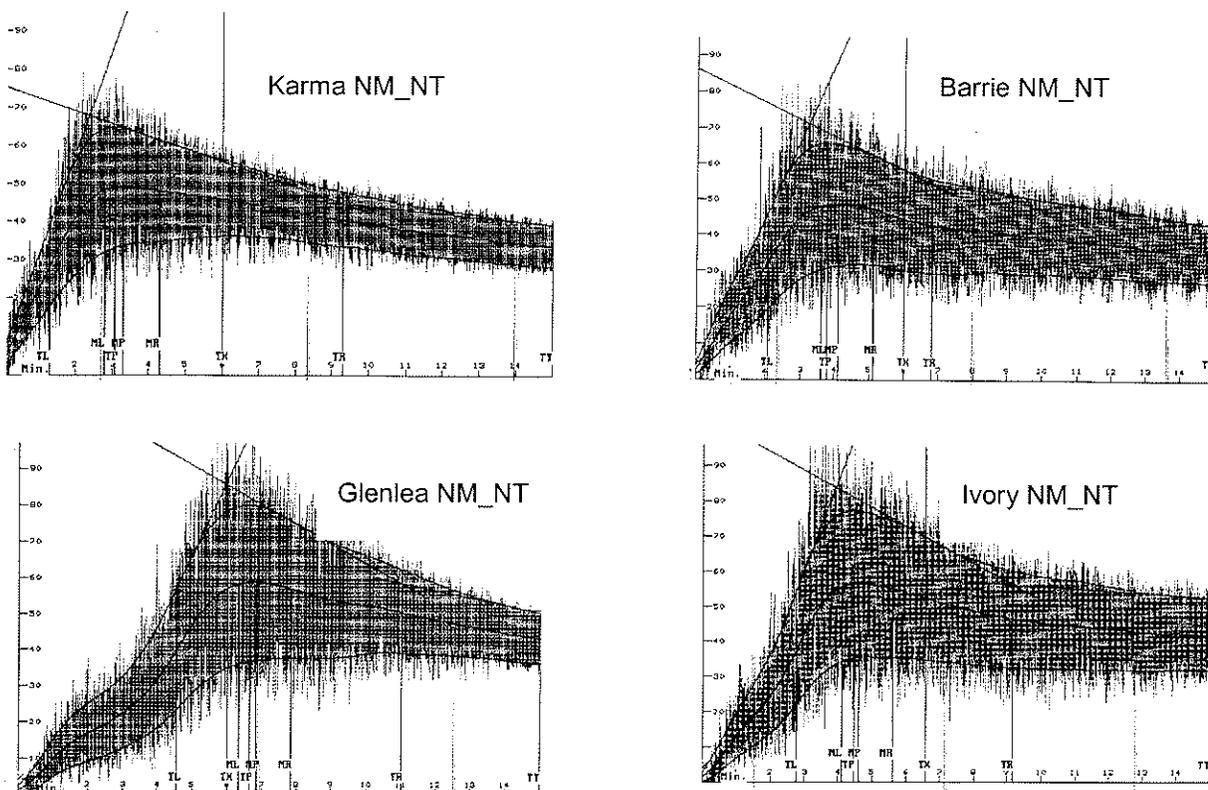


Fig. 4.19 Mixograms for non-treated wheat samples

Table 4.24 The Mixograph Data of Wheat Varieties for Four Micronizing/Tempering Treatments

SAMPLE <sup>1</sup>	Mixograph Parameters			
	MPT <sup>2</sup> (min)	MPH <sup>3</sup> %	PBW <sup>4</sup> %	WIP <sup>5</sup> % Tq*min
AC KARMA NM_NT (1)	4.96	40.8	26.5	130.1
AC KARMA NM_NT (2)	3.30	49.0	30.8	105.9
AC KARMA M_NT (1)	3.25	5.2	6.2	15.5
AC KARMA M_NT (2)	18.75	35.2	22.1	470.3
AC KARMA M_T22% (1)	4.17	11.0	17.9	5.0
AC KARMA M_T22% (2)	2.13	17.5	19.0	23.7
AC BARRIE NM_NT (1)	5.45	43.4	33.8	136.7
AC BARRIE NM_NT (2)	4.12	48.5	33.8	126.4
AC BARRIE M_NT (1)	1.94	5.8	8.3	6.7
AC BARRIE M_NT (2)	2.52	11.8	10.2	18.0
AC BARRIE M_T22% (1)	3.79	9.3	5.8	22.1
AC BARRIE M_T22% (2)	1.97	15.5	14.5	19.9
GLENLEA NM_NT (1)	7.77	49.0	34.8	215.8
GLENLEA NM_NT (2)	6.83	58.6	42.7	205.4
GLENLEA M_NT (1)	12.70	40.2	29.1	250.9
GLENLEA M_NT (2)	1.72	8.6	8.4	10.4
GLENLEA M_T22% (1)	1.37	7.7	11.6	6.4
GLENLEA M_T22% (2)	2.06	13.2	16.2	18.2
AC IVORY NM_NT (1)	4.61	56.4	41.7	141.9
AC IVORY NM_NT (2)	4.34	62.4	44.6	147.3
AC IVORY M_NT (1)	1.73	5.3	7.9	4.7
AC IVORY M_NT (2)	1.94	10.4	9.7	12.5
AC IVORY M_T22% (1)	1.79	10.8	16.2	12.2
AC IVORY M_T22% (2)	1.75	18.6	19.1	21.0

<sup>1</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

<sup>2</sup> MPT: Mixograph peak time.

<sup>3</sup> MPH: Mixograph peak height.

<sup>4</sup> PBW: peak band width.

<sup>5</sup> WIP: work input to peak.

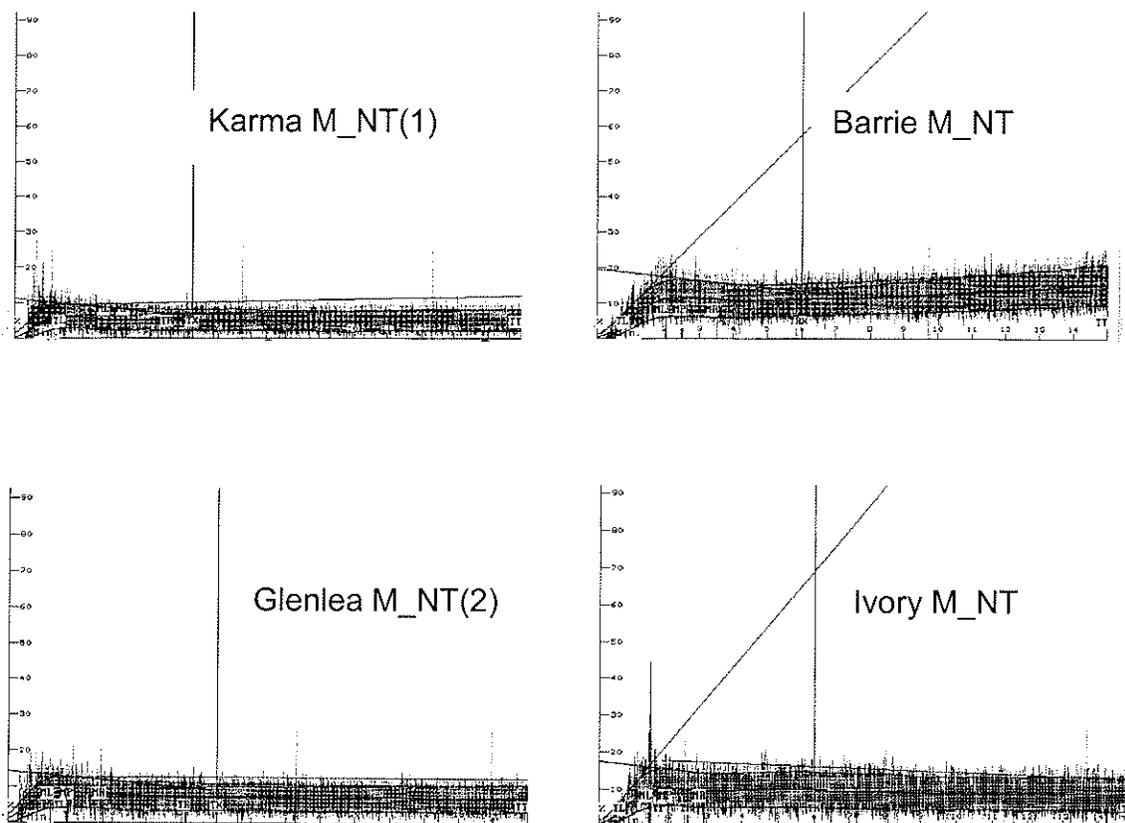


Fig. 4.20 Mixograms for micronized but not tempered wheat samples

The mixograms of M\_NT samples from four varieties are shown in Fig.4.20. There was a dramatic drop in most values for Mixograph parameters, including MPT, MPH, and WIP (Table 4.24, Appendix IX). The results confirmed that micronizing treatments caused wheat protein functionality to deteriorate.

All wheat varieties exhibited similar Mixograph curves after being micronized at 16% or 22% moisture level. Two opposite Mixograph behaviors were observed in two M\_NT Glenlea samples (Fig. 4.21). The dough mixing property of Glenlea M\_NT (2) sample was almost entirely lost compared to the control one. In contrast, the dough property of Glenlea M\_NT (1) sample

appeared to be improved after the treatment, although the MPH value dropped slightly. The MPT value increased from 7.77 min. in control, to 12.70 min. in treated sample. Similar curve was observed in AC Karma M\_NT (2) sample. This result suggested that wheat samples had different capacity to endure heat treatment. This attribute was not correlated with wheat varieties. However, the low initial moisture content might contribute to this result.

In the tempering and sprouting studies, the Mixograph results obtained for NM\_T22% AC Barrie and the sprouted AC Karma samples supported the Farinograph results. Mixograph curves obtained for NM\_T22% AC Barrie were similar to those for non-treated AC Barrie control samples. Sprouted AC Karma wheat had lower MPT value (2.9 min.) compared to sound untreated samples (4.1 min.) (Appendix IX).

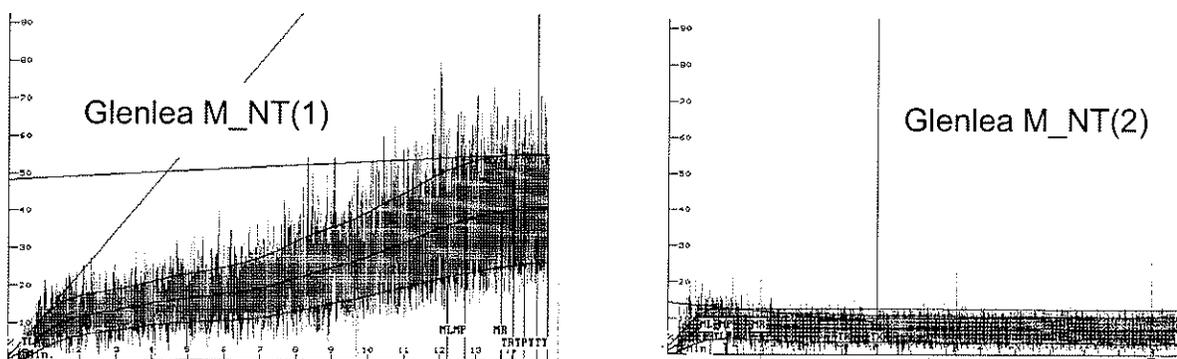


Fig. 4.21 Mixograms of micronized Glenlea samples

### 4.3.3 Micro-scale Extension Test

Micro-scale extension properties, including maximum resistance to extension ( $R_{\max}$ ) and extensibility, were determined to investigate the effects of wheat varieties and micronizing treatments on wheat extensibility behaviors. The extensograms of control samples varied according to wheat varieties (Fig. 4.22). Glenlea, usually considered the strongest wheat, had the largest  $R_{\max}$  Value (24.5 g). In contrast, AC Karma, the relatively weakest wheat as shown in the farinogram, had the smallest  $R_{\max}$  Value (9.5 g) (Table 4.25, Appendix X).

Micronized and tempered samples had much lower extensibility than the control samples. The dramatic changes after treatments were consistent among all wheat varieties (Fig. 4.22). Different extensograms were obtained between two M\_NT Glenlea samples (Fig. 4.23).  $R_{\max}$  Value of Glenlea M\_NT(1) was 39% larger than that of the control (Table 4.25). In the meantime, the extensibility value was lower. This result suggested that the Glenlea M\_NT(1) sample became even stronger after the treatment, which corresponded to the Farinograph and the Mixograph observations.

In the tempering and sprouting studies, the extensograms of NM\_NT and NM\_T22% AC Barrie samples were the same (Fig. 4.24). The dough made from sprouted but untreated AC Karma wheat was very sticky. The average extensibility was much larger (186 mm) compared to that of the sound control samples (131 mm). The mean  $R_{\max}$  Value was dropped from 9.5 g for the sound control sample to 6.1 g for the untreated sprouted samples. The results indicated that the gluten strength became weaker during germination (Appendix X).

Table 4.25 The Micro-scale Extension Data of Wheat Varieties for Four Micronizing/Tempering Treatments

SAMPLE <sup>1</sup>	Extension Parameters		
	R <sub>max</sub> <sup>2</sup> (g)	Extensibility <sup>3</sup> (mm)	Area <sup>4</sup> (g·mm)
AC KARMA NM_NT (1)	9.77	113.48	616.37
AC KARMA NM_NT (2)	9.23	149.49	636.67
AC KARMA M_NT (1)	7.13	14.85	61.71
AC KARMA M_NT (2)	11.66	56.50	307.63
AC KARMA M_T22% (1)	15.32	19.61	82.17
AC KARMA M_T22% (2)	11.75	26.03	70.88
AC BARRIE NM_NT (1)	21.69	106.22	1010.20
AC BARRIE NM_NT (2)	17.56	130.93	1071.15
AC BARRIE M_NT (1)	7.76	27.28	82.53
AC BARRIE M_NT (2)	3.32	87.34	140.02
AC BARRIE M_T22% (1)	8.78	14.34	54.65
AC BARRIE M_T22% (2)	11.13	26.88	67.06
GLENLEA NM_NT (1)	24.52	94.18	1170.87
GLENLEA NM_NT (2)	24.41	119.14	1304.66
GLENLEA M_NT (1)	34.04	67.86	1190.61
GLENLEA M_NT (2)	6.10	56.24	133.62
GLENLEA M_T22% (1)	10.33	13.46	61.51
GLENLEA M_T22% (2)	11.69	27.53	77.95
AC IVORY NM_NT (1)	20.48	112.89	1199.95
AC IVORY NM_NT (2)	22.80	120.83	1214.40
AC IVORY M_NT (1)	9.81	22.04	89.86
AC IVORY M_NT (2)	7.51	39.48	110.12
AC IVORY M_T22% (1)	14.73	13.03	70.65
AC IVORY M_T22% (2)	14.73	25.66	75.64

<sup>1</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T22%: micronized, tempered to 22% moisture.

<sup>2</sup> R<sub>max</sub>: maximum resistance to extension.

<sup>3</sup> Extensibility: extensibility from start until rupture.

<sup>4</sup> Area: area under the curve.

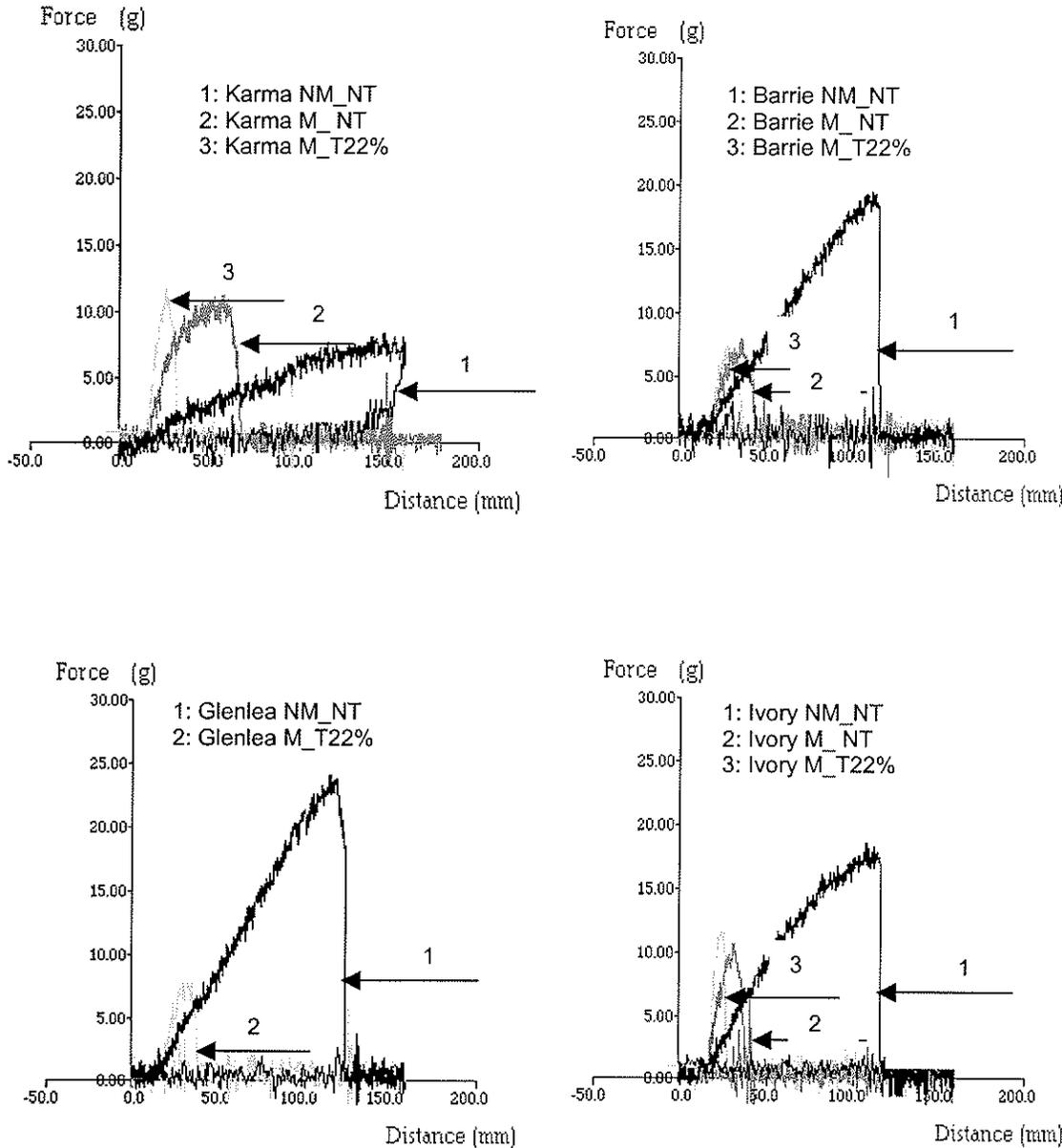


Fig. 4.22 Micro-scale extension curves for wheat samples

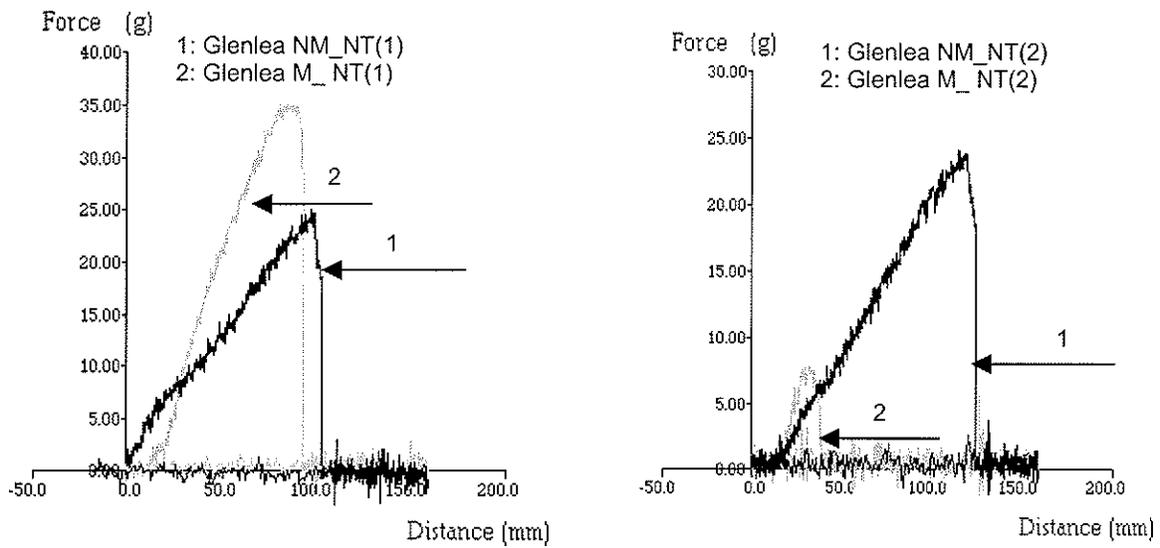


Fig. 4.23 Micro-scale extension curves of Glenlea samples with or without micronizing treatments

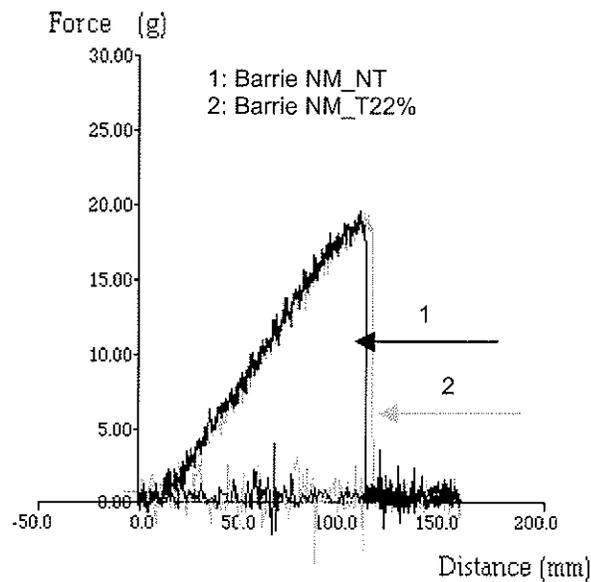


Fig. 4.24 Micro-scale extension curves of AC Barrie before and after tempering to 22% moisture level without micronization

#### 4.4 SUMMARY OF THE RESULTS

Wheat physical characteristics, including kernel weight, kernel diameter, kernel hardness index (HI), and kernel moisture content, were analyzed by the Single Kernel Characteristics System (SKCS 4100). The control wheat samples gave typical single kernel values, with variations according to wheat varieties. A significant change in HI and kernel weights was caused by the tempering pretreatment. Twenty-two percent tempered samples, both with and without micronizing, had softer and heavier kernels than the control samples. The micronizing process alone had no obvious influence on single kernel characteristics. A negative relationship between moisture content and HI was found ( $r = -0.67$ ). A much stronger correlation ( $r = 0.97$ ) was obtained between moisture content and kernel weight for AC Barrie. We concluded that the moisture content has an important effect on single kernel characteristics, in agreement with Gaines et al (1996).

Milling performance was assessed by the yields of flour and bran. Micronization at lower moisture level had no great effect on milling quality. The lowest flour yields and the highest bran contents were derived from the samples micronized at 22% moisture level for all wheat varieties. This was likely due to the additional changes, such as starch gelatinization, in starch and other components, in the micronizing process with high moisture level.

Protein and ash contents of control samples were typical values for such flours (CWB 2000), and varied according to wheat varieties. The ash content of AC Barrie was similar to that of AC Ivory, and significantly lower than Glenlea

and AC Karma. Micronization of 22% tempered samples resulted in a higher ash content than the other three treatments. This result supported our observations on milling performance. Micronization did not change the total protein content of wheat samples.

Protein fractionation analysis was carried out on the basis of solubility. Four protein fractions, monomeric protein (MP), soluble glutenin (SG), insoluble glutenin (IG), and residue protein (RP), were obtained. For the control samples, IG ranged from 25.8 to 32.6% of total proteins. The stronger the wheat gluten the higher proportion of IG. Micronizing treatments significantly reduced the amount of MP and SG proteins among all wheat varieties at all moisture levels. In contrast, the amounts of IG and RP fractions markedly increased after the processing. The extent of the alteration for protein groups was greater when the tempering level of the micronized samples was higher. There were no obvious differences in protein solubility among wheat varieties after micronizing treatments. The increase of IG was highly correlated with the reduction of MP amount ( $r = -0.98$ ).

Size-exclusion chromatography was performed to separate 50% 1-propanol soluble proteins into three sharp peaks: peak 1 (polymeric proteins), peak 2 (gliadins), and peak 3 (albumins-globulins). For the control samples, intervariety variation in peak 1 was found, with a range of 20.1-27.2% in total extractable proteins. The extractable amounts of the three distinct protein groups were markedly and progressively reduced among all treated wheat samples. The amount of polymeric proteins by SE-HPLC was highly correlated with the

proportion of SG in total protein obtained by the fractionation method ( $r = 0.92$ ). Also, individual protein groups responded differently to micronizing process. Polymeric proteins were more susceptible to the treatments than the other two protein groups. Tempering process alone or mild germination did not affect wheat protein solubility.

Electrophoretic patterns derived from High Molecular Weight (HMW) gels, Low Molecular Weight (LMW) gels, and  $\omega$ -gliadin gels, were determined for control and treated samples. The composition of both glutenin subunits and gliadins were identical for micronized and control flour samples. However, the intensity of all protein bands was gradually decreased after the treatments in  $\omega$ -gliadin gels, whereas, the quantities of protein components were increased under the same treatments in LMW gels.

Alpha-amylase activity was determined for sound and sprouted wheat samples. Micronization had some impact on decreasing  $\alpha$ -amylase activity, but  $\alpha$ -amylase level of sprouted AC Karma after the micronization still remained much higher than that of the sound kernels.

The Farinograph, Mixograph, and micro-scale extension test all provide information concerning the rheological behaviors of doughs, which are strongly affected by gluten protein properties (Bushuk 1985). For the control samples, the shape of these dough-testing curves varied according to wheat varieties. The micronization processing dramatically affected the wheat gluten functional properties as seen from the significant drop of dough development time and

stability values in the Farinograph, the distinctive Mixograph curves of wheat samples before and after micronizing treatments, as well as the pronounced reduction in extension parameters. The germination of AC Karma was detrimental to the wheat quality as shown by the decrease in Farinograph water absorption and dough development time, which was in general agreement with other studies (Lukow and Bushuk 1984).

## GENERAL DISCUSSION

The main objective of this research was to expand existing knowledge of the effects of micronization on the physicochemical and rheological properties of wheat. This type of information was the basis to explore new applications of the micronizing technique for agricultural products. Four wheat varieties, AC Karma, AC Barrie, Glenlea, and AC Ivory, were used for this investigation to represent different wheat classes and gluten strength. Comparisons of the effects of micronization on these four varieties provided further information in relation to the optimal utilization of this technology in wheat. Protein composition studies provided a better insight into the structural and molecular changes of wheat during the micronizing process. The investigation of moisture contribution to micronizing effects elucidated the relationship between the moisture level and the micronization, and distinguished their individual effects on this technology. The attempt of using micronization to reduce alpha-amylase level in sprouted wheat was a potential means to treat pre-harvest sprouting wheat.

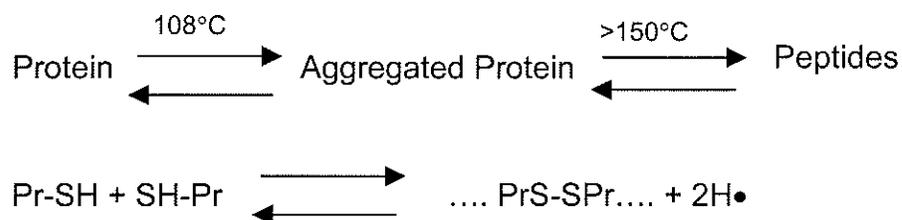
There were two marked changes of micronized wheat. One was the decrease of protein solubility. The micronized wheat with higher moisture level resulted in further reduction of protein solubility by causing proteins more susceptible to denaturation (Arntfield et al 1997). The other was the loss of gluten functionalities observed in the rheological tests. The micronized dough gluten was poorly coherent, short, and lumpy. The cause of these phenomena

was the severe heat treatment associated with micronization, which presumably resulted in protein aggregation and denaturation during the process.

Micronization was basically a short, intense heat processing. The changes in protein solubility and functionality indicated that the heat-induced denaturation of wheat gluten proteins did occur during the micronizing process. Schofield et al (1983) studied the effect of heat on wheat gluten by heating sealed wet glutens in a water bath to the required temperatures for 10 minutes. They reported that the baking performance of gluten declined progressively with heating, and most of its functionality was destroyed by 75°C. The loss of gluten functionality was accompanied by the reduction of gluten extractability in an SDS buffer. In contrast, the extractability of gliadin proteins in 50% 1-propanol was unaffected by heating at temperatures up to 75°C, but decreased markedly after heating at 100°C. Pence et al (1953) indicated the glutenin fraction of gluten was more susceptible to heat than the gliadin fraction. The protein fraction study conducted in this research by SE-HPLC also suggested that there were different rates of reducing solubility among protein groups after micronizing heat treatments (Fig. 4.10). Polymeric proteins were the most susceptible group to the heat-induced micronizing process among all three protein fractions. Conversely, gliadins were the most stable protein group to the heat damage.

Schofield et al (1983) also suggested that the heat caused the conformational changes in gluten complex, particularly in the glutenins. After labeling free sulphhydryl (SH) groups in gluten with [<sup>14</sup>C]-iodoacetamide, they examined SDS extractable proteins with chromatography. The researchers

reported that there was a loss of radioactivity from the glutenin region on heating to 70°C. The heat-produced protein aggregation might be caused by the cross-linking of glutenin molecules by forming new disulfide bonds through the oxidization of the SH groups (Hansen et al 1975). They postulated a reaction scheme as below:



The SDS-PAGE electrophoregrams (Fig. 4.13-4.16) in this study demonstrated quantitative variations of protein classes after micronization, which were convincing evidence that proteins were aggregated by disulphide bonding during micronizing heat treatments.

Mardikar and Schofield (2000) also reached the similar conclusion by blocking free SH groups using iodoacetamide. The new cross-links of glutenin would hinder glutenin molecules interaction with other flour particles, such as starch, which normally leads to coherent dough. Lefebvre et al (2000) studied temperature-induced changes in the rheological behavior of wheat. They observed that irreversible rheological changes occurred at temperatures > 40°C without SH-blocking agent N-ethylmaleimide.

Wadhawan and Bushuk (1989a, 1989b) devitalized commercial gluten by dry heating at 110°C for 20 hours. They reported that devitalization of gluten by

heating led to a substantial decrease in the proportions of both gliadins and soluble glutenins, and a parallel increase in the proportion of residue proteins. The negative correlation between vitality, on the basis of loaf volume, and the relative amount of residue proteins was highly significant ( $r = -0.75$ ,  $P < 0.01$ ), i.e., samples contained more insoluble residue proteins gave poorer baking performance. Their findings may provide explanations to the changes of protein composition and rheological behaviors of the micronized wheat samples in current study. Gluten devitalization might have occurred during the heating associated micronization treatments.

Besides the proteins, the other flour particles, especially starch, might also be impacted by micronization, and consequently have a contribution to the changes of gluten functionality. Fasina et al (1999) studied characteristics of the starch-protein matrix for micronized barley by analyzing scanning electron micrograph pictures. They found that micronization of non-tempered kernels resulted in the swelling (up to 50  $\mu\text{m}$ ) and eventual rupturing of the granules. Whereas micronization of tempered kernels caused the starch granules to melt, indicating starch gelatinization. Although starch studies were not included in this project, in the preliminary study, only wheat micronized at 22% moisture level had obvious starch gelatinization (40%). High moisture content in the seed during gelatinization is critical for the removal of amylose and swelling of the granule during starch gelatinization (Arntfield et al 1997).

The complexity of the gluten structure and composition limited the theoretical inferences of the heat-induced denaturation during micronization.

However, the thesis presented a detailed study on the effects of micronization on various wheat properties, which has practical value in regards to extending the knowledge of this promising technology applied in agricultural products, and gaining further insights into the structure of gluten network. The excellent parallelism of results obtained in the protein fractionation procedure and protein composition analysis by SE-HPLC (Fig. 4.9) indicated that the protein solubility methods could be used with confidence in fundamental protein studies.

## CONCLUSIONS

The major conclusions reached in this research are as follows:

- Micronization significantly affected the protein composition, physical and dough functional properties of all wheat varieties tested.
- Micronization had similar effects on all wheat varieties. The initial difference among varieties had minor impact on the changes resulting from micronizing process.
- For micronized samples, the level of tempering was an important factor that influenced physical properties and protein composition particularly.
- The low initial moisture content appeared to protect wheat from micronizing heat damage.
- The protein composition and dough functionality of non-micronized wheat were not noticeably affected by tempering to 22% moisture level.
- The amount of soluble proteins was lowered by micronization. Whereas, the amount of insoluble glutenin and residue proteins was increased.
- Dough rheological tests were more sensitive than the protein solubility methods for detecting the damage to gluten functionality.
- Micronization did not decrease alpha-amylase activity sufficiently to make it useful for treating sprouted grain.

## RECOMMENDATIONS FOR FUTURE RESEARCH

Recommendations for future research based on this project include:

- To examine the effects of micronization on wheat characteristics at much lower temperature level, and without pre-tempering
- To determine the impact of micronization on other chemical components in wheat, such as starch, dietary fiber, and minor components
- To identify the main factors that are responsible for the vitality of wheat gluten subjected to heating associated treatments
- To investigate the possibility of improving the nutritional value of wheat end products by the addition of micronized flour
- To assess consumer acceptance of micronized foods
- To explore the new applications of micronization in other cereals and oil seeds, such as the elimination of anti-nutritional factors of oat

## LITERATURE CITED

Abe, T. and Afzal, T. M. 1997. Thin-layer infrared radiation drying of rough rice. *J. Agric. Engng. Res.* 67:289-297.

American Association of Cereal Chemists (AACC). 1997. Approved methods of the AACC, 8<sup>th</sup> ed. The Association: St. Paul, MN.

AgritelGrains Ltd., 2002. AC Ivory hard white spring wheat. Web site: <http://www.agritelgrain.com/index/>.

Arntfield, S. D., Scanlon, M. G., Malcolmson, L. J., Watts, B., Ryland, D., and Savoie, V. 1997. Effect of tempering and end moisture content on the quality of micronized lentils. *Food Res. Int.* 30:371-380.

Aussenac, T. and Carceller, J. L. 2000. Use of a one-line fluorescence detection to characterize glutenin fraction in the separation techniques (SE-HPLC and RP-HPLC). Pages:144-147 in: *Wheat Gluten: the 7<sup>th</sup> international workshop gluten 2000*. P. R. Shewry and A. S. Tatham, eds. MPG Books Ltd, Bodmin, Cornwall, UK.

Batey, I. L., Gupta, R. B., and MacRitchie, F. 1991. Use of size-exclusion high performance liquid chromatography in the study of wheat flour proteins: an improved chromatographic procedure. *Cereal Chem.* 68:207-209.

Bietz, J. A. 1985. High performance liquid chromatography: How proteins look in cereals. *Cereal Chem.* 62:201-212.

Blenford, D. E. 1979. Potential applications of micronizing in food processing with special reference to cocoa beans. *Confect. Manuf. Market.* 16:3,5,7.

Blenford, D. E. 1980. Potential applications of micronizing in food processing. *Food Trade Rev.* 50:6-8.

Bloksma, A. H. and Bushuk, W. 1988. Rheology and chemistry of dough. Pages: 131-218 in: *Wheat Chemistry and Technology*. Vol:II Y. Pomeranz ed. AACC Inc. St. Paul, MN.

Botero Uribe, M. E. 1997. Infrared radiation effects on some functional characteristics of wheat flour. Master Thesis, University of Manitoba, Winnipeg.

Bushuk, W. 1985. Rheology: theory and application to wheat flour doughs. Pages: 1-26 in *Rheology of Wheat Products*. H. Faridi ed. AACC, Inc.: St. Paul, MN.

Byers, M., Mifflin, B. J., and Smith, S. J. 1983. A quantitative comparison of the extraction of proteins fractions from wheat grain by different solvents, and of the polypeptide and amino acid composition of the alcohol-soluble protein. *J. Sci. Food Agric.* 34:447-462.

Canadian Wheat Board (CWB). 2000. Grains from western Canada: 1999-2000.

Canadian Wheat Board (CWB). 2001. Grains from western Canada: 2000-01 crop year.

Cenkowski, S. and Sosulski, F. W. 1997. Physical and cooking properties of micronized lentils. *J Food Process Eng.* 20:249-264.

Chen, C. H. and Bushuk, W. 1970. Nature of proteins in triticale and its parental species. I. Solubility characteristics and amino acid composition of endosperm proteins. *Can. J. Plant Sci.* 50:9-14.

Ciaffi, M., Tozzi, L., and Lafiandra, D. 1996. Relationship between flour protein composition determined by size-exclusion high-performance liquid chromatography and dough rheological parameters. *Cereal Chem.* 73:346-351.

Copson D. A. 1975. Theory of microwave heating. Pages: 1-33 in: *Microwave Heating*. 2<sup>nd</sup> ed. Avi publishing company, INC.

Cornell, H. J. and Hoveling, A. W. 1998. *Wheat Chemistry and Utilization*. Technomic publishing company, Inc.

Dachkevitch, T. and Autran, J. 1989. Prediction of baking quality of bread wheats in breeding programs by size-exclusion high-performance liquid chromatography. *Cereal Chem.* 66:448-456.

Daniels, N. W. R. and Frazier, P. J. 1976. Wheat proteins-physical properties and baking function. Pages: 299-315 in *Plant Proteins*. G. Norton ed. Boston: Butterworths, London.

Datta, A. K. 2001. Mathematical modeling of microwave processing of foods: an overview. Pages: 147-187 in: *Food Processing Operations Modeling Design and Analysis*. J. Irudayaraj ed. Marcel Dekker, Inc., New York.

Edwards, R. A., Ross, A. S., Mares, D. J., Ellison, F. W., and Tomlinson, J. D. 1989. Enzymes from rain-damaged and laboratory-germinated wheat I. effects on product quality. *J. Cereal Sci.* 10:157-167.

Ewart, J. A. D. 1972. A modified hypothesis for the structure and rheology of glutenin. *J. Sci. Food Agric.* 23:687-699.

Fasina, O. O., Tyler, R. T., Pickard, M. D., and Zheng, G. H. 1999. Infrared heating of hullless and pearled barley. *J. Food Process. Preserv.* 23:135-151.

Fasina, O. O. and Tyler R. T. 2001. Infrared heating of biological materials. Pages 189-224 in: *Food Processing Operations Modeling Design and Analysis*. J. Irudayaraj ed. Marcel Dekker, Inc., New York.

Fu, B. X. 1996. Biochemical properties of wheat gluten proteins in relation to bread making quality. PhD Thesis, University of Manitoba, Winnipeg.

Fu, B. X. and Sapirstein, H. D. 1996. Procedure for isolating monomeric proteins and polymeric glutenin of wheat flour. *Cereal Chem.* 73:143-152.

Fu, B. X. and Kovacs, M. I. P. 1999. Rapid single-step procedure for isolating total glutenin proteins of wheat flour. *J. Cereal Sci.* 29:113-116.

Gaines, C. S., Finney, P. F., Fleege, L. M., and Andrews, L. C. 1996. Predicting a hardness measurement using the single-kernel characterization system. *Cereal Chem.* 73:278-283.

Ginzburg, A. S. 1969. Drying and thermal treatment of food products by infra-red rays. Pages: 174-309 in: *Application of Infra-red Radiation in Food Processing*. Leonard Hill Books, London.

Graveland, A., Bosveld, P., Lichtendonk, W. J., Moonen, H. H. E., and Scheepstra, A. 1982. Extraction and fractionation of wheat flour proteins. *J. Sci. Food Agric.* 33:1117-1128.

Graveland, A., Bosveld, P., Lichtendonk, W. J., Marseille, J. P., Moonen, H. H. E., and Scheepstra, A. 1985. A model for the molecular structure of the glutenins from wheat flour. *J. Cereal Sci.* 3:1-16.

Gupta, R. B., Batey, I. L., and MacRitchie, F. 1992. Relationships between protein composition and functional properties of wheat flours. *Cereal Chem.* 69:125-131.

Gupta, R. B., Khan, K., and MacRitchie, F. 1993. Biochemical basis of flour proteins in bread wheats. I. Effects of variation in the quantity and size distribution of polymeric protein. *J. Cereal Sci.* 18:23-41.

Gupta, R. B., Popineau, Y., Lefebvre, J., Cornec, M., Lawrence, G. J., and MacRitchie, F. 1995. Biochemical basis of flour properties in bread wheats. II. Changes in polymeric protein formation and dough/gluten properties associated with the loss of low Mr or high Mr glutenin subunits. *J. Cereal Sci.* 21:103-116.

Hansen, L. P., Johnston, P. H., and Ferrel, R. E. 1975. Heat-moisture effects on wheat flour. I. physical-chemical changes of flour proteins resulting from thermal processing. *Cereal Chem.* 52:459-472.

Huebner, F. R., and Wall, J. S. 1976. Fractionation and quantitative differences of glutenin from wheat varieties varying in baking quality. *Cereal Chem.* 53:258-269.

Igbasan, F. A. and Guenter, W. 1996. The enhancement of the nutritive value of peas for broiler chickens: an evaluation of micronization and dehulling processed. *Poult. Sci.* 75:1243-1252.

Janssen, A. M., Vliet, T., and Vereijken, J. M. 1996. Rheological behavior of wheat glutes at small and large deformations. Effects of Gluten composition. *J. Cereal Sci.* 23:33-42.

Kadam, S. S., Smithard, R. R., Eyre, M. D., and Armstrong, D. G. 1987. Effects of heat treatments of antinutritional factors and quality of proteins in winged bean. *J. Sci. Food Agric.* 39:267-275.

Kasarda, D. D. 1989. Glutenin structure in relation to wheat quality. Pages: 277-302 in: *Wheat is Unique*. Y. Pomeranz ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Kobrehel, K. and Bushuk, W. 1977. Studies of glutenin. X. Effect of fatty acids and their sodium salts on solubility in water. *Cereal Chem.* 54:833-839.

Kunerth, W. H. and D'Appolonia, B. L. 1985. Use of the mixograph and farinograph in wheat quality evaluation. Pages:27-50 in *Rheology of Wheat Products*. H. Faridi ed. AACC, Inc.: St. Paul, MN.

Larroque, O. R., Gianibelli, M. C., Sanchez, M.G., and MacRitchie, F. 2000. Procedure for obtaining stable protein extracts of cereal flour and whole meal for size-exclusion HPLC analysis. *Cereal Chem.* 77:448-450.

Lasztity, R. 1996. *The Chemistry of Cereal Proteins*. CRC Press: Boca Raton, FL.

Lee, J. W. and MacRitchie, F. 1971. The effect of Gluten protein fractions on dough properties. *Cereal Chem.* 48:620-625.

Lefebvre, J., Popineau, Y., Deshayes, G., and Lavenant, L. 2000. Temperature-induced changes in the dynamic rheological behavior and size distribution of polymeric proteins for gluteins from wheat near-isogenic lines differing in HMW glutenin subunit composition. *Cereal Chem.* 77:193-201.

Lukow, O. M. and Bushuk, W. 1984. Influence of germination on wheat quality. I. Functional (breadmaking) and biochemical properties. *Cereal Chem.* 61:336-339.

Lukow, O. M., Payne, P. I., and Tkachuk R. 1989. The HMW glutenin subunit composition of Canadian wheat cultivars and their association with bread-making quality. *J. Sci. Food Agric.* 46:451-460.

Lukow, O.M., Hussain, A., and Branlard, G. 1994. Low molecular weight (LMW) glutenin composition of Canadian grown wheat cultivars. *Cereal World.* 39:617.

Lukow, O. M. 1997. Quality screening using the mixograph in Canada. Pages:73-74 in: *The Mixograph Handbook*. C. E. Walker, J. L. Hazelton, and M. D. Shogren, eds. National Manufacturing Division, TMCO, Lincoln, NE.

Lukow, O. M., Suchy, J., and Fu, B. X. 2000. A small scale wheat protein fractionation method using Dumas and Kjeldahl analysis. Pages:227-230 in: *Wheat Gluten: the 7<sup>th</sup> international workshop gluten 2000*. P. R. Shewry and A. S. Tatham, eds. MPG Books Ltd, Bodmin, Cornwall, UK.

MacRitchie, F., Kasarda D. D., and Kuzmicky, D. D. 1991. Characterization of wheat protein fractions differing in contributions to breadmaking quality. *Cereal Chem.* 68:122-130.

MacRitchie, F. 1992. Physicochemical properties of wheat proteins in relation to functionality. *Adv. Food Nutr. Res.* 36:1-87.

MacRitchie, F. 1999. Wheat proteins: characterization and role in flour functionality. *Cereal Foods World.* 44:188-193.

Mardikar, S. H. and Schofield, J. D. 2000. Influence of the redox status of gluten protein SH groups on heat-induced changes in gluten properties. Pages: 227-230 in: *Wheat Gluten: the 7<sup>th</sup> international workshop gluten 2000*. P. R. Shewry and A. S. Tatham, eds. MPG Books Ltd, Bodmin, Cornwall, UK.

Martinan, J. P., Nicolas, Y., Bouguennec, A., Popineau, Y., Saulnier, L., and Branlard, G. 1998. Relationships between mixograph parameters and indices of wheat grain quality. *J. Cereal Sci.* 27:179-189.

McCurdy, S. M. 1992. Infrared processing of dry peas, canola, and canola screenings. *J. Food Sci.* 57:941-944.

Megazyme International Ireland Ltd. 1996. Megazyme alpha-amylase assay procedure. Bray Business Park, Bray, Co. Wicklow, Ireland.

Micronizing Co. Ltd. 2002. Web site: <http://www.micronizing.co.uk/>. Access date: March, 2002.

Nieto-Taladriz, M.T., Perretant, M. R., and Rousset, M. 1994. Effect of gliadins and HMW and LMW subunits of glutenin on dough properties in the F6 recombinant inbred lines from a bread wheat cross. *Theor. Appl. Genet.* 88:81-88.

Ohm, J. B., Chung, O. K., and Deyoe, C. W. 1998. Single-kernel characteristics of hard winter wheats in relation to milling and baking quality. *Cereal Chem.* 75:156-161.

Oliver, J. R. and Allen, H. M. 1992. The prediction of breadbaking performance using the farinograph and extensograph. *J. Cereal Sci.* 15:79-89.

Orfeuil, M. 1987. Infrared radiation heating. Pages 343-389 in: *Electric Process Heating: technologies/equipment/applications*. Battelle Press, Columbus.

Osborne, B. G., Kotwal, Z., Blakeney, A. B., O'Brien, L., Shah, S., and Fearn, T. 1997. Application of the single-kernel characterization system to wheat receiving testing and quality prediction. *Cereal Chem.* 74:467-470.

Payne, P. I., Corfield, K. G., Holt, L. M., and Blackman, J. A. 1981. Correlations between the inheritance of certain high-molecular weight subunits of glutenin and bread-making quality in progenies of six crosses of bread wheat. *J. Sci. Food Agric.* 32:51-60.

Pence, J. W., Mohammad, A., and Mecham, D. K. 1953. Heat denaturation of gluten. *Cereal Chem.* 30:115-126.

Pomeranz, Y. and Williams, P. C. 1990. Wheat hardness: Its genetic, structural, and biochemical background, measurement, and significance. *Advances in Cereal Science and Technology*. X: 471-544.

Rusnak, B. A., Chou, C. L., and Rooney, L. W. 1980. Effect of micronizing on kernel characteristics of sorghum varieties with different endosperm type. *J. Food Sci.* 45:1529-1532.

Sapirstein, H. D. and Fu, B. X. 1998. Intercultivar variation in the quantity of monomeric proteins, soluble and insoluble glutenin, and residue protein in wheat flour and relationships to breadmaking quality. *Cereal Chem.* 75:500-507.

Sarantinos, J. and Black, R. 1996. Effects of micronisation on the chemical and functional properties of chickpeas. *Food Australia*. 48:39-42.

Savage, G. P. and Clark, A. 1988. The effect of micronization on the nutritional value of yellow and brown sorghum. *Nutr. Rep. Int.* 37:829-837.

Scanlon, M. G., Malcolmson, L. J., Arntfield, S. D., Watts, B., Ryland, D., and Prokopowich, D. J. 1998. Micronization pretreatments for reducing the cooking time of lentils. *J. Sci. Food Agric.* 76:23-30.

Schofield, J. D., Bottomley, R. C., Timms, M. F., and Booth, M. R. 1983. The effect of heat on wheat gluten and the involvement of sulphhydryl-disulphide interchange reactions. *J. Cereal Sci.* 1:241-253.

Shewry, P.R., Tatham, A.S., Forge, J., Kreis, M., and Mifflin, B. J. 1986. The classification and nomenclature of wheat gluten proteins: a reassessment. *J. Cereal Sci.* 4:97-106.

Shewry, P. R., Halford, N. G., and Tatham, A. S. 1989. The high molecular weight subunits of wheat, barley and rye: Genetics, molecular biology, chemistry and role in wheat gluten structure and functionality. *Oxford Surveys of Plant Molecular and Cell Biology*. 6:163-220.

Shewry, P. R., Halford, N. G., and Tatham, A. S. 1992. Critical review article: high molecular weight subunits of wheat glutenin. *J. Cereal Sci.* 15:105-120.

Shiau, S. Y. and Yang, S. P. 1982. Effect of micronizing temperature on the nutritive value of sorghum. *J. Food Sci.* 47:965-968.

Shuey, W. C. 1975. Practical instruments for rheological measurements on wheat products. *Rheology of Wheat Products: a Symposium. Cereal Chem.* 52(3, II):42r-81r.

Singh, N., Sekhon K. S., and Nagi, H. P. S. 1987. Laboratory sprout damage and effect of heat treatment on milling and baking properties of Indian wheats. *J. Food Sci.* 52:176-179.

Singh, N. K., Donovan, G. R., Batey, I. L., and MacRitchie, F. 1990a. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. I. Dissolution of total proteins in the absence of reducing agents. *Cereal Chem.* 67:150-161.

Singh, N. K., Donovan, R., and MacRitchie, F. 1990b. Use of sonication and size-exclusion high-performance liquid chromatography in the study of wheat flour proteins. II. Relative quantity of glutenin as a measure of breadmaking quality. *Cereal Chem.* 67:161-170.

Stathopoulos, C. E., Tsiami, A. A., and Schofield, J. D. 2000. Pages: 400-403 in: *Wheat Gluten: the 7<sup>th</sup> international workshop gluten 2000*. P. R. Shewry and A. S. Tatham, eds. MPG Books Ltd, Bodmin, Cornwall, UK.

Suchy, J., Lukow, O. M., and Ingelin, M. E. 2000. Dough microextensibility method using a 2-g mixograph and a texture analyzer. *Cereal Chem.* 77:39-43.

Tatham, A. S., Shewry, P. R., and Belton, P. S. 1990. Structural studies of cereal prolamins, including wheat gluten. *Adv. Cereal Sci. Technol.* 10:1-78.

Toews, R. 2001. Effects of pretreatment and micronization on the cookability and chemical components of green and yellow field peas. Master Thesis, University of Manitoba, Winnipeg.

Vanzetti, R. 1972. Pages 3-29 in: *Practical applications of infrared techniques*. John Wiley & Sons, Inc. New York.

Wadhawan, C. K. and Bushuk, W. 1989a. Studies on vitality of commercial gluten. I. Physical, chemical and technological characteristics. *Cereal Chem.* 66:456-461.

Wadhawan, C. K. and Bushuk, W. 1989b. Studies on vitality of commercial gluten. II. Solubility fractionation, electrophoresis, and fluorescence results. *Cereal Chem.* 66:461-466.

Weegels, P. L., Hamer, R. J., and Schofield, J. D. 1996. Functional properties of wheat glutenin. *J. Cereal Sci.* 23:1-18.

Wrigley, C. W., du Cros, D. L., Fullington J. G., and Karsarda, D. D. 1984. Changes in polypeptide composition and grain quality due to sulfur deficiency in wheat. *J. Cereal Sci.* 2:15-24.

Wrigley, C.W. and Bekes, F. 1999. Glutenin-protein formation during the continuum from anthesis to processing. *Cereal Foods World.* 44:562-565.

Zawistowska, U. 1989. Challenges with alpha-amylase inhibition in sprout-damaged wheat. Pages: 117-129 in: *Wheat is Unique*. Y. Pomeranz ed. Am. Assoc. Cereal Chem.: St. Paul, MN.

Zhao, Y. 2000. The effects of pretreatment and micronization on the quality and cookability of lentils. Master Thesis, University of Manitoba, Winnipeg.

Zheng, G. H., Fasina, O., Sosulski, F. W., and Tyler, R. T. 1998. Nitrogen solubility of cereals and legumes subjected to micronization. *J. Agric. Food Chem.* 46:4150-4157.

**Appendices include the detailed data in this research**

Note:

<sup>1</sup> Each value in the tables is the average of two determinations, except milling performance.

<sup>2</sup> Pooled SD: Pooled standard deviation.

<sup>3</sup> NM\_NT: non-micronized, non-tempered; M\_NT: micronized, non-tempered; M\_T16%: micronized, tempered to 16% moisture; M\_T22%: micronized, tempered to 22% moisture.

**Appendix I** Detailed Data for Single Kernel Characteristics

Table A.1a The Single Kernel Characteristics of Wheat in the Primary Study

SAMPLE	SINGLE KERNEL CHARACTERISTICS			
	Weight (mg)	Diameter (mm)	Hardness Index	Moisture (%)
AC KARMA NM_NT (1)	35.46	3.26	93.79	12.94
AC KARMA NM_NT (2)	37.79	3.31	87.88	10.95
AC KARMA M_NT (1)	35.52	3.24	93.10	12.15
AC KARMA M_NT (2)	36.82	3.31	99.14	9.77
AC KARMA M_T16% (1)	36.75	3.24	79.48	15.52
AC KARMA M_T16% (2)	38.97	3.31	86.01	14.86
AC KARMA M_T22% (1)	38.48	3.35	78.75	16.97
AC KARMA M_T22% (2)	40.05	3.39	81.29	16.85
AC BARRIE NM_NT (1)	32.62	3.17	107.29	13.91
AC BARRIE NM_NT (2)	31.68	3.23	83.29	12.03
AC BARRIE M_NT (1)	31.22	3.15	105.62	10.11
AC BARRIE M_NT (2)	31.49	3.24	100.15	11.13
AC BARRIE M_T16% (1)	32.07	3.19	107.00	13.40
AC BARRIE M_T16% (2)	32.73	3.24	89.04	14.63
AC BARRIE M_T22% (1)	34.75	3.34	90.26	17.03
AC BARRIE M_T22% (2)	34.90	3.40	85.67	16.93
GLENLEA NM_NT (1)	44.16	3.61	96.73	9.99
GLENLEA NM_NT (2)	41.09	3.41	90.71	12.68
GLENLEA M_NT (1)	44.54	3.71	95.91	9.64
GLENLEA M_NT (2)	40.05	3.43	102.24	11.39
GLENLEA M_T16% (1)	45.68	3.65	93.35	13.56
GLENLEA M_T16% (2)	41.05	3.40	95.87	14.91
GLENLEA M_T22% (1)	47.96	3.71	74.31	16.43
GLENLEA M_T22% (2)	41.96	3.43	88.69	16.30
AC IVORY NM_NT (1)	30.69	3.10	101.84	14.15
AC IVORY NM_NT (2)	28.60	3.02	93.56	11.72
AC IVORY M_NT (1)	30.07	3.13	109.98	12.77
AC IVORY M_NT (2)	27.78	3.05	104.75	10.18
AC IVORY M_T16% (1)	30.17	3.12	110.79	13.69
AC IVORY M_T16% (2)	28.89	3.05	109.75	14.58
AC IVORY M_T22% (1)	32.07	3.22	98.73	16.77
AC IVORY M_T22% (2)	29.95	3.12	100.99	16.93
Overall Mean	35.813	3.298	94.874	13.590
Pooled SD	5.504	0.184	9.827	2.450

Table A.Ib The Single Kernel Characteristics of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

SAMPLE	SINGLE KERNEL CHARACTERISTICS			
	Weight (mg)	Diameter (mm)	Hardness Index	Moisture (%)
AC BARRIE NM_NT (1)	32.62	3.17	107.29	13.91
AC BARRIE NM_NT (2)	31.68	3.23	83.29	12.03
AC BARRIE M_NT (1)	31.22	3.15	105.62	10.11
AC BARRIE M_NT (2)	31.49	3.24	100.15	11.13
AC BARRIE M_T22% (1)	34.75	3.34	90.26	17.03
AC BARRIE M_T22% (2)	34.90	3.40	85.67	16.93
AC BARRIE NM_T22% (1)	35.01	3.32	79.41	17.50
AC BARRIE NM_T22% (2)	34.75	3.36	75.38	17.43
Overall Mean	33.303	3.276	90.884	14.509
Pooled SD	1.706	0.092	12.127	3.093

Table A.Ic The Single Kernel Characteristics of AC Karma in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	SINGLE KERNEL CHARACTERISTICS			
	Weight (mg)	Diameter (mm)	Hardness Index	Moisture (%)
SOUND NM_NT (1)	35.46	3.26	93.79	12.94
SOUND NM_NT (2)	37.79	3.31	87.88	10.95
SOUND M_NT (1)	35.52	3.24	93.10	12.15
SOUND M_NT (2)	36.82	3.31	99.14	9.77
SOUND M_T22% (1)	38.48	3.35	78.75	16.97
SOUND M_T22% (2)	40.05	3.39	81.29	16.85
SPROUTED NM_NT (1)	26.83	2.80	75.31	9.61
SPROUTED NM_NT (2)	27.51	2.83	78.93	9.49
SPROUTED M_NT (1)	27.30	2.82	77.69	8.77
SPROUTED M_NT (2)	26.80	2.80	82.90	8.59
SPROUTED M_T22% (1)	28.81	2.85	82.41	16.42
SPROUTED M_T22% (2)	28.62	2.85	81.44	16.46
Overall Mean	32.499	3.068	84.386	12.414
Pooled SD	5.244	0.256	7.431	3.393

**Appendix II** Detailed Data for Milling Performance

Table A.IIa The Milling Performance of Wheat Varieties in the Primary Study

SAMPLE	MILLING PERFORMANCE <sup>1</sup>				
	Yield (%)	Bran (%)	Shorts (%)	RF (%)	BF (%)
AC KARMA NM_NT (1)	75.4	13.2	10.9	21.8	54.2
AC KARMA NM_NT (2)	74.9	14.7	10.2	24.9	50.1
AC KARMA M_NT (1)	75.1	15.6	9.1	20.2	55.1
AC KARMA M_NT (2)	74.8	14.8	9.8	24.9	50.5
AC KARMA M_T16% (1)	74.2	16.1	9.4	20.8	53.7
AC KARMA M_T16% (2)	75.4	14.3	9.5	23.7	52.5
AC KARMA M_T22% (1)	65.3	19.2	15.1	16.0	49.6
AC KARMA M_T22% (2)	73.1	17.6	8.7	24.3	49.5
AC BARRIE NM_NT (1)	70.9	16.6	12.0	21.5	49.9
AC BARRIE NM_NT (2)	72.4	16.5	10.8	25.5	47.2
AC BARRIE M_NT (1)	73.3	16.9	9.5	21.1	52.5
AC BARRIE M_NT (2)	72.3	17.7	9.6	26.7	46.0
AC BARRIE M_T16% (1)	72.5	17.2	9.7	20.8	52.3
AC BARRIE M_T16% (2)	72.8	16.1	9.8	25.3	48.8
AC BARRIE M_T22% (1)	65.5	23.5	10.7	16.3	49.6
AC BARRIE M_T22% (2)	68.9	20.4	9.7	25.0	44.8
GLENLEA NM_NT (1)	71.5	13.6	14.4	20.8	51.2
GLENLEA NM_NT (2)	71.3	15.2	13.3	21.0	50.5
GLENLEA M_NT (1)	74.1	13.8	11.8	21.2	53.2
GLENLEA M_NT (2)	70.2	17.5	11.9	21.1	49.5
GLENLEA M_T16% (1)	73.4	15.7	10.6	21.6	52.1
GLENLEA M_T16% (2)	71.5	14.7	12.4	21.7	51.2
GLENLEA M_T22% (1)	70.7	16.0	13.2	21.9	48.9
GLENLEA M_T22% (2)	67.3	20.0	11.5	22.8	45.6
AC IVORY NM_NT (1)	73.6	14.2	12.0	25.8	48.0
AC IVORY NM_NT (2)	72.8	16.3	10.7	26.4	46.7
AC IVORY M_NT (1)	74.3	15.4	10.0	20.6	54.0
AC IVORY M_NT (2)	73.4	15.6	10.7	23.4	50.3
AC IVORY M_T16% (1)	71.5	15.9	12.3	21.0	50.7
AC IVORY M_T16% (2)	72.2	15.4	11.6	21.8	51.3
AC IVORY M_T22% (1)	65.0	16.5	18.3	18.6	46.6
AC IVORY M_T22% (2)	67.9	19.5	11.7	22.2	46.6
Overall Mean	71.80	16.43	11.28	22.21	50.08
Pooled SD	2.93	2.20	2.00	2.59	2.68

Table A.IIb The Milling Performance of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

SAMPLE	MILLING PERFORMANCE <sup>1</sup>				
	Yield (%)	Bran (%)	Shorts (%)	RF (%)	BF (%)
AC BARRIE NM_NT (1)	70.9	16.6	12.0	21.5	49.9
AC BARRIE NM_NT (2)	72.4	16.5	10.8	25.5	47.2
AC BARRIE M_NT (1)	73.3	16.9	9.5	21.1	52.5
AC BARRIE M_NT (2)	72.3	17.7	9.6	26.7	46.0
AC BARRIE M_T22% (1)	65.5	23.5	10.7	16.3	49.6
AC BARRIE M_T22% (2)	68.9	20.4	9.7	25.0	44.8
AC BARRIE NM_T22% (1)	73.1	17.0	9.7	25.1	48.2
AC BARRIE NM_T22% (2)	71.5	18.0	10.4	25.2	46.4
Overall Mean	70.99	18.33	10.30	23.30	48.08
Pooled SD	2.63	2.44	0.86	3.45	2.50

Table A.IIc The Milling Performance of AC Karma in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	MILLING PERFORMANCE <sup>1</sup>				
	Yield (%)	Bran (%)	Shorts (%)	RF (%)	BF (%)
SOUND NM_NT (1)	75.4	13.2	10.9	21.8	54.2
SOUND NM_NT (2)	74.9	14.7	10.2	24.9	50.1
SOUND M_NT (1)	75.1	15.6	9.1	20.2	55.1
SOUND M_NT (2)	74.8	14.8	9.8	24.9	50.5
SOUND M_T22% (1)	65.3	19.2	15.1	16.0	49.6
SOUND M_T22% (2)	73.1	17.6	8.7	24.3	49.5
SPROUTED NM_NT (1)	71.8	17.3	10.6	26.6	45.5
SPROUTED NM_NT (2)	71.4	17.7	10.6	26.2	45.6
SPROUTED M_NT (1)	73.0	15.9	9.9	28.0	46.2
SPROUTED M_NT (2)	72.3	17.3	9.2	28.0	45.6
SPROUTED M_T22% (1)	69.8	19.1	10.3	24.6	46.0
SPROUTED M_T22% (2)	69.4	19.8	9.9	24.8	45.4
Overall Mean	72.19	16.85	10.36	24.19	48.61
Pooled SD	2.96	2.04	1.63	3.43	3.46

<sup>1</sup> BF: break flour; RF: reduction flour.

**Appendix III Detailed Data for Ash Content**

Table A.IIIa The Ash Content (%) of Wheat Varieties in the Primary Study

VARIETY	TREATMENT							
	NM_NT		M_NT		M_T16%		M_T22%	
	1	2	1	2	1	2	1	2
AC KARMA	0.585	0.506	0.581	0.590	0.626	0.602	0.664	0.658
AC BARRIE	0.478	0.488	0.461	0.452	0.475	0.538	0.489	0.521
GLENLEA	0.587	0.552	0.536	0.507	0.521	0.597	0.564	0.635
AC IVORY	0.421	0.445	0.433	0.467	0.418	0.492	0.463	0.493
Overall Mean				0.5264				
Pooled SD				0.0699				

Table A.IIIb The Ash Content (%) of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

MICRONIZING CONDITION	TEMPERING LEVEL				
	NT		T22%		
	1	2	1	2	
NM	0.478	0.488	0.516	0.483	
M	0.461	0.452	0.489	0.521	
Overall Mean				0.4860	
Pooled SD				0.0239	

Table A.IIIc The Ash Content (%) of AC Karma in the Effects of Micronization on Sprouted Wheat Study

CONDITION	TEMPERING LEVEL					
	NM_NT		M_NT		M_T22%	
	1	2	1	2	1	2
Sound	0.585	0.506	0.581	0.59	0.664	0.658
Sprouted	0.467	0.458	0.540	0.528	0.549	0.564
Overall Mean				0.5575		
Pooled SD				0.0646		

**Appendix IV** Detailed Data for Total Protein Content

Table A.IVa The Total Protein Content (%) of Wheat Varieties in the Primary Study

VARIETY	TREATMENT							
	NM_NT		M_NT		M_T16%		M_T22%	
	1	2	1	2	1	2	1	2
AC KARMA	11.4	11.9	11.4	12.0	11.3	11.8	11.0	11.7
AC BARRIE	13.0	13.1	13.1	12.9	12.9	13.2	12.2	12.6
GLENLEA	10.8	12.8	11.0	12.1	10.8	12.4	10.7	11.9
AC IVORY	14.7	15.5	14.4	15.5	14.8	15.0	14.4	14.8
Overall Mean					12.72			
Pooled SD					1.47			

Table A.IVb The Total Protein Content (%) of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

MICRONIZING CONDITION	TEMPERING LEVEL				
	NT		T22%		
	1	2	1	2	2
NM	13.0	13.1	13.5	13.3	13.3
M	13.1	12.9	12.2	12.6	12.6
Overall Mean	12.96				
Pooled SD	0.41				

Table A.IVc The Total Protein Content (%) of AC Karma in the Effects of Micronization on Sprouted Wheat Study

CONDITION	TEMPERING LEVEL					
	NM_NT		M_NT		M_T22%	
	1	2	1	2	1	2
Sound	11.4	11.9	11.4	12.0	11.0	11.7
Sprouted	12.4	12.4	12.3	12.4	12.2	12.1
Overall Mean	11.93					
Pooled SD	0.47					

**Appendix V** Detailed Data for Protein Fractionation Analysis

Table A.Va The Protein Fractionation Analysis of Wheat in the Primary Study

SAMPLE	PERCENTAGE OF PROTEIN FRACTIONS <sup>1</sup>			
	MP (%)	SG (%)	IG (%)	RP (%)
AC KARMA NM_NT (1)	53.52	11.54	26.82	8.11
AC KARMA NM_NT (2)	54.58	12.88	24.75	7.79
AC KARMA M_NT (1)	43.24	5.79	38.15	12.83
AC KARMA M_NT (2)	49.72	7.29	31.28	11.71
AC KARMA M_T16% (1)	45.83	4.44	36.74	12.99
AC KARMA M_T16% (2)	40.89	2.29	43.17	13.66
AC KARMA M_T22% (1)	30.04	3.10	53.26	13.60
AC KARMA M_T22% (2)	35.18	2.29	50.65	11.87
AC BARRIE NM_NT (1)	55.63	7.33	29.47	7.57
AC BARRIE NM_NT (2)	55.00	7.60	28.59	8.81
AC BARRIE M_NT (1)	50.60	3.33	34.05	12.02
AC BARRIE M_NT (2)	52.29	6.54	30.54	10.64
AC BARRIE M_T16% (1)	49.28	5.11	33.40	12.22
AC BARRIE M_T16% (2)	43.84	2.47	39.98	13.71
AC BARRIE M_T22% (1)	41.21	3.35	41.94	13.50
AC BARRIE M_T22% (2)	38.20	1.60	45.58	14.62
GLENLEA NM_NT (1)	53.79	7.45	32.54	6.22
GLENLEA NM_NT (2)	51.13	9.38	32.58	6.90
GLENLEA M_NT (1)	51.10	5.32	32.69	10.89
GLENLEA M_NT (2)	42.45	3.68	42.40	11.47
GLENLEA M_T16% (1)	42.59	4.17	41.34	11.90
GLENLEA M_T16% (2)	41.47	2.97	43.68	11.88
GLENLEA M_T22% (1)	42.13	3.48	42.09	12.30
GLENLEA M_T22% (2)	37.27	1.13	48.57	13.03
AC IVORY NM_NT (1)	53.34	10.85	29.05	6.77
AC IVORY NM_NT (2)	52.35	7.87	32.35	7.42
AC IVORY M_NT (1)	44.46	4.90	38.24	12.40
AC IVORY M_NT (2)	44.15	3.91	37.76	14.19
AC IVORY M_T16% (1)	47.10	4.83	36.74	11.33
AC IVORY M_T16% (2)	40.18	2.13	43.68	14.01
AC IVORY M_T22% (1)	37.32	1.78	48.12	12.77
AC IVORY M_T22% (2)	37.54	2.88	44.60	14.99
Overall Mean	45.544	5.115	37.963	11.379
Pooled SD	6.738	3.021	7.336	2.545

Table A.Vb The Protein Fractionation Analysis of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

SAMPLE	PERCENTAGE OF PROTEIN FRACTIONS <sup>1</sup>			
	MP (%)	SG (%)	IG (%)	RP (%)
AC BARRIE NM_NT (1)	55.63	7.33	29.47	7.57
AC BARRIE NM_NT (2)	55.00	7.60	28.59	8.81
AC BARRIE M_NT (1)	50.60	3.33	34.05	12.02
AC BARRIE M_NT (2)	52.29	6.54	30.54	10.64
AC BARRIE M_T22% (1)	41.21	3.35	41.94	13.50
AC BARRIE M_T22% (2)	38.20	1.60	45.58	14.62
AC BARRIE NM_T22% (1)	53.86	9.59	28.05	8.50
AC BARRIE NM_T22% (2)	54.54	7.38	30.07	8.00
Overall Mean	50.166	5.840	33.536	10.458
Pooled SD	6.699	2.744	6.634	2.672

Table A.Vc The Protein Fractionation Analysis of AC Karma in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	PERCENTAGE OF PROTEIN FRACTIONS <sup>1</sup>			
	MP (%)	SG (%)	IG (%)	RP (%)
SOUND NM_NT (1)	53.52	11.54	26.82	8.11
SOUND NM_NT (2)	54.58	12.88	24.75	7.79
SOUND M_NT (1)	43.24	5.79	38.15	12.83
SOUND M_NT (2)	49.72	7.29	31.28	11.71
SOUND M_T22% (1)	30.04	3.10	53.26	13.60
SOUND M_T22% (2)	35.18	2.29	50.65	11.87
SPROUTED NM_NT (1)	54.56	10.68	27.87	6.89
SPROUTED NM_NT (2)	54.48	11.97	26.43	7.13
SPROUTED M_NT (1)	52.63	7.97	29.16	10.24
SPROUTED M_NT (2)	53.13	7.31	30.04	9.52
SPROUTED M_T22% (1)	44.54	3.45	39.48	12.53
SPROUTED M_T22% (2)	44.79	2.61	39.74	12.86
Overall Mean	47.534	7.240	34.803	10.423
Pooled SD	8.195	3.868	9.531	2.458

<sup>1</sup> MP: monomeric protein; SG: soluble glutenin; IG: insoluble glutenin; RP: residue protein.

**Appendix VI** Detailed Data for Protein Composition Analysis Using SE-HPLC

Table A.VIa Soluble Protein Fractions of Wheat Using SE-HPLC in the Primary Study

SAMPLE	$10^{-5} \times$ Absolute HPLC Area <sup>1</sup>			
	Peak 1	Peak 2	Peak 3	Total
AC KARMA NM_NT (1)	22.1	42.7	16.9	81.7
AC KARMA NM_NT (2)	20.4	38.2	15.4	74.0
AC KARMA M_NT (1)	8.1	35.4	10.2	53.8
AC KARMA M_NT (2)	16.7	43.5	14.0	74.1
AC KARMA M_T16% (1)	8.9	36.7	12.0	57.7
AC KARMA M_T16% (2)	5.2	34.0	9.7	48.9
AC KARMA M_T22% (1)	1.7	24.7	5.5	31.9
AC KARMA M_T22% (2)	2.5	32.2	7.8	42.4
AC BARRIE NM_NT (1)	17.6	58.4	13.5	89.5
AC BARRIE NM_NT (2)	17.6	54.4	13.6	85.7
AC BARRIE M_NT (1)	9.7	54.2	10.9	74.9
AC BARRIE M_NT (2)	12.6	60.4	12.3	85.2
AC BARRIE M_T16% (1)	9.5	54.1	10.2	73.8
AC BARRIE M_T16% (2)	4.8	51.5	9.2	65.5
AC BARRIE M_T22% (1)	4.0	42.8	8.0	54.8
AC BARRIE M_T22% (2)	2.2	41.5	6.6	50.2
GLENLEA NM_NT (1)	13.6	41.0	11.6	66.1
GLENLEA NM_NT (2)	16.4	45.5	13.9	75.7
GLENLEA M_NT (1)	12.2	40.5	10.3	63.0
GLENLEA M_NT (2)	7.5	44.9	8.5	60.9
GLENLEA M_T16% (1)	4.7	34.7	6.0	45.4
GLENLEA M_T16% (2)	5.1	41.7	7.4	54.2
GLENLEA M_T22% (1)	4.0	32.2	6.4	42.6
GLENLEA M_T22% (2)	2.6	36.0	6.2	44.8
AC IVORY NM_NT (1)	23.1	64.9	13.6	101.6
AC IVORY NM_NT (2)	23.3	68.1	15.9	107.3
AC IVORY M_NT (1)	9.0	54.7	8.7	72.4
AC IVORY M_NT (2)	9.7	59.3	9.5	78.4
AC IVORY M_T16% (1)	11.3	57.6	9.7	78.5
AC IVORY M_T16% (2)	5.4	53.1	8.6	67.0
AC IVORY M_T22% (1)	3.9	44.0	5.9	53.7
AC IVORY M_T22% (2)	4.0	53.0	7.9	64.8
Overall Mean	9.97	46.12	10.17	66.26
Pooled SD	6.60	10.64	3.18	17.58

Table A.VIb Soluble Protein Fractions of AC Barrie Wheat Using SE-HPLC in the Contribution of Tempering to Micronization Effects Study

SAMPLE	$10^{-5} \times$ Absolute HPLC Area <sup>1</sup>			
	Peak 1	Peak 2	Peak 3	Total
AC BARRIE NM_NT (1)	17.6	58.4	13.5	89.5
AC BARRIE NM_NT (2)	17.6	54.4	13.6	85.7
AC BARRIE M_NT (1)	9.7	54.2	10.9	74.9
AC BARRIE M_NT (2)	12.6	60.4	12.3	85.2
AC BARRIE M_T22% (1)	4.0	42.8	8.0	54.8
AC BARRIE M_T22% (2)	2.2	41.5	6.6	50.2
AC BARRIE NM_T22% (1)	19.2	61.5	15.4	96.1
AC BARRIE NM_T22% (2)	19.0	62.5	15.1	96.6
Overall Mean	12.73	54.47	11.92	79.11
Pooled SD	6.83	8.16	3.24	17.83

Table A.VIc Soluble Protein Fractions of AC Karma Using SE-HPLC in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	$10^{-5} \times$ Absolute HPLC Area <sup>1</sup>			
	Peak 1	Peak 2	Peak 3	Total
SOUND NM_NT (1)	22.1	42.7	16.9	81.7
SOUND NM_NT (2)	20.4	38.2	15.4	74.0
SOUND M_NT (1)	8.1	35.4	10.2	53.8
SOUND M_NT (2)	16.7	43.5	14.0	74.1
SOUND M_T22% (1)	1.7	24.7	5.5	31.9
SOUND M_T22% (2)	2.5	32.2	7.8	42.4
SPROUTED NM_NT (1)	24.4	48.3	16.6	89.2
SPROUTED NM_NT (2)	23.9	47.6	17.8	89.2
SPROUTED M_NT (1)	18.5	48.1	15.0	81.6
SPROUTED M_NT (2)	18.7	48.6	16.3	83.7
SPROUTED M_T22% (1)	8.2	44.0	11.9	64.1
SPROUTED M_T22% (2)	6.6	39.7	10.0	56.3
Overall Mean	14.30	41.09	13.11	68.50
Pooled SD	8.35	7.41	3.98	18.82

<sup>1</sup> Arbitrary unit for HPLC peak area was millivolts x centiminutes.

**Appendix VII** Detailed Data for Alpha-amylase Activity

Table A.VIIa The Alpha-amylase Activity (units/g) of Wheat Varieties in the Primary Study

VARIETY	TREATMENT							
	NM_NT		M_NT		M_T16%		M_T22%	
	1	2	1	2	1	2	1	2
AC KARMA	0.034	0.017	0.016	0.011	0.012	0.012	0.008	0.008
AC BARRIE	0.021	0.087	0.007	0.107	0.005	0.035	0.006	0.029
Glenlea	0.023	0.034	0.024	0.038	0.013	0.022	0.008	0.017
AC IVORY	0.025	0.056	0.023	0.057	0.014	0.026	0.009	0.021
Overall Mean					0.0258			
Pooled SD					0.0229			

Table A. VIIb The Alpha-amylase Activity (units/g) of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

MICRONIZING CONDITION	TEMPERING LEVEL			
	NT		T22%	
	1	2	1	2
NM	0.021	0.087	0.243	0.626
M	0.007	0.107	0.006	0.029
Overall Mean			0.141	
Pooled SD			0.211	

Table A. VIIc The Alpha-amylase Activity (units/g) of AC Karma in the Effects of Micronization on Sprouted Wheat Study

CONDITION	TEMPERING LEVEL					
	NM_NT		M_NT		M_T22%	
	1	2	1	2	1	2
Sound	0.034	0.017	0.016	0.011	0.008	0.008
Sprouted	3.602	3.666	3.107	3.274	1.784	1.891
Overall Mean					1.452	
Pooled SD					1.603	

### Appendix VIII Detailed Data for Farinograph

Table A.VIIIa The Farinograph Data of Wheat Varieties in the Primary Study

SAMPLE	Farinograph Parameters <sup>1</sup>			
	FAB_14 (%)	DDT (min.)	STAB (min.)	BDT (min.)
AC KARMA NM_NT (1)	57.6	4.7	4.7	6.6
AC KARMA NM_NT (2)	58.1	3.7	4.5	7.3
AC KARMA M_NT (1)	56.4	1.2	1.6	2.4
AC KARMA M_NT (2)	56.0	2.0	NA <sup>2</sup>	6.6
AC KARMA M_T16% (1)	56.0	1.7	1.4	3.3
AC KARMA M_T16% (2)	54.6	1.3	3.1	4.0
AC KARMA M_T22% (1)	56.1	1.9	13.4	1.9
AC KARMA M_T22% (2)	54.7	1.9	3.7	3.0
AC BARRIE NM_NT (1)	59.2	6.5	12.6	15.0
AC BARRIE NM_NT (2)	58.3	6.5	8.3	11.0
AC BARRIE M_NT (1)	59.2	1.5	1.9	3.3
AC BARRIE M_NT (2)	56.6	2.3	3.0	4.7
AC BARRIE M_T16% (1)	58.5	1.7	1.7	3.1
AC BARRIE M_T16% (2)	57.2	1.2	2.1	3.1
AC BARRIE M_T22% (1)	58.8	0.9	1.1	0.9
AC BARRIE M_T22% (2)	57.6	1.4	1.8	2.1
GLENLEA NM_NT (1)	57.9	20.4	39.0	40.0
GLENLEA NM_NT (2)	58.5	10.4	22.3	25.0
GLENLEA M_NT (1)	57.8	33.9	NA <sup>2</sup>	NA <sup>2</sup>
GLENLEA M_NT (2)	57.1	1.4	1.3	1.9
GLENLEA M_T16% (1)	57.4	1.2	1.4	2.1
GLENLEA M_T16% (2)	57.2	1.7	1.8	2.4
GLENLEA M_T22% (1)	57.9	1.7	2.0	2.5
GLENLEA M_T22% (2)	57.6	2.2	2.5	2.6
AC IVORY NM_NT (1)	60.6	8.3	11.3	16.0
AC IVORY NM_NT (2)	61.1	7.0	9.4	12.6
AC IVORY M_NT (1)	57.2	1.9	19.1	20.0
AC IVORY M_NT (2)	57.6	1.9	3.3	3.9
AC IVORY M_T16% (1)	58.1	1.8	13.0	16.9
AC IVORY M_T16% (2)	55.5	2.0	3.8	4.0
AC IVORY M_T22% (1)	57.2	2.4	22.3	2.4
AC IVORY M_T22% (2)	57.7	1.9	19.1	2.3
Overall Mean	57.54	4.39	7.88	7.51
Pooled SD	1.43	6.63	8.93	8.63

Table A.VIIIb The Farinograph Data of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

SAMPLE	Farinograph Parameters <sup>1</sup>			
	FAB_14 (%)	DDT (min.)	STAB (min.)	BDT (min.)
AC BARRIE NM_NT (1)	59.2	6.5	12.6	15.0
AC BARRIE NM_NT (2)	58.3	6.5	8.3	11.0
AC BARRIE M_NT (1)	59.2	1.5	1.9	3.3
AC BARRIE M_NT (2)	56.6	2.3	3.0	4.7
AC BARRIE M_T22% (1)	58.8	0.9	1.1	0.9
AC BARRIE M_T22% (2)	57.6	1.4	1.8	2.1
AC BARRIE NM_T22% (1)	58.4	6.3	8.7	11.2
AC BARRIE NM_T22% (2)	58.6	6.2	8.5	10.9
Overall Mean	58.34	3.95	5.74	7.39
Pooled SD	0.87	2.62	4.30	5.24

Table A.VIIIc The Farinograph Data of AC Karma in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	Farinograph Parameters <sup>1</sup>			
	FAB_14 (%)	DDT (min.)	STAB (min.)	BDT (min.)
SOUND NM_NT (1)	57.6	4.7	4.7	6.6
SOUND NM_NT (2)	58.1	3.7	4.5	7.3
SOUND M_NT (1)	56.4	1.2	1.6	2.4
SOUND M_NT (2)	56.0	2.0	NA <sup>2</sup>	6.6
SOUND M_T22% (1)	56.1	1.9	13.4	1.9
SOUND M_T22% (2)	54.7	1.9	3.7	3.0
SPROUTED NM_NT (1)	56.0	2.7	2.0	3.7
SPROUTED NM_NT (2)	56.5	2.7	2.1	3.9
SPROUTED M_NT (1)	57.4	1.7	2.0	3.0
SPROUTED M_NT (2)	57.0	2.0	2.5	3.4
SPROUTED M_T22% (1)	58.3	1.7	1.8	2.3
SPROUTED M_T22% (2)	58.5	1.7	1.7	2.5
Overall Mean	56.88	2.33	3.64	3.88
Pooled SD	1.14	0.99	3.43	1.88

<sup>1</sup> FAB\_14: Farinograph water absorption on 14%mb; DDT: dough development time; STAB: stability; BDT: time to breakdown.

<sup>2</sup> NA: not available.

**Appendix IX** Detailed Data for Mixograph

Table A.IXa The Mixograph Data of Wheat Varieties in the Primary Study

SAMPLE	Mixograph Parameters			
	MPT (min.)	MPH %	PBW %	WIP <sup>1</sup> % Tq*min.
AC KARMA NM_NT (1)	4.96	40.8	26.5	130.1
AC KARMA NM_NT (2)	3.30	49.0	30.8	105.9
AC KARMA M_NT (1)	3.25	5.2	6.2	15.5
AC KARMA M_NT (2)	18.75	35.2	22.1	470.3
AC KARMA M_T16% (1)	3.29	4.3	7.0	2.0
AC KARMA M_T16% (2)	3.65	13.1	8.3	33.7
AC KARMA M_T22% (1)	4.17	11.0	17.9	5.0
AC KARMA M_T22% (2)	2.13	17.5	19.0	23.7
AC BARRIE NM_NT (1)	5.45	43.4	33.8	136.7
AC BARRIE NM_NT (2)	4.12	48.5	33.8	126.4
AC BARRIE M_NT (1)	1.94	5.8	8.3	6.7
AC BARRIE M_NT (2)	2.52	11.8	10.2	18.0
AC BARRIE M_T16% (1)	1.75	6.0	6.7	5.2
AC BARRIE M_T16% (2)	2.56	8.0	7.9	15.2
AC BARRIE M_T22% (1)	3.79	9.3	5.8	22.1
AC BARRIE M_T22% (2)	1.97	15.5	14.5	19.9
GLENLEA NM_NT (1)	7.77	49.0	34.8	215.8
GLENLEA NM_NT (2)	6.83	58.6	42.7	205.4
GLENLEA M_NT (1)	12.70	40.2	29.1	250.9
GLENLEA M_NT (2)	1.72	8.6	8.4	10.4
GLENLEA M_T16% (1)	1.77	7.3	10.5	7.6
GLENLEA M_T16% (2)	2.30	13.5	11.1	25.1
GLENLEA M_T22% (1)	1.37	7.7	11.6	6.4
GLENLEA M_T22% (2)	2.06	13.2	16.2	18.2
AC IVORY NM_NT (1)	4.61	56.4	41.7	141.9
AC IVORY NM_NT (2)	4.34	62.4	44.6	147.3
AC IVORY M_NT (1)	1.73	5.3	7.9	4.7
AC IVORY M_NT (2)	1.94	10.4	9.7	12.5
AC IVORY M_T16% (1)	3.01	6.8	7.6	12.0
AC IVORY M_T16% (2)	1.90	12.0	15.7	15.9
AC IVORY M_T22% (1)	1.79	10.8	16.2	12.2
AC IVORY M_T22% (2)	1.75	18.6	19.1	21.0
Overall Mean	3.912	22.038	18.303	70.116
Pooled SD	3.548	18.924	12.000	102.552

Table A.IXb The Mixograph Data in the Contribution of Tempering to Micronization Effects Study

SAMPLE	Mixograph Parameters			
	MPT (min.)	MPH %	PBW %	WIP <sup>1</sup> % Tq*min.
AC BARRIE NM_NT (1)	5.45	43.4	33.8	136.7
AC BARRIE NM_NT (2)	4.12	48.5	33.8	126.4
AC BARRIE M_NT (1)	1.94	5.8	8.3	6.7
AC BARRIE M_NT (2)	2.52	11.8	10.2	18.0
AC BARRIE M_T22% (1)	3.79	9.3	5.8	22.1
AC BARRIE M_T22% (2)	1.97	15.5	14.5	19.9
AC BARRIE NM_T22% (1)	4.70	52.8	38.8	146.1
AC BARRIE NM_T22% (2)	4.62	52.8	37.5	146.3
Overall Mean	3.639	29.988	22.838	77.775
Pooled SD	1.339	21.102	14.348	65.764

Table A.IXc The Mixograph Data of AC Karma in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	Mixograph Parameters			
	MPT (min.)	MPH %	PBW %	WIP <sup>1</sup> % Tq*min.
SOUND NM_NT (1)	4.96	40.8	26.5	130.1
SOUND NM_NT (2)	3.30	49.0	30.8	105.9
SOUND M_NT (1)	3.25	5.2	6.2	15.5
SOUND M_NT (2)	18.75	35.2	22.1	470.3
SOUND M_T22% (1)	4.17	11.0	17.9	5.0
SOUND M_T22% (2)	2.13	17.5	19.0	23.7
SPROUTED NM_NT (1)	3.01	51.9	34.0	94.9
SPROUTED NM_NT (2)	2.87	54.9	34.2	102.7
SPROUTED M_NT (1)	11.24	40.9	25.9	318.2
SPROUTED M_NT (2)	10.91	45.4	28.6	352.7
SPROUTED M_T22% (1)	1.10	15.0	10.6	12.6
SPROUTED M_T22% (2)	1.75	15.8	11.8	19.8
Overall Mean	5.620	31.883	22.300	137.617
Pooled SD	5.287	17.778	9.331	156.275

<sup>1</sup> MPT: mixograph peak time; MPH: mixograph peak height; PBW: peak band width; WIP: work input to peak.

**Appendix X** Detailed Data for Micro-scale Extension Test

Table A.Xa The Micro-scale Extension Data of Wheat Varieties in the Primary Study

SAMPLE	Extension Parameters <sup>1</sup>		
	R <sub>max</sub> (g)	EXTENS (mm)	AREA (g·mm)
AC KARMA NM_NT (1)	9.77	113.48	616.37
AC KARMA NM_NT (2)	9.23	149.49	636.67
AC KARMA M_NT (1)	7.13	14.85	61.71
AC KARMA M_NT (2)	11.66	56.50	307.63
AC KARMA M_T16% (1)	4.60	99.68	134.51
AC KARMA M_T16% (2)	9.85	27.48	74.05
AC KARMA M_T22% (1)	15.32	19.61	82.17
AC KARMA M_T22% (2)	11.75	26.03	70.88
AC BARRIE NM_NT (1)	21.69	106.22	1010.20
AC BARRIE NM_NT (2)	17.56	130.93	1071.15
AC BARRIE M_NT (1)	7.76	27.28	82.53
AC BARRIE M_NT (2)	3.32	87.34	140.02
AC BARRIE M_T16% (1)	7.66	14.81	60.19
AC BARRIE M_T16% (2)	9.41	103.14	123.32
AC BARRIE M_T22% (1)	8.78	14.34	54.65
AC BARRIE M_T22% (2)	11.13	26.88	67.06
GLENLEA NM_NT (1)	24.52	94.18	1170.87
GLENLEA NM_NT (2)	24.41	119.14	1304.66
GLENLEA M_NT (1)	34.04	67.86	1190.61
GLENLEA M_NT (2)	6.10	56.24	133.62
GLENLEA M_T16% (1)	10.17	20.05	74.71
GLENLEA M_T16% (2)	9.58	28.57	66.36
GLENLEA M_T22% (1)	10.33	13.46	61.51
GLENLEA M_T22% (2)	11.69	27.53	77.95
AC IVORY NM_NT (1)	20.48	112.89	1199.95
AC IVORY NM_NT (2)	22.80	120.83	1214.40
AC IVORY M_NT (1)	9.81	22.04	89.86
AC IVORY M_NT (2)	7.51	39.48	110.12
AC IVORY M_T16% (1)	8.35	29.31	84.02
AC IVORY M_T16% (2)	12.87	26.88	79.56
AC IVORY M_T22% (1)	14.73	13.03	70.65
AC IVORY M_T22% (2)	14.73	25.66	75.64
Overall Mean	12.773	57.350	362.425
Pooled SD	6.788	43.445	455.208

Table A.Xb The Micro-scale Extension Data of AC Barrie Wheat in the Contribution of Tempering to Micronization Effects Study

SAMPLE	Extension Parameters <sup>1</sup>		
	R <sub>max</sub> (g)	EXTENS (mm)	AREA (g·mm)
AC BARRIE NM_NT (1)	21.69	106.22	1010.20
AC BARRIE NM_NT (2)	17.56	130.93	1071.15
AC BARRIE M_NT (1)	7.76	27.28	82.53
AC BARRIE M_NT (2)	3.32	87.34	140.02
AC BARRIE M_T22% (1)	8.78	14.34	54.65
AC BARRIE M_T22% (2)	11.13	26.88	67.06
AC BARRIE NM_T22% (1)	21.78	113.53	1114.44
AC BARRIE NM_T22% (2)	15.93	119.15	858.92
Overall Mean	13.494	78.209	549.871
Pooled SD	6.788	47.628	501.800

Table A.Xc The Micro-scale Extension Data of AC Karma in the Effects of Micronization on Sprouted Wheat Study

SAMPLE	Extension Parameters <sup>1</sup>		
	R <sub>max</sub> (g)	EXTENS (mm)	AREA (g·mm)
SOUND NM_NT (1)	9.77	113.48	616.37
SOUND NM_NT (2)	9.23	149.49	636.67
SOUND M_NT (1)	7.13	14.85	61.71
SOUND M_NT (2)	11.66	56.50	307.63
SOUND M_T22% (1)	15.32	19.61	82.17
SOUND M_T22% (2)	11.75	26.03	70.88
SPROUTED NM_NT (1)	5.48	192.23	810.15
SPROUTED NM_NT (2)	6.74	179.67	675.18
SPROUTED M_NT (1)	13.64	76.08	472.33
SPROUTED M_NT (2)	13.08	83.85	530.81
SPROUTED M_T22% (1)	11.15	30.08	88.18
SPROUTED M_T22% (2)	11.28	28.41	74.88
Overall Mean	10.519	80.857	368.913
Pooled SD	2.962	63.968	284.887

<sup>1</sup> R<sub>max</sub>: maximum resistance to extension; EXTENS: extensibility from start until rupture; AREA: area under the curve.

**Appendix XI** The ANOVA for Single Kernel Characteristics

Table A.XIa The ANOVA for Single Kernel Weight

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	10.5973	10.5973	3.26	0.0912
Variety	3	841.9331	280.6444	86.27	<0.0001
Treatment	3	35.8107	11.9369	3.67	0.0366
VarietyxTreatment	9	2.3219	0.2580	0.08	0.9997
Error	15	48.7991	3.2533		
Corrected Total	31	939.4621			

Table A.XIb The ANOVA for Single Kernel Diameter

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	0.0226	0.02258	2.47	0.1369
Variety	3	0.8183	0.2728	29.83	<0.0001
Treatment	3	0.0556	0.01853	2.03	0.1534
VarietyxTreatment	9	0.0166	0.0018	0.20	0.9899
Error	15	0.1371	0.0091		
Corrected Total	31	1.0503			

Table A.XIc The ANOVA for Single Kernel Hardness Index

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	44.8760	44.8760	0.98	0.3375
Variety	3	1147.6522	382.5507	8.37	0.0017
Treatment	3	812.2100	270.7366	5.92	0.0071
VarietyxTreatment	9	303.2482	33.6942	0.74	0.6713
Error	15	685.7509	45.7167		
Corrected Total	31	2993.7372			

Table A.XId The ANOVA for Single Kernel Moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr>F
Block	1	0.3220	0.3220	0.24	0.6347
Variety	3	2.5910	0.8637	0.63	0.6063
Treatment	3	158.0188	52.6729	38.48	<0.0001
VarietyxTreatment	9	4.5402	0.5045	0.37	0.9330
Error	15	20.5347	1.3690		
Corrected Total	31	186.0067			