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RELATIONSHIP BETWEEN FLOUR NON-STORAGE PROTEINS AND COOKIE-BAKING QUALITY IN SOFT WHEAT FLOURS

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ABSTRACT

RELATIONSHIP BETWEEN FLOUR NON-STORAGE PROTEINS AND COOKIE-BAKING QUALITY IN SOFT WHEAT FLOURS

BY

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Wheat flour quality is often influenced by its protein composition. Sodium dodecyl sulfate polyacrylamide gel electrophoresis of the polypeptide compositions of albumin and globulin proteins extracted from eight different soft wheat varieties indicated the presence of polymeric proteins within these fractions. Studies on the effects of the soft wheat albumins and globulins on cookie baking quality in 32 soft wheat flours suggested that diameters of sugar-snap cookies was influenced by flour globulin content. The results of baking experiments involving a poor cookie flour supplemented by globulin from a good cookie flour suggests qualitative differences among soft wheat flour globulins.

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1. INTRODUCTION

Wheat is one of the major cereal crops cultivated in the United States; the 1992 production was estimated at about 67 million metric tons (US Wheat 1992). Wheat grown in the United States can be categorized into three major types, depending on the hardness of the wheat kernel. These categories are:

(i) Durum wheat, which has extremely hard kernels and is normally milled into coarse granules, called semolina, used for pasta production.

(ii) Hard wheat, which has a kernel texture not as hard as that of durum wheat, and is normally milled into flour for bread production.

(iii) Soft wheat, which has soft-textured kernels, is often unsuitable for making leavened bread, and is normally used in the manufacture of products like cookies, cakes, pastries and soup thickeners.

Soft wheat production in the United States in 1992 was about 19 million metric tons. This accounted for about 28% of the year's total wheat production (US Wheat 1992). Soft wheat flour is considered best suited for such products as cakes, cookies, crackers, pastries and noodles because of its properties of fine flour particle size, low protein content and lower levels of damaged starch granules when

compared to hard wheat flour (Finney 1989). A problem often encountered by users of soft wheat flour is the wide variation in product quality that can occur between different lots of flour while using the same product formulation (Faridi et al 1987). To counteract this, manufacturers of soft wheat products blend different flours and mill streams or change their product formulations in order to maintain the quality characteristics of their products. A possible cause of this problem is the large number of soft wheat varieties under cultivation, which exhibit wide variation in their milling and baking properties (Finney et al 1987). Therefore, there is a need for thorough screening of soft wheat varieties, to identify those that give consistent quality in specific products (Clements 1987).

In order to fully understand the effect of variety on product quality, it is essential to investigate the role played by various flour components in determining flour quality. Flour protein has been shown to be one of the important factors affecting flour quality, particularly for hard wheats. What is not fully understood is the individual roles played in soft wheat flour quality by different classes of flour proteins such as the glutenins, gliadins, albumins and globulins.

The objective of this study was to investigate possible relationships between soft wheat flour albumins and globulins (non-storage proteins) and soft wheat flour quality, as determined by cookie-baking potential. This study was conducted in two phases; phase one involved the comparison of the levels of non-storage proteins among soft wheat varieties with known quality attributes. Phase two involved the evaluation of flour quality for a separate collection of soft wheat varieties. Furthermore, in this second phase, globulin fractions were extracted from two flours that exhibited major differences in their cookie-baking potentials. These extracts were used to study the effects of increasing levels of flour globulin on cookie diameter, as well as the effect of globulin from a good cookie flour on a poor cookie flour.

2. LITERATURE REVIEW

2.1 Introduction

The relationship between flour quality performance and flour composition has long been of interest to cereal scientists. Elucidating the physical and chemical properties of individual flour components, and the types of interactions among these different components of wheat flour, is essential to understanding what constitutes a good quality flour. Further knowledge of these aspects related to flour quality raises the potential for new innovations in quality testing. Better understanding of the relationship between flour composition and end-product quality could also be significant in helping end users of soft wheat make appropriate modifications to their production processes.

2.2 Soft Wheat

The three different types of wheat (soft, hard and durum wheats) exhibit inherent genetic differences in the number of chromosomes within their nuclei (Williams 1986). Durum wheats are tetraploid species; they have four sets of the seven chromosomes that comprise the complete wheat

genome, for a total of 14 pairs of chromosomes. Hard and soft wheats are hexaploid species; they possess 21 pairs of chromosomes comprised of six sets of the seven chromosomes. Among the hexaploid wheats, a wide range in kernel hardness is observed. The texture of the kernel is an important factor influencing the end uses of the wheat, since it determines flour properties such as particle size and levels of damaged starch granules.

Biffen (1908) distinguished between hard and soft wheats by crushing their grains on an iron plate. Hard wheats gave coarse granular particles while soft wheats gave a fine powder. Symes (1969), while studying the expression of kernel hardness in near isogenic lines of hexaploid wheats, observed that kernel texture was an inheritable character and concluded that a single gene was responsible for determining kernel hardness. Stenvert and Kingswood (1977) studied the physical structure of starch and protein from hard and soft wheat kernels. They observed that the protein matrix within the hard wheat kernels showed a continuous structure while the protein in soft wheat kernels had a discontinuous matrix structure. They came to the conclusion that the protein in hard wheats is more strongly bound to starch than in soft wheats. Recently, Greenwell and Schofield (1986) described a 15kDa starch granule protein band (determined by polyacrylamide gel

electrophoresis) that was prominent in soft wheat varieties and absent or faint in hard wheats. What relationship this protein might have with kernel texture is yet to be fully understood.

2.3 Wheat Proteins

Protein is one of the major components of soft wheat flour. Protein content in soft wheat flours ranges between 7 to 10% (Kaldy and Rubenthaler 1987). Flour proteins can be separated into different classes which have various biochemical and physico-chemical properties. Osborne (1907) described a scheme for the separation of wheat proteins into four major classes, based on the sequential extraction of flour with a variety of solvents:

(i) Albumins: extractable in distilled water.

(ii) Globulins: extractable in dilute salt solutions.

(iii) Gliadins: extractable in 70% aqueous ethanol.

(vi) Glutenins: extractable in dilute acids and alkali

Chen and Bushuk (1970) modified the Osborne procedure by simultaneously extracting the albumins and globulins from hard wheat in 0.5M NaCl, then separating these two fractions by dialyzing the extract against distilled water to precipitate the globulins.

Another method for extracting the albumins and globulins from wheat flour was described by Pence and Elder (1953). In this procedure albumins were extracted from flour with distilled water or 0.1% phosphate buffer, and the extract was made to 0.8M ammonium sulfate to precipitate the pentosans. The supernatant was then made to 1.74M ammonium sulfate and another precipitate was collected. This precipitate was resuspended in water and reprecipitated once again to obtain the albumins. This method had the advantage of removing most of the pentosans that were present in aqueous extracts of flour. However the modified Osborne procedure is still the most widely employed for the fractionation of total flour proteins.

The gliadins and glutenins are the storage proteins of wheat flour, and together they account for about 80% of the total flour protein. They are characterized by their insolubility in water and relatively high levels of the non-polar amino acids proline and glutamine (Chen and Bushuk 1970). The gliadins and glutenins have the ability to associate through disulfide bonds, hydrogen bonds and hydrophobic interactions, forming a cohesive and elastic protein network called gluten (Graveland and Henderson 1987). Formation of gluten is important to many of the functional properties of wheat flour, and it is absolutely essential for bread production.

The albumins and globulins account for the non-storage proteins of wheat. They comprise mostly metabolic proteins from the wheat endosperm. Chen and Bushuk (1970) estimated that the albumins and globulins in a hard wheat flour constituted about 17% of flour protein. Fullington et al (1983) reported that the levels of the non-storage proteins in flour were influenced by environment, and that the proportion of non-storage proteins relative to storage proteins decreased as flour protein content increased. Previously the albumins and globulins were considered incapable of forming high-molecular-weight (HMW) aggregates like the storage proteins, and because of this their role in influencing wheat flour quality may have been overlooked. However, some investigations on albumins and globulins have revealed that they are indeed capable of forming HMW aggregates (Singh and Shepherd 1985; Gupta et al 1991). This has implications concerning the contributions of the albumins and globulins to flour quality properties. The next section reviews research conducted on the albumin and globulin proteins of wheat flour.

2.4 Studies on Albumins and Globulins

Pence and Elder (1953) studied the nature of albumin and globulin proteins from soft wheat flours. Based on the

results of moving boundary electrophoresis, they determined that the albumin fraction was a heterogeneous mixture that contained at least four polypeptides with similar molecular weights but different electrophoretic mobilities. They also observed a disparity between the average molecular weights, in NaCl and dissociating reagents such as salicylate and urea, for albumin proteins prepared by ammonium sulfate precipitation. When albumins were dissolved in the dissociating reagents, average molecular weight was 17kDa, but when the dissociating reagents were removed by dialysis, the average molecular weight was observed to be 28kDa. This appeared to suggest the possible formation of aggregates by albumin proteins in wheat flour.

Silano et al (1969) studied the polypeptide composition of the albumin and globulin fractions of wheat flour using discontinuous electrophoresis. They reported the presence of 14 polypeptide bands for the albumin fraction and 15 bands for the globulin fraction, with widely varying electrophoretic mobilities. This supported the observation of Pence and Elder (1953) that the salt-soluble proteins comprise a heterogeneous mixture of polypeptides.

Pence and Elder (1953) and Holme (1962) had observed that some of the protein in water extracts of flour was strongly associated with the pentosan fraction during ammonium sulfate fractionation of the flour water-solubles.

Figueroa and Khan (1993) investigated the presence of glycoproteins in albumin fractions from soft, hard and durum wheats. They observed the co-elution of proteins and carbohydrates during size exclusion chromatography for certain fractions in all three types of wheat. The co-migration of albumin proteins and carbohydrate was also observed during polyacrylamide gel electrophoresis. The authors thus concluded that certain albumin proteins are covalently linked to carbohydrate. This suggests the possibilities of non-covalent interactions between such albumin prosthetic groups and flour macromolecules such as starch. Gupta et al (1991) studied the total flour proteins in hard wheat varieties using gel exclusion chromatography, two-step, and diagonal electrophoreses. They observed the presence of HMW albumins that co-eluted with the low-molecular-weight (LMW) glutenins during gel exclusion chromatography. Electrophoretic patterns of these albumins under reduced and unreduced conditions in diagonal electrophoresis indicated that these were polymeric proteins linked via disulfide bonds.

In addition to the albumins, research on the globulin proteins has also turned up evidence suggesting that this class of proteins is capable of forming HMW polymers. Singh and Shepherd (1985) reported the presence of three HMW globulin bands that appeared during sodium dodecyl sulfate

polyacrylamide gel electrophoresis (SDS-PAGE) of total wheat proteins. Resolution of these proteins by 2D-electrophoresis confirmed that they were tetrameres bound together by disulfide bonds.

Terada et al (1978) reported the existence of a HMW globulin fraction that appeared to polymerize at pH 9.5. From the results of SDS-PAGE analysis of soft wheat flour globulins, they observed that a band labeled 9 diminished after the globulin fraction was incubated at pH 9.5, while a new band of lower mobility labeled as band 11 appeared. They determined that band 11 disappeared and band 9 was restored following reduction of the globulin fraction with 2-mercaptoethanol, a disulfide reducing agent. It was also observed that the concentration of free thiol groups in the globulin fraction was reduced following incubation at pH 9.5. Based on these observations they concluded that the protein fraction in the so-called band 9 had associated by disulfide bonds to form band 11.

The evidence that albumin and globulin proteins are capable of forming structural polymers suggests that they could have important effects on the physico-chemical properties of a flour and its products. Bernadin and Kasarda (1973) theorized that the globulin proteins could be included in the gluten protein network, and could help stabilize extensive protein networks in dough due to their

amphipathic properties of possessing both polar and non-polar regions.

2.5 Soft Wheat Flour Quality

Flour quality represents the flour's potential to produce end-products with desirable properties. Due to the wide variety of products made from soft wheat flour, it is difficult to determine what constitutes a good quality soft wheat flour, since a flour that performs satisfactorily in one product might perform poorly in another. However, there are some flour properties which are widely accepted to be indicative of good soft wheat flour quality. The following reviews these flour quality parameters and their relevance to flour quality prediction.

2.5.1 Milling Quality

The milling properties of wheat are influenced by the texture of the wheat grain. Softer wheats produce flours with smaller particle sizes, and lower levels of damaged starch which are often desirable for optimum quality in soft wheat products. Parameters often measured to determine soft wheat milling quality are: breakflour yield, endosperm separation index, and ash content.

2.5.1.1 Breakflour Yield

This is a measure of the quantity of flour recovered after the wheat has passed through the first set of break rolls. This is a good indicator of the hardness of the wheat, with softer textured wheats producing higher quantities of break flour (Gaines 1985).

2.5.1.2 Endosperm Separation Index

This milling quality parameter was described by Yamazaki and Andrews (1982). It is defined as the amount of endosperm remaining attached to the bran after the break and first reduction passes. This is considered a measure of the ease with which the endosperm is separated from the bran. Flours with lower endosperm separation indices are considered better suited for soft wheat products.

2.5.1.3 Flour Ash Content

This is an indirect measurement of the amount of bran contaminating the flour, since the endosperm has a much lower mineral content than the bran. High levels of bran are often considered deleterious to soft wheat flour quality.

2.5.2 Flour Physico-Chemical Properties

These are measurements of the functional properties of the flour macromolecular constituents. Factors such as water absorption, and functional properties of flour proteins and starch can be determined by means of these tests.

2.5.2.1 Alkaline Water Retention Capacity

This test was developed by Yamazaki (1953) to predict the suitability of a soft wheat flour for cookie baking. The test measures a flour's ability to retain alkaline water against a centrifugal force. Flours that retained less water were considered better suited for cookie baking. This was further buttressed by the observation of Yamazaki (1955) that flours containing higher levels of hydrophilic polymeric constituents bake cookies with smaller diameters.

2.5.2.2 Alveograph Test

The use of the alveograph to determine the suitability of wheat flour for bread-baking was described by Chopin (1927). The alveograph measures the extent to which a developed piece of dough can be stretched before it ruptures. The test involves the formation of a sheet of dough, which is then blown into a bubble, simulating

conditions during leavening of bread dough. Physical properties of the dough measured are:

(i) The maximum overpressure (P) which is the initial ordinate of the curve. This represents the tensile strength at the initial stage of dough deformation.

(ii) The length of the curve (L) which represents the extensibility of the dough.

(iii) The area under the curve (W) which represents the amount of work done before rupture of the dough bubble. In a recent study, Bettge et al (1989) reported that cookie diameter could be predicted from an algorithm based on flour protein content and alveograph P values.

2.5.2.3 Sedimentation Test

This test measures the swelling potential of a flour in a solution of lactic acid. The extent of swelling is related to the flour's gluten protein strength and protein content. Soft wheat flours normally have weak gluten protein strengths. Sedimentation values below 20 ml are indicative of good quality soft wheat flours (AACC 1983).

2.5.2.4 Falling Number Assay

This test measures the time in seconds it takes a plunger to fall vertically through a set distance in a heated flour-water slurry. The rate of the plunger's fall

is related to the viscosity of the slurry. The falling number assay is widely employed for the qualitative determination of alpha-amylase activity and degree of sprouting in cereals. The viscosity of a flour-water mixture at temperatures above the gelatinization temperature for the starch is mainly dependent on the physico-chemical properties of the starch (Varriano-Marston et al 1982). Alpha-amylase cleaves the starch polymer into smaller polysaccharide units, causing a reduction in the viscosity of the flour-water mixture. Test results may also be affected by starch granule state, as cleavage of intact starch granules occurs at a much slower rate than for damaged starch granules and gelatinized starch.

2.5.3 Baking Quality of Soft Wheat Flour

Although testing for certain flour functional properties might give an indication of a flour's performance in a particular end product, the best test would be one where the conditions simulate those actually applied to the flour during processing. Baking tests give the closest approximation between laboratory testing and actual end use for soft wheat flours. Three types of laboratory baking tests widely employed for testing of soft wheat flours are:

2.5.3.1 Sugar-Snap Cookie Baking Test (AACC 1983, Method 10-50D)

This test is extensively used to assess the value of a flour for a wide variety of soft wheat products, including cookies and crackers. Cookie diameter is the main parameter considered. Flours that produce cookies with larger diameters are considered better suited for soft wheat products. Other parameters considered are the spread factor (ratio of cookie diameter to thickness), cookie texture, and surface cracking.

2.5.3.2 Wire-Cut Cookie Baking Test (AACC 1992, Method 10-54)

The wire-cut cookie baking test was developed as an alternative to the sugar-snap cookie baking test, which had high levels of sucrose and low levels of water in its formula. This was found to be incompatible with most commercial cookie-baking formulas that had lower levels of sugar and higher levels of water (Gaines 1993). The wire-cut cookie formula is more consistent with the formulations of commercially produced cookies, than the sugar-snap cookie formula, by containing lower levels of sucrose and more water. As for the sugar-snap cookie test, the major parameter considered is cookie diameter. Cookie texture may also be determined from this test.

2.5.3.3 Layer Cake Baking Test (AACC 1983, Method 10-90)

This test is also employed to predict flour quality for soft wheat products. Cake volume and crumb structure are the parameters measured. Flours that produce large cakes with a fine crumb structure are considered suitable for most soft wheat applications.

2.6 Protein Quality

Protein quality as it relates to wheat flour quality can be regarded as the interplay between flour protein content and the polypeptide composition of the flour. The term protein quality was described by Orth and Bushuk (1972) to explain the variations in bread-baking potential among hard wheat varieties. They determined that the levels of acetic acid insoluble glutenins in flour appeared to be a varietal characteristic, and were positively correlated with bread volume. Since then, a number of investigations have been conducted into the associations between the specific polypeptide composition of flour proteins and flour quality.

Bushuk and Zillman (1978) described a lactic acid polyacrylamide gel electrophoresis (Acid-PAGE) procedure for the separation of gliadin proteins, for purposes of variety identification. This could also be used to screen wheat varieties for characteristics known to be associated with

good quality. Ng and Bushuk (1987) developed a SDS-PAGE procedure for resolving and comparing the glutenin subunits from wheat flour proteins. This procedure was intended for the identification and nomenclature of the glutenin subunits, but can also be employed for varietal identification and quality determination. Payne et al (1987) studied the HMW glutenins of wheat varieties, using SDS-PAGE. They reported that certain HMW glutenin subunits (5 + 10, 17 + 18, 7 + 8) were associated with good bread-baking quality, and another set of HMW glutenin subunits (2 + 12, 6 + 8) were associated with poor bread-baking quality.

Caldwell and Kasarda (1978) reported that it was possible to discriminate between different species of wheat, using Acid-PAGE of their non-storage proteins. Clements (1990) investigated the application of polyacrylamide gel electrophoresis of non-storage proteins as a means of soft wheat flour quality prediction. Non-storage proteins extracted from four different soft wheat flours were analyzed using two different running conditions: either 10-20% acrylamide gradient gels buffered at pH 5.3-6.0 with potassium acetate/Beta-alanine acetate, or 8% acrylamide gels buffered at pH 8.0-8.5 with Tris-HCl/Tris glycine. Under these conditions the polypeptide bands segregate based on their molecular weights and relative charges. This produces banding patterns that may be used for varietal

identification. However, the author was unable to observe any direct associations between the banding patterns and soft wheat quality.

A recently introduced method for the analysis of flour polypeptides and their quality relationships is reversed-phase high performance liquid chromatography (Bietz and Huebner 1987). This method has the advantage over polyacrylamide gel electrophoresis of being faster, having better resolution, and allowing quantification of the polypeptide fractions.

Soft wheat quality, for most practical purposes, was long regarded as being in less demand for attention than hard wheat quality. As a result of this, most investigations of flour protein quality have focused on hard wheats with an emphasis on bread-baking quality. Studies on wheat protein quality have been mainly concentrated on the glutenin and gliadin proteins, especially in hard wheats, because of their importance in bread-baking. With increasing recognition of soft wheats as a separate class with different quality requirements, different approaches to determine protein quality properties as they relate to soft wheat will be required.

2.7 Fractionation and Reconstitution Baking Studies

A major area of research on wheat has been to determine the effect of flour composition on end product quality (MacRitchie et al 1990). The major chemical classes constituting wheat flour are: proteins, carbohydrates and lipids (Bushuk 1986). The quality of various end-products can be influenced to varying extents by the nature of these flour constituents. One method of investigation that has been useful for studying the effect of flour composition on end-product quality is fractionation and reconstitution baking experiments (MacRitchie 1985). This normally involves the fractionation of flour into five fractions, namely: lipid, water-soluble, gluten, starch and starch tailings fractions. These fractions are then recombined in different proportions, or specific fractions may be left out. The results of such treatments can give an indication of the effects of these different flour components on baking quality.

Results of flour fractionation and reconstitution studies of soft wheat quality by various researchers have increased understanding in this area of wheat chemistry and baking technology. Yamazaki (1955) studied the effects of the five flour fractions on the diameters of sugar-snap cookies baked from reconstituted flours. It was observed

that increasing the levels of the starch tailings fraction in reconstituted flours caused significant reductions in cookie diameter. Based on the high alkaline water retention capacity observed for the starch tailings fraction, it was concluded that its reduction of cookie diameter was due to its hydrophilic nature. Sollars (1956) showed that the water-soluble fraction of soft wheat flour caused considerable reductions in the diameters of cookies baked from reconstituted flours. In a subsequent investigation, Sollars (1959) separated the water-soluble fraction of soft wheat flours into two chemical classes: pentosans and proteins. Reductions in cookie diameters were observed when the levels of the water soluble pentosans and albumin proteins in flour were raised.

Yamazaki et al (1977) studied the effects of the five soft wheat flour fractions on cookie diameter, while varying their levels relative to each other in reconstituted flours. They concluded that the starch tailings and gluten fractions depress cookie diameter. In contrast to the findings of Sollars (1956; 1959), Yamazaki et al (1977) reported that the association of the water-soluble fraction with the gluten and the starch tailing fractions nullified their cookie-spread depressing effects.

It is obvious from the results of these investigations that flour quality can be influenced by its composition.

Recent information suggests that the effect of the flour components on water mobility within the cookie dough could be partly responsible for their effect on cookie baking (Gaines et al 1988; Gaines and Finney 1989). More information about the relationship between flour composition and flour quality, and the differences in this regard among different varieties could be useful for the development of better quality soft wheat varieties.

2.8 The Role of Flour Proteins in Cookie-Baking

To investigate the effects of proteins on the baking quality of soft wheat flour, it is important to know the different physical and chemical changes that occur to flour proteins during baking. In the initial stages of baking, shortening melts and action of the leavening system causes expansion of the cookie dough in all directions. At some point during baking, a rapid increase in the viscosity of the cookie dough occurs. At this point the cookie stops spreading. This rapid increase in the viscosity of the cookie dough must be related to flour quality, since different flours using the same cookie dough formulation produce cookies with different diameters (Hoseney et al 1988).

The tendency of storage proteins to form gluten has been reported, by Finney et al (1950) and Yamazaki and Donelson (1976), to have cookie-spread depressing effects. Doescher et al (1987) suggested that the rapid increase in viscosity observed during cookie baking was due to the formation of a gluten protein matrix that then underwent a transition into a glass polymer. They concluded that the difference between a good quality cookie flour and a poor quality cookie flour was that the poor quality flour's gluten underwent the transition sooner and at a lower temperature. Gaines (1990) studied the effect of sulfhydryl oxidizing, reducing and blocking agents on cookie-baking quality. The author reported that N-ethylmaleimide, a sulfhydryl blocking agent and dithiothreitol, a disulfide cleaving agent had significant cookie-spread increasing effects. This supports the postulation by Doescher et al (1987) that the formation of an extensive protein matrix occurred in cookie doughs during baking.

Most investigations of the role of flour proteins in cookie baking have been concentrated on storage proteins. The effect of non-storage proteins in cookie baking has been largely neglected. However, some quality-related effects could be suggested for this group of proteins. Many of the proteins present in the albumin and globulin fractions are known to have enzymatic properties (Wrigley and Bietz 1988).

Of the enzymes present in wheat flour, the alpha- and beta-amylases have the most significant effects with regards to bread-baking quality (Drapron and Godon 1987). Gaines and Finney (1989) studied the effects of commercial enzyme preparations on sugar-snap cookie diameter. Enzymes having cellulase, protease, beta-glucosidase, beta-glucanase and cellobiase activities were used in their study. They found that cookie diameter was increased for both hard wheats and soft wheats, when cellulase or protease enzyme preparations were included in the doughs. This suggested that flour polypeptides with enzymatic properties similar to those used in their investigation could have similar effects on a flour's cookie-baking quality.

3. MATERIALS and METHODS

3.1 Materials

3.1.1 Wheat Samples

Thirty-two soft wheat flours representing eight varieties were obtained from the USDA Soft Wheat Quality Laboratory (SWQL) in Wooster, Ohio, for use in phase one of this study. There were four flour samples from different locations and/or crop years per variety. Milling and baking data for these flour samples (breakflour yield, alkaline water retention capacity and sugar-snap cookie diameter) were also supplied by the SWQL. Another set of eight flour samples, representing eight soft wheat varieties harvested in 1993 were employed in the second phase of this study. The different varieties utilized in these two phases are listed in Table 1.

3.1.2 Reagents and Chemicals

Coomasie brilliant blue R, sucrose, sodium chloride, methyl green, glycine and TRIS(tris hydroxylmethyl amino methane) were from Sigma chemical company (St. Louis, MO). Sodium dodecyl sulfate (SDS), acrylamide and bisacrylamide were from Boehringer Mannheim company, Indianapolis, IN.

TABLE 1. Soft Wheat Varieties Used in This Study

Soft Red	Soft White	Club
Argee[a]	Augusta[a]	Hyak[b]
Caldwell[a]	Chelsea[b]	Tres[b]
Cardinal[a]	Frankenmuth[ab]	
Dynasty[b]	Madsen[b]	
Excel[b]	Stephens[b]	
Pioneer 2550[a]		
Pioneer 2555[a]		
Tyler[a]		

[a]Varieties studied in phase one
[b]Varieties studied in phase two

Sodium hydroxide was from Mallincroft company, Sweden. All chemicals were of reagent grade.

3.2 Methods

3.2.1 Grain Milling

Flours studied in phase one were obtained from wheat milled on an Allis Chalmers mill. Breakflour yield was recorded as the amount of flour obtained after the break passes. For the samples studied in phase two; whole wheat flours for the falling number assays were obtained by milling wheat samples on a Udy Cyclone mill. Flours for other tests conducted during phase two of this study were obtained by milling on a Brabender Junior Quadrumat mill.

3.2.2 Falling Number Determination

Falling number values were determined for the eight whole wheat flours milled from the soft wheat samples utilized in phase two of the study. Measurements were conducted according to the procedure described in the Falling Number Operations Manual, Perten Company, Huddinge, Sweden.

3.2.3 Alkaline Water Retention Capacity

This was conducted according to method 56-10 of the Approved Methods of the AACC (1983).

3.2.4 Flour Sedimentation Test

Flour sedimentation tests were conducted on flour samples studied in phase two, according to method 56-61A described in the Approved Methods of the AACC (1983).

3.2.5 Alveograph Test

The alveograph test was conducted on flour samples studied in phase two according to method 54-30 described in the Approved Methods of the AACC (1983).

3.2.6 Protein Content Determination

Protein contents of flour, and of the albumin and globulin fractions recovered during fractionation of flour proteins were determined by measurement of total nitrogen by the micro-Kjeldahl procedure, AACC Approved Method 46-13 (1983). Nitrogen content of samples was multiplied by a factor of 5.7 to obtain protein contents.

3.2.7 Flour Ash Content Determination

Ash contents were determined for all the flour samples used in this study according to method 08-01 described in the Approved Methods of the AACC (1983).

3.2.8 Cookie Baking

Sugar-snap cookies were baked from flours studied in phase one according to AACC Approved Method 10-50D (1983). Wire-cut cookies were baked in phase two of the study according to the AACC Approved Method 10-54 (1990).

3.2.9 Protein Fractionation

Flour proteins were fractionated according to the modified Osborne fractionation procedure of Chen and Bushuk (1970). The albumin and globulin proteins were extracted from flour, with 0.5M NaCl. Separation of the albumins from the globulins was accomplished by dialyzing the extract against deionized distilled water for 48 hours to precipitate the globulins. The flour residue was also collected. All recovered fractions were freeze-dried and stored at $4^{o}C$. Protein contents of the albumin and globulin proteins were determined by the micro-Kjeldahl procedure AACC Approved Method 46-13 (1983).

3.2.10 Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (SDS-PAGE)

Polyacrylamide gel electrophoresis in the presence of SDS was conducted according to the procedure of Ng and Bushuk (1987). Apparatus was a Hoefer Scientific vertical slab gel unit, model SE 600, with a Biorad computer-controlled electrophoresis power supply, and a water bath for maintaining running temperature at 20°C. Running time was reduced by four hours to prevent low molecular weight polypeptides from running off the gel. Total flour proteins were analyzed to test for variety homogeneity of flour samples. SDS-PAGE analysis of the polypeptide compositions of albumin and globulin proteins extracted from the flour samples were also carried out, under both reducing and non-reducing conditions to investigate possible differences that may be related to flour quality.

3.2.11 Lactic Acid Polyacrylamide Gel Electrophoresis (Acid-PAGE)

The homogeneity of flour samples representative of the same variety was determined by Acid-PAGE analysis of their gliadin proteins according to Khan et al (1985). Equipment was the same as described for SDS-PAGE analysis.

3.2.12 Statistical Analyses

Analysis of variance and correlation studies were carried out using the Super ANOVA statistical package (Berkeley, CA).

4. RESULTS AND DISCUSSION

The results of the investigations conducted during phases one and two of this study are presented and discussed in this chapter in sections 4.1 and 4.2, respectively.

4.1 Results From Phase One of the Study

In phase one of this study, 32 soft wheat flours representing eight varieties were analyzed for their milling qualities, physico-chemical properties and sugar-snap cookie-baking potentials. In addition, determinations of the albumin and globulin contents of these flours and their polypeptide compositions were carried out.

4.1.1 Baking and Flour Quality Parameters

Results of the determinations of flour quality parameters (breakflour yield, moisture, ash and protein contents, alkaline water retention capacity, and sugar-snap cookie diameter) for the 32 soft wheat flours studied in phase one are listed in Appendix I. Mean values of these parameters for the varieties studied are listed in Table 2.

Breakflour yield is often used as a measure of wheat kernel texture. Softer textured wheats are normally

Table 2. Flour Quality Parameters[a] of the Eight Varieties Studied in Phase One

Variety	Breakflour yield (%)	Moisture content (%)	Ash content (%)	Protein content (%)	AWRC[d] (%)	Cookie diameter (cm)
Argee[b]	33.1 ± 3.0	11.9 ± 1.5	0.40 ± 0.04	9.4 ± 1.2	49.3 ± 0.2	9.2 ± 0.2
Augusta[c]	30.8 ± 6.0	13.0 ± 0.7	0.40 ± 0.06	9.4 ± 0.1	49.4 ± 2.7	8.8 ± 0.1
Caldwell[b]	32.9 ± 5.2	12.9 ± 0.9	0.33 ± 0.03	8.7 ± 0.9	52.7 ± 0.7	8.8 ± 0.1
Cardinal[b]	27.8 ± 3.1	11.3 ± 0.8	0.33 ± 0.03	9.4 ± 1.4	51.0 ± 1.2	8.9 ± 0.1
Frankenmuth[c]	29.4 ± 3.4	12.1 ± 0.7	0.39 ± 0.04	9.8 ± 0.7	49.7 ± 0.5	8.9 ± 0.2
Pioneer 2550[b]	31.5 ± 6.1	12.2 ± 1.5	0.33 ± 0.03	9.0 ± 1.1	52.2 ± 2.1	9.0 ± 0.1
Pioneer 2555[b]	38.3 ± 3.2	12.4 ± 0.6	0.33 ± 0.01	8.6 ± 0.2	51.3 ± 0.6	9.0 ± 0.2
Tyler[b]	31.2 ± 4.9	11.9 ± 1.4	0.30 ± 0.02	9.4 ± 1.4	52.8 ± 1.7	8.6 ± 0.2

[a]Varietal means of flour quality parameters
[b]Soft red winter wheat
[c]Soft white winter wheat
[d]Alkaline water retention capacity

expected to produce higher quantities of breakflour (Gaines 1985). Wide variations were observed for breakflour yield in this study, with values ranging between 24.3 and 42.1%.

Flour moisture content was between 10.4 and 14.0%. Moisture content is important to flour quality for economic reasons. A flour containing high levels of moisture sets the purchaser at a disadvantage, and would have less storage quality than one with lower moisture.

Flour ash content was observed between 0.26 and 0.44%--well within the 0.48% limit recommended for ash content of cookie flours (Mailhot and Patton 1988). Ash content is normally utilized as a general indicator of milling quality. As flour extraction from the wheat increases so does the amount of bran in the flour. This is indicated by an increase in flour ash content.

Flour protein content obtained for the 32 flour samples showed wide variations, with values ranging between 7.0 and 11.3%. Soft wheat flours are normally preferred to contain low levels of protein; usually less than 10% for cookie flours (Mailhot and Patton 1988). Results obtained in this study are similar to those reported by Kaldy and Rubenthaler (1987); they had observed a range of 7.2 to 10.1% for flour protein content in a study of 20 soft wheat flours.

Alkaline water retention capacity (AWRC) is a flour quality parameter used for predicting a flour's cookie-

baking potential. Flours with lower AWRC values are considered better suited for cookie baking than those with higher AWRC values. The AWRC values of the flour samples studied varied between 47.0 and 55.3%.

The diameters of sugar-snap cookies are widely used as indicators of soft wheat flour quality. Sugar-snap cookie diameters for the flours studied varied between 8.7 and 10.3 cm. Varietal differences were observed in this study, with the soft red winter wheats producing larger cookies than the soft white winter varieties ($p < 0.05$).

4.1.2 Albumin and Globulin Contents

A white fibrous hygroscopic material was recovered following freeze-drying of the Osborne albumin fractions. The globulin fractions gave a brownish crystalline material. Protein contents of the Osborne albumin fractions ranged between 30.0 and 48.9%, while protein contents of the globulin fractions varied between 52.1 and 74.5%. The total amount of protein present in the recovered albumin and globulin fractions are reported in Table 3 as percentages of flour and of flour protein. The levels of the non-storage proteins (albumins and globulins) ranged between 12.6 and 23.8% of flour protein. As reported by Fullington et al (1983), the amounts of the non-storage proteins in flour increased at higher levels of flour protein, while the

Table 3. Non-storage Protein[a] Contents of 32 Soft Wheat Flours

Flour sample	Flour Albumin (%)	Flour Globulin (%)	Flour non-storage proteins (%)	$\left[\frac{\text{Albumin}}{\text{Flour protein}}\right]^b$	$\left[\frac{\text{Globulin}}{\text{Flour protein}}\right]^c$	$\left[\frac{\text{Non-storage proteins}}{\text{Total proteins}}\right]^d$
Argee	1.47	0.30	1.77	19.86	4.09	23.95
Argee	1.49	0.44	1.93	14.47	4.29	18.76
Argee	1.50	0.42	1.91	15.75	4.38	20.13
Argee	1.41	0.30	1.71	15.02	3.19	18.21
Augusta	1.46	0.49	1.95	17.81	5.97	23.78
Augusta	1.40	0.45	1.85	14.88	4.76	19.63
Augusta	1.17	0.48	1.65	12.19	5.00	17.20
Augusta	1.34	0.33	1.67	13.97	3.41	17.38
Caldwell	1.43	0.33	1.77	17.69	4.10	21.79
Caldwell	1.65	0.37	2.02	18.15	4.05	22.20
Caldwell	1.38	0.25	1.64	18.19	3.32	21.51
Caldwell	1.58	0.48	2.06	16.11	4.86	20.97
Cardinal	1.19	0.24	1.43	14.93	2.98	17.91
Cardinal	1.18	0.28	1.45	12.66	2.97	15.62
Cardinal	1.28	0.39	1.67	12.44	3.77	16.21
Cardinal	1.44	0.44	1.88	12.78	3.89	16.67
Frankenmuth	1.53	0.39	1.92	18.18	4.61	22.79
Frankenmuth	1.47	0.24	1.70	14.96	2.43	17.39
Frankenmuth	1.26	0.58	1.84	12.51	5.72	18.23
Frankenmuth	1.16	0.58	1.74	11.57	5.79	17.36
Pioneer 2550	1.33	0.30	1.63	16.03	3.65	19.68
Pioneer 2550	1.31	0.39	1.70	14.29	4.21	18.49
Pioneer 2550	1.34	0.42	1.76	13.28	4.19	17.47
Pioneer 2550	1.35	0.36	1.71	16.07	4.23	20.31
Pioneer 2555	1.28	0.39	1.68	14.42	4.40	18.82
Pioneer 2555	0.80	0.46	1.26	9.03	5.26	14.29
Pioneer 2555	0.71	0.39	1.10	8.12	4.46	12.58
Pioneer 2555	1.33	0.45	1.78	16.36	5.51	21.87
Tyler	1.03	0.29	1.32	13.71	3.92	17.63
Tyler	1.04	0.33	1.36	14.81	4.64	19.45
Tyler	1.27	0.37	1.64	12.10	3.53	15.63
Tyler	1.32	0.38	1.70	14.07	4.05	18.12

[a]Albumin and globulin proteins
[b]Albumin content of total flour proteins (%)
[c]Globulin content of total flour proteins (%)
[d]Combined albumin and globulin content of flour proteins (%)

proportion of non-storage proteins to total flour protein decreased as flour protein content increased. Figures 1 and 2 illustrate the relationships between flour protein content and flour non-storage protein content, and between flour protein content and the fraction of it present as non-storage protein, respectively. Figure 3 shows the distribution of cookie diameters at different levels of flour globulin.

4.1.3 Statistical Studies

Correlation studies were conducted between the different flour quality parameters measured and sugar-snap cookie diameter. Correlation studies were also carried out between flour albumin and globulin measurements and sugar-snap cookie diameter. For the purposes of correlating flour levels of non-storage proteins with cookie diameters, sugar-snap cookie diameters were corrected to 9% protein according to Kaldy and Rubenthaler (1987), in order to nullify the effects of unusually low or high levels of flour protein. Significant statistical correlations observed are shown in Table 4.

Breakflour yield was positively correlated with cookie diameter ($r=0.341$, $p < 0.05$). This corresponds with other observations (Gaines 1985; Kaldy and Rubenthaler 1987) that wheats producing greater quantities of breakflour bake

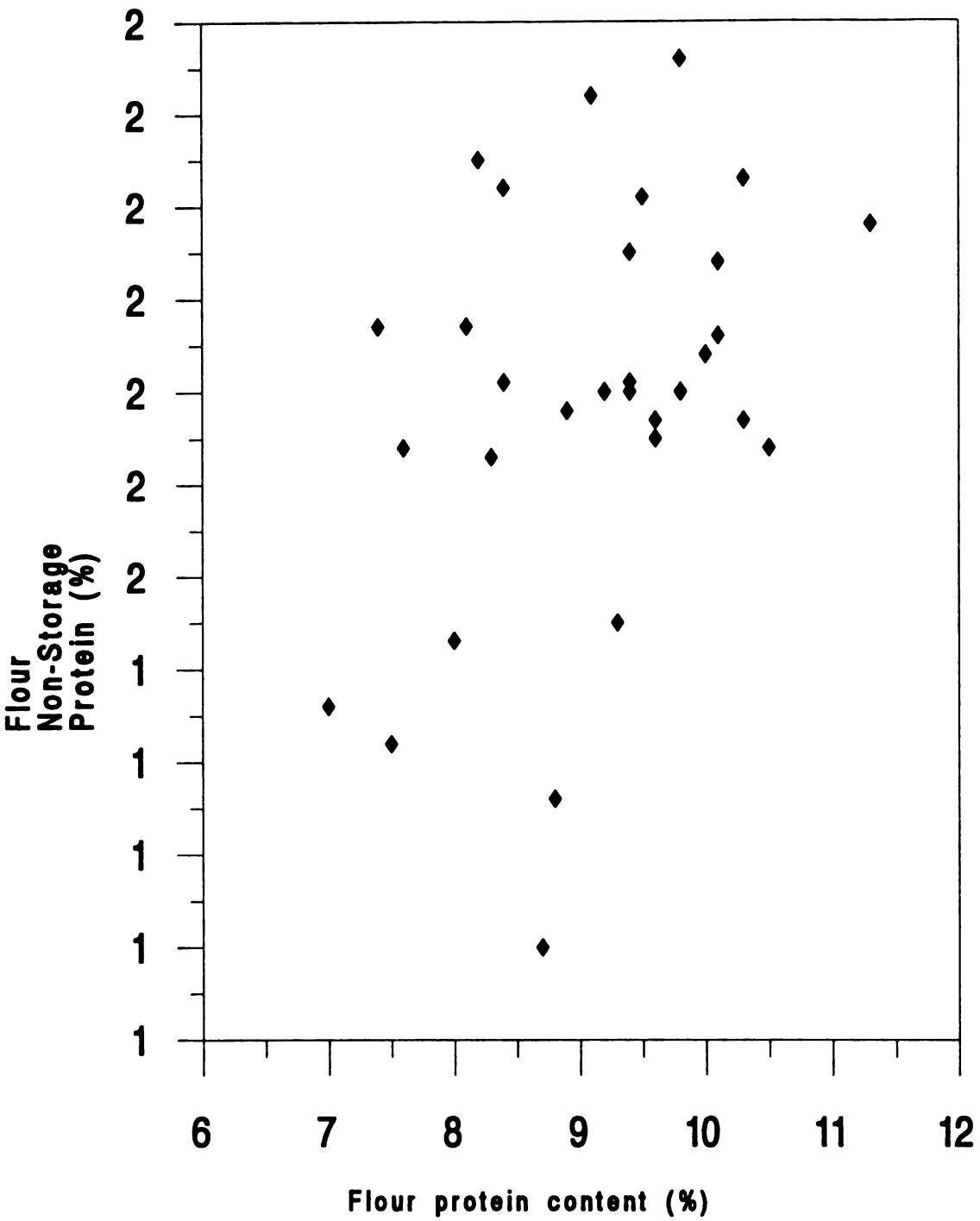


Figure 1. Relationship between flour protein content and flour non-storage protein content (albumins and globulins) of 32 soft wheat flours

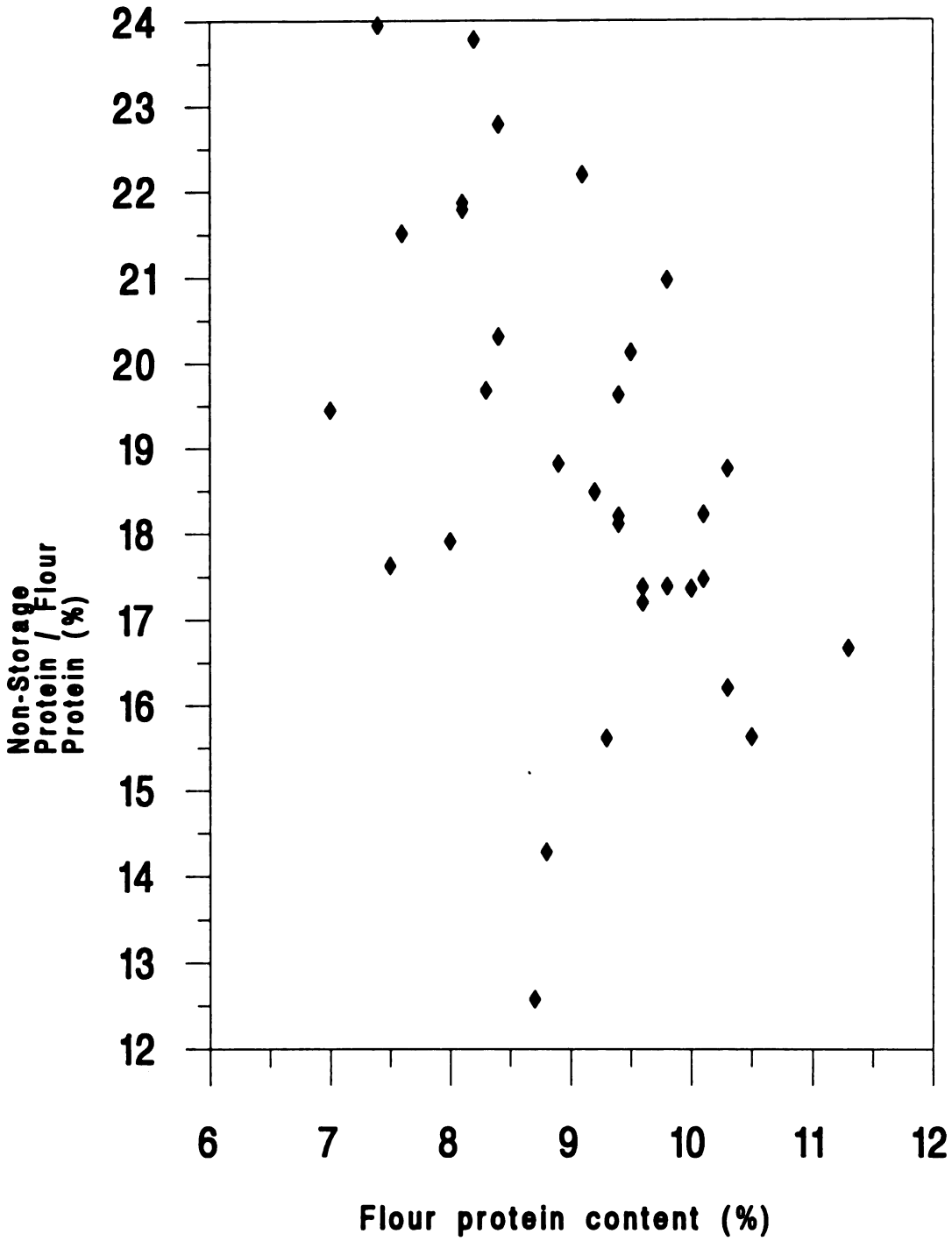


Figure 2. Relationship between flour protein content and the relative proportion of non-storage proteins

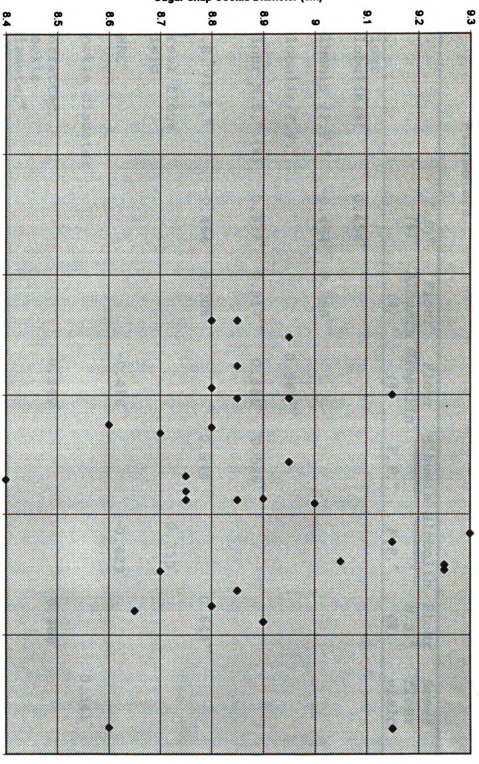


Figure 3. Distribution of sugar-snap cookie diameters at different levels of flour globulin content

Table 4. Correlation Coefficients Among Flour Protein Fractions and Quality Parameters

	F.P.[a] (%)	*Flour Albumin (%)*	*Flour Globulin (%)*	*Albumin / F.P.[a]*	*Globulin / F.P.[a]*	*Flour N.S.[b] (%)*	*Break flour yield*	*AWRC[c]*
Flour Globulin(%)	0.456*							
Albumin /F.P.[a]	-0.464**	0.769***						
Globulin/F.P.[a]			0.869***					
Flour N.S.[b] (%)	0.372*	0.917***	0.366*	0.588**				
N.S.[b]/F.P.[a]	-0.466**	0.708***		0.948***		0.644**		
Break flour yield					0.359*			
AWRC[c]			-0.488**		-0.429*			
Cookie diameter							0.341*	
Corrected cookie diameter[d]			0.349*			0.388*		-0.390*

[a]Flour protein content
[b]Non-storage protein
[c]Alkaline water retention capacity
[d]Cookie diameter corrected to 9% protein according to Kaldy and Rubenthaler (1987)
*, **, *** significant at 0.05, 0.01 and 0.001 levels, respectively

larger cookies. Breakflour yield was also positively correlated with flour globulin content ($r=0.361$, $p < 0.05$). Flour protein content was positively correlated with flour levels of non-storage proteins ($r= 0.372$, $p < 0.05$), and negatively with the proportion of non-storage proteins to the total flour protein ($r= -0.466$, $p < 0.01$). No significant correlations were observed between flour protein content and flour quality parameters such as breakflour yield, AWRC, and cookie diameter.

Alkaline water retention capacity values were negatively correlated with flour levels of globulin ($r= -0.488$, $p <0.01$) and the globulin fraction of flour protein ($r= -0.429$, $p <0.01$). This suggests that increasing the flour content of globulins reduces the flour's hydration capacity. AWRC was also negatively correlated with breakflour yield. This was to be expected, since wheats producing more breakflour will not be subjected to extensive grinding by the reduction rolls, thus producing flour with low levels of damaged starch granules, which absorb less water.

Sugar-snap cookie diameters, after correction to 9% protein, were positively correlated with flour globulin levels ($r= 0.349$, $p < 0.05$). Corrected cookie diameters were also positively correlated with flour levels of the non-storage proteins ($r= 0.388$, $p < 0.01$). Significant

statistical correlations were observed between sugar-snap cookie diameters and the globulin and non-storage protein contents of the flours. However, no significant statistical correlation was observed for the albumin fraction, which makes up the major portion of the non-storage proteins. It could be that the effects of the albumin and globulin fractions on cookie diameter are additive.

4.1.4 Electrophoretic Analyses of Albumins and Globulins

Flour samples used in phase one were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and lactic acid polyacrylamide gel electrophoresis (Acid-PAGE) of total flour proteins to test for homogeneity of samples obtained from the same variety. Similar polypeptide band patterns were obtained in all cases for all four samples representative of the same variety. These are presented in Appendix II for SDS-PAGE analysis and Appendix III for Acid-PAGE analysis.

The polypeptide compositions of the Osborne albumin and globulin fractions of 32 soft wheat flours were each analyzed by SDS-PAGE under reduced conditions (protein extracts were treated with 2-mercaptoethanol) and unreduced conditions (without 2-mercaptoethanol). Similar polypeptide banding patterns were observed among the 32 soft wheat

flours, for each of the reduced and unreduced albumins as well as for each of the reduced and unreduced globulins.

Differences were observed in the number of polypeptide bands between extracts run under reduced conditions and those run under unreduced conditions. For the unreduced albumin proteins, over 30 polypeptide bands were observed, while about 39 polypeptide bands were observed for the reduced albumins. As an example, Figure 4 depicts SDS-PAGE patterns (A = unreduced, B = reduced) of albumin fractions from 12 flour samples. It appeared that two low mobility bands in the unreduced samples were replaced by five polypeptide bands in the high molecular weight region (HMWR). At least one low molecular weight band designated by (**) with greater mobility than any of the bands observed in the unreduced samples was also observed. Differences were also evident in the polypeptide band patterns of globulins in SDS-PAGE, between reduced and unreduced samples. Figure 5 depicts SDS-PAGE patterns (A = unreduced, B = reduced) of globulin fractions from the same 12 flour samples illustrated for the albumins. For the globulins, 32 polypeptide bands were observed under unreduced conditions. It was also observed that large amounts of protein did not enter the gel under unreduced conditions, evident from the heavily stained bands present at the top of the separating gels. Following reduction with 2-mercaptoethanol, the heavy

bands at the top of the separating gel were greatly diminished. At least six new polypeptide bands were observed in the high molecular weight region (HMWR).

These results indicate that polypeptide associations, most likely through disulfide bonding, may occur between certain polypeptides present within each of the albumin and globulin fractions. Evidence of such disulfide interactions have been reported by various authors (Singh and Shepherd 1985; Terada et al 1978; Gupta et al 1991).

Figure 4. SDS-PAGE patterns of the albumin fraction from 12 soft wheat flours.
1 = Frankenmuth, 2 = Pioneer 2555, 3 = Tyler

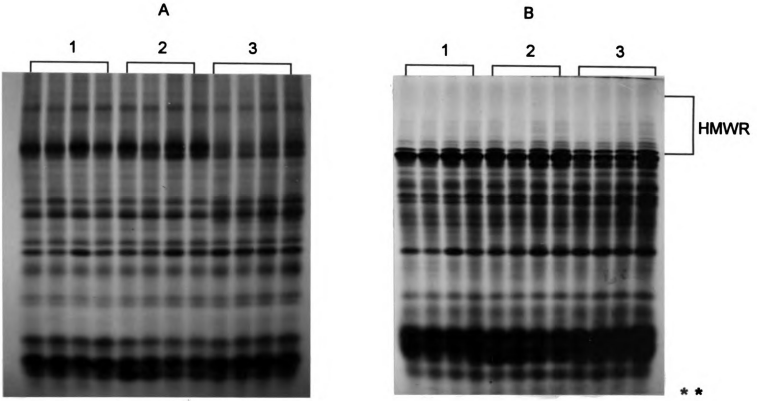


Figure 4

Figure 5. SDS-PAGE patterns of the globulin fraction from 12 soft wheat flours.
1 = Frankenmuth, 2 = Pioneer 2555, 3 = Tyler

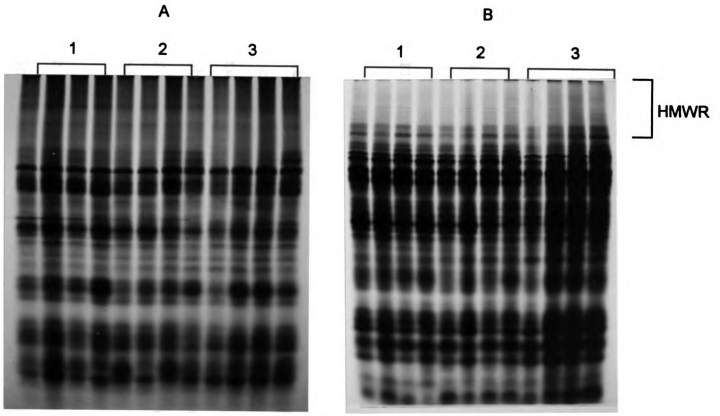


Figure 5

4.2 Results from Phase Two of the Study

During the second phase of this research project, eight different soft wheat flours were studied. Flour quality was evaluated by physico-chemical and baking tests. Based on wire-cut cookie diameters, two flours, one good quality and one poor quality, were selected to study the effect of changes in globulin content on cookie diameter. Results of these investigations are discussed in the next two sections.

4.2.1 Flour Milling Properties

4.2.1.1 Flour Yield

Flours from eight different wheat varieties, were milled on a Brabender Junior Quadrumat mill. Flour yield was between 60.1 and 69.3% for flours studied (Table 5). The highest flour yield was obtained for the club wheat variety Hyak and the lowest flour yield was observed for the soft white variety Frankenmuth.

4.2.1.2 Flour Moisture

Flour moisture varied between 13 and 14% for the flours studied (Table 5). Wheat samples had been tempered to 15% moisture according to the AACC Approved Method (1983). This

TABLE 5. Milling and Flour Quality Parameters

Variety	Flour yield (%)	Flour moisture (%)	Falling number (seconds)	Flour protein (%)	Sedimentation volume (ml)	AWRC[a] (%)
Chelsea	63.1	13.0	320	7.4	15.2	65
Dynasty	65.4	13.7	335	8.7	21.0	63
Excel	63.4	13.2	354	8.0	21.1	69
Frankenmuth	60.1	13.9	362	7.7	15.0	67
Hyak	69.3	13.7	333	9.0	26.5	66
Madsen	65.1	14.0	277	9.6	23.0	66
Stephens	63.4	13.4	402	10.5	24.5	61
Tres	64.1	13.0	357	9.4	13.2	62
Mean	64.2	13.5	343	8.8	19.9	65
s.d.[b]	2.6	0.4	36	1.1	4.9	3

[a]Alkaline water retention capacity
[b]Standard deviation

means flour moisture contents were 1-2% less than moisture contents of the wheat.

4.2.2 Physico-Chemical Properties of Flours

4.2.2.1 Falling Number Determination

Falling number values ranged between 277 and 402 seconds (Table 5). The highest value was obtained for the variety Stephens, while Madsen gave the lowest values. All the flours studied had falling number values greater than 250, the minimum acceptable value for soft wheat flours (Mailhot and Patton 1988). This indicates that all wheat samples used were sound.

4.2.2.2 Flour Protein Content

The protein contents of the flours studied ranged from 7.4 to 10.5% (Table 5). Interestingly the highest and lowest protein contents were observed for the soft white varieties Stephens and Chelsea, respectively.

4.2.2.3 Sedimentation Volume

Flour sedimentation volumes obtained ranged between 13.2 and 26.5 ml (Table 5). None of the flours studied here exhibited any strong gluten strength based on the results of this test. Khelifi and Branlard (1992) had reported a range

of 27 to 60 ml for flour sedimentation volumes in a study of protein qualities among several wheat varieties. In this study, the highest sedimentation volume was observed for Hyak and the lowest was for Tres; both club wheat varieties.

4.2.2.4 Alkaline water retention capacity

The AWRC values recorded for the eight flours studied ranged between 61 and 69% (Table 5). These values were rather high compared to those normally reported for soft wheats in the literature. This could be due to differences in equipment among laboratories. Similar problems with elevated results for AWRC had been reported by Nemeth (1993).

4.2.2.5 Alveograph results

Investigations of the physical properties of flour doughs were conducted using the alveograph. The results of all alveograph parameters are presented in Table 6. These are:

(i) The P value: this is a measure of the dough's tensile strength. The P values observed ranged between 54.5 and 71.8 mm. High P values are generally indicative of strong doughs.

(ii) The L value: this is a measure of dough extensibility. The L values obtained ranged from 59.8 to 91.0 mm. Higher L values generally indicate more extensible doughs.

(iii) The P/L value: this is the ratio of dough strength to extensibility. The P/L ratios observed were between 0.60 and 0.99.

(iv) The W value: this is the work carried out to form the dough bubble before it ruptures. The work of deformation varied between 112.5 and 169.4 Joules in the samples studied.

(v) The G value (the swelling index): this is the square root of the volume of air needed to rupture the dough bubble. These values ranged between 17.1 and 21.2 cm^3 in the flours studied.

4.2.3 Baking Results

4.2.3.1 Wire-Cut Cookie Baking

The diameters of wire-cut cookies varied between 7.6 and 8.4 cm for the eight soft wheat varieties (presented in Table 7). The largest cookie diameters were obtained for the soft white varieties: Chelsea and Frankenmuth. On the other hand, another soft white variety, Madsen produced the smallest cookies. A large disparity was also observed in the diameters of cookies baked from the club wheat varieties.

TABLE 6. Alveograph Data

Variety	Alveograph				
	P[a] (mm)	L[b] (mm)	W[c] (Joules)	G[d] (cm^3)	(P/L)[e]
Chelsea	70.1	70.8	150.4	18.7	0.99
Dynasty	58.0	79.8	169.4	19.8	0.73
Excel	67.7	74.8	153.7	19.2	0.90
Frankenmuth	57.9	59.8	112.5	17.1	0.97
Hyak	66.0	82.0	163.5	21.0	0.81
Madsen	71.8	84.3	160.2	20.3	0.85
Stephens	54.5	91.0	138.7	21.2	0.60
Tres	56.1	76.3	121.0	19.3	0.74

[a]Alveograph P value (maximum overpressure)
[b]Alveograph L value (measure of dough extensibility)
[c]Work of deformation
[d]Swelling index
[e]Ratio of dough strength to dough extensibility

Table 7. Wire-cut Cookie Baking Data[a]

Variety	Cookie Diameter (cm)	Cookie Thickness (cm)
Chelsea	8.4	1.0
Dynasty	8.0	1.1
Excel	8.2	1.1
Frankenmuth	8.4	1.1
Hyak	7.8	1.1
Madsen	7.6	2.3
Stephens	8.0	1.1
Tres	8.3	1.0

[a]Average value for two cookies

Relatively large cookies (8.3 cm in diameter) were obtained from Tres, while Hyak produced much smaller cookies (7.8 cm) in diameter.

4.2.3.2 Effects of Globulin Proteins on Cookie Diameter

Wire-cut cookies were baked from Madsen flour supplemented with 30 mg of globulin extracted from either Madsen or Chelsea flours. Wire-cut cookies baked from untreated Madsen flour as control had an average cookie diameter of 7.3 cm. Identical results were also observed for cookies baked from Madsen flour supplemented with Madsen globulin. However in the case of cookies baked from Madsen flour supplemented with Chelsea globulin, average cookie diameter observed was 7.5 cm. This was an improvement of 0.2 cm in cookie diameter, over the control cookies and those supplemented with Madsen globulins.

4.2.4 Statistical Analysis of Results

All flour quality parameters measured were correlated with each other. Significant statistical correlations ($p < 0.05$) observed between related tests are presented in Table 8.

4.2.4.1 Flour Milling

Flour yield was negatively correlated with wire-cut cookie diameter ($P < 0.05$). Harder textured wheats normally give higher flour yields and bake smaller cookies, which probably explains this observation. Flour yield also showed very significant correlations ($P < 0.05$) with alveograph W and G values.

4.2.4.2 Flour Physico-Chemical Properties

Flour protein content was positively correlated with flour sedimentation volumes. This is as expected, since a flour's swelling potential is related to its protein content and gluten strength. Significant correlations were also observed among the flour quality parameters that measure properties of flour proteins. Flour sedimentation volume showed highly significant positive correlations ($P < 0.05$) with alveograph L, W, and G values. The AWRC values were positively correlated with alveograph P values ($p < 0.05$).

4.2.4.3 Cookie Baking

Cookie diameter was negatively correlated ($p < 0.05$) with such flour quality parameters as flour yield and flour sedimentation volume. Wire-cut cookie diameter was also negatively correlated ($p < 0.05$) with alveograph L and G values.

No significant statistical correlation was obtained between wire-cut cookie diameter and AWRC, the parameter normally used for predicting sugar-snap cookie-baking potential for soft wheat flours. The lack of correlation between wire-cut cookie diameters and AWRC values could be due to the differences in formula water added between this test and the sugar-snap cookie-baking test. Other factors, such as the unusually high AWRC values obtained, may be responsible for this discrepancy.

TABLE 8. Correlation Coefficients Among Flour Physico-Chemical and Baking Properties

	Flour yield (%)	*Falling number (sec)*	*Flour protein (%)*	*Sedimentation volume (ml)*	*AWRC[a] (%)*	*Alveograph*			
						P[b]	*L[c]*	*W[d]*	*G[e]*
Sedimentation (ml)									
AWRC[a] (%)									
Flour yield (%)									
Alveograph P[b]		-0.793*							
Alveograph L[c]			0.857**	0.741*					
Alveograph W[d]	0.708*								
Alveograph G[e]	0.756*		0.786*	0.814*			0.969***		
Alveograph (P/L)[f]			-0.884**		0.784*		-0.792*		-0.730*
Cookie diameter[g]	0.691*			-0.813*			-0.727*		-0.754*
Cookie thickness[g]									

[a]Alkaline Water Retention Capacity
[b]Alveograph P values
[c]Alveograph L values
[d]Alveograph work of deformation
[e]Alveograph swelling index
[f]Ratio of dough strength to extensibility
[g]Determined from the wire-cut cookie baking test (AACC method, 10-54)
*, **, *** Significant at 0.05, 0.01 and 0.001 levels, respectively

5. GENERAL DISCUSSION

Investigations of soft wheat flour quality have revealed that flour quality is often influenced by the variety that was the source. Studies of the effects of different constituents of soft wheat flour on cookie baking have shown that flour protein plays an important part in soft wheat flour quality, as it relates to cookie baking. Previous investigations of the role of flour proteins in cookie baking have tended to concentrate on the effects attributable to the storage proteins. This is understandable, since most studies of wheat quality have been conducted on hard wheats, and gluten proteins have been shown to be very important in hard wheat quality. However, most quality requirements for soft wheat flours are distinctly different from those for hard wheat flours. Bearing this in mind, it is necessary to investigate the contributions of the other classes of wheat flour proteins (non-storage proteins) to soft wheat quality.

This study was conducted to investigate the possible effects of soft wheat flour non-storage proteins on cookie baking quality. The study was conducted in two phases. In phase one the globulin and albumin contents of 32 soft wheat flours were determined. The associations between these and commonly employed determinants of soft wheat flour quality

were investigated. Electrophoreses in the presence of sodium dodecyl sulfate (SDS-PAGE) of the albumin and globulin fractions obtained from these flours were also carried out, in order to detect any qualitative differences that may be present.

In phase two, several quality parameters were determined for a set of eight different soft wheat varieties. Based on the diameters obtained from the wire-cut cookie-baking test, two soft wheat flours, one of good quality and one of poor quality were selected to study the effect of the exchange of flour protein fractions, determined from phase one experiments to have quality related-effects.

The results of tests conducted during phase one indicated that many factors are involved in determining soft wheat flour quality. The diameters of sugar-snap cookies were influenced by the kernel texture of the wheat, as determined by measurements of the breakflour yield. Differences were observed in cookie-baking ability among the different soft wheat varieties studied. Cookie diameter was also influenced by flour protein content. These results are consistent with the normal requirements which expect soft wheats to have high breakflour yields and low protein contents.

Investigation of the relationship between soft wheat albumins and globulins and cookie-baking potential yielded some interesting results. Flour globulin content was positively correlated with sugar-snap cookie diameter, especially when corrections were made for the confounding effects of too high or too low flour protein contents. Flour globulin content was also negatively correlated with alkaline water retention capacity (AWRC) values. The relative contents of globulins in flour protein were positively correlated with breakflour yield and negatively with AWRC. These observed associations between flour globulin and soft wheat quality parameters suggest the globulin proteins may have a functional role in soft wheat flour quality. No relationships were observed between flour albumin measurements and any of the soft wheat quality parameters. When the relationships between these flour quality parameters and the measurements of albumins and globulins combined (i.e., non-storage proteins) were studied, results similar to those obtained for the globulins were observed.

Electrophoretic studies of the polypeptide compositions of the albumins and globulins, among eight different varieties, indicated that the polypeptide compositions within each of these two classes of proteins were fairly homogenous. However, it is possible that quantitative

differences were present which were not detectable by the method employed.

In phase two, the qualities of eight soft wheat flours were evaluated by means of tests measuring the flours' physico-chemical properties and wire-cut cookie-baking potentials. The results of these tests indicated that flour properties such as sedimentation volume, and alveograph L and G values could be useful as predictors of soft wheat flour quality, particularly for cookie baking. The baking quality of wire-cut cookies was used to evaluate the value of these tests in soft wheat quality testing.

To test the observation from phase one experiments, that flour globulin levels could influence cookie-baking quality, cookie-baking studies with globulin proteins were conducted using the poor quality cookie flour milled from the variety Madsen. No change in cookie diameter was observed when 30 mg of globulin extracted from Madsen flour was added to the cookie formula. When the same amount of globulin extracted from a good quality cookie flour (Chelsea) was added to the Madsen flour, a 0.2 cm increase in cookie diameter was observed. This suggests there may be some qualitative differences between the globulin proteins of different soft wheat varieties that can affect the cookie baking properties of such flours.

It is important to mention that a more complete study in phase two should also include sugar-snap cookie baking in the study of the effects of addition of extracted globulins to the cookie formula. Due to the limited quantities of globulin extractable from flour, and the lengthy time required to obtain enough quantities for baking studies, only the wire-cut cookie formula was chosen as an example to demonstrate the potential effects of globulins on cookie-baking quality of soft wheat flours.

Results of this study show that the cookie-baking potential of soft wheat flours are affected by a number of factors that can be linked to inherent properties of the wheat variety. Cookie-baking quality was mostly influenced by factors such as: flour protein content, the gluten protein strength (as determined from flour sedimentation volumes), and alveograph parameters.

Results of the measurements of flour globulin in phase one suggested that flour globulin content might have some direct effect on cookie baking. Studies of the effect of directly adding globulins to cookie flours suggested that increased flour globulin can have effects on cookie diameter. Differences in the diameter effects of globulins from two different flours were also observed.

6. SUMMARY

1. Soft wheat is a commodity that is important to certain sections of the food industry. Users of soft wheat flour often complain about lack of uniform quality among different lots of flour recieved from millers. It has been suggested that wide variations in flour quality among the soft wheat varieties under cultivation are responsible. Studies of the relationship between flour composition and quality are desireable, in order to understand the basis for these varietal differences.

2. This study investigated the associations between the content and composition of soft wheat non-storage proteins (albumins and globulins) and flour quality. Flour quality was evaluated based on physico-chemical and baking tests.

3. Flour quality parameters such as alkaline water retention capacity (AWRC), breakflour yield, and cookie diameter were correlated with flour globulin. No associations were observed between flour albumins and these flour quality parameters.

4. Results of sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) showed that polypeptide compositions of the albumins and globulins were fairly homogenous, among the eight different varieties studied. Results from samples run under both reduced and unreduced

conditions suggest the presence of polymeric proteins within the albumin and globulin fractions.

5. Cookie-baking studies were used to compare the effects of globulins extracted from a good quality cookie flour and a poor quality cookie flour. Results suggested that some qualitative factor capable of influencing cookie-baking quality may be present in the globulin fraction.

6. It is important to bear in mind that since all the soft wheat samples studied here were not cultivated at the same location, environmental factors may be responsible for some of the quality variations observed.

APPENDICES

APPENDIX I

Appendix I

Flour Quality Determinations

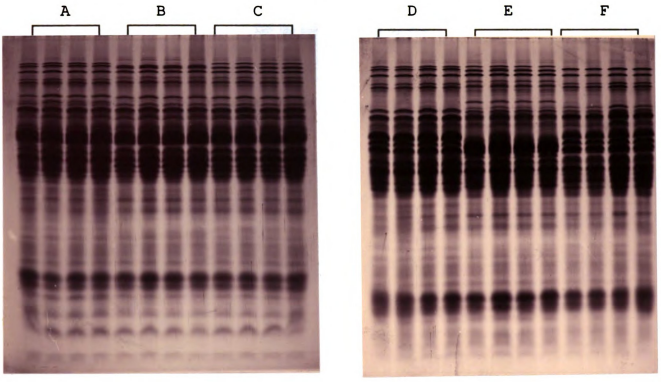
Variety	Breakflour yield (%)	Moisture content (%)	Ash content (%)	Protein content (%)	AWRC	Cookie diameter (cm)
Argee	37.20	13.73	0.329	7.76	49.00	8.95
	30.30	10.43	0.411	10.66	49.40	9.25
	33.40	12.47	0.403	9.64	49.40	9.30
	31.30	11.14	0.422	9.52		9.15
Augusta	38.80	13.99	0.415	8.47	47.00	8.90
	24.30	12.66	0.313	9.68	52.40	8.70
	31.00	12.55	0.440	9.90	48.90	8.65
	29.20	12.63	0.412	9.43		8.80
Caldwell	38.20	13.67	0.336	8.38	53.40	8.70
	25.90	13.61	0.344	9.35	52.00	8.75
	32.20	11.99	0.286	7.67	53.20	8.95
	35.10	12.15	0.362	9.52	52.30	8.80
Cardinal	26.80	10.37	0.336	7.64		8.80
	31.80	11.09	0.320	9.01	49.90	8.85
	24.30	12.31	0.296	9.82	52.30	8.75
	28.20	11.52	0.351	10.98	50.70	9.05
Frankenmuth	28.40	12.12	0.400	8.78	49.50	8.85
	28.70	11.35	0.395	9.70		8.85
	26.30	13.08	0.324	10.26	50.30	8.60
	34.20	11.82	0.419	10.30	49.40	9.15
Pioneer 2550	29.70	10.59	0.315	7.67	52.10	8.85
	39.00	11.34	0.343	9.30	51.00	8.90
	32.80	13.62	0.368	10.31	50.70	9.15
	24.50	13.42	0.310	8.77	55.30	8.95
Pioneer 2555	34.90	11.75	0.329	8.84	51.80	9.00
	36.60	12.49	0.321	8.60	50.90	8.85
	39.50	13.05	0.338	8.81	51.80	8.85
	42.10	12.12	0.324	8.32	50.80	9.25
Tyler	29.50	13.47	0.316	7.56	55.10	8.80
	31.10	11.25	0.311	7.10	52.80	8.60
	26.20	12.48	0.256	10.61	52.20	8.40
	37.90	10.35	0.332	9.06	51.00	8.75

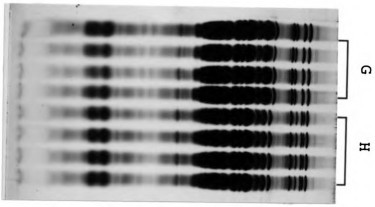
AWRC = Alkaline Water Retention Capacity

APPENDIX II

Appendix II

SDS-PAGE patterns of total proteins from 32 flour samples

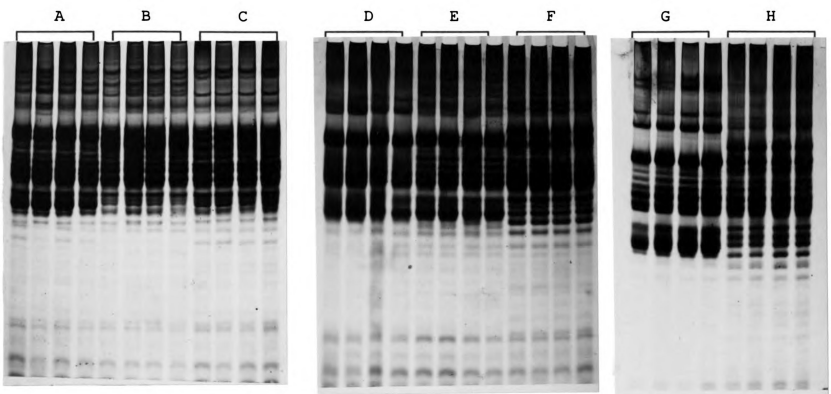



G
H

APPENDIX III

Appendix III

Acid-PAGE patterns of gliadin proteins from 32 flour samples



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