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ISOLATION AND QUANTIFICATION OF STORAGE PROTEINS IN U.S. SOFT WHEAT FLOURS AND THEIR RELATIONSHIP TO RHEOLOGICAL AND BAKING PROPERTIES

By

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ABSTRACT

ISOLATION AND QUANTIFICATION OF STORAGE PROTEINS IN U.S. SOFT WHEAT FLOURS AND THEIR RELATIONSHIP TO RHEOLOGICAL AND BAKING PROPERTIES

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A simple and effective quantification method for determination of high-molecular-weight glutenin subunits (HMW-GS, or A subunits) and low-molecular-weight (LMW) glutenin subunit groups (B and C subunits) was developed. This method provides a reliable tool for simultaneous quantification and identification of glutenin subunits by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with densitometry using a known quantity of glutenins as a quantitative standard.

The four gliadin subgroups (ω-, γ-, β- and α-gliadins) in 17 soft wheat patent flours and seven straight-grade flours were identified and quantified by acid-polyacrylamide gel electrophoresis (Acid-PAGE) coupled with densitometry using a known quantity of gliadin standard. The HMW-GS and LMW glutenin subunit groups in these flours were identified and quantified by the method developed. Flour rheological properties were evaluated by alveograph, farinograph and mixograph tests. Japanese-type sponge cakes and AACC

sugar-snap cookies were made to evaluate the flour's baking performances. Of the cultivars studied, patent flours were significantly lower in protein content and the relative quantities of individual gliadin subgroups than straight-grade flours. Patent flours also showed significantly weaker dough properties and better cookie-baking quality than straight-grade flours. Significant negative correlations were found between the relative quantities of ω- and γ-gliadins and Japanese sponge cake volume and between the relative quantity of α-gliadins and the sugar-snap cookie diameter per unit flour protein. Patent flours containing subunit 1 of HMW-GS exhibited significantly weaker dough properties and better Japanese sponge cake-baking and sugar-snap cookie-baking qualities than those containing subunit 2* of HMW-GS. The quantities of extractable B and C subunits appeared to have positive and negative effects on Japanese sponge cake-baking quality, respectively. On the other hand, the quantities of extractable B subunits in patent flours and straight-grade flours each correlated positively with sugar-snap cookie-baking quality. The ratio of the quantities of B subunits to C subunits may be an important parameter in relation to the quality of soft wheat products.

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CHAPTER 1. INTRODUCTION

Wheat is one of the major cereal crops cultivated in the United States. The US wheat production in 1994 was about 67 million metric tons, one third of which was soft wheat (Anonymous 1994). Soft wheat flour has been utilized in a wide range of commercial products (such as biscuits, cakes, cookies, crackers, doughnuts, pancakes, pastries, pie crusts, pretzels, noodles, wafers, waffles, etc.) because of its properties of fine flour particle size, low protein content and low starch damage (Finney 1989).

Much research has been conducted in the past to develop procedures for soft wheat quality evaluation (Yamazaki 1953, Pinchney et al 1957, Rasper et al 1986), however, no tests have been as satisfactory as baking tests. The diameter of a sugar-snap cookie or the volume of a cake is perhaps the most commonly used indicator for soft wheat flour baking quality (Finney 1989), although other product characteristics (e.g., texture, appearance and color) are of importance as well. The absence of a physicochemical test to reliably predict soft wheat baking quality is due to a number of reasons; there is a lack of knowledge of what factors are responsible for, or contribute to, baking quality; and the end products from soft wheat are so diverse. Thus, an "ideal" flour for one product or class of products would not be ideal for another (Finney 1989).

Flour protein has been shown to be one of the important factors affecting flour end-use quality for hard wheats. What is not fully understood is the individual roles played by different types of flour proteins such as albumins, globulins, gliadins and glutenins, in soft wheat flour quality.

Storage proteins, also called gluten proteins, consist of two major types of proteins: gliadins and glutenins. They account for about 70% of the total wheat flour proteins (Kasarda et al 1976). Gluten is formed when flour is wetted with water and interaction occurs between the gliadins and glutenins (Wrigley and Bietz 1988). Gluten proteins are important to flour end-use properties because of their unique ability to form viscoelastic doughs. In general, gliadins are believed to contribute to dough extensibility and glutenins to dough strength and elasticity (Wall 1979). The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (GS) have been reported as important indicators for various dough characteristics of hexaploid wheat flours (Kruger et al 1988, Payne et al 1988, Gupta et al 1989, Gupta and MacRitchie 1994). Variations in the relative quantity and type of glutenin subunits in a wheat cultivar are responsible for the amounts and size distribution of glutenin polymers, which were reported to most likely account for the combined effects of individual glutenin subunits on dough strength (Gupta et al 1993, Gupta and MacRitchie 1994, Gupta et al 1995). The relative quantities of some gliadin subgroups have also been reported to be linked to dough rheological properties and breadmaking quality (Branlard and Dardevet 1985, Wieser et al 1994).

Previous research has indicated that low gluten content and weak gluten strength are generally desired for good sugar-snap cookie baking (Gaines and Finney 1989, Kulp and Olewnik 1989, Gaines 1990, Kaldy et al 1993, Souza et al 1994). However, little is known about the variation in the relative quantity of each protein fraction in gluten in relation to the soft wheat flour end-use quality.

Therefore, in the research described here, the effects of variations in the type of HMW-GS, and of the quantities of HMW and LMW glutenin subunits and gliadin subgroups on soft wheat flour rheological and baking properties have been studied.

The objectives of this study were:

1. To develop a simple and effective method for quantifying glutenin subunits in wheat flours.
2. To investigate the effects of the relative quantities of gliadin subgroups on soft wheat flour rheological properties and baking performance.
3. To determine potential quality markers from HMW-GS for soft wheat products and to investigate the variation in the relative quantities of glutenin subunits in relation to soft wheat flour rheological and baking properties.

The following document includes a Literature Review (Chapter 2), three major studies (Chapters 3-5), a Summary and General Discussion (Chapter 6), Recommendations for Future Research (Chapter 7), and an Appendix. The format for the three papers is according to *Cereal Chemistry*. Quality data of the 17 soft wheat cultivars studied are presented in the Appendix in a *Cereal Chemistry* paper format.

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CHAPTER 2. LITERATURE REVIEW

CLASSIFICATION AND NOMENCLATURE OF WHEAT FLOUR PROTEINS

Osborne (1907) was one of the first to develop a sequential procedure for fractionating wheat flour proteins based on their solubilities in various solvents. In this procedure, sequential extraction of flour with different solvents yields albumin (extractable in water), globulin (extractable in saline), gliadin (extractable in 70% to 90% aqueous alcohol), and glutenin (extractable in dilute aqueous acid or alkali) fractions. Although the flour protein fractions obtained by this method are cross-contaminated, it has been used for many years by cereal chemists to investigate the structural and functional properties of cereal proteins (Bushuk 1981).

In addition to the five classes (albumins, globulins, gliadins, soluble glutenins and insoluble glutenins) of wheat proteins defined by a modified Osborne fractionation method (Chen and Bushuk 1970), a new class of endosperm proteins, the triplet proteins or "triticins", was found by Singh and Shepherd (1985). These proteins are neither glutenin nor gliadin groups based on solubility, but seem to be globulin storage proteins (MacRitchie et al 1990).

Gliadins are mainly single polypeptides (monomers) that associate by hydrogen bondings and hydrophobic interactions (Wrigley and Bietz 1988). Their molecular weights range from about 30,000 to 80,000. In contrast, glutenins are high molecular weight polymers with molecular weights reaching several millions (MacRitchie et al 1990). Gluten is formed when wheat flour is wetted with water and interaction occurs between the gliadins and glutenins (Wrigley and Bietz 1988).

The gliadin proteins have been further divided into four subgroups, ω-, γ-, β- and α-gliadins, in order of increasing mobility on acid polyacrylamide gel electrophoresis (Jones et al 1959, Woychick et al 1961). The molecular weights of γ-, β, and α-gliadins fall in the range of 30,000-40,000, and those of ω-gliadins in the range of 40,000-80,000 (Shewry et al 1986).

Bushuk and Zillman (1978) proposed a modified nomenclature system for gliadins based on a single prominent band in the reference variety Marquis which was assigned the arbitrary mobility of 50. For example, gliadins 42 and 45 are two gliadin bands which have relative mobilities of 42 and 45, respectively, with respect to the specific gliadin band 50 according to the Bushuk-Zillman nomenclature.

Glutenins are likewise classified into high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight glutenin subunits (LMW-GS) when separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) after reduction by a reducing agent (Payne and Corfield 1979, Jackson et al 1983). The HMW-GS have the lowest mobility with molecular weights ranging from 95,000 to 136,000 (Shewry et al 1986). The LMW-GS, also called aggregated gliadins, have molecular weights in the range of 36,000-44,000 (Bietz and Wall 1972). However, LMW-GS overlap in mobility with many other endosperm proteins, especially gliadins, on conventional one-dimensional SDS-PAGE gel. This complicates the electrophoretic separation of total flour proteins into their respective groups.

A numerical nomenclature (subunits 2, 5, 10, 12, etc.) was proposed by Payne and Lawrence (1983) to identify HMW-GS (A subunits) on the basis of their

mobilities on SDS-PAGE. On the other hand, due to the large number of and the complexity of separation of LMW-GS on SDS-PAGE, only two main groups of subunits were initially designated, namely B subunits and C subunits with B subunits having lower mobilities than C subunits. Later, a D group of LMW-GS, which contains GS with mobilities between those from A and B groups on SDS-PAGE, was reported (Jackson et al 1983).

Shewry et al (1986) proposed a different classification and nomenclature of wheat gluten proteins based on their molecular genetics. These authors suggested that all gluten proteins should be called "prolamins" because they are rich in glutamine and proline and are soluble in aqueous alcohol under appropriate conditions. The prolamins are then subgrouped into HMW prolamins, sulfur-rich prolamins (S-rich prolamins), and sulfur-poor prolamins (S-poor prolamins). The HMW prolamins are equivalent to the HMW-GS, while the S-poor prolamins are equivalent to the ω-gliadins. The S-rich prolamins include at least three types of wheat proteins, namely the α+β gliadins, the γ-gliadins and the LMW-GS, all of which are rich in sulfur amino acids (2-3%). Although glutenins and gliadins are similar in amino acid composition, glutenins are typified by higher glycine, and gliadins by higher proline, contents (MacRitchie et al 1990).

GENETICS OF WHEAT STORAGE PROTEINS

Common wheat is a hexaploid species, containing 3 genomes A, B, and D. Each genome has 7 chromosome pairs (namely 1, 2, ..., 7). Genetic studies have

shown that the structural genes controlling the syntheses of various classes of wheat storage proteins are located on specific chromosomes (1A, 1B, 1D, 6A, 6B, 6D). It was found that genes coding for the gliadin proteins are on the short arms of homologous chromosomes of groups 1 and 6 (Mecham et al 1978, Brown and Flavell 1981). All of the ω-gliadins, most of the γ-gliadins and a few of the β-gliadins are controlled by genes (Gli-1) on the short arm of group 1 chromosomes, whereas all of the α-gliadins, most of the β-gliadins and some γ-gliadins are encoded by genes (Gli-2) on the short arms of group 6 chromosomes. The Gli-1 loci comprise genes located at Gli-A1, Gli-B1, and Gli-D1, and the Gli-2 loci at Gli-A2, Gli-B2, and Gli-D2 (Singh and Shepherd 1988).

The genes encoding the HMW-GS are located on the long arms of chromosomes 1A, 1B, and 1D (Lawrence and Shepherd 1980). Their locations have been designated Glu-A1, Glu-B1, and Glu-D1, respectively (Payne and Lawrence 1983). For any wheat cultivar, 3 to 5 HMW-GS are evident upon separation by SDS-PAGE under reduced conditions: one or none is encoded by genes at the Glu-A1 locus, one or two by genes at the Glu-B1 locus, and two by genes at the Glu-D1 locus (Payne et al 1980).

Contrary to the HMW-GS, the LMW-GS were found to be coded for by genes on the short arms of chromosomes 1A, 1B, and 1D (Brown et al 1979, Jackson et al 1983). Glu-3 has been designated for the genes encoding LMW-GS (McIntosh et al 1989). Since the locations of Glu-3 genes on the short arms of group 1

chromosomes are adjacent to those of the Gli-1 genes, there are close genetic linkages between these two kinds of genes (Payne et al 1987a).

ISOLATION AND CHARACTERIZATION OF WHEAT STORAGE PROTEINS

Isolation and characterization of various flour proteins have been achieved by various techniques. Among them, electrophoresis and gel chromatography are commonly used methods. Acid-polyacrylamide gel electrophoresis (Acid-PAGE) has been often used in "fingerprinting" or identifying wheat cultivars for variety certification or for specific end-uses (Bietz and Wall 1980, Jones et al 1982, Lookhart et al 1982, Khan et al 1983, Khan et al 1985, Clements 1987, Lookhart and Albers 1988, Ng et al 1988, Pomeranz et al 1989, Clements 1990). Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has also been widely used in hard wheat studies for varietal identification (Okada et al 1988, Ng et al 1988), for potential end-use quality estimation (Khan and Bushuk 1979, Ng and Bushuk 1988, Khan et al 1989), and for molecular weight determination (Butaki and Dronzek 1979, Ng and Bushuk 1989). Isoelectric focusing, which separates proteins or polypeptides on the basis of differences in ionized amino acid content has shown itself a powerful tool in wheat protein analysis. Khan and Bushuk (1979) employed this technique to isolate individual subunits of glutenin from gel filtration fractions. A relatively new technique, free-flow preparative isoelectric focusing, has been used for fractionating unreduced (Ng et al 1989) and reduced wheat storage proteins

(Curioni et al 1990). Recently, capillary electrophoresis has been reported to effectively separate wheat storage proteins (Bietz 1993, Werner et al 1993).

Gel chromatography, including gel permeation chromatography, ion-exchange chromatography, hydrophobic interaction chromatography, and high-performance liquid chromatography (HPLC), has also proven to be valuable for isolation and characterization of wheat storage proteins. In the past decade, much work has been done using reversed-phase HPLC (RP-HPLC) to analyze hard wheat proteins and predict their breadmaking quality (Huebner and Bietz 1984, 1987; Kruger et al 1988; Lookhart and Albers 1988; Scanlon et al 1990, Lew et al 1992).

Ultracentrifugation is another useful separation method which separates proteins based on their different molecular weights. This technique was used by Finney et al (1982) and Jones et al (1983) to study the physical and biochemical properties of hard wheat protein fractions.

The HMW-GS and LMW-GS have been shown to be important markers of dough characteristics of bread wheat (Kruger et al 1988, Payne et al 1988, Gupta et al 1989, Gupta and MacRitchie 1994). Therefore, the isolation and characterization of individual glutenin subunits appear to be very important in understanding the relationship between glutenin composition and flour end-use quality. The HMW-GS can be easily separated by one-dimensional (1-D) SDS-PAGE, but it is difficult to obtain reliable separation of LMW-GS by means of a conventional 1-D SDS-PAGE method used for HMW-GS analysis, because their mobilities overlap with those of gliadins and some albumins and globulins. Two-dimensional (2-D) electrophoresis

techniques have been effectively employed to fractionate all the storage proteins (Payne et al 1985), and are especially useful for LMW-GS; however, they are tedious and time-consuming. Simplification of separation methods for glutenin analysis is therefore of interest.

Several papers have been published on developing simplified 1-D electrophoresis separation methods for both HMW and LMW subunits of glutenin in wheat (Khelifi and Branlard 1991, Gupta and MacRitchie 1991, Singh et al 1991, Zhen and Mares 1992). In their methods, gliadins were first removed by 50% 1-propanol (Singh et al 1991) or dimethylsulfoxide (DMSO) (Gupta and MacRitchie 1991) or Acid-PAGE gel (Khelifi and Branlard 1991) before glutenins were extracted so that the final glutenin extract was relatively without contamination. Mostly recently, based on the extraction protocol established by Singh et al (1991), Melas et al (1994) developed a simple and rapid method for preparing large quantities of relative pure HMW-GS and LMW-GS from wheat through a selective precipitation by acetone. This new modified procedure seems to be quite efficient at extracting relatively pure glutenins from flour.

RELATIONSHIP OF STORAGE PROTEINS TO DOUGH AND BREADMAKING PROPERTIES

There are significant relationships between each class of gluten proteins, specifically, gliadins, HMW-GS, and LMW-GS, and dough properties (MacRitchie et al 1989). The elasticity and strength of the bread dough are strongly affected by the

type and quantity of HMW-GS. Variation in dough properties and breadmaking quality among wheat varieties has shown to result largely from allelic differences in HMW-GS (Payne et al 1984, Lawrence et al 1987, Ng and Bushuk 1988). For example, the chromosome 1D-encoded HMW-GS 5+10 impart greater elasticity and strength to doughs than their allelic counterparts, 2+12. Later studies have shown that LMW-GS composition seems to be as important as that of HMW-GS to dough properties (MacRitchie et al 1990). On the other hand, the gliadins are generally thought to be responsible for the viscous properties of wheat gluten and the extensibility of flour doughs (Wall 1979).

Payne et al (1987b) proposed a "Glu-1 quality score" to estimate breadmaking and British biscuitmaking qualities for each wheat cultivar based on SDS-sedimentation volumes. Wheat cultivars which are associated with larger SDS-sedimentation volumes are considered more suitable for breadmaking. Table I lists the quality score assigned to each of the commonly occurring subunits or subunit pairs used to generate a Glu-1 quality score for each cultivar (Payne et al 1987b). A maximum score of 10 and a minimum of 3 is possible for any wheat cultivar based on the presence or absence of specific HMW-GS in the cultivar. For example, a wheat cultivar containing subunits 1 (Glu-A1), 17+18 (Glu-B1), and 5+10 (Glu-D1) has a Glu-1 quality score of 10; and a wheat cultivar containing no subunits from Glu-A1, subunit 7 (Glu-B1), and subunits 4+12 (Glu-D1) has a Glu-1 quality score of 3.

Table I

Quality Scores Assigned to Individual HMW Glutenin Subunits or Subunit Pairs (Payne et al 1987b)

	Gene Locus		
Score	Glu A1	Glu B1	Glu D1
4	–	–	5+10
3	1	17+18	–
3	2*	7+8	–
2	–	7+9	2+12
2	–	–	3+12
1	null	7	4+12
1	–	6+8	–

Contrary to HMW-GS, the association of LMW-GS and dough properties was little studied until recent years. Unusual solubility, tendency to aggregate, and overlap with other proteins in 1-D SDS-PAGE systems make them more difficult to analyze than HMW-GS and gliadins. However, studies on the functionality of LMW-GS to dough properties and breadmaking have been accelerated since the development of a two-step one-dimensional SDS-PAGE method (Singh and Shepherd 1988, Gupta and Shepherd 1989). Previous results confirmed that some

LMW-GS are related to significant differences in physical dough properties and breadmaking quality (Gupta et al 1989, MacRitchie et al 1989).

Gupta et al (1989) found that the LMW glutenin allele (Glu-A3m) affected more largely dough extensibility and resistance to extension than did the HMW subunit allele (Glu-A1b) in a study of 56 F_2-derived F_6 families from a cross involving two wheats of contrasting quality type. Gupta and MacRitchie (1994) quantitated and qualitated the variation in LMW-GS and HMW-GS in wheat and related them to dough properties. They concluded that different amounts of LMW-GS produced by LMW glutenin alleles at the Glu-A3 and Glu-B3 loci determined the quantity and size distribution of the polymeric proteins (glutenins), while the alleles at the Glu-B1 and Glu-D1 loci yielded HMW-GS of similar quantities and were associated only with the size distribution of the polymeric proteins.

Because the genes coding for LMW-GS (Glu-3) and some gliadins (Gli-1) all reside on the short arms of group 1 chromosomes, there is a close linkage between genes of LMW-GS and genes for gliadins. The association of gliadin alleles and baking quality has been assumed to reflect mainly the relationship of LMW glutenin alleles and quality (Payne et al 1987a). Payne et al (1991) indicated that allelic variation at the Glu-1 loci (coding for HMW-GS) and Gli-loci (coding for LMW-GS, ω-gliadins and γ-gliadins) affects greatly the balance of elasticity and extensibility in doughs, leading to differences in baking performance. They believed that at the Gli-1 loci the LMW-GS were the causal proteins responsible for the differences in breadmaking qualities. Other results showed that the quantity of the LMW-GS was

more effective than that of gliadins in determining dough strength (Gupta 1993). Further studies on variation in LMW-GS composition would permit a more accurate quality assessment of wheat. Gupta et al (1991) suggested that allelic variation in LMW-GS is important for explaining dough quality differences in bread wheats.

Studies on the relationship of gliadin proteins to wheat quality was stimulated by the observation that certain gliadins, namely 42 and 45 defined according to Bushuk and Zillman (1978), were associated with dough weakness and strength, respectively, in durum wheats (Kosmolak et al 1980 and references therein). Huang and Morrison (1988) concluded in their study of 40 Chinese and eight British varieties that gliadin bands 44.5 and 45.0 were generally associated with strong gluten, and gliadin bands 41.0 and 45.5 with weak gluten. In a series of studies, Wrigley and coworkers (Wrigley 1980, Wrigley et al 1981) also correlated specific gliadins with the quality characteristics of grain hardness and dough strength on the basis of analysis of 79 varieties (mainly Australian wheats). In order to avoid the possible confusion resulting from pedigree relationships, Branlard and Dardevet (1985) collected 70 wheats differing widely in quality from 14 countries and found that 10 out of 55 gliadin components studied were significantly positively related to the breadmaking quality, and 8 components were significantly negatively related to the breadmaking quality.

Though more information is now available concerning the contribution of specific gliadins to breadmaking quality, unlike the gliadins of durum wheats or the HMW-GS of bread wheats, there has not been much agreement about which

gliadins are involved. The reasons for this may be due to different fractionation methods, different nomenclature of gliadins, diverse genotypes of wheat used in various studies, and varying evaluation methods (MacRitchie et al 1990).

SOFT WHEAT FLOUR QUALITY

Flour quality has different meanings to farmers, millers and bakers. In this dissertation, only the milling, physicochemical, dough rheological and baking properties will be discussed. Among these, the baking quality is the most important end-use parameter. Most soft wheat products require flour that has relatively low protein content (8.5-10.0%), low alkaline water retention capacity (AWRC), weak mixing properties and low damaged starch levels (Finney 1989). It should be kept in mind that little documental information is available about the suitability of soft wheat flour for making most products except for cookies and cakes. The following reviews some major properties of soft wheat flours which are thought to relate to the quality of flour end products.

Break Flour Yield

This is the percentage of flour recovered from break rolls in the mill. Break flour has superior quality to straight-grade flour (a composite of all streams of flours produced on the mill) in terms of its finer particle size and lower starch damage. Break flour yield is a good indicator of both wheat hardness and flour particle size. A higher break flour yield indicates a softer wheat kernel texture. The softer kernel

soft wheat can produce baked products of tender texture and is considered desirable for all soft wheat products (Finney 1989). The relative hardness of wheat is determined by its genotype and environmental factors.

Falling Number

Falling number is the time, in seconds, required for a viscometer-stirrer to fall a fixed distance through a hot, aqueous flour suspension being liquefied by enzymes present in a flour sample, in a standardized apparatus. Falling number measurement of whole wheat flour or white flour has been widely used to determine α-amylase activity and degree of preharvest sprouting. Higher α-amylase activity in wheat will cleave starch polymers faster, leading to quicker reduction in the viscosity of aqueous flour suspension and a lower falling number value. Any wheat with a falling number value below 200 is considered to have high amylase activity and be sprout damaged (Anonymous 1988).

Alkaline Water Retention Capacity (AWRC)

The AWRC is the amount of alkaline water held by flour at 14% moisture basis against a centrifugal force. This test was first developed by Yamazaki (1953) to predict flour quality for sugar-snap cookies (SSCs). It was designed to simulate the role of soft wheat flours in SSC baking, where the cookie dough is in alkaline condition. Flours which hold alkaline water poorly against centrifugal force are considered better suited for SSC baking. However, for today's soft wheats, AWRC

values do not show the same close association with cookie diameter as they did earlier, perhaps due to changes in breeding materials and farming practices (Finney 1989).

Zeleny Sedimentation Test

This test measures the volume of the "sediment", consisting mainly of swollen gluten and starch, after suspending flour in a lactic acid-isopropanol solution for 5 min (AACC 1992). The sedimentation volume is influenced by the quantity and quality of the gluten. Soft wheat flours usually have low gluten content and weak gluten strength and therefore, low sedimentation values.

Starch Pasting Viscosity

The amylograph is a recording viscometer that may be used primarily to determine the effect of α-amylase on viscosity of flour as a function of temperature. The viscosity of the starch gel is reduced by the action of α-amylase during heating of slurry. The peak viscosity is an index of α-amylase activity present in the flour, the lower peak viscosity indicating higher levels of α-amylase activity (Shuey and Tipples 1982). However, the peak viscosity is also influenced by the amount of damaged starch because it is readily attacked by α-amylases (Dengate 1984). The peak viscosity has proven to be an important quality parameter to Japanese-type

sponge cake baking since a low peak viscosity shows potential harm to sponge cakes by dropping of cake centers during cooling (Nagao et al 1976).

Dough Rheological Tests

It is generally accepted that the rheological properties of doughs play an important role in relation to the quality of baked products. The rheological instruments that have been extremely useful in quality control are the recording dough mixers such as the alveograph, farinograph and mixograph. The properties exhibited by a dough being evaluated by these instruments can be used to predict the wheat's behavior in the bakery. For soft wheat products, a dough exhibiting weak strength is generally desired. However, results derived from physical dough-testing instruments for soft wheat flours cannot be interpreted in the same way as results from hard wheat flours since the rheological properties of a soft wheat flour are not just the opposite of those of hard wheat flours (Hoseney et al 1988).

Cookie and Cake-Baking Tests

None of the measurements discussed above is a direct indicator of a flour's baking quality. Baking tests remain the ultimate quality tests. However, the evaluation of soft wheat flour quality is complicated by the diversity of soft wheat products produced and the large number of flour blends available (Hoseney et al 1988). Sugar-snap cookie and white layer cake (high-ratio cake) baking tests are two commonly used official American Association of Cereal Chemists (AACC)

methods (AACC 1992) for the evaluation of soft wheat quality. Japanese-type sponge cake-making (Nagao et al 1976) is an unofficial test to evaluate a flour's suitability for Japanese soft wheat products. Flours that produce larger diameter cookies or bigger volume cakes (high-ratio cakes and Japanese sponge cakes) are thought to have better flour quality, though the texture of products should also be considered.

ROLES OF SOFT WHEAT FLOUR PROTEINS ON COOKIE- AND CAKE-BAKING QUALITIES

It is generally believed that the protein content of flours for soft wheat products should be relatively low. Protein contents of 8.5-9.0% are often specified for cookie flours. In the preparation of dough for sugar-snap cookies, the gluten does not develop during mixing because of the competition for water among sugars, salts, and pentosans. The proteins, however, appear to become functional during baking and help form the basis for the cookie structure (Hoseney et al 1988, Gaines 1990). Likewise, in the preparation of high-ratio cake batters, the gluten is not developed into a cohesive mass, such as that in a bread dough, in the mixing step. Three factors appear to contribute to this: first, the flour protein is usually low in cake flours; second, the sugar competes for the water and slows gluten development; and, third, the basic dough pH also slows gluten development (Hoseney et al 1988).

The effects of soft wheat flour protein content on the quality of cookies and cakes have been studied. Generally speaking, protein content of a soft wheat has a

negative but weak correlation with cookie diameter (Yamazaki 1954, Yamazaki and Lamb 1962, Abboud et al 1985, Gaines 1985, Kaldy et al 1993). The larger diameter sugar-snap cookies are normally made from softer textured wheats, which in general yield lower protein flours and have lower alkaline water retention capacity (AWRC) values. Similarly, some negative correlations have been found between cake volume and flour protein content (Gaines and Donelson 1985, Kaldy and Rubenthaler 1987). Larger volume Japanese-type sponge cakes (Nagao et al 1976) and white layer cakes (Gaines 1985) are usually made from softer textured wheat cultivars with lower protein content, and finer flour particle size.

In the initial stages of cookie baking, the shortening melts, reducing the viscosity of the dough. At the same time, the sugar solution content increases as a result of dissolution of sugar in water, leading to increases in fluidity and spreading of the dough as a function of gravity. As the temperature of the cookie dough increases, the shortening system becomes active and helps to expand the dough in all directions. At some point during baking, a rapid increase in the viscosity of the dough occurs, which slows down and finally stops the spreading of cookies. Because starch is not gelatinized during cookie baking, the increase in viscosity is presumably due to the properties of the flour proteins (Hoseney 1986).

The fact that the protein content seems not to be important if the flour is milled from a good-quality soft wheat cultivars indicates that the protein quality could be more important than quantity (Hoseney et al 1988). There have been two theories regarding the mechanism by which cookie doughs stop spreading during

baking (Doescher et al 1987, Slade et al 1989), both of which involve the quality of flour proteins as a critical function. Doescher et al (1987) suggested that the rapid increase in dough viscosity during cookie baking is due to the formation of a continuous gluten network after its glass transition temperature is reached. They found that the difference between a good-quality and a poor-quality cookie flour lies in the glass transition temperature of their respective gluten, with poor-quality cookie flour being lower in the gluten glass transition temperature than good-quality cookie flour. In contrast, Slade et al (1989) theorized that the glass transition temperature of wheat gluten is not involved in the sugar-snap baking mechanism. Instead, they suggested that a poor-quality cookie flour produces a dough having a three-dimensional, self-supporting and elastic network that first expands upon heating and then shrinks when above glass transition temperature. A good-quality cookie dough exhibits viscous expansion during heating and then collapse above glass transition temperature. Presumably, soft wheat cultivars differ in those rheological properties.

Gaines (1990) studied the effects of sulfhydryl oxidizing, reducing and blocking agents on sugar-snap cookie-baking quality. He reported that L-cysteine, a reducing agent, and N-ethylmaleimide (NEMI), a sulfhydryl blocking agent, had significant effects on increasing cookie diameter. This supports the assumption of Doescher et al (1987) that gluten network is formed during cookie baking.

Gaines and Finney (1989) reported the effects of various cellulases and proteases on cookie spread and cookie dough consistency. Their results showed that the papaya protease had the greatest function in increasing cookie spread and

improving top grain of baked cookies. The papaya protease, which has extensive exo- and endoproteolytic activities, can produce substantial degradation of gluten proteins, leading to decreased dough viscosity and prolonged expanding time.

It is a common practice to improve the high-ratio cake-baking properties of soft wheat flours by chlorination. It has been reported that the solubility of flour proteins increases as a result of the dispersing, hydrolytic, and oxidative actions of chlorine (Tsen and Kulp 1971). It was speculated that with the action of chlorine, the solubilized proteins in cake batters might be more suitable than the insoluble proteins for building the proper cake structure, and that they might also act more effectively as complex-forming agents and thus permit better incorporation of shortening (Tsen and Kulp 1971). However, one reason that flour for making Japanese-type sponge cakes (JSCs) is not chlorinated is because of the differences in cake formula. Chlorinated flours reduce the quality of JSCs (Worthington 1994). Similarly, chlorine treatment of flour reduces its sugar-snap cookie-baking quality (Donelson 1990).

The fractionation and reconstitution method has been widely used in soft wheat studies since the pioneering work of Yamazaki (1950) on cookie flours. Sollars (1956) used acetic acid to fractionate flour into four fractions and found that the water-soluble fraction gave a small but consistent effect of decreasing cookie spread and that the effects of gluten on cookie spread was erratic. Later, Donelson and Wilson (1960) employed this technique to study cake flour baking quality and found that gluten had the greatest effect on cake volume and structure. In a later

study, Donelson (1988) found that when the gluten fraction was removed from the flour, a significant increase in cookie spread occurred in every case, but he did not comment on the texture and flavor of the baked products. Kaldy et al (1993) also showed that the amount of gluten was negatively correlated with cookie diameter. However, these studies dealt only with the quantity of some protein fractions or crude gluten. The contribution of each gluten fraction in soft wheat flour to product quality has been little studied.

Pomeranz et al (1989) reported that gliadin patterns of soft wheats change during baking. Acid-PAGE results showed that heating increased the solubility of ω-gliadins and decreased the solubility of most other gliadins. Results from HPLC indicated that highly hydrophobic gliadins were more reactive in good than in poor cookie flours during baking.

The effects of HMW-GS on soft wheat baking quality have been preliminarily investigated. The Glu-1 quality score was found to be negatively correlated with British biscuit-making quality (Payne et al 1987b), however, the individual HMW-GS (except subunit pair 13+19) and Glu-1 quality score did not correlate with AACC SSC-baking quality (Souza et al 1994, Lookhart et al 1993). Souza et al (1994) calculated glutenin rank sum (GRS), a modified scoring system of Payne et al (1987b), of each cultivar they studied and found that the GRS was negatively correlated with sugar-snap cookie diameter. However, this correlation was greatest in the year with the lowest average flour protein content and least in the year with the highest average protein content.

The review of the literature presented above clearly indicates that the storage proteins of soft wheat have certain functionality in soft wheat products. Further determination of the actual roles played by each gluten fraction in soft wheat product quality is of special importance to breeders for early screening of genetic lines, and to millers and bakers for selecting certain desired qualities for their respective needs. The present study was designed to extend the previous investigations by adding more precise information about the relationship of storage proteins of U.S. soft wheat cultivars to their rheological and baking properties.

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CHAPTER 3. QUANTIFICATION OF GLUTENIN SUBUNITS BY SEQUENTIAL ACETONE PRECIPITATION AND BY SDS-PAGE COUPLED WITH DENSITOMETRY USING A KNOWN QUANTITY OF GLUTENINS AS A STANDARD

Quantification of Glutenin Subunits by Sequential Acetone Precipitation and by SDS-PAGE Coupled with Densitometry Using a Known Quantity of Glutenins as a Standard

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ABSTRACT

Glutenin subunit groups (high-molecular-weight glutenin subunits, HMW-GS; low-molecular-weight glutenin subunits, LMW-GS) were quantified by a sequential acetone precipitation method, and by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with densitometry using a known quantity of extractable glutenin proteins as a quantitative standard. The average quantity of extractable HMW-GS analyzed in 17 soft wheat patent flours was 8.46% and 7.26% of flour protein by the sequential acetone precipitation and densitometric methods, respectively, whereas the average quantity of extractable LMW-GS was 15.29% of flour protein by the sequential acetone precipitation method and 17.07% of flour protein by the densitometric method. The mean total quantities of extractable glutenin subunits in the 17 flour samples determined by these two methods were 23.75% and 24.33%. There were no significant differences between the two methods ($p>0.05$) in the quantities of the total glutenins determined. However, the quantities of HMW-GS, LMW-GS and total glutenin subunits determined by each of the procedures were highly correlated. The densitometric quantification of glutenin subunit groups with the aid of a known quantity of glutenin proteins as a quantitative standard was shown to be an effective method because of its speed, small sample size, reliability, and simultaneous quantification and characterization of glutenin subunits.

INTRODUCTION

The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (GS) have been reported as important markers of dough characteristics of hexaploid wheat flours (Kruger et al 1988, Payne et al 1988, Gupta et al 1989, Gupta and MacRitchie 1994). Wheat cultivars vary in the quantity and type of glutenin subunits responsible for the amounts and size distribution of glutenin polymers (Gupta et al 1993, Gupta and MacRitchie 1994). Therefore, quantification of glutenin subunits can provide a better understanding of their roles in determining flour end-use quality.

Glutenin subunits were initially classified into A, B, and C groups based on their mobilities (A, lowest; C, highest) in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gel (Payne and Corfield 1979). Later, a D group of LMW-GS, which contains GS with mobilities between those from A and B groups on SDS-PAGE, was reported (Jackson et al 1983). The A subunits include all the HMW-GS, while B, C, and D subunits form collectively the LMW-GS. On SDS-PAGE, D subunits have the lowest mobilities among the LMW-GS, whose mobilities are similar to those of ω-gliadins on SDS-PAGE. The B subunits have slightly lower mobilities than α-, β-, and γ-gliadins, and C subunits have mobilities similar to those of α-, β-, and γ-gliadins on SDS-PAGE.

Separation of HMW-GS has been carried out by reversed-phase high-performance liquid chromatography (RP-HPLC) (Kruger et al 1988, Marchylo et al 1989, Sutton 1991, Kawka et al 1992, Andrews et al 1994, Gupta and

MacRitchie 1994), SDS-PAGE (Payne et al 1981, Payne and Lawrence 1983, Ng and Bushuk 1987), and acid-polyacrylamide gel electrophoresis (Acid-PAGE) (Morel 1994), however, the separation and identification of LMW-GS has been done mostly by SDS-PAGE (Gupta and Shepherd 1990, Gupta and MacRitchie 1991, Singh et al 1991, Zhen and Mares 1992). More recently, Acid-PAGE has also been used to separate both HMW-GS and LMW-GS (Morel 1994).

Reversed-phase-HPLC (Kruger et al 1988, Marchylo et al 1989, Gupta and MacRitchie 1991, 1994, Andrews et al 1994) and densitometric analysis of electrophoretic gels (Brunori et al 1991, Kolster and van Gelder 1991, Kolster and Vereijken 1994, Mosleth et al 1994, Peltonen and Virtanen 1994) have been used for quantification of glutenin subunits. The RP-HPLC has distinguished itself to be an accurate and potentially automated tool for the quantification of individual HMW-GS in a glutenin extract. However, the identification of individual glutenin subunits by RP-HPLC requires use of SDS-PAGE. Densitometric analysis of SDS-PAGE measures the intensity of each band on electrophoresis gels, after staining, by scanning each band through a visible light. Therefore, the densitometric analysis of SDS-PAGE can provide simultaneous identification and quantification of glutenin subunits.

There is some concern about accuracy of the densitometric approach since factors, such as type of proteins, gel uniformity, and staining and destaining times of gels, could affect the reproducibility and accuracy of results (Kolster et al 1992). On the other hand, a SDS-PAGE densitometric approach

can be a viable tool to simultaneously identifying and quantifying glutenin subunits if some modifications can be made to improve its accuracy. In the present study, the objectives were (1) to quantify HMW-GS and LMW-GS determined by a modified sequential acetone precipitation method, and HMW-GS and LMW-GS determined by SDS-PAGE coupled with a densitometric method using a known quantity of glutenin proteins as a standard, and (2) to compare these two quantification methods.

MATERIALS AND METHODS

Chemicals and Reagents

Acrylamide (>99% purity), dithiothreitol (DTT), sodium dodecyl sulfate (SDS) and Tris were from Boehringer Mannheim Corporation (Indianapolis, IN). Coomassie Brilliant Blue R250, N,N'-methylenebisacrylamide, glycine, Pyronin Y, 2-mercaptoethanol, N,N,N',N'-tetramethyl ethylene diamine (TEMED) and molecular weight markers for SDS-PAGE (MW: 205 kDa, 116 kDa, 97.4 kDa, 66 kDa, 45 kDa and 29 kDa) were from Sigma Chemical Company (St. Louis, MO). Ammonium persulfate (crystal), acetone, hydrochloric acid, 2-propanol and trichloroacetic acid (TCA) were from J.T. Baker Inc. (Phillipsburg, NJ). Distilled and deionized water was used throughout the study.

Wheat Samples

Seventeen soft wheat cultivars harvested in 1992 or 1993 (Table I) were selected for this study. These cultivars cover all classes of soft wheats produced in the U.S.. The samples were milled on a Miag-Multomat mill to obtain patent flours at a 45% extraction rate. The protein contents of these samples ranged from 6.7% to 8.9% (Table I).

Preparation of Extractable Glutenin Subunit Groups by Sequential Acetone Precipitation Method for Quantitative Measurements

Two solutions were used for the preparation of glutenin subunits: solution A was 60% ethanol, and solution B was a 50% 2-propanol solution containing 0.08 M Tris-HCl buffer (pH 8.0). Figure 1 outlines the preparation procedures according to Melas et al (1994) with some modifications. A five gram flour sample was first dispersed in 250 ml of solution A and mixed for 30 min at room temperature. This step was to remove most of the gliadins, albumins and globulins from the flour. The sample solution was then centrifuged at 25,000 x g (20^{o}C) for 6 min and the residue was recovered. The extraction process was repeated one more time. The final residue was used for the extraction of glutenin subunits. Twenty-five ml of solution B containing 1% (w/v) dithiothreitol (DTT) was added into this residue. After suspension, the mixture was sonicated in a water bath (FS 14H, Fisher Scientific, Pittsburgh, PA) for 1 min to increase protein extractability (Singh et al 1990) and extraction of glutenin subunits was

further conducted in a 65°C water bath for 1 hr with shaking. The mixture was centrifuged as above and the supernatant recovered. The extraction was repeated one more time in 10 ml of extracting solution B for 30 min. The supernatants were pooled, and 30 ml of solution B containing 1.4% (v/v) 4-vinylpyridin was added to alkylate the proteins for 30 min at 65°C, after which the entire mixture was centrifuged as above. The supernatant was then precipitated sequentially by adjusting the acetone concentration to 40% and then 80% to obtain HMW-GS and LMW-GS, respectively, in the solution and precipitates were separated by centrifugation as above. The precipitates were washed twice with distilled and deionized water and centrifuged to remove Tris, after which they were freeze-dried and their contents determined by micro-Kjeldahl using the conversion factor of 5.7 and reported as percentage of total flour protein (14% m.b.). The preparation procedure was done in duplicate. The above preparation is herewithin referred to as the sequential acetone precipitation method.

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

Glutenin subunit groups obtained by the above sequential acetone precipitation method were identified by SDS-PAGE. Sample solutions for applying on SDS-PAGE gels were obtained by combining about 2.0-2.5 mg of a precipitate with 0.5 ml of a solution C comprised of 20% glycerol, 6 M urea, and 25 mM acetic acid, together with 0.5 ml of SDS sample buffer (Ng and Bushuk

1987), and heating in a boiling water bath for 2.5 min. Ten μl of a heated sample solution was loaded per well on the gels. The electrophoresis running conditions were according to Ng and Bushuk (1987) except that the running time was increased to 24 h for better resolution. Staining and destaining conditions were according to Sapirstein and Bushuk (1985).

Preparation of Extractable Glutenins for SDS-PAGE and Densitometric Analyses

The preparation of glutenin proteins is outlined in Figure 2. Most of the gliadins, albumins and globulins were first removed from the flour by adding 1 ml of solution A to a 20 mg flour sample in a micro-centrifuge tube, vortexing for 30 min at 20°C, then centrifuging for 10 min at 14,000 x g (20°C). The process was repeated one more time. The residue was then suspended in 0.1 ml of solution B containing 1% (w/v) DTT. The mixture was sonicated in a water bath for 1 min, extracted in a 65°C water bath with shaking for 1 hr, centrifuged as above, and the supernatant collected. The residue was further extracted with 0.05 ml of solution B containing 1% DTT, vortexed for 30 min, and centrifuged as above. The supernatants were pooled and 0.15 ml of solution B containing 1.4% (v/v) 4-vinylpyridin was added. After 30 min of alkylation in a 65°C water bath, 1.2 ml acetone was added into the solution (to a total of 80% acetone in the solution) to precipitate the total glutenins. The glutenin precipitate was then obtained by centrifugation as above. The precipitate was dried in a 60°C oven for 5 min,

then solubilized in 100 μl of solution C, after which 100 μl of SDS sample buffer was added. The sample solution was subsequently heated in a boiling water bath for 2.5 min. Based on the estimated protein content in the sample solution, 16 μl of the heated sample solution was loaded onto the SDS-polyacrylamide gels per well. The electrophoresis running conditions were the same as described previously. Gel electrophoresis was performed in duplicate. After staining and destaining the gels, the protein bands were subjected to densitometric analysis as described below. The above preparation is herewithin referred to as the densitometric method.

Determination of the Relative Dye Staining Sensitivities of HMW-GS and LMW-GS by Densitometer

High-molecular-weight glutenin subunits and LMW-GS of three cultivars, Augusta, Caldwell and Frankenmuth, prepared by the sequential acetone precipitation method, were used to determine relative dye staining sensitivities. Each of the two glutenin subunit groups (2.5 mg) of each cultivar was solubilized in 0.5 ml solution C, after which 0.5 ml SDS sample buffer was added into each of the solutions. The sample solutions were then heated in a boiling water bath for 2.5 min. On each gel, two wells per sample were loaded with a 5 μl aliquot each of respective HMW-GS or LMW-GS. A total of four SDS-PAGE gels were run.

After destaining the gels, the electrophoretic patterns were analyzed by a GS 300 Transmittance/Reflectance Scanning Densitometer with GS 365W software (Hoefer Scientific Instruments, San Francisco, CA) on wet gels. The respective staining sensitivities of HMW-GS and LMW-GS on each gel were calculated by dividing the respective quantities of HMW-GS and LMW-GS loaded on the gel by their respective areas on the densitograms; the lower the value, the more sensitive to Coomassie stain the glutenin subunit group. For the three cultivars used, the average ratio of the staining sensitivity of HMW-GS to that of LMW-GS was 1.3 (range 1.21-1.37). This average ratio value was used as a conversion factor for more accurate quantification of glutenin subunit groups during analysis of densitometric data.

Preparation of Glutenin Proteins as a Quantitative Standard for Densitometric Analyses

Glutenins of cultivar Frankenmuth were used as a protein standard for quantification. Total HMW and LMW glutenin subunit groups of Frankenmuth were prepared by the sequential acetone precipitation method, except that the acetone concentration was adjusted immediately to 80% (i.e., bypassing the 40% acetone precipitation step) to obtain total glutenins. Glutenins (2.3 mg based on micro-Kjeldahl analysis) were solubilized in 0.5 ml of solution C, after which 0.5 ml SDS sample buffer was added into the solution. The sample solution was then heated in a boiling water bath for 2.5 min. The protein

concentration in the solution was 2.3 μg/μl. A 10 μl aliquot of this standard was loaded into each of two wells per gel.

Quantitative Analysis of Glutenin Subunits by Densitometer

The quantities of A, B, and C subunits were calculated from their respective areas on the densitograms. A known quantity of glutenin proteins of Frankenmuth run on the same gel was used as an internal standard to calculate the quantity of protein per unit area of densitogram for each group of glutenin subunits based on the staining sensitivity ratio (1.3) of HMW-GS to LMW-GS. Because the D subunit region on SDS-PAGE contained only a few faint bands, indicating very small quantities of subunits present, the amount of D subunits was not determined. All analyses were scanned twice and average results were calculated and protein content reported as percentage of flour protein. For a cultivar, the coefficient of variation (CV) of staining intensity was 9.2% for A subunits, 3.3% for B subunits, and 9.5% for C subunits from lanes among eight gels; and the CV of the quantity of glutenin subunit groups determined was 7.4% for A subunits, 3.5% for B subunits, and 7.8% for C subunits from lanes among eight gels when a known quantity of glutenin standard loaded on each gel was used to normalize the densitogram areas of each glutenin subunit group.

Statistical Analyses

Data were subjected to ANOVA, paired "t" test, and correlation analyses on the Microsoft Excel program (Cambridge, MA).

RESULTS AND DISCUSSION

Quantification of HMW-GS and LMW-GS Extracted by the Sequential Acetone Precipitation Method

Albumins, globulins, and gliadins must be removed from a flour sample prior to extracting glutenins in order to obtain a relatively pure fraction. It has been reported that 60% ethanol is one of the most efficient aqueous ethanols for removing gliadins, albumins and globulins from flour (Wieser et al 1994). The relative amount of albumins and globulins extracted by 60% ethanol is about 35% of the amount of gliadins extracted (Wieser et al 1994). Flour is easier to suspend in 60% ethanol than in the 50% propanol solutions used in other studies (Singh et al 1991, Melas et al 1994). Additionally, it has been reported that 50% propanol extracts glutenins more efficiently than does 60% ethanol (Zhen and Mares 1992), which prompted the use of 60% ethanol in the present study to optimize gliadin removal while preserving glutenin solubility. Furthermore, extraction of gliadins by 50% propanol or 70% ethanol at high temperature (60^{0}C) could remove some high-molecular-weight polypeptides (MW > 60,000 Da) aside from gliadins (Byers et al 1983, Kruger et al 1988, Zhen and Mares 1992), leading to a lower amount of extractable glutenins in the

residue following gliadin extraction. Preliminary experiments with our flour samples showed that pre-extraction of gliadins with 50% 1-propanol or 2-propanol at 60^{0}C greatly reduced the amount of extractable HMW-GS up to 43% and that of LMW-GS up to 47% compared with the results of pre-extraction at room temperature (data not shown). Thus, the removal of albumins, globulins, and gliadins from these flour samples was conducted using 60% ethanol at room temperature to minimize the loss of glutenin proteins.

Precipitation of HMW-GS and LMW-GS by varying the acetone concentration in a protein mixture was first proposed by Melas et al (1994) to prepare large quantities of pure LMW-GS. Table I shows the quantities of HMW-GS and LMW-GS in flour proteins of 17 patent flour samples determined by this method. The average quantities of extractable HMW-GS and LMW-GS in these samples accounted for 8.46% and 15.29% of flour protein, respectively. The quantity of total extractable glutenin subunits ranged from 18.47% to 26.12% of flour protein with a mean value of 23.75%. However, HMW-GS obtained by this procedure were contaminated by some LMW-GS as seen from electrophoretic results (Figure 3, lanes 2, 5, 8 and 11), therefore, the quantities of HMW-GS measured might be slightly higher than their actual values and those of LMW-GS might be slightly lower than their actual values. For cultivar Clark in Figure 3 (lanes 10 and 11), more than five bands were visible in the HMW-GS area, which may be a result of HMW-GS variants or a potential mixture and/or biotype of the cultivar.

Quantification of Extractable Glutenin Subunit Groups by Densitometer

Although the staining sensitivities of HMW-GS and LMW-GS varied from gel to gel, the ratios of the staining sensitivities of HMW-GS to those of LMW-GS remained fairly constant at 1.3, indicating that LMW-GS have higher affinities for Coomassie Brilliant Blue R250 than HMW-GS. In other words, the quantity of HMW-GS per unit densitogram area is 1.3-fold higher than that of LMW-GS per unit densitogram area. These dye-binding differences may be attributed to the different amino acid compositions of various proteins (Stoschek 1990, Eynard et al 1994). This average ratio (1.3) was used to calculate the respective quantities of HMW-GS and LMW-GS per unit densitogram area based on the total densitogram peak areas produced by a known quantity of glutenin proteins of Frankenmuth run on the same gel.

The SDS-PAGE patterns of glutenin subunits of eight cultivars are shown in Figure 4. A typical densitometric reading of an SDS-PAGE pattern (one lane) is shown in Figure 5. The quantity of each group of glutenin subunits quantified by densitometer is listed in Table II. In the literature, quantitative results of glutenin subunits determined by RP-HPLC (Kruger et al 1988, Marchylo et al 1989, Sutton 1991, Andrews et al 1994) or densitometer (Kolster and Van Gelder 1990, Kolster et al 1992) have been expressed as peak area (Kruger et al 1988, Andrews et al 1994), relative proportion of total HMW-GS area (%) (Sutton 1991, Marchylo et al 1989), relative proportion of total storage protein area (%) (Sutton 1991, Marchylo et al 1989), or in absorbance units (Kolster and

Van Gelder 1990, Kolster et al 1992). Kolster and Vereijken (1994) converted Coomassie Brilliant Blue absorbance values of their HMW-GS to the absolute quantities of subunits using conversion factors. In the present study, an aliquot of known quantity (2.3 μg/μl) of a pure total glutenin subunit mixture prepared by the acetone precipitation method was loaded on each gel to normalize the densitogram areas of respective HMW-GS and LMW-GS on the same gel using an average staining sensitivity ratio (1.3) of HMW-GS to LMW-GS. An absolute quantity of protein for each band could then be calculated more accurately. The quantities of HMW-GS determined by this method are comparable to those reported by Kolster and Vereijken (1994).

It should be mentioned that the present study is the first to report using a known quantity of glutenin subunit proteins to quantify other glutenin subunits. Since the dye-binding ability of Bovine Serum Albumin (BSA) protein is greatly different from those of various classes of wheat flour proteins (albumins, globulins, gliadins and glutenins) (Eynard et al 1994), use of BSA to accurately quantify the glutenin proteins in each band on SDS-PAGE gels was not possible (data not shown). Therefore, a known quantity of glutenin proteins was employed in the present study as a quantitative standard under the premise that HMW-GS or LMW-GS from different wheat cultivars would have similar binding abilities with Coomassie Brilliant Blue R250. Inclusion of a known quantity of glutenin proteins in each gel as a quantitative standard also reduced the CV of the quantities of each glutenin subunit group determined among different gels for

a cultivar. This was because the method of normalization of glutenin subunit densitogram areas by a quantitative standard on the same gel reduced variations in staining intensity results caused by gel non-uniformity, and staining and destaining conditions.

When data from Tables II and I are compared, the estimated quantities of A subunits (% of patent flour protein) obtained by the densitometric method (Table II) were significantly lower than their counterparts from the precipitation method (Table I), and conversely, the average of the total LMW-GS (B + C subunits) in Table II was significantly higher than that in Table I. These statistical comparisons are reported in Table III. However, there were no significant differences between the averages of the total amount of extracted glutenin subunits (A+B+C) determined by these two methods (Table III).

When using the densitometer, B and C subunits can be measured separately. Among the three groups of subunits, statistically significant differences were observed with B subunits higher than A subunits and C subunits in amounts (Table II). The ratio of LMW-GS (B + C subunits) to HMW-GS (A subunits) was about 2.5. However, this value is still lower than those reported for bread wheats (Shewry et al 1992, Bushuk 1994). The differences among these may be due to variations in extraction methods, quantification techniques, cultivars and types of flour samples. Since gliadins have similar mobilities to the LMW-GS, the presence of gliadins in LMW-GS would increase the ratio of LMW-GS to HMW-GS. In the present method, the gliadins were

mostly removed by 60% ethanol. However, pre-extraction with 60% ethanol, similar to 50% 1-propanol, can remove not only gliadins but also some glutenins consisting of a relatively larger amount of LMW glutenin subunits and a smaller proportion HMW glutenin subunits from wheat flour proteins (Kruger et al 1988). Additionally, the extraction method used here cannot extract all of the glutenin proteins from the flours (Byers et al 1983, Kruger et al 1988). Also, the differences in LMW-GS to HMW-GS ratios among various classes of wheats need to be further investigated. Furthermore, in the present study, patent flour of 45% extraction rate was used for protein fractionation, and it is very likely that the protein composition differs from that of the straight-grade flour used for bread production or cookie-making.

Quantitative Comparison of Results From the Two Methods

Table III shows the comparative results of the two methods and Table IV lists the correlation coefficients for individual groups of glutenin subunits determined by these two methods. Although the results from the two methods showed some differences in the quantities of extractable HMW-GS and LMW-GS, the total quantity of extracted glutenin subunits determined was similar (Table III). In addition, HMW-GS, LMW-GS, and total glutenin subunits determined by each of the procedures were highly significantly correlated (Table IV).

These results indicate that the densitometric method using a known quantity of glutenin proteins for the quantification of other glutenin subunits is reliable, and compares well with the sequential acetone precipitation method (Table III). However, the densitometric method has many advantages over the sequential acetone precipitation method since it can determine quantities of not only A subunits, total LMW-GS, and total glutenin subunits, but also of B subunits, C subunits, and even individual glutenin subunits. Quantification of each glutenin subunit would allow us to further study their functions in flour end-use quality (Kolster et al 1992). Along with the use of SDS-PAGE, which allows easy identification of glutenin subunits via reference cultivars, this technique was further effectively adapted in our subsequent studies to relate both the quantity and quality of extractable glutenin subunits and gliadins in flour proteins to flour rheological and baking properties. Other important features that the densitometric method offers are its faster speed of determination and the requirement for only small amounts of material (20 mg) for analysis, both of which would be great assets for breeding programs screening early generation lines.

SUMMARY

Extractable glutenin subunit groups of 17 soft wheat patent flour samples with 45% extraction rate were quantitatively determined by the sequential acetone precipitation method and by the method of SDS-PAGE coupled with

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densitometry using a known quantity of glutenin proteins as a quantitative standard. The affinity of B+C subunits for Coomassie Brilliant Blue R250 is 1.3-fold higher than that of A subunits. There was no significant difference between the two methods in the total quantities of glutenin subunits measured. Among the three groups of glutenin subunits extracted, B subunits accounted for the highest amount in flour protein, followed by A subunits, with C subunits the lowest in amount, on the basis of the densitometric method. The ratio of extractable LMW-GS to HMW-GS was about 2.5 for these flours. Use of a known quantity of glutenin proteins as a quantitative standard for densitometric analysis of other glutenin subunits on SDS-PAGE gel improved the accuracy of this measurement. The method of SDS-PAGE coupled with densitometry and using a known quantity of glutenin standard can be an effective method due to its convenience, reliability, and the ability to simultaneously analyze multiple samples for protein quantification and identification. This technique may be very helpful for quick evaluation of wheat quality based on the composition and quantity of proteins and in breeding programs for screening early generation lines.

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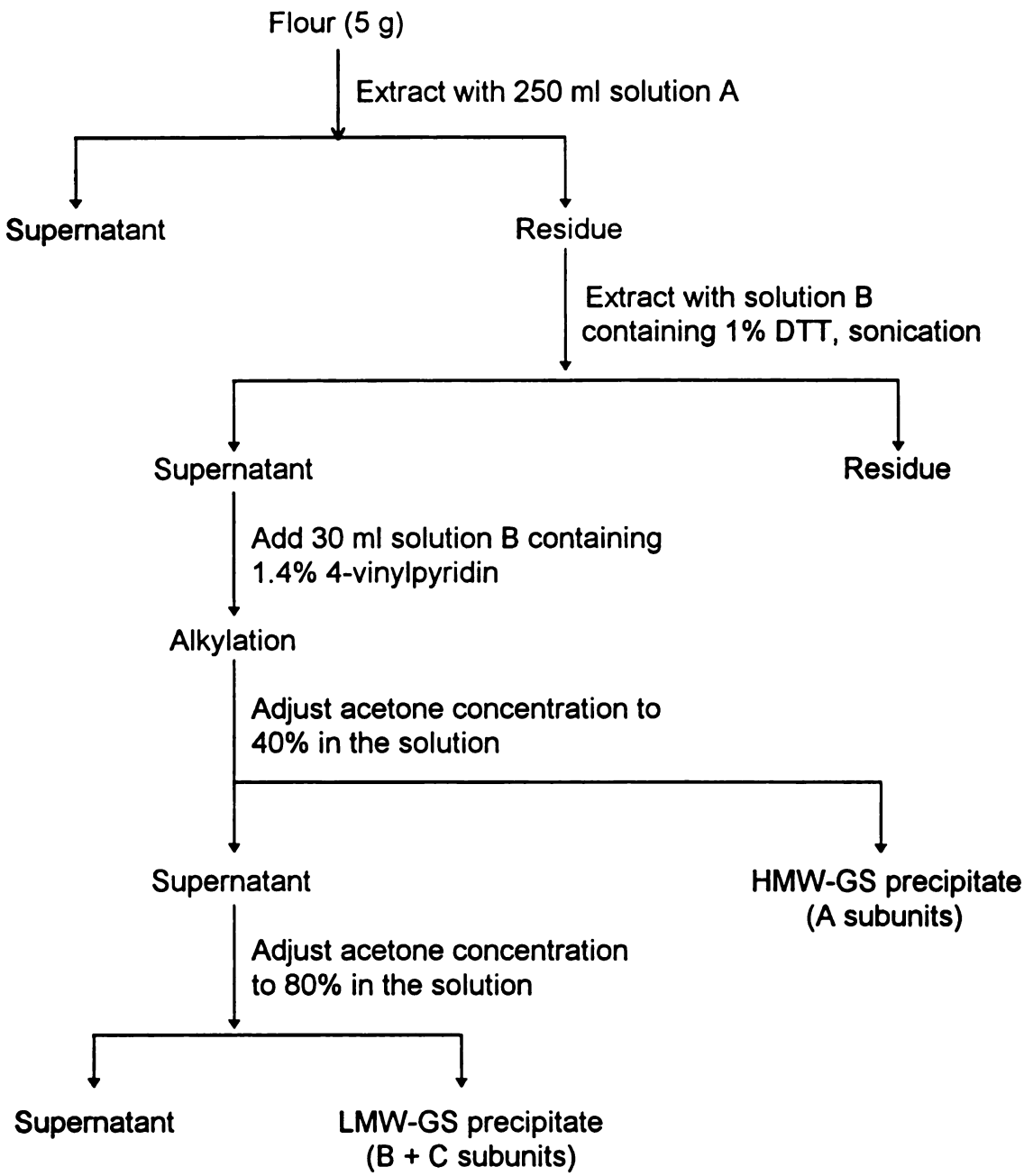

Figure 1. Schematic outline of the sequential acetone precipitation procedure to obtain high-molecular-weight glutenin subunit (HMW-GS or A subunits) and low-molecular-weight glutenin subunit (LMW-GS or B+C subunits) groups. This procedure is based on Melas et al (1994) with some modifications. Solution A is 60% aqueous ethanol solution, solution B is a 50% 2-propanol solution containing 0.08 M Tris-HCl buffer (pH 8.0), and DTT is dithiothreitol. For details, see text.

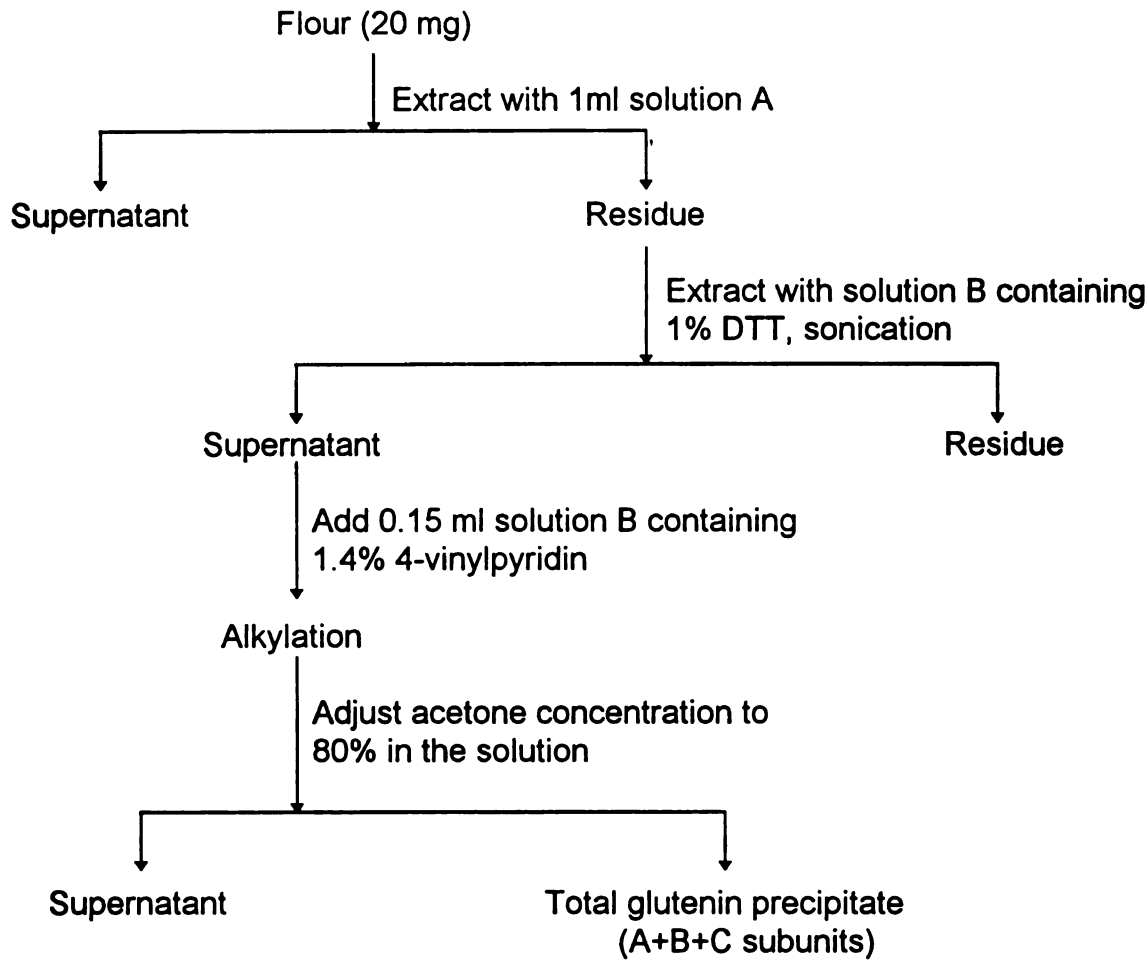

Figure 2. Schematic outline of the extraction procedure to obtain relatively pure total glutenin proteins (A+B+C subunits) for densitometric analysis. Solution A is 60% aqueous ethanol solution, solution B is a 50% 2-propanol solution containing 0.08 M Tris-HCl buffer (pH 8.0), and DTT is dithiothreitol. For details, see text.

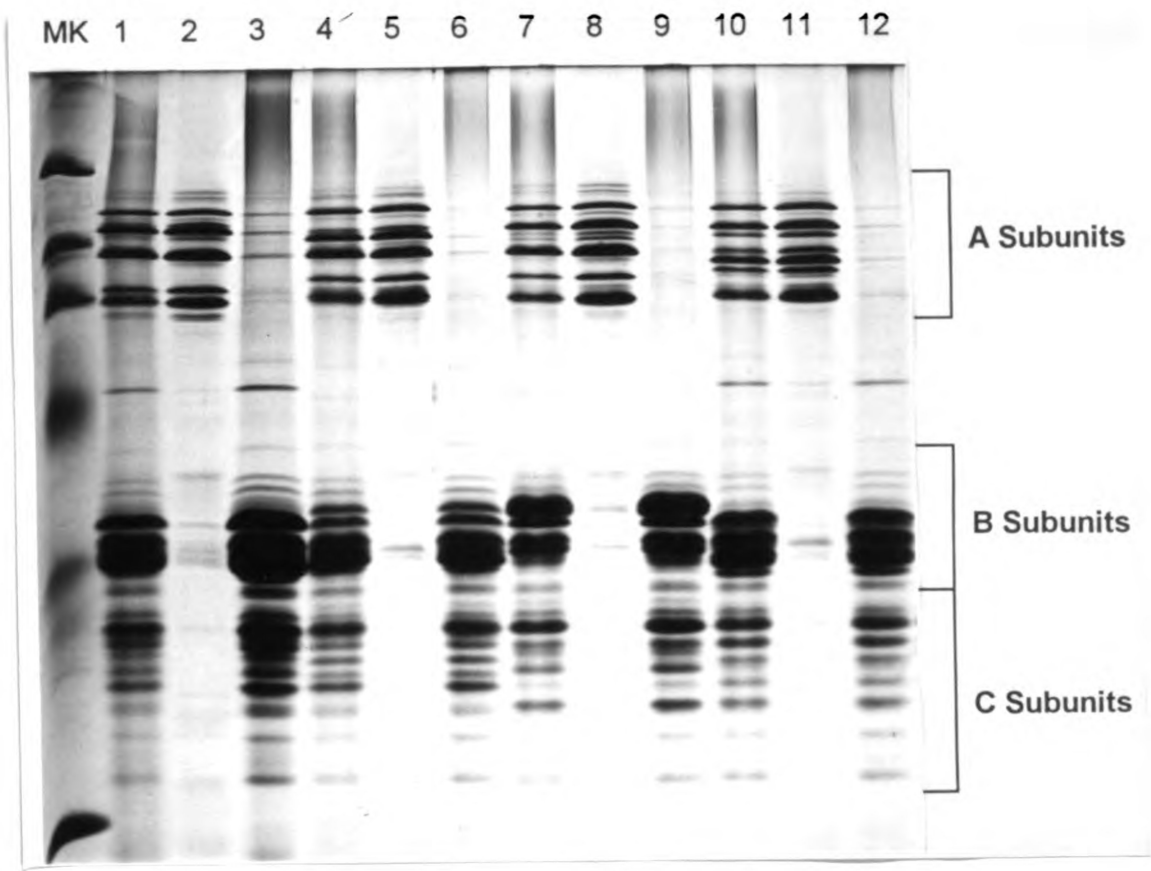

Figure 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of high-molecular-weight glutenin subunit (HMW-GS or A subunits) and low-molecular-weight glutenin subunit (LMW-GS or B + C subunits) groups obtained by sequential acetone precipitation (40% and 80% acetone, respectively). Total glutenin subunits of each cultivar precipitated directly from the extracts of glutenins by 80% acetone were included as controls. The wheat cultivars are: Augusta (lanes 1-3), Caldwell (lanes 4-6), Chelsea (lanes 7-9), and Clark (lanes 10-12). Lanes 1, 4, 7, and 10 = total glutenins; lanes 2, 5, 8, and 11 = HMW-GS groups; lanes 3, 6, 9, and 12 = LMW-GS groups. MK = Molecular markers with molecular weights (from top to bottom) of 205 kDa, 116 kDa, 97.4 kDa, 66 kDa, 45 kDa and 29 kDa.

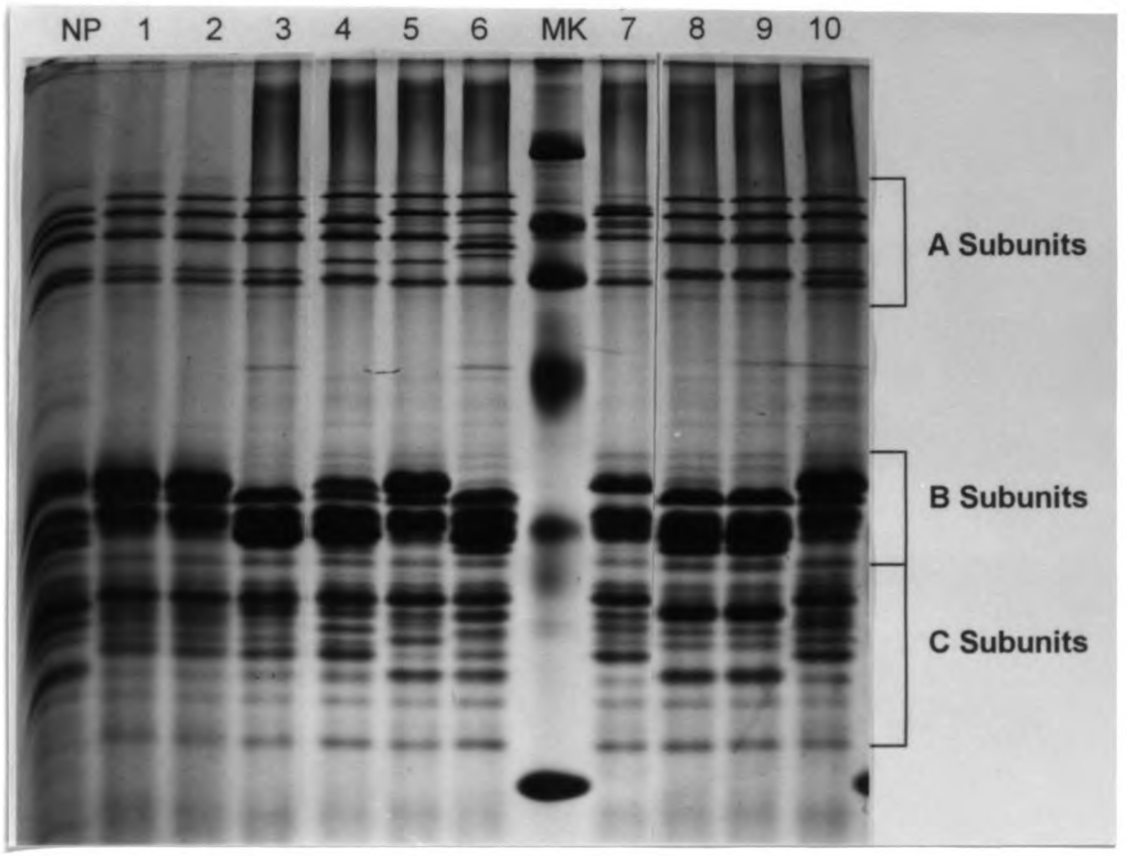

Figure 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of total glutenin subunits obtained from 80% acetone precipitation of eight soft wheat cultivars for densitometric analysis. The wheat cultivars are: Augusta (lane 3), Caldwell (lane 4), Chelsea (lane 5), Clark (lane 6), Crew (lane 7), Dynasty (lane 8), Excel (lane 9), and Frankenmuth (lane 10). NP = Neepawa (a Canadian cultivar used as a marker for high-molecular-weight glutenin subunits). Lanes 1 and 2 = glutenins of cultivar Frankenmuth used as protein standards for glutenin subunit quantification. MK = Molecular markers with molecular weights (from top to bottom) of 205 kDa, 116 kDa, 97.4 kDa, 66 kDa, 45 kDa and 29 kDa. A subunits = high-molecular-weight glutenin subunits; B and C subunits = low-molecular-weight glutenin subunits.

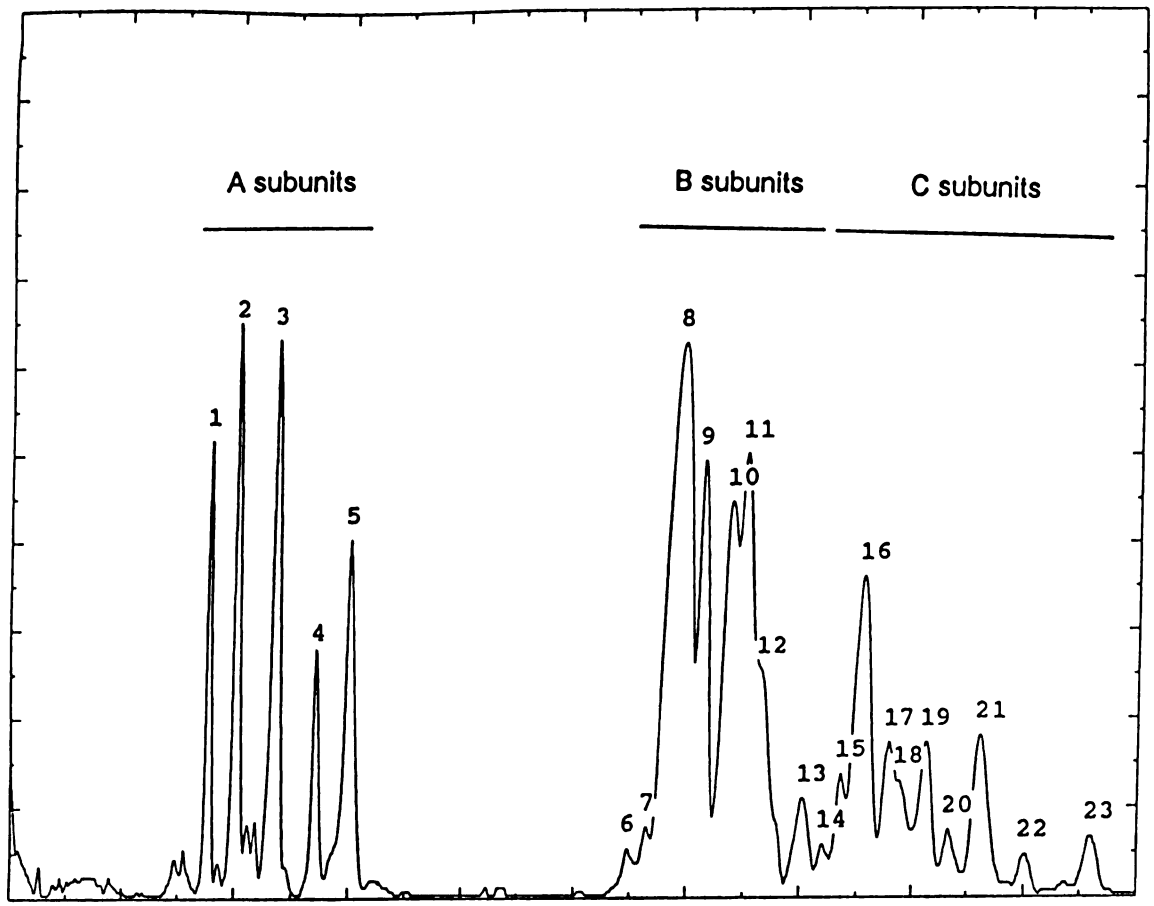

Figure 5. A densitometric reading of glutenin subunits from cultivar Chelsea. Peaks 1-5 are high-molecular-weight glutenin subunits (A subunits), peaks 6-13 are B subunits, and peaks 14-23 are C subunits. B and C subunits are low-molecular-weight glutenin subunits.

Table I

Quantity of Extractable HMW-GS[a] and LMW-GS[a] in 17 Patent Flour Samples Determined after Sequential Acetone Precipitation Method

Cultivar	Flour Protein (%)[c]	A Subunits[b] (%)[d]	B + C Subunits[b] (%)[d]	A+B+C Subunits[b] (%)[d]	(B+C)/A[b]
Augusta[e]	7.1	7.22	16.24	23.46	2.25
Caldwell[f]	7.6	9.61	16.50	26.11	1.72
Chelsea[e]	7.2	8.27	14.68	22.95	1.78
Clark[f]	7.3	9.57	15.78	25.35	1.65
Crew[e]	7.2	9.40	13.70	23.10	1.46
Dynasty[f]	7.9	8.00	16.54	24.54	2.07
Excel[f]	7.3	9.89	15.50	25.39	1.57
Frankenmuth[e]	8.2	8.20	15.84	24.04	1.93
Freedom[f]	7.8	9.16	14.85	24.01	1.62
Hyak[g]	6.7	9.18	16.94	26.12	1.85
Kmor[e]	7.8	10.36	14.12	24.48	1.36
Lewjain[e]	8.1	8.27	16.78	25.05	2.03
Madsen[e]	8.9	7.49	13.84	21.33	1.85
Malcom[e]	7.7	7.25	15.48	22.73	2.14
Rely[g]	8.2	9.84	15.55	25.39	1.58
Stephens[e]	8.3	6.91	14.36	21.27	2.08
Tres[g]	8.7	5.27	13.20	18.47	2.50
Mean	7.8	8.46	15.29	23.75	1.85

[a] HMW-GS = high-molecular-weight glutenin subunits; LMW-GS = low-molecular-weight glutenin subunits.
[b] A subunits = HMW-GS; B + C subunits = total LMW-GS; A+B+C = total glutenin subunits; (B+C)/A = LMW-GS to HMW-GS ratio.
[c] 14% moisture basis.
[d] Percentage of patent flour protein (14% m.b.).
[e] Soft white wheat.
[f] Soft red wheat.
[g] Club wheat.

Table II

Quantity of Glutenin Subunits Per Group Determined by Densitometer

Cultivars	A Subunits[a] (%)[b]	B Subunits[a] (%)[b]	C Subunits[a] (%)[b]	B+C Subunits[a] (%)[b]	A+B+C Subunits[a] (%)[b]	(B+C)/A
Augusta	6.98	10.83	6.18	17.01	23.99	2.44
Caldwell	8.47	11.07	6.86	17.93	26.40	2.12
Chelsea	6.67	11.51	5.70	17.21	23.88	2.58
Clark	7.45	11.67	7.32	18.99	26.44	2.55
Crew	7.19	9.60	6.44	16.04	23.23	2.23
Dynasty	7.12	11.48	6.41	17.89	25.01	2.51
Excel	7.65	10.86	6.29	17.15	24.80	2.24
Frankenmuth	7.33	11.54	7.73	19.27	26.60	2.63
Freedom	8.12	9.14	7.26	16.40	24.52	2.02
Hyak	7.55	10.59	7.18	17.77	25.32	2.35
Kmor	11.28	10.10	6.35	16.45	27.73	1.46
Lewjain	8.95	10.72	7.06	17.78	26.73	1.99
Madsen	5.83	8.03	6.19	14.22	20.05	2.44
Malcom	6.23	9.54	6.80	16.34	22.57	2.62
Rely	7.51	9.64	8.26	17.90	25.41	2.38
Stephens	5.23	9.23	7.21	16.44	21.67	3.14
Tres	3.87	7.58	7.87	15.45	19.32	3.99
Mean[c]	7.26 a	10.18 b	6.89 a	17.07	24.33	2.45

[a] Abbreviations same as Table I except no distinction between B and C subunits on Table I.
[b] Percentage of patent flour protein (14% m.b.).
[c] Values within this row with different letters are significantly different at the 5% level.

Table III

Comparison of the Mean Quantities of Extractable Glutenin Subunit Groups Determined by Precipitation and Densitometric Methods

Parameters	Precipitation (%)[c]	Densitometer (%)[c]	t[a,b]
Extractable glutenin subunit groups			
HMW-GS[d]	8.46	7.26	5.25***
LMW-GS[d]	15.29	17.07	8.39***
Total glutenin subunits	23.75	24.33	2.11 ns

[a] Paired "t" test between two groups of data.
[b] ***, significant at the 0.1% level; ns, not significant at the 5% level.
[c] Percentage of patent flour protein (14% m.b.), each value is the mean of 17 samples.
[d] HMW-GS = high-molecular-weight glutenin subunits; LMW-GS = low-molecular-weight glutenin subunits.

Table IV

Correlation Coefficients[a] for the Quantities of the Extracted Groups of Glutenin Subunits for 17 Patent Flour Samples Determined by Two Methods

Parameter[b]	A	BC	ABC	A'	B'	C'	B'C'	A'B'C'
A	1							
BC	0.281	1						
ABC	0.833***	0.765***	1					
A'	0.807***	0.357	0.747***	1				
B'	0.466	0.717**	0.726***	0.472	1			
C'	-0.085	0.079	-0.017	-0.179	-0.238	1		
B'C'	0.408	0.739***	0.700**	0.363	0.846***	0.316	1	
A'B'C'	0.762***	0.634**	0.877***	0.869***	0.769***	0.047	0.777***	1

[a] **, ***, significant at the 1% and 0.1% levels, respectively.

[b] A, BC, ABC = A subunits (HMW-GS), B + C subunits (total LMW-GS), and A + B + C subunits (total glutenin subunits), respectively, determined by the sequential acetone precipitation method; A', B', C', B'C', A'B'C' = A subunits (HMW-GS), B subunits (LMW-GS), C subunits (LMW-GS), B + C subunits (total LMW-GS), and A + B + C subunits (total glutenin subunits), respectively, determined by the densitometric method.

CHAPTER 4. RHEOLOGICAL AND BAKING PROPERTIES OF U.S. SOFT WHEAT FLOURS. I. EFFECTS OF QUANTITY OF GLIADIN SUBGROUPS

Rheological and Baking Properties of U.S. Soft Wheat Flours.

I. Effects of Quantity of Gliadin Subgroups

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ABSTRACT

The gliadin subgroups (ω-, γ-, β-, and α-gliadins) in 17 soft wheat patent flours and seven straight-grade flours were identified and quantified by acid-polyacrylamide gel electrophoresis (Acid-PAGE) coupled with densitometry. Flour rheological properties were evaluated by alveograph, farinograph and mixograph tests. Japanese-type sponge cakes and AACC sugar-snap cookies were made to evaluate the flours' baking performances. The results showed that the percentages of individual gliadin subgroups in flour protein were significantly lower in the patent flours than in the straight-grade flours. Statistical analyses revealed that the patent flours exhibited significantly weaker dough properties and better cookie-baking quality than their counterpart straight-grade flours. The quantities of some individual gliadin subgroups and total gliadins were shown to affect the various rheological properties. Significant negative correlations were found between the quantities (%) of ω-gliadins, γ-gliadins and total gliadins and cake volume, and between the quantities (%) of ω-gliadins and total gliadins and cake volume per unit flour protein. The quantities (%) of α-gliadins and total gliadins also correlated negatively with the cookie diameter per unit flour protein.

INTRODUCTION

Gluten proteins, also called storage proteins, consist of two major types of proteins: gliadins and glutenins. They account for about 70% of the total wheat flour proteins (Kasarda et al 1976). Gluten is formed when wheat flour is wetted with water and interaction occurs between the gliadins and glutenins (Wrigley and Bietz 1988). Gluten proteins are important in determining flour end-use properties because of their unique ability to form viscoelastic doughs. In general, gliadins are believed to contribute to dough extensibility and glutenins to dough strength and elasticity (Wall 1979).

Previous research has shown that low gluten content and weak gluten strength are generally desired for good sugar-snap cookie baking (Gaines and Finney 1989, Kulp and Olewnik 1989, Gaines 1990, Kaldy et al 1993, Souza et al 1994). However, little information is available regarding the contribution of each protein fraction in gluten to soft wheat flour end-use quality.

Quantification of gliadin subgroups (ω-, γ-, β-, and α-gliadins) has been carried out using reversed-phase high-performance liquid chromatography (RP-HPLC), but no values for the actual amount of each gliadin subgroup have been reported except for peak areas of chromatogram regions (Wieser et al 1994). Additionally, in RP-HPLC the α- and β-gliadins cannot be separated well in some cases (Wieser et al 1994) in contrast to acid-polyacrylamide gel electrophoresis (Acid-PAGE) by which all gliadin subgroups are well separated (Lookhart and Albers 1988, Bushuk and Sapirstein 1990).

Quantitative determination of gliadin subgroups and glutenin subunits by electrophoresis coupled with densitometry has been widely applied (Branlard and Dardevet 1985, Brunori et al 1990, Gupta and MacRitchie 1994, Kolster and Vereijken 1994, Mosleth et al 1994, Peltonen and Virtanen 1994, Hou and Ng 1995). Its popularity for determining the protein content is due to its speed, small sample size, convenience and reliability (Hou and Ng 1995). This method was, therefore, adapted in the present study with some modifications to quantify the gliadin subgroups in soft wheat flour proteins. A known quantity of modified Osborne gliadins of a soft wheat flour was used as a quantitative standard. The objectives of the present study were (1) to determine the relative quantities of gliadin subgroups present in soft wheat patent flours and straight-grade flours by Acid-PAGE coupled with densitometry, (2) to compare the differences in rheological and baking properties between patent flours and straight-grade flours milled from the same cultivars, and (3) to investigate the relative quantity of gliadin subgroups in relation to patent flour rheological properties and baking performance.

MATERIALS AND METHODS

Wheat Samples

Seventeen soft wheat cultivars harvested in 1992 or 1993 were selected for this study. These cultivars cover all classes of soft wheats produced in the U.S.. The samples were milled on a Miag-Multomat Mill to obtain patent flours of

45% extraction rate. The protein contents of these flour samples ranged from 6.7% to 8.9% (Yamamoto et al 1995, also see Appendix I). Seven of the 17 cultivars (Augusta, Caldwell, Chelsea, Dynasty, Freedom, Hyak and Lewjain) were also milled on a Chopin Mill to obtain straight-grade flours. The protein contents of these flours ranged from 7.4% to 8.8%.

Chemicals and Reagents

Acrylamide (>99% purity) was from Boehringer Mannheim Corporation (Indianapolis, IN). Ascorbic acid, Commassie Brilliant Blue R250, ferrous sulfate, N,N'-methylenebisacrylamide and silver nitrate were from Sigma Chemical Company (St. Louis, MO). Ammonium persulfate (crystal), hydrogen peroxide (30%, AR) and trichloroacetic acid (TCA) were from J.T. Baker Inc. (Phillipsburg, NJ). Aluminum lactate was from Fluka Chemika-Biochemika (CH-9470 Buchs/Switzerland). Lactic acid (85%, ACS grade) and potassium hydroxide were from Columbus Chemical Industries Inc. (Columbus, WI). Distilled and deionized water was used throughout the study.

Gliadin Preparation and Acid-PAGE

The extraction of gliadin proteins from flours was performed according to the following procedure. One hundred mg of flour was placed into a microcentrifuge tube, after which 0.4 ml of 60% ethanol was added. The 60% ethanol was shown to be one of the most efficient ethanol solutions for gliadin

extraction (Wieser et al 1994). The extraction was started by first vortexing for 2 min, followed by immersing the tube in a 40°C water bath with shaking for 30 min. During this period, the sample was vortexed four times at 0, 10, 20, and 30 min. The contents were then centrifuged for 6 min at 14,000 x g at room temperature, and the supernatant was collected. This extraction process was repeated one more time. In the third extraction process, 0.2 ml of 60% ethanol was used, while other conditions remained the same. The three supernatants were pooled and ethanol was partially evaporated in an air circulating oven at 30°C until the total liquid volume was reduced by half. The partially concentrated supernatant was frozen after addition of a small quantity of distilled and deionized water, and then freeze-dried. The freeze-dried material was redissolved in 250 μl of 60% ethanol at 40°C, and underwent 1 min of ultrasonication in a sonicating water bath at room temperature to give a sample solution. Two hundred fifty μl of extract dilution solution (40% w/v sucrose and 0.5% w/v methyl green dye in pH 3.1 0.25% w/v aqueous aluminum buffer) was finally added into the sample solution for Acid-PAGE analysis.

Gliadins of cultivar Pioneer 2555 were extracted by 60% ethanol from the residue after removing albumins and globulins by 0.5 M NaCl solution at 4°C according to the modified Osborne sequential fractionation method (Chen and Bushuk 1970). This protein fraction was then used as a quantitative standard for quantification of gliadin subgroups by Acid-PAGE coupled with densitometry. The protein content of the modified Osborne gliadin fraction was determined by

the micro-Kjeldahl method (AACC 1992). The concentration of gliadin proteins in the standard solution was 5.42 μg/μl. Additionally, a Canadian cultivar, Neepawa, was fractionated and prepared according to Sapirstein and Bushuk (1985) and used as a reference for the identification of gliadin subgroups.

Acid-PAGE at pH 3.1 was performed according to Lafiandra and Kasarda (1985) with some modifications. Electrophoresis was carried out in 1.5 mm thick gels (18 cm wide, 16 cm long) with a vertical electrophoresis apparatus (Hoffer Scientific Instruments, San Francisco, CA) at a constant current of 45 mA/gel for a total of 3 hr and 15 min including 1 hr prerunning before preparing stacking gel. After completion of a run, gels were removed from glass plates and stained in a container with a staining solution of 4% v/v stock dye solution (1% w/v Coomassie Brilliant Blue R250 in 95% v/v aqueous ethanol) in 12% w/v aqueous trichloroacetic acid (TCA) (Sapirstein and Bushuk 1985) for 18 hr. Gels were then rinsed in water containing a few drops of Triton X-100 to remove surface stains before gel scanning for gliadin quantification and photography.

In the present study, all except two wells on each gel were loaded with 10 μl of a sample solution; a 10 μl aliquot of gliadin standard solution was loaded into each of the two remaining wells. Electrophoresis was carried out twice for each sample on separately run gels.

Identification and Quantification of Gliadin Subgroups by Densitometer

After rinsing the gels, the electrophoretic patterns were analyzed by a GS 300 Transmittance/Reflectance Scanning Densitometer with GS 365W software (Hoefer Scientific Instruments, San Francisco, CA). Gliadin patterns on the electrophoregrams were divided into four major groups: ω-, γ-, β-, and α-gliadins based on the method of Bushuk and Sapirstein (1990).

The relative quantity (called quantity hereinafter) of each group of gliadins was calculated from their respective areas on the densitograms with the aid of an "internal standard", a known quantity of the modified Osborne gliadin fraction of cultivar Pioneer 2555 on the same gel. The "internal standard" was used to calculate the quantity of protein per unit area of densitogram for each gliadin subgroup. Each PAGE pattern was scanned twice, using duplicate gels, and the mean values (n=4) of the quantity of each gliadin subgroup were reported as percentage of flour protein.

Flour Quality Evaluation

Data of flour protein content determinations, flour alveograph, farinograph and mixograph tests, Japanese-type sponge cakes and micro sugar-snap cookies for the 17 cultivars were obtained from an earlier study (Yamamoto et al 1995, see Appendix I). Flour protein content determinations, mixograph and micro sugar-snap cookie-baking tests of the seven straight-grade flours were conducted according to AACC Approved Methods (AACC 1992).

Statistical Analyses

Data were subjected to ANOVA, paired "t" test, and correlation analyses on a Microsoft Excel program (Cambridge, MA).

RESULTS AND DISCUSSION

Acid-PAGE of Gliadin Proteins from Soft Wheat Cultivars

Figure 1 shows the Acid-PAGE patterns of gliadin proteins for the 17 patent flour samples used in this study. Genotypical differences were evident among the cultivars except for two cultivars, Dynasty (lane 8) and Excel (lane 9). These two cultivars have very similar gliadin banding patterns.

Based on the relative mobility of gliadin proteins of the Canadian cultivar Neepawa on Acid-PAGE, the gliadins of each cultivar were divided into four subgroups, ω-, γ-, β-, and α-gliadins (Bushuk and Sapirstein 1990). There seem to be clear boundaries between adjacent gliadin subgroups, as seen from Acid-PAGE (Fig. 1).

Quantification of Gliadin Subgroups in Soft Wheat Flours

The quantities of each gliadin subgroup determined for the 17 patent flour samples are listed in Table I. Significant differences were observed among quantities of the four gliadin subgroups in patent flour, with the β-gliadins present in highest quantity (15.24% of flour protein), followed by γ-gliadins

(13.60% of flour protein), then α-gliadins (9.21% of flour protein), and the ω-gliadins present in the lowest quantity (5.06% of flour protein). These observations are generally consistent with the results of Wieser et al (1994) using RP-HPLC, except that they combined α- and β-gliadins together for their analyses, and those of Branlard and Dardevet (1985) using densitometry coupled with Acid-PAGE for bread wheat flours. In the present study, the percentage of total gliadins in the patent flour protein ranged from 36.35% to 52.10% with an average of 43.11% (Table I).

Table II shows the ranges of quantities of gliadin subgroups present in seven straight-grade flours. The mean values determined for the ω-, γ-, β-, and α-gliadins in these seven flour samples were 6.17%, 17.28%, 19.15% and 10.65% of flour proteins, respectively. The total quantity of gliadins varied from 44.15% to 59.52% of flour protein with an average of 53.25%. Statistical results indicated that the quantities of each gliadin subgroup and total gliadins were significantly higher in seven straight-grade flours than in their counterpart patent flours (Table II). These differences may be caused by the different extraction rates of these two types of flours.

Mixograph and Sugar-Snap Cookie Baking Properties of Seven Soft Wheat Straight-Grade Flours

Table III shows the minimum, maximum and mean values of mixograph parameters and sugar-snap cookie-baking quality of the seven straight-grade flours. The ranges for mixograph parameters measured were: peak time 2.5-3.8 (min), peak height 33.0-43.2 (mm), stability time 3.2-7.9 (min) and tolerance 5.0-13.0 (mm). Their mean values were 3.1 (min), 38.9 (mm). 5.1 (min) and 8.4 (mm), respectively. The sugar-snap cookie diameter varied from 8.08 to 8.68 (cm) with a mean value of 8.43 (cm), the cookie thickness was 7.5 to 8.4 (mm), and the spread factor (ratio of diameter to thickness) was 9.9 to 11.5. The values derived from mixograms for these flours seemed to be within the ranges of the 17 patent flours described by Yamamoto et al (1995), however, the average cookie diameter was smaller, and cookie thickness and spread factor larger than those of patent flours.

Further statistical analyses were conducted to compare the quality differences between patent flour and straight-grade flour samples from the seven cultivars (Table IV). In addition to their significantly higher content of each gliadin subgroup and total gliadins as shown in Table II, the straight-grade flour samples, as expected, are significantly higher in flour protein content (Table IV) confirming the results of previous studies (Farrand 1974, Finney et al 1981). Farrand and Hinton (1974) reported that there is a protein gradient from inner to outer endosperms with outer endosperm having much higher protein content

than inner endosperm. Patent flour, being milled mainly from inner endosperm, was expected to be lower in protein content. It is perhaps because of these differences, the straight-grade flour showed significantly larger mixograph peak height and shorter peak time than did patent flour. However, mixograph stability and tolerance were not significantly different between these two types of flours. In another study (Yamamoto et al 1995) using the same 17 soft wheat cultivars, we found that soft wheat patent flours exhibiting longer mixograph peak times and shorter peak heights could produce bigger diameter sugar-snap cookies. It was therefore anticipated that the straight-grade flours would make smaller sugar-snap cookies, and in fact they did produce cookies with significantly smaller cookie diameter and spread factor and larger thickness than did patent flours (Table IV). Since the straight-grade flour had higher protein content and produced a smaller diameter cookie, the cookie diameter per unit flour protein for straight-grade flour was significantly smaller than that for patent flour.

For good sugar-snap cookie baking, a soft wheat flour with lower starch damage and smaller particle size is desired (Yamazaki 1959a, 1959b, Gaines et al 1988, Yamamoto et al 1995). Since patent flour has finer particle size and less damaged starch than straight-grade flour (Finney 1989), it is also expected to be of better cookie- baking quality.

Relationship Between Quantities of Gliadin Subgroups and Soft Wheat Patent Flour Quality

Table V shows the significant correlation coefficients between patent flour properties and relative amounts of gliadin subgroups and total gliadins present in the flour protein. The amount of β-gliadins correlated negatively with alveograph W value, indicating that they may have a function of weakening the dough. The amounts of α-gliadins and total gliadins correlated negatively with mixograph peak time and stability values, whereas the amounts of ω-gliadins, α-gliadins and total gliadins were positively correlated with mixograph peak height values. A significant negative correlation was also found between the amount of ω-gliadins and mixograph tolerance value. In addition to these correlations, the amounts of γ-gliadins and total gliadins were significantly positively correlated with farinograph peak time, and the γ-gliadins negatively correlated with farinograph tolerance index value as well. These results indicate that the quantities of these gliadin subgroups could affect the dough rheological properties, verifying some results reported previously (Branlard and Dardevet 1985, Wieser et al 1994).

Yamamoto et al (1995), using the same set of materials, reported that good quality Japanese-type sponge cakes and sugar-snap cookies were made from soft wheat patent flours with longer mixograph peak times and stability times and shorter mixograph peak height values. For soft wheat flours, a lower

farinograph water absorption was also associated with larger spread sugar-snap cookies (Nemeth et al 1994, Yamamoto et al 1995) and shorter farinograph peak time was associated with good quality Japanese-type sponge cakes (Yamamoto et al 1995). Since the quantities of certain gliadin subgroups in the flour protein could be important in determining dough rheological properties (Table V), they could also influence the flour baking qualities. Indeed, it was found that the Japanese-type sponge cake volume correlated negatively with quantities of ω-gliadins, γ-gliadins and total gliadins in the flour protein (Table V). When the influence of total flour protein content on flour baking quality was eliminated by dividing cake volume by total flour protein content, the cake volume per unit flour protein was still significantly negatively correlated with the amounts of ω-gliadins and total gliadins in the flour protein. Although the cookie spread did not directly relate to the amounts of any of the gliadin subgroups nor to total gliadins in the flour protein, the amounts of α-gliadins and total gliadins in the flour protein correlated negatively with cookie diameter per unit flour protein (Table V). This seems to be supported by data showing that the patent flour had better cookie-baking quality than straight-grade flour (Table IV), since the straight-grade flour protein contained significantly higher amount of gliadins than patent flour (Table II). Even though the flour protein content also demonstrated significant correlations with mixograph properties, it did not significantly correlate with flour baking quality (Yamamoto et al 1995), indicating that not the quantity of total flour protein but the relative amounts of gliadin subgroups and glutenin subunits,

and the type of glutenin subunits (Hou et al 1995) are important in determining the soft wheat flour end-use quality.

Further confirmation of the effects of the certain gliadin subgroups on soft wheat baking qualities could be realized by addition of each of those gliadin subgroups to a base flour to do a baking test. Newly developed techniques for separation of large quantities of undenatured gliadin fractions would make this investigation feasible (Weegels et al 1994). This kind of fortification study would also help us to clarify whether the negative effects of the quantities of gliadin proteins on soft wheat baking quality was caused by the relative quantity of some low-molecular-weight glutenin subunits (LMW-GS). It was found using the same set of materials that the quantity of extractable C subunits (a group of LMW-GS) significantly correlated negatively with sponge cake volume and cake volume per unit flour protein (Hou et al 1995). When the quantities of gliadin subgroups of the 17 patent flours obtained from the present study were correlated with the quantity of extractable C subunits for the same 17 flour samples from our other study (Hou et al 1995), it was interestingly noticed that the quantity of total gliadins was positively correlated with the quantity of extractable C subunits ($r=0.531$, $P<0.05$). However, this incidental correlation should be further confirmed with more cultivars.

Results from the present gliadin study and the glutenin study in our companion paper (Hou et al 1995) for the same 17 patent flours indicated that both the quantities of certain gliadin subgroups and the type and quantities of

glutenin subunits are functional in flour end-use quality. The balance of elasticity and extensibility in doughs was said to be determined by the type of glutenin subunits (Payne et al 1991) and by the quantities of glutenin subunits and gliadins (Gupta and MacRitchie 1994). Therefore, any factors that break this balance would alter dough properties, leading to detrimental results in baking quality.

SUMMARY

Gliadin subgroups of 17 soft wheat patent flours of 45% extraction rate and seven straight-grade flours were quantified by Acid-PAGE coupled with densitometry. The rheological and baking properties of these flours were evaluated. The determined percentages of individual gliadin subgroups and total gliadins in flour protein were significantly lower in the patent flours than those in straight-grade flours, which may result from the different extraction rates of these two types of flours. Significant differences were also observed in rheological and baking properties between patent flours and straight-grade flours. Patent flours had significant lower protein contents, longer mixograph peak times and shorter mixograph peak height values than their counterpart straight-grade flours. Accordingly, patent flours exhibited better sugar-snap cookie-baking quality. The quantities of some individual gliadin subgroups or total gliadins were shown to affect various rheological properties. Significant negative correlations were found between the percentage of ω-gliadins, γ-

gliadins or total gliadins in flour protein and cake volume, and between the percentage of ω-gliadins or total gliadins and cake volume per unit flour protein. The percentage of α-gliadins or total gliadins in flour protein also correlated negatively with the cookie diameter per unit flour protein.

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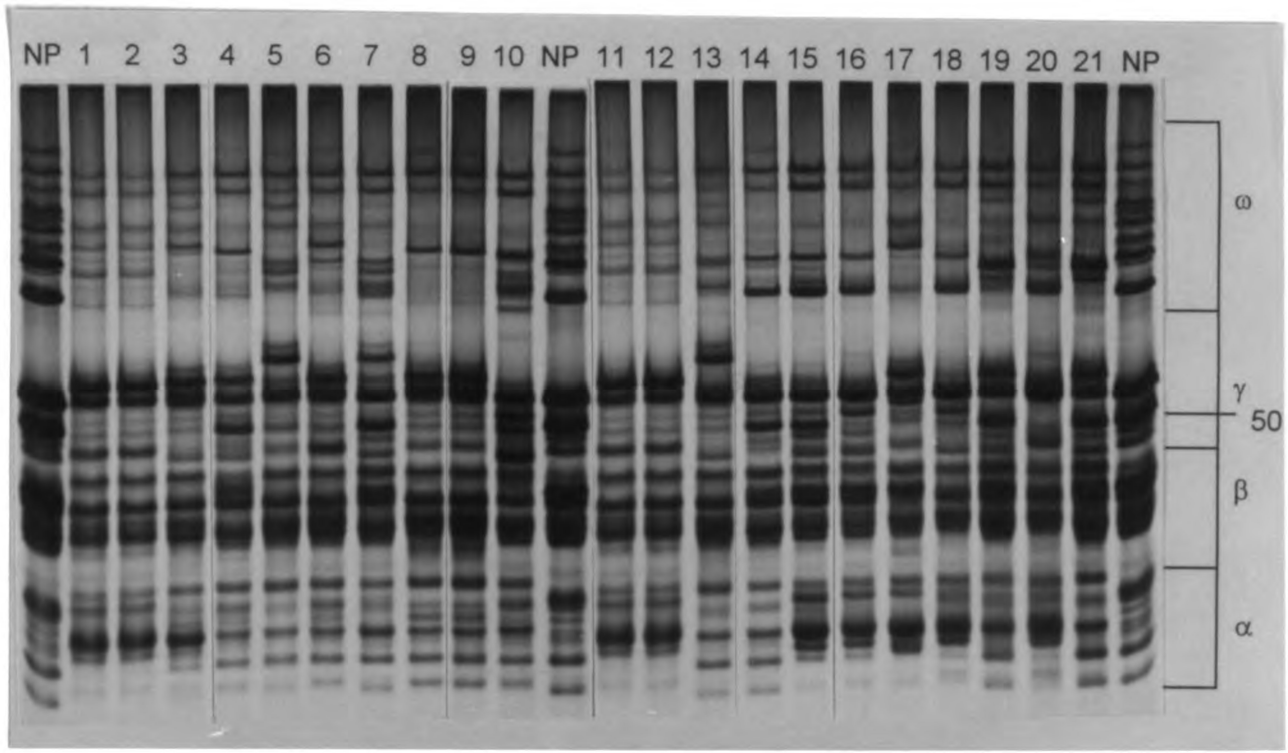

Figure 1. Acid-PAGE patterns of 17 soft wheat cultivars. Lanes 1, 2, 11, and 12=protein standard (cultivar Pioneer 2555) for gliadin subgroup quantification; 3=Augusta; 4=Caldwell; 5=Chelsea; 6=Clark; 7=Crew; 8=Dynasty; 9=Excel; 10=Freedom; 13=Frankenmuth; 14=Hyak; 15=Kmor; 16=Lewjain; 17=Madsen; 18=Malcom; 19=Rely; 20=Stephens; 21=Tres; NP=Neepawa (reference). 50=Reference band of cultivar Neepawa. ω, γ, β, and α indicate ω-, γ-, β-, and α-gliadin subgroups, respectively, based on the method of Bushuk and Sapirstein (1990).

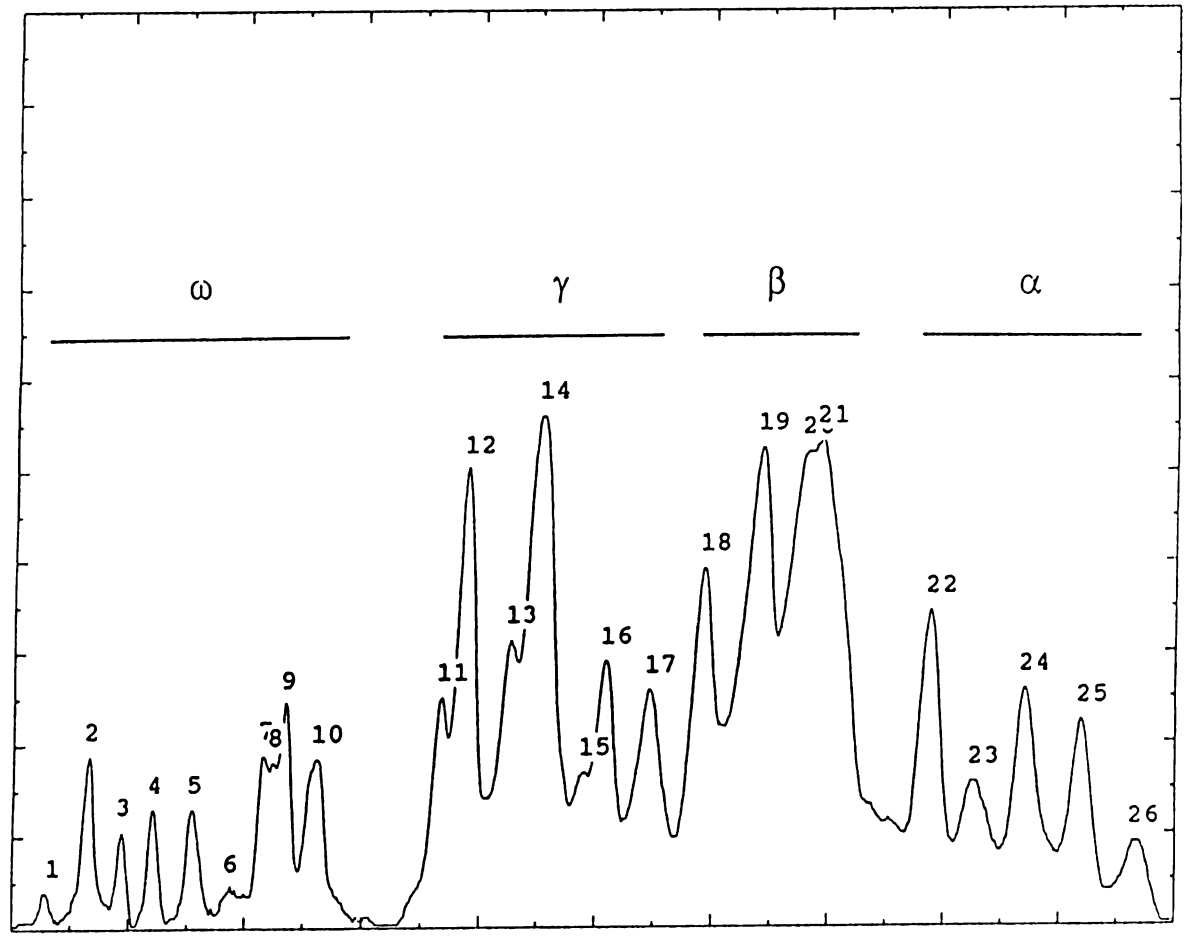

Figure 2. Densitometric readings of gliadin proteins from cultivar Chelsea. Peaks 1-10 are ω-gliadins; peaks 11-17 are γ-gliadins; peaks 18-21 are β-gliadins; and peaks 22-26 are α-gliadins.

Table I

Quantities of Gliadin Subgroups Determined by Densitometric Method for 17 Soft Wheat Patent Flours

Cultivar	ω[a] (%)[b]	γ[a] (%)[b]	β[a] (%)[b]	α[a] (%)[b]	Total Gliadins (%)[b]
Augusta[c]	3.02	13.07	11.25	9.80	37.14
Caldwell[d]	3.92	12.56	13.41	7.35	37.24
Chelsea[e]	4.23	12.55	14.56	6.66	38.00
Clark[d]	4.38	14.10	14.30	8.21	41.00
Crew[e]	5.70	14.70	19.49	8.22	48.10
Dynasty[d]	2.20	11.12	15.94	7.10	36.35
Excel[d]	3.48	12.59	16.00	8.94	41.00
Frankenmuth[c]	4.43	12.80	17.85	8.60	43.68
Freedom[d]	7.63	15.36	14.80	8.47	46.26
Hyak[e]	4.59	13.82	13.96	6.01	38.39
Kmor[e]	8.03	14.14	14.92	11.72	48.80
Lewjain[e]	5.02	15.03	13.28	9.08	42.40
Madsen[e]	4.47	12.97	13.84	9.89	41.17
Malcom[e]	5.37	14.08	14.79	12.50	46.74
Rely[e]	7.05	16.78	16.95	11.32	52.10
Stephens[e]	5.93	11.60	13.42	13.62	44.57
Tres[e]	6.64	13.89	20.30	9.02	49.86
Mean[f]	5.06 a	13.60 c	15.24 d	9.21 b	43.11

[a] ω, γ, β, and α indicate ω-, γ-, β-, and α-gliadins, respectively.
[b] Percentage of patent flour protein (14% m.b.).
[c] Soft white wheat.
[d] Soft red wheat.
[e] Club wheat.
[f] Mean values with different letters within this row are significantly different at the 5% level.

Table II

Comparative Results of Quantities of Gliadin Subgroups Determined by Densitometric Method in Straight-Grade and Patent Flours From Seven Soft Wheat Cultivars

Gliadin Subgroups	Straight-Grade Flours			Patent Flours	
	Minimum (%)[c]	Maximum (%)[c]	Mean (%)[c]	Mean (%)[d]	t[a,b]
ω-gliadins	4.18	9.61	6.17	4.37	16.25***
γ-gliadins	13.99	19.32	17.28	13.36	5.36**
β-gliadins	16.06	21.26	19.15	13.89	6.04***
α-gliadins	8.37	13.57	10.65	7.78	7.08***
Total gliadins	44.15	59.52	53.25	39.40	8.50***

[a] Paired "t" test between means of two groups of data.
[b] **, ***, significant at the 1% and 0.1% levels, respectively.
[c] Percentage of straight-grade flour protein (14% m.b.); each value is the mean of seven samples.
[d] Percentage of patent flour protein (14% m.b.); each value is the mean of seven samples.

Table III

Mixograph Data and Sugar-Snap Cookie-Baking Quality of Seven Soft Wheat Straight-Grade Flours

Quality Parameter	Minimum	Maximum	Mean
Mixogram			
Peak time (min)	2.5	3.8	3.1
Peak height (mm)	33.0	43.2	38.9
Stability (min)	3.2	7.9	5.1
Tolerance (mm)	5.0	13.0	8.4
Sugar-Snap Cookie-Baking			
Cookie diameter (cm)	8.08	8.68	8.43
Cookie thickness (mm)	7.5	8.4	8.0
Spread factor	9.9	11.5	10.55

Table IV

Comparative Results of Mixograph Data and Sugar-Snap Cookie-Baking Quality for Two Sets of Flours from Seven Cultivars

Parameter[a]	Patent Flour	Straight-Grade Flour	
	Mean		$t^{b,c}$
FP (%)[d]	7.5	8.3	3.44*
MPT (min)	4.1	3.1	3.27*
MPH (mm)	36.1	38.9	2.60*
MS (min)	5.6	5.1	0.61 ns
MT (mm)	6.1	8.4	2.15 ns
SSCD (cm)	8.74	8.43	6.62***
SSCD/% FP	1.17	1.03	4.76**
SSCT (mm)	7.0	8.0	3.87**
SSCSF	12.59	10.55	4.32**

[a] Abbreviations: FP = flour protein; MPT = mixograph peak time; MPH = mixograph peak height; MS = mixograph stability; MT = mixograph tolerance; SSCD = sugar-snap cookie diameter; SSCT = sugar-snap cookie thickness; SSCSF = sugar-snap cookie spread factor.
[b] Paired "t" test between means of two groups of data.
[c] *, **, and ***, significant at the 5%, 1% and 0.1% levels, respectively; ns, not significant at the 5% level.
[d] 14% moisture basis.

Table V

Correlation Coefficients[a] of Quantities of Gliadin Subgroups in Patent Flour of 17 Soft Wheat Samples to Flour Rheological and Baking Properties

Properties[b]	ω[c] (%)[d]	γ[c] (%)[d]	β[c] (%)[d]	α[c] (%)[d]	Total Gliadins (%)[d]
AW ($x10^{-4}$ J)			-0.495*		
MPT (min)				-0.501*	-0.623**
MPH (mm)	0.569*			0.532*	0.613**
MS (min)				-0.569*	-0.631**
MT (mm)	-0.561*				0.572*
FPT (min)		0.617**			
FT (B.U.)		-0.528*			
JSCV (ml)	-0.553*	-0.521*			-0.570*
JSCV/% FP	-0.496*				-0.596*
SSCD/% FP				-0.543*	-0.511*

[a] *, **, significant at the 5% and 1% levels, respectively.
[b] Abbreviations are the same as those in Table IV, and AW = alveograph strength (W).
[c] ω, γ, β, and α indicate ω-, γ-, β-, and α-gliadins, respectively.
[d] Percentage of patent flour protein (14% m.b.).

CHAPTER 5. RHEOLOGICAL AND BAKING PROPERTIES OF U.S. SOFT WHEAT FLOURS. II. EFFECTS OF TYPE AND QUANTITY OF GLUTENIN SUBUNITS

Rheological and Baking Properties of U.S. Soft Wheat Flours.

II. Effects of Type and Quantity of Glutenin Subunits

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ABSTRACT

The high-molecular-weight glutenin subunits (HMW-GS) and low-molecular-weight (LMW) glutenin subunit groups (B and C subunits) in 17 soft wheat patent flours and seven straight-grade flours were identified and quantified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with densitometry using a known quantity of glutenins as a quantitative standard. Flour rheological properties were evaluated by alveograph, farinograph and mixograph. Japanese-type sponge cakes and AACC sugar-snap cookies were made to evaluate the flours' baking performances. Patent flours containing subunit 1 of HMW-GS showed significantly smaller alveograph P and P/L values and farinograph water absorption value, and larger alveograph L and G values than patent flours containing subunit 2*. Additionally, patent flours containing subunit 1 produced significantly larger volume Japanese sponge cakes and bigger diameter sugar-snap cookies. The Glu-1 quality score was positively correlated with dough strength parameters, but not with baking qualities. The quantities of individual glutenin subunit groups in flour protein were shown to affect the various rheological properties. The quantity of B subunits was significantly positively correlated with the qualities of sponge cakes and sugar-snap cookies, while the quantity of C subunits was significantly negatively correlated with the quality of sugar-snap cookies. The ratio of the quantities of B subunits to C subunits in

flour protein may be an important parameter in relation to the qualities of soft wheat products.

INTRODUCTION

Glutenin proteins are very large polymeric molecules consisting of high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (GS) linked through intra- and inter-disulfide bonds. The relative size distribution of glutenin proteins is most likely responsible for all the individual and combined effects of different glutenin subunits on dough strength (Gupta and MacRitchie 1994). The variation in the amounts and size distribution of the glutenin polymers is caused by the quantity and/or type of the subunits produced during protein synthesis (Gupta et al 1993, Gupta and MacRitchie 1994). Accordingly, both the type and the quantity of individual glutenin subunits are important to dough properties and flour end-use qualities (Branlard and Dardevet 1985, Payne et al 1987, Ng and Bushuk 1988, Marchylo et al 1989, Sutton 1991, Gupta and MacRitchie 1994). One of the most widely used scoring systems for evaluating a flour's breadmaking potential, the Glu-1 quality score, was proposed by Payne et al (1987) based on the composition of HMW-GS in flour. This scoring system was derived from the SDS-sedimentation volumes of 250 wheat cultivars, and scores can range from a minimum of 4 to a maximum of 10 for any of the common wheat cultivars.

Previous research has shown that for soft wheat flour, gluten proteins as a whole affect the quality of sugar-snap cookies (Gaines and Finney 1989, Kulp and Olewnik 1989, Gaines 1990, Kaldy et al 1993, Souza et al 1994). Soft

wheat products generally require flour with low protein content and weak gluten strength (Bettge et al 1989, Souza et al 1994).

The effects of HMW-GS on soft wheat baking quality have been reported. The Glu-1 quality score was found to be negatively correlated with British biscuit-making quality (Payne et al 1987), however, the individual HMW-GS (except subunit pair 13+19) and their quality scores did not correlate with sugar-snap cookie-baking quality (Souza et al 1994, Lookhart et al 1993). It is likely that high flour protein content masks the effects of individual HMW-GS on baking quality. The influences of glutenin subunits on cookie-baking quality are more apparent when combined effects of HMW-GS are considered (Souza et al 1994). This combined effect, expressed as a glutenin rank sum (GRS) was calculated according to a modified scoring system of Payne et al (1987). The results showed the GRS was negatively correlated with sugar-snap cookie diameter, however, Souza et al (1994) examined several years of data, and this correlation was greatest in the year when the average flour protein content was lowest, and least in the year when the average flour protein content was highest (Souza et al 1994).

There is little information available regarding the contributions of the quantities of HMW-GS and LMW-GS to soft wheat end-use qualities. The quantity of glutenin subunits determines partially the relative size distribution of the glutenin proteins, which was reported could alone account for all the allelic differences in dough strength (Gupta and MacRitchie 1994). Quantification of

glutenin subunits in soft wheats would allow us to better understand their functionality, if any, in baking quality.

The objectives of the present study were: (1) to quantify the individual HMW-GS and glutenin subunit groups (A, B and C subunits) defined by Payne and Corfield (1979) in 17 soft wheat patent flours by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) coupled with densitometry using a known quantity of glutenins as a quantitative standard, and (2) to relate the presence or absence of certain HMW-GS and the quantities of extractable glutenin subunits to dough rheological and baking properties.

MATERIALS AND METHODS

Wheat Samples

Seventeen soft wheat cultivars harvested in 1992 or 1993 were selected for this study. These cultivars cover all classes of soft wheats produced in the U.S.. The samples were milled on a Miag-Multomat mill to obtain patent flours of 45% extraction rate. The protein contents of these flour samples ranged from 6.7% to 8.9% (Yamamoto et al 1995, see Appendix I). Seven of the 17 cultivars (Augusta, Caldwell, Chelsea, Dynasty, Freedom, Hyak and Lewjain) were also milled on a Chopin Mill to obtain straight-grade flours. The protein contents of these flours varied from 7.4% to 8.8% (Hou et al 1995).

Chemicals and Reagents

Chemicals and reagents were the same as described by Hou and Ng (1995).

Identification of High-Molecular-Weight Glutenin Subunits and Their Glu-1 Scores

Total proteins were extracted from flour according to Ng and Bushuk (1987). SDS-PAGE was conducted in 1.5 mm thick gels (18 cm wide, 16 cm long) according to Ng and Bushuk (1987) on a vertical electrophoresis unit (Hoefer Scientific Instruments, San Francisco, CA). The nomenclature of HMW-GS was based on the method of Payne and Lawrence (1983). The Glu-1 quality score of each cultivar was calculated according to Payne et al (1987).

Preparation and Quantification of Extractable Glutenin Subunits

Preparation and quantitative determination of individual HMW-GS or glutenin subunit groups (A, B, and C subunits) were carried out according to the procedures described by Hou and Ng (1995).

Soft Wheat Flour Quality Evaluation

Data of flour protein content determinations, alveograph, farinograph and mixograph tests, Japanese-type sponge cakes and micro sugar-snap cookies of

17 patent flour samples were obtained from an earlier study (Yamamoto et al 1995, see Appendix I). Data of flour protein content determinations, mixograph and micro sugar-snap cookie baking tests of the seven straight-grade flours were obtained from Hou et al (1995).

Statistical Analyses

Data were subjected to ANOVA, student's "t" test, and correlation analyses on the Microsoft Excel program (Cambridge, MA).

RESULTS AND DISCUSSION

Determination of High-Molecular-Weight Glutenin Subunit Compositions by SDS-PAGE

Electrophoretic patterns of HMW-GS of the 17 soft wheat cultivars analyzed are shown in Figure 1. Table I lists the HMW-GS compositions of the 17 cultivars. Subunit 12', whose mobility is between those of subunits 9 and 10, has similar mobility on SDS-PAGE to subunit 10' reported by Lookhart et al (1993). This 12' is present only with subunit 2 in the cultivars analyzed, and presumed to be analogous to subunit 12, thus it was assigned the number 12'. It should be noted that in the report by Lookhart et al (1993), subunit 10' was also present in association with subunit 2. The quality scores of glutenin subunits encoded by genes on each chromosome (1A, 1B, and 1D) are also shown on

Table I except for subunit pair 6+7 and subunit 6, whose quality scores are not available. Furthermore, the same score for 2+12' as 2+12 has been used in the analysis. The Glu-1 scores of these 17 cultivars ranged from 6 to 10 according to Payne et al (1987). It can be noticed from Table I that most of the soft wheat cultivars analyzed (15 out of 17) contained subunit 2 instead of subunit 5, presence of the latter being an indicator of a strong wheat flour (Greene et al 1988, Lafiandra et al 1993).

Results of Quantitative Determination of Extractable Glutenin Subunits in Soft Wheat Flours

Table II shows the quantities of extractable HMW-GS present in two or more of the 17 patent flour samples and the quantities of three groups of glutenin subunits (A, B and C subunits) in these patent flours. Among the 10 glutenin subunits, subunits 10 and 7 were present in the largest quantities (1.93% of patent flour protein), while subunit 8 was present in the least quantity (0.66%) (Table II). Subunits 2 and 5, which were reported to have opposite effects on dough strength (Lafiandra et al 1993), showed close relative quantities in the patent flour protein (1.57% vs. 1.75%).

The quantity of A subunits ranged from 3.87 to 11.28% of patent flour protein, with a mean value of 7.26%; the quantity of B subunits varied from 7.58 to 11.67% of patent flour protein, with an average of 10.18%; and the quantity of C subunits ranged from 5.70 to 8.26%, with a mean of 6.89% (Table II).

Statistical analyses showed that B subunits were significantly higher in quantity than either A or C subunits among the three groups of glutenin subunits in patent flour proteins studied. The quantities of LMW-GS (B and C subunits) and total glutenin subunits (A, B and C subunits) in patent flour proteins accounted for about 17% and 24%, respectively.

The average quantities of extractable A, B and C subunits of seven straight-grade flour samples tested were 6.94%, 11.36% and 6.54% of flour protein, respectively. No significant differences ($P>0.05$) were found between the quantities of each of these glutenin subunit groups and total glutenin subunits present in straight-grade flours and their respective counterpart subunit groups in patent flours (see Appendix II).

Relationship of the HMW-GS and Their Quality Scores to the Quality Parameters of Patent Flours

Table III lists the significant correlation coefficients of specific HMW-GS and their quality scores to patent flour rheological parameters and baking results. Among five HMW-GS or subunit pairs, subunits 1 and 2* are of particular importance, because subunit 1 was positively correlated with Japanese-type sponge cake volume ($r=0.487$, $P<0.05$) and sugar-snap cookie diameter ($r=0.821$, $P<0.001$), while subunit 2* was negatively correlated with both the cake volume ($r=-0.494$, $P<0.05$) and cookie diameter ($r=-0.771$, $P<0.001$). Subunit 1 also correlated positively with alveograph extensibility (L)

and swelling index (G) values, and negatively with alveograph stability (P/L, ratio of tenacity to extensibility) and farinograph water absorption values. In contrast, subunit 2* showed positive correlations with alveograph P and P/L values, and farinograph water absorption. These results are consistent with those of Branlard and Dardevet (1985) who found that subunit 2* was positively correlated with alveograph P value and that subunit 1 was positively correlated with alveograph L value. Previous results with the same set of samples showed that the larger diameter sugar-snap cookies and bigger volume Japanese-type sponge cakes are generally positively correlated with alveograph L and G values, and negatively with alveograph P, the ratio P/L, and farinograph water absorption values (Yamamoto et al 1995). It can be inferred that subunit 1 is beneficial and subunit 2* detrimental to the qualities of Japanese-type sponge cakes and sugar-snap cookies.

In their study, Payne et al (1987) assigned the same quality score (3) to subunits 1 and 2* based on their higher SDS-sedimentation volumes. Therefore, the presence of subunit 1 or 2* is usually an indicator of a strong hard wheat for good breadmaking (Payne et al 1987). In a recent study calculating glutenin rank sum (GRS) to predict the soft wheat baking quality of sugar-snap cookies, Souza et al (1994) assigned a rank of 4 to subunit 1 or 2*, the same rank as for subunit pair 13+19 or 5+10. They assumed that the best alleles for breadmaking would be the worst for pastry quality. However, results from the present study using patent flours indicated that subunits 1 and 2* in soft wheats did not play

the same roles in Japanese-type sponge cake baking and sugar-snap cookie baking as those same subunits in hard wheats play in breadmaking. Use of Payne's quality score based on HMW-GS (Payne et al 1987) to predict the quality of soft wheat products may not be as satisfactory as it has been for breadmaking.

No significant correlations were found among subunits 6, 2+12 (or 2+12'), and 5+10 and patent flour baking quality, however, they all showed various relationships with dough rheological properties (Table III). For example, subunit pair 2+12 (or 2+12') had significantly negative correlations with alveograph P and W (strength) values, and mixograph peak time and stability value, while subunit pair 5+10 showed significantly positive correlations with each of those parameters. These results are consistent with previous findings that subunits 5+10 generally produce stronger doughs and subunits 2+12 weaken the dough strength (Payne et al 1981, Branlard and Dardevet 1985, Ng and Bushuk 1988, Lafiandra et al 1993). Subunit 6 was shown to be negatively correlated with alveograph L, G, and W values and positively correlated with farinograph peak time. No report is available to confirm or refute this information.

A recent study by Souza et al (1994) showed that individual HMW-GS did not have any significant correlations with sugar-snap cookie diameter except for subunit pair 13+19, whose presence seemed to have an adverse effect on cookie diameter. In 51 soft white spring wheats they analyzed, the largest factor in determining cookie diameter was flour protein content. In contrast, the protein

contents of the 17 patent flours used in the present study did not significantly correlate with cookie diameter or cake volume (Yamamoto et al 1995). This may be due to several reasons: some cultivars used in their study were from the same pedigrees and had the same HMW-GS compositions and, therefore, the effect of total flour protein content on cookie-baking quality might be dominant over individual HMW-GS; additionally, the flour protein contents in their samples (average 9.6%) were higher than those in our patent flours (average 7.9%) with 45% extraction rate; and finally, the effect of allelic variation of subunits on cookie diameter was probably masked in their study by the high protein content since low protein content flour is generally desirable for making soft wheat products. In their study (Souza et al 1994), it was found that when all the HMW-GS were combined to calculate the GRS, cookie diameter had greatest negative correlation with GRS score in the year with the lowest average flour protein content. These results indicate that there may exist some interactions between HMW-GS and flour protein content in determining the soft wheat baking potential.

The relationship of quality scores of HMW-GS encoded by genes on each chromosome (1A, 1B and 1D) and of Glu-1 score to flour quality parameters are also presented in Table III (only significant correlation coefficients are listed). It can be seen that quality scores of 1A, 1B, 1D, and Glu-1 score correlated positively with most of the rheological parameters (alveograph P and W, mixograph peak time and stability, and farinograph water absorption). These

results are generally in agreement with previous findings (Randall et al 1993). However, none of these quality scores were significantly correlated with cookie diameter or cake volume (Table III). The Glu-1 quality score was intended for predicting breadmaking quality of hard wheats, and may not be useful in relating HMW-GS compositions of soft wheats to baking quality (Lookhart et al 1993) unless necessary modifications are made based on the actual relationships of individual HMW-GS to a specific baking quality. For example, in their study, Souza et al (1994) found that the presence of subunit pair 13+19 (44.2% of their samples contained this subunit pair) in a cultivar reduced corrected cookie diameter by an average of 0.01 cm. Accordingly, they assigned a rank of 4 to them, the same score as for subunit pair 5+10 and subunits 1 and 2*, and found that significantly negative correlations existed between the GRS scores and sugar-snap cookie diameter.

The student's "t" test was used to examine the effects of the subunit 1 vs. 2*, and subunit pair 2+12 (or 2+12') vs. 5+10 on various quality parameters of flours (Table IV). No significant difference was noticed in patent flour protein content between flours containing subunits 1 and 2*. However, the quantity of subunit 1 was significantly higher than that of subunit 2* in the patent flours studied (1.02% vs. 0.85% of flour protein). Even so, patent flour samples containing subunit 2* showed significantly higher alveograph P and P/L, and farinograph water absorption values, and lower alveograph L and G values, cake volumes and cookie diameters than flours containing subunit 1. These results

indicated that subunit 1 is qualitatively weaker than subunit 2* in these patent flours and, therefore, is good for sponge cake and cookie baking.

When compared with subunit 2 (present in subunit pair 2+12 or 2+12'), the presence of subunit 5 (present in subunit pair 5+10) exerts significantly greater effects on dough rheological properties (Table IV). The protein contents did not differ significantly between flours containing subunit 2 and those containing subunit 5. The average quantities of the two subunits in the patent flours were also not significantly different (1.57% and 1.75% of flour protein for subunits 2 and 5, respectively), confirming previous results that the quantities of subunits 2 and 5 were the same in wheat flours (Gupta and MacRitchie 1994). However, flours with subunit 5 demonstrated significantly higher alveograph P and W values, and mixograph peak time and stability values than those with subunit 2. These results further confirm that subunit 5 is an indicator of strong flours, and subunit 2 an indicator of weak flours (Lafiandra et al 1993).

Subunit 5, compared with subunit 2, contains an additional cysteine residue that could have a significant effect on the formation of glutenin polymers by promoting a differential cross-linking (Greene et al 1988). In their paper, Gupta and MacRitchie (1994) reported that subunit pair 5+10 and 2+12 were present in similar quantities, but they differed in their ability to form different sizes of polymeric proteins (native glutenins). Subunit pair 5+10 exert a greater effect on dough strength by producing a greater proportion of larger-sized glutenin polymers than do subunit pair 2+12. The difference in polymerizing

behavior of these subunit pairs results mainly from structural differences in subunits 5 and 2. In the present study, only two cultivars contained the subunit pair 5+10. Even though significant differences were observed in some rheological properties between flours containing subunit pair 5+10 and subunit pair 2+12 (or 2+12') (Table IV), no significant differences were observed on flour baking quality. More cultivars containing the subunit pair 5+10 should be selected to further determine the different effects of subunit 2 versus 5 on soft wheat flour end-use quality.

Relationship of Quantities of Extractable Glutenin Subunits to Patent Flour Quality

Correlations between the quantities of individual HMW-GS and the quality parameters of patent flours were examined. The quantity of subunit 2* was positively correlated with mixograph tolerance value ($r=0.833$, $P<0.05$), indicating the higher quantity of subunit 2* would make the flour stronger, leading to poor sponge cake- and cookie-baking qualities. Contrary to subunit 8, the quantity of which correlated negatively with mixograph peak height ($r=-0.993$, $P<0.01$), farinograph peak time ($r=-0.954$, $P<0.05$) and stability values ($r=-0.982$, $P<0.05$), the quantity of subunit 9 positively correlated with mixograph peak height value ($r=0.878$, $P<0.05$), but negatively correlated with mixograph stability value ($r=-0.939$, $P<0.05$). These results suggest that both the type and quantity of certain HMW-GS could be responsible for the structure and

molecular size distribution of polymeric proteins, which were reported to most likely account for the combined effects of individual glutenin subunits on dough strength (Gupta et al 1993, Gupta and MacRitchie 1994). However, no significant correlation was observed between the quantity of individual HMW-GS and flour baking quality.

Table V lists the significant correlation coefficients for the quantities of glutenin subunit groups (A, B and C subunits) to flour quality parameters. The total quantity of extractable HMW-GS (A subunits) in patent flour protein correlated positively with alveograph W value, but did not correlate with flour baking quality. Further examination of their functionality is needed before any statement could be made whether the quantity of A subunits has any effect on soft wheat flour baking quality. However, the quantities of extractable LMW glutenin subunit groups (B and C subunits) showed various influences on some of the rheological and baking properties of flours (Table V). The quantity of extractable B subunits in patent flour protein correlated positively with mixograph peak time and negatively with farinograph water absorption value. Positive correlation was found between the quantity of extractable B subunits and cookie diameter. When the influence of flour protein content was eliminated, the quantity of extractable B subunits strongly correlated positively with cookie diameter per unit flour protein and cake volume per unit flour protein. This result was further confirmed by studying the sugar-snap cookie-baking quality of seven straight-grade flour samples. The quantity of extractable B subunits (% of flour

protein) was significantly correlated with the diameter of sugar-snap cookies per unit flour protein ($r=0.773$, $P<0.05$).

In contrast to B subunits, the quantity of extractable C subunits showed opposite effects on flour quality. As shown in Table V, the quantity of extractable C subunits in patent flour protein correlated positively with farinograph peak time, and negatively with cake volume and cake volume per unit flour protein. Additionally, the ratio of the quantities of B subunits to C subunits was positively correlated with alveograph L and mixograph peak time values, and negatively with mixograph peak height and farinograph water absorption values. Strong positive correlations were observed between the qualities of Japanese sponge cakes and sugar-snap cookies and the ratio of the quantities of B to C subunits in flour proteins. Results from sugar-snap cookie baking of seven straight-grade flours confirmed that the ratio of the quantities of B subunits to C subunits was highly positively correlated with cookie diameter per unit flour protein ($r=0.842$, $P<0.05$). These results indicated that the proper balance of B and C subunits may be important to the quality of soft wheat products. The relative quantities of B and C subunits may determine the strength of glutenin proteins, and hence affect soft wheat baking quality.

It was also noticed (Table V) that the total quantities of extractable LMW-GS in patent flour protein correlated positively with mixograph peak time, and negatively with farinograph water absorption value. No significant relationship was found between the quantity of LMW-GS and flour baking quality. The ratio

of LMW-GS to HMW-GS was found to be negatively correlated with alveograph W value, indicating that the relative amount of LMW-GS to HMW-GS is very important to flour strength (Table V). The total quantities of extractable glutenin subunits in flour proteins are also linked to the flour strength because they correlated positively with the alveograph W and mixograph peak time values. However, their influences were not strong enough to significantly affect the flour baking quality.

In the present study, the LMW glutenin subunits have been classified into two groups: B subunits and C subunits. However, within the B and C subunits, a total of over 40 different subunits have been detected, with each cultivar exhibiting 7 to 16 subunits (Gupta and Shepherd 1990). These subunits have been assigned to three groups which are genetically controlled by the Glu-3 locus on the short arms of chromosomes 1A, 1B and 1D (Gupta and Shepherd 1988, 1990). The allelic variation at the Glu-3 locus of bread wheat has been linked to physical dough properties (Gupta et al 1989, Metakovsky et al 1990, Gupta et al 1994, Gupta and MacRitchie 1994). Certain LMW glutenin subunits have also been identified as affecting biscuit-making quality (Morel, cited by Autran 1993). The variation in the quantities of certain LMW-GS were also shown to affect the molecular size distribution and/or quantity of the glutenin proteins (Gupta and MacRitchie 1994). Therefore, further identification and quantification of LMW-GS encoded by genes on each Glu-A3, Glu-B3 and Glu-D3 locus, along with consideration of HMW-GS compositions, would help us

better understand the functionality of glutenin proteins in soft wheat end-use quality. Eventually, it may be possible to manipulate the glutenin protein compositions aiming at breeding of soft wheats for specific products.

SUMMARY

High-molecular-weight glutenin subunits and low-molecular-weight glutenin subunit groups (B and C subunits) of 17 soft wheat patent flours and seven straight-grade flours were identified and quantified. Rheological and baking tests (Japanese-type sponge cakes and AACC sugar-snap cookies) were conducted to evaluate flour quality. Preliminary results revealed that both the type and quantity of certain HMW-GS are important to flour quality. The quantity of extractable B subunits in flour protein was positively correlated with Japanese-type sponge cake-baking and AACC sugar-snap cookie-baking qualities, and the quantity of extractable C subunits in flour protein correlated negatively with cake-baking quality. The ratio of the quantities of B subunits to C subunits may be an important parameter for evaluating soft wheat end-use quality. Results from straight-grade flours confirmed that the quantity of extractable B subunits and the ratio of the quantities of B subunits to C subunits positively correlated with sugar-snap cookie diameter per unit flour protein.

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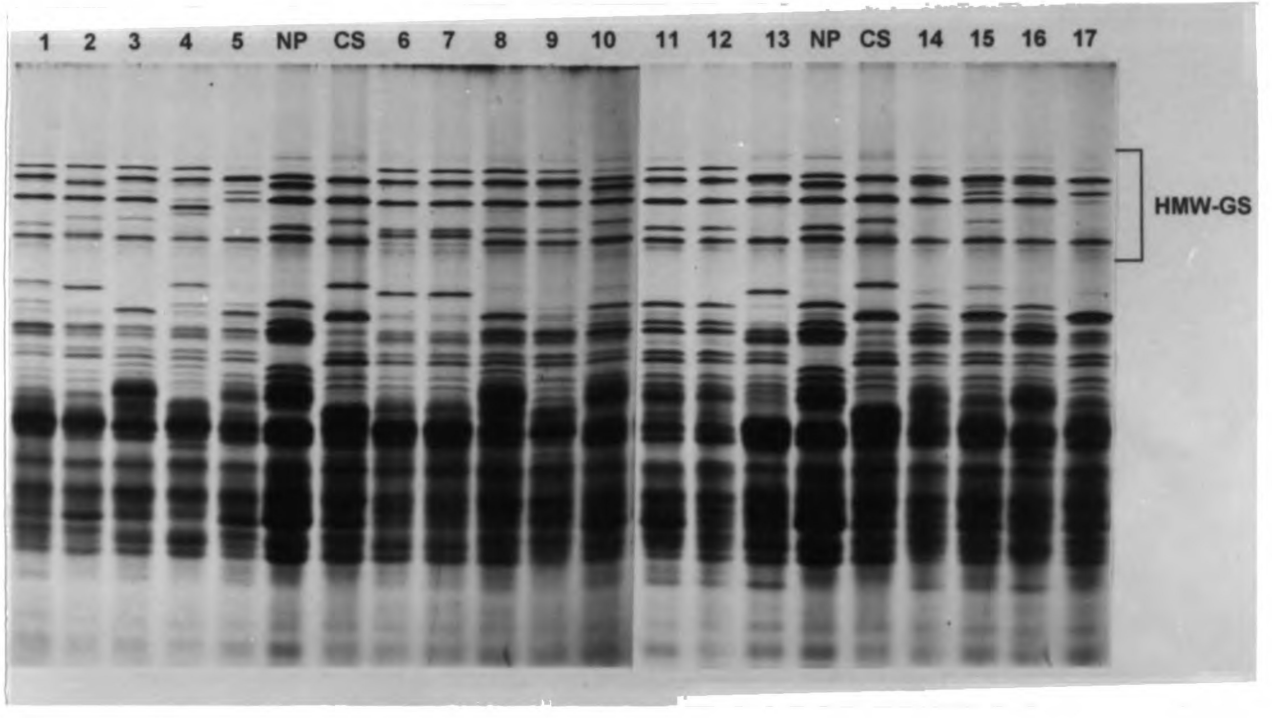

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretic patterns of high-molecular-weight glutenin subunits (HMW-GS). Wheat cultivars are: Augusta (lane 1), Caldwell (lane 2), Chelsea (lane 3), Clark (lane 4), Crew (lane 5), Dynasty (lane 6), Excel (lane 7), Frankenmuth (lane 8), Freedom (lane 9), Hyak (lane 10), Kmor (lane 11), Lewjain (lane 12), Madsen (lane 13), Malcom (lane 14), Rely (lane 15), Stephens (lane 16), and Tres (lane 17). NP = Neepawa (a Canadian cultivar used as a marker for HMW-GS). CS = Chinese Spring (a marker for HMW-GS).

Table I

High-Molecular-Weight Glutenin Subunits (HMW-GS)[a] and Their Quality Scores from 17 Soft Wheat Cultivars

Cultivar	HMW-GS			Glu-A1 Score[b]	Glu-B1 Score[b]	Glu-D1 Score[b]	Glu-1 Score[b]
	Glu-A1	Glu-B1	Glu-D1				
Augusta[c]	1	7+9	2+12	3	2	2	7
Caldwell[d]	1	7+8	5+10	3	3	4	10
Chelsea[c]	1	7+8	2+12	3	3	2	8
Clark[d]	1	28+29	2+12	3	3	2	7
Crew[e]	2*	6+7	2+12	3	?[f]	2	?
Dynasty[d]	1	7+9	2+12'	3	2	2	7
Excel[d]	1	7+9	2+12'	3	2	2	7
Frankenmuth[c]	1	7+9	2+12	3	2	2	7
Freedom[d]	1	7+9	2+12	3	2	2	7
Hyak[e]	2*	7+8	5+10	3	3	4	10
Kmor[c]	1	7+9	2+12	3	2	2	7
Lewjain[c]	1	7+9	2+12	3	2	2	7
Madsen[c]	2*	7	2+12	3	1	2	6
Malcom[c]	2*	7	2+12	3	1	2	6
Rely[e]	2*	6+7+8[g]	2+12	3	2	2	7
Stephens[c]	2*	7	2+12	3	1	2	6
Tres[e]	null	6	2+12	1	?[f]	2	?

[a] The nomenclature was based on the method of Payne and Lawrence (1983).
[b] Quality scores of high-molecular-weight glutenin subunits were calculated according to Payne et al (1987).
[c] Soft white wheats.
[d] Soft red winter wheats.
[e] Club wheats.
[f] ?: not assigned.
[g] Potential mixture and/or biotype of the cultivar.

Table II

Quantities of Individual High-Molecular-Weight Glutenin Subunits (HMW-GS) and Glutenin Subunit Groups in 17 Patent Flour Samples Determined by Densitometric Method

Glutenin Subunit	Minimum (%)[a]	Maximum (%)[a]	Mean (%)[a,b]
HMW-GS[c]			
1 (10)	0.77	1.21	1.02
2* (6)	0.73	0.99	0.85
2 (15)	1.10	2.40	1.57
5 (2)	1.71	1.78	1.75
6 (3)	0.76	2.05	1.38
7 (15)	1.01	2.86	1.93
8 (4)	0.47	0.82	0.66
9 (5)	0.71	1.63	1.09
10 (2)	1.49	2.37	1.93
12 (13)	0.95	2.04	1.44
Glutenin subunit groups[d]			
A subunits	3.87	11.28	7.26 a
B subunits	7.58	11.67	10.18 b
C subunits	5.70	8.26	6.89 a
B+C	14.22	9.27	17.07
A+B+C	19.32	27.73	24.33

[a] Percentage of patent flour protein (14% m.b.).
[b] Values within this column with different letters are significantly different at the 5% level.
[c] Values in parentheses are numbers of observations.
[d] A subunits = HMW-GS; B and C subunits = low-molecular weight glutenin subunits; A + B + C = total glutenin subunits.

Table III

Correlation Coefficients[a] for the Presence of Certain High-Molecular-Weight Glutenin Subunits (HMW-GS) and Their Quality Scores with Soft Wheat Patent Flour Rheological and Baking Qualities

Parameter[b]	HMW-GS					Quality Scores			
	1	2*	6	2+12 or 2+12'	5 +10	1A	1B	1D	Glu-1
P (mm)		0.560*		-0.548*	0.548*			0.548*	
L (mm)	0.593*		-0.531*						
P/L	-0.611**	0.588*							
G	0.599*		-0.562*						
W ($x10^{-4}$ J)			-0.527*	-0.692**	0.692**	0.491*		0.692**	0.671**
MPT (min)	0.501*			-0.637**	0.637**		0.663**	0.637**	0.750**
MS (min)				-0.741***	0.741***		0.519*	0.741***	0.725**
FWA (%)	-0.721**	0.713**					-0.599*		
FPT (min)			0.538*						
JSCV (ml)	0.487*	-0.494*							
SSCD (cm)	0.821***	-0.771***							

[a] *, **, and ***: significant at the 5%, 1%, and 0.1% levels, respectively.
[b] Abbreviations: P = alveograph tenacity; L = alveograph extensibility; G = alveograph swelling index; MPT = mixograph peak time; MS = mixograph stability; FWA = farinograph water absorption; FPT = farinograph peak time.
JSCV = Japanese-type sponge cake volume ; SSCD = sugar-snap cookie diameter.

Table IV

Comparative Results of the Effects of Four High-Molecular-Weight Glutenin Subunits on Some Quality Parameters of 17 Soft Wheat Patent Flour Samples

Parameters[b]	1 (n=10)[a]	2* (n=6)[a]		2 (n=15)[a]	5 (n=2)[a]	
	Mean		t[c,d]	Mean		t[c,d]
FP (%)	7.7	8.0	0.87 ns	7.8	7.2	1.64 ns
SQ (g/100g FP)	1.02	0.85	2.58*	1.57	1.75	1.93 ns
P (mm)	29.6	39.5	2.41*	31.0	46.1	2.54*
L (mm)	142.3	111.1	2.40*	128.3	119.8	0.36 ns
P/L	0.22	0.38	2.93*	0.26	0.41	1.68 ns
G	26.4	23.2	2.43*	24.9	24.2	0.30 ns
W ($x10^{-4}$ J)	105.5	103.2	0.16 ns	93.5	157	3.71**
MPT (min)	3.7	2.6	1.82 ns	2.8	5.4	3.20**
MS (min)	4.2	3.9	0.30 ns	3.4	8.0	4.28***
FWA (%)	49.5	52.3	4.09**	50.5	50.8	0.19 ns
JSCV (ml)	1162	1119	2.19 *	1146	1143	0.09 ns
SSCD (cm)	8.74	8.35	5.41***	8.58	8.62	0.18 ns

[a] Values in parentheses are numbers of observations.

[b] Abbreviations are the same as those in Table III, FP = flour protein, and SQ = subunit quantity.

[c] Student's t test.

[d] *, **, ***: significant at the 5%, 1%, and 0.1% levels, respectively; ns: not significant at the 5% level.

Table V

Correlation Coefficients[a] for Quantities (%)[b] of Glutenin Subunit Groups in 17 Soft Wheat Patent Flours with Flour Rheological and Baking Qualities

Parameter[c]	A[d]	B[d]	C[d]	B/C[d]	BC[d]	BC/A[d]	ABC[d]
L (mm)				0.540*			
W ($x10^{-4}$ J)	0.500*					-0.505*	0.542*
MPT (min)		0.662**		0.582*	0.518*		0.593*
MPH (mm)				-0.509*			
FWA (%)		-0.662**		-0.508*	-0.649**		
FPT (min)			0.553*				
JSCV (ml)			-0.514*	0.547*			
JSCV/FP (ml/%)		0.613**	-0.518*	0.732***			
SSCD (cm)		0.558*		0.674**			
SSCD/FP (cm/%)		0.662**		0.736***			

[a] *, **: significant at the 5% and 1% levels, respectively.
[b] Percentage of patent flour protein (14% m.b.).
[c] Abbreviations are the same as those in Table III, and FP = flour protein.
[d] A = high-molecular-weight glutenin subunits; B = B group of low-molecular-weight glutenin subunits; C = C group of low-molecular-weight glutenin subunits; BC = total low-molecular-weight glutenin subunits; B/C = ratio of B group of low-molecular-weight glutenin subunits to C group of low-molecular-weight glutenin subunits; BC/A = ratio of low-molecular-weight glutenin subunits to high-molecular-weight glutenin subunits; ABC = total glutenin subunits.

CHAPTER 6. SUMMARY AND GENERAL DISCUSSION

The high-molecular-weight (HMW) and low-molecular-weight (LMW) glutenin subunits (GS) have been reported as important indicators for various dough characteristics of hexaploid wheat flours (Kruger et al 1988, Payne et al 1988, Gupta et al 1989, Gupta and MacRitchie 1994). Variations in the quantity and type of glutenin subunits in a wheat cultivar are responsible for the amounts and size distribution of glutenin polymers, which have been said to most likely account for the combined effects of individual glutenin subunits on dough strength (Gupta et al 1993, Gupta and MacRitchie 1994, Gupta et al 1995). The relative quantities of some gliadin subgroups have also been found to affect dough rheological properties (Branlard and Dardevet 1985, Wieser et al 1994).

Gluten proteins are functional in preparation of various soft wheat products (Gaines and Finney 1989, Kaldy et al 1993, Souza et al 1994). However, little information is available regarding the effect of relative quantity of each gluten fraction on the quality of soft wheat products. In this dissertation the Japanese-type sponge cake-baking and AACC sugar-snap cookie-making qualities of 17 U.S. soft wheat cultivars were studied in relation to differences in the relative quantities of the gliadin subgroups and glutenin subunit groups and the type of HMW-GS.

To study variation in the quantity of glutenin subunits effectively, a simple and effective method must be developed for a reliable quantification of the subunits present in the flour. The reversed-phase high-performance liquid chromatography (RP-HPLC) has distinguished itself to be an accurate and potentially automated tool for this purpose, however, the identification of individual glutenin subunits by RP-

HPLC requires use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) for initial calibration. On the other hand, densitometric analysis of SDS-PAGE can provide simultaneous identification and quantification of glutenin subunits. However, there is some concern about accuracy of the densitometric approach since factors, such as type of proteins, gel uniformity, and staining and destaining times of gels, could affect the reproducibility and accuracy of results (Kolster et al 1992). Accordingly, a modified densitometric method was developed and is described in **Chapter 3**. In this new method, a known quantity of glutenin proteins was employed as a quantitative standard. Inclusion of a known quantity of glutenin proteins in each gel as a quantitative standard reduced the coefficient of variance of the quantities of each glutenin subunit group determined among different gels for a cultivar. This was because the method of normalization of glutenin subunit densitogram areas by a quantitative standard on the same gel compensated for variations in staining intensity caused by gel non-uniformity, and staining and destaining conditions. Within glutenin proteins, the staining sensitivities of HMW-GS and LMW-GS with Coomassie Brilliant Blue R250 were different. The affinity of LMW-GS for Coomassie Brilliant Blue R250 is 1.3-fold higher than that of HMW-GS. This may be due to their different amino acid compositions. Quantitative data from densitometric analyses of glutenin subunits are in accordance with those of micro-Kjeldahl measurements.

The effects of relative quantity of gliadin subgroups on soft wheat flour rheological and baking properties have not been investigated. In **Chapter 4**, the

relative quantities of gliadin subgroups in 17 soft wheat patent flours and seven straight-grade flours were determined by acid-polyacrylamide gel electrophoresis coupled with densitometry using a known quantity of gliadins as a quantitative standard. The flour protein contents, relative quantities of each gliadin subgroup and total gliadins were significantly higher in straight-grade flours than in their counterpart patent flours. As a result, patent flours showed significantly longer mixograph time, shorter mixograph height and larger sugar-snap cookie diameter than did patent flours. These differences likely resulted from the different extraction rates of these two types of flours. Correlation results confirmed that the relative quantities of some gliadin subgroups and total gliadins affected the various rheological properties and baking quality of patent flours. Significant negative correlations were found between the relative quantities of ω- and γ-gliadins and sponge cake volume and between the relative quantity of α-gliadins and the corrected cookie diameter by flour protein content. These results indicated that selecting soft wheat cultivars containing relatively low gliadin contents would be suitable for better end-use quality.

Chapter 5 describes investigation into variation in the type of HMW-GS and in the relative quantities of HMW-GS and LMW glutenin subunit groups of 17 soft wheat patent flours in relation to soft wheat rheological and baking properties. Patent flours containing subunit 1 showed significantly weaker dough rheological properties and better Japanese sponge cake-baking and sugar-snap cookie-baking qualities than patent flours containing subunit 2*. These results indicated that

subunit 1 is qualitatively weaker than subunit 2* in the patent flours analyzed and, therefore, is good for baking. Since subunit 5 contains an additional cysteine residue that could have a significant effect on the formation of glutenin polymers by promoting a differential cross-linking (Greene et al 1988), patent flours with subunit 5 exhibited significantly stronger dough rheological properties than those with subunit 2. Accordingly, selecting soft wheat cultivars containing subunits 1 and 2 could potentially produce better quality sponge cakes and sugar-snap cookies.

It was shown from correlation results that the relative quantity of individual HMW-GS had some influences on dough properties. The quantity of extractable B and C subunits in flour protein appeared to have positive and negative effects on the qualities of Japanese sponge cakes and sugar-snap cookies, respectively. The quantity of extractable B subunits in patent flours and straight-grade flours each positively correlated with sugar-snap cookie-baking quality. The ratio of the quantities of B subunits to C subunits may be more useful for the evaluation of soft wheat end-use quality. Further identification and quantification of individual LMW-GS would help us better understand the functionality of glutenin proteins in soft wheat end-use quality. Eventually, it may be possible to manipulate the genes coding for glutenin subunits for desired glutenin compositions and the level of gene expression for proper amount of each group of glutenin subunits, aiming at breeding of soft wheats for specific products.

Results from gliadin and glutenin studies for the same 17 soft wheat patent flours indicated that both the relative quantities of gliadin subgroups and the type

and relative quantities of glutenin subunits are functional in flour end-use quality. The proper balance of elasticity and extensibility in doughs was believed to be determined by the type of glutenin subunits (Payne et al 1991) and by the relative quantities of gliadins and glutenins (Gupta and MacRitchie 1994); therefore, any factors that break this balance would alter dough properties, leading to detrimental results in baking quality. A mathematical model could be developed if more cultivars were tested to predict cake- or cookie-baking potential for any soft wheat cultivar by analyzing its gluten composition. The results of the present study should be of value to breeders for early screening of genetic lines for soft wheat products, and to millers and bakers for selecting certain desired qualities for their specific needs.

The major new findings of these studies are as follows:

- A simple and effective method for simultaneous quantification and identification of glutenin subunits has been developed. This method was the first to use a known quantity of glutenins as a quantitative standard for quantification by densitometric analysis.
- Of the cultivars studied, patent flours are significantly lower in their relative quantities of each gliadin subgroup and total gliadins, weaker in dough properties and better in sugar-snap cookie-baking quality than straight-grade flours.
- The relative quantities of ω-gliadins, γ-gliadins and total gliadins are negatively correlated with sponge cake volume, and the relative quantities of

α-gliadins and total gliadins are negatively correlated with the corrected cookie diameter by flour protein content.

- Soft wheat flours containing subunit 1 of HMW-GS are more desirable for making good quality sponge cakes and sugar-snap cookies than flours containing subunit 2*.
- The quantities of B and C subunits show significantly positive and negative correlations, respectively, with Japanese sponge cake-baking quality, while the quantity of B subunits had a significantly positive correlation with sugar-snap cookie-baking quality.
- The ratio of the quantities of B subunits to C subunits may be more useful for the evaluation of soft wheat end-use quality.

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CHAPTER 7. RECOMMENDATIONS

Recommendations for future research are as follows:

1. To analyze more cultivars from different years and locations for developing quality prediction models for various soft wheat products.
2. To confirm the functionality of relative quantity of each gliadin subgroup in soft wheat end-use quality by fortification studies.
3. To identify low-molecular-weight glutenin subunits and determine potential quality markers for soft wheat products.
4. To isolate and physicochemically characterize the B group and the C group of low-molecular-weight glutenin subunits which are associated with soft wheat end-use quality.

APPENDICES

Appendix I

Comparative Studies of Rheological Properties and Baking Qualities of Various Types of Soft Wheats Grown in the United States

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ABSTRACT

Seventeen soft wheat cultivars from four types of U.S. soft wheats, eastern soft white winter (ESWW), western soft white winter (WSWW), club, and soft red winter (SRW) wheats, were selected for comparison of their milling, physicochemical and rheological properties and their suitabilities for making Japanese-type sponge cakes (JSCs) and AACC sugar-snap cookies (SSCs). The texture characteristics of JSCs were determined with Texture Profile Analysis (TPA). Patent flours of SRW and ESWW wheats had less starch damage, smaller particle size and higher viscograph peak viscosity than the other two types of wheats. Likewise, SRW and ESWW wheat patent flours exhibited weaker alveograph and mixograph properties, and produced larger volume JSCs and bigger diameter SSCs. However, SRW wheats may be the most suitable for JSC making since cakes made from them were softer and less chewy in texture. Correlation analyses indicated that decreasing flour particle size is a very important parameter related to improving quality of both JSCs and SSCs, while starch damage is more detrimental to SSC quality. Results from the present study also showed that the alveograph and mixograph are very useful tools for evaluation of soft wheat quality for cake and cookie baking.

INTRODUCTION

Soft wheat flour has been used for a wide range of commercial products. In the U.S., the sugar-snap cookie and layer cake baking tests (AACC 1992) are usually used to evaluate soft wheat baking quality. A soft wheat flour which can produce large spread cookies or big volume cakes is usually considered a good quality flour for soft wheat products. However, it should be recognized that an ideal flour for one class of products may not be ideal for another (Finney 1989).

Most soft wheats milled in Japan have been imported from the U.S., and are mainly U.S. western white wheats (Nagao 1989). The flour for making Japanese-type sponge cakes is specifically milled from 100% U.S. western white wheats. Japan has not imported U.S. soft red winter wheats for many years because of price, availability and potential risks for making Japanese soft wheat products.

A recent survey of U.S. soft wheat quality indicated that the protein contents of U.S. soft white wheats (western and eastern soft white wheats) and club wheats had increased over the last 13 years, being higher than those of soft red winter wheats since 1988, while the protein contents of soft red winter wheats had remained almost constant during the same period (U.S. Wheat 1980-1992). Most Japanese milling and baking companies have been concerned about the increases in protein contents of U.S. western white wheats due to the direct negative influence on the quality of Japanese cakes. For the

Japanese sponge cake, low flour protein content is usually desirable (Nagao et al 1976).

There has been little information in the past 18 years comparing the qualities of different types of soft wheats grown in the U.S., especially as to their suitabilities for making Japanese-type sponge cakes (JSCs). The objectives of the present study were (1) to compare the milling, physicochemical and rheological properties of four types of U.S. soft wheats and (2) to compare their end-use properties for JSCs and AACC sugar-snap cookies (SSCs).

MATERIALS AND METHODS

Wheat Samples

Seventeen soft wheat cultivars harvested in 1992 or 1993 from the states of Washington, Michigan, Ohio and Indiana were selected for this study. These cultivars cover four types of soft wheats produced in the U.S.: three eastern soft white winter (ESWW) wheats (Augusta, Chelsea and Frankenmuth); five western soft white winter (WSWW) wheats (Kmor, Lewjain, Madsen, Malcom and Stephen); four club wheats (Crew, Hyak, Rely and Tres); and five soft red winter (SRW) wheats (Caldwell, Clark, Excel, Dynasty and Freedom). These samples were cleaned using a Carter Dockage Tester. The cleaned wheats were tempered to 15% moisture and milled on a Miag-Multomat mill to obtain patent flours at a 45% extraction rate.

Milling Quality

Wheat samples were analyzed for test weight and flour yield. The moisture contents of wheat and flour, and flour ash content were determined according to the AACC Approved Methods (AACC 1992). Flour particle size was determined by a Coulter Counter Analyzer according to the Operator's Handbook (Anonymous 1986). Starch damage was determined with a Chopin SD4 apparatus (Dubois 1988).

Protein Content and Other Physicochemical Analyses

Total nitrogen content of flour and wheat was determined by the micro-Kjeldahl method (AACC 1992). Protein content was calculated by multiplying the nitrogen content by the conversion factor 5.7 and expressed based on 14% moisture basis. Alkaline water retention capacity of flour and flour Zeleny sedimentation volumes were determined according to AACC Approved Methods (AACC 1992).

Flour Starch Pasting Properties

Flour samples were analyzed by Brabender Viscograph-E to determine effect of α-amylase on flour viscosity as a function of temperature according to the Amylograph Handbook (Shuey and Tipples 1982). A mixture of flour (65 g) and distilled water (450 ml) were heated in the viscograph-E from 30^0C to 95^0C

at a rate of temperature increase of 1.5°C/min, held at 95°C for 15 min, and cooled to 50°C at the same rate as the temperature increase. Viscosity was measured at a torque of 700 cm.gf (gf = gram of force) and recorded at a chart speed of 30 cm/hr. The pasting parameters obtained from a pasting curve were defined according to Dengate (1984). However, only peak viscosity, breakdown viscosity and breakdown from each pasting curve were reported in the present study.

Flour Rheological Measurements

Farinogram values (water absorption, peak time, stability time, and mixing tolerance index) were obtained from a 15-min farinograph mixing curve for 50 g of flour (14% m.b.) according to the AACC Approved Method (AACC 1992). Alveogram values (tenacity P, extensibility L, stability P/L, swelling index G, and work W) were also determined according to the AACC Approved Method (AACC 1992) using a Chopin Alveograph with hydrostatically controlled airflow. Dough mixing properties were measured using 35 g of flour (14% m.b.) with a National Manufacturing mixograph according to the AACC Approved Method (AACC 1992). Mixograph tolerance was defined as the width, in millimeters, of the mixograph curve two minutes past peak time (Graybosch et al 1993). Mixograph stability time was defined as the interval, in minutes, between the arrival time and departure time from the center line of the mixograph curve (Pyler 1988).

Flour Baking Quality Evaluation

Japanese-type sponge cakes and AACC sugar-snap cookies were baked to evaluate the flour's baking performances. All sponge cakes and cookies were made from each of the patent flours.

Sponge Cake Testing Method

Sponge cakes were prepared by the formula and procedure described by Nagao et al (1976) with some modifications. The cake formula and ingredients for one cake are: flour (as-is moisture basis), 150 g; sugar (fine-granulated sucrose), 150 g; fresh whole egg, 150 g; and distilled water, 60 ml. The Hobart mixer (model N-50) with three speed settings (1, 2 and 3) was equipped with a 5-qt bowl and a wire whip for creaming sugar, whole egg and water. Whole eggs (without shells) were weighed into a mixing bowl and fast beat with a hand whip for 10 sec, after which the same amount of sugar was added into eggs. The mixture was warmed up to 45^{0}C in a water bath with slow mixing by hand whip to dissolve the sugar. At this point, the mixture was mixed in the Hobart mixer at medium speed (No. 2 setting) for 6-7 min until the specific gravity of the egg-sugar batter reached 0.28 ± 0.01 g/cm^3, at which point the mixer speed was reduced to low (No. 1 setting) for 1 min while 60 ml water was added. The mixing bowl was then removed from the mixer, after which sifted flour was added into the cream. The cream-flour mixture was subjected to mixing by hand with a wooden spoon until the cake batter specific gravity reached 0.50 ± 0.01 g/cm^3. At this stage, the cake batter temperature was between 25-30^{0}C. The cake

batter (320 g) was poured into a circular baking pan (inside diameter, 6 in.; depth, 2 in.) with parchment paper lining bottom and inner side wall. The surface of the cake batter was smoothed with a plastic spatula, after which the pan was put into a rotary oven (National Manufacturing Co., Lincoln, NE) to bake at 180^{0}C for 30 min. The remaining cake batter was used for measuring its viscosity with a Brookfield viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA). After baking, the cake was removed from the pan immediately and cooled for more than 1 hr at room temperature before packaging. Cake volume was determined with a rapeseed displacement method and graded according to the AACC Approved Method (AACC 1992). A larger cake volume is considered to be an indicator of a better quality flour.

Sugar-Snap Cookie Testing Method

Sugar-snap cookies were baked according to the micro method of AACC Approved Method (AACC 1992). The diameter and thickness of the cookies were measured. Cookies with greater diameter indicate better flour quality.

Texture Profile Analysis of Japanese-Type Sponge Cakes

Instron (model 4202, Instron Corp., MA) was employed to determine the texture characteristics of cakes the day after baking. The texture profile analysis (TPA) was used to obtain the force-time curves of sponge cake samples with the Instron connected to a computer with Instron Series XII software (Cyclic Test). The operation procedures were based on the methods of Szczesniak (1963) and

Vovan et al (1982). A 0.635 cm diameter plunger, attached to the Instron, compressed a cake twice in sequence to a depth of 40% of cake thickness. The Instron was operated with a crosshead speed of 20 cm/min, chart speed of 20 cm/min and a full scale load of 2 N. These operating conditions yielded the lowest variation coefficients for cake firmness, cohesiveness, adhesiveness and elasticity (springiness) (Vovan et al 1982). Analyses of the force-time curve led to the extraction of six textural parameters: four measured (hardness, cohesiveness, adhesiveness and elasticity) and two calculated from the measured parameters (gumminess and chewiness) (Bourne 1978).

Statistical Analyses

Data were subjected to ANOVA and correlation analyses on Minitab (Minitab Inc., PA) and Microsoft Excel (Cambridge, MA) programs, respectively.

RESULTS AND DISCUSSION

Milling Quality Data and Patent Flour Physicochemical Properties

Table I shows some of the quality parameters related to milling and flour physicochemical properties for the soft wheat cultivars used in this study. Test weight values ranged from 56.4 to 62.6 lb/bu, with limited variation among all samples. No significant differences were observed among the four types of wheats. There were not many differences in flour ash contents among cultivars (0.27% to 0.40%) (Table I) and differences were not significant among the four

types of wheats. However, flour starch damage due to the milling process varied greatly from 9.7 ucd for cv. Excel to 18.4 ucd for cv. Madsen (Table I). Soft red winter wheats had significantly lower starch damage than the other three types of wheats ($P<0.05$), while WSWW had significantly higher starch damage than any of the other three types of wheats ($P<0.05$). Since flour starch damage is related to wheat hardness, flour with higher starch damage usually has larger particle size (Nemeth et al 1994). As expected, in addition to the wide variations in flour particle size, flour of cv. Madsen, which had the highest amount of damaged starch, also had the largest particle size (63.0 μm) (Table I). Soft red winter wheats also produced the smallest mean particle size among the four types of wheats (data not shown), which is consistent with their low starch damage as discussed above. These results indicate that of the cultivars tested, SRW wheats were the softest, while club wheats may be the hardest wheats used in the present study since the hardest wheats produced the largest particle size flours and the most damaged starch, and vice versa.

Flour protein contents were relatively low, ranging from 6.7% for Hyak to 8.9% for Madsen (Table I) since flours were extracted at a 45% rate for this study. It was also found that flour protein contents did not differ significantly among the four types of wheats ($P>0.05$).

Alkaline water retention capacity (AWRC) of flour samples ranged from 50.2 to 60.5% (Table I). AWRC was originally considered an important parameter for assessing a soft wheat flour because of its inverse relationship

with SSC diameter (Yamazaki 1953). However, for today's soft wheats, AWRC values do not show the same close association with cookie diameter as they did earlier, perhaps due to changes in breeding materials and practices (Finney 1989). Mean flour AWRC values of the four types of wheats were not significantly different from each other ($P>0.05$). Zeleney sedimentation volumes of these flour samples ranged from 10 to 26 ml (Table I), all of which are indicative of weak flours.

Starch Pasting Characteristics

Flour starch pasting characteristics obtained from the viscograms are presented in Table I. The peak viscosity range was 200 to 1090 B.U.. The peak viscosity indicates the highest viscosity yielded by the starch during the gelatinization process under the given conditions of the test (Shuey and Tipples 1982). It is also interpreted as an index of α-amylase activity present in the flour, the lower peak viscosity indicating higher levels of α-amylase activity (Shuey and Tipples 1982). However, the peak viscosity is also influenced by the amount of damaged starch because it is readily attacked by α-amylases (Dengate 1984). The peak viscosity has proven to be an important quality parameter to JSC baking since a low peak viscosity indicates potential harm to JSCs by the dropping of cake centers during cooling (Nagao et al 1976). Breakdown viscosity, which measures the degree of disintegration of the starch granules (Mazurs et al 1957) or of paste stability (Olkku and Rha 1978), varied

from 110 to 870 B.U. (Table I). The stability of the hot paste can be practically reported as the difference between the peak viscosity and breakdown viscosity (Shuey and Tipples 1982). This difference is termed breakdown (Dengate 1984), which in the current study ranged from 30 to 280 B.U. (Table I).

Both the viscograph peak viscosity and breakdown showed significant differences among the four types of wheat flours ($F=3.85$, $P<0.05$ and $F=11.67$, $P<0.001$, respectively), whereas the breakdown viscosity was the same among the four types of wheat flours ($F=1.89$, $P>0.05$). Soft red winter wheat flours had the highest peak viscosities indicating the least α-amylase activity and perhaps the least damaged starch, which is consistent with the results of starch damage measurement. Unlike SRW wheats, flours of WSWW wheats had the highest starch damage and the smallest peak viscosities among the four types of wheat flours (data not shown). However, the pasting stabilities of SRW flours were the lowest in contrast to WSWW wheat flours, as indicated by their viscograph breakdown values (data not shown).

Patent Flour Rheological Properties

Table II lists the alveograph test results of 17 patent flour samples. The ranges for the alveograph flour properties are: 21.3-56.7 mm for tenacity (P), 69.5-188.1 mm for extensibility (L), 0.11-0.56 for stability (P/L), 18.5-30.4 for swelling index (G), and 43-173 $x10^{-4}$ J for strength (W). All these samples exhibited properties typical of soft wheat flours. Significant differences were

observed in L ($F=3.44$, $P<0.05$), P/L ($F=3.44$, $P<0.01$) and G ($F=3.71$, $P<0.05$) values among the four types of wheat flours, while P and W values were not significantly different among the four types of wheat flours ($P>0.05$). Soft red winter wheat flours had significantly larger L and G values than club wheat flours ($P<0.05$) indicating that doughs of SRW wheat flours were more extensible and stretchable than those of club wheat flours. Both SRW and club wheat flours were not significantly different from ESWW and WSWW wheat flours in L and G values ($P>0.05$). In contrast to their larger L and G values, SRW wheat flours had smaller P/L values than club wheat flours ($P<0.05$).

Farinograph and mixograph test results are shown in Table III. The ranges of farinograph properties are: 48.6-55.0% for water absorption, 0.7-2.0 min for dough development time (peak time), 1.0-4.3 min for stability, and 90-190 B.U. for mixing tolerance index. Mixograph properties of the 17 samples are: peak time 0.8-5.5 min; peak height 32-48.5 mm; stability 1.4-9.5 min; and tolerance 3.0-11.5 mm.

Significant differences in farinograph water absorption ($F=4.60$, $P<0.05$) and mixograph peak height ($F=4.94$, $P<0.05$) were also observed among the four types of wheat flours. Variation in farinograph water absorption is generally recognized to be contributed to by two major factors, protein content and starch damage (Farrand 1969). Therefore, higher farinograph water absorption values of WSWW wheat flours may be caused mainly by their higher levels of damaged starch since their protein contents were not significantly higher than the other

three types of wheat flours ($P>0.05$). Similarly, lower water absorption values of ESWW flours resulted mainly from their lower levels of damaged starch. No significant differences ($P>0.05$) in farinograph peak time, stability and mixing tolerance values were attributable to specific types of wheat flours.

Mixograph peak height of a soft wheat flour can be used as an indicator of protein strength (Finney et al 1987). Accordingly, SRW wheat flours with significantly shorter mixograph peak height values ($P<0.05$) would be weaker in protein strength than WSWW wheat flours with significantly longer peak height values. Similar to the dough farinograph properties, mixograph peak time, stability and tolerance values did not differ significantly among the four types of wheat flours ($P>0.05$).

Japanese-Type Sponge Cake and AACC Sugar-Snap Cookie Baking Results

Patent flour of 45% extraction rate has been found to be ideal for JSC baking. Flour samples in the current study produced JSCs whose volumes ranged from 1088 to 1218 ml; batter viscosity from 7.0 to 11.0 cp; and cake score from 61 to 84 (Table IV). The patent flour quality was also evaluated by making AACC SSCs. The cookie diameter ranged from 8.24 to 9.01 cm; cookie thickness from 5.8 to 8.4 mm and cookie spread factor from 10.0 to 15.3 (Table IV). Greater volume sponge cakes and larger spread cookies indicate better flour quality.

In Japan, cake flour is milled from 100% U.S. western white wheat (Nagao 1989). Earlier, club wheats were found to produce better quality sponge cakes and cookies than soft white wheats even though their protein contents were slightly higher than those of soft white wheats (Nagao et al 1977). Results from the present study showed that significant differences existed in JSC volumes ($F=3.69$, $P<0.05$) and SSC diameters ($F=4.27$, $P<0.05$) among the four types of wheat flours. Furthermore, SRW wheat flours produced significantly larger volume cakes than did club wheat flours ($P<0.05$), while cakes made from ESWW, WSWW and club wheat flours were not significantly different in their volumes ($P>0.05$). With regard to cookie quality, both ESWW and SRW wheat flours produced significantly larger diameter cookies than WSWW and club wheat flours did ($P<0.05$). There was no significant difference ($P>0.05$) in diameter between cookies made from WSWW and club wheat flours, nor for cookies made from ESWW and SRW wheat flours. The cake scores and cookie spread factors were also the same among the four types of flour ($P>0.05$).

Results from the present study also indicated that sponge cake and cookie baking qualities of club and WSWW wheats were inferior to those of SRW wheat (data not shown). These results may suggest that some U.S. SRW wheats may be alternative choices for Japanese milling and baking companies due to their better suitabilities for JSC making. However, Japan has not imported U.S. SRW wheats for many years due to their high price and low availability. Their reputation for higher protein contents than any other type of

soft wheats, even though this was prior to 1986, might also prevent Japan from considering importing U.S. SRW wheats.

Sponge Cake Texture Measurements

Aside from cake volume, the textural characteristics of cakes are also important to cake quality because of their direct relationship to consumer acceptance. Table V shows the textural profile analysis (TPA) results of sponge cakes. Six parameters were derived from each TPA curve. The firmness, adhesiveness, cohesiveness, elasticity, gumminess and chewiness values of sponge cakes tested ranged from 0.732 to 1.075 N, -0.001 to -0.003 J, 2.322 to 3.951, 59.449 to 84.895%, 1.811 to 4.248 N and 38.307 to 63.769 N.mm, respectively. Significant differences were found in cake firmness ($F=3.91$, $P<0.05$) and chewiness ($F=5.22$, $P<0.01$) among the four types of wheats, but not in cake adhesiveness, cohesiveness, elasticity and gumminess ($P>0.05$). Cakes made from WSWW, club and SRW wheat flours were significantly softer in texture than those made from ESWW flours ($P<0.05$), while cakes made from club and SRW wheat flours were significantly less chewy than those made from ESWW and WSWW wheat flours ($P<0.05$). It was also noticed that cakes made from club wheat flours were less cohesive, less gummy, and significantly less chewy ($P<0.05$) than those made from WSWW wheat flours, and were similar in firmness and elasticity ($P>0.05$) to those made from WSWW wheat flour. This could explain why club wheats were presumed to make better quality Japanese-

type sponge cakes than WSWW wheats even though the club wheats produced smaller volume sponge cakes.

Soft red winter wheat flours produced JSC textures similar to those from club wheat flours. Cakes made from SRW wheat flours also had significantly larger volumes than those made from club wheat flours ($P<0.05$), accordingly, SRW wheats could be more beneficial than any other type of wheat for making overall high quality JSCs.

Relationship Between Flour Particle Size or Starch Damage and Other Quality Parameters of Patent Flours

Flour particle size was positively correlated with starch damage, alveograph P/L value, farinograph water absorption value, and negatively correlated with alveograph L and G values, mixograph peak time, JSC volume, cake volume per unit flour protein, SSC diameter, cookie diameter per unit flour protein and cookie spread factor (Table VI). Flour starch damage also correlated positively with AWRC, alveograph P and P/L values, farinograph water absorption value, and negatively with mixograph tolerance value, viscograph peak viscosity, breakdown viscosity and breakdown values, and SSC diameter (Table VI). These results are in good agreement with those of Nemeth et al (1994) except that they did not perform a sponge cake test. A softer kernel wheat will produce flour with less damaged starch, leading to less water absorption by the flour and to a higher viscograph peak viscosity for the flour.

According to Nagao et al (1976), soft wheat flour with small hydration capacity, fine particle size and high amylograph viscosity was desirable for making JSCs and AACC SSCs. Therefore, flours of small particle sizes and less starch damage would make good quality AACC SSCs as confirmed by the present study and elsewhere (Gaines et al 1988, Nemeth et al 1994). However, flour particle size is more important than starch damage to the quality of JSCs based on the present findings.

Relationship Between Japanese-Type Sponge Cake or AACC Sugar-Snap Cookie Baking Qualities and Quality Parameters of Patent Flours

Japanese-type sponge cake volume was significantly positively correlated with alveograph L value, and negatively correlated with flour particle size, alveograph P and P/L values, mixograph peak height and farinograph peak time (Table VII). When the influence of flour protein content was eliminated, the sponge cake volume per unit flour protein correlated significantly and positively with mixograph peak time and stability time, and negatively with flour particle size and mixograph peak height (Table VII). These results indicate that the alveograph is perhaps a better tool than either mixograph or farinograph to evaluate flour properties that may relate to JSC quality. On the other hand, the mixograph is a more precise tool for evaluating flour properties that may relate to corrected cake volume by flour protein content.

Significant correlations were also observed between sponge cake chewiness and Zeleney sedimentation volume ($r=0.557$, $P<0.05$), and between sponge cake elasticity and viscograph breakdown value ($r=-0.537$, $P<0.05$). These results indicate that the properties of flour proteins and starch play roles in affecting the cake texture.

The SSC diameter was significantly positively correlated with alveograph L and G values and mixograph peak time, and negatively correlated with flour particle size, starch damage, alveograph P/L value, mixograph peak height and farinograph water absorption value (Table VII). These results are consistent with those of Nemeth et al (1994). Similar to JSC baking, SSC diameter per unit flour protein was significantly positively correlated with mixograph peak time and stability time, and negatively correlated with flour particle size and mixograph peak height (Table VII). There was also a significant negative correlation between cookie spread factor and flour particle size ($r=-0.494$, $P<0.05$). The prediction of cookie baking quality of soft wheat flours can be achieved by either alveograph or mixograph measurement. However, cookie diameter per unit flour protein could only be related to mixograph measurement. Therefore, for proper evaluation of the soft wheat baking quality of a flour for sponge cakes or cookies, both alveograph and mixograph tests should be employed. It should be emphasized that the flour particle size is a very important quality parameter to both JSC and SSC qualities as indicated by their significant inverse correlations (Table VII). Flour protein content did not correlate significantly with JSC volume

or SSC diameter for the 17 wheat flours used in the present study ($P>0.05$), indicating that the quality rather than quantity of flour proteins may be functional in end-use quality of soft wheat flours.

SUMMARY

Results of this study identified some important differences among ESWW, WSWW, club and SRW wheats grown in the U.S.. The soft red winter and ESWW wheats appeared to be more suitable for making JSCs and AACC SSCs than the WSWW and club wheats used in this study. This may be related to the smaller particle size and less damaged starch of the SRW and ESWW wheat flours. Soft red winter wheats could be considered the most suitable for JSCs if the sponge cake textures are also considered in addition to the large cake volumes. Correlation analyses indicated that flour particle size is one of the most important properties to flour baking qualities. Flour starch damage affected more the quality of cookies than of sponge cakes. The sponge cake textures can also be influenced by the properties of flour protein and starch. The alveograph is a good tool for the evaluation of flours used for making JSCs and SSCs, and the mixograph is an alternate choice, especially for relating flour properties to corrected cake volume and cookie diameter by flour protein content.

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Table I

Milling Qualities, Flour Protein Contents, Alkaline Water Retention Capacities (AWRC), Zeleny Sedimentation Volumes and Starch Viscograph Pasting Properties of 17 Soft Wheat Cultivars

Wheat Cultivar	Test Weight (lb/bu)	Flour Ash[a] (%)[b]	Flour Starch Damage[a] (UCD)[c]	Flour Particle Size[a] (μm)	Flour Protein[a] (%)[b]	Flour AWRC[a] (%)[b]	Flour Sedimentation Volume[a] (ml)	Peak Viscosity (B.U.)	Breakdown Viscosity (B.U.)	Breakdown (B.U.)
ESWW[d]										
Augusta	56.4	0.29	12.3	41.6	7.1	55.4	17	740	615	125
Chelsea	60.0	0.35	14.3	39.1	7.2	55.0	14	715	585	130
Frankenmuth	60.0	0.35	10.5	48.6	8.2	50.2	21	1040	870	170
WSWW[d]										
Kmor	60.6	0.37	15.6	31.9	7.8	55.0	26	595	560	35
Lewjain	61.3	0.28	16.3	42.8	8.1	58.8	21	370	330	40
Madsen	62.6	0.37	18.4	63.0	8.9	57.8	19	320	265	55
Malcom	59.4	0.33	16.9	53.8	7.7	56.3	14	610	580	30
Stephen	62.3	0.32	18.4	60.2	8.3	58.5	13	360	275	85
CLUB										
Crew	60.5	0.39	14.5	53.4	7.2	52.2	13	910	830	80
Hyak	61.8	0.30	18.1	57.2	6.7	60.4	24	200	110	90
Rely	59.5	0.40	14.2	47.0	8.2	53.4	17	810	755	55
Tres	60.2	0.38	16.1	60.5	8.7	60.5	10	770	695	75
SRW[d]										
Caldwell	59.8	0.36	13.3	31.9	7.6	58.1	21	860	730	130
Clark	60.8	0.36	10.1	40.3	7.3	52.5	15	1090	810	280
Dynasty	58.7	0.27	10.5	28.9	7.9	57.2	17	840	640	200
Excel	58.8	0.30	9.7	30.5	7.3	55.3	16	840	720	120
Freedom	60.3	0.28	15.4	45.2	7.8	55.4	16	655	455	200

[a] Values are means of two duplicates.
[b] Percentage of flour weight (14% m.b.).
[c] An arbitrary unit used in the Chopin/Dubois method.
[d] ESWW = eastern soft white winter; WSWW = western soft white winter; SRW = soft red winter.

Table II

Alveograph Test Results

Wheat Cultivar	P[a] (mm)	L[a] (mm)	P/L[a]	G[a]	W[a] (x 10^4 J)
ESWW[b]					
Augusta	21.5	132.5	0.16	25.5	86
Chelsea	23.1	166.0	0.14	28.6	92
Frankenmuth	31.9	128.0	0.25	25.2	125
WSWW[b]					
Kmor	33.3	153.7	0.22	27.6	111
Lewjain	44.4	108.6	0.41	23.1	128
Madsen	37.7	146.1	0.26	26.8	111
Malcom	36.7	110.0	0.33	23.3	88
Stephen	33.2	106.5	0.31	22.9	88
CLUB					
Crew	35.5	69.5	0.51	18.5	62
Hyak	56.7	100.7	0.56	22.2	173
Rely	37.1	133.5	0.28	25.7	97
Tres	24.2	75.2	0.32	19.2	43
SRW[b]					
Caldwell	35.4	138.8	0.26	26.2	141
Clark	29.8	110.5	0.27	23.3	90
Dynasty	21.3	188.1	0.11	30.4	98
Excel	22.0	146.0	0.15	26.8	73
Freedom	33.2	150.7	0.22	27.1	111

[a] P, tenacity; L, extensibility; P/L, the ratio of tenacity to extensibility; G, swelling index; and W, flour strength.

[b] Abbreviations are the same as those in Table I.

Table III

Farinograph and Mixograph Test Results

	Farinograph				Mixograph			
Wheat Cultivar	Water Absorption (%)	Peak Time (min)	Stability (min)	Tolerance Index (B.U.)	Peak Time (min)	Peak Height (mm)	Stability (min)	Tolerance (mm)
ESWW[a]								
Augusta	48.6	0.7	1.3	190	4.3	33.0	6.5	5.0
Chelsea	48.6	1.0	1.7	155	3.3	37.0	3.0	6.0
Frankenmuth	48.9	1.0	3.0	160	2.5	45.1	3.5	8.0
WSWW[a]								
Kmor	48.9	1.0	2.0	160	2.8	48.5	1.8	4.1
Lewjain	51.4	1.1	1.7	115	3.7	43.0	3.5	6.0
Madsen	55.0	1.0	2.6	110	0.8	48.0	3.0	7.0
Malcom	52.4	1.0	1.8	130	2.4	40.0	2.8	5.0
Stephen	53.3	1.0	2.0	150	2.1	44.0	2.5	4.0
CLUB								
Crew	51.8	1.0	2.5	150	2.3	40.5	3.1	6.0
Hyak	52.2	1.0	1.6	150	5.5	36.0	9.5	8.0
Rely	49.2	2.0	3.5	100	2.2	44.0	2.5	5.0
Tres	51.0	1.0	1.3	160	1.1	43.0	1.4	3.0
SRW[a]								
Caldwell	49.4	0.8	1.0	165	5.3	33.0	6.4	7.0
Clark	49.3	1.0	3.5	120	3.0	40.2	4.0	7.8
Dynasty	49.0	1.0	3.0	130	3.5	35.0	4.7	6.0
Excel	50.1	1.0	2.0	190	5.0	32.0	3.3	11.5
Freedom	50.4	1.0	4.3	90	3.3	36.0	5.5	5.0

[a] Abbreviations are the same as those in Table I.

Table IV

Japanese-Type Sponge Cake and AACC Sugar-Snap Cookie Baking Results[a]

Wheat Cultivar	Cake Volume (ml)	Batter Viscosity (cp x 1000)[b]	Cake Score[c]	Cookie Diameter (cm)	Cookie Thickness (mm)	Cookie Spread Factor[d]
ESWW[e]						
Augusta	1205	7.0	73	8.74	6.6	13.3
Chelsea	1185	7.2	73	9.01	6.2	14.6
Frankenmuth	1095	8.9	72	8.60	6.5	13.2
WSWW[e]						
Kmor	1122	8.6	83	8.64	6.7	12.9
Lewjain	1123	8.4	61	8.75	7.1	12.3
Madsen	1115	7.5	65	8.24	7.5	11.0
Malcom	1185	9.9	71	8.41	7.5	11.2
Stephen	1128	8.6	73	8.26	7.7	10.7
CLUB						
Crew	1098	11.0	84	8.46	7.0	12.1
Hyak	1098	7.6	77	8.35	7.3	11.4
Rely	1088	9.1	83	8.38	7.0	12.0
Tres	1143	9.3	73	8.45	7.5	11.3
SRW[e]						
Caldwell	1188	7.3	72	8.88	7.6	11.8
Clark	1150	9.8	79	8.44	8.4	10.0
Dynasty	1218	7.7	82	8.69	7.5	11.6
Excel	1180	9.4	75	8.89	5.8	15.3
Freedom	1155	8.5	69	8.78	6.7	13.1

[a] Values are means of three replicates.
[b] CP = centipoises.
[c] Determined according to AACC Approved Methods (AACC 1992).
[d] The ratio of cookie diameter to thickness.
[e] Abbreviations are the same as those in Table I.

Table V

Textural Profile Analysis Results of Japanese-Type Sponge Cakes[a]

Wheat Cultivar	Firmness[b] (N)	Adhesiveness[b] (J)	Cohesiveness[b]	Elasticity[b] (%)	Gumminess[b] (N)	Chewiness[b] (N.mm)
ESWW[c]						
Augusta	0.968	-0.002	2.711	77.662	2.627	52.636
Chelsea	0.871	-0.003	2.672	81.704	2.324	49.346
Frankenmuth	1.072	-0.003	2.679	84.701	2.875	63.197
WSWW[c]						
Kmor	0.866	-0.003	2.929	82.062	2.527	53.776
Lewjain	1.000	-0.003	3.017	80.840	3.030	63.769
Madsen	0.832	-0.002	3.046	78.462	2.538	51.720
Malcom	0.775	-0.003	2.950	83.038	2.284	49.259
Stephen	0.732	-0.003	2.937	81.316	2.121	44.822
CLUB						
Crew	0.855	-0.003	2.678	84.245	2.281	49.997
Hyak	0.802	-0.002	2.964	78.429	2.374	48.430
Rely	0.931	-0.003	2.665	84.895	2.478	54.642
Tres	0.775	-0.003	2.380	80.036	1.838	38.307
SRW[c]						
Caldwell	0.828	-0.002	3.326	77.565	2.491	50.032
Clark	1.075	-0.001	3.951	59.449	4.248	47.211
Dynasty	0.809	-0.003	2.792	82.359	2.237	47.822
Excel	0.839	-0.003	3.037	81.184	2.545	53.741
Freedom	0.777	-0.003	2.322	83.993	1.811	39.404

[a] Values are means of three replicates.
[b] Definitions are according to Bourne (1978).
[c] Abbreviations are the same as those in Table I.

Table VI

Correlation Coefficients[a] of Flour Particle Size and Starch Damage with Other Quality Parameters of Patent Flours

Quality Parameter[b]	Particle Size (μm)	Starch Damage (UCD)[c]
Starch damage	0.701**	1.0
AWRC	--	0.597*
P	--	0.610**
L	-0.649**	--
P/L	0.545*	0.542*
G	-0.645*	--
MPT	-0.585*	--
MT	--	-0.509*
FWA	0.769***	0.746***
VPV	--	-0.847***
VBV	--	-0.763***
VB	--	-0.680**
JSCV	-0.553*	--
JSCV/FP	-0.499*	--
SSCD	-0.779***	-0.510*
SSCD/FP	-0.521*	--
SSCSF	-0.494*	--

[a] *, **, ***, significant at the 5%, 1% and 0.1% levels, respectively; --, not significant at the 5% level (data not listed).

[b] Abbreviations are the same as those in Table II, and MPT = mixograph peak time; MT = mixograph tolerance; FWA = farinograph water absorption; VPV = viscograph peak viscosity; VBV = viscograph breakdown viscosity; VB = viscosity breakdown; JSCV = Japanese-sponge cake volume; SSCD = sugar-snap cookie diameter; FP = flour protein; SSCSF = sugar-snap cookie spread factor.

[c] An arbitrary unit used in the Chopin/Dubois method.

Table VII

Correlation Coefficients[a] of Japanese-Type Sponge Cake and Sugar-Snap Cookie Test Results with Quality Parameters of Patent Flours

Quality Parameter[b]	JSCV[b]	JSCV/FP[b]	SSCD[b]	SSCD/FP[b]
Particle size	-0.553*	-0.499*	-0.779***	-0.521*
Starch damage	--	--	-0.510*	--
P	-0.639**	--	--	--
L	0.492*	--	0.522*	--
P/L	-0.650**	--	-0.535*	--
G	--	--	0.513*	--
MPT	--	0.760***	0.577*	0.807***
MPH	-0.692**	-0.826***	-0.590*	-0.750***
MS	--	0.585*	--	0.581*
FWA	--	--	-0.667**	--
FPT	-0.490*	--	--	--

[a] *, **, ***, significant at the 5%, 1% and 0.1% levels, respectively; --, not significant at the 5% level (data not shown).

[b] Abbreviations are the same as those in Tables II and VI, and MPH = mixograph peak height; MS = mixograph stability; FPT = farinograph peak time.

Appendix II

Comparative Results of Quantities (%)[a] of Each Glutenin Subunit Group and Total Glutenin Subunits Present in Seven Respective Straight-Grade Flours and Patent Flours

Glutenin Subunit Group[b]	Straight-Grade Flour	Patent Flour	$t^{c,d}$
	Mean		
A subunits	6.94	7.69	1.94 ns
B subunits	11.36	10.76	0.86 ns
C subunits	6.54	6.66	0.61 ns
A+B+C subunits	24.84	25.12	0.24 ns

[a] Percentage of flour protein (14% m.b.).

[b] A subunits = high-molecular-weight glutenin subunits; B and C subunits = two groups of low-molecular-weight glutenin subunits; A+B+C = total glutenin subunits.

[c] Paired "t" test between means of two groups of data.

[d] ns, not significant at the 5% level.