Quantification of Glutenin Subunits by Sequential Acetone Precipitation and by Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis (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%, respectively. 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.

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 achieved 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 PAGE (A-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, A-PAGE has also been used to separate both HMW-GS and LMW-GS (Morel 1994).

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RP-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 because 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, an 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 to: 1) 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) 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 trichlor-

oacetic 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 United States. The samples were milled on a Miag-Multomat mill to obtain patent flours at a 45% extraction rate. The protein contents range of these samples was 6.7–8.9% (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 5-g 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 15,000 × *g* (20°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. Solution B (25 ml) containing 1% (w/v) dithiothreitol (DTT) was added into this residue. After suspension, the mixture was sonicated in a water bath (FS14H, 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; 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% mb). The preparation procedure was done in duplicate. This preparation is herewithin referred to as the sequential acetone precipitation method.

SDS-PAGE

Glutenin subunit groups obtained by the sequential acetone precipitation method were identified by SDS-PAGE. Sample solutions for applying on SDS-PAGE gels were obtained by combining ≈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 m*M* 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. A heated sample solution (10 µl) was loaded per well on the gels. The electrophoresis running conditions were as in Ng and Bushuk (1987), except that the running time was increased to 24 hr for better resolution. Staining and destaining conditions were as in 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 microcentrifuge tube, vortexing for 30 min at 20°C, then centrifuging for 10 min at 14,000 × *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 of acetone was added into the solution (to a total of 80% acetone in the solution) to precipitate the total glutenins. The glutenin precipitate was

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

Cultivar	Flour Protein (14% mb)	Subunits[b,c]			(B+C)/A[b]
		A	B + C	A + B + C	
Augusta[d]	7.1	7.22	16.24	23.46	2.25
Caldwell[e]	7.6	9.61	16.50	26.11	1.72
Chelsea[d]	7.2	8.27	14.68	22.95	1.78
Clark[e]	7.3	9.57	15.78	25.35	1.65
Crew[f]	7.2	9.40	13.70	23.10	1.46
Dynasty[e]	7.9	8.00	16.54	24.54	2.07
Excel[e]	7.3	9.89	15.50	25.39	1.57
Frankenmuth[d]	8.2	8.20	15.84	24.04	1.93
Freedom[e]	7.8	9.16	14.85	24.01	1.62
Hyak[f]	6.7	9.18	16.94	26.12	1.85
Kmor[d]	7.8	10.36	14.12	24.48	1.36
Lewjain[d]	8.1	8.27	16.78	25.05	2.03
Madsen[d]	8.9	7.49	13.84	21.33	1.85
Malcolm[d]	7.7	7.25	15.48	22.73	2.14
Rely[f]	8.2	9.84	15.55	25.39	1.58
Stephens[d]	8.3	6.91	14.36	21.27	2.08
Tres[f]	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 = HMW-GS; B + C = total LMW-GS; A+B+C = total glutenin subunits; (B+C)/A = LMW-GS to HMW-GS ratio.
[c] Percentage of patent flour protein (14% mb).
[d] Soft white wheat.
[e] Soft red wheat.
[f] Club wheat.

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. This preparation is herewithin referred to as the densitometric method.

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

HMW-GS 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 of 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 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 GS300 transmittance/reflectance scanning densitometer with GS365W 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 the 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 of 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.

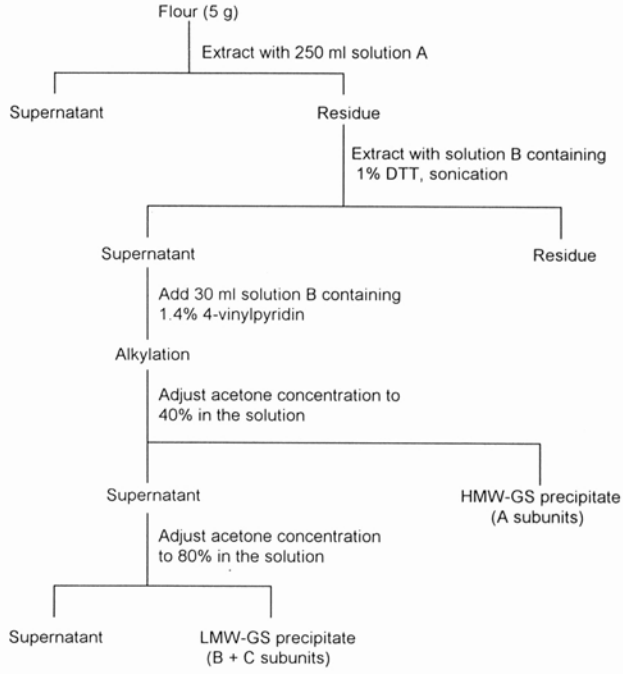

Fig. 1. Schematic outline of the sequential acetone precipitation method to obtain high molecular weight glutenin subunit (HMW-GS or A subunits) and low molecular weight glutenin subunit (LMW-GS or B + C subunits) groups. Method based on Melas et al (1994) with some modifications. Solution A is 60% aqueous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

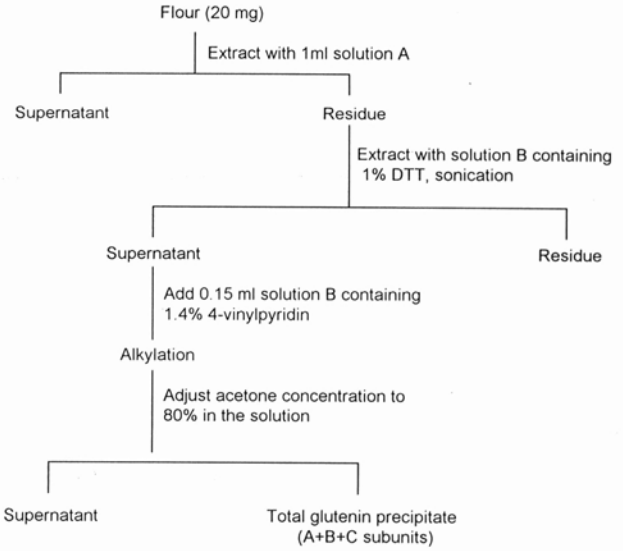

Fig. 2. Schematic outline of the extraction method to obtain relatively pure total glutenin proteins (A + B + C subunits) for densitometric analysis. Solution A is 60% aqueous ethanol solution, solution B is a 50% 2-propanol solution containing 0.08*M* Tris-HCl buffer (pH 8.0). DTT = dithiothreitol.

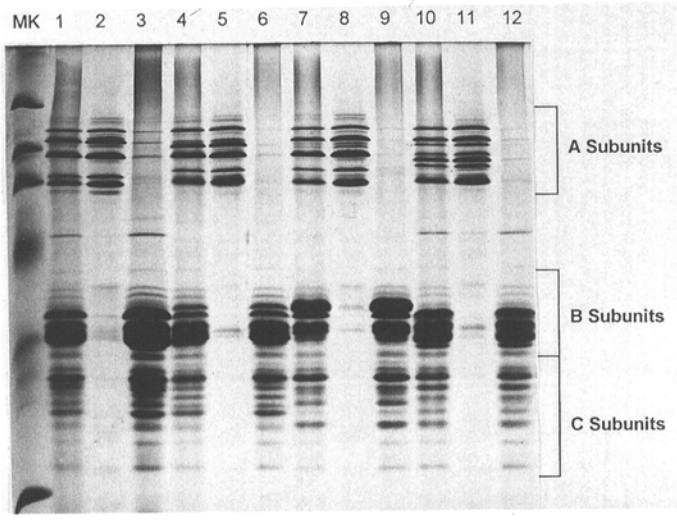

Fig. 3. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of high molecular weight glutenin subunit (HMW-GS or A subunits) and low molecular weight glutenin subunit (LMW-GS or B + C subunits) groups obtained by sequential acetone precipitation (40 and 80% acetone, respectively). Total glutenin subunits of each cultivar precipitated directly from the extracts of glutenins by 80% acetone were included as controls. Wheat cultivars: 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 (from top to bottom) 205, 116, 97.4, 66, 45, and 29 kDa.

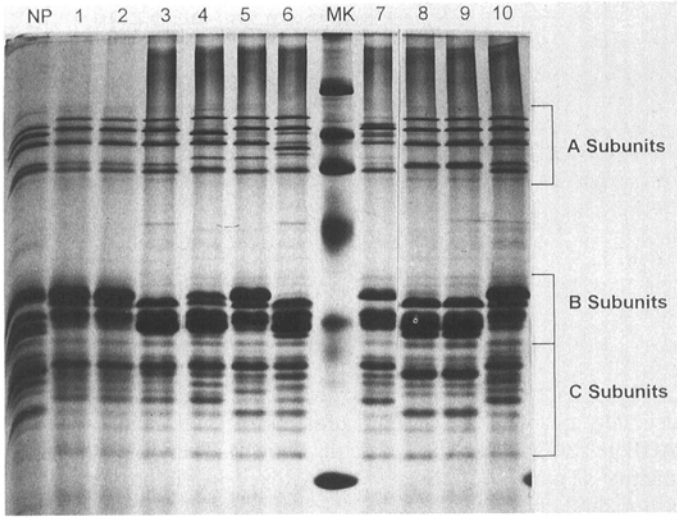

Fig. 4. Sodium dodecyl sulfate polyacrylamide gel electrophoresis patterns of total glutenin subunits obtained from 80% acetone precipitation of eight soft wheat cultivars for densitometric analysis. Wheat cultivars: Augusta (lane 3), Caldwell (lane 4), Chelsea (lane 5), Clark (lane 6), Crew (lane 7),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

Statistical Analyses

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

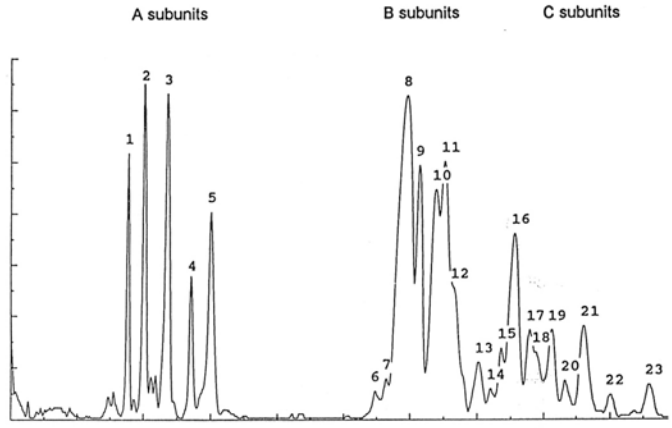

Fig. 5. Densitometric reading of glutenin subunits from cultivar Chelsea. Peaks 1–5 are high molecular weight glutenin subunits (A subunits); peaks 6–13 are B subunits; peaks 14–23 are C subunits. B and C subunits are low molecular weight glutenin subunits.

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 before extracting glutenins 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 ≈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°C) could remove some high molecular weight polypeptides (>60,000) 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 preextraction of gliadins with 50% 1-propanol or 2-propanol at 60°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 (Fig. 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

TABLE II
Quantity of Glutenin Subunits per Group Determined by Densitometer[a]

Cultivar	Subunits[b] A	B	C	B+C	A+B+C	(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
Malcolm	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]	7.26a	10.18b	6.89a	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% mb).
[c] Values within this row with different letters are significantly different at the 5% level.

may be a result of HMW-GS variants or a potential mixture 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 do 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 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).

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

Parameter	Precipitation[a]	Densitometer[a]	t^b
Extractable GS groups[c]			
HMW-GS	8.46	7.26	5.25***[d]
LMW-GS	15.29	17.07	8.39***[d]
Total GS	23.75	24.33	2.11ns[e]

[a] Percentage of patent flour protein (14% mb). Each value is the mean of 17 samples.
[b] Paired t test between two groups of data.
[c] HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.
[d] Significant at the 0.1% level.
[e] Not significant at the 5% level.

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 nonuniformity, 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

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

	Sequential Acetone Precipitation[b]			Densiometric Method[c]				
Parameter	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] **, *** are significant at the 1% and 0.1% levels, respectively.
[b] A subunits (HMW-GS), B + C subunits (total LMW-GS), and A + B + C subunits (total glutenin subunits), respectively. HMW-GS = high molecular weight glutenin subunits; LMW-GS = low molecular weight glutenin subunits.
[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.

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. Because 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, preextraction 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-GS and a smaller proportion HMW-GS 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 ratios of LMW-GS to HMW-GS 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, as 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 a 45% extraction rate were quantitatively determined by the sequential acetone precipitation method and by the method of SDS-PAGE coupled with 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 ≈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 because of 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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