Relationships of Quantity of Gliadin Subgroups of Selected U.S. Soft Wheat Flours to Rheological and Baking Properties

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ABSTRACT

The gliadin subgroups (ω-, γ-, β-, and α-gliadins) in 17 soft wheat patent flours from four wheat classes and seven straight-grade flours were identified and quantified by acid polyacrylamide gel electrophoresis (A-PAGE) coupled with densitometry using a known quantity of modified Osborne gliadins as a quantitative standard. Flour rheological properties were evaluated by alveograph, farinograph, and mixograph tests. Japanese-type sponge cakes (JSC) and AACC sugar-snap cookies (SSC) were made to evaluate the flours' baking performances. The results showed that the quantity of α-gliadins was wheat class related. The percentages of individual gliadin subgroups in flour protein were significantly lower in the patent flours than in counterpart straight-grade flours. The quantities of certain gliadin subgroups and total gliadins have various associations with flour rheological properties, and JSC and SSC end-use quality for each class of wheat.

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, for a soft wheat flour, 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 done 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 can not be separated well in some cases (Wieser et al 1994) in contrast to acid polyacrylamide gel electrophoresis (A-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 1991, 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 gliadin subgroup or glutenin subunit 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 selected soft wheat patent flours and straight-grade flours by A-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 four classes of soft wheats produced in the United States: three eastern soft white winter (ESWW) wheats, five western soft white winter (WSWW) wheats, four club wheats, and five soft red winter (SRW) wheats, as described previously (Yamamoto et al 1996). The samples were milled on a Miag-Multomat mill to obtain short patent flours of 45% extraction rate. The protein contents of these flour samples ranged from 6.7 to 8.9%. Seven of the 17 cultivars (Augusta, Caldwell, Chelsea, Dynasty, Freedom, Hyak, and Lewjain) also were 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 (Indianapolis, IN). Ascorbic acid, Coomassie Brilliant Blue R250, ferrous sulfate, N,N'-methylenebisacrylamide, and silver nitrate were from Sigma Chemical (St. Louis, MO). Ammonium persulfate (crystal), hydrogen peroxide (30%, AR), and trichloroacetic acid (TCA) were from J. T. Baker (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 (Columbus, WI). Distilled and deionized water was used throughout the study.

Gliadin Preparation and A-PAGE

The extraction of gliadin proteins from flours was performed according to the following procedure. Flour (100 mg) was placed into a microcentrifuge tube, after which 0.4 ml of 60% ethanol was added. The 60% ethanol is one of the most efficient ethanol

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solutions for gliadin extraction (Wieser et al 1994). The extraction was started by 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 centrifuged for 6 min at 14,000 × *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. Extract dilution solution (250 µl) (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 A-PAGE analysis. The gliadin extraction was prepared twice from each sample.

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 A-PAGE coupled with densitometry. The protein content of the modified Osborne gliadin fraction was determined by the micro-Kjeldahl method (AACC 1995). 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.

A-PAGE at pH 3.1 was performed according to Lafiandra and Kasarda (1985) with some modifications. Electrophoresis was performed in gels 1.5-mm thick (18-cm wide, 16-cm long) with a vertical electrophoresis apparatus (Hoefer 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 the 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 performed twice for each gliadin extract on separately run gels.

Identification and Quantification of Gliadin Subgroups by Densitometry

After rinsing the gels, the electrophoretic patterns were analyzed by a transmittance/reflectance scanning densitometer (GS 300, Hoefer Scientific Instruments, San Francisco, CA) with GS 365W software. 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 (1991).

The relative quantity (called quantity hereinafter) of each group of gliadins was calculated from the 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. The PAGE pattern of each gliadin extract (two gliadin extracts for each flour sample) was scanned once, 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

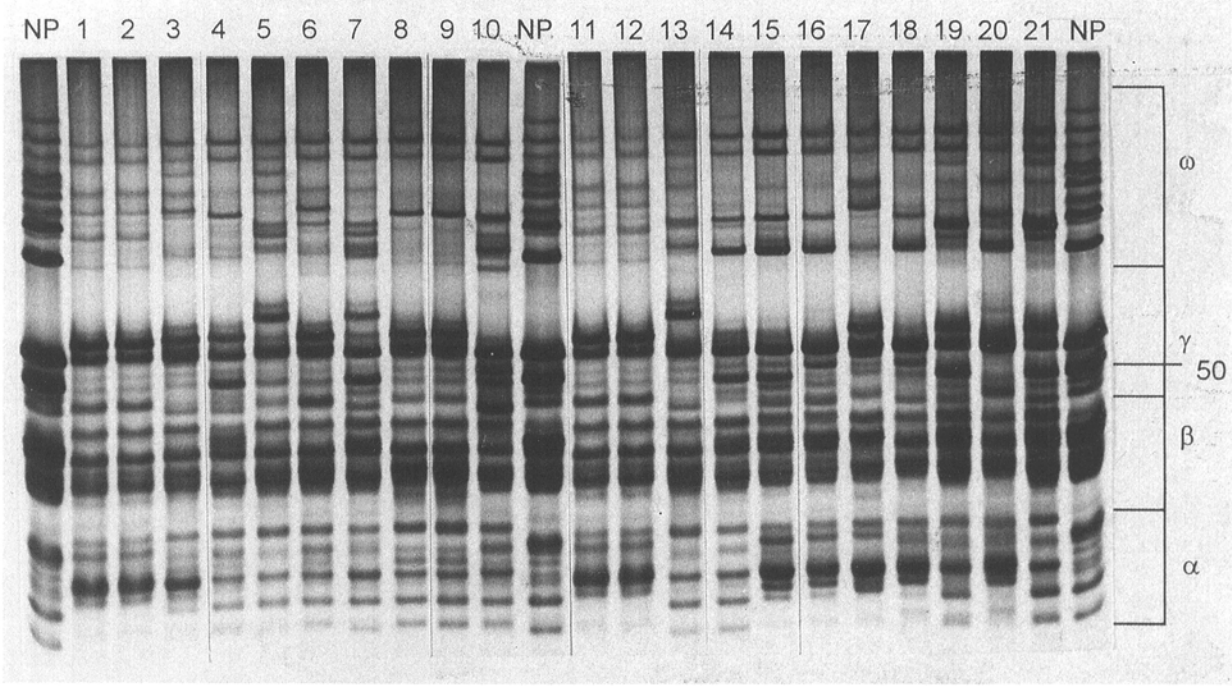

Fig. 1. Acid polyacrylamide gel electrophoretic patterns of 17 soft wheat cultivars. Lanes 1, 2, 11, and 12 = protein standards (cv. Pioneer 2555) for gliadin subgroup quantification; 3 = Augusta; 4 = Caldwell; 5 = Chelsea; 6 = Clark; 7 = Crew; 8 = Dynasty; 9 = Excel; 10 = Freedom; 13 = Frankenmuth; 14 = Hyak; 15 = Kmor; 16 = Lewjain; 17 = Madsen; 18 = Malcolm; 19 = Rely; 20 = Stephens; 21 = Tres; NP = Neepawa (reference). 50 = Reference band of cultivar Neepawa. ω, γ, β, and α indicate gliadin subgroups, respectively, based on the method of Bushuk and Sapirstein (1991).

(JSC), and micro sugar-snap cookies (SSC) for the 17 cultivars were obtained from an earlier study (Yamamoto et al 1996). Flour protein content determinations, mixograph, and micro SSC-baking tests of the seven straight-grade flours were conducted according to standard methods (AACC 1995).

Statistical Analyses

Data were subjected to one-way analysis of variance (ANOVA) using the Minitab program (Minitab Inc., PA), and to paired *t*-test and correlation analyses using a Microsoft Excel program (Cambridge, MA).

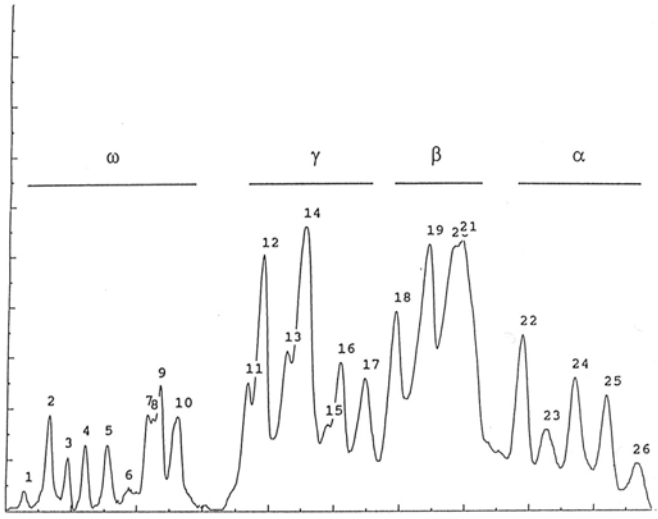

Fig. 2. Densitometric readings of gliadin proteins from cultivar Chelsea. Peaks 1–10 are ω-gliadins; peaks 11–17 are γ-gliadins; peaks 18–21 are β-gliadins; and peaks 22–26 are α-gliadins.

RESULTS AND DISCUSSION

A-PAGE of Gliadin Proteins from Soft Wheat Cultivars

Figure 1 shows the A-PAGE patterns of gliadin proteins for the 17 patent flour samples used in this study. Genotypic 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 A-PAGE, the gliadins of each cultivar were divided into four subgroups: ω-, γ-, β-, and α-gliadins (Bushuk and Sapirstein 1991). There were clear boundaries between adjacent gliadin subgroups, as seen from A-PAGE (Fig. 1).

Quantification of Gliadin Subgroups in Soft Wheat Flours

The typical densitometric readings of gliadin proteins are shown in Figure 2. The quantities of each gliadin subgroup determined for the 17 patent flour samples from four wheat classes are listed in Table I. The results from ANOVA revealed that the means of the quantities of α-gliadins were significantly different among wheat classes. This result suggests that α-gliadin content in flour protein is wheat class related. For each wheat class and combined sample, significant differences were also observed among quantities of the four gliadin subgroups in patent flour, with the β-gliadins present in highest quantity, followed by γ-, then α-, and ω-gliadins present in the lowest quantity. 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 analyses. These observations are also generally consistent with the results of Branlard and Dardevet (1985) using densitometry coupled with A-PAGE for bread wheat flours. In the present study, the percentage of total gliadins in the 17 patent flour proteins ranged from 36.35 to 52.10% with an average of 43.11%.

Table II shows the ranges of quantities of gliadin subgroups present in seven straight-grade flours. The mean values deter-

TABLE I
Quantities (%) of Gliadin Subgroups Determined by Densitometry for 17 Soft Wheat Patent Flours[a]

	Gliadins				
Cultivar[b]	**ω**	**γ**	**β**	**α**	**Total**
ESWW					
Augusta	3.02 ± 0.03	13.07 ± 0.25	11.25 ± 0.12	9.80 ± 0.33	37.14
Chelsea	4.23 ± 0.29	12.55 ± 0.55	14.56 ± 0.56	6.66 ± 0.14	38.00
Frankenmuth	4.43 ± 0.35	12.80 ± 1.11	17.85 ± 1.54	8.60 ± 0.85	43.68
Mean	3.89	12.81	14.55	8.35bc	39.61
WSWW					
Kmor	8.03 ± 0.54	14.14 ± 0.95	14.92 ± 1.37	11.72 ± 0.61	48.80
Lewjain	5.02 ± 0.15	15.03 ± 1.32	13.28 ± 0.62	9.08 ± 0.28	42.40
Madsen	4.47 ± 0.42	12.97 ± 1.27	13.84 ± 0.71	9.89 ± 0.90	41.17
Malcolm	5.37 ± 0.57	14.08 ± 1.45	14.79 ± 1.90	12.50 ± 1.05	46.74
Stephens	5.93 ± 0.51	11.60 ± 0.70	13.42 ± 1.28	13.62 ± 0.99	44.57
Mean	5.76	13.56	14.05	11.36a	44.74
Club					
Crew	5.70 ± 0.30	14.70 ± 1.01	19.49 ± 1.74	8.22 ± 0.16	48.10
Hyak	4.59 ± 0.43	13.82 ± 1.10	13.96 ± 1.41	6.01 ± 0.35	38.39
Rely	7.05 ± 0.73	16.78 ± 1.75	16.95 ± 1.80	11.32 ± 0.96	52.10
Tres	6.64 ± 0.64	13.89 ± 1.42	20.30 ± 1.95	9.02 ± 1.01	49.86
Mean	6.00	14.80	17.68	8.64b	47.11
SRW					
Caldwell	3.92 ± 0.35	12.56 ± 1.27	13.41 ± 1.56	7.35 ± 0.49	37.24
Clark	4.38 ± 0.18	14.10 ± 0.42	14.30 ± 1.20	8.21 ± 0.16	41.00
Dynasty	2.20 ± 0.16	11.12 ± 0.51	15.94 ± 1.11	7.10 ± 0.15	36.35
Excel	3.48 ± 0.29	12.59 ± 0.43	16.00 ± 1.52	8.94 ± 0.35	41.00
Freedom	7.63 ± 0.51	15.36 ± 0.67	14.80 ± 0.45	8.47 ± 0.25	46.26
Mean	4.32	13.15	14.89	8.01c	40.37

[a] Percentage of patent flour protein (14%, mb). Values are means ± standard deviation of four measurements. Mean values with different letters in a column are significantly different at the 5% level (Tukey's pairwise comparisons).
[b] ESWW = Eastern soft white winter; WSWW = western soft white winter; SRW = soft red winter.

mined 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, total gliadins, and flour proteins were statistically significantly higher in seven straight-grade flours than in counterpart patent flours. However, the differences in flour protein content (0.8%) were much smaller than those in the quantities of each gliadin subgroup (1.8–5.3%) and total gliadins (13.9%) between these two types of flours. These differences may be caused by the different extraction rates of these two types of flours.

Comparison of Mixograph and SSC-Baking Properties of Seven Soft Wheat Short Patent Flours and Straight-Grade Flours

The quality differences between short patent flour and straight-grade flour samples from the seven cultivars are shown in Table III. The straight-grade flour showed significantly shorter mixograph peak time and larger peak height than did patent flour. However, mixograph stability and tolerance values were not significantly different between these two types of flours. In another study (Yamamoto et al 1996) 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 SSC. It was, therefore, anticipated that the straight-grade flours would make smaller SSC, and, in fact, they did produce cookies with significantly smaller cookie diameter and spread factor and greater thickness than did patent flours (Table III). Because 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 smaller than that for patent flour. The small difference in flour protein content between these two types of flours may not be solely responsible for the variations in mixing properties and cookie-baking quality.

Association of Quantities of Gliadin Subgroups with Soft Wheat Patent Flour Quality

Table IV shows the correlation coefficients between patent flour properties and the quantities of gliadin subgroups and total gliadins present in the flour protein for each class of wheat. In ESWW wheat flours, the total gliadin content had strong positive correlations with the alveograph *W* value and flour protein content, and negative correlations with JSC-baking quality. Because the protein content of ESWW wheat flours also had strong positive correlations with the *W* value and farinograph water absorption value, it might contribute to the association of the quantity of total gliadins with flour rheological and baking properties.

In WSWW wheat flours, the quantity of ω-gliadins was negatively correlated with mixograph stability value, and the quantity of α-gliadins was negatively correlated with the *W* value, suggesting that α-gliadins could weaken the dough strength in this class of flours. In terms of club wheat flours, the quantity of ω-, γ- and β-gliadins showed stronger associations with flour rheological and baking qualities. The quantity of ω-gliadins correlated positively with mixograph peak height, and negatively with JSC volume per unit flour protein; the quantity of γ-gliadins was positively correlated with farinograph peak time and negatively with farinograph tolerance index; and the quantity of β-gliadins correlated negatively with the *W* value, and positively with SSC diameter, suggesting that the β-gliadins may be beneficial to flour cookie-baking quality. In addition, the quantity of total club wheat gliadins was positively correlated with mixograph peak height, and negatively correlated with mixograph stability time. The quantities of γ-gliadins and β-gliadins in SRW flour proteins were negatively correlated with JSC volume and the *P* value, respectively.

Results from the present study suggested that both the quantities of certain gliadin subgroups and the total flour protein were functional in flour for JSC and SSC end-use quality, and that the degree of association of each gliadin subgroup content with flour rheological and baking properties differed from one wheat class to another. These findings deserve further confirmation with more varieties selected from each wheat class. The balance of elasticity and extensibility in doughs has been 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 potentially detrimental results in baking quality.

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

	Straight-Grade Flours[a]			Patent Flours[b]	
Gliadin Subgroup	Minimum	Maximum	Mean	Mean	t[c]
ω	4.18	9.61	6.17	4.37	16.25***
γ	13.99	19.32	17.28	13.36	5.36**
β	16.06	21.26	19.15	13.89	6.04***
α	8.37	13.57	10.65	7.78	7.08***
Total	44.15	59.52	53.25	39.40	8.50***
Flour protein (%)[d]	7.4	8.8	8.3	7.5	3.44*

[a] Percentage of straight-grade flour protein (14%, mb). Each value is mean of seven flour samples.
[b] Percentage of patent flour protein (14%, mb). Each value is mean of seven flour samples.
[c] Paired *t*-test between means of two groups of data. *, **, and *** are significant at the 5, 1, and 0.1% levels, respectively.
[d] 14% mb.

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

	Mean		
Parameter[a]	Patent Flour	Straight-Grade Flour	t[b]
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] MPT = mixograph peak time; MPH = mixograph peak height; MS = mixograph stability; MT = mixograph tolerance; SSCD = sugar-snap cookie diameter; FP = flour protein; SSCT = sugar-snap cookie thickness; SSCSF = sugar-snap cookie spread factor (ratio of cookie diameter to thickness).
[b] Paired *t*-test between means of two groups of data; *, **, and *** = significance at 5, 1, and 0.1%, respectively; ns = not significant at the 5% level.

TABLE IV
Correlation Coefficients of Quantities[a] of Gliadin Subgroups in Patent Flours of 17 Soft Wheat Samples to Flour Rheological and Baking Properties[b,c]

	ESWW (n = 3)	WSWW (n = 5)	Club (n = 4)	SRW (n = 5)
ω-Gliadins (%) and				
MPH (mm)	0.834	0.375	0.993**	0.269
MS (min)	−0.965	−0.894*	−0.895	0.315
JSCV (ml)	−0.736	−0.099	0.229	−0.758
JSCV/FP (ml/%)	−0.708	0.392	−0.974*	−0.674
FP (%)	0.672	−0.581	0.910	0.116
γ-Gliadins (%) and				
FPT (min)	−0.877	0.621	0.957*	0.202
FTI (BU)	0.933	−0.281	−0.965*	−0.598
JSCV (ml)	0.192	0.175	−0.595	−0.928*
FP (%)	−0.104	−0.495	0.294	−0.158
β-Gliadins (%) and				
P (mm)	0.928	−0.493	−0.947	−0.921*
W ($\times 10^{-4}$ J)	0.928	−0.298	−0.991**	−0.780
SSCD (cm)	−0.334	0.098	0.959*	0.098
FP (%)	0.903	−0.583	0.648	0.115
α-Gliadins (%) and				
W ($\times 10^{-4}$ J)	−0.008	−0.913*	−0.545	−0.602
MPT (min)	0.437	−0.145	−0.733	0.001
MPH (mm)	−0.194	−0.246	0.944	0.003
MS (min)	0.865	−0.623	−0.795	−0.584
SSCD/FP (cm/%)	−0.223	0.101	−0.796	0.630
FP(%)	0.053	−0.362	0.759	−0.607
Total gliadins (%) and				
W ($\times 10^{-4}$ J)	1.000**	−0.384	−0.813	−0.197
MPT (min)	−0.893	0.329	−0.923	−0.375
MPH (mm)	0.978	−0.037	0.983*	0.271
MS (min)	−0.490	−0.811	−0.959*	−0.101
FPT (min)	0.601	−0.420	0.550	0.446
JSCV (ml)	−0.999*	0.416	0.165	−0.799
JSCV/FP (ml/%)	−1.000**	0.763	−0.894	−0.459
SSCD/FP (cm/%)	−0.978	0.728	−0.834	0.071
FP (%)	0.999*	−0.826	0.814	−0.093
FP (%) and				
W ($\times 10^{-4}$ J)	0.998*	0.193	−0.716	0.458
MPT (min)	−0.875	−0.715	−0.857	−0.247
MPH (mm)	0.969	0.476	0.896	−0.123
MS (min)	−0.456	0.352	−0.840	0.590
FWA(%)	0.997*	−0.777	−0.686	−0.117

[a] Percentage of patent flour protein (14% mb)
[b] * and ** = Significant at the 5 and 1% levels, respectively. Values in parentheses are numbers of observations in 17 flour samples.
[c] ESWW = Eastern soft white winter; WSWW = western soft white winter; SRW = soft red winter; MPT = mixograph peak time; MPH = mixograph peak height; MS = mixograph stability; SSCD = sugar-snap cookie diameter; FP = flour protein (14%, mb); JSCV = Japanese sponge cake volume; FPT = farinograph peak time; FTI = farinograph tolerance index; P = alveograph tenacity; W = alveograph strength; FWA = farinograph water absorption.

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