

Swelling Index of Glutenin Test. I. Method and Comparison with Sedimentation, Gel-Protein, and Insoluble Glutenin Tests

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ABSTRACT

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Molecular weight distribution of wheat proteins is primarily responsible for the viscoelastic properties of flour dough. Furthermore, the amount of SDS insoluble proteins (mainly high molecular weight glutenin) plays the major role. We have developed a simple test to determine the swelling power of glutenin (swelling index of glutenin or SIG) for predicting dough properties and end-use quality. Flour samples (40 mg) were hydrated in distilled water and then allowed to swell in nonreducing solvents (SDS, lactic acid, or mixtures of the two) followed by low speed centrifugation. The SIG was calculated as the weight of the residue divided by the original sample weight. The SIG test was compared with the results from other small-scale tests for 20 flour samples. SIG tests showed highly significant correlations with the gel protein and insoluble glutenin test ($r \geq 0.85$, $r \geq 0.93$, $P < 0.001$, respectively) and significant correlations with SDS and Zeleny sedimentation tests ($r \geq 0.74$, $r \geq 0.72$, $P < 0.001$, respectively). The swelling capacity of glutenin depended on

swelling time and mixing intensity in nonreducing solvents. Swelling curves obtained from SIG values versus different swelling time can be divided into three distinct stages: swelling, swollen, and breakdown. These stages may reflect soluble and insoluble glutenin contents and quality among different cultivars. SIG test values for short swelling time and low mixing intensity were significantly correlated to gel protein content and SDS-sedimentation values ($r = 0.96$, $r = 0.90$, $P < 0.001$, respectively). SIG test values for long swelling time and high mixing intensity were significantly correlated to insoluble glutenin content ($r = 0.96$, $P < 0.001$). The difference of swelling condition (time and mixing intensity) among these small-scale methods is the reason for their different correlations with insoluble glutenin content. Because large numbers of samples can be analyzed in a short time with excellent reproducibility, the SIG test may be a useful screening test in a breeding program, predicting the quantity and quality of insoluble glutenin.

Protein quantity and quality are important attributes in the variation of the breadmaking quality of wheat flour (Finney and Barmore 1948). The insoluble glutenin content, which is considered to be a key expression of protein quality, is directly related to breadmaking quality (Orth and Bushuk 1972; Gupta et al 1992; Bean et al 1998; Sapirstein and Fu 1998). Glutenin has the important property of swelling in various nonreducing solvents (dilute acetic acid, lactic acid, and SDS) and the swelling volume appears to be directly related to quantity and quality of glutenin present (Weegels et al 1996). Therefore, small-scale tests used to predict breadmaking quality are based on glutenin swelling capacity or directly on insoluble glutenin content.

The Zeleny (1947) and SDS-sedimentation tests (McDermott and Redman 1977) measure the sedimentation volume of a suspension of flour in dilute lactic acid and in SDS-lactic acid solvents, respectively. The sedimentation volume depends mainly on the amount and the swelling characteristics of the glutenin, since other proteins such as gliadin are soluble in the Zeleny and SDS test solutions (Echert et al 1993). The SDS-sedimentation test correlates better with loaf volume than the Zeleny sedimentation test (Axford et al 1979). Therefore, a number of modified SDS-sedimentation tests have been developed (Kovacs 1985; Pena et al 1990) and are widely used in predicting dough properties and breadmaking qualities in the early stages of wheat breeding programs (Weegels et al 1996).

The determination of gel protein, also called glutenin macropolymer (Weegels et al 1994), is a more direct test in which the swollen insoluble glutenin forms a gel-like layer on top of the starch pellet when SDS extracts are centrifuged (Graveland et al 1979). The high centrifugation speed separates the starch from the swollen glutenin and the weight of the remaining gel protein is an index of the quantity of insoluble glutenin present (Graveland et al 1982).

The most direct means of determining the insoluble glutenin content is by a sequential extraction procedure (Orth and Bushuk 1972; Orth and O'Brien 1976; Fu and Sapirstein 1996) but the protein fractionation and Kjeldahl determinations make it a complex and

time-consuming process. In a recent report, monomeric protein and soluble glutenin were extracted with 50% 1-propanol and the remaining protein pellet analyzed by nitrogen combustion (Bean et al 1998), a method that is quicker than Kjeldahl.

Although the glutenin swelling characteristics are the basis of sedimentation and gel protein tests, little is known of their role in these tests. In this report, the effects of various swelling conditions and various swelling solvents on glutenin swelling properties were investigated, and a test to determine the swelling power of glutenin (swelling index of glutenin or SIG) was developed. The test used only a small sample size (which is important in breeding programs) and had the added advantages of being simple and quick. The implication of differences among Zeleny, SDS sedimentation, gel protein, and SIG tests is presented on the basis of glutenin swelling properties and glutenin fractions (soluble and insoluble glutenin).

MATERIALS AND METHODS

Wheat Flours and Small-Scale Tests

A set of 20 cultivars was chosen to represent a wide range of dough rheological properties and breadmaking quality. They were field-grown in 1996 at Manitoba, Canada. Grain samples were tempered to 15 and 16% moisture content according to grain hardness, and milled into straight-grade flour on a Buhler laboratory mill.

The SDS-sedimentation test was conducted according to Kovacs (1985). The Zeleny sedimentation test was performed as specified by Approved Method 56-60 (AACC 2000). The gel protein was determined based on the procedure of Graveland et al (1979). Flour (1.5 g) was suspended in 15 mL of distilled water by intermittent vigorous hand-shaking for 20 min and then 15 mL of 3% SDS was added. The mixture was swollen for another 20 min at $24 \pm 1^\circ\text{C}$ with intermittent vigorous hand-shaking. The suspension was centrifuged at $60,000 \times g$ for 30 min at 15°C , and the clear supernatant was discarded. The gel layer was recovered using a spatula and weighed. The gel protein was calculated as the weight of the gel divided by the original sample weight.

Determination of Soluble and Insoluble Glutenin in Flour

Proteins were fractionated according to a modified procedure of Fu et al (1998), and the protein fractions were determined by measuring the turbidity of the protein precipitate with trichloroacetic acid based on a simple assay for the determination of proteins in solu-

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tion (Choi et al 1993). This procedure classified the proteins into three fractions: monomeric protein, soluble glutenin, and insoluble glutenin. After removing all the monomeric proteins using the method of Fu and Kovacs (1999) from 100 mg of flour, the soluble glutenin was extracted with 3×1.0 mL of 40% (v/v) 1-propanol for 30 min each time. The suspensions were centrifuged at $15,000 \times g$ for 5 min, and the three supernatants were pooled. Similarly, insoluble glutenin was extracted from the residue with 3×1.0 mL of 40% (v/v) 1-propanol containing 0.2 % (w/v) dithiothreitol (DTT) at 60°C for 30 min each time, and the three supernatants were pooled after $15,000 \times g$ for 5 min. A mixture of 40% 1-propanol with or without reducing agent is more efficient than 50% 1-propanol for extracting soluble glutenin or insoluble glutenin (Wang et al 1998). Subsamples (0.4 mL) of supernatant for soluble or insoluble glutenin determinations were mixed with 4 mL of 40% (w/v) trichloroacetic acid. After standing for 35 min, the turbidity was determined at 590 nm. Calibration curves for soluble and insoluble glutenin were prepared based on standard wheat flour (cultivar Genesis) extracts obtained in the same procedure as described above. Protein content of soluble and insoluble glutenin fractions obtained from Genesis was determined by Approved Method 46-13 ($N \times 5.7$) (AACC 2000). The calibration curves were developed by diluting the known protein content fractions with 40% 1-propanol. Absorption values at 590 nm for soluble and insoluble glutenin were converted to protein concentration, expressed in terms of the percentages of flour.

SIG Test in SDS-Lactic Acid (SIG-SDSLA)

Flour (40 mg) was weighed into a 1.5-mL preweighed plastic microcentrifuge tube and 0.6 mL of distilled water was added. The tubes were quickly capped and mixed thoroughly using a single-tube vortex stirrer (Vortex Genie 2, Scientific Industries, Bohemia,

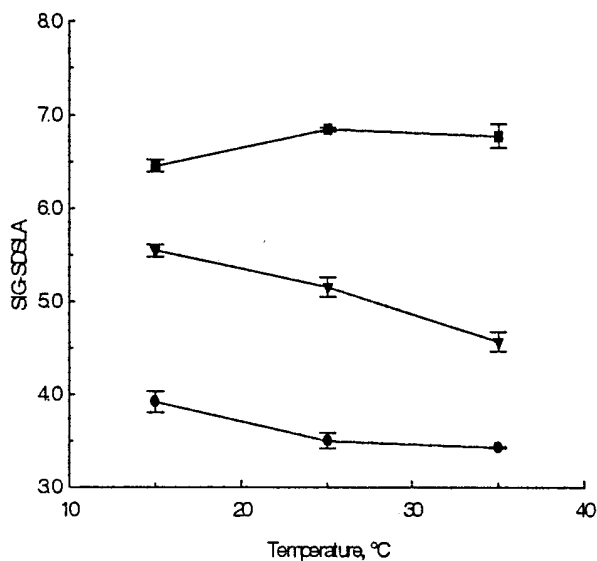


Fig. 1. Effect of temperature on swelling index of glutenin (SIG) in SDS-lactic acid (SIG-SDSLA) tests. Error bars indicate standard deviations ($n = 2$). Suneca (■), Banks (▼), Alpha 16 (●).

NY) for 5 sec. The contents of the tubes were then mixed in a thermomixer (Eppendorf model 5436, Brinkmann Instruments, Westbury, NY) at 1,400 rpm for 20 min with a 5-sec vortexing at 10 and 20 min. After the hydration, 0.6 mL of SDS-lactic acid stock solution as described in Approved Method 56-70 (AACC 2000) was added and the tubes were vortexed for 5 sec. The tubes were then placed in the thermomixer at 1,400 rpm for 20 min with a 5-sec vortexing at 10 and 20 min, followed by immediate centrifugation at $300 \times g$ for 5 min (Micromax model, International Equipment Co., Needham Height, MA). The bulk of the supernatant and foam on the surface was removed quickly with a 1-mL syringe connected to a water aspirator filter pump, and the residue was recentrifuged at $300 \times g$ for 2 min. The remaining supernatant was then drawn off with the syringe equipped with a needle, taking care not to contact the residue's surface. The tubes and precipitates were weighed and the SIG was calculated as the weight of the swollen precipitate divided by the original sample weight (14% moisture base).

SIG Test in Dilute Lactic Acid (SIG-LA)

The procedure was similar to the SIG-SDSLA procedure except that initially 0.8 mL of distilled water was added to 40 mg of flour, the thermomixer mixing time was 10 min followed by the addition of 0.4 mL isopropanol-lactic acid stock solution prepared according to Approved Method 56-60 (AACC 2000) with another 10-min thermomixer mixing period and a final centrifugation at $100 \times g$.

SIG in SDS (SIG-SDS)

The procedure was similar to SIG-SDSLA except that the centrifugation speed was $1,000 \times g$ and the SIG-SDSLA stock solution was replaced by 3% SDS.

Statistical Analysis

All measurements are averages of two determinations. Statistical analyses were performed using the data analysis tools of Microsoft Excel 97.

RESULTS AND DISCUSSION

Influence of Various Factors on SIG Value

The influence of mixing intensity, the ratio of solvent-to-flour, hydration and swelling time, and centrifugation speed, and temperature on the SIG tests were investigated using strong, medium and weak gluten type cultivars (farinograph dough development time 3.1, 4.3, and 8.6 min for Alpha 16, Banks, and Suneca, respectively). Table I shows the effects of mixing intensities (mixing in the thermomixer combined with vortexing vs. hand-shaking) on the SIG. The strong cultivar Suneca required high mixing intensity (vortexing) to obtain its higher SIG value, while the medium and weak Banks and Alpha 16 cultivars only needed low mixing intensity (hand-shaking). This indicates that the glutenin in a strong cultivar is tolerant to vigorous mixing condition. The decreased SIG values of Banks and Alpha 16 under high mixing intensity may be due to some of the insoluble glutenin (under low mixing intensity) becoming soluble, a phenomenon also seen during the fractionation of gel protein (Graveland et al 1979). Temperature effects on the SIG tests were also cultivar-dependent: SIG values increased for strong

TABLE I
Effects of Mixing Intensity on the SIG-SDSLA and SIG-SDS Tests^a

	SIG-SDSLA			SIG-SDS		
	Suneca	Banks	Alpha 16	Suneca	Banks	Alpha 16
Vortexing and mixing	6.65	4.45	3.28	7.15	4.46	3.80
Hand-shaking ^b	6.55	5.60	4.21	6.95	5.53	4.40
LSD ^c	0.10	0.10	0.10	0.15	0.15	0.15

^a SIG-SDSLA = swelling index of glutenin test in SDS-lactic acid solution; SIG-SDS = swelling index of glutenin test in SDS solution.

^b Vortexing and mixing in thermomixer were replaced by hand-shaking and resting.

^c Least significant difference at 5% level, based on analysis of variance. Difference between two means exceeding this value is significant.

cultivars but decreased for weak ones at high temperature (Fig. 1). Therefore, the SIG test should only be performed at a constant room temperature ($24 \pm 1^\circ\text{C}$) to avoid the different responses to temperature with different cultivars. The use of a correction formula for SIG values at different temperatures may not be valid because different cultivars have different temperature responses.

All three cultivars showed higher SIG values as hydration time increased (Fig. 2). On the other hand, the hydration in the procedure adds to the convenience and precision of the method because flour easily suspends in water; thus avoiding sticky, inner-dried particles that occur during direct swelling by SDS.

The SIG values of the three cultivars had different responses to increased swelling time (Fig. 3). The SIG values for the strong cultivar (Suneca) increased during the first 15 min of swelling and then stayed constant for any additional swelling time. The SIG values

of the weak cultivar Alpha 16 dropped rapidly during the first 5–15 min and then more slowly with prolonged swelling time. Interestingly, the SIG-SDSLA for Banks increased to a peak after 5 min and then dropped dramatically. This phenomenon was seen during the FY sedimentation test (Kruger and Hatcher 1995) where values increased for strong cultivars but decreased for weak ones over time. The different changes among the three cultivars of wheat may be due to different solubility rates of their glutenin. The glutenin of a strong cultivar is solubilized at a slower rate than that of weak cultivars during prolonged SDS extraction (He et al 1991). To obtain maximum variation of SIG value among cultivars, 20 min of swelling time was chosen for a routine SIG test.

The SIG test is also affected by the solvent-to-flour ratio (Fig. 4). The SIG values are higher with a lower ratio because the solvent is unable to efficiently extract all of the soluble glutenin and a portion

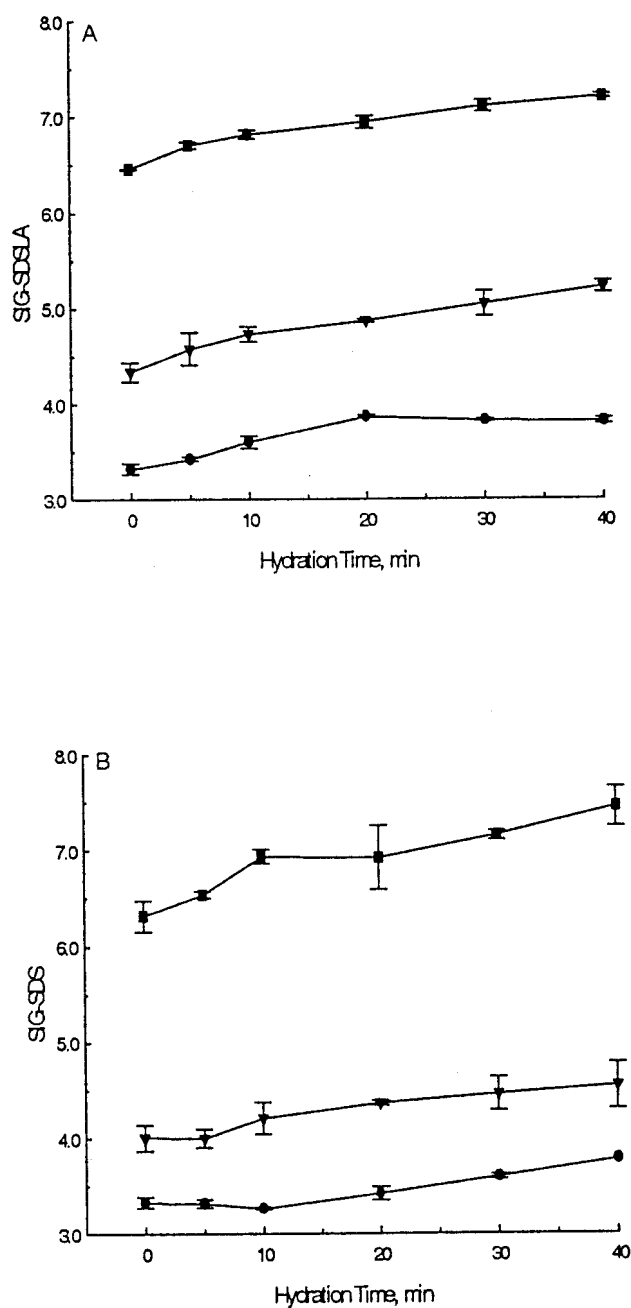


Fig. 2. Effect of hydration time on swelling index of glutenin (SIG) in SDS-lactic acid (SIG-SDSLA) (A) and SIG-SDS (B) tests. Error bars indicate standard deviations ($n = 2$). Suneca (■), Banks (▼), Alpha 16 (●).

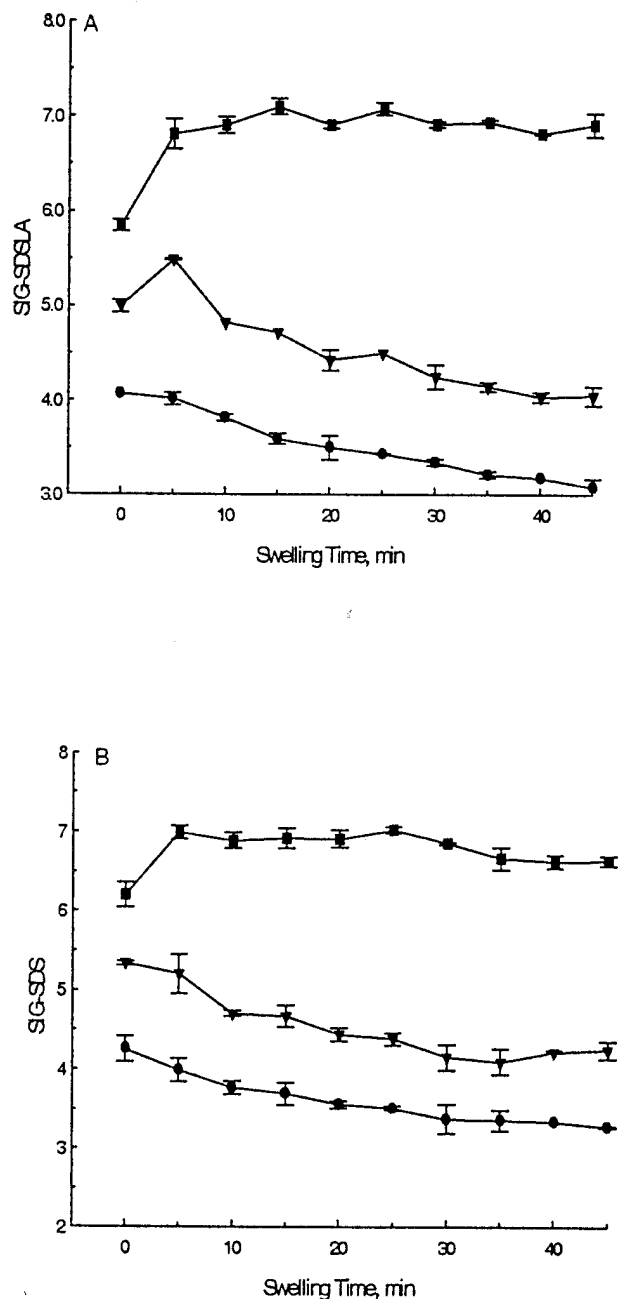


Fig. 3. Effect of swelling time on swelling index of glutenin (SIG) in SDS-lactic acid (SIG-SDSLA) (A) and SIG-SDS (B) tests. Vortexing for 5 sec at beginning and ending of swelling period, and vortexing for 5 sec at 10-min intervals. Error bars indicate standard deviations ($n = 2$). Suneca (■), Banks (▼), Alpha 16 (●).

of the soluble glutenin remains swollen in the residue. A sample size of 35–45 mg seems optimal when 1.2 mL of solvent is used for swelling glutenin.

As expected, SIG values decreased as centrifugation force increased (results not shown). To maximize differentiation among cultivars, a low centrifuge force is recommended in the SIG test but it is difficult to separate supernatant from residues if the centrifuge force is too low. Exact centrifuge force is critical in achieving reproducible SIG values between tests.

Our results showed that glutenin swelling is influenced by the mixing intensity, the ratio of solvent-to-sample, and the swelling time and temperatures. The strong swelling treatment (high mixing intensity, high solvent-to-sample ratio, high temperature and long swelling time) allow efficient extraction of the soluble glutenin and results in complete swelling of the insoluble glutenin. Optimizing the test conditions enhances the differentiation of SIG values

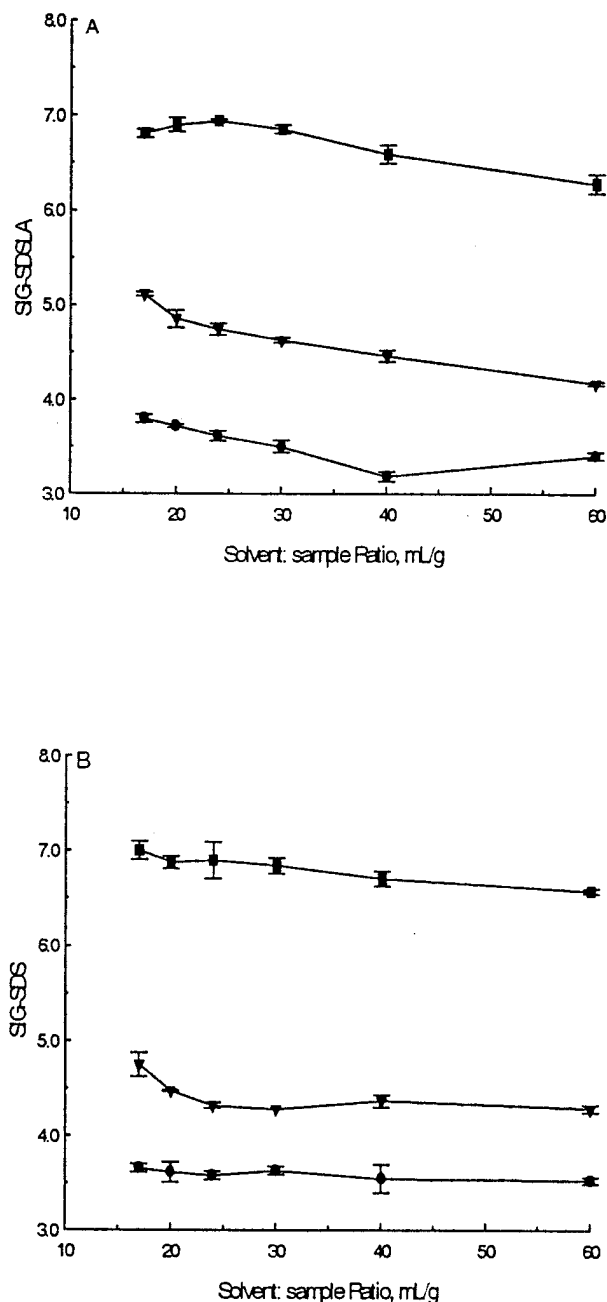


Fig. 4. Effect of solvent-to-flour ratio on swelling index of glutenin (SIG) in SDS-lactic acid (SIG-SDSLA) (A) and SIG-SDS (B) tests. Error bars indicate standard deviations ($n = 2$). Suneca (■), Banks (▼), Alpha 16 (●).

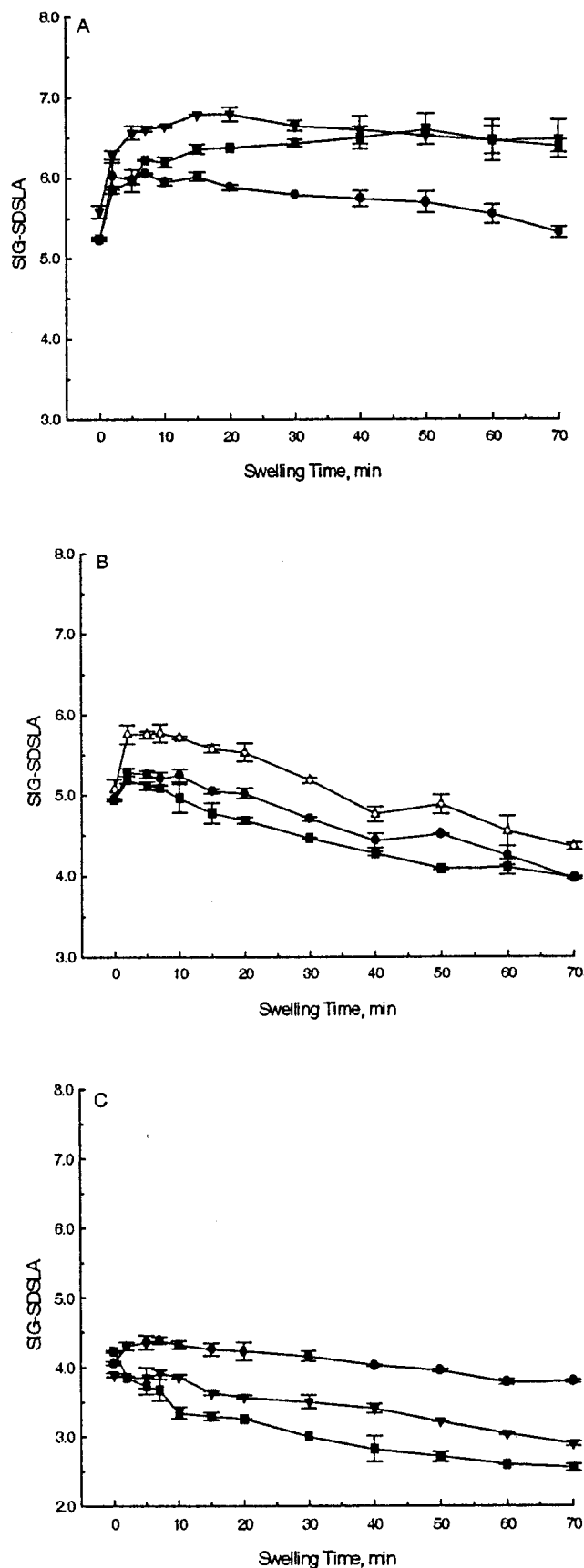


Fig. 5. Swelling curves for three groups of samples. A, Strong cultivars Glenlea (■), Suneca (▼), Sunco (●). B, Medium cultivars Banks (■), Tingalen (▼), Torres (●). C, Weak cultivars Fielder (■), Alpha 16 (▼), 90W950 (●). Vortexing for 5 sec at beginning and ending of swelling period, and vortexing for 5 sec at 10-min intervals. Error bars indicate standard deviations ($n = 2$).

among cultivars, and this differentiation reflects the insoluble glutenin content among cultivars.

The three solvents used in the SIG tests gave swelling volumes of glutenin in the order of SIG-SDS > SIG-SDSLA > SIG-LA. A solvent containing SDS is recommended because the swelling volume of insoluble glutenin is greater in SDS than in lactic acid and the separation of the supernatant from the swollen glutenin by syringe is also easier. The highest SIG values were obtained using 1.5% SDS as the solvent. However, the swollen glutenin of some samples was so watery that a higher centrifugation speed was required to separate the supernatant from the residue and, for this reason, we recommend a solvent composed of SDS and lactic acid.

Reproducibility of the SIG Test

The reproducibility of the SIG test with the three solvents was determined using the three wheat cultivars with high, medium, and low SIG values in 24 replicates under optimum conditions (Table II). The coefficients of variation (CV %) were 1.16–2.97 in the SIG-SDSLA, 2.28–7.57 in SIG-SDS, and 0.97–2.04 in SIG-LA. The stronger gluten type cultivar Suneca with the highest SIG values and firm residue gave the best reproducibility.

Correlations Between SIG-SDSLA, SIG-LA, SIG-SDS, Gel Protein, SDS-sedimentation, Zeleny Sedimentation, and Insoluble Glutenin Content

The relationship between SIG tests, sedimentation tests, and gel protein with percentage of insoluble glutenin in flour (IG/F) are shown in Table III. All of the correlation coefficients were positive and significant. The SIG-SDSLA, SIG-SDS, and SIG-LA had a strong linear correlation with IG/F. However, the sedimentation volumes (SDS and Zeleny) had lower correlation coefficients with IG/F. The correlation of SDS-sedimentation with IG/F in this study was higher than that obtained by Blackman and Gill (1980) but similar to Dachkevitch and Autran (1989). The order of correlation of these tests with IG/F was SIG > gel protein > sedimentation. Although sedimentation, gel protein, and SIG tests are based on the quantity and quality of insoluble protein, the results suggest that SIG tests are more closely related to insoluble glutenin content than gel protein or sedimentation tests. Sedimentation tests are the poorest predictors of insoluble glutenin content in these three tests. The correlation coefficients among small-scale tests are listed in Table III. Strong linear relationships were found between different SIG tests, while two sedimentation tests displayed a good linear relationship as expected.

The correlation coefficients also showed that gel protein fell between SIG and sedimentation tests. Mixing intensity and centrifugation force account for the differences obtained from sedimentation, gel protein, and SIG tests. The low mixing intensity in the sedimentation test may not be adequate to extract all of the soluble glutenin, and the insoluble glutenin may not swell fully in the sedimentation tests. The suspending property of swollen insoluble protein probably affects the sedimentation volume. The centrifugation in SIG test forces the swollen insoluble glutenin and starch particles together, eliminating possible effects of different glutenin suspending properties among the cultivars. The high mixing intensity and long swelling time in the SIG test provide conditions similar to that in the IG/F test. Consequently, the SIG test mainly measures the swelling capacity of insoluble glutenin and reflects the content of insoluble glutenin.

Effects of Swelling Property of Glutenin on Sedimentation, Gel Protein, and SIG Tests

To investigate the performance of glutenin in the swelling processes, SIG-SDSLA values for 20 wheat cultivars were determined with different swelling times (0–70 min). According to SDS-sedimentation value, there were three strong cultivars (SDS volume >80 mL), three weak cultivars (SDS volume <60 mL), and three mediate cultivars (SDS volumes almost identical) in 20 test samples (Table IV). The swelling curves are presented in Fig. 5. The process of glutenin swelling in the nonreducing solvent can be divided into three stages. In the first stage (swelling stage), the glutenin began to swell to peak volume. The glutenin from strong wheat needs a long time to reach peak volume, while the weak wheat immediately reached peak volume. In the second stage (swollen stage), the swollen glutenin maintained its state and had peak SIG value. The glutenin from strong wheat kept its peak state much longer than that of weak wheat. In the third stage (breakdown stage), the glutenin loses swelling ability, probably because part of the insoluble glutenin starts to dissolve.

The different cultivars have totally different behaviors in the three stages. Generally, the swelling process of a strong cultivar is longer than that of a weak cultivar in both the swelling and swollen stages, while in the breakdown stage, the value of SIG falls a little (Glenlea and Suneca in Fig. 5A). Sunco had the highest SDS sedimentation value among the three strong cultivars (Table IV), but its insoluble glutenin content was low compared with that of Glenlea and Suneca. On the other hand, the range of SIG values of the three cultivars was similar to the range of insoluble glutenin content. Furthermore, the insoluble glutenin contents of both Suneca and Glen-

TABLE II
Reproducibility of SIG Tests on Three Diverse Wheat Cultivars^{a,b}

	SIG-SDSLA			SIG-SDS			SIG-LA		
	Suneca	Banks	Alpha 16	Suneca	Banks	Alpha 16	Suneca	Banks	Alpha 16
Mean	6.80	4.60	3.67	7.05	4.62	3.81	4.81	3.90	3.38
SD	0.0792	0.0839	0.1090	0.1600	0.3500	0.1340	0.0468	0.1490	0.0691
CV (%)	1.16	1.82	2.69	2.28	7.57	3.51	0.97	3.81	2.04

^a SIG-SDSLA = swelling index of glutenin test in SDS-lactic acid solution; SIG-SDS = swelling index of glutenin test in SDS solution; SIG-LA = swelling index of glutenin test in lactic acid solution.

^b SD = standard deviation; CV = coefficient of variation ($n = 24$).

TABLE III
Correlation Coefficients Between SIG Test, Sedimentation Tests, and Gel Protein^{a,b}

	SIG-SDSLA	SIG-SDS	SIG-LA	Gel Protein	SDSV	Zeleny	IG/F
SIG-SDSLA	1.00	0.99***	0.97***	0.93***	0.74***	0.72***	0.96***
SIG-SDS		1.00	0.96***	0.86***	0.74***	0.72***	0.95***
SIG-LA			1.00	0.85***	0.74***	0.75***	0.93***
Gel Protein				1.00	0.83***	0.79***	0.85***
SDSV					1.00	0.90***	0.64**
Zeleny						1.00	0.63**

^a SIG-SDSLA = swelling index of glutenin test in SDS-lactic acid solution; SIG-SDS = swelling index of glutenin test in SDS solution; SIG-LA = swelling index of glutenin test in lactic acid solution; SDSV = SDS sedimentation volume.

^b **, *** = significant at 1 and 0.1%, respectively.

lea were almost the same, but their swelling curves were different. It took ≈20 min for Suneca to reach peak value, while Glenlea required almost 50 min. This indicates that the quality of glutenin (strength) also affects the swelling properties of glutenin. An extra long time for swelling of Glenlea could be related to its extra strong gluten properties (farinograph dough development time 15.6 min). In the medium group, the three cultivars had almost the same value of SDS-sedimentation, but SIG values and insoluble glutenin contents were different (Table IV). Although the SIG values at 0 swelling time of the three cultivars were almost the same, Timgalen, which had a higher insoluble glutenin content, had higher SIG values during the swelling stage than the other two cultivars with lower insoluble glutenin content (Fig. 5B and Table IV). SIG values are, therefore, mainly dependent on insoluble glutenin content. In the weak cultivar group, the swelling and swollen stages were short, and for some cultivars, the SIG method was not able to detect a swelling and swollen stage (Fielder in Fig. 5C). The SIG value of Fielder, which had the highest SDS-sedimentation value and lowest IG/F among the three weak cultivars (Table IV), dropped sharply as swelling time increased. The high soluble glutenin content in Fielder could account for the high SDS sedimentation volume and sharp decrease in the swelling curve (Table IV). We postulate that the soluble glutenin swelled quickly at short swelling time and low mixing intensity (as swelling condition in SDS sedimentation test and 0 swelling time) and then dissolved in SDS solution as swelling time increased. Because the insoluble glutenin content was low for Fielder, the increase resulting from completely swollen insoluble glutenin could not compensate for the bigger decrease resulting from dissolving soluble glutenin, inducing the sharp decrease of SIG value. The ratio of insoluble-to-soluble glutenin in 90W950 was higher than that in Fielder (Table IV), explaining why 90W950 had a lower sedimentation value and SIG value at 0 min than those of Fielder. On the other hand, as swelling time increased, the decrease of SIG value of 90W950 was small (Fig. 5C) because the soluble glutenin content was lower than that of other weak cultivars. Although mechanisms that determine the solubility and swelling ability of glutenins are not well known, we presume that content and quality of glutenin are the

main determinants of the swelling property of glutenin. The content of glutenin, especially insoluble glutenin, governs the SIG value determined under optimum conditions, and glutenin quality may control the glutenin swelling curve. SIG can be used to evaluate flour quality between cultivars with a wide range of quality. For the cultivars with a narrower range of insoluble glutenin content, the difference between Suneca and Glenlea, for example, swelling curves differentiate the quality of glutenin (Wang and Kovacs 2001a,b). Comparing the differences of glutenin content and SIG value between 90W950 and Torres, Torres had a higher SIG value and similar insoluble glutenin content compared with that of 90W950. In this case, the difference may be attributed to a different content of soluble glutenin and different swelling properties of insoluble glutenin. Overall, the sedimentation tests are determined by both soluble glutenin and insoluble glutenin content because the mixing intensity is too low for dissolving the soluble glutenin and the soluble glutenin also contributes to the sedimentation volume.

Flour particle size influences the swelling capacity of glutenin. The large particle size of semolina had a lower SIG value than that from ground semolina (Wang and Kovacs 2001b). It is well known that flour from hard wheats contains more damaged starch than flour from soft wheats. However, Schlesinger (1964) reported that the effect on Zeleny sedimentation values of increased starch damage by ball-milling of previously tested flours was negligible. Similarly, correlations of damaged starch and particle size index with SIG values were not significant. When SIG values were determined with SDS solvent containing 2 mM DTT, the correlation coefficient of the SIG values with the IG/F was significantly lower ($r = 0.27$), as compared to values obtained without DTT ($r = 0.96$, $P < 0.001$). The range of SIG-SDSLA values from 20 samples changed from 3.00–6.81 (without reducing agents) to 1.75–2.04 (with 2 mM DTT). The results of the subtraction of SIG values (with 2 mM DTT) from SIG values without reducing agents were highly significantly correlated with IG/F ($r = 0.91$, $P < 0.001$). Apparently, the possible effect of damaged starch and flour size is negligible in the SIG test, and the results suggest that insoluble glutenin is the main factor responsible for SIG values.

TABLE IV
Comparison of SDSV, SIG-SDSLA, Insoluble Glutenin Content, and Soluble Glutenin Content for Three Groups of Samples^a

	Strong Cultivars			Medium Cultivars			Weak Cultivars			LSD ^b
	Glenlea	Suneca	Sunco	Banks	Timgalen	Torres	Fielder	Alpha 16	90W950	
SDSV	80	85	86	70	71	70	51	43	49	
SIG-SDSLA	6.34	6.81	5.89	4.61	5.20	4.73	3.00	3.50	4.13	0.20
IG/F	3.95	4.00	3.37	2.70	3.27	2.67	1.76	2.15	2.63	0.21
SG/F	1.24	1.67	1.39	1.88	1.88	1.71	1.80	1.19	0.72	0.29

^a SDSV = SDS sedimentation volume; SIG-SDSLA = swelling index of glutenin test in SDS-lactic acid solution; IG/F = the percentage of insoluble glutenin in flour; SG/F = percentage of soluble glutenin in flour.

^b Least significant difference at 5% level, based on analysis of variance. Difference between two means exceeding this value is significant.

TABLE V
Correlation Coefficients of SIG-SDSLA (with different swelling time) with Gel Protein, Sedimentation Tests, and Insoluble Glutenin Content^{a,b}

Swelling Time (min) ^c	Gel Protein	SDSV	Zeleny	IG/F
0	0.96***	0.90***	0.85***	0.80***
2	0.97***	0.81***	0.80***	0.90***
5	0.96***	0.78***	0.79***	0.92***
7	0.95***	0.77***	0.75***	0.94***
10	0.94***	0.77***	0.78***	0.94***
15	0.93***	0.76***	0.73***	0.95***
20	0.93***	0.74***	0.72***	0.96***
30	0.90***	0.76***	0.72***	0.96***
40	0.86***	0.73***	0.70***	0.95***
50	0.86***	0.77***	0.71***	0.94***
60	0.86***	0.75***	0.70***	0.93***
70	0.84***	0.74***	0.69***	0.93***

^a SDSV = SDS sedimentation volume; IG/F = the percentage of insoluble glutenin in flour.

^b *** significant at 0.1%.

^c Vortexing for 5 sec at beginning and ending of swelling period, and vortexing for 5 sec at every interval of 10 min.

Correlation coefficients of SIG with sedimentation tests were higher at short swelling time than at long swelling time (Table V). Gel protein was strongly related to SIG-SDSLA at a swelling time of 0–7 min. When swelling time increased, the correlation coefficients of SIG-SDSLA with insoluble glutenin content increased. Mixing intensity and swelling time were the main reasons for differences between the tests, and additional information about protein quality can be obtained from SIG tests. The SIG value obtained at short swelling time and low mixing intensity is a good predictor of glutenin quality which corresponds to sedimentation and gel protein tests, and the SIG value obtained at long swelling time and high mixing intensity is a good predictor of insoluble glutenin content.

Comparison of SIG and Sedimentation Test Methodology

SIG and sedimentation tests are simple and can quickly predict the quality of wheat, but the advantage of the SIG test over the sedimentation test is the high correlation with insoluble glutenin content. The sedimentation boundary is difficult to identify in some cultivars because the supernatant is too turbid. To get a clear supernatant in the SDS sedimentation test, an increase in concentration of lactic acid is needed. This problem is overcome in SIG tests by centrifugation. The sedimentation tests require strict conditions, especially the sedimentation time in the Zeleny test. When the sedimentation volume is recorded at different times, the results vary. On the other hand, the conditions are easily controlled in SIG tests and the SIG procedure does not require a set of uniform and scaled leak-proof tubes that have to be carefully cleaned after each use. Compared with determinations of gel protein and insoluble glutenin, the SIG test can be conducted quickly with simple equipment, and 24 samples can be tested at one time. Among the various small-scale quality test methods, SIG tests require the smallest amount of sample, an important consideration for wheat breeders.

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