# A Modified Screening Test for Rapid Estimation of Gluten Strength in Early-Generation Durum Wheat Breeding Lines[1]

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ABSTRACT

Gluten strength influences the firmness of cooked pasta. A sodium dodecyl sulfate-microsedimentation test (MST) using 1 g of ground wheat was developed for use in plant breeding to determine relative gluten strength of early-generation durum lines. The MST was compared to an existing sedimentation procedure requiring 6 g of wheat and to micromixograph measurements. Each of these tests was performed, and protein content and cooked spaghetti firmness were calculated for eight durum wheat cultivars grown at six locations in North Dakota. Statistical analyses showed that the MST was superior to the other factors tested for predicting cooked spaghetti firmness. The MST alone accounted for 53% of the variation in cooked firmness. When combined with wheat protein content, the MST accounted for 71% of the variation in cooked firmness. The MST requires small amounts of material (maximum 1 g of ground sample), solutions and equipment, and it is simple to perform, fast, selective, and reproducible when used to screen for gluten strength in durum wheat cultivars. The MST also has proved useful to commercial buyers selecting for or monitoring shipments of durum wheat or semolina on the basis of gluten strength.

Gluten strength in durum wheat influences dough-mixing properties of durum semolina and cooking quality of spaghetti. Grzybowski and Donnelly (1979) showed that cooking quality was affected by protein quantity and quality, particularly with respect to cooked spaghetti firmness and stability, although high protein content alone did not necessarily guarantee optimum cooking quality.

Quick and Donnelly (1980) evaluated a sodium dodecyl sulfate (SDS)-sedimentation test developed by McDermott and Redman (1977). They compared this test, which required 6 g of wheat, to the micromixograph test of Bendelow (1967), which required about 25 g of wheat per test and reported that 65% of variation in the micromixogram score was attributed to its association with sediment volume. Their data showed correlation coefficients near zero between protein content and sediment volumes or mixogram score, indicating essentially no association among the traits. Dexter et al (1980) investigated an SDS-sedimentation test to determine its suitability as a rapid, small-scale test for predicting durum wheat gluten strength, and to compare it to the micromixograph method. These workers reported that SDS sedimentation showed much greater cultivar interaction than mixograph development time, which suggested that SDS sedimentation would be the more reliable method of the two for screening out weak gluten lines in the Canadian durum wheat breeding program. Statistical analysis showed that SDS sedimentation and wheat protein accounted for more than 40% of the variability of spaghetti cooking quality, equivalent to a correlation coefficient of about 0.65 ($n = 90$), which was thought capable of significantly improving spaghetti cooking quality.

A more recent report by Dexter et al (1981) showed that although there was a positive linear relationship between gluten strength and baking strength for a series of Canadian durum wheats and Canadian common wheats, gluten of intermediate strength was best for good spaghetti cooking quality. Nonetheless, when durum wheats were considered alone, there was a significant correlation between SDS sedimentation and cooking quality.

Because of the established relationship between gluten strength and cooking quality, cereal technologists and plant breeders have tried to incorporate techniques for determining gluten strength of early-generation lines in their breeding programs. The amount of seed available for testing in early generations normally is a limiting factor. Dexter et al (1980) mentioned using a scaled down SDS-sedimentation test requiring 1 g of ground grain, although the exact procedure was not described.

In this article, a 1-g SDS-microsedimentation test (MST) that has been used in our laboratory for the past two years is described, and its advantages when used to screen out weak gluten cultivars in early-generation durum breeding lines are discussed. The procedure described is now being used by commercial grain buyers, plant breeders, and independent testing laboratories.

## MATERIALS AND METHODS

Three durum cultivars were tested initially: the named varieties Vic and Calvin, and the experimental selection ND 7618, which exhibited strong, weak, and intermediate dough mixing strength, respectively, based on micromixograph tests. The wheat samples were ground on a Udy mill (Udy Analyzer Co., Boulder, CO) equipped with a 1.0-mm sieve. A stock solution was prepared fresh daily and contained a 1:48 ratio of 85% lactic acid–water (1:8, v/v) and sodium dodecyl sulfate (2% solution). The lactic acid solution and the 2% solution of SDS (Matheson Coleman and Bell Manufacturing Chemists) were prepared several days in advance. A 1-g portion (as-is basis) of each ground sample was placed into a standard clear-glass test tube (150 mm long by 16 mm o.d., 14 mm i.d.). Distilled water (4 ml) was added to the ground sample in the test tube, and the contents were mixed at high speed for 2 sec (or until thoroughly mixed) using a variable-speed, single-tube vortex mixer. After soaking 5 min, the contents of the tube were again mixed for 2 sec with the vortex mixer. Five minutes later, the stock solution (12 ml) was added to the mixture in the tube, the tube was stoppered, inverted 10 times, and placed in an upright vertical position. After 10 min, the height of the interface line between the solid and the liquid was measured in millimeters. Sedimentation tests were done at room temperature (19–22° C).

After the test procedure had been established, five named durum cultivars—Cando, Coulter, Edmore, Hercules, and Rugby, which historically had shown a given mixing pattern—were chosen to test the selectivity and reproducibility of the MST procedure as a predictor of micromixograph mixing patterns. Micromixograms were obtained on a micromixograph (National Manufacturing Co., Lincoln, NE) for each of the five cultivars by using 10 g (as-is basis) of semolina (Vasiljevic et al 1977), 5.8 ml of distilled water, and a spring setting of 8. Mixogram curves were scored on a scale of 1 to 8, with higher numerical values indicating a stronger mixing pattern (Quick and Donnelly 1980). Ten replicate MST determinations were made for each of the five cultivars, using ground wheat whole meal. In addition, MST replicates were obtained for a blend (1:1, w/w) of Rugby and Edmore. Wheat total protein and 6 g SDS-sedimentation (eight replicates, Quick and Donnelly 1980) were also measured for each individual sample.

To establish whether the described procedure could be improved

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to obtain greater differentiation between MST values, the influence of varying settling time, temperature, SDS and lactic acid concentrations were studied. The study, done on the durum cultivars Rugby, Hercules, and Edmore, represented weak, intermediate, and strong gluten types, respectively.

To study the effect of environment on the MST procedure, eight cultivars grown at six locations in North Dakota were tested. Wheat protein content, mixograms, and wheat MST and SDS-sedimentation were measured. The samples were also milled into semolina, and the semolina was reground into flour on the Udy grinder (1.0-mm sieve) before testing with the MST procedure. A 15-min final settling time was used when testing these samples. The semolina samples were processed into spaghetti, cooking quality was measured (Dick et al 1982), and the data were analyzed statistically. Means and ranges of the quality measurements were determined for each growing location. Simple correlation coefficients between each quality measurement were also calculated for each growing location. Homogeneity of the correlation coefficients among the six locations was established by using the chi-square test. The data were then pooled, and correlation coefficients were calculated for the pooled data.

TABLE I
Reproducibility of Microsedimentation Test (MST)[a] and SDS-Sedimentation Test (SDST)[b]

| Cultivar | Wheat Protein[c] (%) | Semolina Mixogram (unit) | MST Value[d] Mean (mm) | MST Value[d] S.D.[e] (mm) | MST Value[d] CV[f] | SDST Value[g] Mean (mm) | SDST Value[g] S.D. (ml) | SDST Value[g] CV |
|---|---|---|---|---|---|---|---|---|
| Rugby | 14.4 | 2 | 23.9 | 0.57 | 2.4 | 21.3 | 0.46 | 2.2 |
| Cando | 12.8 | 3 | 28.8 | 0.92 | 3.2 | 25.8 | 0.71 | 2.8 |
| Hercules | 14.8 | 6 | 38.5 | 0.85 | 2.2 | 30.5 | 0.53 | 1.7 |
| Coulter | 13.9 | 7 | 44.4 | 1.96 | 4.4 | 34.8 | 0.46 | 1.3 |
| Edmore | 14.2 | 8 | 54.3 | 1.64 | 3.0 | 34.6 | 1.06 | 3.1 |

[a] 1 g wheat whole meal.
[b] 6 g wheat whole meal.
[c] As-is moisture basis.
[d] Based on 10 replicate determinations.
[e] Standard deviation.
[f] Coefficient of variation.
[g] Based on eight replicate determinations.

TABLE II
Microsedimentation Test (MST) Value Differentiation (ΔMST) with Varying Sodium Dodecyl Sulfate (SDS) Concentration[a]

| Cultivar | MST (mm)[b] Mean | MST (mm)[b] Range | MST Values (mm)[c] at SDS Concentration (%) of 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|---|
| Rugby | 24 | 4 | | | | | | |
| | | | 0 | 12 | 11 | 10 | 12 | 11 |
| Hercules | 34 | 15 | | | | | | |
| | | | 3 | 11 | 11 | 12 | 11 | 8 |
| Edmore | 43 | 23 | | | | | | |

[a] 1.3*N* lactic acid.
[b] MST for the respective cultivars at all six SDS concentrations tested.
[c] Hercules MST value minus Rugby MST value, and Edmore MST value minus Hercules MST value.

TABLE III
Microsedimentation Test (MST) Value Differentiation (ΔMST) with Varying Lactic Acid Concentration[a]

| Cultivar | MST (mm)[b] Mean | MST (mm)[b] Range | MST Values (mm)[c] at Acid Normality of 0.1[d] | 0.2 | 0.5 | 0.7 | 0.9 | 1.3 | 1.6 |
|---|---|---|---|---|---|---|---|---|---|
| Rugby | 25 | 7 | | | | | | | |
| | | | 23 | 20 | 17 | 14 | 14 | 9 | 12 |
| Hercules | 40 | 20 | | | | | | | |
| | | | 21 | 20 | 15 | 15 | 11 | 14 | 15 |
| Edmore | 57 | 27 | | | | | | | |

[a] Two percent sodium dodecyl sulfate.
[b] MST for the respective cultivar at all seven acid concentrations tested.
[c] Hercules MST value minus Rugby MST value, and Edmore MST value minus Hercules MST value.
[d] Interface line was very difficult to see.

## RESULTS

Table I shows the relationship between the means of the 10 replicate MST and eight replicate SDS-sedimentation test determinations with the micromixogram scores. Although the standard deviation of the MST replicates appeared to increase as the mean value increased, the coefficient of variation remained low for all mean levels obtained, indicating good reproducibility for the procedure.

The mean value of the three MST replicate determinations for the Rugby-Edmore blend was 38 mm, or almost exactly halfway between the respective means of Rugby (23.9) and Edmore (54.3) alone. This indicates a strong additive effect on MST values when wheat of different mixing strengths are blended together.

Table II shows the influence on MST values with varying SDS concentrations at constant lactic acid concentration. The mean values show the relative gluten strengths of the cultivars tested. Comparison of the ranges of MST values shows that the strong gluten types are much more responsive to change in SDS concentration than are the weak gluten types. Differentiation in MST values among the cultivars at 1% SDS concentration was virtually impossible. However, at SDS concentrations from 2–6% differentiation was very good and quite uniform. These data suggest that SDS concentrations of 2% are ideal for the MST method previously described and that higher concentrations could be used but certainly are not necessary.

Table III shows the influence on MST values with varying concentrations of lactic acid at constant SDS concentration. The very large range in MST values obtained for the stronger gluten types indicates they had much greater responsiveness to change in lactic acid concentration than the weak gluten types. Relative differences in values among the cultivars were greatest at low acid concentration, but did not appear to stabilize until concentrations of 0.7*N* or higher were used. These data indicate that the lactic acid concentration (1.3*N*) we used previously was within acceptable limits. If there is any doubt about the age or condition of the lactic acid, it should be either refluxed or substituted with fresh solution.

Comparison of the means in Tables II and III indicates that MST values obtained are slightly more sensitive to changes in concentration of lactic acid than they are to changes in SDS concentration. However, after the minimum SDS concentration requirement is satisfied, the lactic acid concentration can be optimized.

Table IV shows the influence of solution temperature on absolute MST values and on the ability to differentiate among cultivars with different gluten strengths. MST values decrease as the temperature increases, although the relative differences

TABLE IV
Influence of Solution Temperature on Microsedimentation Test (MST) Values[a]

| Cultivar | MST Values (mm) at Solution Temperature (°C) of[b] 15.6 | 21.1 | 26.7 | 29.4 | 35.0 | 40.6 |
|---|---|---|---|---|---|---|
| Rugby | 28 | 22 | 23 | 21 | 22 | 21 |
| | (12) | (13) | (13) | (9) | (8) | (7) |
| Hercules | 40 | 35 | 36 | 30 | 30 | 28 |
| | (15) | (17) | (14) | (12) | (10) | (8) |
| Edmore | 55 | 52 | 50 | 42 | 40 | 36 |

[a] Two percent sodium dodecyl sulfate; 1.3*N* lactic acid.
[b] ΔMST values in parenthesis: Hercules MST minus Rugby MST and Edmore MST minus Hercules MST.

TABLE V
Means and Ranges of Quality Measurements for Eight Durum Wheat Cultivars Grown at Six North Dakota Locations[a]

| Cultivar | Wheat Protein (W)[b] (%) | | Mixogram Score (X) (unit) | | MST Value (Y)[c] (mm) | | SDST Value (Z)[c] (ml) | | MSTS Value (V)[c] (mm) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Mean | Range | Mean | Range | Mean | Range | Mean | Range | Mean | Range |
| Cando | 14.3 | 12.3–15.6 | 3.8 | 3–5 | 28.7 | 25–33 | 25.2 | 22–27 | 24.0 | 21–29 |
| Coulter | 15.1 | 12.6–17.6 | 6.8 | 6–8 | 51.9 | 44–63 | 37.7 | 35–41 | 32.8 | 29–39 |
| Crosby | 15.2 | 12.2–18.3 | 3.3 | 2–5 | 27.4 | 22–31 | 23.7 | 21–28 | 21.7 | 19–25 |
| Lloyd | 14.5 | 12.3–16.7 | 7.0 | 5–8 | 53.5 | 44–62 | 39.3 | 33–46 | 35.6 | 30–41 |
| Rolette | 15.3 | 12.9–17.8 | 2.5 | 2–3 | 24.2 | 19–29 | 23.1 | 20–27 | 19.7 | 16–22 |
| Rugby | 15.0 | 11.7–17.5 | 2.2 | 2–3 | 24.4 | 18–30 | 22.2 | 19–25 | 19.7 | 17–24 |
| Vic | 14.9 | 12.7–17.0 | 6.8 | 6–8 | 52.4 | 38–62 | 39.1 | 30–53 | 37.3 | 30–44 |
| Ward | 15.3 | 12.6–17.4 | 2.2 | 1–3 | 25.4 | 17–32 | 22.8 | 15–29 | 20.1 | 15–24 |

[a] Locations were Casselton, Dickinson, Fargo, Langdon, Minot, and Williston. Correlation coefficient (r) of individual values for pooled data ($n = 48$) were: X vs Y = 0.918**; X vs Z = 0.685**; X vs W = 0.048; X vs V = 0.934**; W vs Y = 0.199; W vs Z = −0.243; W vs V = 0.037; Y vs Z = 0.585**; Y vs V = 0.939**; Z vs V = 0.700**.
[b] 14% moisture basis.
[c] MST = microsedimentation test, 1 g wheat whole meal; MSTS = reground semolina microsedimentation test, 1 g reground semolina; SDST = SDS-sedimentation test, 6 g wheat whole meal.

between cultivars remain quite stable until solution temperatures above 27° C are used. Though testing in a water bath would have been most accurate, it was not absolutely necessary for our purposes because of the relatively stable ambient temperatures (19–25° C) in our laboratory and because we always include check samples in our screening tests to obtain relative comparisons within a test set. If testing is to be done under conditions of extreme temperature variation, however, either a water bath should be used or a temperature conversion chart should be established.

Variations in final settling time (data not shown) indicated that the reading after 15 min was preferrable to the reading after 10 min because the rate of settling stabilized after 15 min so that there was a drop of no more than 1 mm per additional 5 min. Therefore, all subsequent measurements in this study were made after 15 min of settling time.

Means and ranges of quality measurements for eight durum wheat cultivars grown at six locations are given in Table V. These data represent samples with a wide range in protein content and gluten strength. Though wheat protein content varied widely for all cultivars among the six locations, none of the other factors tested showed a significant correlation with wheat protein. Mixogram score was significantly correlated with all of the sedimentation tests ($P < 0.01$); reground semolina MST (MSTS) with a value of 0.934** and MST with a value of 0.918** showed the higher correlations (Table V). Analysis of variance (data not shown) substantiated the work by Dexter et al (1980), which showed that wheat sedimentation had significant differences among cultivars and across locations, whereas the mixogram measurement was affected by cultivars but not by locations. Maximum $r^2$ improvement regression analysis (Table VI) for spaghetti cooked firmness (dependent variable) showed the best one-variable model to be MST, which by itself accounted for 53% of the variation in firmness. MST and wheat protein showed the best two variable coefficient of determination ($r^2$) of 0.713 for cooked firmness, which is the same value obtained for the best three-variable model of MST, wheat protein, and mixogram score. These results indicate that it is not necessary to measure both microsedimentation (MST or MSTS) and mixogram score as a predictor of cooked firmness, but one or the other in addition to wheat protein or semolina protein. Though spaghetti cooked firmness or tenderness is perhaps not a totally adequate measure of cooking quality (Matsuo and Irvine 1971), it correlates well with taste panel scores (Walsh 1971).

TABLE VI
Maximum $r^2$ Improvement Regression Analysis for Spaghetti Cooked Firmness (Dependent Variable)[a]

| Number in Model | Variable in Model[b] | $r^2$ |
|---|---|---|
| 1 | SDST | 0.089 |
| 1 | WP | 0.316 |
| 1 | SP | 0.324 |
| 1 | MX | 0.365 |
| 1 | MSTS | 0.407 |
| 1 | MST | 0.532 |
| 2 | MX, SDST | 0.389 |
| 2 | MSTS, MX | 0.408 |
| 2 | WP, SDST | 0.517 |
| 2 | MST, MX | 0.559 |
| 2 | MX, WP | 0.649 |
| 2 | MX, SP | 0.660 |
| 2 | MSTS, SP | 0.704 |
| 2 | MST, WP | 0.713 |
| 3 | MX, WP, SDST | 0.652 |
| 3 | MSTS, MX, SP | 0.704 |
| 3 | MST, MX, WP | 0.713 |

[a] Data from eight durum wheat cultivars grown at six locations.
[b] WP = wheat protein; SP = semolina protein; MST = microsedimentation test, 1 g wheat whole meal; MSTS = microsedimentation test, 1 g reground semolina; SDST = SDS-sedimentation test, 6 g wheat whole meal; MX = mixogram score.

## DISCUSSION

The secret of success of the MST procedure is related, of course, to SDS and lactic acid concentration, but the reproducibility of the test appears to be most dependent on the rapid, thorough mixing of the sample obtained when a vortex-type mixer is used. Hand mixing and mechanical tilt-type mixers were tried, but neither gave satisfactory results. The hand method gave adequate mixing but poor reproducibility between runs and operators (results not shown), which was probably related to inconsistent mixing and to uneven lag times between mixing when several samples were tested in a series or batch. Tilt-type mechanical mixing was not vigorous enough to mix the sample completely.

The MST analysis can be used for a single sample, or it can be done on a batch or group-sample basis. When several samples are tested as a batch, it is desirable to use a common test tube rack in such a way that all samples of the batch are inverted simultaneously just before the final 10-min rest period. Ten samples per batch work well, although larger numbers of samples can be used if desired. Previous studies have used graduated cylinders to measure sedimentation, but we chose to use test tubes because of their lower cost and ease of handling. Disposable test tubes also can be purchased, and these are more conducive for use in a multiple-tube vortex mixer, which works well when large numbers of samples need to be tested. Although the type of container is not of great importance, the containers must be of the same dimensions and have a uniform testing diameter so that direct relative analytical comparisons can be obtained between samples.

Other factors to consider are moisture and protein content of the

sample. Because the first samples tested in this study were grown, harvested, and stored under the same conditions, moisture content was not considered important for these tests. However, it might be desirable to use exact weights on a given moisture basis when comparing samples grown under unknown or widely diverse conditions. This study has shown that protein content of durum wheat is not as important as gluten strength in determining inherent cooking quality of spaghetti as long as protein is present in amounts so as not to be a limiting factor. Measurement of protein content is most important for establishing the minimum protein yield of a given cultivar, but because protein content has not been a critical limiting factor in the current durum breeding program at North Dakota State University, it is usually not measured until the $F_5$ generation. On the other hand, gluten strength, which is considered to be a primary factor in the present breeding program, is estimated as early as the $F_2$ generation using the MST. This is not to imply, however, that protein content is not considered important.

The MST procedure described has the advantages of requiring small amounts of sample (maximum 1 g of ground sample), solutions and equipment, and is simple to perform, fast, selective, and reproducible when used to screen for gluten strength in durum wheat cultivars. In addition, the required equipment is relatively inexpensive and unsophisticated, and the procedure can be done at room temperature in most laboratories, which eliminates the need for a water bath to control the temperature of the samples. The small sample requirement of the MST is definitely an advantage to the plant breeder when only small amounts of seed are available for testing. The test has also proved useful to commercial buyers who require a fast, reproducible indicator when selecting for or monitoring shipments of durum wheat or semolina on the basis of gluten strength. The strong additive effect of MST for cultivar blends makes the test suitable for testing commercial samples. One large commercial firm uses the MST in several of its elevators when selecting durum for gluten strength, and to cut costs the company developed a temperature correction table so that it was not necessary to perform the MST in a water bath at each testing site.

Although there has not been a need to analyze for samples less than 1 g within the present structure of the durum breeding program, a sedimentation-type procedure could probably be done on ground material much less than 1 g using test tubes with smaller diameters or Westergren-type blood tubes in combination with the MST procedure.


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