A Modified Micro Sedimentation Test for Screening Early-Generation Wheat Selections[1]

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ABSTRACT

A modified micro sedimentation test is described which employs the following basic modifications: 1) A 0.40-g. sample is used; 2) the lactic acid reagent is added immediately after dispersion of the flour in hydration water; 3) after 5 min. of mixing, the samples are centrifuged, the supernatant liquid discarded, and the residues redispersed in lactic acid; 4) the total volume of solution in the redispersion is 10 ml.; 5) the hydrated material is allowed to settle for 10 min. before a reading is taken. This test correctly classified 96% of the bread types, 100% of the soft club types, and 64% of the soft common types. Two-thirds of the soft common types which were misclassified were of high protein content, and consequently overlapped with poor-quality bread types.

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Market classes and subclasses represent inherent differences in flour quality, and wheat breeders in the Pacific Northwest recognize that new wheat varieties should have flour quality characteristics which conform to the traditional varieties.

Wheat-breeding programs in this region include all market classes and subclasses other than durum; consequently, the early-generation material sent to us for screening is extremely variable in flour quality characteristics. There is, therefore, a real need for a simple, rapid, and objective method for separating bread types, soft common types, and soft club types from this material and obtaining an estimate of the gluten quality.

Although we have been able to judge some important milling characteristics of thousands of F_3 selections since the development of the micromilling technique of Seeborg and Barmore (1), no entirely suitable method has been available for evaluating these widely variable selections.

It is a well-known fact that variations in gluten quality are for the most part genetically controlled, and that protein content is influenced more by environmental conditions than by variety. Since the sedimentation test reflects differences in both protein content and gluten quality, it appeared to offer the best possibilities for modification and adaptation to the task of screening early-generation selections.

Zeleny et al. (2) reported using his standard sedimentation test (3), which was later modified by Pinckney et al. (4), for early-generation screening; and indicated that the test could be reliably scaled down to one-tenth and applied to part of the wheat from a single plant, leaving sufficient wheat for planting. Recently Greenaway et al. (5) reported using the micro method suggested by Zeleny, but employed a modification used earlier by Kitterman[3] which eliminated part of the "ceiling effect" (6).

Over 100 modifications of the standard micro Zeleny sedimentation test were studied. The micro test reported here evaluated wheats grown in this area more correctly than our micro version of the standard Zeleny procedure. It has been used by this laboratory since 1962 to screen thousands of early-generation samples annually.

The basic modifications are as follows: 1) A 0.40-g. sample is used instead of 0.32 g.; 2) the lactic acid reagent is added immediately after dispersion of the flour in hydration water; 3) after 5 min. of mixing, the samples are centrifuged, the supernatant liquid discarded, and the residue redispersed in lactic acid; 4) the total volume of solution in the redispersion is 10 ml.; 5) the hydration material is allowed to settle for 10 min. before a reading is taken.

MATERIALS AND METHODS

Apparatus

1. Mill: The 5-g. mill (7), modified by the addition of a second screen of 135-mesh stainless steel.

2. Pipets: Three Cornwall[4] continuous pipets, equipped with automatic

[3]Paper presented at Pacific Northwest Section's 29th annual meeting, Seattle, June 1963: "A modified micro sedimentation test for flour quality"; also, personal communication (Sept. 11, 1963) with M. H. Neustadt, Grain Div., Consumer and Marketing Service, USDA, Agr. Research Center, Beltsville, Md.

[4]Mention of trade names of materials and equipment does not constitute endorsement by the Department of Agriculture.

two-way valves and mounted on ring stands, are used for rapid dispensing of solutions. The pipets used to dispense the lactic acid are fitted with stainless-steel two-way valves.

3. Cylinders: In routine testing, breakage of glass cylinders proved to be excessive. The cylinders used in this work were cut from clear Plexiglas tubing which has a 3/8-in. bore and a 1/16-in. wall thickness. Discs 1/2-in. in diameter were cut from 1/8-in. sheet Plexiglas and cemented to one end of the tubes. The over-all length is 192 mm. The cylinders are calibrated by pipetting 10 ml. of water into each cylinder and carefully marking the position of the meniscus. These cylinders cost $0.50 each, including labor, and there is virtually no breakage. Precision-bore Plexiglas tubing is desirable if it can be obtained.

4. Mixers: A rocker mixer similar to that described by Pinckney et al. (4) was scaled down in size to fit the Plexiglas cylinders, and geared to make 16 complete oscillations per min. An Adams Cyclo Mixer, Model A-400, was used to make the first dispersion of flour in the hydration water.

5. Centrifuge: Any suitable centrifuge equipped with a tachometer and an autotransformer control may be used.

6. Suction tube: A 12-in. length of Plexiglas tubing with a 3-mm. bore, connected to an aspirator pump for quick removal of the supernatant lactic acid.

7. Timer: A GraLab, Model 167.

8. Cylinder racks: Three special racks designed for holding the cylinders during the weighing, testing, and reading operations.

9. Measuring rule: A centimeter rule, 14 cm. long with divisions in mm.

Reagents

1. The lactic acid reagent and hydration water are the same as outlined in ref. 8, Method 56-61.

2. Volume adjusting solution: mix thoroughly 1 part of lactic acid reagent and 2 parts of hydration water.

Samples

The samples used in this study were aliquots from 210 samples of commercial varieties which were routinely grown by plant breeders in this area and sent to this laboratory for routine milling and flour quality tests. All market classes (other than durum) are represented among the 27 commercially important varieties selected. Each variety is represented by seven or eight samples ranging from low to high protein content. These varieties have been routinely tested for many years in this laboratory and their quality is well established.

Tempering

Vacuum tempering is now used instead of the method reported by Seeborg (1). The early-generation material is tempered in 3 by 5-in. paper coin envelopes in a large vacuum chamber provided with a free water surface. The chamber is evacuated to 0.1 mm. of mercury with a suitably protected pump, sealed off, and allowed to come to equilibrium vapor pressure. The samples

hydrate to moisture of 14.5 to 15.5% in 42 to 45 hr. For this study, ten 10-g. aliquots of each of the 210 samples were tempered in this manner.

Milling

The addition of 135-mesh stainless-steel sieves to the 5-g. mill permits the recovery of approximately 1 g. of flour from 5 g. of wheat. The flour samples are air-dried in the coin envelopes at room temperature for 96 hr. before testing; this permits them to dry to a uniform moisture level. For this work, the flour from the 10 aliquots of each of the 210 samples was composited, blended, and air-dried.

Modified Micro Sedimentation Test: Procedure

1. Weigh 0.40 g. of flour into the 10-ml. cylinders.
2. Hold cylinders in a horizontal position and tap on the bench top a few times to jar the flour into a wedge shape for easy dispersion, then place in a rack which holds them at a 30° angle.
3. A 20-sec. interval between samples permits 15 samples to be tested at a time. Start timing and simultaneously add 6 ml. of hydration water from a Cornwall pipet. Stopper the cylinder and mix on Adams Cyclo Mixer for 7 to 8 sec. to completely disperse flour. About 13 sec. will have elapsed up to this point. Unstopper the cylinder, add 3.0 ml. of lactic acid reagent immediately from another Cornwall pipet, then stopper the cylinder and place in the rocker-mixer. Prepare the 15 samples in this manner—one every 20 sec.
4. Remove the cylinders one at a time from the rocker after each has mixed for 5 min., and when all have finished mixing, centrifuge for 3 min. at 130 to 140 relative centrifugal force (r.c.f.). Allow the centrifuge to come up to required speed without forcing, and leave the cylinders under the specified r.c.f. for about 1 min. The centrifuge may be braked to a quick stop without disturbing the residue. Standard cylinders with bases may be centrifuged with a No. 976 head and No. 353 cups made by the International Equipment Co.
5. Remove the cylinders from the centrifuge. Take three or four at a time, unstopper, remove the supernatant liquid with the suction tube, and place the cylinders in a rack. With a little practice the supernatant liquid may be removed rapidly without removing any residue.
6. After centrifugation, the swollen residues vary in volume, and 7.5 ml. of solution is all that can be added rapidly from a pipet without going over the 10 ml. vol. in the case of high-protein bread wheat samples. Start the timing again and simultaneously add 7.5 ml. of the volume-adjusting solution from a Cornwall pipet. Bring vol. up to 10 ml., dispensing volume-adjusting solution from a wash bottle, stopper cylinder, shake vigorously by hand to thoroughly disperse residue, and place in rocker-mixer. Treat each of the 15 cylinders in this manner at 20-sec. intervals.
7. After 5 min., remove cylinders from rocker-mixer and immediately place upright in a rack.
8. At end of exactly 10 min., measure height of sediment to nearest 0.1 cm.

Our Micro Version of Standard Zeleny Test

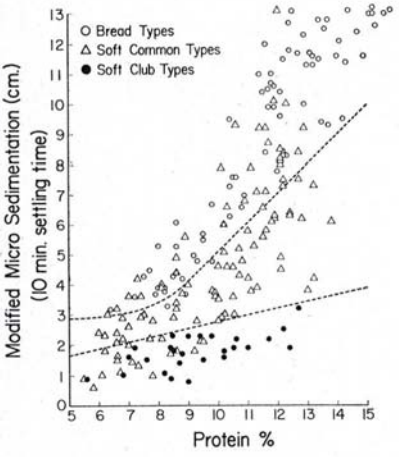
In order to make a valid comparison, the same flour samples were subjected to the authors' micro version of the standard Zeleny sedimentation test. This micro version is simply a one-tenth scale version of the standard Zeleny, except that a 0.40-g. sample and 10 ml. total liquid volume were used, as in the Modified Micro test. The cylinders and equipment used were the same as in the latter test. Thus the only difference between the two micro methods is the centrifugation and second dispersion; and the effect of this modification can be directly compared.

Protein Determination

The protein content of the flours used in this study was determined on a 0.5-g. sample, both by the Udy (9) dye-binding method and by the Kjeldahl method on an "as-is" moisture basis. The correlation of the two methods is 0.95.

RESULTS AND DISCUSSION

Generally, more than one market class or subclass is represented in the pedigree of the F_3 selections sent to us. We are therefore faced with the problem of trying to separate selections that are inherently variable in flour quality. As may be seen in Fig. 1, many samples with similar sedimentation



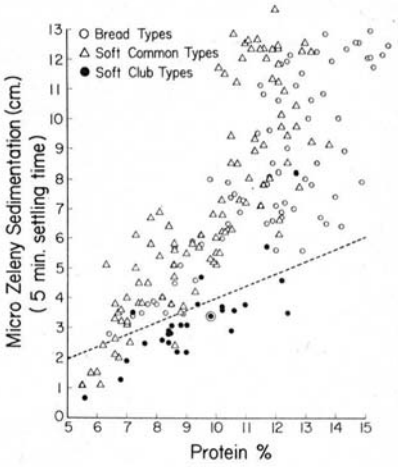


Fig. 1 (left). Ten-minute modified micro sedimentation data vs. flour protein.

Fig. 2 (right). Ten-minute data from the authors' micro version of the standard Zeleny test vs. flour protein.

values have considerably different protein contents. Conversely, many samples with similar protein content have widely different sedimentation values. At any particular sedimentation level below 10.5 cm., either bread types and soft common types, or soft common and soft club types with considerably different protein contents are found. Except for an occasional anomalous high-protein soft common wheat, only bread wheats with superior gluten quality have sedimentation values above 10.5 cm. with this modified test. Except

for these strong bread wheats with sedimentation values above 10.5 cm., we cannot separate this extremely variable early-generation material with only a sedimentation test, nor with only a protein test; both are needed.

The upper dashed line in Fig. 1 delineates the area which includes all the bread wheats except for three borderline samples. Burt, McCall, and Wanser are representative of these bread wheats. We have designated this upper area as the "bread type" area. We consider all soft common wheats which overlap into the "bread type" area as being misclassified. High-protein soft common wheats which overlap into the "bread type" area overlap with bread types which have either suffered gluten damage or are of inherently weak gluten quality. In the low-protein range some low-protein bread types overlap with some soft common types.

The middle area has been designated the "soft common" area. Gaines, Nugaines, Brevor, and Marfed are characteristic of the soft common wheats which predominate in this area. All soft club types such as Omar, Moro, and Elgin fall into the "soft club" area at the bottom. Some soft common wheats have gluten quality comparable to the soft club wheats, and thus they also fall into the "soft club" area. Within each particular area of the graph the sedimentation value indicates the relative gluten strength of the samples in that area.

Only three of the 80 bread wheats were misclassified as soft common types; all three are low-protein samples. At this protein level we expect to have some overlapping with soft types. Of 103 soft common samples, 37 were erroneously evaluated as bread types; 21 of these were in the high-protein range where we expect some overlapping with bread types of inferior gluten quality. Although 18 of the 103 soft common samples classified as soft club types, this is not really considered to be a misclassification, since some soft common wheats have gluten quality comparable to that of the soft club types. All 27 club samples classified correctly.

Analysis of variance of the data in Fig. 1 shows the following standard deviations: 0.48 cm. for bread types; 0.35 cm. for soft common types; 0.19 cm. for soft club types. Occasionally a variation of 3.0, 1.5, and 0.7 cm. for these three types, respectively, may be encountered, but this usually will not change the classification of the samples.

Figure 2 is a scatter diagram of sedimentation values obtained for the same samples with our micro version of the standard Zeleny test. Although the majority of soft club types may be separated out, it is very evident that bread types are badly intermingled with soft common types throughout most of the protein range. Obviously, this test is not satisfactory for classifying the wheats used in this investigation, since there is very poor differentiation between the bread types and the soft common types. Note in Fig. 2 the many high-protein soft common types that are erroneously shown to have values as high as those of the strongest bread wheats. Data obtained routinely in our laboratory with Buhler flour from these same high-protein soft common wheats reveal that the standard macro Zeleny method also erroneously measures them as being equal in gluten quality to strong bread wheats. This is

further confirmed by data on high-protein soft common wheats accumulated in this laboratory over many years.

Pinckney et al. (4) reported that the sedimentation values of the high-protein soft wheats they studied were below the minimum they established for good-quality bread wheats. Apparently they did not encounter high-protein soft wheats similar to those grown in this region.

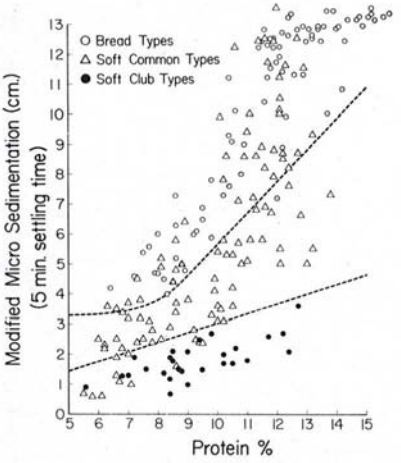
Correlation coefficients and regression equations for the data in Figs. 1 and 2 are compared in Table I. Although the soft club data from our micro

TABLE I. CORRELATION COEFFICIENTS AND REGRESSION EQUATIONS FOR SEDIMENTATION VALUES VS. PROTEIN

Wheat Types	Modified Micro		Micro Zeleny[a]	
	r_{xy}	Y =	r_{xy}	Y =
Bread types	0.90	1.334X–6.416	0.90	0.954X–4.432
Soft common types	0.80	0.895X–3.964	0.80	1.103X–4.530
Soft club types	0.70	0.226X–0.324	0.80	0.545X–1.986

[a] Authors' micro version of the standard Zeleny test. All sedimentation values measured at 10-min. settling time.

version of the standard Zeleny test has a slightly higher "r" value than the corresponding data from our modified micro test, the standard error of estimate is nearly twice as great for our micro Zeleny data: 0.707, compared to 0.448 for our modified micro data.



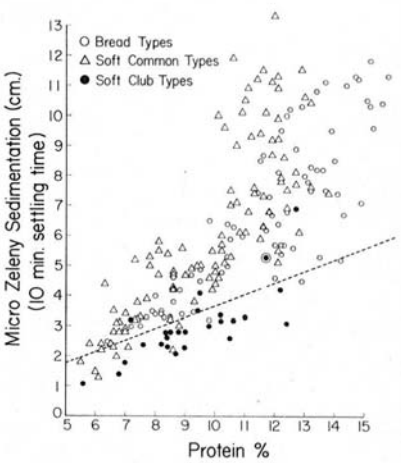


Fig. 3 (left). Five-minute modified micro sedimentation data vs. flour protein.

Fig. 4 (right). Five-minute data from the authors' micro version of the standard Zeleny test vs. flour protein.

Five-minute settling-time data are plotted in Fig. 3 merely to show the advantage of the 10-min. settling-time used in the Fig. 1 data. The longer settling-time gives better separation between the high-protein soft common and the superior-gluten bread types.

Five-minute settling-time data from our micro Zeleny test are plotted

in Fig. 4 for comparison with the 10-min. settling-time data in Fig. 2. A 10-min. settling time does not improve the micro Zeleny test.

Gortner and Doherty (10) have reported that certain inorganic salts have a decreasing effect on water imbibition by gluten. Linko and Chin (11) have shown that the addition of certain inorganic salts, and the use of tap water during the hydration period, decreased the sedimentation value significantly. They suggested that these salts inhibit water-imbibition and swelling by interfering with the formation of hydrogen bonds.

The viscosity effects measured by the MacMichael test (8, Method 56-80) are produced by the same gluten-swelling phenomenon that takes place in the sedimentation test, and Gortner and Sharp (12) have reported that removal of water-soluble ash markedly increased flour viscosity.

The improvement achieved with our modified micro sedimentation test appears to be due to the removal of soluble ash components.

Acknowledgment

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