Micropycnometer Measurement of Single-Kernel Density of Healthy, Sprouted, and Scab-Damaged Wheats[1]

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ABSTRACT

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Samples from four market lots of hard red winter and soft red winter wheat containing sprout- and scab-damaged kernels were used to test a prototype single-kernel density micropycnometer. Fifteen kernels for each damage type and an equal number of healthy kernels were weighed to the nearest 0.01 mg, then measured for volume to the nearest 1.0 µL. Volume measurements for all kernels were performed three consecutive times with the micropycnometer, then kernels were evaluated for weight, size, moisture, and hardness using a Single Kernel Characterization System. The structure of the sampling plan and the goals of the study indicated that a mixed-model statistical analysis was needed. The fixed effects were wheat class and type of kernel, and the random effects included lot, the interaction of lot with class and kernel type, kernels within each lot, and repeated measures of single-kernel density. Results indicated that variability of the three measurements per kernel did not depend on type of kernel or class of wheat. The standard deviation for repeated density measurements was 0.0029 g/cm^3. Kernel-to-kernel variability changed depending on the type of kernel; healthy and sprout-damaged kernels showed similar variability in density, whereas scab damaged kernels had a variance about four to five times higher. Type of kernel significantly affected mean density; healthy kernels averaged 1.28 g/cm^3, sprout-damaged kernels averaged 1.19 g/cm^3, and scab-damaged kernels averaged 1.08 g/cm^3. Wheat class did not exert a significant influence on single-kernel density. Attempts to predict single-kernel density using kernel weight, size, moisture, and hardness found no relationships of practical importance.

Damage from preharvest sprouting and scab presents a major economic problem in wheat (*Triticum aestivum*) production. Preharvest sprouting causes harvest losses, reduced test weight, loss of seed viability, and reduced flour quality resulting from protease and α-amylase enzymatic activity (Sorrells et al 1989, Moot and Every 1990). Scab-damaged kernels are characterized by a dull, lifeless, chalky appearance and usually contain visible fungus in the germ or in the crease. Scab results from field infection by *Fusarium graminearum* or related species during anthesis through kernel filling. Infected wheat heads produce shriveled kernels and, in many instances, the mycotoxin deoxynivalinol.

Tkachuk et al (1991a,b) reported that conditioning wheat lots using a gravity table removed most sprout- and scab-damaged kernels in the lighter fractions. Rapid identification of low density sprout- and scab-damaged kernels by density separation techniques could enable wheat millers or grain elevator operators to predict the amount of damaged kernels that would be removed during cleaning.

The development of a density gradient column in the early 1960's is the first reported attempt to measure single-kernel density for wheat. Peters and Katz (1962) separated kernels in a solution of carbon tetrachloride and cyclohexane, thereby providing a density gradient ranging between 1.25 and 1.46 g/cm^3. They reported that kernels of a homogeneously selected sample of hard spring wheat (cultivar Lee) varied in density from 1.29 to 1.41 g/cm^3 at 12% moisture. However, their work did not investigate the effect of different forms of damage on kernel density or the role of wheat class on the variation of individual kernel density.

Technological advances toward an objective grain inspection system have resulted in the rapid characterization of single-kernel wheat properties. The Single Kernel Characterization System (SKCS) weighs each kernel before determining the size, moisture, and hardness during crushing at a rate of 110 kernels per minute (Martin et al 1993). Further automation of the SKCS could include image analysis and spectral analysis of kernel chemical components. The addition of diameter and length through image analysis combined with the SKCS measurement of kernel weight could permit the prediction of single-kernel density.

In view of these technological advances and the interest in exploring new uses for the SKCS, the first objective of this research was to investigate the effects of sprout and scab damage on single-kernel density and explore the relationship between density and single-kernel weight, size, moisture, and hardness. These two types of damage occur in the field; thus, the use of the SKCS to rapidly detect the amount of damaged wheat in a lot would benefit the grain industry, particularly during harvest rush. Single-kernel density measurement using a micropycnometer could serve as a reference point from which the SKCS could be tested and calibrated. Therefore, the second objective of this study was to investigate the precision of an experimental micropycnometer.

MATERIALS AND METHODS

Wheat Samples

Samples of market wheat that were graded by the Federal Grain Inspection Service (FGIS/GISPA) field office staff in Kansas City, MO, were used for the study. These samples consisted of four lots of hard red winter wheat (HRW) and four lots of soft red winter wheat (SRW), each of which contained healthy, sprout-damaged, and scab-damaged kernels.

Sprout- and scab-damaged kernels were separated from nondamaged kernels by FGIS personnel. Fifteen kernels for each damage type and an equal number of healthy kernels were selected from all eight lots. Kernels were weighed individually to the nearest 0.01 mg before taking three consecutive kernel volume measurements.

Micropycnometer

An apparatus was fabricated to measure the volume of single wheat kernels based on liquid displacement (Fig. 1). Red gage manometer oil, obtained from Dyer Instruments of Michigan City, IN, was selected because of its low volatility, surface-wetting characteristic, and specific gravity of 0.826. A clear Lucite cup was connected to syringes with small tubing. A 500-µL syringe was used to fill the cup and replenish lost oil. The cup had a removable point gage mounted on top for locating the height of the oil surface. A fixture connected the cup and tubing to a 100-µL

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precision syringe with the plunger fastened to a rack and pinion drive. A linear digital indicator tracked the syringe plunger position and was used to measure displaced oil. Contact between the gage and oil surface was observed through a 25× stereomicroscope under illumination by a high-intensity lamp.

Liquid displacement was determined as follows. First, the 100-µL plunger position was adjusted to zero displacement. Oil contained in the 500-µL syringe was injected through the cup bottom to a level that contacted the gage. The gage was observed and adjusted to a zero setting. After a reference level was established for the filled cup, the digital indicator was set to zero. Next, the plunger was pulled from the syringe to lower the oil level in the cup by ≈75 µL, the gage was removed, the subject kernel was placed in the cup, and the gage was replaced. Then the plunger was pushed back into the syringe while the oil level inside the cup was observed through the microscope. When the oil surface contacted the gage, thus indicating a filled cup, the digital indicator reading was recorded as the volume displaced by the kernel. Two additional readings were taken without removing the kernel from the cup. For these additional readings, the oil level was lowered to the bottom of the kernel then returned to the gage point. Kernels were in contact with the oil for ≈2 min, after which they were placed on paper towels to wick off the surface layer of oil. Throughput was ≈15 kernels per hour.

Three 3.175-mm diameter (16.76-µL) precision ball bearings were used as reference volumes to calibrate the digital indicator reading. The use of three spheres provided a means to check the linearity of the system as determined by linear regression.

Statistical Analysis

The structure of the sampling plan and the goals of the study indicated that a mixed-model analysis was needed (Littell et al 1996). The fixed-effect factors, for which mean densities at different levels were to be compared, were the class of wheat (HRW and SRW) and the type of kernel (healthy, sprout- and scab-damaged). Random-effect factors, which might contribute to the variability of the density, were the lots within each class; the lot × type interaction; the kernels within each lot, class, and type; and the three measurements from each kernel.

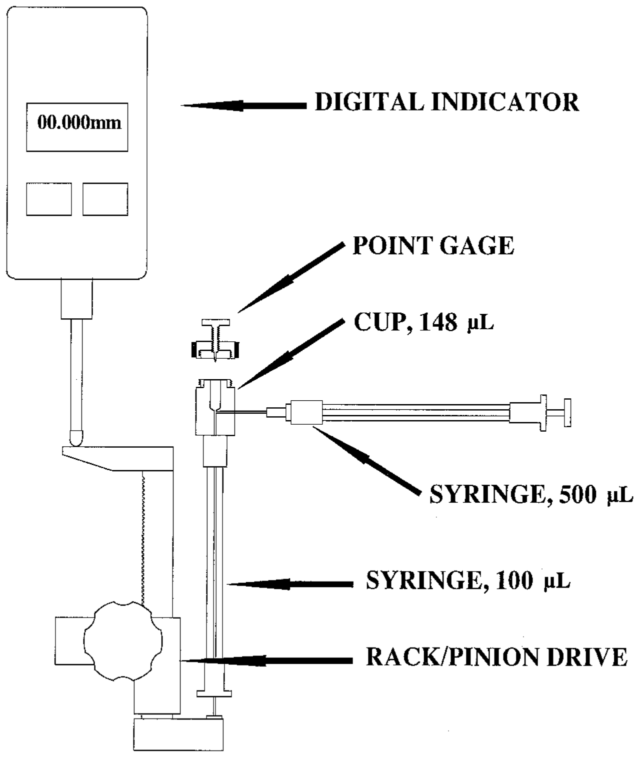

Fig. 1. Diagram of the 100-µL micropycnometer.

Variance components for random effects and means for fixed effects were estimated jointly using PROC MIXED in SAS (SAS Institute, Cary, NC). Furthermore, the possibility of heterogeneous variances was investigated by fitting separate variance components to subgroups as: 1) separate variances for the lots and lot × type interaction for the two classes of wheat; 2) separate variances of the kernels from different classes and lots; and 3) separate variances for the three measurements on kernels from different classes and lots. With one exception, tests for potential heterogeneity were performed using likelihood ratio test statistics computed from pairs of runs with and without the separate variance components. Heterogeneous variance components were retained in the model where they were significant; otherwise, a homogeneous variance was assumed, and a single variance component was used for all subgroups.

For each kernel, the sample variance of the three measurements was computed. These variances were treated as a response variable, and tests for differences due to class of wheat and type of kernel were performed using ANOVA methods. If no significant effects were found, a homogeneous variance of measurements was assumed for all subsequent models.

Once the heterogeneity structure was completely determined, likelihood ratio tests were used to test whether any of the homogeneous variance components can be removed from the model completely. Nonsignificant components were eliminated.

Following modeling of the variance structure, the tests for fixed effects were performed using *F*-tests. Where significant, means were separated using *t*-tests.

TABLE I
Fixed Effects Test for Wheat Class; Type of Kernel Damage (Healthy, Sprouted, Scab-Damaged); and Their Interaction on Single-Kernel Density Measured by a Micropycnometer

Source	*F*-Value	Probability
Class	0.05	0.8309
Type	69.80	0.0001
Class × type	0.88	0.4419

TABLE II
Mean and Standard Deviation (SD) Values for Single-Kernel Density (g/cm^3) Measured by a Micropycnometer by Wheat Class and Type of Kernel Damage

Class × Type[a]	Mean	SD
HRW × healthy	1.278	0.066
HRW × sprouted	1.201	0.068
HRW × scab-damaged	1.069	0.155
SRW × healthy	1.280	0.084
SRW × sprouted	1.183	0.075
SRW × scab-damaged	1.094	0.157

[a] HRW = hard red winter wheat; SRW = soft red winter wheat.

TABLE III
Single-Kernel Density (SKD) Regression Models for Wheat Class and Damage (Healthy, Scab-Damaged, and Sprouted)

Class and Type[a]	Regression Model (SKD =)[b]	R^2
HRW healthy	1.27 + 0.0032 SKWT – 0.0082 SKMST	0.34
HRW sprouted	1.19 + 0.0032 SKWT – 0.0082 SKMST	0.27
HRW scab-damaged	No regression	
SRW healthy	No regression	
SRW sprouted	No regression	
SRW scab-damaged	1.003 + 0.0034 SKHARD	0.27

[a] HRW = hard red winter wheat; SRW = soft red winter wheat.

[b] SKWT = single-kernel weight; SKMST = single-kernel moisture; SKHARD = single-kernel hardness.

Backward stepwise regression was performed using single-kernel data of weight, size, moisture, and hardness measured by the Perten 4100 SKCS (Reno, NV) to predict single-kernel density.

RESULTS AND DISCUSSION

Variance Components for Random Effects

The analysis of variance components for random effects indicated that the three kernel measurements did not vary significantly ($P > 0.10$) between kernel type (healthy, sprout-, and scab-damaged) and class (HRW and SRW), and no significant type × class interaction occurred. Furthermore, the standard deviation among the three kernel measurements across all kernel types and class performed by the micropycnometer was 0.0029 g/cm^3. These results indicate that the micropycnometer provided a high degree of precision and single-kernel density measurements were consistently repeatable, regardless of wheat class or kernel damage.

The kernel-to-kernel variation in density within a lot was influenced significantly by the type of damage. Specifically, the likelihood ratio tests revealed a significantly greater kernel-to-kernel variation in density ($P < 0.05$) for scab-damaged kernels as compared to healthy kernels and sprout-damaged kernels.

Lot-to-lot variability did not contribute significantly to the variance component model. Therefore, complete removal of the lot effect from the model was possible.

Fixed Effects for Single-Kernel Density

The type of kernel (healthy vs. damaged) had a significant effect on single-kernel density. No significant class or class × type interaction was observed (Table I).

Healthy kernels had an average kernel density of 1.28 g/cm^3, sprout-damaged kernels had an average density of 1.19 g/cm^3, and scab-damaged kernels had an average density of 1.08 g/cm^3. Standard error values for the least squares means were 0.007 for healthy and sprout-damaged kernels and 0.017 for scab-damaged kernels. These values indicate that the means for each kernel type were significantly different from one another.

The comparatively lower density values for scab-damaged wheat are related to activity of the invading organism. Bechtel et al (1985) observed that *F. graminearum* is an aggressive invader of wheat kernels, destroying starch granules, storage protein, and cell walls. Although the fungus was most prevalent in aleurone and pericarp tissues, hyphae extended throughout the starch endosperm.

Seitz and Bechtel (1985) observed a wide variation in the intensity of invasion by *F. graminearum* between lightly and severely damaged kernels. All scab-damaged kernels measured for single-kernel density were selected (picked) by Federal Grain Inspection personnel during official inspections. Although a minimum criteria for scab damage exists within the official grain inspection system, a kernel-to-kernel variation in the level of invasion for the damaged kernels likely explains the comparatively larger variation in density between individual scab-damaged kernels.

Sprout-damaged kernels displayed an intermediate density (1.19 g/cm^3) as compared to healthy and scab-damaged kernels. Sprout damage is identified in the U.S. Grain Grading Standards for wheat by any of the following: the germ cover is broken open with a sprout showing at the top; the germ cover is broken open with a sprout showing; the sprout is broken off leaving no germ cover over the socket area; or the sprout is broken off leaving part of the germ cover over the socket area. Physiological and structural changes during sprouting include activation of hydrolytic and proteolytic enzymes and cell wall deterioration. MacGregor (1982) reported for Klages barley that protein and cell wall degradation preceded α-amylase attack of starch granules and that this activity began at the proximal end of the kernel. When examining the role of the pericarp in control of germination and dormancy of wheat, Woodbury and Wiebe (1982) observed that changes in moisture content of polymers would result in shrinkage or swelling. They reasoned that these structural changes within a kernel "have a memory", which may subtend germinability and dormancy. A similar argument may explain why sprout-damaged kernels possessed a lower average kernel density when compared to healthy kernels.

Instrument Precision and Accuracy

The precision (repeatability) of the single-kernel micropycnometer was high (0.0029 g/cm^3 standard deviation). Any kernel change between the three consecutive volume readings such as swelling, shrinking, oil absorption, moisture change, or bubble presence did not significantly affect the density measurement. Standard deviations for single-kernel density ranged between 0.066 g/cm^3 for healthy HRW wheat samples and 0.157 g/cm^3 for scab-damaged SRW wheat samples (Table II).

The single-kernel micropycnometer tended to provide kernel density values below those reported for the gas pycnometer. In previous reports, bulk kernel density measurements using a gas (helium) pycnometer averaged 1.40 g/cm^3 (Chang 1988, McGaughey et al 1990). This difference can be explained partially by void spaces in a kernel that can be occupied by helium atoms but not by the molecules of a liquid. Kernel weight per unit volume displaced in helium is larger than the kernel weight per unit volume displaced in a liquid. Average kernel density measured in a carbon tetrachloride and cyclohexane solution ranged between 1.27 and 1.38 g/cm^3 (Peters and Katz 1962). Nondamaged kernels were separated by a categorical system which included kernels with some degree of damage and a lower density. The result was an average density for healthy kernels that agreed with the low end of the carbon tetrachloride and cyclohexane solution range.

Regression Analysis

Separate regression models were developed for class (HRW and SRW) and type of kernel damage (Table III). Models developed for density of HRW healthy and sprout-damaged kernels included the same regression parameters for single-kernel weight and moisture. However, the intercepts of the two models were significantly different. This was due to the significant difference in the mean values for single-kernel density of healthy and sprouted kernels. No significant regressors were identified for predicting kernel density of scab-damaged HRW or of healthy and sprout-damaged SRW samples. Single-kernel hardness was related significantly to single-kernel density for scab-damaged SRW wheat.

The regression models developed using weight, size, moisture, and hardness failed to explain much of the variability in single-kernel density (Table III). This was due to the large variation of single-kernel density within each class × type group. The degree in overlap of single-kernel density likely preclude this as a singular objective test for differentiating healthy, sprout-, and scab-damaged kernels. An objective method for measuring damage would permit a shift from the present categorical system (damaged, nondamaged) to one that measures a continuum between healthy and damaged kernels. While work by Tkachuk et al (1991a) supports the use of density as a means for separating damaged kernels, they also report the presence of scab-damaged kernels (in small amounts) in the high-density fractions.

The use of near-infrared reflectance spectral analysis to identify sprout damage (as determined by Rapid Visco-Analyzer prediction of falling number) is reported to be good (Shashikumar et al 1993). Addition of spectral analysis to the SKCS could augment the predictive ability of an objective system that identifies the type and intensity of kernel damage and should be explored.

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