Effect of Wheat Moisture Content on Hardness Scores Determined by Near-Infrared Reflectance and on Hardness Score Standardization

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ABSTRACT

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Near-infrared reflectance instrumentation provides an empirically measured scale for wheat hardness. The hardness scale is based on the radiation-scattering properties of meal particles at 1,680 and 2,230 nm. Hard wheat meals usually have larger particle sizes than soft wheat meals. The objective of this study was to determine the sensitivity of near-infrared reflectance wheat hardness measurements to moisture content, and to make the hardness score (HS) independent of moisture by correcting hardness measurements for the actual moisture content of measured samples. Forty wheat cultivars composed of hard red winter, hard red spring, soft red winter, and soft white winter were used. Wheat kernel sample groups were stored at 20, 40, 60, and 80% rh. After equilibration, the samples were ground, and the meal was analyzed for HS and moisture. Averaged across wheat samples and relative humidity treatments, HS were 48, 50, 54, and 65 for 20, 40, 60, and 80% rh, respectively. HS from storage at 80% rh (13.4% meal moisture) were higher ($P < 0.05$), and HS from storage at 20% rh (9.3% meal moisture) were lower ($P < 0.05$) than the control values, which had an intermediate meal moisture content (11%). Within each class of wheat, HS increased as moisture content increased. An algorithm was developed to correct HS to 11% moisture. The correction provided HS that were nearly independent of moisture content.

Near-infrared reflectance (NIR) instrumentation has been used to distinguish between hard and soft wheat hardness. NIR spectra are markedly affected by mean particle size (Williams 1979). When particle size increases, the logarithm (base 10) of the reciprocal of reflectance ($1/R$) increases at every wavelength in the NIR spectrum (Norris et al 1989). As a result, log $1/R$ values for hard wheats are greater than those for soft wheats. Williams (1979) reported correlations between NIR spectra and hardness of wheat as assessed by the particle size index. Norris et al (1989) developed a hardness scale using NIR data at 1,680 and 2,230 nm, but their approach differed from that of Williams and Sobering (1986) in that the NIR-based hardness scale was not independently defined. Their scale had a hardness score (HS) that ranged from about 20 for soft wheat to about 120 for hard wheat. The wavelengths of 1,680 and 2,230 nm were chosen to maximize the precision of HS determinations. The equation coefficients had to be adjusted for each measurement configuration. A standardization was accomplished with 10 standard wheat samples maintained and distributed by the Federal Grain Inspection Service (FGIS). The procedure reported by Norris et al (1989) resulted in method 39-70A of the American Association of Cereal Chemists (AACC 1983): "Wheat Hardness as Determined by Near-Infrared Reflectance."

After receipt by users, the 10 standard wheat hardness samples from FGIS may attain various moisture contents, depending on handling, storage, ambient temperature, and humidity. Wheat moisture content and wheat meal particle size have influenced the bias and slope of NIR calibration equations for the measurement of protein and moisture content (Williams 1975, Williams and Thompson 1978). Norris et al (1989) reported that the hardness method was relatively insensitive to moisture, and that HS changed less than five units for samples with moisture content between 10 and 13%. However, standardization and test samples may attain moisture contents that exceed the range investigated by Norris et al (1989). Therefore, the objective of this study was to investigate the effect of wheat kernel moisture content on the HS determined by AACC method 39-70A and to generate an algorithm for making the HS independent of sample moisture content.

MATERIALS AND METHODS

Wheats Cultivars and Sampling

Forty cultivars representing four wheat classes were obtained from the FGIS. They consisted of 10 hard red winter (HRW), 10 hard red spring (HRS), 10 soft red winter (SRW), and 10 soft white winter (SWW) wheats. HRW and SWW wheats were pure cultivar market samples from the FGIS field offices at Wichita, KS, and Moscow, IN, respectively. HRS samples were five market samples from the Wichita, KS, FGIS field office, as well as the cultivars Amadon, Len, Columbus, and Coteau from North Dakota and an unknown cultivar from Souris River Grain Seed Plant in Newburg, ND. SRW samples were seven market samples from the Moscow, IN, FGIS field office and two cultivars of Caldwell and Reeds 104 from Missouri. Each of the cultivar samples was divided into four groups of wheat kernel samples (75 g) that were stored in different controlled relative humidity environments.

Relative Humidity Storage Conditions

Storage conditions were controlled using four stainless steel desiccator cabinets (Fisher Scientific, Pittsburgh, PA) located in a room maintained between 45 and 50% rh at 25°C. The relative humidity in each cabinet was controlled by saturated salt solutions. Potassium acetate, sodium iodide, sodium bromide, and ammonium sulfate solutions established the relative humidity at 20, 40, 60, and 80%, respectively (Greenspan 1977). Desiccator cabinets were equipped with internal fans that continuously circulated the air.

A 50-g subsample from each of the four 75-g wheat kernel samples was spread in individual aluminum wire-screen containers, placed in the cabinets, and exposed directly to controlled environments on all sides of the containers. At each relative humidity environment studied, one container each of hard and soft wheat of known moisture content and sample weight was weighed weekly to determine the gain or loss of water. The time needed for all samples to reach equilibrium (i.e., constant weight) was three weeks. Kernels were transferred to open-topped glass jars three days before removal from the relative humidity environments.

Cabinet relative humidity was monitored with an animal membrane humidity indicator (Bacharach Instrument Co., Pittsburgh, PA). Indicators were calibrated over H_2SO_4 solutions of known density in cabinets at 25°C. Instrument accuracy was 3% rh, based on solution density and humidity values in the *Handbook of*

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Chemistry and Physics (Weast and Astle 1972). Cabinet and room temperature were monitored with a laboratory mercury thermometer.

NIR HS and Whole Kernel Moisture Measurements

The procedure for sampling, storing, and analyzing the wheat kernel samples is shown in Figure 1. A laboratory-equilibrated subsample (25 g) was designated as a control; it was analyzed for HS and moisture before the remaining 50-g subsample was stored.

From each control subsample, 15 g was ground (1 g/sec) in a Tecator Cyclone mill equipped with a 1.0-mm screen and packed in NIR sample cells. NIR spectra were taken with an NIRSystem 6250 scanning monochromator. Spectra consisted of 700 data points from 1,100 to 2,498 nm at 2-nm intervals. Data were recorded as the log (1/*R*) and transferred to a minicomputer (DEC Microvax) for analysis. Wheat HS was determined by AACC method 39-70A. The remaining 10 g from each control subsample was analyzed for moisture by the Karl Fischer paste extraction method (Windham 1989).

On the same day that the 25-g control subsamples of the 40 cultivars were analyzed for HS and moisture, 50-g subsamples were stored in experimental relative humidity environments. After equilibration, subsamples were analyzed for HS and whole kernel moisture as previously described. That protocol was followed for storage at each relative humidity environment studied. Two sets of all 40 samples were processed per week. The 10 wheat hardness standardization samples obtained from FGIS (as prescribed in AACC method 39-70A) were divided into four groups. One hardness standardization group was ground and scanned with each control group at each relative humidity environment to provide four replicates. The replicates were used to make a slope and bias correction to the two-term model of Norris et al (1989, equation 3) and to determine the precision of the HS measurement. The control HS averaged across wheat classes were compared with the HS after storage at 20, 40, 60, and 80% rh. Data were treated by a two-way analysis of variance (SAS 1987).

Wheat Meal Moisture Measurement

After the determination of HS, three samples were randomly selected from each class of wheat within each relative humidity storage group ($N = 48$) for NIR moisture calibration. In addition, five samples were randomly selected from each relative humidity storage group ($N = 20$) for equation verification. Samples were analyzed for moisture using the Karl Fischer paste extraction method (Windham 1989). Moisture content of the calibration samples ranged from 9.0 to 13.9%. Moisture data were linearly regressed against the log (1/*R*) values at 1,940 and 2,310 nm. The regression equation was:

$$M = 9.79 + 67.06X_1 - 62.36X_2 \qquad (1)$$

where M is the percent moisture and X_1 and X_2 are the log (1/*R*) values at 1,940 and 2,310 nm, respectively. Those wavelengths are available on most fixed-filter NIR instruments and are currently used by the FGIS. Standard error of calibration and equation performance were 0.084 and 0.097% moisture, respectively. That equation was used to predict the moisture content of all ground wheat samples.

Validation of HS Moisture Correction

Wheat samples from 20 known pure cultivars for four wheat classes were obtained from the USDA Soft Wheat Quality Laboratory, Wooster, OH. The HRS samples were the cultivars Wheaton, Butte 86, Guard, Stoa, and Veery. The HRW samples were the cultivars Lancer, Shawnee, Mit, Bounty 203, and Newton. The SRW samples were the cultivars Caldwell, Adena, Becker, Hillsdale, and Keiser. The SWW samples were the cultivars Yorkstar, Augusta, Frankenmuth, Geneva, and Tecumseh. Wheats were subdivided into three groups and stored at 20, 50, and 80% rh as previously described. The saturated salt solution used to control the relative humidity at 50% was magnesium nitrate (Greenspan 1977). Wheat samples were analyzed for kernel moisture, meal moisture, and HS as previously described. HS was compared with HS uncorrected for moisture and with HS corrected for moisture after equilibrium storage at 20 and 80% rh. Wheats stored at 50% rh were designated as validation controls.

RESULTS AND DISCUSSION

Moisture contents of wheat stored at different relative humidity environments are shown in Table I. Across cultivars, the control samples, which were received from FGIS and sealed with no special humidity storage treatments, had an average whole kernel moisture content of 11.7% and an average wheat meal moisture content of 11.0%. The moisture content of whole kernels placed in the 40 and 20% rh environments decreased by 1.1 and 2.4%, respectively. Conversely, in the 60 and 80% rh environments, whole kernel moisture content increased 1 and 5.1%, respectively. The range in moisture content obtained by storage at different relative humidity environments is in agreement with Pixton and Warburton (1971). For all samples, at least 80% of the total moisture change occurred in only seven days. The percent moisture of wheat meals was not significantly different ($P < 0.05$) from the moisture content of the whole kernels for the control, 20% rh, and 40% rh environments. However, the moisture contents of the wheat meal samples placed in the 60 and 80% rh environments decreased 1.2 and 3.4%, respectively.

The precision of the HS measurement, as indicated by the mean standard deviation (SD) of the predicted HS from the four groups of FGIS standardization samples, was 2.4 HS units. The mean SD of the four groups that were analyzed on the same day was 1.8 HS units. All standardization samples were ground and reanalyzed after three months of storage in moisture-proof pouches. It was assumed that no significant changes in the grinder and spectrophotometer had taken place. The SD of the additional measurement for a single standardization sample ranged from 1.1 to 2.0 HS units, indicating that the equation repeatability

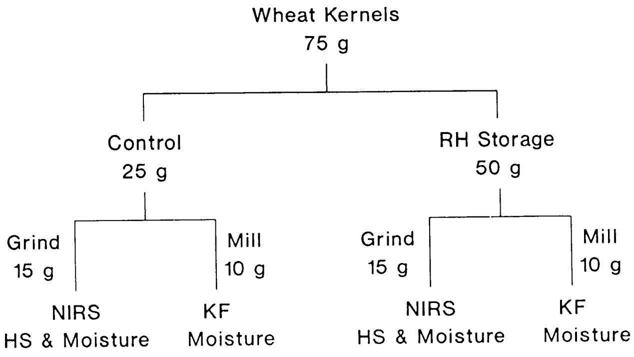

Fig. 1. Flow chart of procedure for sampling, storing, and analyzing each wheat group. HS = hardness score, RH = relative humidity treatment, NIRS = near-infrared reflectance spectrum, KF = Karl Fischer paste extraction method (Windham 1989).

TABLE I

Mean and Standard Deviation of Moisture Content (%) of Wheat Kernels[a] and Meals[b] Stored at Different Relative Humidity (rh) Environments

	Whole Kernel		Wheat Meal	
Treatment	Mean	SD[c]	Mean	SD
Control[d]	11.7	1.2	11.0	0.7
20% rh	9.3	0.2	9.3	0.2
40% rh	10.6	0.3	10.3	0.2
60% rh	12.7	0.4	11.5	0.2
80% rh	16.8	0.4	13.4	0.4

[a] Determined by Karl Fischer paste extraction method (Windham 1989).

[b] Determined by near-infrared reflectance.

[c] Standard deviation.

[d] Mean of the four control subsamples.

was precise and restandardization was not required.

Mean HS across relative humidity treatments for the four wheat classes were significantly different ($P < 0.01$). Mean HS for the SRW, SWW, HRW, and HRS wheat classes were 24, 31, 68, and 91 units, respectively. Comparison of mean HS of the four control groups with the HS after kernel storage at 20, 40, 60, and 80% rh (Table II) indicated that HS of the four wheat classes from the 80% rh environments were different ($P < 0.05$) from control HS.

Figure 2 shows the relationship between HS of the four relative humidity treatments versus the HS of the control samples. Precise agreement between treated and control sample HS is depicted by the diagonal line. The actual regression of control HS against relative humidity treatment HS was:

$$Y = 3.04 + 0.98x \tag{2}$$

Deviations from the ideal regression line were due to a decrease in HS for samples stored at 20% rh and an increase for samples stored at 80% rh (Table II). The NIR log ($1/R$) spectrum of samples increased when moisture content increased. For example, the 80% rh sample had an increase in the log ($1/R$) spectrum, whereas the 20% rh sample had a decrease in log ($1/R$) spectrum. Change in the spectrally determined HS was due neither to wheat classes nor to spectral changes in the water absorption band. It may be that it was the result of the moisture-induced change in particle size during grinding. Preliminary results suggest that higher moisture wheat produced meal with larger average particle size and less NIR scattering, hence, reflectance decreased.

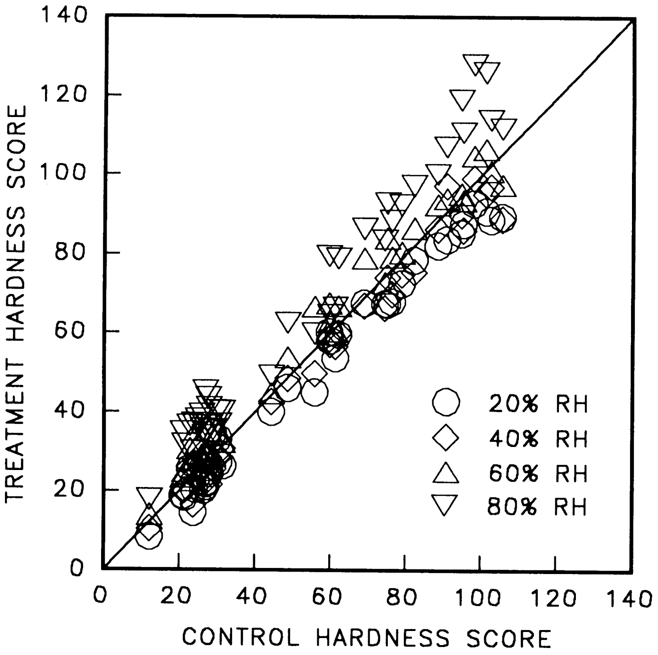

Fig. 2. Hardness scores of 40 wheat samples from four wheat classes, each stored at four relative humidity (RH) environments, compared with control (untreated) hardness scores.

Effects of wheat meal moisture content (from humidity storage treatments) on HS for each class of wheat are shown in Figure 3. The standard error of the regression equations were 0.20, 0.43, 0.47, and 0.47 for HRS, HRW, SWW, and SRW, respectively. Within each class of wheat, as moisture content increased, HS increased. Hard wheats increased linearly to a greater extent than did the soft wheats. That relationship provided the means for developing a moisture-independent HS, by observing that the rate of change of HS with moisture content was different for hard versus soft wheat (Fig. 3). The slope increased monotonically with HS. In Figure 4, the slope from the four wheat classes in Figure 3 is associated with the HS at 11% meal moisture content. The standard error of the slope of the regression was 0.002.

The 11% moisture level was chosen as the standard moisture content to which all HS were corrected because 11% moisture was the average moisture content found after grinding standardization samples. That was also the approximate meal moisture content after grinding kernels with 12% moisture. The information contained in Figure 4 allowed all HS to be corrected to 11% moisture by creating a family of regression lines of the type shown in Figure 3; each class has its own slope. That relationship is expressed mathematically as:

$$\mathrm{HS_{cor}} = \mathrm{HS} - \mathrm{slope}\,(\%M - 11) \tag{3}$$

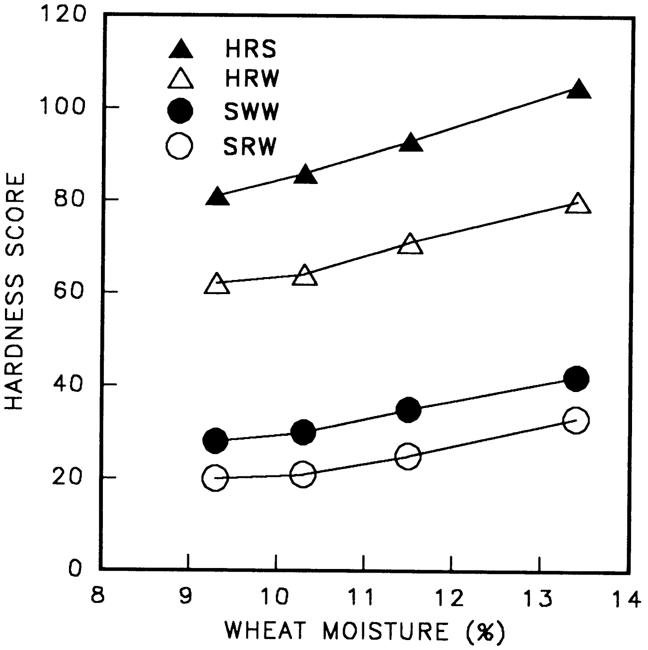

Fig. 3. Effect of wheat moisture content (%) on hardness score. Mean of wheat samples; 10 hard red winter (HRW), 10 hard red spring (HRS), 10 soft red winter (SRW), and 10 soft white winter (SWW) wheats. Best straight line fit for wheat classes are HRS $y = 28.51 + 5.67x$, $R^2 = 0.99$; HRW $y = 17.79 + 4.64x$, $R^2 = 0.97$; SWW $y = -6.89 + 3.64x$, $R^2 = 0.98$; SRW $y = -10.51 + 3.14x$, $R^2 = 0.96$.

TABLE II

Hardness Scores[a] of Wheat Kernels from Four Wheat Classes[b] Stored at Different Relative Humidity (rh) Environments

Storage Treatment	SRW		SWW		HRW		HRS	
	Mean	SD[c]	Mean	SD	Mean	SD	Mean	SD
Control	23.9	5.3	29.2	6.5	67.0	10.4	90.7	14.5
20% rh	20.1	6.7	27.8	5.1	63.0	9.5	81.2	14.5
40% rh	20.6	4.4	29.3	4.9	63.7	9.2	85.8	15.3
60% rh	25.3	4.6	33.7	4.5	71.3	10.9	93.0	12.0
80% rh	32.9	5.7	41.0	3.9	80.4	12.5	106.0	20.2

[a] Least significant difference = 4.5 ($P < 0.05$).

[b] SRW = soft red winter; SWW = soft white winter; HRW = hard red winter; HRS = hard red spring.

[c] Standard deviation.

where HS_{cor} = HS corrected to 11% moisture; slope = $2.34 + 0.036\ HS_{cor}$ (the best linear fit to the slope data of Fig. 4); and $\%M$ = moisture content of the meal, determined from NIR log $(1/R)$ data. The corrected HS are achieved by solving for HS_{cor}. The equation used to correct all HS was:

$$HS_{cor} = HS - 2.34\,(\%M - 11)\ /\ 1 + 0.036\,(\%M - 11) \quad (4)$$

The results of correcting HS to 11% meal moisture are shown in Figure 5. The mean HS across relative humidity treatments for the four wheat classes were different ($P < 0.01$), as was found with uncorrected HS. However, the corrected HS were not different, due to kernel storage at the four relative humidity treatment environments. Mean corrected HS (averaged across cultivars) was 53, 55, 53, 54, and 55 units for the control and samples stored at 20, 40, 60, and 80% rh, respectively. Linear regression of corrected control HS against corrected treatment HS ($Y = 1.25 + 1.00x$) had an intercept and slope not greatly different from 0.0 and 1.0, respectively. On the average, samples from 20% rh, with a meal moisture content of 9.3% and an uncorrected HS of 48, had a corrected HS of 55 at 11% meal moisture. Conversely, samples from 80% rh, with a meal moisture of 13.4% and an uncorrected HS of 65, gave a corrected HS of 55 at the 11% meal moisture.

The moisture correction equation was validated using the set of 20 cultivars obtained from the USDA Soft Wheat Quality Laboratory, Wooster, OH, described previously. Wheat samples stored at 50% rh were designated as validation controls. Those samples had a mean whole kernel moisture content, meal moisture content, and uncorrected HS of 11.8%, 10.9%, and 46 units, respectively. Wheat samples stored at 20% rh had a mean whole kernel moisture content, meal moisture content, and HS of 9.5%, 9.6%, and 42 units, respectively. Wheat samples stored at 80% rh had a mean whole kernel moisture content, mean meal moisture content, and HS of 17.2%, 14.0%, and 55 units, respectively. The uncorrected HS from 20 and 80% rh were significantly different ($P < 0.05$) from those of 50% rh. However, corrected HS of 48 and 43 units from storage at 20 and 80% rh, respectively, were not significantly different ($P > 0.05$) from those of 50% rh (46 units), determined at 10.9% meal moisture. Linear regression of 50% rh HS against corrected 20 and 80% rh, $y = -2.54 + 1.03(x)$, had an intercept and slope not significantly different ($P > 0.05$) from 0.0 and 1.0, respectively. Standard error was 0.022.

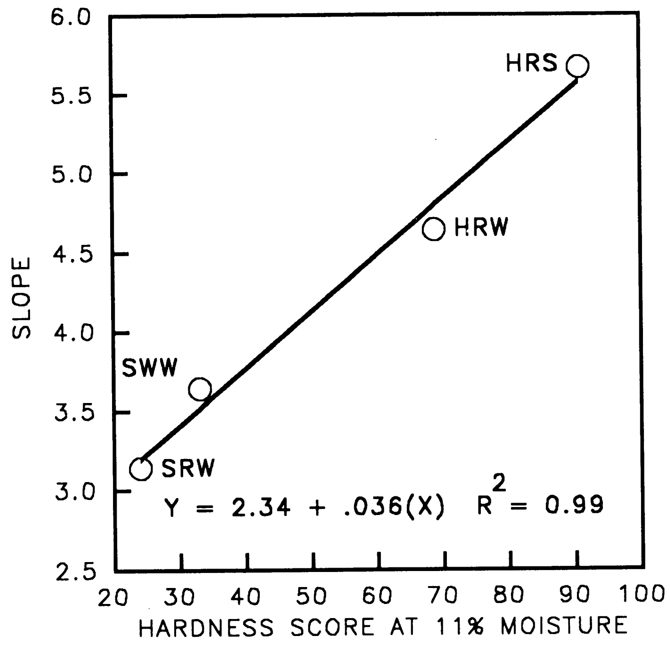

Fig. 4. Relationship of slope from Fig. 3 vs. hardness score at 11% meal moisture for four classes of wheat. Equation is the best straight line fit for the data. HRW = hard red winter; HRS = hard red spring; SRW = soft red winter; SWW = soft white winter.

In addition to the correction eliminating the influence of whole kernel moisture content on HS, precision of the measurement was improved. The four control groups ground and analyzed over a two-week period had a mean (across cultivars) meal moisture contents of 10.6, 11.5, 10.7, and 11.0%. The variation in moisture was possibly caused by a change in laboratory humidity over the two-week period of sample analysis. The precision of the measurement (average standard deviation) for the four control groups was 2.8 uncorrected hardness units. Correction of HS for moisture improved the precision of the measurement to 2.2 hardness units. Norris et al (1989) stressed the importance of uniform kernel flow into and out of the grinding chamber to reduce the SD of HS for replicated grinds. It is likely that some of the error in HS between laboratories reported by Norris et al (1989) was caused by changes in kernel moisture content.

AACC method 39-70A recommends that HS measurement be monitored over time with both hard and soft wheat samples to assess the constancy of grinder and instrument performance. However, if the local humidity varies significantly, and the samples are not stored in moisture-proof containers, HS may fall outside the quality control chart limits. In addition, the method recommends equilibration of unground samples to the local testing humidity. Variation in local humidity could thus cause variation in kernel moisture content over time. The HS samples are positively or negatively biased, depending on whether the samples were higher or lower in moisture content than the standardization samples. However, the influence of kernel moisture content on HS can be eliminated by the moisture correction procedure.

CONCLUSIONS

As written, the AACC method 39-70A for wheat hardness is unnecessarily affected by wheat samples with kernel moisture contents that vary more than 1%. That method was successfully modified to give HS independent of kernel moisture content by correcting HS to a fixed moisture level of 11.0%. Corrected HS for each wheat class show no significant differences for samples with moisture contents from 9 to 14%. Kernel moisture content

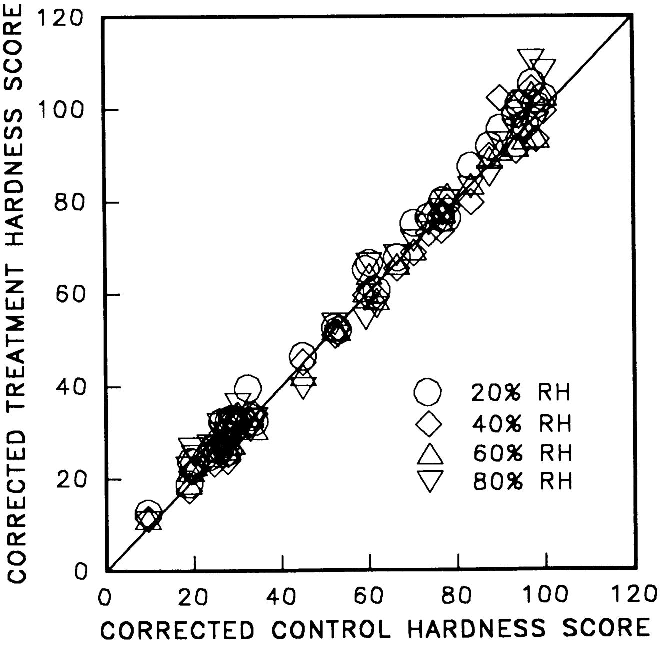

Fig. 5. Hardness scores corrected to 11% moisture for wheat samples from four wheat classes, each stored at four relative humidity (RH) environments, compared with corrected control (untreated) hardness scores. HRW = hard red winter; HRS = hard red spring; SRW = soft red winter; SWW = soft white winter.

can easily be assessed spectroscopically in a manner similar to that for HS determinations. Corrected HS (HS_{cor}) is:

$$HS_{cor} = HS - 2.34\ (\%M - 11)\ /\ 1 + 0.036\ (\%M - 11)$$

In addition, the cause of the variation of the standard method HS with kernel moisture content was determined. HS increased with increasing kernel moisture content. HS depends on meal particle size because the log $(1/R)$ spectra in the NIR depend on particle size. It is likely that meal particle size increases with increasing kernel moisture content; this is the subject of a separate study. Even though differences in moisture content may also affect kernel hardness, a major cause of fluctuations in the precision of the HS measurement is sample-to-sample variability in wheat moisture, particularly for grain with high moisture.

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