Relationship Between Single Wheat Kernel Particle-Size Distribution and Perten SKCS 4100 Hardness Index

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ABSTRACT

The Perten Single Kernel Characterization system is the current reference method for determination of single wheat kernel texture. However, the SKCS 4100 calibration method is based on bulk samples. The objective of this research was to develop a single-kernel hardness reference based on single-kernel particle-size distributions (PSD). A total of 473 kernels, drawn from eight different classes, was studied. Material from single kernels that had been crushed on the SKCS 4100 system was collected, milled, then the PSD of each ground single kernel was measured. Wheat kernels from soft and hard classes with similar SKCS hardness indices (HI 40–60) typically had a PSD that was expected from their genetic class. That is, soft kernels tended to have more particles at <21 µm than hard kernels after milling. As such, a combination of HI and PSD gives better discrimination between genetically hard and soft classes than either parameter measured independently. Additionally, the use of SKCS-predicted PSD, combined with other low level SKCS parameters, appears to reduce classification errors into genetic hardness classes by ≈50% over what is currently accomplished with HI alone.

The Single Kernel Characterization System (SKCS) developed by Martin et al (1993) and commercialized by Perten Instruments as the SKCS-4100 is a widely used instrument for measuring single wheat kernel weight, moisture, diameter, and hardness, and is one of the standards for measuring wheat kernel texture, or degree of hardness (Approved Method 55-31; AACC International 2000). Other common and approved methods for measuring texture, or hardness, of samples is the particle-size index from ground samples (AACC Approved Method 55-30), which involves grinding a sample and passing it through a 75-µm sieve (#200 U.S. mesh); and near-infrared reflectance spectroscopy measured from ground samples (AACC Approved Method 39-70A). The NIR method primarily responds to light scattering caused by different particle sizes of the ground sample. Soft wheat will have more particles at <41 µm and a peak in the particle-size distribution (PSD) at ≈25 µm (Harland 1994). An advantage that the SKCS has over other kernel texture methods is that it utilizes measurements from each kernel analyzed and reports the mean and variance of all measurements from a sample. This is useful for understanding the range of properties within a sample and determining whether a sample might contain a mixture of soft and hard classes of wheat.

Wheat hardness is an important indicator of milling and end use quality (reviewed by Morris and Rose 1996). Ohm et al (1998) reported a negative correlation between milling score and SKCS hardness index (HI) standard deviation, indicating that uniform kernel hardness improves milling performance. Hardness is also correlated with bread loaf volume (Ohm et al 1998) and dough viscosity (Moss 1980; Oda et al 1980). Oda et al (1980) also showed a negative correlation between noodle quality and HI. The genetic basis of the soft and hard wheat classes is well resolved at the genetic level and involves the expression of the puroindoline genes (Morris 2002). As such, all soft wheats are genetically the same and carry the *Pina-D1a/Pinb-D1a* haplotype. Genes at other loci confer additional kernel texture variation. Hard wheats, on the other hand, may carry different mutations in either puroindoline a or b, and these different mutations confer smaller differences in kernel hardness (Morris 2002).

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SKCS hardness index calibration was developed using 10 samples of wheat designated by the Federal Grain Inspection Service (FGIS) as wheat hardness reference samples (WHRS). These samples comprise three pure cultivars of hard red winter, two pure cultivars of hard red spring, two pure cultivars of soft red winter, and three pure cultivars of soft white wheat (Morris and Massa 2003). The SKCS is calibrated to give a 300 kernel mean hardness value of 75 (dimensionless) for the five hard samples, and 25 for the five soft samples (Martin et al 1993). The National Institute of Standards and Technology (NIST) maintains a supply of these samples for future calibrations. As the supply diminishes, the stock is replenished with new samples and the calibration target values of 75 and 25 are adjusted based on estimates of hardness based on NIR methods (NIST 2004). Thus, single kernel hardness is based on a calibration obtained from bulk samples. Furthermore, calibration is based on wheat samples that eventually change as supplies are exhausted. There remains a need for a single kernel calibration of hardness that can be performed on any representative sample at any time and that will yield repeatable results.

As mentioned earlier, the other two AACC Approved Methods for kernel hardness are based on PSD after some form of grinding the samples. Hardness is likely an indication of the adhesion strength between the starch granules with surrounding protein (Simmonds et al 1973). Barlow et al (1973), Mattern (1988), and Moss et al (1980) used microscopy to explain differences in the resulting PSD of hard and soft wheat during milling. Hard wheat kernels fracture along cell walls during milling, leading to larger particles. In contrast, soft wheat kernels have intercellular voids (Glenn and Saunders 1990), leading to more random fracture patterns, which releases smaller starch granules. This leads to different flour PSD between hard and soft classes as noted by Harland (1994).

Several techniques can be used to determine PSD including laser light scattering, microscopy, sieving, sedimentation analysis, permeability of a powder column, and the electrical-sensing zone technique. The different techniques measure different parameters and each has its advantages and disadvantages; therefore the choice of technique will depend largely on the application. Laser diffraction particle sizing (LDS) reduces the analysis time to minutes per sample with results tabulated into volume, number, and surface area percentage. Algorithms employed by LDS systems are based on the Mie theory, which predicts the angular scattering intensity of a smooth, internally homogeneous sphere of known refractive index, illuminated by light of a given wavelength and polarization. Approved Method 55-40 (AACC International 2000) makes use of this LDS methodology to quickly analyze flour particles suspended in isopropyl alcohol.

Grain inspectors have observed that in the U.S. Pacific Northwest (PNW) region, discriminating soft white wheat from hard white wheat has become increasingly difficult; that is, PNW hard white cultivars frequently resemble soft white cultivars and vice versa. This is partially due to a lessening of the morphological differences between these classes of wheat. The confounding of morphological lines is a result of breeding programs that exploit crosses of soft wheat by hard wheat into the progeny. While this is an effective approach from a plant breeder's perspective, it makes visual classification more difficult for grain inspectors.

Most market classes of wheat have cultivars that contain both hard and soft wheat in their lineage, and soft white cultivars are no exception. Some of these soft cultivars are sold into traditional soft wheat markets; however, SKCS hardness indices are higher than those expected from older white wheat cultivars and soft red winter classes (Engle and Morris 2005). Compounding the problem are indications that the SKCS is not as effective at discriminating soft white wheat from hard white wheat (at an acceptable accuracy) as it is in discriminating soft red wheat from hard red wheat. As a result, it has become difficult to determine, using the Perten SKCS 4100, whether a sample contains pure soft white wheat, pure hard white wheat, or a mixture of the two. Hard white cultivars from the southern Great Plains appear different enough from their PNW counterparts, and discerning them from soft white cultivars is much less problematic.

This study had two main objectives: 1) to develop a method of calibrating the Perten SKCS 4100 based on single-kernel PSD; and 2) to determine whether wheat cultivars with intermediate hardness values have a PSD that follows genetic class. If this were the case, it would be desirable to analyze the low level SKCS data so that kernels could be more accurately classified into genetic classes.

MATERIALS AND METHODS

Briefly, single kernels were processed in a Perten SKCS 4100 set so that the raw crush profiles and all low-level data were saved for each kernel. The crushed material exiting the SKCS was caught and milled further in a single pair of mill rolls from a Quardramat Jr. mill. Next, the particle-size distribution (PSD) of the milled material from each kernel was determined by a laser diffraction particle-size analyzer. Finally, the SKCS low-level parameters and raw crush profiles were analyzed to develop a calibration to predict an attribute of the PSD that described most of the variance of each PSD. The predicted PSD attribute and SKCS data were then used to classify kernels into the respective genetic hardness classes.

TABLE I
Source of Samples, SKCS 4100 Hardness Index (HI) Classification, and Specific Class

Source	HI	Classification	Specific Class
Hard cultivars			
Arapahoe (NIST)	57	Hard	HRW
Blanca Grande (WWQL)	54	Hard	HWS
Blanca Grande (USW)	68	Hard	HWS
Hard Alpowa (WWQL)	59	Mixed	HRW
ID377S (WWQL)	78	Hard	HWS
Klasic (WWQL)	53	Hard	HWS
Len (NIST)	78	Hard	HRS
Newton (NIST)	59	Hard	HRW
Platte (USW)	70	Hard	HWW
Tam 105 (NIST)	71	Hard	HRW
Trego (USW)	67	Mixed	HWW
Yecora Rojo (NIST)	59	Hard	HRS
Soft cultivars			
Alpowa (USW)	34	Soft	SWS
Brundage (USW #1)	34	Soft	SWW
Brundage (USW #2)	22	Soft	SWW
Cardinal (NIST)	12	Soft	SRW
Eltan (USW)	31	Soft	SWW
Madsen (WWQL)	59	Hard	SWW
Madsen (NIST)	33	Soft	SWW
Madsen (USW #1)	44	Mixed	SWW
Madsen (USW #2)	27	Mixed	SWW
Malcolm (NIST)	24	Soft	SWW
Titan (NIST)	15	Soft	SRW
Tres (NIST) (club)	27	Soft	Club

Single Kernel Wheat Samples

Wheat samples representing a wide range of hardness values were obtained. The 10 wheat hardness reference cultivars of the U.S. National Institute of Standards and Technology (NIST), which represent four hard and four soft wheat classes, were included. The four hard classes were hard red spring (HRS), hard red winter (HRW), hard white spring (HWS), and hard white winter (HWW). The four soft classes were soft red winter (SRW), soft white spring (SWS), soft white winter (SWW), and club. In addition to the 10 NIST samples, a total of 14 additional samples were obtained from the Western Wheat Quality Lab (WWQL) in Pullman WA, Washington State Crop Improvement Association (WSCIA) in Pullman WA, and the U.S. Wheat Associates (USW) office in Portland, OR. Table I summarizes the list of wheat samples, source of samples, and the resulting SKCS 4100 wheat hardness classification. Based on the SKCS 4100 HI results, there were nine soft, four mixed, and 11 hard wheat samples included in this sample set. Twenty kernels from each sample were randomly selected for grinding and for PSD analysis.

Four samples of Madsen, a soft white winter wheat cultivar, were included in the sample set as this cultivar is frequently classed as "mixed" or "hard" by the SKCS 4100. As shown in Table I, the average HI range was 27–59 and different samples were classified as hard, mixed, and soft. Because these four samples, supposedly of the same cultivar, had a large range of HI, gliadins from the Madsen samples were extracted and analyzed by capillary electrophoresis (CE) as described in Bean and Lookhart (2000) to ensure that they all belonged to the same cultivar.

Instrumentation

Individual kernel crushing. The SKCS 4100 was modified to enable collection of the kernel material after crushing each kernel. These modifications included removing the front cover, removing the two bolts that fasten the singulator-weighing-crushing unit to the SKCS 4100, and raising this unit ≈3 cm to facilitate collection of the crushed material. A container was placed directly below the crushing mechanism to collect the crushed material.

The crushing procedure involved six steps: 1) weighing a single kernel with a precision balance to the nearest 1/10 mg (40SM-200A, Precisa balances, Switzerland); 2) dropping the single kernel into the singulator and allowing the kernel to be automatically picked up and dropped in the SKCS weigher bucket and crushed in the normal operating manner of the machine; 3) collecting crushed material as it falls into a container placed underneath the SKCS crushing wheel; 4) lightly brushing the area where the crushed kernel exits the SKCS to recover as many residual crushed particles as are left in the instrument; 5) weighing collected crushed material to determine the percentage of each kernel recovered after SKCS crushing; kernels and associated data with <90% recovery or >100% recovery were not used for further analysis; 6) the SKCS 4100 instrument was cleaned thoroughly with compressed air between samples. This procedure was tested on five kernels from three independent samples with an average hardness of 0.1, 43.6, and 69.1. The average kernel recovery range was 97–98% with standard deviations of recovery of 1–3.1%. Soft kernels were slightly more prone to lower recoveries; more soft kernels did not achieve 90% recovery (data not shown).

Crushed kernel milling. A hand-cranked mill was fabricated (Fig. 1) that simulates the last two rolls (R10 and R12 at a 0.003 in. [0.076 mm] roll gap) of the Quadrumat Jr. mill. These rollers were selected so that these procedures could be duplicated on similar mills. The rolls, bearings, and shafts were removed from the mill and placed in an aluminum block fabricated to hold the bearings. The gap between the rolls was adjusted by rotating the eccentric bearings. On the opposite side of the rolls, the two shafts were coupled with straight spur gears, one with 32 teeth and the other with 80 teeth (TS2032-20 and TS2080-20, Browning, Maysville, KY). These gears provided a mill roll speed ratio of 2.5:1 between two rolls. This ratio was chosen to create shearing as the material was milled, as is typical in most milling operations. A hand-crank was attached to the shaft with the 32 tooth gear. The crank was used to power the rolls at a rate of 55–65 rpm. For each kernel, the crank was completely rotated five revolutions.

The crushed material from the SKCS 4100 was placed on the rolls and manually cranked at ≈60 rpm to grind the material in the mill. A brush was used to collect all materials that adhered to the rollers and mill surfaces onto a collection container below the rolls. The ground sample was weighed and then transferred to a microcentrifuge tube that was kept sealed before measurement of PSD. The mill was thoroughly cleaned with compressed air in between each sample.

Particle-size measurement of ground single kernels. A laser particle counter (LS 13-320, Beckman/Coulter, Miami, FL) was used for measuring the PSD of each ground single kernel. A modified Approved Method 55-40 (AACC International 2000) in a universal liquid module with an integrated sonicator were used for all analyses.

Before initial analysis, the system was aligned (then realigned every hour) and background analysis completed. Obscuration levels for the single kernel samples were ≈5–6% and was somewhat lower than the optimum 8% for the universal liquid module.

The ground sample was placed in a microcentrifuge tube filled with isopropyl alcohol. Three standard operating methods (macros) were written to analyze each sample with a resultant 20 PSD profiles collected for each sample: 1) after the sample was placed into the instrument, five separate analyses were performed, each for a duration of 5 sec; 2) repeat analysis on the same sample with the addition of sonication at the maximum output level of 8 to break up any clumps of the sample material; and 3) repeat of 10 more analyses, each with a 5-sec duration, on the sample after sonication. Preliminary experiments indicated that the last PSD reading, the 10th after sonication, was the most repeatable and followed what is expected from very soft and hard kernels. This was the only PSD used for calibrations with SKCS parameters.

Data analysis. The PSD >373 μm was quite variable and no trend between the wheat class, hardness, and the PSD for particles >373 μm was observed. Particles >373 μm are likely to be removed by sieving during commercial milling operations anyway. As such, the PSD >373 μm was removed and the percentages of the PSD were recomputed using the sum of all the PSD percentages <373 μm. There was also an unexplained artifact shown as an upward slope in the cumulative PSD at 2–5 μm in the PSD of ≈10% of the kernels. This occurred on both hard and soft kernels. Thus, data in the PSD at 2–5 μm were not used for computing the cumulative PSD. The cumulative PSD of 5–40 μm were transformed into principal components so that the entire peak at 25 μm would be included. The first principal component (with the highest eigenvalue) explained 99% of all the variance in the cumulative PSD. Stepwise regression was used to select one single value in the cumulative PSD with the highest correlation to the first principal component. The selected feature was the cumulative percentage of particles >21 μm ($CPSD_{21}$). This PSD value explains >99% of the variance of the principal component. Given that $CPSD_{21}$ is more intuitive and simpler to use than the principal component, this was the PSD feature chosen as the new reference for single kernel hardness.

Crush force histograms, discrete Fourier transforms of the raw crush force profile, normalized crush profiles, spectrograms of the crush profile, and crush profile models using the summation of three Gaussians were used to build regression equations to estimate the cumulative PSD at 21 μm. Fourier transforms, histograms, spectrograms, and Gaussian modeling did not improve the calibrations over what could be accomplished with the SKCS low level parameters and normalized crush profiles (data not shown). Thus, only the low level parameters that the SKCS already generated and normalized crush force profiles were used.

The raw crush profiles were normalized so that they all had the same length (128 data points) and were divided by the dry weight of the kernel. This normalized the higher forces required to crush larger kernels and adjusted for the softening effect of higher moisture contents.

The particle-size estimate developed by Williams et al (1998) was applied to this data and the estimate was used as a potential feature. This particle-size estimate used SKCS parameters to estimate the particle-size index of whole wheat flour passed through a 75-μm sieve. The SKCS parameters used for this estimate were: 1) *peak force* of the raw crush profile; 2) XCON (logarithm of the ratio of force and conductance computed at the time of maximum force); 3) ln(*temperature*), (temperature is taken of the crescent by a thermocouple); 4) *gompB*^{9} (a modeling parameter related to the shape of the histogram of crush profile slope values); 5) (kernel weight)^2; 6) *Dy* fraction 25–30 (area under crush profile slope histogram 25–30); 7) (crush area × XCON)$^{1/2}$ where crush area is the area under the raw crush profile.

In addition, all SKCS low level data were used (Martin et al 1993) including logarithms, square roots, squared values, and some cross products of weight, moisture, peak force, conductance, and crush area, as well as crush profile slope histogram fractions (Dy fractions).

Stepwise regression was used to select a small subset of features best able to estimate $CPSD_{21}$. Variable selection and regression model calibrations were performed on a training set that consisted of a random selection of half the data. The regression model was tested on a validation set that contained the other half of the data. The selection method was based on adding or deleting variables from the model that resulted in the largest reduction of mean square error of prediction (MSE). After prediction of $CPSD_{21}$, this feature and other SKCS features were used to develop classification models to discriminate genetically hard kernels from soft kernels. Stepwise Discriminant analysis was used as the feature selection and classification method.

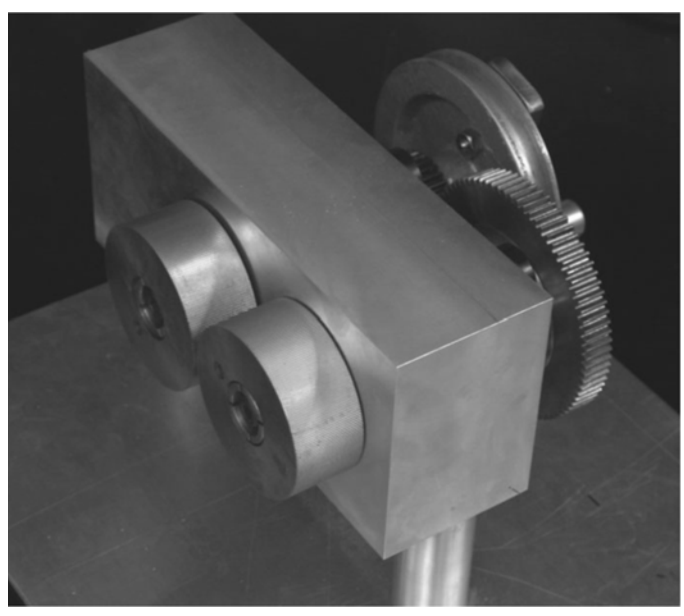

Fig. 1. Hand-cranked mill used to further process kernels after crushing in the SKCS 4100 system.

RESULTS AND DISCUSSION

Average PSD and average cumulative PSD for each wheat class are shown in Figs. 2 and 3, respectively. These have the characteristics of hard and soft wheat as reported by Harland (1994). The soft kernels generally have a higher percentage of small particles <10 μm and a peak that was mostly absent on hard kernels centered at ≈25 μm.

Figure 4 is a scatterplot of $CPSD_{21}$ against the SKCS HI. As expected, genetically soft kernels have larger $CPSD_{21}$ values. There are many genetically hard and soft kernels with hardness values in the 40–60 range that overlap in hardness. Using HI alone, kernels from these two classes would be inseparable. However, the soft and hard classes can be separated on the basis of $CPSD_{21}$ value. This indicates that, even though the compression forces required to crush these kernels may be similar, as indicated by HI, the genetically hard and soft kernel fragments are different, as indicated by $CPSD_{21}$.

The $CPSD_{21}$ measurement does differ considerably from the 75-μm sieve, which is used in Approved Method 55-30 (AACC International 2000) for determining wheat hardness. However, cumulative PSD values at 75 μm do not discriminate genetic classes nearly as well as $CPSD_{21}$ does. Using the cumulative distribution at 75 μm to classify kernels into genetic classes, a classification error rate of 15.6% was achieved (data not shown). In contrast, using $CPSD_{21}$ to classify kernels achieved an error rate of 9.3%. As can be seen from Fig. 2, hard kernels begin to have more particles than soft kernels above ≈45 μm. If cumulative PSD values >45 μm are used, then the discrimination between the hard

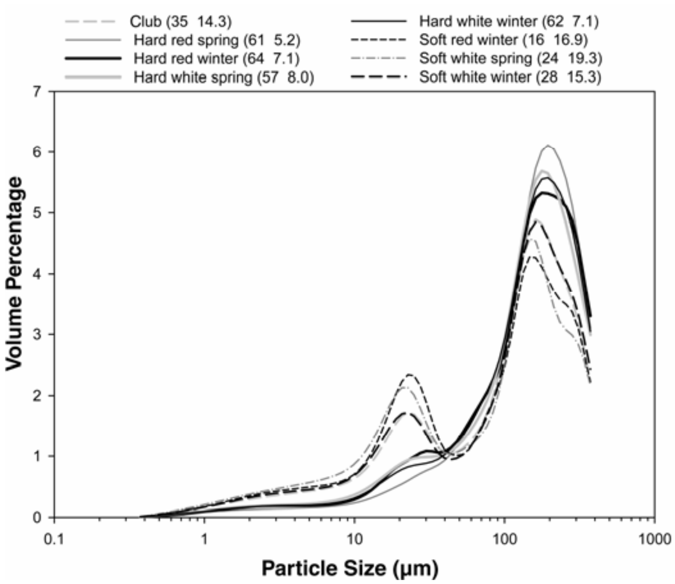

Fig. 2. Average particle-size distribution for each wheat class. Average hardness index and $CPSD_{21}$ values are listed after each class in the legend. Note that the soft classes have a pronounced peak at ≈25 μm, whereas the hard classes have only a small or no peak in this area. Also note that the soft classes have more particles >10 μm.

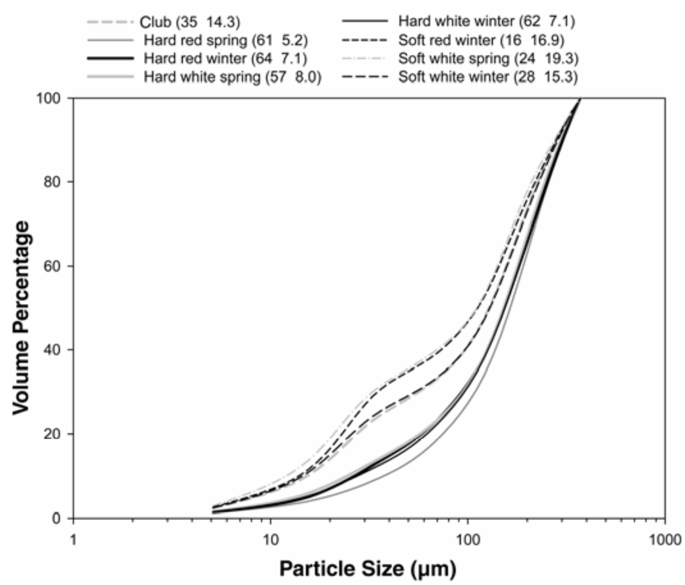

Fig. 3. Average cumulative particle-size distribution for each wheat class. Average hardness index and $CPSD_{21}$ values are listed after each class in the legend.

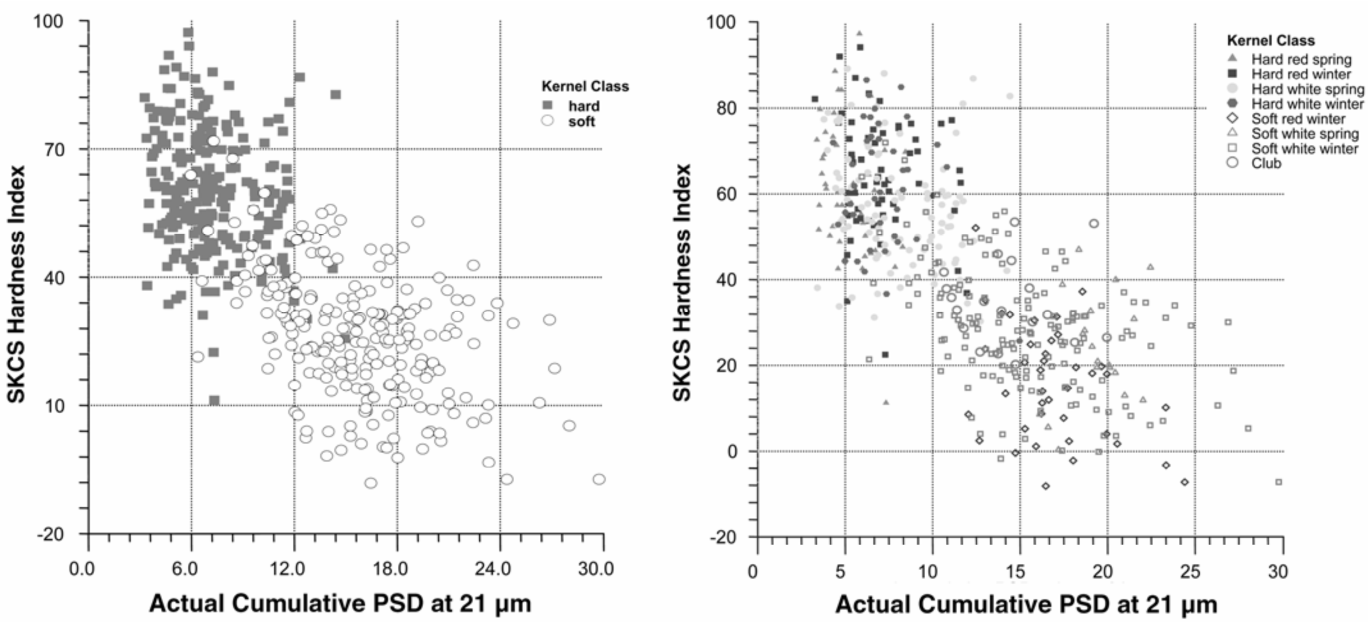

Fig. 4. Scatter plot of SKCS hardness and actual cumulative PSD value at 21 μm. Note that the soft kernels have larger $CPSD_{21}$ values, this trend holds even at hardness values of 40–60, where there are data points from both hardness classes.

TABLE II
Mean Moisture, Weight, and SKCS Hardness Index for All Cultivars Studied

Sample Description[a]	Moisture		Weight		SKCS HI	
	Mean	SD	Mean	SD	Mean	SD
Hard cultivars						
Arapahoe (NIST)	9.2	0.9	46.0	5.3	57.2	13.8
Blanca Grande (WWQL)	8.9	1.4	42.0	6.1	50.4	11.5
Blanca Grande (USW)	9.2	1.3	47.5	7.9	60.2	14.9
Hard Alpowa (WWQL)	9.6	1.0	50.1	5.2	56.1	8.9
ID377S (WWQL)	9.2	1.3	46.1	4.0	68.4	14.0
Klasic (WWQL)	9.9	1.4	47.7	6.1	51.1	10.6
Len (NIST)	10.2	1.1	39.2	3.9	73.5	10.4
Newton (NIST)	10.0	1.0	35.6	4.3	66.0	13.4
Platte (USW)	9.6	1.0	36.3	4.8	62.6	14.3
Tam 105 (NIST)	9.3	1.5	33.3	3.8	69.8	10.6
Trego (USW)	9.6	0.9	37.4	6.1	61.3	14.8
Yecora Rojo (NIST)	9.7	0.7	57.8	6.7	49.4	11.1
Average	9.5	1.1	43.3	5.4	60.5	12.4
Soft cultivars						
Alpowa (USW)	9.9	1.1	33.6	4.6	24.0	12.7
Brundage (USW #1)	10.5	1.3	39.3	5.0	18.3	13.7
Brundage (USW #2)	10.6	0.9	48.3	8.3	23.7	10.1
Cardinal (NIST)	10.7	1.1	48.0	7.4	20.4	14.3
Eltan (USW)	9.7	1.7	33.2	5.5	28.6	12.0
Madsen (WWQL)	8.7	1.3	39.3	4.0	47.3	11.3
Madsen (NIST)	10.0	1.0	51.3	7.6	27.4	14.4
Madsen (USW #1)	10.1	1.4	43.9	7.2	36.6	11.1
Madsen (USW #2)	10.2	1.4	42.6	5.9	21.6	11.0
Malcolm (NIST)	10.5	0.9	56.5	7.9	22.9	7.9
Titan (NIST)	9.9	1.1	40.9	6.6	10.9	11.3
Tres (NIST) (Club)	10.9	0.9	45.4	5.6	34.7	9.7
Average	10.1	1.2	43.5	6.3	26.4	11.6

[a] USW, U.S. wheats, Portland, OR; WWQL, Western Wheat Quality Lab, Pullman, WA; WSCIA, Washington State Crop Improvement Association, Pullman, WA; NIST, National Institue of Standards, Gaithersburg, MD.

TABLE III
Classification Performance for Each Cultivar Using SKCS Hardness and by Cumulative Single-Kernel Particle-Size Distributions (PSD) at 21 μm[a]

Sample Description[b]	HI Class No. of Kernels		$CPSD_{21}$ Class No. of Kernels		$CPSD_{21}$	
	Hard	Soft	Hard	Soft	Mean	SD
Hard cultivars						
Arapahoe (NIST)	16	4	20	0	6.4	1.7
Blanca Grande (WWQL)	13	7	15	5	10.1	1.9
Blanca Grande (USW)	17	3	17	3	7.5	3.1
Hard Alpowa (WWQL)	18	2	20	0	7.6	1.5
ID377S (WWQL)	18	2	20	0	5.1	1.1
Klasic (WWQL)	14	6	17	3	9.8	1.7
Len (NIST)	20	0	20	0	4.6	1.3
Newton (NIST)	19	1	17	3	8.2	2.4
Platte (USW)	17	3	20	0	6.4	1.7
Tam 105 (NIST)	20	0	20	0	6.5	1.4
Trego (USW)	17	2	18	1	7.8	2.0
Yecora Rojo (NIST)	15	5	20	0	5.8	1.3
Average of all hard cultivars	85.4%	14.6%	93.7%	6.3%	7.1	1.8
Soft cultivars						
Alpowa (USW)	1	19	0	20	19.3	1.7
Brundage (USW #1)	0	20	0	20	19.7	4.2
Brundage (USW #2)	1	19	2	18	16.2	3.2
Cardinal (NIST)	1	18	0	19	15.4	2.1
Eltan (USW)	2	17	0	19	19.4	4.4
Madsen (WWQL)	10	10	11	9	10.9	3.2
Madsen (NIST)	3	17	8	12	12.7	2.3
Madsen (USW #1)	2	16	6	12	12.7	2.2
Madsen (USW #2)	0	19	1	18	15.9	3.6
Malcolm (NIST)	0	20	2	18	14.7	3.5
Titan (NIST)	0	20	0	20	18.2	3.0
Tres (NIST) (club)	2	17	4	15	14.3	2.7
Average of all soft cultivars	9.4%	90.6%	14.5%	85.5%	15.8	3.0

[a] Kernels classed as soft had HI < 46 or $CPSD_{21}$ > 11.5.
[b] USW, U.S. wheats, Portland, OR; WWQL, Western Wheat Quality Lab, Pullman, WA; WSCIA, Washington State Crop Improvement Association, Pullman, WA; NIST, National Institue of Standards, Gaithersburg, MD. Source of each sample is shown in parentheses.

and soft kernels will obviously be reduced. Kernels with $CPSD_{21}$ values ≤11.5 were considered hard, while kernels with greater $CPSD_{21}$ values were classed as soft. The cutoff of 11.5 was chosen because this is where the value of zero intersected the correlation line between the PSD principal component and $CPSD_{21}$. Table II compares the classification accuracies using SKCS HI and $CPSD_{21}$ for each cultivar studied.

As shown in Table III, the Madsen sample from the WWQL had the highest classification error rate when using SKCS HI. The mean of $CPSD_{21}$ for the WWQL Madsen sample was 10.9, indicating that the PSD data for at least half of these kernels was more similar to hard kernels. As shown in Table II, the moisture of the Madsen WWQL sample was substantially lower than other samples studied. While the very low moisture (such as 8.7%) could have increased the HI, it should not have changed its PSD.

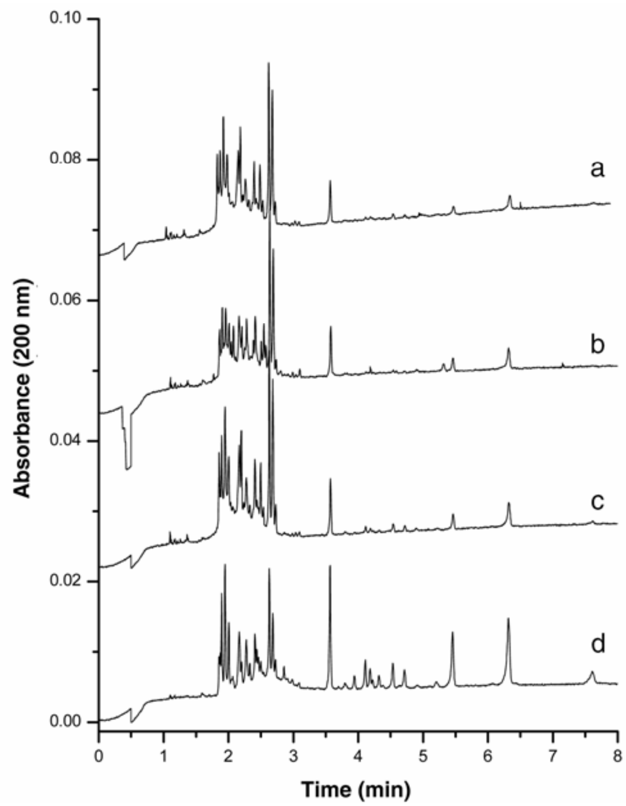

Fig. 5. Capillary electrophoresis separations of gliadins from four Madsen samples: a) USW #1, b) USW #2, c) NIST, and d) WWQL.

Analysis of gliadins from the four Madsen samples showed the WWQL sample had a different pattern than did the other Madsen samples (Fig. 5d). The most obvious difference was in the late-migrating ω-gliadins (4–5 min), but other minor differences were noted in the α/β-gliadins (1.5–3 min). Madsen USW #2 (Fig. 5b) also had slight differences in the α/β region compared with the USW #1 and NIST samples (Fig. 5a and c, respectively). Gliadin fingerprinting is an accepted technique for identifying and differentiating wheat cultivars and CE has been used to successfully differentiate wheat cultivars from all classes of wheat (Bean and Lookhart 2000). Thus, differences in the CE patterns of the Madsen samples indicated that the USW #2 and WWQL were biotypes of Madsen or were contaminated with other wheat cultivars. As a result of the CE investigation and low moisture content of the Madsen WWQL sample, data from the Madsen WWQL sample was not included in any further analysis or calibration.

Among the hard kernel cultivars, the Blanca Grande sample from WWQL had the highest classification error rate, with seven of the 20 kernels being classified as soft by HI and five classified as soft by $CPSD_{21}$. Thus, the HI is classifying these kernels based on physical properties that do not agree with genetic class. Also, the Klasic sample had six kernels classified as soft by the SKCS, while the PSD indicates that only three should be classed as soft. The NIST Yecora Rojo sample is another example where the SKCS and PSD do not match. Yecora Rojo are large kernels (Table II) and perhaps the SKCS over-compensates for large kernels when computing hardness.

Stepwise feature selection was applied to select SKCS features that were best able to estimate $CPSD_{21}$. The stepwise procedure selected eight features as listed in Table IV. Multiple regression was used with these eight features to estimate $CPSD_{21}$ from the single kernel cumulative PSD. A scatterplot of predicted and actual $CPSD_{21}$ is shown in Fig. 6. The validation set had R^2 = 0.73 and MSE = 2.98. As seen in Table IV, the independent variable $gompB^9$ was the most significant variable contributing to the regression model. This variable describes the histogram of slope values computed from the raw SKCS crush profile (Martin et al 1993). This indicates that large and small changes in crush force are important

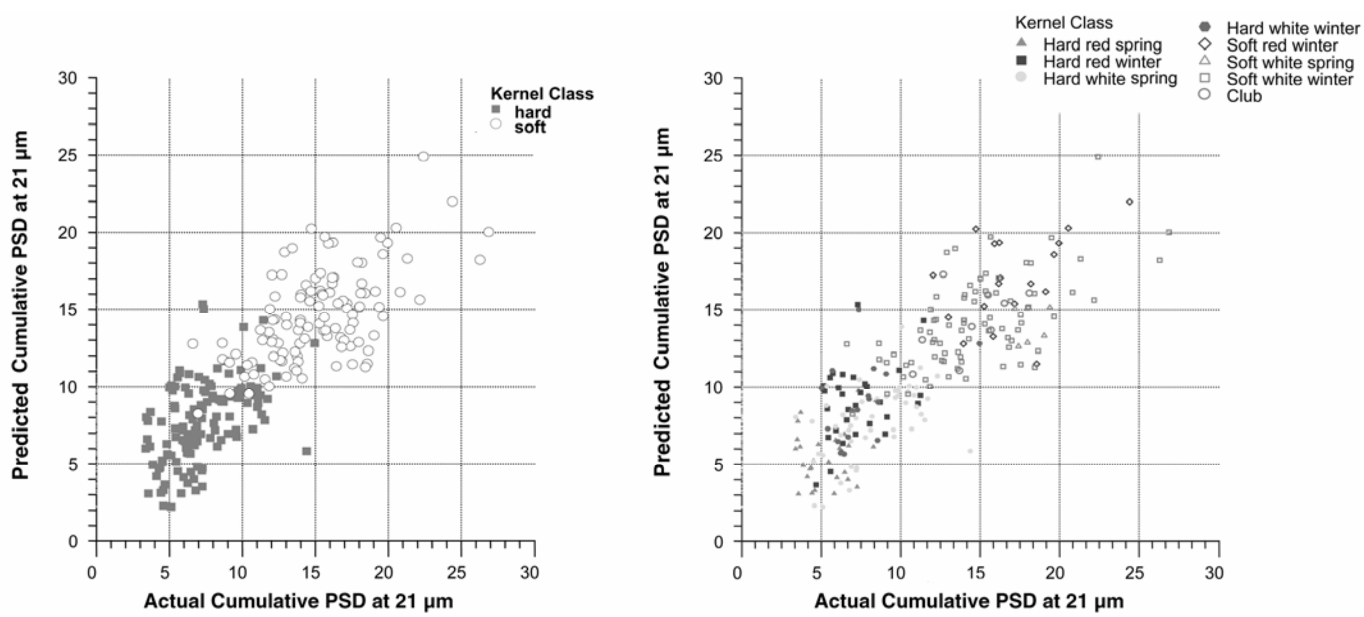

Fig. 6. Scatter plot of predicted and actual $CPSD_{21}$ values, validation set only. R^2 = 0.73 and MSE = 2.98 between actual and predicted values.

factors in predicting $CPSD_{21}$. It is well recognized that the more abrupt fracturing of the hard kernels causes an increased count of large and moderate slope values in the crush profile (Martin et al 1993).

Classification into Genetic Hardness Class by Discriminant Analysis

Discriminant analysis was used to classify kernels into genetic hardness class (soft or hard) based on various PSD and SKCS features. The stepwise selection showed that Predicted $CPSD_{21}$, weight^2, and points 32 and 75 of the normalized crush profile as the best subset of features for classifying kernels as hard or soft. As summarized in the Table V, using a combination of predicted $CPSD_{21}$ with low level SKCS features, the classification errors can be reduced by ≈50% when compared with classifications using HI alone. From Table V, it appears that predicted $CPSD_{21}$ is a slightly better feature for classifying kernels into genetic hardness classes than the PSI estimate using SKCS features (Williams et al 1998). The PSI estimate from William et al (1998) used a 75-μm sieve according to Approved Method 55-30 (AACC International 2000). However, as discussed earlier, use of the cumulative PSD at 75 μm will decrease classification accuracy, as the harder kernels begin to have more particles at >40 μm. While the cumulative PSD value at 75 μm and the PSI after passing flour through a 75-μm sieve are quite different measures, these data suggest that the 75-μm sieve may not be optimal for discriminating hard and soft wheat classes. Also, as discussed earlier, a combination of SKCS crush profile features with $CPSD_{21}$ offer the best classification accuracies than either feature type alone.

Table VI shows the classifications made for each subclass when using predicted $CPSD_{21}$, weight^2, and points 32 and 75 of the normalized crush profile, and compares these classifications when using HI alone. Comparing the classifications made using predicted PSD and raw crush profile parameters with those made with HI alone, it is evident that the four combined features increase classification accuracies compared with HI alone for club, HRS, HWS, SWS, and SWW, while there is little improvement for HRW, HWW, and SRW. Thus, even though HWW was not included in the original calibration of the SKCS, the additional methods used in this study do not greatly improve classification accuracy of HWW. However, classification accuracy for SWS and SWW are improved so that samples containing soft white and hard white classes may be better identified.

Figure 7 displays average normalized crush profiles of the different hardness classes. During crushing of a single kernel, a force is exerted until the kernel first fractures. This is displayed between points 1 and 15 of the crush profiles (Fig. 7). However, it is likely that the soft kernels break down into smaller particles during this initial kernel fracture. Between points 15 and 40, the kernel fragments undergo further crushing as the gap between rotor and crescent of the SKCS decreases; however, this requires less force than the initial fracturing. After point 40, small fragments are slowly broken down until they exit the rotor/crescent. In the region between point 40 and the rotor/crescent exit, the hard classes increase the crush force at a faster rate, leading to a higher and broader peak. This is likely caused by more moderately sized particles remaining from the hard classes. In contrast, the small particles from soft classes do not require as much crush force to break down, leading to a lower peak overall and, in particular, lower forces between points 65 and 90 that lead up to the peak, as

TABLE IV
Selected SKCS Variables for Estimating $CPSD_{21}$ in the Order Selected and Contribution to Model R^2 and MSE

Variable	Model R^2	MSE
gompB[9]	0.53	3.83
Crush point 76	0.60	3.56
ln(weight)	0.64	3.36
$(\text{Crush area})^{1/2}$	0.69	3.12
Location of peak force	0.72	3.00
Crush point 114	0.73	2.95
Crush point 86	0.74	2.91
Crush point 37	0.75	2.87

TABLE V
Comparison of Classification Accuracies Using Various SKCS and PSD Parameters

	Classification Accuracies					
	Training Set		Validation Set		Avg of Training and Validation Sets	
Independent Variables	Hard	Soft	Hard	Soft	Hard	Soft
SKCS Hardness Index (HI)	90%	90%	89%	92%	90%	91%
Actual $CPSD_{21}$	87%	93%	97%	90%	95%	89%
SKCS cumulative PSI estimate (Williams et al 1998)	88%	89%	89%	92%	88%	91%
$CPSD_{21}$	93%	86%	94%	94%	93%	90%
HI + $CPSD_{21}$	91%	93%	94%	93%	93%	92%
Predicted $CPSD_{21}$ + weight^2, and points 32 and 75 of the normalized crush profile	93%	94%	95%	97%	95%	96%
HI + actual $CPSD_{21}$	96%	95%	98%	94%	97%	95%

TABLE VI
Classifications Using Predicted $CPSD_{21}$, Weight^2, and Points 32 and 75 of the Normalized Crush Profile and Hardness Index Alone[a]

	Classification Based on Predicted PSD and SKCS Crush Profile Features		Classification Based on HI Alone		
Wheat Class	Hard	Soft	Hard	Soft	Total Kernels
Club	0%	100%	21%	79%	19
Hard red spring	98%	3%	90%	10%	40
Hard red winter	93%	7%	93%	7%	60
Hard white spring	94%	6%	86%	14%	100
Hard white winter	92%	8%	92%	8%	59
Soft red winter	3%	97%	3%	97%	39
Soft white spring	0%	100%	5%	95%	20
Soft white winter	6%	94%	10%	90%	136

[a] Results organized by class and include both the training and validation sets.

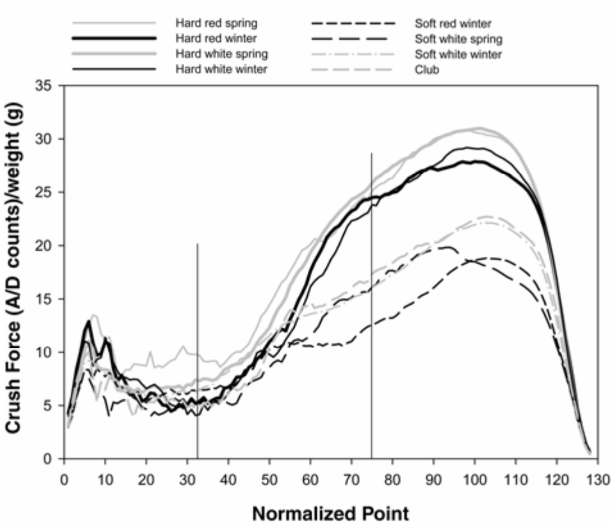

Fig. 7. Average normalized crush profiles for all wheat classes studied. Note that the hard wheat classes have higher values at the beginning of the last peak, whereas the soft classes have a lower slope leading up to the last peak. Vertical lines denote points 32 and 75 selected to better discriminate between hard and soft classes.

shown in Fig. 7. For predicting $CPSD_{21}$, points 37, 76, 86, and 114 of the crush profile were chosen by the stepwise regression procedure. Additionally, for classifying soft and hard wheat classes, points 32 and 75 of the normalized crush profiles were chosen. Points 30 through 100 lie in the portion of the crush profile where the smaller particles are crushed. This region of the crush profile may be the most significant for discriminating soft and hard classes because it is working with kernel fragments rather than the whole kernel, as is the case at the beginning of the crush profile. As the whole kernel is being compressed, it may be subject to variations due to kernel orientation and morphology. In contrast, in the middle of the crush profile leading up to the final peak, the smaller kernel pieces may be more immune to variance caused by kernel orientation and shape.

The experimentation and data presented lay the groundwork for a single kernel calibration of the Perten SKCS 4100 based on PSD rather than the current method of assigning hardness values to the NIST set. A future calibration would need to comprise a larger data set consisting of a controlled range of kernel moisture contents. Additionally, the method may be more repeatable if the mill rolls were powered by a motor rather than a hand crank to maintain more consistent roll speeds. Another source of error could be the presence of large bran material still present after milling. It is unknown what effect this has on the resulting PSD. If large bran particles remain in the PSD, the profiles will fluctuate widely with respect to the volume percent in the 1,000–2,000 µm size ranges. These bran particles vary in size and cannot be broken up with sonication in the laser diffraction instrument. This may be eliminated by sieving the material before measuring PSD by laser diffraction. It is unknown what effect large bran pieces may have on the SKCS. Another source of error that is harder to control is the loss of material in the SKCS and through milling. Samples that did not have at least 90% recovery were excluded. Approximately 5% of samples had <90% recovery and it is not known if this biased the data set. Genetically soft samples tended to have slightly lower recovery rates and more of them did not achieve 90% recovery. This was probably due to the smaller particles sticking to the SKCS and mill rolls. However, hard kernels had variable recovery rates with some <90%, probably due to kernel orientation in the SKCS as it was crushed. Abnormal orientation of hard kernels can cause shattering and a large portion of the kernel may get lodged in the machine.

CONCLUSIONS

Particle-size distributions (PSD) were measured for a total of 473 kernels of various classes and cultivars after they were run through the Perten SKCS 4100 system. It appears that the most useful information in the PSD for discriminating between hard and soft wheat classes was at 5–45 µm. The soft kernels had a strong tendency to have a higher percentage of smaller particles, particularly at <25 µm. The cumulative PSD value at 21 µm explained most of the kernel to kernel variance in the PSD.

Wheat kernels from soft and hard classes that have overlapping SKCS hardness values, have a strong tendency to have a PSD more in line with what is expected from the genetic class (Fig. 4). After milling, soft kernels tend to have more particles at <21 µm than hard kernels, even though SKCS hardness values may be similar. As such, combining HI and PSD gives better discrimination between genetically hard and soft classes than either parameter measured independently. Additionally, use of SKCS predicted PSD combined with other low level SKCS parameters appears to reduce classification errors into genetic hardness classes by ≈50%, to an overall average error rate of 4.5 versus 9.5% when HI is used alone.

It appears that hardness classification performance can be improved, overall, by 50% for this data set over the use of a simple HI by using additional data generated by the SKCS. It should be noted that a robust calibration for classification of kernels into correct hardness classes may require a more comprehensive data set than that used in this study.

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