VARIATION IN SINGLE KERNEL HARDNESS WITHIN THE WHEAT SPIKE

by

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Abstract

Variation in wheat kernel hardness is influenced by several factors including genetic expression and environmental conditions. However, these factors explain only a portion of the observed variation. Thus, there are unknown contributors to this important physical property. The following experiments investigated growing locations between farms and within the spike as a source of variation. Four commercial varieties of Hard Red Winter (HRW) wheat were chosen for evaluation; Jagger, Jagalene, Overley, and 2137. In total, 374 wheat spikes were collected from three farms participating in the Kansas State University Research and Extension- 2007 Crop Performance Tests (KSCPT). For analyses, each kernel was removed and cataloged by spikelet and floret position. A total of 10,240 kernels were uniquely identified by variety, farm, plot, spike, spikelet and floret position. Using the single kernel characterization system (SKCS), kernels were crushed to determine the hardness, diameter, weight, and moisture content. The variability of each measured attribute was greatest between spikes of a given variety. Measured attributes exist in gradients along the spike, with the top and bottom portions being most variable. This research broadens our knowledge of wheat kernel variation, and results from this experiment may contribute to improved methods for single kernel analysis.

Contents

List of Figures

List of Tables

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To my parents Tarry and Linda, I owe a debt of gratitude for teaching me that anything is possible through hard work and perseverance. To my brothers I owe years of support and friendship, thanks for helping me keep it in perspective.

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Chapter 1

Introduction

Kernel hardness is an important measurable attribute of wheat that has been correlated to it's chemical and genetic make-up. The evaluation of wheat kernel hardness has been used in predictions of flour yield and gives early indication of baking performance (Pomeranz and Williams, 1990). Factors influencing kernel hardness include variety and environment, however the total variation in hardness has yet to be explained (Morris et al., 2005). Understanding the variation in hardness will contribute to advancements in uniformity for hardness, which can improve the accuracy of predictions made from kernel hardness. To understand the basis of kernel hardness we begin by describing early kernel development.

Wheat kernel development

It was shown in a study done by Ries and Everson (1973), that characteristics of the planted seed are passed on to offspring. In the study, seeds were separated by size and protein content before being planted. The results show that larger seeds with higher protein content led to more vigorous offspring, measured by growth rate and kernel yield. The study concluded that large seeds were more abundant in assimilates required for early growth, leading the researchers to believe that the increased assimilate, not genetic effects, were responsible for the observed improvements. This study shows that certain attributes of a developing wheat plant are influenced by the previous generation.

In winter wheat varieties seeds must go through a period of freezing called vernalization which triggers the growth response and influences early development. With a successful vernalization period the seed will begin to develop as day length and temperature increase (Curtis et al., 2002). After sprouting, individual structures develop from cells called meristem, shown in figure 1.1.

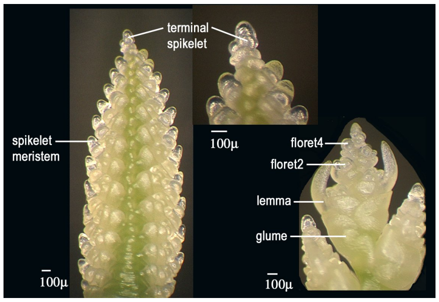

Figure 1.1: Wheat spike in early development (Wheat, 2007)

The development of spike structures and the sequence in which they appear is described by Kirby (1974). Two important structures, the spikelet and floret, make up the adult wheat spike. The spikelet, which develops first, is a grouping of florets attached to the rachis at a single point. The mature spikelet will contain 3-4 fertile florets, each containing the reproductive organs necessary for kernel development. The mature wheat spike will contain 15-18 spikelets attached on alternating sides of the rachis, which can be seen in figure 1.2.

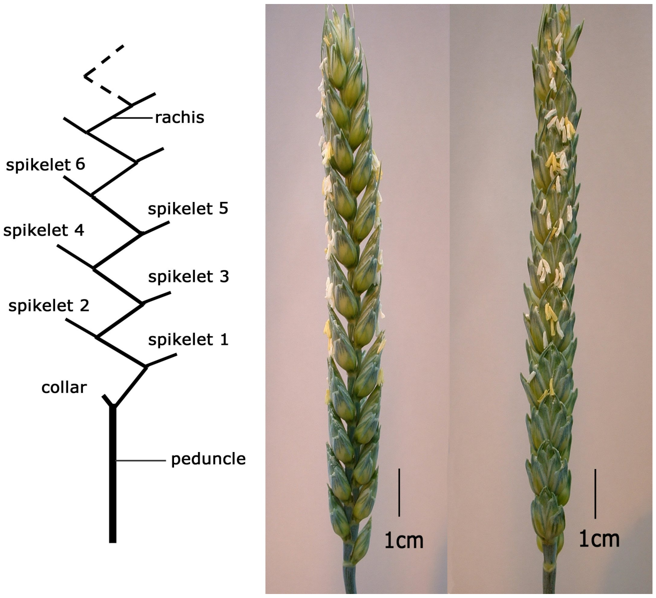

Figure 1.2: Diagram of the Wheat Spike (Wheat, 2007)

In early stages of development spikelets appear along the spike in sequence, with spikelets in the center portion appearing first. Each additional spikelet forms successively closer to the top and bottom of the spike. This pattern described by Kirby (1974), is one that will be repeated throughout various phases of development. New spikelets continue to develop along the spike until the appearance of the terminal spikelet, at which time no additional spikelets emerge. The terminal spikelet stage is important, as it influences the potential kernels that a spike may contain. The time required to reach the terminal spikelet stage is dependant on day length and temperature (Rawson, 1970), meaning more spikelets, and potentially more kernels will develop from seeds that sprout early in the season. Spike development up to this point has taken place underground, and during the next phase, the spike will emerge from the soil.

As the stem elongates the structures of the wheat spike continue to develop, and eventually the reproductive organs within the floret reach maturity. During this 5-10 day period called anthesis, pollination occurs and early kernel development begins. As with previous phases of development the time at which a floret reaches anthesis follows a sequence, beginning with the central spikelets (Rawson and Evans, 1970).

Within the spikelet the bottom most floret labeled floret 1 in figure 1.3, will reach anthesis first followed by the second and then third. In most spikelets three to four florets will develop kernels, however as many as five or more may be fertile. These fertile florets remain fertile until the lower grains in the spikelet begin to develop, at which time the remaining florets will die. This process described by Rawson and Evans (1970), appears to be a survival mechanism, for when a lower floret fails to set grain the more apical florets will compensate with later kernel development.

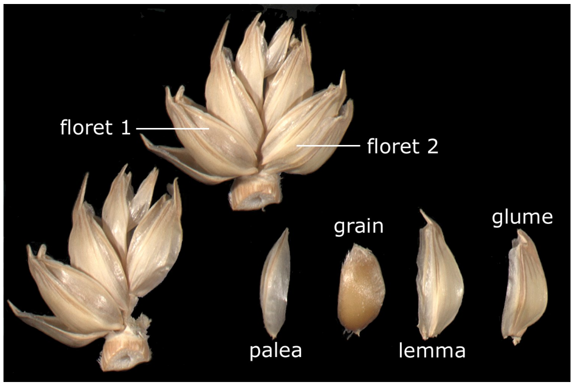

Figure 1.3: Spikelet structure showing florets. (Wheat, 2007)

After the basal floret in the central spikelet reaches anthesis the sequence of spikelets reaching anthesis proceeds towards the terminal spikelet at the tip of the spike. Once the terminal spikelet reaches anthesis floret 1 of spikelet 7 will reach anthesis and progression continues towards the base (Evans et al., 1972). The time from anthesis is important to wheat quality, because plant death and dessication occur simultaneously for all kernels. Meaning basal kernels in the central spikelets have a longer period for kernel filling and protein synthesis than apical spikelets. Studies of spike development included research investigating growth rate in the spike. In this research Rawson and Evans (1970), reported that second floret kernels in all spikelets exhibited the highest growth rate of any position. Investigating differences at different positions in the spike has been an approach taken by several scientists.

Studies measuring positional effects

In early work done by Ali et al. (1969), researchers analyzed kernels at various locations along the wheat spike. After dividing the spike into three equal sections they found the lowest third of the spike contained the highest protein content while decreasing towards the top. Results of this study were recently confirmed by Bramble et al. (2002), who investigated variance components of protein content in wheat. In this work, Bramble analyzed the top middle and bottom portion of the spike using Single Kernel Near Infrared Reflectance (SKNIR). With the use of this advanced technology, samples could be analyzed efficiently, and the study was able to included multiple farms and varieties. Results of this work confirm significant differences for protein content between top, middle, and bottom portions of the spike, as well as between farms and varieties. Although these studies investigated the spike after being divided into thirds, other work has shown gradients at the single kernel level as well.

During their growth rate experiments Rawson and Evans (1970), reported that kernels from the second floret to be larger in diameter and heavier than other kernels of the same spikelet. This finding was confirmed by Kirby (1974), who added that apical spikelets had significantly lower kernel weight than other kernels in the spike. In an effort to further explain differences by kernel position

Calderini and Ortiz-Monasterio (2003), investigated nutrient supply to individual kernels throughout the spike. In this study they found that concentrations of Mg, Ca, K, P, and S decreased in kernels of the 3rd and 4th florets. This along with previous research provides evidence that gradients exist for various measurable attributes. Studies have yet to detail the relation between kernel position and hardness however inferences can be made from past research.

Wheat kernel attributes related to their hardness

In a study done by Gwirtz et al. (1996), several pure varieties of wheat were separated by kernel size into small, medium, and large fractions. it was found that protein content, as a percent of kernel weight, was highest for small kernels and lowest for the large fraction . In this experiment pearling value was obtained as a test of hardness, and the results show the smallest kernels having the highest pearling value and the largest having the lowest pearling value. In a separate study investigating protein content and milling yield, Gwirtz (1998) found that protein content had a direct positive correlation to SKCS and NIR hardness values. The studies done by Gwirtz show that kernel size and it's chemical components have an effect on kernel hardness, however other contributors have been found to exist.

Environmental factors related to kernel hardness were discussed by Pomeranz et al. (1985), in a study that compared hardness values for several varieties grown at locations around the world. The findings from this experiment show NIR hardness to be positively correlated to protein content in wheat. However, time to grind, particle size index and abrasion reveal only a weak negative correlation. Results from this experiment suggest that the environment may have less impact on variation of hardness than the variety alone. It also maintains that the protein content within a single variety is more telling of hardness, than protein content in general.

The genetic basis of kernel hardness was described by Morris (2002), in a review of puroindoline protein. In this review Morris details that soft wheat varieties carry genetic coding for two proteins related to hardness, puroindoline-a and puroindoline-b. It is believed that these proteins interact with starch granules in a way that imparts softness to kernel texture (Swan et al., 2006). In hard wheat varieties mutation in the genetic code for puroindoline protein disrupts it's synthesis. Several different mutations have been found, which result in either the absence of puroindoline-a or mutation of puroindoline-b. It seems as though protein synthesis within a kernel effects hardness from an early point in development. Research done by Turnbull et al. (2003), found grain hardness could be determined as early as five days post anthesis. Moreover, hard and soft varieties could be distinguished by kernel hardness from this point to maturity. In this experiment kernel hardness was measured using the Single Kernel Characterization System (SKCS), however other methods have been employed to determine kernel hardness.

Methods of determining kernel hardness

Methods approved by the American Association of Cereal Chemists (AACC), have proved reliable in determining kernel hardness. Two methods, Near Infrared Reflectance (NIR) and Particle Size index (PSI) involve grinding the wheat sample. NIR Hardness, Approved Method 39-70A (AACC, 2000a), uses the principle of light scattering to determine a hardness score.

Ground particles from soft wheat tend to be smaller than those from hard wheat, which scatter light in a unique and detectable way. In the PSI test, Approved Method 55-30 (AACC, 2000b), a ground sample is passed through a 75μ sieve. The hardness score is denoted by the percentage of material passing through the sieve. This method uses similar logic as the NIR method, in that ground particles from soft wheat tend to be smaller than those of hard wheat, which is detected as an increased percentage through the sieve. Results from both grinding methods are cumulative of the sample analyzed, which confounds any single kernel information available. This limitation can be overcome by using the SKCS 4100, Approved Method 55-31 (AACC, 2000c), which collects data on individual kernels.

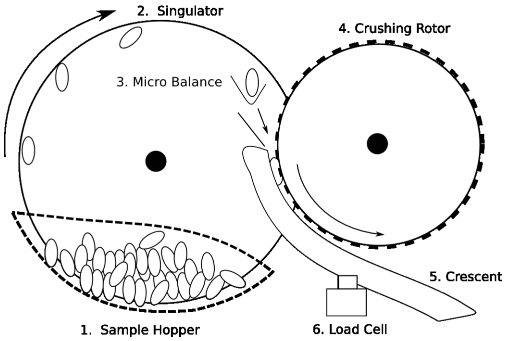

Figure 1.4: Diagram of the SKCS (Miller, 2008)

The SKCS shown in figure 1.4, generates a report of sample statistics after analyzing 300 kernels. The report includes mean and standard deviation for kernel weight, diameter, hardness index, and moisture content. After removing light impurities the wheat sample is loaded into the sample hopper shown in figure 1.4. The procedure begins when a single kernel is picked up by the singulator and placed in the micro-balance to determine weight. The micro-balance takes repeated measurements of the kernel weight before reporting a value, and discharging the kernel into the rotor and crescent. As the kernel is crushed, the crescent is deflected a small distance equal to the diameter of the kernel, recorded in millimeters (mm). Deflection of the crescent is met with resistance from a load cell which measures force required to crush the kernel. This measurement, recorded over time, is used to generate a crush profile for each kernel. The single kernel crush profile is shown in figure 1.5.

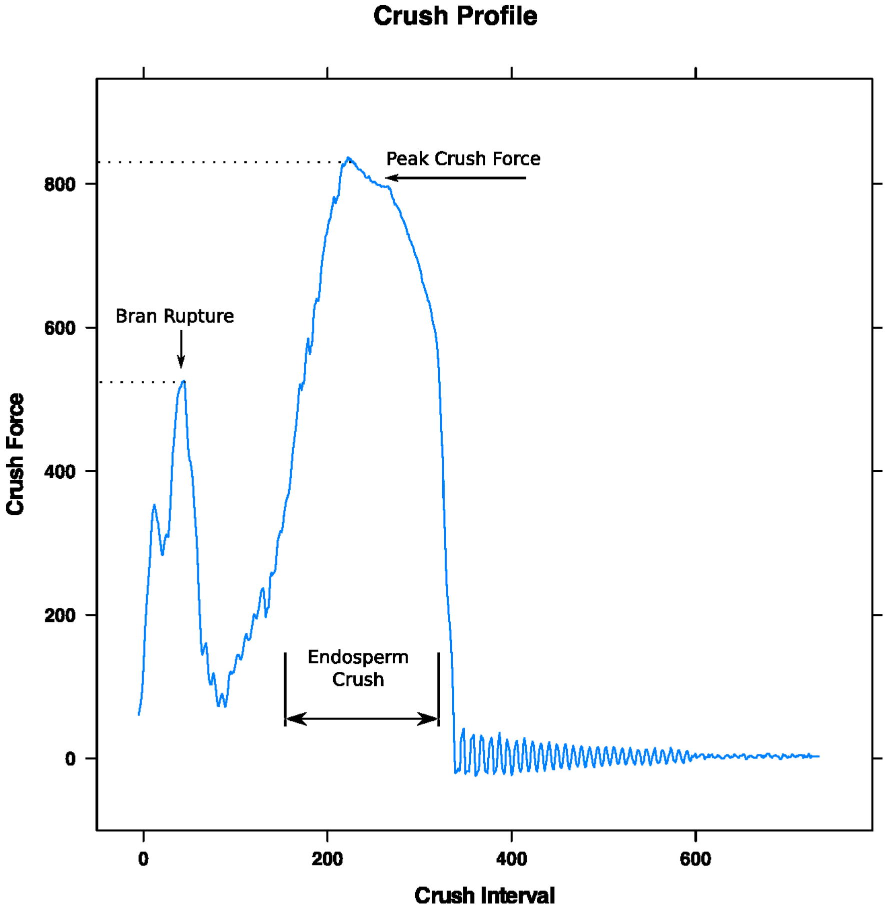

Figure 1.5: SKCS Crush profile generated from raw crush data (Miller, 2008)

At the point of peak force, moisture content (%M.C.) is determined by conductance, and the value is recorded. Each measurement, including the crush profile are used to calculate hardness index (HI). The full equation for HI is largely unpublished, however Osborne and Anderssen (2003) document several calculations in their review of the SKCS. The hardness index value is based on an arbitrary scale developed for NIR hardness. Using this scale, hard wheat will have a mean score near 75 HI, with soft wheat near 25 HI. Values are not normalized, and individual kernel values are allowed to fall continuously along the scale. SKCS hardness is well correlated with NIR hardness and PSI (Maghirang and Dowell, 2003)(Pearson et al., 2007), and is widely accepted as the standard method for measurement of single kernel hardness (Osborne and Anderssen, 2003). Historically single kernel analysis was done on small samples, due to the labor involved. However, with the development of the SKCS studies have grown larger, and researchers are beginning to investigate the natural variation in wheat kernel hardness.

Research objective

Research has shown that kernel hardness is successful in predicting flour yields, determining genetic make-up, and assessing overall wheat quality (Morris et al., 2001; Bettge and Morris, 2000; Ohm et al., 1998). It is clear that uniformity in wheat kernel hardness will strengthen these predictions and improve processes effected by kernel hardness. The typical range of hardness values are represented in the Federal Grain Inspection Service (FGIS) calibration samples for the SKCS 4100. This sample set includes wheat from both hard and soft classes and contains varieties with range of mean hardness between -6 to 80.3 HI. Each sample is verified to be pure, however standard deviations range from ±12.7 to ±20.8 HI (Maghirang and Dowell, 2003). In a commercial blend such as that found at a mill or elevator, genetic class may be the only known information about the wheat's origin.

Wheat is bought and sold as a commodity, and limited information is retained after leaving the farm. Sampling and grading are performed as wheat is purchased with no restriction to the amount of blending before that point. The effect of wheat pooling is described by Gwirtz et al. (1997), in an experiment showing that blended grain retains many of the characteristics of it's origin, increasing the variability of the resulting blend. It is not well documented how much variation in hardness results from commingling of grain. Research has shown that over 70% of the variability in hardness can be attributed to genetic effects (Morris et al., 2005), but the total variation has yet to be described.

In order to better understand the variation observed in wheat, this experiment will explain the single kernel variation in hardness attributed to varieties in the same class, as well as location of the kernel during development. This research proposes to describe the gradients of hardness observed along the length of the spike, as well as patterns of hardness within the spikelet.

Design of experiment

Studies aiming to describe variation often use hierarchical design. In a hierarchical experiment, sampling occurs at increasingly specific locations to describe the most relevant sources of variation. This type of analysis was used by (Bramble, 2001), when looking at variance components for protein content in wheat. In our study the top level of sampling (farms) will contain 3 observations, from this point we sample several varieties, which are nested within farms. In this experiment each variety was planted in replicated plots, which become a factor nested in variety and farm. Within a plot are the individual wheat plants which are collected for analysis. Wheat heads were chosen randomly within plots, which is another source of variation to consider. This factor named spike is nested in all of the preceding factors. All factors to this point are considered random factors in the experiment.

Statistically, a random factor originates from a larger population of possible factors such as varieties, farms etc. When defining a factor as random, only limited inferences can be made about the factor. However, by generalizing a factor as random, the findings can be applied to the population from which it originates. In the case of this experiment it is more important to describe variation attributed to fields, than to compare the differences between field-1 and field-2.

Four commercial varieties of Kansas hard red winter wheat (crop year 2007) and three growing locations were chosen for analysis in this experiment. Samples were collected from varieties 2137, Jagger, Jagalene, and Overley at three locations Ashland, Belleville, and Everest. These farms, and others, are maintained by Kansas State University- Research and Extension, in support of the Kansas Crop Performance Tests (KSCPT), a report published for use by farmers and growers in the state. Each location is planted in a randomized complete block design with replication of varieties in plots. The seed used in the crop performance trial meets or exceeds the standards for certified seed, ensuring genetic purity within each variety (Lingenfelser, 2007). Wheat spikes were chosen at random with no preference given to main or secondary tillers. One single spike was chosen from each plant from the inner rows of the plot, minimizing the risk of cross contamination. In our experiment we are interested about fixed kernel positions in the wheat spike. For this reason we choose the spikelet and floret position within the spike to be a fixed factor location. By doing this we can perform specific tests to determine the differences in kernel hardness at points along the spike. For this we use SAS SOFTWARE^{TM}(SAS, 2000-2004), SAS PROC MIXED. The proc mixed procedure allows for statistical analysis to be performed on models that contain both fixed and nested random effects.

Chapter 2

Materials and Methods

Wheat Selection and Quality

Of the 10,240 kernels collected, the number observed for each variety and farm were unequally distributed and a table showing the count by location can be seen in table 2.1.

	Ashland $N = 4549$	Belleville $N = 2138$	Everest $N = 3553$
variety : 2137	26% (1192)	0% (0)	27% (958)
Jagalene	23% (1051)	27% (587)	18% (644)
Jagger	27% (1240)	26% (549)	29% (1041)
Overley	23% (1066)	47% (1002)	26% (910)

Table 2.1: Sample size N, by variety and location

The number of kernels collected from a particular farm or variety reflected the overall quality of the wheat. In a general overview of quality, the wheat from Ashland had a healthy appearance and contained kernels with larger weight and diameter on average. Belleville and Everest each suffered damage due to weather and disease which were both considered in the analysis. A table showing mean values for the measured attributes can be seen in table 2.2. As noted above Belleville was missing variety 2137, resulting in fewer total kernels.

	N	Hardness (HI)	Diameter (mm)	Weight (mg)	Moisture (%MC)
Farm					
Ashland	4549	75.59	2.51	24.73	9.60
Belleville	2138	71.02	2.26	18.56	13.22
Everest	3553	77.19	2.46	23.32	10.08
Overall					
	10240	75.19	2.44	22.95	10.52

Table 2.2: Average values for measured attributes by farm.

Naming scheme

To perform a statistical analysis with growing location as a factor, each identifiable location was recorded. As wheat spikes were collected from the field each was labeled by farm, variety and plot. Each wheat spike contained approximately 35 kernels which were collected for analysis. To complete this analysis, individual kernels were removed and identified by location in the spike. The structured nature of the wheat spike forms a natural coordinate system, yielding a unique location for every kernel. The naming of kernel location followed a convention developed by Wilhelm and McMaster (1996) which is demonstrated in Figure 2.1.

Using this naming scheme, each kernel was uniquely identified using all location factors. Generically, the name of a single kernel was Farm$_X$- Variety$_X$- Plot$_X$- Spike$_X$- Spikelet$_X$- Floret$_X$.

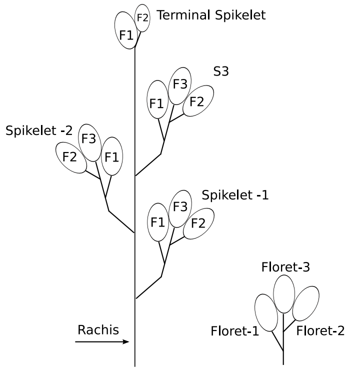

Figure 2.1: Floret naming convention

The order of collection began at the base of the head continuing towards the apex, starting with the first fertile spikelet identified (S1). Within the spikelet the first kernel removed was floret one (F1) proceeding sequentially towards floret four (F4). Tools used for kernel removal and storage can be seen in figure 2.2. After visually identifying the kernel position, the sharp end of the dissection tool was used to remove the kernel from the enclosing glume structures. After recording location, kernels were removed and stored in uniquely identified cells of a pill box also seen in figure

2.2. Cataloged kernels were processed individually using the SKCS 4100 to determine the hardness, weight, diameter and moisture content.

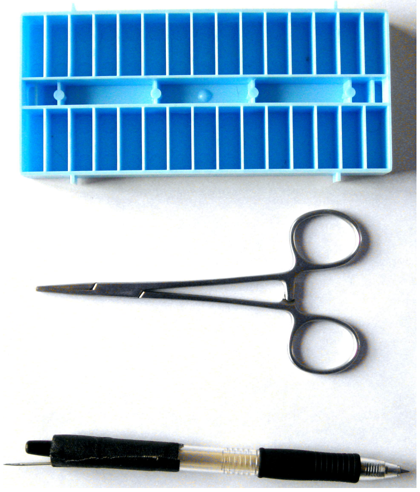

Figure 2.2: Top: Pill box, middle: Medical style forceps, Bottom: Ink pen with needle attached

Data Collection

The default parameters were SKCS modified from the standard procedure to facilitate single kernel data collection. The standard procedure generates test statistics based on a 300 kernel sample. The output summary lists mean and standard deviation values of measured attributes with additional charting of the data. In this experiment the sample size was reduced to 30 kernels per session, chosen to coincide with the number of kernels per pill box. Individual kernel data stored by the SKCS is identified by sample session (pill box) and the order that kernels are crushed (1-30). This systematic approach to crushing and naming allowed for efficient processing of individual kernels. A Visual Basic macro was written in MS Excel to align raw SKCS data with kernel data recorded during dissection.

Under default settings the SKCS will reject a kernels weighing less than 12g or having diameter less than 1mm. The goal for this experiment was to characterize the wheat spike, therefore parameters were set to allow the lowest measurable weight, diameter, and hardness values. Even so, some of the kernels collected were too thin and light to be measured, resulting in lost data. Spikes containing a large percentage of shriveled kernels were identified as SH so that the missing values due to shriveling could be linked to a particular origin.

Wheat quality characteristics were provided by the KSCPT and are outlined in table A.1 found in the Appendix A.1. This table describes the quality of the chosen varieties at locations throughout the state of Kansas. The 2007 wheat crop was generally poor, with only 32% of the planted acreage listed as good to excellent, 40% poor to very-poor, and 28% was listed in fair condition. Two of the main factors were freeze damage and wet conditions, which killed some of the early tillers, and promoted disease such as fusarium head blight (Lingenfelser, 2007).

After processing individual kernels statistical analyses were performed using location factors: farm, variety, plot, spike, spikelet and floret. During the dissection process other observations were recorded describing the condition of the wheat spike. These observations were coded as a potential source of variation. The coding for these factors became Blight (B), Some Blight (SB), normal or No Blight (NB) and shriveled (SH). Other factors were created post hoc, and included combinations of the spikelet and floret position such as top-middle-bottom (TMB).

Statistical analysis

Statistical analysis was done with SAS PROC MIXED, SAS PROC GLM SAS (2000-2004), and the statistical analysis package R©(R, 2008). The full model was reduced to a smaller set of significant factors using a stepwise procedure. The reduced model below shows the significant terms and their interactions using non-standard notation, random factors are listed within parentheses. The mixed effects model allows for variance components to be determined from the random portion of the model, while making linear predictions from the fixed effects portion.

$Hardness = spikelet + floret + blight + (farm + farm : variety + farm : variety : plot + farm : variety : plot : head + farm : variety : plot : head : spikelet)$

Kernel location within the spike, and the wheat condition were tested for statistical significance using means comparison under PROC GLM. This test uses Tukey's Honestly Significant Differences (HSD), which holds family-wise error to .05%.

Chapter 3

Results and Discussion

Variance components

The statistical measure of variance is equal to the squared deviation for error or simply standard deviation squared (σ^2). Because discussion using squared values is difficult to understand, variances have been converted to standard deviation, which is reported in units of interest. In this study the overall standard deviation for hardness was found to be ±19.24 HI, with values ranging from 5.82 HI to 141.4 HI. This value is consistent with the standard deviation in hardness found in a commercial blend (Gwirtz, 2008). Statistical analyses show that factors related to growing location explained 39.9% of the total variance. Previously published data has shown that 75-90% of hardness variation can be explained by genetic factors alone. It was suggested by Morris et al. (2005), that a phenotypic response to environment may trigger the expression of minor genes related to kernel texture. Even though it isn't proved by our experiment, statistically, the variation explained by these two studies overlap. Indicating that a portion of our observed variation in hardness may be a phenotypic response to environment. Without further testing it is difficult to indicate what portion of our model describes the phenotypic response if any. The components of variation for all attributes are given in table 3.1.

Figure 3.1 shows the distribution of each attribute by farm. It is clear that hardness values between farms are nearly equal in distribution. This observation is quantified in table of variance

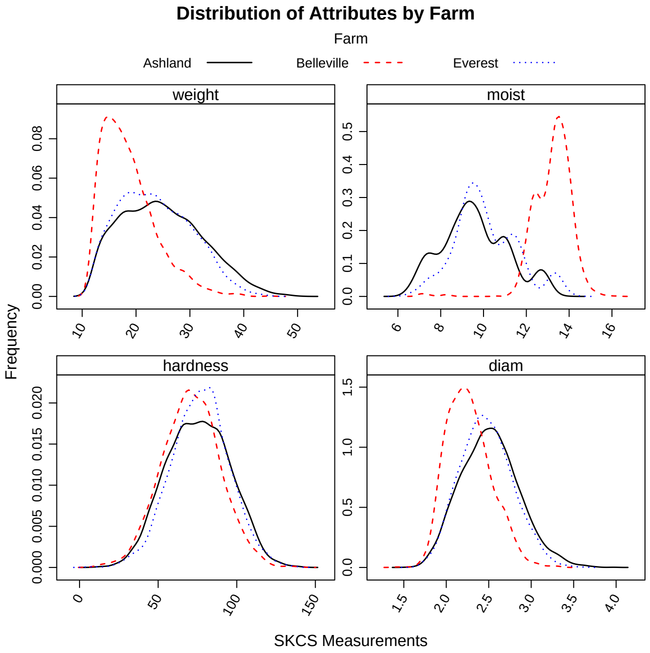

Figure 3.1: Distribution of attributes by farm

components, which reports almost zero variation between farms for hardness. However, when examining the distributions for weight and diameter we observe farms to represent nearly 15% of the variation for those attributes. When examining the distribution at Belleville we see weight and diameter values are skewed left. To further investigate this observation, variety 2137 was removed from Ashland and Everest so each farm was equally represented.

The resulting distribution (not shown) appeared nearly identical, confirming the skewness to be a farm level effect. By using varieties from the same wheat class (Hard Red Winter) we are able to distinguish differences related to variety. In figure 3.2 it is shown again that hardness is less effected by variety than weight and diameter. The genetic relationship to hardness was shown by (Morris, 2002), and further evidence of this can be implied by our study. By investigating the distributions for Jagger and Jagalene, we observe them to be more alike than the other varieties. This importance of this observation is that Jagalene is a genetic hybrid of Jagger, and the hard wheat variety Abilene. We can theorize then that similarities between these varieties may be attributed to genetic factors versus an unobserved influence.

From the table of variances we see that variety makes up the second largest source of variation

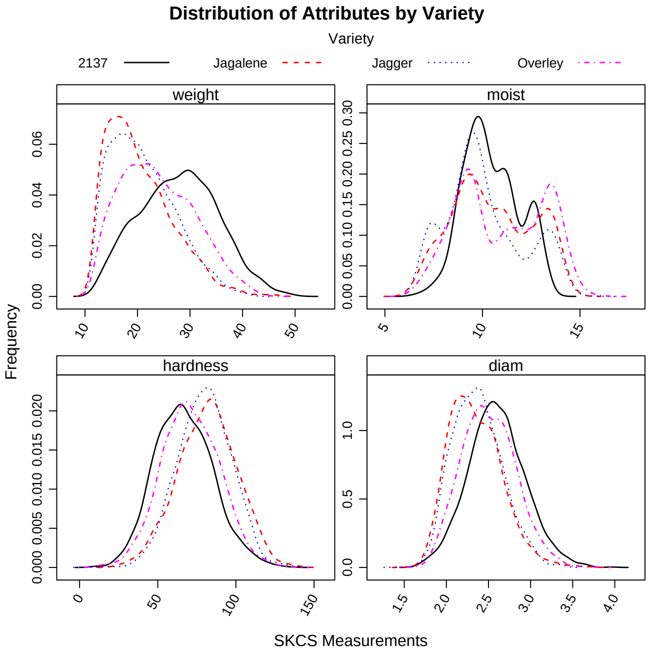

Figure 3.2: Distribution of attributes between varieties (Miller, 2008)

equal to ±6.95 HI or 13.07% of the total variation for hardness. This finding is true for diameter and weight, with variety accounting for 9.86% and13.5% of the total variation for those attributes respectively. Spike to spike variation was found to be the largest source of variation for all attributes. In terms of kernel hardness this variation equated to ±8.6 HI, accounting for nearly 20% of the total variation. This source was responsible for 18.8% of the variability in diameter and nearly 25% of the variability for weight. Variability within the spike was the primary focus in this study, and the detailed analysis follows in the remaining sections.

	Hardness			Diameter			Weight			Moisture		
Source:	SD	% of Explained	% of Total	SD	% of Explained	% of Total	SD	% of Explained	% of Total	SD	% of Explained	% of Total
Farm	0.016	0.00%	0.00%	0.120	26.77%	13.42%	2.782	23.68%	15.06%	1.900	65.29%	95.63%
Farm:Variety	6.955	32.90%	13.07%	0.103	19.67%	9.86%	2.637	21.28%	13.53%	0.474	4.06%	23.84%
Farm:Variety:Plot	3.151	6.75%	2.68%	0.062	7.04%	3.53%	1.447	6.41%	4.08%	0.192	0.67%	9.68%
Farm:Variety:Plot:Spike	8.600	50.30%	19.98%	0.142	37.50%	18.80%	3.533	38.22%	24.30%	1.279	29.58%	64.37%
Farm:Variety:Plot:Spike:Spikelet	3.844	10.05%	3.99%	0.070	9.02%	4.52%	1.844	10.41%	6.62%	0.150	0.41%	7.57%
Total Explained	12.126	100.00%	39.72%	0.233	100.00%	50.14%	5.716	100.00%	63.58%	30.607	100.00%	1540.15%
Unexplained Deviation(residual)	14.769		58.93%	0.225		46.82%	3.863		29.04%	0.459		23.07%
Total Standard Deviation	19.240		100%	0.328		100.00%	7.168		100.00%	1.987		100.00%

Table 3.1: Variance components shown in standard deviation (SD)

Gradients along the spike

Kernel hardness along the spike follows a natural gradient where the top and bottom kernels are typically harder than those in the center of the spike, and is shown in figure 3.3. The opposite trend is observed for weight and diameter, with the top and bottom having the smallest kernels. To test the statistical significance of this observation a new factor was created by dividing the spike into thirds based on the number of spikelets present.

	N	Hardness	Diameter	Weight	Moisture
Top	3448	75.43^{A}	2.40^{A}	21.62^{A}	10.44^{A}
Middle	3056	73.79^{B}	2.49^{B}	24.19^{B}	10.54^{B}
Bottom	3362	75.70^{A}	2.47^{C}	23.74^{C}	10.58^{B}
Overall					
	9866	75.01	2.45	23.14	10.52

Table 3.2: Mean values for SKCS measurements by top, middle, bottom.

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^{ABC} Values with matching letters are not significantly different p <.05

Using SAS PROC GLM, all pairwise comparisons were tested between top middle and bottom portions of the spike. The results of the comparisons shown in table 3.2 , include comparisons for each attribute. In the comparisons for kernel hardness we find that the top and bottom are not significantly different, with the center portion having significantly softer kernels. A close examination of kernel weight and diameter shows that the top yields the smallest kernels followed by the bottom portion, and the middle producing significantly larger, heavier kernels.

In the study done by Bramble et al. (2002), it was determined that the bottom portion had the highest protein decreasing towards the top. His findings, together with our data indicate that certain areas of the spike may yield kernels that are alike in physical attributes, yet significantly different in chemical composition. The comparison between the top middle and bottom are based on pooling the kernels, however more differences are revealed when we narrow the focus to the spikelet level.

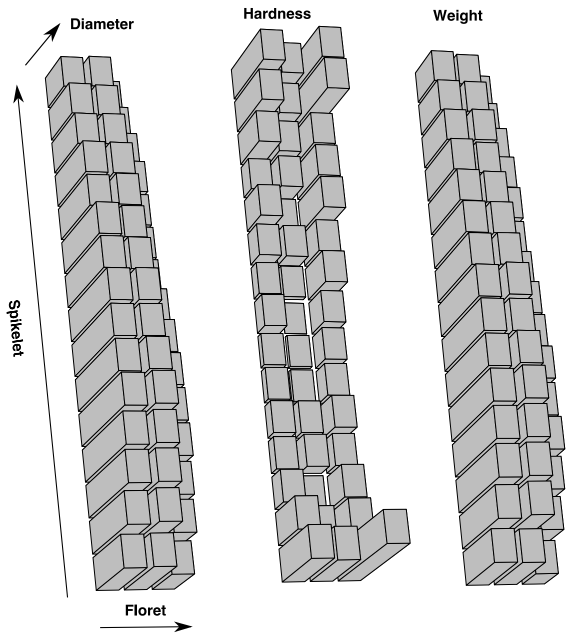

Figure 3.3: Bar graph depicting mean values at positions in the spike(Miller, 2008)

Spikelet to spikelet

In growth rate experiments done by Rawson and Evans (1970) it was shown that the sequence of spikelet formation was mimicked in the pattern of florets reaching anthesis, and then again with grain set and development. When detailing the attributes along the spike, it may not be surprising that patterns emerge, which appear to follow the patterns of growth.

We begin by looking at a graph of mean kernel diameter for each position along the spike shown in figure 3.4. It is apparent from this graph that the incremental change in kernel diameter is nearly identical in the first and second floret. Tests of this hypothesis are shown in table 3.3 and results

	N	Hardness	Diameter	Weight	Moisture
Floret					
1	4164	75.46^{A}	2.47^{A}	23.52^{A}	10.53^{A}
2	3772	73.94^{B}	2.49^{A}	24.02^{B}	10.55^{A}
3	1930	76.15^{C}	2.33^{B}	20.58^{C}	10.45^{B}
Overall					
	9866	75.01	2.45	23.14	10.52

Table 3.3: Mean values for SKCS measurements by floret

[ABC] Values with matching letters are not significantly different $p < .05$

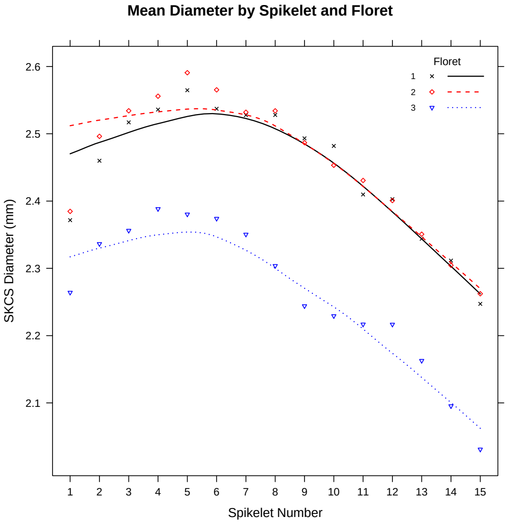

Figure 3.4: Mean kernel diameter by spikelet and floret

for diameter indicate no significant difference between the first and second floret. The third floret is clearly smaller than the first and second however, the incremental change in diameter follows in parallel with the first two florets.

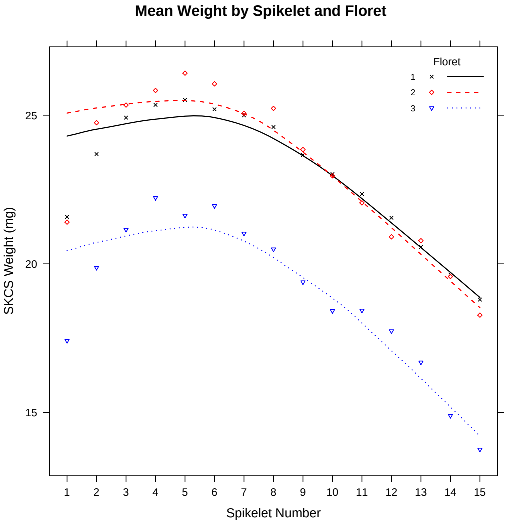

Figure 3.5: Mean kernel weight by spikelet and floret

Kernel weight shown in figure 3.5 follows a trend similar to that of diameter with central kernels being heavier on average. However in the analysis we find that the second floret has a significantly larger kernel weight that the first or second floret. This pattern fits well with research by Evans et al. (1972) showing that second floret kernels develop more rapidly than other kernels. Following the pattern of grain set described by Evans, we may theorize that time from anthesis provides a slight advantage to kernel development. From the center of the spike to the top, first floret kernels are equal if not slightly larger than second floret kernels. These early setting grains would presumably have the longest period of kernel filling before plant death and dessication. Further tests could confirm, but rapid growth of second floret kernels will likely out pace the first floret kernels, but given enough time the first floret kernel may catch up. In the lower portion of the spike the second floret grows quickly, and before the first floret can catch up plant death occurs leaving these kernels smaller on average. Even still the second floret has a significantly larger kernel weight than the first and third florets. Kernel hardness along the spike shown in figure 3.6, follows a trend opposite of weight and diameter, with the hardest values occurring in the upper and lower spikelets.

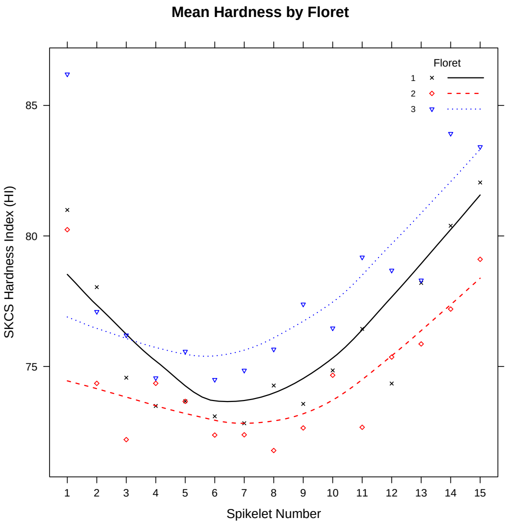
Mean Hardness by Floret

Figure 3.6: Mean kernel hardness along the spike

The interesting observation for hardness is the significant difference in hardness between the first and second floret. This hardness value is likely telling of the chemical differences between the two florets. All though no analytical testing was done at the single kernel level, prior studies have reported protein content of the second floret (Jie et al., 2005; Ali et al., 1969; Bremner, 1972), to be greater than other floret positions. In either case the similarities in weight and dimensions between first and second florets indicate that internal structure may results in differences between positions.

Fusarium Head Blight

The weather conditions at Belleville and Everest favored to the growth of Fusarium Head Blight which had a deleterious effect on the grain quality from those locations, a table showing the percentage of blight and other conditions observed can be seen in table 3.4.

	Ashland	Belleville	Everest
2137	0	0	116
Jagalene	0	18	175
Jagger	0	63	48
Overley	0	234	343

Table 3.4: Frequency of Fusarium head blight by farm and variety

It should be noted that 68% of the observed blight occur ed at Everest, with the remaining 32% occurring in Belleville. The occurrence of blight had a significant effect ($p<.05$) of lowering kernel weight and hardness values for the infected kernels. The average hardness of normal kernels at Everest was 79.03 HI compared to 70.00 HI for the kernels containing blight. The Overall hardness at Everest was 77.19 HI, meaning that occurrence of blight effectively lowered the overall mean hardness by two points. Tables listing the mean values with tests of significance are listed in table 3.5.

Results indicate that infected kernels have a significantly lower hardness, diameter and weight than kernels that have no symptoms of blight. Kernels displaying mild characteristics of blight (SB) were not significantly different than the kernels showing blight but did have a larger weight,

	N	Hardness	Diameter	Weight	Moisture
Condition					
Blight	949	69.46^{A}	2.32^{A}	19.36^{A}	11.04^{A}
No Blight	7746	75.18^{B}	2.49^{B}	24.16^{B}	10.41^{AB}
Some Blight	610	75.05^{AB}	2.37^{A}	20.98^{A}	11.20^{B}
Shriveled	561	82.12^{C}	2.24^{A}	17.79^{A}	10.45^{AB}
Overall					
	9866	75.01	2.45	23.14	10.52

Table 3.5: Mean values for SKCS measurements by condition

[ABC] Values with matching letters are not significantly different $p <.05$

diameter and hardness score. The practical significance of this finding is that conventional cleaning practices are assumed to remove most of the blighted kernels, either by density or size separation, and based on the results it appears that the differences between blighted and healthy kernels may be enough to make an effective separation. The risk to the system are the kernels that display only mild characteristics of blight. These kernels have similar weight, diameter, and texture and may pass through the system without detection. One observation that was noted in the experiment was the pattern of blight within the spikelet. In spikelets that contained blight, the first floret had blight 100% of the time. However, it was frequently observed in spikelets effected by blight where the third and fourth floret kernels were normal in appearance. The blight seemed to attack kernels at different levels of intensity. Some kernels were completely fused to the outer glume resulting in a soft but compact mass overtaking the entire spikelet. In other more mild cases, the kernels were soft but intact and could be collected for analysis. In heads that were mildly effected by blight the effects seemed to lessen towards the apex of the spikelet. There were no patterns of blight in terms of top middle or bottom portions.

In this study, variation in moisture content was largely dependant on the day in which the sample was processed. Even though samples were crushed within one day of being dissected, data collection took place over a long period of time. The shift in moisture content by day, was driven by relative humidity and moisture absorption from the atmosphere. All though moisture content does effect kernel hardness the algorithm used by the SKCS will partially correct the hardness score to account for moisture. Moreover, secondary analysis of crush day, moisture content, and hardness proved that absorptivity did not significantly impact hardness measurement.

Chapter 4

Conclusion

Through this research it has been shown repeatedly that patterns govern the wheat kernel through the many phases of development. In general, the grains of the center portion were the most developed kernels in the spike, with quality diminishing towards the base and tip. within the spikelet the first and second floret were most similar in weight and diameter, however they were not equal in hardness, with the second kernel being significantly softer than the first and third. The pattern of the third floret paralleled the first and second giving indication time, rather than potential, limited the size of that kernel .

In general, kernels infected by blight had a lower hardness than healthy kernels, however infected kernels with only surface characteristics of blight displayed normal hardness. These kernels may pose a risk, as they may be retained even after cleaning

The largest source of variation for all attributes was found between spikes, accounting for up to 25% of the total variation. The variation within the spike accounted for little over 3%, meaning that spike to spike variation was a shift in mean hardness per spike, with each spike having a similar internal distribution . More investigation into this phenomenon may help to remove a large amount of variability in wheat.

Future Work

The research answered questions about gradients of hardness within the spike and the variation of hardness due to growing location. Understanding the natural variation in hardness gives insight to improved methods of single kernel analysis. In single kernel analyses that are destructive, it may be helpful to select from the spike rather than a bulk sample. Secondary analysis can be performed on neighboring kernels to provide supportive information to the experiment.

Gradients in the wheat spike described by this and other experiments, may be influential to the milling industry. We typically remove the smallest kernels during the cleaning process, which are shown to be the most variable kernels. However, research has shown that this fraction would include the portion of the spike containing the highest protein content. An in depth study of the kernels taken from the top and bottom of the head, may reveal a characteristic that can be exploited for mechanical separation of the two.

It was shown in this study that 70% of kernels exist in the top and bottom portion of the spike, which is also the most variable region. Breeding programs that focus on developing this part of the spike, could bring improvements in grain yields and reduce variability.

Future research from this study would be to investigate the variation in protein at the single kernel level, coupled with SKCS data. Previous research has suggested that vascular effects are involved in the varied trans-location of nutrients to the spike. Breeding programs may target this, to ensure even levels of assimilate, which would likely promote uniformity within the spike.

Understanding the trigger for anthesis, and ensuring that florets to reach anthesis simultaneously may be an approach to improving variability in wheat. Moreover, improvements to grain yield may be seen by targeting the third floret kernel. This kernel appears to have equal potential to become fully developed, given enough time for kernel filling.

Appendix A

Supporting Tables

Farm	VARIETY	Class	HI	σHI	Moist (%MC)	σMC	Wt. (mg)	σWt	Diam (mm)	σDiam	NIR-MC	NIR-Prot	Prot14%MB
Columbus	2137	MIXED	53	17.3	12.9	0.4	24.5	7.1	1.97	0.44	11.51	10.66	10.36
Everest	2137	MIXED	52.7	18.3	13.6	0.3	31.7	9.9	2.3	0.5	11.74	11.38	11.09
Columbus-S	2137	MIXED	50.1	21.2	12.7	0.4	23.7	7.4	1.93	0.45	11.27	11.1	10.76
Garden City	2137	HARD	57.8	14.2	12.3	0.3	24.2	6.1	1.85	0.37	10.89	14.54	14.03
Garden City-Irr	2137	HARD	68.5	18.6	13.5	0.4	28.8	7.2	2.09	0.45	11.38	11.28	10.95
Hesston	2137	HARD	57.4	17.7	12.3	0.3	21.8	5.6	1.83	0.37	10.93	10.77	10.4
Belleville	2137	MIXED	54.1	19.1	12.4	0.4	32.3	8.6	2.33	0.49	11.08	11.44	11.06
Colby	2137	HARD	75.9	14.5	11.7	0.3	30.3	6.6	2.18	0.39	10.25	11.61	11.12
Colby	2137	HARD	77.4	12	12	0.3	30.1	7.6	2.18	0.44	10.39	12.24	11.75
Everest	Jagalene	HARD	71	22	13.3	0.4	26.2	9	2.11	0.49	11.54	12.76	12.41
Columbus-S	Jagalene	MIXED	54.1	29	12.3	0.5	21.4	6.4	1.82	0.4	10.88	11.82	11.4
Garden City	Jagalene	HARD	74	15.9	12.4	0.3	25.2	7.2	2.06	0.41	10.92	14.77	14.26
Garden City-Irr	Jagalene	HARD	79.8	16.3	13.4	0.4	27.2	7	2.18	0.41	11.27	12.47	12.08
Hesston	Jagalene	HARD	65.8	20.3	12.4	0.4	19.8	5.8	1.73	0.38	10.73	12.62	12.16
Belleville	Jagalene	HARD	69.3	16.9	12.6	0.3	27.4	8.4	2.16	0.48	10.9	12.63	12.19
Colby	Jagalene	HARD	83.6	16.1	11.5	0.2	30.5	7.3	2.32	0.39	10.25	11.62	11.14
Colby	Jagalene	HARD	82.2	13.7	11.7	0.3	30	8.1	2.28	0.49	10.26	12.87	12.33
Everest	Jagger	HARD	63.6	18.2	13.5	0.4	26.8	9.4	2.11	0.51	11.42	12.63	12.26
Columbus	Jagger	HARD	59.8	15.8	12.7	0.4	23.2	6.4	1.96	0.39	11.31	11.87	11.51
Columbus-S	Jagger	MIXED	57.3	20.7	12.6	0.4	22.3	6.3	1.9	0.4	11.18	11.96	11.58
Garden City	Jagger	HARD	68.5	16.5	12.4	0.4	24.9	8.7	1.98	0.44	10.86	16.79	16.2
Garden City-Ir	Jagger	HARD	75.6	16.8	13.2	0.4	24.5	6.7	1.98	0.43	10.98	13.28	12.83
Hesston	Jagger	HARD	68.7	17.5	12.3	0.4	18.2	5.4	1.63	0.3	10.94	12.9	12.45
Belleville	Jagger	HARD	69.2	19.4	12.8	0.4	24.4	7.7	1.97	0.42	10.91	12.43	12
Colby	Jagger	HARD	79.2	13.9	11.4	0.3	27.5	7	2.12	0.41	10.14	11.69	11.19
Colby	Jagger	HARD	83.1	13.8	11.6	0.3	26.5	7.9	2.09	0.44	9.87	13.26	12.65
Everest	Overley	MIXED	56.9	20.2	13.6	0.3	30.9	10.4	2.4	0.56	11.37	12.47	12.1
Columbus	Overley	HARD	58	19.3	12.8	0.5	24.1	7.7	2.03	0.47	11.12	12.79	12.37
Garden City	Overley	HARD	60.1	16.1	12.5	0.3	29.5	8.9	2.22	0.44	10.77	15.33	14.77
Garden City-Irr	Overley	HARD	61	16.4	13	0.3	34.1	7.8	2.5	0.48	11.18	13.64	13.2
Hesston	Overley	HARD	66.3	20.3	12.6	0.4	21.4	6.4	1.82	0.36	10.82	11.44	11.03
Belleville	Overley	HARD	62.7	17.3	12.5	0.4	30.1	8.8	2.33	0.52	11.09	12.45	12.04
Colby	Overley	HARD	70.9	14.2	11.3	0.5	34	7.1	2.48	0.48	9.87	12.33	11.76
Colby	Overley	HARD	68.5	13.4	11.2	0.3	37.3	7.9	2.64	0.43	10.01	13.57	12.97

Table A.1: KSCPT results crop year 2007 all counties

	2137				Jagalene				Jagger				Overely			
	Hardness	Diam	Wt.	%MC	Hardness	Diam	Wt.	%MC	Hardness	Diam	Wt.	%MC	Hardness	Diam	Wt.	%MC
Farm																
Ashland	61.81	2.64	29.05	10.58	82.03	2.42	22.08	9.24	84.58	2.37	21.05	9.02	74.18	2.63	26.80	9.54
Belleville	-	-	-	-	77.54	2.20	17.42	12.86	71.92	2.14	16.04	13.29	66.71	2.36	20.60	13.39
Everest	73.53	2.52	25.74	10.28	85.08	2.35	20.35	10.54	79.55	2.46	23.13	9.68	72.74	2.48	23.09	9.98
Overall																
	67.03	2.58	27.58	10.45	81.74	2.34	20.39	10.54	80.27	2.36	20.84	10.09	71.23	2.49	23.58	10.97

Table A.2: Mean values for SKCS measurements by farm and variety

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