# Single Kernel Protein Variance Structure in Commercial Wheat Fields in Western Kansas

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## **ABSTRACT**

This research was undertaken to quantify the structure of protein variation in a commercial hard red winter (HRW) wheat (Triticum aestivum L.) production system. This information will augment our knowledge and practices of sampling, segregating, marketing, and varietal development to improve uniformity and end-use quality of HRW wheat. The allocation of kernel protein variance to specific components in southwestern Kansas was performed by a hierarchical sampling design. Sources of variability included Field, Plot (plots within a field), Row (rows within a plot), Plant (plants within a row), Head (heads within a plant), Position (spikelets at a specific position on a head), Spikelet (spikelets within a position), and Kernel (kernels within a spikelet). Individual kernels (10 152) were collected from 46 fields planted to one of four cultivars: Jagger, 2137, Ike, or TAM 107. Kernels were evaluated for protein concentration by a single kernel characterization system equipped with a diode array near-infrared (NIR) spectrometer. For the cultivars 2137 and Ike, all sources of variability except Spikelet were statistically significant (P < 0.05). For Jagger, all sources except Row were significant and for TAM 107, variation attributed to Field and Plant were not significant. Field and Plot sources of variability contributed the greatest amount of variance within the hierarchy for Jagger, 2137, and Ike. For TAM 107, Plot was the greatest source of variability. The least squares means were calculated for the fixed effect Position. Jagger, Ike, and 2137 showed a significant protein gradient in which the highest protein concentration occurred at the base of the head and the lowest protein content at the top. For TAM 107, the greatest protein content was found at the base. Results of this study provide a benchmark for future efforts to improve wheat consistency through breeding and crop management. The protein variance structure described during this study also defines practical limits for managing and marketing protein content in HRW.

QUALITY-ORIENTED MARKETING of hard red winter wheat offers farmers and grain handlers additional value compared with a commodity-based marketing system. Policy and institutional changes that drive this marketing approach include formation of producer marketing groups, the Federal Agriculture Improvement and Reform Act of 1996 (FAIR) which empowers farmers to select crops that provide the greatest revenue, and the Grain Quality Acts of 1986 and 1990 which contain congressional mandates to develop technology that rapidly assesses grain quality. Additionally, the abolishment of governmental purchasing groups in many coun-

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tries has increased the demand for improved wheat quality by export customers (Dexter and Preston, 2001).

A key element of the Grain Quality Acts involves the development of rapid quality detection systems such as the single kernel characterization system (SKCS) and whole kernel NIR technology. Osborne et al. (1997) evaluated the SKCS 4100 as a means of measuring wheat kernel weight, hardness, moisture content, and sample uniformity and found it performed satisfactorily during harvest in Australia. Baker et al. (1999) developed a wheat segregation strategy to be used at elevators in which the SKCS and whole grain NIR are used to predict a composite milling and baking yield within 60 s.

Wheat consistency is an important element of quality (Arizmendi and Herrman, 2001). In a protein-based segregation system, protein variability (consistency) within a field could influence sampling methodology and wheat segregation at the first collection point. Defining the protein variability structure in commercial wheat production systems can assist wheat breeders in their selection process by establishing a protein uniformity benchmark before release. Additionally, this information can provide a baseline from which crop physiologists can examine factors that determine protein variability.

Levi and Anderson (1950) reported protein variability within specific production units: plots within fields, plants within rows, heads within plants, and spikelets within heads. Their analysis included direct protein measurements of randomly sampled kernels. To determine the protein variability within a plot, they made direct protein measurements of randomly selected kernels from four test plots (0.05 and 0.1 ha) of two different cultivars, Red Bobs and Marquis, at three locations in Canada. To assess protein variability within a row, they sampled individual kernels from 68 wheat plants of the cultivar Thatcher within a 3.05-m row in a test plot. A third set included individual kernels sampled from 24 spikes in the above sample set. For all components (plots, rows, spikes, and spikelets), the range, distribution, and standard deviation of protein content were cal-

Malloch and Newton (1934) investigated protein variability within a field as a function of soil heterogeneity. Over two successive years (1930 and 1931) a field of a single cultivar (Red Bobs in 1930 and Marquis in 1931) was selected that was determined to be "reasonably level and did not show obvious variation in soil." Just before harvest of the main crop, they hand-cut 50 5.5-m rows at locations throughout the field. Kernels from each of these "plots" were combined. The average protein content for the field was 148 g kg<sup>-1</sup> with a standard deviation of 8.3 g kg<sup>-1</sup> and a range of 42 g kg<sup>-1</sup> (167 g kg<sup>-1</sup>–125 g kg<sup>-1</sup>) in 1930 and an average of 144 g kg<sup>-1</sup>

with a standard deviation of 5.6 g kg<sup>-1</sup> and a range of 24 g kg<sup>-1</sup> (129–153 g kg<sup>-1</sup>) in 1931.

Both studies were limited in scope because they relied on a small number of samples and cultivars that are no longer under production. In this research, we investigated the extent of protein variability as it existed in fields of HRW wheat under commercial production in Kansas.

### **MATERIALS AND METHODS**

Single kernel samples of HRW wheat were collected from 46 fields under commercial production in two counties (Stanton and Kearney) in southwest Kansas just before the 2000 harvest. There were four cultivars of HRW wheat included in the fields samples: Jagger, 2137, Ike, and TAM 107. These cultivars represented the top four cultivars under production in southwest Kansas in 2000 (Kansas Agricultural Statistics, 2000).

The experimental design for this study was based on a hierarchical sampling procedure comprised of seven sources of variability. These sources of variability included Field, Plot (plots within a field), Row (rows within a plot), Plant (plants within a row), Head (heads within a plant), Position [spikelets at a specific position on a head (top, middle, base)], Spikelet (spikelets within a position), and Kernel (kernels within a spikelet). In total, there were 46 fields sampled with three plots in each field, three rows within each plot, two plants within each row, two heads from each plant, three positions within each head, two spikelets within each position on a head (for one randomly chosen head per field), and two kernels from each spikelet. Table 1 summarizes the sources of variability and number of samples taken within each component for each cultivar. In total, 10 152 wheat kernels were used in the variance component analysis.

Sampling occurred just before harvest at the point when kernels were in the hard dough stage. In most instances, fields were sampled 1 to 2 d before harvest. Field selection was based on producer cooperation. Selection of the plots within each field was conducted by means of random plot selection methods outlined in the 2000 Wheat Objective Yield Survey Interviewer Manual (USDA, 2000). Each plot consisted of three adjacent rows with two adjacent plants in each row. Individual heads were sampled at random from each plant, kept intact and uniquely identified by field, plot, row, and plant location. Two kernels were removed from spikelets on each head with the spikelets identified by their relative position on the head: base (B), middle (M) and top (T). The B spikelets were the bottom-most spikelets that contained a minimum of two kernels. The T spikelets occurred closest to the top of the head and contained at least two kernels. And the M spikelets were the spikelets occurring equidistant from the B and T spikelets. Kernels removed from the spikelets

Table 1. Sources of variation and number of kernels evaluated by cultivar.

Component	Number	Jagger	2137	Ike	Tam 107	
Field	47	13	14	11	8	
Plot	3	39	42	33	24	
Row	3	117	126	99	71	
Plant	2	234	251	197	142	
Head	2	468	502	394	284	
Position	3	1404	1506	1182	852	
Spikelet	3/Field	1443	1548	1215	873	
Kernel	2	2886	3094	2426	1746	

were uniquely identified relative to one another within the spikelet.

To measure individual kernel protein concentration, spectral data were collected with the Perten SKCS 4170, which contains a NIR diode-array near-infrared spectrometer (Perten Instruments, Springfield, IL). The spectrometer measures absorbance at 400 to 1700 nm using an array of silicon (7-nm resolution) and indium-gallium-arsenide (11-nm resolution) sensors. The kernels were introduced individually by hand into the detection area of the spectrometer. The spectrometer performed eight spectral scans per kernel and recorded the average. The protein prediction model was created from a 500 kernel reference sample with protein values measured with the Leco (Leco Corp., St. Joseph, MI) combustion nitrogen analyzer. The standard error for cross validation for the NIR prediction model is 0.93% with an  $r^2 = 0.9$  (Bramble, 2001, unpublished data). The prediction model for individual protein content yielded good results, especially in the middle protein ranges (100–200 g  $kg^{-1}$ ).

We analyzed the variance components for single kernel protein using SAS procedures for mixed models (SAS Institute, Cary, NC). Least squares means for the fixed effect Position were computed and compared by F-tests and least significant differences (LSD). Field, Plot, Row, Plant, Head, Head  $\times$  Position, and Spikelet were treated as random effects. Variance components for each random effect were tested for significance by F-tests based on expected mean squares in PROC GLM. Restricted maximum likelihood (REML) estimates of the variance components and their standard errors were obtained by means of PROC MIXED.

## **RESULTS**

In this study, we employed a hierarchical sampling framework for the analysis of protein variance of wheat within a commercial production system. Tests of significance for the sources of variability are presented in Table 2. Declaration of significance indicates that, within the hierarchical design, the respective source contributed to the total protein variability. Jagger displayed significance for all sources of variability except Row (P = 0.056), while Ike and 2137 displayed significance for all sources of variability except Spikelet. For TAM 107, Field and Plant were not significant (P > 0.05) sources of variation.

Variance estimates and standard errors are presented in Table 3 for each cultivar by Field, Plot, Row, Plant, Head, Position, and Spikelet (note: Kernel falls within the residual measurement). The hierarchical design resulted in the greatest amount of data available for Kernel and decreased through the component hierarchy to Field (Table 1). This results in a higher calculated standard error for Field variance estimates and makes inter-

Table 2. P-values for tests of significance for each of the variance components by cultivar.

Component	2137	Ike	Jagger	Tam 107	
Field	0.0036	0.0028	0.0051	0.2527	
Plot	0.0001	0.0001	0.0001	0.0001	
Row	0.0392	0.0031	0.0557	0.0153	
Plant	0.0014	0.0011	0.0021	0.4268	
Head	0.0001	0.0001	0.0001	0.0003	
<b>Head</b> × <b>Position</b>	0.0001	0.0001	0.0001	0.0021	
Spikelet	0.5890	0.7254	0.0359	0.0001	

	2137		Ike		Jagg	Jagger		Tam 107	
	Variance	SE	Variance	SE	Variance	SE	Variance	SE	
	g kg <sup>-1</sup>								
Field	244	141	228	153	192	115	85	157	
Plot	307	87	253	83	225	70	526	194	
Row	16	10	29	12	22	14	28	14	
Plant	30	11	25	11	49	16	28	13	
Head	73	09	64	8	105	11	43	10	
<b>Head</b> × <b>Position</b>	36	04	19	4	4	3	90	8	
Spikelet	0.0		10	3	9	2	1	5	
Residual	86	03	66	2	76	3	76	4	

Table 3. Variance estimates and standard errors by component for each cultivar.

pretation of these results less certain. The standard error associated with the other sources of variability was low relative to the variance estimate, and inferences regarding these components are more reliable.

# Field and Plot

For Jagger (Table 3), the variance estimate for Field was 192 g kg<sup>-1</sup> protein with a standard error of 115 g kg<sup>-1</sup>. The large standard error results, in part, from the small number of fields (13). For Plot, the estimated variance was 225 g kg<sup>-1</sup> with a standard error of 70 g kg<sup>-1</sup>. The standard error decreased while the variance estimate increased compared with the results for Field. Ike and 2137 displayed a similar variance structure as Jagger with lower Field variance estimates (Ike = 228g kg<sup>-1</sup> and 2137 = 244 g kg<sup>-1</sup>) and an increase in the Plot (Ike = 253 g kg<sup>-1</sup> and 2137 = 307 g kg<sup>-1</sup>). In TAM 107, Plot explained the greatest source of protein variance and was substantially higher than that of the other cultivars (526 g kg<sup>-1</sup> with a standard error of 194 g kg<sup>-1</sup>). Levi and Anderson (1950) calculated the standard deviation of four test plots in their study and found it to be  $14~g~kg^{-1}$  protein.

Our study and the work of Levi and Anderson (1950) report higher within-field protein variation than did Malloch and Newton (1934). A difference between our study and theirs was the large number of randomly selected fields under a wide range of commercial production practices and soil types. The Stanton County soil survey (USDA, 1961) indicated three different major soil types within the study area and within fields there were as many as four subclasses of soil.

#### **Row and Plant**

For Jagger, we found a sharp decrease in the variance estimate for Row (22 g kg<sup>-1</sup> with a standard error of

14 g kg $^{-1}$ ) and Plant (49 g kg $^{-1}$  with a standard error of 16 g kg $^{-1}$ ) compared with the Field and Plot variance estimates. Levi and Anderson (1950) did not calculate a row-to-row standard deviation; however, they did calculate a standard deviation of 6.0 g kg $^{-1}$  for 68 plants in one 3.05-m row. Similar to Jagger, Ike and 2137 displayed a sharp decrease for the Row and Plant estimates (Ike = 29 and 25 g kg $^{-1}$  and 2137 = 16 and 30 g kg $^{-1}$ , respectively).

# **Head and Spikelet**

The Jagger Head protein variance (105 g kg<sup>-1</sup>) was greater than that observed for Plant and Row as were the Head protein variances for Ike (64 g kg<sup>-1</sup>) and 2137 (73 g kg<sup>-1</sup>). Levi and Anderson (1950) observed a "heads within a plant" standard deviation of 11.0 g kg<sup>-1</sup>. For the Spikelet component, the variance estimate was 9 g kg<sup>-1</sup> for Jagger with a standard error of 2 g kg<sup>-1</sup>. This is a large drop in variance over Head and differs from Levi and Anderson (1950), who calculated a 11 g kg<sup>-1</sup> standard deviation for variability within a head and is possibly due to the Position effect.

# **Position**

In addition to estimating the variance within an individual head, mean protein concentration was calculated for the fixed effect Position (Table 4). For Jagger, Ike, and 2137, a protein trend was found with the highest protein content occurring in the bottom-most spikelet and decreasing toward the top-most spikelet. For 2137 and Ike, all three positions varied significantly from one another (P < 0.05). With Jagger, a similar trend was present, however, the bottom and middle spikelets did not differ significantly in protein content (P = 0.07). With TAM 107, the middle spikelet had a mean of 139.0 g kg<sup>-1</sup> and the top 142.5 g kg<sup>-1</sup>, which differs in trend

Table 4. Least square means for kernel protein content by position on the head by cultivar.

	Jagger		Ike		TAM 107		2137	
Position†	LS Mean	Significance‡	LS Mean	Significance	LS Mean	Significance	LS Mean	Significance
	g kg <sup>-1</sup>		g kg <sup>-1</sup>		g kg <sup>-1</sup>		g kg <sup>-1</sup>	
T	138.7	a	134.6	a	142.4	b	129.0	a
M	146.9	b	138.6	b	139.4	a	130.1	b
В	147.7	b	140.7	c	145.0	c	131.6	c

 $<sup>\</sup>dagger$  T = Top; M = Middle; B = Bottom position on wheat head.

<sup>‡</sup> LSD 0.05.

from the other cultivars; however, the bottom-most spikelet had the greatest protein content.

Levi and Anderson (1950) measured protein concentration for each individual spikelet on three plants. They found in seven of nine heads the top two spikelets had "decidedly lower protein content than the remaining spikelets." Levi and Anderson made the observation that "the means suggest that protein concentration tends to decrease from about the eighth spikelet [counted from the top] to the top spikelet."

## **DISCUSSION**

The mechanism that regulates grain yield and protein concentration (there is typically a negative relationship between the two) is the availability of nitrogen, followed by redistribution of nitrogen within the plant (del Molino, 1992). He concluded that 53% of the variance in protein content was assignable to the field effect, whereas fertilization rates only accounted for 18.4%. The standard deviations for protein content within each field ranged from 10.4 to 21.6 g kg<sup>-1</sup>, indicating considerable within-field variation. McNeal and Davis (1954) report that differences in vegetative growth accounted for most of the variance in grain protein content. Del Molino (1992) reached this same conclusion and stated that grain protein concentration mainly depends on the ratio of nitrogen accumulated in the vegetative parts from anthesis to grain production. Within fields in southwestern Kansas there is considerable variation in topography, soil type, proximity to irrigation source, and many other climatic and production factors. Each of these factors has the potential to affect the pattern of localized plant growth and the final protein concentrations among plots in a given field.

In the present study, the variance attributed to Row and Plant was small but significant. Austin et al. (1977) reported that variation in the amount of nitrogen in the leaves was the major cause of variation in total plant nitrogen. Further, the regulation of the nitrogen uptake by the grain depends on nitrogen supply whether by transfer from the vegetative parts or contributed from the roots (Spiertz and Ellen, 1978).

The protein variability within the head (Position) observed in our research is supported by additional wheat physiology studies. In studying head development in spring wheat, Kirby (1974) noted that the weight of an individual grain in the head is dependent upon the spikelet and floret position within the head. The distribution of the weight of individual grains within the ear might be due to insufficient nutrients to support the potential growth of all the florets. Under these conditions, the available nutrients might be allocated in accordance to physical factors.

Stoddard (1999) found the largest kernels in spikelets in the middle region of the head with the smallest kernels at the bottom and top of the head. Grain nitrogen concentration as a percentage of total grain mass was constant across the entire head but grain nitrogen content, when measured directly, showed a similar trend

as grain mass, with the bottom and top spikelets having lower nitrogen concentration than the middle region of the head (Stoddard, 1999).

Rawson and Evans (1970) investigated possible mechanisms for variation in nutrients within a head as a function of competition for assimilates both between and within spikelets. They found significantly higher growth rates in the central spikelets than in the lower and upper spikelets. Using <sup>14</sup>C, they found that spikelets in the upper half of the ear received progressively less carbon closer to the top of the ear.

# **CONCLUSION**

The experimental design enabled us to assign random effects for all sources of variability except Position. Consequently, we can draw inferences about protein variability in a commercial wheat production system. On the basis of study results, one could design a field sampling system to quantify protein concentration and better assign a confidence interval on protein measurements within and between fields. This type of information provides insight into the ability to measure wheat protein concentration within a field by means of on-line NIR technology in harvesting equipment, at the country grain elevator, and within the grain trade. Currently, the grain trade assigns a protein premium schedule at 2.0 g kg<sup>-1</sup> (0.2%) concentration increments which is lower than the Position (within a head) protein differences observed in this study.

Three of the wheat cultivars (Jagger, Ike, and 2137) exhibited a similar trend in protein variability within the hierarchical design. TAM 107 deviated substantially from these three cultivars and exhibited less desirable end-use properties (McCluskey et al., 2001). As single kernel protein measurement capabilities become more readily available to wheat breeders, the application of this technology and information contained in this study can help establish protein uniformity benchmarks to improve consistency and end-use quality.

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