This chapter provides a description of the testing procedures used to analyze the corn and sorghum samples collected for this report. Suggestions for how to interpret the test results presented and descriptions of the statistical tests utilized are also provided. Additional resources on the developments in rapid testing technologies for grain are available at the U.S. Grains Council web site (www.grains.org).

CHAPTER 5
TESTING METHODOLOGY AND INTERPRETATION OF RESULTS

The following sections describe the corn quality tests performed as part of this report. All tests were performed by the Identity Preserved Grain Laboratory in Champaign, Illinois, unless otherwise indicated.

Tests Performed on All Samples

BCFM (Broken corn and foreign material) was determined by sieving the entire sample as received. Material passing through a 12/64 inch round-hole sieve plus any foreign material retained by the sieve was considered to be BCFM and was expressed in %.

Total damage was determined by Champaign Grain Inspection, Inc., using a split sample of approximately 250 g. Damage was determined by picking a 250 g sample using FGIS inspection procedures and was expressed in % total damage. Total damage includes damage from mold, insects, heat, sprouting, etc.

Test weight was determined using an uncleaned sample following FGIS procedures except that a pint cup had to be used because of the relatively small sample sizes received. It was expressed in lb/bu. High test weight value can indicate many different characteristics, including adequate growing season weather for full kernel filling or that the variety grown has higher proportions of hard vitreous endosperm relative to soft endosperm.

True density was determined by pre-weighing 100 kernels and placing them in a Micromeritics helium-air comparison pycnometer which measured volume in cm³. True density was calculated as mass of the 100 kernels divided by their volume, g/cm³. True densities typically ranged from 1.2–1.35 g/cm³ at “as is” moisture of about 12–15%. Typically pycnometers give smaller volumes than other liquid displacement methods that are sometimes used. The smaller volume is measured because the helium gas penetrates into pore spaces of corn samples much more extensively than liquids penetrate. The smaller volumes will, therefore, result in slightly higher density than when using liquid displacement methods.

1,000-kernel weights were determined from the kernel weight of 100 kernels tested for true density. The 100-kernel weights were multiplied by 10 and were expressed in grams. Typically 1,000-kernel weights range from 240 to 390 g with the larger values indicating a large-sized kernel. Large uniform-sized kernels often enable higher flaking grit yields in dry milling.

Average kernel volume was determined from the pycnometer volumes obtained for 100 kernels. It was calculated as the 100-kernel volume in cm³/100. Kernel volumes usually range from 0.18–0.3 cm³ per kernel for small and large kernels, respectively.

Percent thins were determined by sieving 250 g through a 20/64 inch round-hole sieve. The sieve was shaken from side to side 30 times. The percentage of thins represented 100 times the weight of the material passing through the sieve divided by 250 g and was expressed in %. Percentages of thins can range from 1% (very large kernels) to 90% (very small kernels). Dry millers prefer corn with 30% or less thins. Thins from 30–50% are normally considered to be in an acceptable range for regular dent corn.

Stress cracks were determined by performing two 100-kernel tests on whole kernels. The 100 kernels were placed with their germ side down on top of a light table which allowed light to pass through the vitreous endosperm of the kernel. Kernels were sorted into four categories: (1) no cracks, (2) 1 crack, (3) 2 cracks, or (4) more than 2 cracks. Stress crack percentage was expressed as all kernels containing 1, 2, or more than 2
cracks divided by 100 kernels. High stress crack percentages lead to more breakage in handling and often indicate that high-temperature heated air drying or rapid cooling was used.

**Stress crack index (SCI)** provides a weighting to indicate severity of stress cracking. SCI was defined by the following equation: (1 × % of kernels with 1 stress crack) plus (3 × % of kernels with 2 stress cracks) plus (5 × % of kernels with more than 2 stress cracks). The SCI can range from 0–500, with a high number indicating numerous multiple stress cracks in a sample, which is undesirable for most uses.

**Chemical composition** tests for protein, oil, starch, and fiber were determined using a 250 g sample in a whole-kernel Foss Grainspec Near Infrared Transmittance (NIT) instrument. The NIT was calibrated to chemical tests and the standard error of predictions for protein, oil, starch, and fiber were 0.25%, 0.18%, 0.84%, and 0.10%, respectively. All values were expressed at 0% moisture (dry basis). The fiber figure that is presented is for crude fiber versus total fiber.

Analysis of the NIT data on all the samples shows that the sum of the NIT components (protein, oil, starch, and fiber) is approximately 86% to 88%. The difference between the total of the reported components and the total dry matter (100%) is attributable largely to other unreported components such as ash, sugar, remaining fiber, etc. and any prediction errors in the reported NIT components. Other references for corn composition cite ash at 1.4%, sugar at 2.6%, and remaining fiber at 9.5%. (Rooney and Serna-Saldivar, 1987)

**Moisture** was determined using a Motomco 919 moisture meter equipped with a Model 3086 computer containing the calibration charts.

**Tests Performed for Food Grade/Dry Milling Use**

Three additional tests were performed on the samples of white, hard endosperm/food grade, and a portion of the producer, elevator, and export corn. They included the following:

**Germ-to-grit volume ratio** was determined on 15–20 kernels that were moistened overnight so that the germ and pericarp could be manually removed with a knife. The pericarp was discarded. The germs were separated from the remaining grit pieces and both were dried at 70°C (158°F) overnight to remove remaining moisture. The germs were placed in the smallest cup of a helium-air comparison pycnometer to measure volume. Similarly, the volume of the grits was obtained. The ratio of the germ volume to the grit volume was calculated. Typically, germ-to-grit volume ratios are in the range of 0.10–0.24 or 10–24%. A lower germ-to-grit volume ratio indicates smaller germs and relatively more endosperm, which is desirable for dry millers.

The **pericarp removal** test is important because in alkaline cooking any remaining pericarp catches on cutting wires of sheeting devices and causes poor quality chip products. Pericarp presence also affects color and texture and gives a non-uniform product appearance. A method to evaluate the ease of pericarp removal was given by Serna-Saldívar et al. (1991). Basically, corn was boiled for 20 minutes at 98–100°C (208.4–212°F) in a lime (3.33 g CaO/liter water) solution, cooled in tap water for 1 minute, immersed in a May-Gruenwald solution consisting of eosine, methyl blue and methanol which dyes any remaining pericarp a blue color. The sample of about 20 kernels was rinsed and visually scored from 1–5 with 1 given for pericarp totally removed and 5 for all pericarp still attached. Pericarp removal varies significantly by corn hybrid and growing location and soft endosperm corn typically loses pericarp more easily than hard corn.

The **vitreous endosperm** test involved visually determining the percent of vitreous endosperm in two replications of 20 kernels per replication. The kernel was placed germside up on a light box and the kernel was divided into regions. Each region was categorized for percentage of vitreous endosperm. It is a subjective test based on the criteria used.

**Tests Performed on High Oil, Nutritionally Enhanced, and Some Commodity Corn**

The amino acid profiles were performed by CN Laboratories in Courtland, Minnesota.

**Amino acid profiles** were generated for high oil, nutritionally enhanced, and some elevator corn samples. The amino acids were determined by the AOAC procedure, which involves ion exchange chromatography following either acid hydrolysis or, in the case of sulfur amino acids, performic acid hydrolysis.

**Tests Performed on Waxy Corn**

**Waxy purity** was performed on the waxy samples only. It was determined by counting two replications of 100 kernels, moistening them overnight, and then cutting off a small portion from the crown end of the kernel. The kernels were sprayed with a 0.5% iodine solution. Waxy corn contains nearly 100% amylopectin starch which turns a brownish color temporarily after expo-
sure to iodine. However, corn that contains amylose starch stains a blue or violet color which remains permanently. If 5% or less of the kernels stain blue or violet, the sample was considered to be waxy; however, it is preferable to have less than 2% of the kernels stain blue or violet.

**Milling Tests**

Wet and dry milling tests were performed by the Department of Agricultural Engineering at the University of Illinois, Urbana-Champaign.

The **100 g wet milling test** is a small-scale test which estimates the amount of starch and other coproducts which are recoverable with a conventional wet milling process. The test procedure is documented in Eckhoff et al. (1996). A 100 g sample of corn is steeped for 24 hours at 52°C (126°F) in a 200 ml solution containing 0.2% sulfur dioxide (put in as sodium metabisulfite) and 0.55% lactic acid. After steeping, the volume of steepwater remaining and the corn volume was measured prior to grinding the steeped kernels in a blender with dulled blades to release the germs. The resulting mash was sieved on a U.S. No. 7 sieve to retain germ and coarse fiber. The product was dried and the coarse fiber was separated from the germ by aspiration. The material passing through the sieve was finely ground using a Quaker City mill and sieved on a 200-mesh screen to recover cellular fiber. Remaining starch and protein were separated by tabling. Data was reported as a dry basis percentage of the original sample dry weight.

**Starch recovery** is the ratio of starch yield to the starch content multiplied by 100.

The **dry milling** test is an estimate of how the corn sample will fractionate in a conventional degeminating dry milling facility. Samples of approximately 1 kilogram were tempered for 18 minutes after the addition of 8.5% (by weight) water to the corn. The tempered corn was then passed through a horizontal drum degeminator, where the corn was impacted resulting in partial separation of the germ and fiber from the endosperm pieces. The product was dried one hour at 120°F (49°C) to approximately 15% moisture. A 10-15 g subsample was removed and used to determine a moisture content by drying at 265°F (130°C) for two hours. The remaining material was sieved using a laboratory box sifter. The largest sized fraction (+5) was roller milled and aspirated at 0.4-0.5 inches of water vacuum to remove the pericarp fraction. The “heavy” material was sifted on a 10-mesh screen to remove the “flattened” germ particles. The remaining endosperm fraction was weighed and identified as “large (flaking) grit”. The -5 portion was also rolled, aspirated and sifted. The “lifts” from the aspirator were added to the pericarp fraction. The “heavies” were sifted on a 10 mesh sieve. The +10 germ particles were added to the germ fraction and the -10 endosperm portion was sifted on a 24 mesh sieve. The +24 particles are identified as “small grit” and the -24 as “fines”. Data was reported on a dry basis percentage of the original sample dry weight.

**Interpreting Wet Milling Results**

Wet milling results are based upon a laboratory analysis which utilizes equivalent steeping and milling procedures for all samples. No effort was made to optimize the steeping conditions or milling procedures for individual samples. The steeping conditions used (24 hours, 0.2% sulfur dioxide, 0.55% lactic acid and 52°C) were selected as being reasonable operational parameters for yellow dent corn of average milling characteristics. Some phenotypes, such as high amylose and flint, are generally milled with longer steep times. Some yellow dent hybrids would also have increased starch yields if steeped longer. Longer steep times tend to moderate observed differences between hybrids.

Product quality factors such as protein content in the starch, gluten protein content, (RVA) Rapid Visco Analyzer or Amylograph starch characterization, and germ oil content were not measured because of the added cost and small amount of sample available for testing. However, the product quality factors are important and should be evaluated before selecting specific hybrids for wet milling applications.

The yields presented are representative of the process used and are not intended to indicate anticipated commercial wet milling yields. Results from this laboratory procedure correlate with commercial yields but absolute yield values observed in the wet milling plant will vary from facility to facility depending upon equipment and processing parameters used and may be more or less than yield values determined by the laboratory procedure. Some co-products’ yield values will be significantly different from industrial yields. Gluten meal will be lower in protein and higher in total yield for the 100 g procedure due to the use of tabling instead of centrifuges and the lack of recycling in the laboratory procedure. Steepwater solids will generally be lower for the 100 g procedure because of the use of process water as steepwater in commercial facilities.
The values reported represent single replicate tests. The standard deviations of measuring each mill product are listed in Table 5.1. Standard deviation indicates the precision of the test procedure to replicate results and are based upon several experiments with a large number of replicates for each sample.

### Table 5.1 Representative Ranges of Product Yields from Laboratory Wet Milling of Yellow Dent Corn

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Normal Yield Range (%)</th>
<th>Std. Dev. (%)</th>
<th>Representative Values (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steepwater</td>
<td>3.0 – 4.5</td>
<td>0.1</td>
<td>4.3</td>
</tr>
<tr>
<td>Germ</td>
<td>3.5 – 6.0</td>
<td>0.3</td>
<td>5.2</td>
</tr>
<tr>
<td>Fiber</td>
<td>10.0 – 16.0</td>
<td>0.5</td>
<td>10.2</td>
</tr>
<tr>
<td>Starch</td>
<td>64.0 – 69.0</td>
<td>0.4</td>
<td>67.3</td>
</tr>
<tr>
<td>Gluten</td>
<td>9.5 – 15.0</td>
<td>0.3</td>
<td>11.3</td>
</tr>
<tr>
<td>Total Solids</td>
<td>98.0 – 100</td>
<td>0.4</td>
<td>98.3</td>
</tr>
</tbody>
</table>

Data from Eckhoff et al. (1996)

**Interpreting Dry Milling Results**

Dry milling results are based upon a laboratory analysis which uses equivalent temper times and fractionation methods. No effort has been made to optimize product yields or value for any of the samples tested. Dry milling yield results are presented as a relative indication of the suitability of the sample for production of dry milled products. Yield values presented are not intended to be indicative of the values anticipated in a commercial dry milling facility because mills vary as to their equipment and process flows. The yield values may be higher or lower in commercial facilities but the relative ranking of yield among samples will correlate with commercial yields.

The degerminator used is a horizontal drum degerminator, which has been widely used in experimental laboratory testing of small samples at Iowa State University, University of Illinois, Kansas State University, University of Nebraska, and the National Center for Utilization of Renewable Resources-USDA. These degerminators have all been built from the same set of prints and are used because there is no commercially available laboratory scale corn degerminator.

The nomenclature to describe the size of endosperm pieces resulting from the degermination of the samples is based on the size of screen used in the separation. For example, the size fraction +5 represents grits which will be retained on a 5 -mesh screen. Mesh numbers represent the number of screen openings per inch in each direction on the screen; i.e., a -5 mesh screen has five openings per square inch of screen. The product between the 5 and 24 are small, hard pieces of endosperm. This percentage indicates the portion of 5 product that could be utilized as low fat (higher value) grit or meal. The -24 “fines” would be of higher fat content and be utilized as animal feed or other lower valued products. Many U.S. processors discard this fraction with the livestock feed since they have observed that this product increases prime product oil content.

The main parameters of interest are the yields of large grits (+5), small grits (+24), and fines (-24). Germ yield may also indicate how well the corn degeminate which affects the fat content of the grit products. Roller milling all fractions reduces the particle size of the endosperm while increasing the size of germ particles. These germ pieces contain oil and “flatten out” instead of breaking apart. Since the endosperm decreases and the germ increases in size, the germ can be sifted from the endosperm. Increased yield of large and small grits is preferred. Larger endosperm pieces from the degerminator tend to have lower fat content. Hominy feed is a mixture of the germ fraction (oil extracted or unextracted), the pericarp fraction and any unsalable high-fat meal and/or flour (-24). Effort is made to minimize this feed fraction.

Product quality factors such as grit oil content, attached pericarp on the grits, or germ fraction oil content were not measured because of the added cost and small amount of sample available for testing. These quality factors are important to the dry miller and should be evaluated before making specific hybrid selections.

Results shown represent single determinations. Standard deviations of yield measurements vary with grit size and product yield (Pan, 1992). Representative values for soft, medium, and hard endosperm yellow dent corn and ranges for dry milling yields are shown in Table 5.2.

### Table 5.2 Representative Ranges of Dry Milling Yields from a Horizontal Drum Degerminator

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Endosperm Hardness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Soft</td>
</tr>
<tr>
<td>Large grits (+5), %</td>
<td>23.3</td>
</tr>
<tr>
<td>Small grits (+24), %</td>
<td>29.3</td>
</tr>
<tr>
<td>Fines (-24), %</td>
<td>32.2</td>
</tr>
<tr>
<td>Total endosperm, %</td>
<td>84.7</td>
</tr>
<tr>
<td>Pericarp, %</td>
<td>6.9</td>
</tr>
<tr>
<td>Germ, %</td>
<td>8.5</td>
</tr>
</tbody>
</table>

The horizontal drum degerminator has been shown to rank hybrids in a similar manner to the Beall degerminator (the standard degerminator used by the U.S. dry milling industry).
Testing Methodology and Interpretation of Results for Sorghum

The following sections describe the sorghum quality tests performed as part of this report. All tests were performed by the Cereal Quality Laboratory at Texas A&M University, unless otherwise indicated.

Tests Performed on All Samples

Samples were graded by FGIS personnel in League City, Texas, using standard procedures for sorghum. Samples from export shipments were obtained by FGIS in League City from those used to determine the official grade. All the producer and commercial elevator samples were sent to FGIS in League City for official grade and grading factor determinations.

Samples were analyzed for proximate components, physical and milling properties by the Texas A&M Cereal Quality Laboratory. Chemical composition included moisture, oil, ash, protein, starch, NFE (nitrogen free extract) and crude fiber.

Chemical composition. NIR determinations of oil, moisture and protein were accomplished using a Perstorp NIR Systems Model 6500 instrument on ground grain. Protein was also run using the Dumas procedure which combusts the sample and measures the total nitrogen produced. The conversion factor was % nitrogen times 6.25. All values were expressed on a dry basis. Standard AACC procedures were used to determine crude fiber (extraction in standard base and acid), ash (ignition) and oil (ether extraction).

Starch was analyzed by completely gelatinizing the ground samples followed by enzymatic conversion to glucose which was determined colorimetrically. The percentage of glucose was converted to the starch percent.

For analysis the samples were cleaned; sound kernels were ground using an UDY grinder with a 1.0 mm screen. All samples were placed in cold storage during and between analysis. In general, the samples of grain were of excellent quality because of good conditions during maturation and harvesting of the 1999 crop.

The true density of the grain was measured by a nitrogen gas displacement multipycnometer model MVP-1 (Quantachrome Corp., Syosset, NY). Eighty grams of clean representative sample were used; the test was performed at a cell reference pressure of 15-17 psi. Volume and density of samples were calculated. The density was expressed in g/cm³. Density of sorghums ranges from 1.2 to 1.4 g/cm³. The higher the density the harder the grain is under most conditions. It is an index of the relative amounts of hard to soft endosperm in the kernel.

The test weight or bulk density of uncleaned sorghum was determined with a quart container according to FGIS standard procedures. Test weight was expressed in pounds per bushel. Test weight indicates the extent of grain plumpness and other factors that affected the grain during and post maturation. It also is an indirect measure of soundness of the kernels and is related to foreign material, moisture content and other conditions.

Thousand kernel weight was determined by weighing 1,000 whole, sound kernels of sorghum. It was expressed in grams. It indicates the relative size of the kernels which is affected by conditions during and post maturation of the grain.

Color of the grain and milled fractions was determined with a Minolta Chroma Meter Model CR-310, using a large sample adapter. L, a and b values were reported in duplicate. The L value is an index of the lightness of the sample where black is 0 and white is 100. The a value measures the red (+a) or green (-a) color while b indicates yellow (+b) and blue (-b), respectively. These methods are quite repeatable with a low coefficient of variability. Some of the sorghums have a yellow endosperm which shows up after milling to remove the pericarp while others have a white endosperm which gives a lighter color with less yellow hue.

Grain color is determined genetically and by the environment. Genetically, sorghums have red, white or colorless and yellow (lemon) pericarps while the endosperm is yellow or white. The testa of kernels with B1B2 S genes is reddish brown or purple and contains condensed tannins. Taken together the appearance of the kernel is determined by these factors. Kernel appearance should not be used to judge class of sorghum with the exception of the white class where color is restricted to white with no more than 2% kernels of color. The white class cannot have a pigmented testa. In addition, the secondary plant color varies from tan to red, black or purple. A food sorghum has a white pericarp and tan plant color with light color glumes. This reduces the adverse effects of mild weathering on grain color and milling yields and color of products.
contains any color grain that does not have a pigmented testa while white contains only white or colorless kernels without a pigmented testa. The tannin sorghums have a pigmented testa. Thus, sorghum often has a blend of yellow, white and red or reddish brown appearing kernels. Some users often feel that they are receiving mixed sorghum but that is not the case.

Most of the samples analyzed in this study were blends of white, yellow endosperm and red sorghums. However, they did not have pigmented testa so the sorghum class was correctly applied. The pericarp color does not affect the nutritional value of sorghums without a pigmented testa. However, the color of the mixed feed ration is affected by grains with high levels of red or brownish red color. The appearance of mixed formula feeds is sometimes important to certain feeders who do not like a dark colored ration. Thus, sorghum users should be aware that kernels can differ in color and appearance and still be classed as sorghum. White sorghums must contain 98 or more of white kernels.

**Single kernel hardness values** were obtained for the samples using the Perten Single Kernel Characterization system on clean kernels of sorghum. The values reported are the mean force required to crush 200 or more kernels. These hardness values are related to the yields of decorticated grain obtained with the Tangential Abrasive Dehulling Device (TADD). (Decorticated grain is grain that has had the outer layers removed usually by application of abrasive or attrition milling applied to the outside of the kernel.) Single kernel hardness testing methodology has only recently been applied to sorghum. It is affected by the relative proportion of the hard to soft endosperm in the sorghum kernel, kernel size and shape, moisture content and other factors. Moisture and temperature were controlled in these experiments.

**TADD hardness/sorghum milling behavior.** An estimation of endosperm hardness and milling behavior of sorghums was determined by abrasive milling using a TADD. The TADD has an aluminum oxide abrasive disk and is fitted with a 12-hole base for sorghum samples. Each hole was filled with 20 g of sample and milling was for 4 minutes to measure hardness. Decorticated grain is collected with a sample collector attached to a vacuum machine and weighed. TADD hardness (% weight removed after decorticating for 4 minutes) can be calculated as: (initial weight - decorticated grain weight) x (100/initial weight). The loss of material during abrasion subtracted from 100 gives the percent of dehulled grain obtained. This method is an effective method to assess relative dehulling properties of sorghum samples.

Milling evaluations were performed using the TADD and a Roll Over PRL Decorticator by milling the grain for varying time intervals. The yield of decorticated grain, the color of the grain and other properties were determined and used to adjust milling yields to comparable color which gives an excellent comparison among different types of sorghum.

**Phenolic compounds.** A modified Folin-Ciocalteu method was used to determine total phenols in sorghum grain samples. This method measures the redox potential of the phenolic compounds. Clean grain samples were milled in a UDY mill through a 1 mm screen before analysis. Total phenols were extracted by 20–30 ml of 1% HCl in methanol at room temperature using a 0.15–0.30 g sample for 2 hours in a shaker (Hahn, et al., 1984). The samples were centrifuged at 2000 rpm for 5 minutes. The supernatant (0.2 ml) was reacted with 0.4 ml Folin-Ciocalteu reagent and 0.9 ml of 0.5 M ethanolamine for 20 minutes at room temperature. Absorbance was read using a spectrophotometer at 600 nm. Gallic acid (0, 50, 100, 150 and 200 ppm) was used as the standard curve. The results were expressed as mg of gallic acid equivalents per gram of sample oven dry weight. All sorghums contain phenols but only those with a pigmented testa or subcoat contain condensed tannins. The pigmented testa is determined genetically during development of new sorghum cultivars. It has been discriminated against by the USA sorghum industry. Sometimes high levels of phenols are obtained because the glumes of sorghum contain significant levels of phenols. The analysis is on clean grain free of glumes.

**Chlorox bleach test.** The test was performed to determine presence of sorghum kernels with pigmented testa. It involves using KOH and NaOCl to remove the sorghum pericarp so that the colored testa can be identified. 15 g KOH, 15 g sorghum and 70 ml bleach were added to a flask which was heated at 60°C for 15 minutes with gentle stirring. The KOH/NaOCl mixture was removed: the seeds were rinsed with warm water, blotted dry and the percentage of kernels with a pigmented testa were counted. Reference standards of tannin sorghum and nontannin sorghum were used to insure that the test was properly conducted. This test will clearly determine if the sorghum sample contains tannin sorghum and at what level in the mixture.
Condensed tannins and anthocyanins. Modified vanillin-HCl method was used (Hahn and Rooney, 1984). This method is based on the ability of flavanols to react with vanillin in the presence of mineral acids to produce a red color. Ground samples (0.15–0.3 g) were extracted with 8 ml of 1% HCl in methanol for 20 minutes at 30°C in a water bath. The samples were centrifuged at 2000 rpm for 4 minutes. The supernatant (1.0 ml) was reacted with 5 ml vanillin reagent (0.5% vanillin + 2% HCl in methanol) for 20 minutes at 30°C. Blanks were run with 4% HCl in methanol in place of the vanillin reagent and subtracted. Absorbance was read at 500 nm and determined by subtracting blank readings from sample readings. Anthocyanin content was estimated using the blank readings (Hahn et al., 1984). The reference curve was developed by using commercial catechin to prepare a standard curve. The results were expressed as the mg of catechin equivalents per gram of sample dry weight. When the blanks are subtracted, this method gives a relative index of the amount of condensed tannins present in the grain. It will give only a trace amount of condensed tannins in sorghums that do not have a pigmented testa or undercoat. Catechins vary in properties so it is almost impossible to compare values across laboratories unless great care is taken to standardize the procedures and the same catechin standards are used. Tannic acid is not acceptable as a standard for sorghum tannins since there are no tannic acids (hydrolyzable tannins) in sorghum.

Statistical Tests Performed

Statistical tests were used to compare the sample results data from the 1999 and 1998 crops to determine if there were statistically significant differences in the means and variability of the two sets of data. These tests help answer questions such as the following:

1. Are the average true density values for the white corn samples from 1999 and 1998 significantly different?

2. Was there a significant change in the variability of stress cracks in the commodity samples from 1999 to 1998?

To answer these questions scientifically, statistical tests that are based on the characteristics of the data (i.e., mean and standard deviation) are employed. A quick view of the average corn moisture content may indicate they are different but it will not show if the difference is significant.

Test for Comparing Means

The t-test was used to determine if the means (averages) of the sample results were significantly different between 1999 and 1998. The t-test is based on the following equation (Dowdy and Wearden, 1991):

\[ t = \frac{\bar{y}_1 - \bar{y}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} \]

where \( s_p^2 = \frac{(n_1 - 1)s_1^2 + (n_2 - 1)s_2^2}{n_1 + n_2 - 2} \) and

\[ \bar{y} \] is the mean, \( s^2 \) is the variance (standard deviation squared), and \( n \) is the number of observations.

Once the t value has been calculated, it is compared to statistical tables to determine if the two means are significantly different and at what level of significance. When comparative data is shown in the tables of the report, means that are significantly different at the 95% confidence level are indicated. Significantly different at the 95% confidence level implies that we are 95% confident that the data generated in the test samples represent the “true” average of the corn and that the difference observed between the 1999 and 1998 corn crop for the specific item being measured represents a true difference and not a sampling error.

Test for Comparing Variances

The F-test was used to determine if the variances (standard deviation squared) of the sample results were significantly different between 1999 and 1998. The variance or standard deviation is a measure of variability in the sample results. The F-test is based on the following equation (Dowdy and Wearden, 1991):

\[ F = \frac{s_1^2}{s_2^2} \]

where \( s_1^2 \) is the variance of the first data set and \( s_2^2 \) is the variance of the second data set.

Once the F value has been calculated, it is compared to statistical tables to determine if the two variances are significantly different and at what level of significance. As with the means, when comparative data is shown in the tables of the report, variances that are significantly different at the 95% confidence level are indicated. Significantly different at the 95% confidence level implies that we are 95% confident that the data generated in the test samples represent the “true” average of the
corn and that the difference observed between the 1999 and 1998 corn crop for the specific item being measured represents a true difference and not a sampling error.

**Correlation Coefficients**

It is often useful to summarize the relationship between two datasets with correlation coefficients. We may want to know if there is a positive or negative relationship between test weights and dry milling yields. When test weights increase, do milling yields increase or decrease? In addition, we would like to know the strength of the relationship. Correlation coefficients provide answers to these questions.

Correlation coefficients provided in this report were calculated using the following equation:

\[
Corr(X,Y) = \frac{Cov(X,Y)}{Stdev(X) \times Stdev(Y)}
\]

where Stdev(X) and Stdev(Y) denote the standard deviation of X and Y,

and:

\[
Cov(X,Y) = \frac{\sum_{i=1}^{n} (X_i - \bar{X})(Y_i - \bar{Y})}{n-1}
\]

The correlation is always between -1 and +1. A positive correlation indicates that two variables are positively correlated which means they move in the same direction. When the value of one variable goes up the other goes up and when the value of one variable goes down the other goes down. Conversely, two variables that are negatively correlated move in opposite directions. The closer the correlation is to -1 or +1, the stronger the correlation.