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## Quality Characteristics of Soft Wheat Cultivars with Allis Milling

Milling quality is a highly heritable genetic trait. Milling-quality score consists of straight-grade flour yield, endosperm separation index (ESI) and friability. Other milling quality parameters are also generated from the Allis-Chalmers milling data. Data represent millings from a modified Allis-Chalmers mill of “shrivel-free” grain from various locations and/or crop years (1975-2008). Every effort has been adopted to insure that milling-quality data are representative of the cultivar. Nonetheless, there is always a measure of uncertainty in data representing a singularly milled cultivar. Known standard cultivars that are contained within a set are milled and then compared to the previous milling information for those cultivars. The break-flour yield, test weight and 1000-kernal weight for an individual sample are not especially useful parameters, but comparing the break-flour yields, test weights and 1000-kernel weights of the various known standards can be utilized to establish confidence in verification of the named standards provided in a set.

### Grain Handling

#### Grain Production

Historic varieties dating to 1808 and likely earlier were acquired through the National Small Grains Collection (located in Aberdeen, Idaho, and formerly in Beltsville, Maryland). The historic varieties are grown with contemporary cultivars and plant characteristics are compared with recorded plant descriptions to confirm the identity of the various varieties. The SWQL grows 100 to 200 cultivars/varieties each year in forty-square-foot plots.

#### Grain Cleaning and Sizing

Prior to 1985, most of the shriveled grain was removed mechanically utilizing a modified Carter-Day dockage tester or an air-flow scourer. However, some shriveled grain could be present in the remaining sample. In 1985, the Carter-Day was further modified to remove shriveled kernels by air aspiration. The ability to remove shrunken grain was greatly enhanced, but the processing time increased.

In 1989, a large air-aspirator was fabricated by the SWQL that reduced cleaning time significantly and removed shriveled kernels. In 2002, the SWQL began to re-evaluate cultivars that were tested prior to 1989 and update the milling information if needed. That effort was mostly completed in the summer of 2006.

Every cultivar designated for Allis milling is mechanically sized into three or four fractions on a SWQL-modified Carter-Day Dockage Tester and then aspirated. A maximum of 2500 grams can be aspirated at one time. Air flow is electronically adjustable and the lower density shriveled grain within each sized fraction is removed. Visual inspection through a lighted magnifier is used to ascertain that only sound grain remains. Once aspiration of the wheat has been completed, the clean sized fractions are blended. Test weight, 1000-kernel weight and moisture are determined prior to milling.

### **Weather and Environment**

Weather damaged cultivars that produce diminished milling quality can be difficult to identify if known standards are not incorporated within the field trial. In the northern soft wheat region, wet weather at or near harvest time occurred most years from 1990 to 2000 and again in 2003. Some cultivars prominent during that decade produced milling quality data unreflective of their true genetic potential. After a specific cultivar is identified that produced “invalid” milling data, that milling information will be replaced with the updated analysis. A cultivars’ revised milling score could increase by as much as two standard deviations.

An “off color” flour can appear in wheats which are genetically “white” when there is an excessive quantity of wet weather at harvest time. A yellowish flour color sometimes occurs in cultivars that are normally white when the environment “produces” a coarser granulating flour than normal.

Wet weather at harvest time will lower test weights and grain density, and can greatly increase the softness of the kernel so that the flour produces larger cookie spread, although milling-yield potential is not affected. Throughput at the 1st-break rolls is diminished with weathered wheat. However, since the wheat is softer, break-flour yield increases and less middling stock is passed to the reduction rolls. That would result in reduced energy required to power the rolls with less wear on the roll surface. More throughput could possibly be realized with softer-weathered wheat versus coarser type wheat if a double 1st-break system were employed.

Excessively wet weather at harvest time can damage wheat for milling quality. Sprouted wheat (after aspiration) can possess higher test weights than unsprouted wheats. After aspiration to remove shriveled grain, a sprouted wheat may have a test weight in excess of 60# / bushel compared to weathered, unsprouted, non-shriveled wheat with 57# / bushel test weight. Alpha-amylase activity may be present despite a lack of visual evidence of sprouting.

Moderate infection from leaf diseases apparently does not affect milling properties once damaged (shriveled) kernels have been removed, however, baking quality of sugar snap cookies may be affected.

## **Milling Methods**

### **Allis Mill**

The Allis-Chalmers mill was acquired in 1909 by the Ohio Agricultural Experiment Station. Chester Evans, a practical miller, was put in charge of the milling operation and baking plant. Mr. Evans came to the station from Williams Brothers Milling, Kent, Ohio. Apparently the Allis-Chalmers mill was donated to the Soft Wheat Quality Laboratory around 1937. The mill was extensively modified during the early 1970's: self-aligning, double-row roller bearings, and extensions manufactured for the roll spacing control arms. A one-inch movement of the control arm around a twenty-four inch radius is equal to one thousandth of an inch (25 microns) change in roll separation. The standard deviation for flour yield of duplicate millings is 0.15%.

Kernel weight is determined on each cleaned sample and grain volume weight measured. Following grain measurements, samples are tempered to 15% moisture. Tempered grain is milled on the SWQL Allis-Chalmers flour mill using the AACC method 26-32 as modified by Yamazaki and Andrews (1982)<sup>1</sup>. The Allis-Chalmers mill is a long flow experimental milling system with adjustable roll gaps. Grain is initially milled with 6 break roll passes then reduced in 7 reduction roll passes to produce straight grade flour. The roll settings, sifting screen sizes, and mill flow were as diagramed in Yamazaki and Andrews (1982).

For each grain sample, straight grade flour yield and break flour yield are recorded.

### **Data Analysis and Interpretation of Allis Milling**

Since milling quality is a highly heritable genetic trait, excluding weather damaged examples; a single sample likely will produce representative milling yield, ESI and friability. Also, lactic acid solvent retention capacity values within a milling system are highly heritable in all published genetic studies of wheat. However, test weight, kernel weight, break flour yield, cookie baking, flour protein and ash can be influenced significantly by environmental variations. Usually, mean data from three millings will yield quality assessments that are more representative of those traits that are less stable. The number of samples included in the computation of the average is specified for each cultivar. A cultivar that has been composited from several locations/crop years may produce quality data more nearly reflects its genetic nature. Cultivars listed in the tables that have a "c" beside the "number for the average" indicate that a composite sample has been milled to generate the quality data.

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<sup>1</sup> Yamazaki, W.T. and L.C. Andrews. 1982. Experimental milling of soft wheat cultivars and breeding lines. *Cereal Chem.* 59:41-45.

### **Miag Multomat Mill**

The Miag Multomat Mill is a pneumatic conveyance system consisting of eight pair of 254 mm diameter x 102 mm wide rolls, and ten sifting passages. Three pair are corrugated and employed as break rolls and five pair are smooth rolls utilized in the reduction process. Each sifting passage contains six separate sieves. The two top sieves for each of the break rolls are intended to be used as scalp screens for the bran. The third break sieving unit of the Soft Wheat Quality Laboratory (SWQL) Miag Multomat Mill was modified so that the top four sieves are employed to scalp bran. That modification increased the final bran sieving surface by 100% and essentially eliminated any loss of flour. Thus, the mill very closely approximates full scale commercial milling.

**Experimental Milling Procedure:** All SRW varieties are tempered to a 14.0% moisture level. Generally tempered wheat is held for at least 24 hours in order for the moisture to equilibrate throughout the grain. Wheat is introduced into the first break rolls at a rate of 54.4 Kg/hour (120 #/hour). Straight grade flour is a blend of ten flour streams, the three break flour streams and the five reduction streams, plus the grader flour from the break streams and the duster flour from the reduction streams. The straight grade flour mean volume diameter is about 50 microns with ash content usually between 0.42% and 0.52%.

Flour generated by the (SWQL) Miag Multomat Mill very nearly represents that of commercially produced straight grade flour. Bran, head shorts, tail shorts and red dog are by-products which are not included with the flour. Flour yields vary between 70% and 78% which is variety dependent due to milling quality differences and/or grain condition. Sprouted and/or shriveled kernels negatively impact flour production. Recovery of all mill products is usually about 99%. Least significant differences for straight grade flour yield and break flour yield are 0.75% and 0.82%, respectively.

### **Quadrumat Junior Flour Mill**

#### **Micro Milling Method**

Based on average whole grain moisture determination of a subset of the group to be milled, samples are tempered to 15% moisture. Sample preparation for moisture determination uses the low speed Tag-Heppenstall corrugated rolls that have a roll speed differential of 1:1. Tempered grain samples are milled after 48 hours to allow for equal water distribution throughout the kernel.

Samples are milled in a control temperature and humidity room (19 – 21 C and RH 55% - 60%). Milling is conducted on a modified Quadrumat Junior flour mill. Prior to sample analysis, mill should be operating, warm, and equilibrated (36 C + /- 1.0) when mill has equilibrated. Standard sample size for micro milling is 80 g, although other sample sizes can be used. Tempered grain is milled and the product recovered for sifting on a Great Western Sifter Box. The sifter should have 40 mesh and a 94 mesh screen to separate mill product into bran (above 40), midds (between 40 and 94) and flour (through 94 screen and recovered in the flour pan on the bottom).

To calculate softness equivalent (a modified particle size index), the weights of the bran and midds are recorded. The midds are added back to the flour that passed through the 94 mesh screen to produce the final flour product for analysis.

### **Advanced Milling Method**

Midds from micro milling method are further processed as reduction milling on a second Quadrumat Junior mill and sieved as for the micro milling method using an 84 mesh screen to produce baking quality flour. Standard sample size for advanced milling is 200 g, and grain samples are tempered individually to 15% moisture prior to milling. Milled flour is passed through an 84 mesh screen and combined with flour from the micro milling for baking.

Because samples are tempered individually to 15%, the formulas for advanced milling yield are calculated without the adjustment to 15% moisture.

### **Milling Tests**

**Endosperm Separation Index (ESI)** was calculated as described by Yamazaki and Andrews (1982). ESI is the estimated endosperm adhering to bran and bran pieces after the third through fifth break passes and first reduction pass, expressed as a percentage based on the weight of milled grain divided into the flour recovered in the break rolls after the second break stream and the reduction rolls after the first break. Lower ESI values indicate better bran separation from endosperm and better milling quality than higher ESI values.

The quantities of final bran plus four other bran-rich fractions obtained at an intermediate stage of milling are recorded and essentially represent all of the bran. The bran (14.5%) and the germ (2.5%) are subtracted to yield endosperm remaining attached to the bran. The lower that value is, the better the separation was between endosperm and bran. Thus, a lower ESI value indicates better wheat for milling since less energy is required to produce straight-grade flour.

**Friability** Gaines et al., 2000<sup>2</sup>, estimated the ease with which mill stock is reduced to flour. Friability is calculated by dividing the weight of flour recovered during milling by the summed weight of mill stock passed through all roll stands, break and reduction, after the first break. The earlier in the break and reduction process that flour is recovered, the lower the weight of mill stock that passes to the later break and reduction rolls. Higher values of friability indicate better milling efficiency and reduced energy requirements to recover flour.

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<sup>2</sup> Gaines, C.S., P.L. Finney, and L.C. Andrews. 2000. Developing agreement between very short flow and longer flow test wheat mills. *Cereal Sci.* 77:187-192.

Friability is the tendency of the wheat endosperm conglomerates to reduce to flour as a result of corrugated and smooth roll action. The cumulative quantity of stock entering the rolls (usually 20 streams) and the percent of flour extracted from the stock relate to the total energy consumed by the milling process. A higher percentage of friability means that less energy is required per unit of flour extraction.

Friabilities above 30.5% are rare and only exceptionally good-milling wheats fall into this category. Those cultivars displaying friabilities below 27% usually reflect very poor reduction of middling stock on the smooth rolls.

Poor milling-quality cultivars produce middling stocks which do not release flour well after being crushed on the smooth rolls, resulting in higher quantities of carry-over to subsequent reduction rolls. Cultivars that have reduced milling properties due to “weathering” do not reduce well on the smooth rolls and the endosperm and bran do not separate well on the corrugated rolls.

Milling a cultivar with a friability of 25% compared to one of 30% would produce about a 15% increase in the amount of stock entering the corrugated and smooth rolls of the SWQL Allis-Chalmers mill. When milling 60,000 # (1000 bu) of wheat per hour, the quantity passing thru the SWQL mill (not including 1st break) would be 179,000 # of stock for the cultivar with lower friability compared to 156,000 # for the cultivar with higher friability. The cultivar with friability of 25% would also yield about 3.5% less flour.

### **Flour yield**

Flour yield “as is” is calculated as the bran weight (over 40 weight) subtracted from the grain weight, divided by grain weight and times 100 to equal “as is” flour yield. Flour yield is calculated to a 15% grain moisture basis as follows: Flour moisture is regressed to predict the grain moisture of the wheat when it went into the Quad Mill using the formula  $\text{Initial grain moisture} = 1.3429 \times (\text{flour moisture}) - 4$ . The flour yields are corrected back to 15% grain moisture after estimating the initial grain moisture using the formula  $\text{Flour Yield}_{(15\%)} = \text{Flour Yield}_{(\text{as is})} - 1.61\% \times (15\% - \text{Actual flour moisture})$ .

### **Softness Equivalent**

Softness Equivalent (as is) is calculated from the fraction of mill product that is in the mids, with smaller amounts of mids correlating to smaller particle size, greater break flour yield, and greater softness equivalent. The mids weight (over 94) is subtracted from the unadjusted flour yield to calculate the quantity of fine flour that passed through the 94 mesh, which is divided by the unadjusted flour yield and multiplied by 100%. Softness Equivalent at 15% grain moisture is calculated using the estimated grain moisture prior to milling (see milling formulas). The softness equivalents are adjusted to 15% grain moisture with the formula  $\text{Softness Equivalent}_{(15\%)} = \text{Softness Equivalent}_{(\text{as is})} - 1.08\% \times (15\% - \text{Actual flour moisture})$ .

**Flour yield adjustment**<sup>3</sup> based on flour particle size 52% is subtracted from the actual softness equivalent. That difference is multiplied times 0.17% which is the change in flour yield per percentage point change in softness equivalent. Therefore, Adjusted Flour Yield= Flour Yield<sub>(15%)</sub> + (Softness Equivalent<sub>(15%)</sub> - 52%)<sup>4</sup>.

**Mill Score:** Mill score represents a standard adjustment based on flour yield by comparing the test cultivar to a check. The check cultivar produces a score that can be used as a handicap against its traditional expected yield, and the test cultivar mill score is adjusted to the same degree as the check. This method relates test cultivars providing a score that is independent of the environmental influences. The mill score standard deviation will be about 1.43 when evaluating cultivars and test lines that have been grown and harvested together.

## Kernel and Whole Wheat Tests

**Test Weight:** (AACC Method 55-10) Weight per Winchester bushel of cleaned wheat subsequent to the removal of dockage using a Carter-Day dockage tester. Units are recorded as pounds/bushel (lb/bu) and kilograms/hectoliter (kg/hl).

**1000 Kernel Weight:** Units are recorded as grams/ 1000 kernels of cleaned wheat. There is little difference between 1000-kernel weight and milling quality when considering shriveled-free grain. However, small kernelled cultivars that have 1000-kernel weight below 30 grams likely will have reduced milling yield of about .75%.

**Single Kernel Characterization System (SKCS):** (AACC Method 55-31) SKCS distribution showing % soft (A), semi-soft (B), semi-hard (C), and hard (D); SKCS hardness index; SKCS moisture content; CKCS kernel size; and SKCS kernel weight; along with standard deviations.

**Whole Wheat Moisture:** (AACC Method 44-15A) Air-oven method.

**Whole Wheat Crude Protein:** Nitrogen combustion analysis using Elementar Nitrogen Analyzer. Units are recorded in % protein converted from nitrogen x 5.7 and expressed on 12% moisture basis.

**Whole Wheat Falling Numbers:** (AACC Method 56-81B) Units are expressed in seconds using the Perten Falling Numbers instrument.

**Whole Wheat - Amylase Activity:** (AACC Method 22-06) Units are expressed in alpha amylase activity as SKB units/gram (@ 25°C).

<sup>3</sup> On the small Quad Mill, coarser type soft wheats will appear to mill better than they should and conversely, softer type soft wheats will have suppressed "as is" flour yields.

<sup>4</sup> Micro milling adjustments were developed by Lonnie Andrews with Patrick Finney and Charles Gaines. Additional details are included in the Standard Operating Procedures for the Soft Wheat Quality Laboratory

## Flour Tests

**Flour Moisture:** (AACC Method 44-15A) Units are expressed as % of flour.

**Flour Crude Protein:** Protein determined by NIR using a Unity NIR instrument calibrated by nitrogen combustion analysis using Elementar Nitrogen Analyzer. Units are recorded in % protein converted from nitrogen x 5.7 and expressed on 14% moisture basis.

Flour protein differences among cultivars can be a reliable indicator of genetic variation provided the varieties are grown together, but can vary from year to year at any given location. Flour protein from a single, non-composite sample may not be representative. Based on the Soft Wheat Quality Laboratory grow-outs, protein can vary as much 1.5 % for a cultivar grown at various locations in the same ½ acre field.

Flour protein of 8% to 9% is representative for breeder's samples and SWQL grow-out cultivars. As flour protein increases, the expansive capability of the cookie during the baking process decreases. Flour protein is negatively correlated to cookie diameter ( $r = -0.62$ ,  $p < 0.0001$ ) with the cookie shrinking 0.4 cm for every 1 percentage point increase in protein<sup>5</sup>. The effect of flour protein on cookie size is related in part to increased water absorption due to greater protein content, however the amount of cookie shrinkage is greater than that explained by increased water absorption alone.

**Protein quality** is an evaluation of "elasticity" or gluten strength and is not the same as protein quantity. A cultivar possessing a low quantity of protein could still exhibit strong gluten strength. Gluten strength is thought to be a desirable characteristic for cracker production. Gluten strength is measured using a mixograph and is graded on a scale of 1-8, with 1 as weakest and 8 as strongest gluten. Evaluation of gluten strength using the mixograph or farinograph is difficult for soft wheat flours that are 8.5% protein and lower. Since the representative protein range for breeders' samples is 8-9%, many of these flours are not adequately evaluated using the mixograph or farinograph methods. The Lactic Acid SRC, which does not require mixing action to assess gluten, tends to be a better measurement of protein quality when evaluating soft wheats. Lactic acid hydrates the native matrix of insoluble polymeric protein (IPP) present in the flour.

**Flour Ash:** (AACC Method 08-01) Basic method, expressed on 14% moisture basis.

**Flour Falling Numbers:** (AACC Method 56-81B) Units are expressed in seconds using the Perten Falling Numbers instrument. Numbers above 400 seconds reflect factors other than alpha amylase activity (such as particle size). The correlation between alpha amylase activity and falling number is best for samples with falling number values between 200 and 300 seconds. For cake flours and batters, 350 seconds is a common minimum value. For breakfast cereals or cookies and other high sugar products values of 250 seconds are more common cut-off values.

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<sup>5</sup> Correlations and prediction models cited in this section are based on 2289 samples milled at the Soft Wheat Quality Laboratory in 2007 and 2008 on the Quadrumat Advanced milling system.

**Flour Amylase activity:** (AACC Method 22-06) Units are expressed in  $\alpha$ - amylase activity as SKB units/gram (@ 25°C).

**Flour micro Alpha Amylase activity:** (Adapted by Mary Gutierri) The new method adapts AACC Method 22-02 using the Ceralpha K-CERA (Megazyme) alpha amylase assay procedure for measuring alpha amylase activity at higher throughput in a microwell plate. All reagents, controls and precautions are as described in the Megazyme manual. Units are expressed as described as Ceralpha Units per gram (CU/g). The new assay is described completely at the end of this section as the **New Micro Assay for Flour Alpha Amylase Activity.**

**Solvent Retention Capacity Test (SRC):** (Flour Lactic Acid, Sucrose, Water, and Sodium Carbonate Retention Capacities AACC Method 56-11)  
Units are expressed as %.

**Water SRC** is a global measure of the water affinity of the macro-polymers (starch, arabinoxylans, gluten, and gliadins). It is often the best predictor of baked product performance. Water SRC is correlated to Farinograph water absorption but does not directly measure the absorption of the glutenin macropolymer hydration during mixing as does the Farinograph. Water SRC is negatively correlated to flour yield and softness equivalent among flour samples milled on the Quad advanced flour mill ( $r=-0.43$  and  $r=-0.45$ , respectively). Lower water values are desired for cookies, cakes, and crackers with target values below 51% on small experimental mills and 54% on commercial or long-flow experimental mills.

**Sucrose SRC** is a measure of arabinoxylans (also known as pentosans) content, which can strongly affect water absorption in baked products. Water soluble arabinoxylans are thought to be the fraction that most greatly increases sucrose SRC. Sucrose SRC probably is the best predictor of cookie quality with sugar snap cookie diameters decreasing by 0.07 cm for each percentage point increase in sucrose SRC. The negative correlation between wire-cut cookie and sucrose SRC values is  $r=-0.66$  ( $p<0.0001$ ). Sucrose SRC typically increases in wheat samples with lower flour yield ( $r=-0.31$ ) and lower softness equivalent ( $r=-0.23$ ). The cross hydration of gliadins by sucrose also causes sucrose SRC values to be correlated to flour protein ( $r=0.52$ ) and lactic acid SRC ( $r=0.62$ ). Soft wheat flours for cookies typically have a target of 95% or less when used by the US baking industry for biscuits and crackers. Sucrose SRC values increase by 1% for every 5% increase in lactic acid SRC. The 95% target value can be exceeded in flour samples where a higher lactic acid SRC is required for product manufacture since the higher sucrose SRC is due to gluten hydration and not to swelling of the water soluble arabinoxylans.

**Sodium carbonate SRC** is a very alkaline solution that ionizes the ends of starch polymers increasing the water binding capacity of the molecule. Sodium carbonate SRC increases as starch damage due to milling increases. Sodium carbonate is an effective predictor of milling yield and is negatively correlated to flour yield on the Quad advanced milling system ( $r=-0.48$ ,  $p<0.0001$ ). It also is one of several predictors of cookie diameter ( $r=-0.22$ ,  $p<0.0001$ ). Normal values for good milling soft varieties are 68% or less.

**Lactic acid SRC** measures gluten strength. Typical values are below 85% for “weak” soft varieties and above 105% or 110% for “strong” gluten soft varieties. See the above discussion of protein quality in this section for additional details of the lactic acid SRC. Lactic acid SRC results correlate to the SDS-sedimentation test. The lactic acid SRC is also correlated to flour protein concentration, but the effect is dependent on genotypes and growing conditions. The SWQL typically reports a protein-corrected lactic acid SRC value to remove some of the inherent protein fluctuation not due to cultivar genetics. Lactic acid is corrected to 9% protein using the assumption of a 7% increase in lactic acid SRC for every 1% increase in flour protein. On average across 2007 and 2008, the change in lactic acid SRC value was closer to 2% for every 1% protein.

**Flour Damaged Starch:** Chopin SDMatic starch damage instrument using the supplied AACC calibration. Starch damage is a measure of the damage to the starch granule occurring during the milling process.

## Experimental Baked Product Tests

**Wire Cut Cookie:** (AACC Method 10-53, Macro Method)

This method determines the texture (hardness) of the cookies. The use of high-fructose corn syrup and lower sucrose concentration allows for a texture more similar to standard commercial cookie formulations. Differences in hardness reflect differences in flour quality, with softer cookie texture produced with better soft wheat quality.

**Baking Quality of Cookie Flour:** (AACC method 10-52, Micro Method)

See new method presented in this document. Diameter and stack height of cookies baked according to this method are measured and used to evaluate flour baking quality. All data reported in this report were produced using the accepted method prior to December, 2008.

Cookie spread determined within a location is a reliable indicator of the source cultivar’s genetic characteristics. However, cookie spread, unlike milling quality, is greatly influenced by environmental conditions. An absolute single value for cookie spread could be misleading. Within a location the single value is significantly important in comparison to known standards. The average cookie spread for three different examples of a cultivar is representative of that wheat.

Cultivars with larger cookie spreads tend to release moisture efficiently during the baking process due to lower water absorption while cultivars yielding smaller diameter cookies tend to be higher in water absorption and hold the moisture longer during baking.

The best single predictor of cookie diameter is sucrose SRC. The strong negative correlation of sucrose SRC to cookie diameter ( $r=-0.66$ ,  $p<0.0001$ ) has led to its adoption in lieu of baking cookies for most samples. The best prediction model for cookie diameter among grain samples milled on the Quadrumat advanced system uses a combination of sucrose SRC, softness equivalent, and flour protein ( $R^2=0.61$ ). These three measures are combined into the baking quality score used in Quad Micro milling with the baking quality score favoring lower sucrose SRC and flour protein and greater softness equivalent values.

Cultivars that possess excellent milling properties nearly always produce large diameter cookie spreads. Poor milling cultivars nearly always produce smaller cookie spreads. Cultivars that are very soft in granulation usually produce good cookie spreads.

## **AACCI Method 10-52 Baking Quality of Cookie Flour - Micro Method**

First Approval December, 2008

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### Objective:

In North America, a “cookie” is a product similar to what is internationally known as a “biscuit”. Cookie quality of flour is determined by the interaction among endogenous components of the flour and the ingredients in the mix. This method establishes a carefully controlled competition for water among the various components and ingredients, the results of which are manifest as differing cookie diameters. Larger diameter cookies are preferred and an indicator of good pastry-making and specifically cookie-baking potential. The method is also useful to evaluate other flour types, various flour treatments and other factors, such as ingredients, that affect cookie geometry.

### Apparatus

1. National cookie dough micromixer, with head speed of 172 rpm and special cookie dough bowl.
2. Electric mixer, with timer control (Hobart or Kitchen-Aide, with paddle attachment.
3. Aluminum cookie sheet. See note 1.
4. Rolling pin, 5.7 - 7 cm (2.25 - 2.75 in.) diameter. If wood, check for wear to edges from use and replace if necessary.
5. Cookie cutter, 60 mm inside diameter.
6. Small plastic spatula, ground flat at end, with notch cut to fit cookie dough bowl and mixing head pins.
7. Thermometer and humidity meter / hygrometer (see note 2).
8. Baking oven, reel or rotary, electrically heated and capable of maintaining temperature of  $205^{\circ}\text{C} \pm 2^{\circ}$  ( $400^{\circ}\text{F} \pm 4^{\circ}$ ). See note 3.
9. Measuring calipers (large enough to measure 22 cm)

### Reagents

1. Solution A. 0.95 M sodium bicarbonate (79.8 g dissolved in water to make 1L).
2. Solution B. 1.9 M ammonium chloride / 1.52 M sodium chloride (101.6 g and 88.8 g respectively, dissolved in water to make 1 L).
3. Sucrose. Any brand of “Baker’s Special” sugar: sugar passing through a US No.30 sieve (595  $\mu\text{m}$  openings) only. Particle size affects solubility.
4. Shortening. Non-trans fat, vegetable shortening not containing methyl silicone of medium consistency (e.g. Crisco non-trans fat shortening).
5. Nonfat dry milk. To pass through a US No. 30 sieve (595  $\mu\text{m}$  openings).

AACCI Method 10-52  
New Method - Baking Quality of Cookie Flour (cont'd.)

Procedure

The total formulation amounts of each cookie pair are listed in Table I.

1. Sift dry ingredients (sucrose, nonfat dry milk, dry sodium bicarbonate; Table II for sufficient creamed mass for different batch sizes, 21-46 cookie pairs; 37.60 g for each pair) together until well-mixed. Cream these ingredients together with shortening using Hobart or Kitchen-Aide mixer, using a paddle attachment, on low speed 1 min, then scrape bowl and paddle; on medium speed 1 min, then scrape; on high speed 30 sec, then scrape; and on high speed 30 sec. Weigh 37.60 g portions of this creamed mass for each cookie-pair to be baked.
2. Scrape measured creamed mass into cookie dough mixing bowl (National cookie dough micro-mixer, using a cookie dough bowl; head speed 172 rpm). Add water as shown in Tables I and III: add 4.0 mL solution A, 2.0 mL solution B, and additional water (use water amount in Table III for appropriate flour moisture; 8.7 mL total water per cookie pair). Mix 3 min (stopping mixer and scraping after first few sec if shortening is stuck on side of bowl) and scrape with small spatula.
3. Add 40 g flour (14% mb, weight per Table III) to mixing bowl. Mix a total of 25 sec. as follows: Mix for the first 10 sec while tapping side of bowl. Scrape dough from mixer and bowl pins; scrape outer edge and bottom of bowl, pushing dough between pins several times. Mix 5 sec and scrape as just described. Mix 5 sec and scrape. Mix 5 sec and scrape mixer pins.
4. Gently scrape dough from bowl, gently form into a single dough mass and cut with spatula into two equal portions. Transfer to a room-temperature cookie sheet with gauge strips. Roll to thickness with one forward and one backward stroke of rolling pin. Cut dough with cookie cutter, discard excess dough, and remove cutter.
5. Immediately place in oven and bake for 10 min. Remove sheet from oven. Cool 5 min and remove cookies from baking sheet.
6. After cookies have cooled to room temperature (at least 30 min), measure cookie diameter using calipers, or image analysis. Lay two cookies edge-to-edge and measure width. Rotate one cookie 90°, the other 45°. Measure again. Rotate both cookies 90° and measure again. Repeat. Average the four readings and divide by two to obtain average diameter of one cookie.

## New Method - Baking Quality of Cookie Flour (cont'd.)

## Notes

1. Aluminum cookie sheets made of 3003-H14 aluminum alloy, 2.0 mm (0.08 in) thick, 30.5 X 40.6 cm (12 X 16 in) or 25.4 X 33.0 cm (10 X 13 in), or other sizes required to accommodate oven doors and shelves. Cookie sheets should be manufactured with gauge strips fastened to the long edges of the sheets (gauge strips made of the same alloy as the sheets, 7 mm (0.275 in) thick and the length of the baking sheets). New sheets should be conditioned by lightly greasing and placing in hot oven for 15 min, cooling, and repeating the process two or three times. Cookie sheets should have excess grease wiped off after each cookie pair is baked. Cookie sheets should be washed while warm in water (without use of soap or detergent) and wiped dry after each bake.
2. Dough consistency, stickiness and cookie spread are affected by temperature and humidity. Room and ingredient temperature and humidity should be maintained at constant level among bakes ( $21^{\circ}\text{C} \pm 1^{\circ}$  ( $70^{\circ}\text{F} \pm 2^{\circ}$ ) and 30 - 50% are recommended, respectively). Consistent environmental conditions are more important in a lab than adherence to a particular level, within reason.
3. Oven should have a hearth consisting of ceramic-fiber-reinforced structural alumina refractory product (6.4 mm (0.25 in)) thick as shelf liner cut to dimensions of and placed on the steel baking shelf. Oven shelves consisting of wire mesh baking surface are also suitable and may not need shelf liner (to prevent excessive bottom browning).
4. For relatively consistent mixing action, recommended cream mass batch size is 21 - 46 units. Obtain amounts of sugar, nonfat dry milk, sodium bicarbonate and shortening from Table I.
5. Oven should be heated to temperature with oven shelves turning. Bake "dummy" cookies out of scrap dough or extra flour to condition the oven before beginning a test bake, at the beginning of a baking series, or if the oven has not been used for 15 min or longer.

## References

1. Bettge, A.D. and Kweon, M. 2009. Report on collaborative testing of AACCI Method 10-52: Baking quality of cookie flour - micro method. *Cereal Foods World*.
2. Finney, K.F., Yamazaki, W.T., and Morris, V.H. 1950. Effects of varying quantities of sugar, shortening and ammonium bicarbonate on the spreading and top grain of sugar-snap cookies. *Cereal Chem.* 27:30.
3. Gaines, C.S. 1982. Influence of ambient temperature, humidity and flour moisture content on stickiness and consistency in sugar-snap cookie doughs. *Cereal Chem.* 59:507.
4. Kissell, L.T., Marshall, D.B., and Yamazaki, W.T. 1971. Effect of variability in sugar granulation on the evaluation of flour cookie quality. *Cereal Chem.* 50:255.

## New Method - Baking Quality of Cookie Flour (cont'd.)

**Table 1. AACCI Method 10-52 Ingredient amounts per cookie pair**

Ingredient	Amount
Flour (14% mb)	40 g
Sucrose	24 g
Nonfat dry milk	1.2 g
NaHCO <sub>3</sub>	0.40 g
NaHCO <sub>3</sub> (in Soln A)	0.32 g (in 4 mL)
NH <sub>4</sub> Cl (in Soln A)	0.20 g (in 2 mL)
NaCl (in Soln B)	0.18 g
Shortening	12.0 g
Added Water <sup>1</sup>	2.7 mL

<sup>1</sup>Based on moisture of flour, adjusted water was added (see table 3)

**Table 2. AACCI Method 10-52 Ingredient weights for batch preparation.****Ingredient weights (g) for preparing creamed mass for different batch sizes**

Ingredient	20	25	30	35	40	45
Sucrose <sup>1</sup>	504.0	624.0	744.0	864.0	984.0	1104.0
Nonfat dry milk	25.2	31.2	37.2	43.2	49.2	55.2
Sodium bicarbonate	8.4	10.4	12.4	14.4	16.4	18.4
Shortening	252.0	312.0	372.0	432.0	492.0	552.0

**Table 3. AACCI Method 10-52 Calculated amounts of flour and added water for cookie test formula.**

Flour moisture (%)	Added Water (g or mL)	Flour (g)	Flour moisture (%)	Added Water (g or mL)	Flour (g)
9.1	4.9	37.8	12.1	3.6	39.1
9.2	4.9	37.8	12.2	3.5	39.2
9.3	4.8	37.9	12.3	3.5	39.2
9.4	4.7	38.0	12.4	3.4	39.3
9.5	4.7	38.0	12.5	3.4	39.3
9.6	4.6	38.1	12.6	3.3	39.4
9.7	4.6	38.1	12.7	3.3	39.4
9.8	4.6	38.1	12.8	3.3	39.4
9.9	4.5	38.2	12.9	3.2	39.5
10.0	4.5	38.2	13.0	3.2	39.5
10.1	4.4	38.3	13.1	3.1	39.6
10.2	4.4	38.3	13.2	3.1	39.6
10.3	4.3	38.4	13.3	3.0	39.7
10.4	4.3	38.4	13.4	3.0	39.7
10.5	4.3	38.4	13.5	2.9	39.8
10.6	4.2	38.5	13.6	2.9	39.8
10.7	4.2	38.5	13.7	2.8	39.9
10.8	4.1	38.6	13.8	2.8	39.9
10.9	4.1	38.6	13.9	2.7	40.0
11.0	4.0	38.7	14.0	2.7	40.0
11.1	4.0	38.7	14.1	2.7	40.0
11.2	4.0	38.7	14.2	2.6	40.1
11.3	3.9	38.8	14.3	2.6	40.1
11.4	3.9	38.8	14.4	2.5	40.2
11.5	3.8	38.9	14.5	2.5	40.2
11.6	3.8	38.9	14.6	2.4	40.3
11.7	3.7	39.0	14.7	2.4	40.3
11.8	3.7	39.0	14.8	2.3	40.4
11.9	3.7	39.0	14.9	2.3	40.4
12.0	3.6	39.1	15.0	2.2	40.5

### **New Micro Assay for Flour Alpha Amylase Activity**

Adapted by Mary Guttieri for the Soft Wheat Quality Laboratory

The new method adapts the AACC Method 22-02 using the Ceralpha K-CERA (Megazyme) alpha amylase assay procedure for higher throughput to determine flour alpha amylase activity in a microwell plate. All reagents, controls and precautions are as described in the Megazyme manual.

#### ***Required Materials***

- Ceralpha Alpha Amylase Kit (AACC Method 22-02)
- 50 mL conical centrifuge tubes
- Centrifuge with rotor to spin 50 mL conical tubes at 1000 xg
- Analytical balance
- Microplate reader and plate (510 nm)
- Vortex mixer
- Water bath at 40° C
- Multichannel repeating pipette

#### ***Ceralpha Substrate and Stopping Reagent***

Ceralpha substrate is prepared as described and stored frozen (-20°C) in 1 mL aliquots in microcentrifuge tubes.

Additional Stopping Reagent is prepared using 1% w/v sodium phosphate tribasic dodecahydrate in distilled water adjusted to pH11.

#### ***Enzyme Extraction***

1. Accurately weigh 3.0 g of ground grain or flour into a 50 mL conical centrifuge tube.
2. Add 20.0 mL of 1X Extraction Buffer solution (pH 5.4) to each tube and mix vigorously.
3. Allow enzyme to extract over 20 minutes in a 40°C water bath, with occasional mixing.
4. Centrifuge 1,000 x g for ten minutes.
5. Assay enzyme activity within two hours.

#### ***Reaction Blank***

A single set of triplicate Reaction Blanks (non-enzymatic control) is prepared as follows for each batch of samples being analyzed.

1. 0.3 mL of stopping reagent
2. 20 µL of substrate solution at the start of the reaction time.
3. 20 µL of any enzyme preparation in the sample set.

The mean absorbance of the non-enzymatic control is subtracted from all assays conducted during that day to establish the background or blank absorbance.

### ***Assay Procedure***

1. Dispense 20  $\mu\text{L}$  aliquots of Ceralpha Reagent Solution into a microtiter plate and pre-incubate the tubes and contents at 40°C for 5 min. Dispense 3 aliquots for each enzyme extract (assay each extract in triplicate).
2. To each well containing Ceralpha Reagent solution (20  $\mu\text{L}$ ), add 20  $\mu\text{L}$  of wheat  $\alpha$ -amylase extract directly to the bottom of the well at 30 second intervals.
3. Incubate at 40°C for exactly twenty min from time of addition.
4. Following the 20 min incubation period, add exactly 0.3 mL of Stopping Reagent.
5. Read the absorbance of the solutions and the reaction blank at 400 nm against 340  $\mu\text{L}$  distilled water.

## Milling Formulas Used for SWQL Reports

### Micro Milling

#### Grain Moisture Estimate

Grain moisture =  $1.3429 \times (\text{flour moisture}) - 4$

#### Estimated Flour Yield Corrected to 15% Moisture

Flour Yield<sub>(15%)</sub> =  $\text{Flour Yield}_{(\text{as is})} - 1.61\% \times (15\% - \text{Actual flour moisture})$

#### Softness Equivalent (SE)

$SE_{(\text{as is})} = ((\text{GW} - \text{Bran}) - \text{Mids}) / (\text{GW} - \text{Bran})$

Where:

SE = Softness Equivalent

GW = Weight of grain milled

Bran = Weight of milled product that remains above a 40 mesh screen

Mids = Weight of mill product through a 40 mesh and remaining above a 94 mesh screen.

#### Softness Equivalent at 15% grain moisture (SE<sub>15%</sub>)

$SE_{(15\%)} = SE_{(\text{as is})} - 1.08\% \times (15\% - \text{Actual flour moisture})$

#### Flour yield adjustment<sup>6</sup>

Adjusted Flour Yield =  $\text{Flour Yield}_{(15\%)} + 0.17 \times (\text{Softness Equivalent}_{(15\%)} - 52\%)$

#### Milling Quality Score (MQS)

$MQS = MF + (5.0144 \times \text{Adjusted Flour Yield}) - 292.6425$

Where:

MF = Allis Milling Score -  $(5.0144 \times \text{SAFY}) - 292.6425$

Allis Milling Score = Mill score from Allis database for the quality standard designated for the group.

SAFY = Adjusted Flour Yield for the quality standard designated for the trial as measured in the trial being evaluated.

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<sup>6</sup> On the small Quad Mill, coarser type soft wheat samples will appear to mill better than they should and conversely, softer type soft wheat samples will have suppressed "as is" flour yields. When compared to soft wheat samples with lower softness equivalents, wheat samples with higher softness equivalents typically require greater break roll milling to completely separate endosperm from bran. Micro milling adjustments were developed by Lonnie Andrews with Patrick Finney and Charles Gaines. Additional details are included in the Standard Operating Procedures for the Soft Wheat Quality Laboratory.

**Baking Quality Score (BQS)**

$$BQS=BF+ (33.3333*CS)-526.667$$

Where:

BF = Allis Baking Score – SCS

CS = Cookie Score = (-0.145 x Flour Protein) + (-0.07 x Sucrose SRC) + (0.049 x SE) + 21.9

SCS = Standard Cookie Score – cookie score for the quality standard designated for the trial as measured in the trial being evaluated.

Allis Baking Score = Allis baking score for the quality standard as determined in the Allis Milling Database.

**Advanced Flour Milling**

All formulas for Advanced milling are the same as Micro milling with the exception of Baking Quality Score.

**Baking Quality Score (BQS)**

$$BQS = (33.33333 \times \text{Cookie Diameter}) - 526.667 + BF$$

Where:

BF = Baking Factor = Allis Bake Score - (33.33333 x SCD)-526.667

Allis Baking Score = Allis baking score for the quality standard as determined in the Allis Milling Database.

SCD = Standard Cookie Diameter – cookie diameter for the quality standard designated for the trial as measured in the trial being evaluated.

**Allis-Chalmers Flour Milling**

**Recovery Weight**

$$\text{Recovery Wt.} = \text{Bran Wt.} + \text{Red Dog Wt.} + \text{Shorts Wt.} + \text{Straight Grade Wt.}$$

**Flour Yield**

$$\text{Flour yield} = \text{Straight Grade Wt.} / \text{Recovery Wt.}$$

**Endosperm Separation Index (ESI)**

$$ESI = [(\text{Bran Wt.} + \text{Red Dog Wt.} + \text{Shorts Wt.}) / \text{Recovery Wt}] - 17.7$$

**Friability**

Friability = (Summed weight of material milled by 2<sup>nd</sup> to 6<sup>th</sup> Break and 1<sup>st</sup> to 7<sup>th</sup> Reduction)/Weight of straight grade flour

Tom says (Straight Grade Weight)/Summed weight of material milled by 2<sup>nd</sup> to 6<sup>th</sup> Break and 1<sup>st</sup> to 7<sup>th</sup> Reduction)

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<sup>7</sup> In practice the recovery weight is estimated at 98% of milled weight

**Allis Softness Equivalent (Allis SE)**

Allis SE = Break Flour % + 21%

**Allis Milling Score**

Allis milling score =  $33.3 - [80 - \text{Allis straight grade flour yield}] \times 3.7$   
+  $33.6 + [(6 - \text{ESI}) \times 2.8]$   
+  $33 - [32 - \text{Friability}] \times 3.3$

**Allis Baking Score**

Allis Baking Score =  $(33.33333 \times \text{Cookie Diameter}) - 526.66$

## Quality Genotyping

The markers listed below and other published assays for wheat evaluation can be referenced at the Wheat Cap website under the "MAS protocols" section.

<http://maswheat.ucdavis.edu/index.htm>

SSR markers were accessed from the GrainGenes website:

<http://wheat.pw.usda.gov/GG2/index.shtml>

**Table 4. Commonly used PCR markers for testing wheat quality at the Soft Wheat Quality Laboratory**

Primers	Sequence	Product	RXN
	<b><u>High Molecular Weight Glutenins</u></b>		
GluA1	(Zhang, 2003)		
AxFwd	ATGACTAAGCGGTTGGTTCTT	1,200	58°C
Ax2* (reverse)	ACCTTGCTCCCCTTGTCTTT		
Ax1 (reverse)	ACCTTGCTCCCCTTGTCTTG		
GluD1	(Guttieri, 2008), (Wan, 2005)		Touch
DxL_151 (forward)	AGGATTACGCCGATTACGTG		Down*
Dx2R (reverse)	AGTATGAAACCTGCTGCGGAG	664	2+12
Dx5R (reverse)	AGTATGAAACCTGCTGCGGAC		5+10
GluB1	(Z.A., 2006)		
Glu1By8_F5	TTAGCGCTAAGTGCCGTCT	527	64°C
Glu1B_R5	TTGTCCTATTTGCTGCCCTT		
Glu1By9_F1	TTCTCTGCATCAGTCAGGA	662 / 707	59°C
Glu1B_R3	AGAGAAGCTGTGTAATGCC		
Glu1By9_F7	TACCCAGCTTCTCAGCAG	0/2/3	59°C
Glu1B_R6	TTGTCCCGACTGTTGTGG	bands	
Glu1By9_F2	GCAGTACCCAGCTTCTCAA	0/2/3	62°C
Glu1B_R2	CCTTGTCTTGTGTTGTTGCC	bands	
<b><u>Bx7 over-expression</u></b>	(Guttieri, 2008)		
Bx7oe_L1	GCGCGCTCAACTCTTCTAGT	404 / 447	64°C
Bx7oe_R1	CCTCCATAGACGACGCACTT		

Primers	Sequence	Product	RXN
	<b><u>Low Molecular Weight Glutenins</u></b>		
	(Zhang W. G., 2004)		
GluA3F1	GTACGCTTTTGTAGCTTGTGC	1,414	59°C
GluA3R1	TCCATCGACTAAACAACGGAGA		
GluA3F1	As above	1,346	59°C
GluA3R2	GATGCCAACGCCTAATGGCACAC		
GluA3F1	As above	596	59°C
GluA3aR	TGGTGGTTGTTGTTGTTGCTACA		
GluA3bF	CACAATTTTCACAGCAACAGCAG	823	59°C
GluA3bR	GGCACATTGACACTACACATTG		
GluA3bF	As above	196	59°C
GluA3cR	TTGGTGGCTGTTGTGAAGACGA		
GluA3dF	ACCAGTTATTCATCCATCTGCTC	488	59°C
GluA3dR	GTGGTTTCGTACAACGGCTCG		
GluA3eF	CAATGAAAACCTTCCTCGTCTG	1,151	59°C
GluA3R2	As above		
GluA3F1	As above	1,101	59°C
GluA3fR	GTTGCTGCTACAACACTGCTGTA		
GluA3gF	CAGCAGCCACCACATTCGCAA	861	59°C
GluA3R2	As above		
Gliadins	(Ma, 2003)		
GligDF1	AAGCGATTGCCAAGTGATGCG	264	56°C
GligDR1	GTTTGCAACACCAATGACGTA		
GligDF1	AAGCGATTGCCAAGTGATGCG	270	56°C
GligDR2	GCAAGAGTTTGCAACAGCG		
<b><u>Rye translocation</u></b>	(de Froidmond, 1998)		Touch
O11B3	GTTGCTGCTGAGGTTGGTTC		Down*
O11B5	GGTACCAACAACAACAACCC	412	
SECA2	GTTTGCTGGGAATTATTTG		
SECA3	TCCTCATCTTTGTCCTCGCC	632	
1B/1R & 1A/1R	(Saal, 1999)		
SCM9_L_M13	CACGACGTTGTAAAACGACTGACAACCCCTTTCCCTCGT	227/243	Tailed
SCM9_R	TCATCGACGCTAAGGAGGACCC		Reaction*

Primers	Sequence	Product	RXN
<b><u>Waxy - GBSS</u></b>	(Nakamura, 2002)		
AFC	TCGTGTTTCGTCGGCGCCGAGATGG	425, 455, 497	65°C
AR2	CCGCGCTTGTAGCAGTGGAAAGTACC		
BDFL	CTGGCCTGCTACCTCAAGAGCAACT	370, 389,	65°C
BRD	CTGACGTCCATGCCGTTGACGA	410,408	
BDFL	CTGGCCTGCTACCTCAAGAGCAACT	1,731, 2,307	65°C
DRSL	CTGTTTCACCATGATCGCTCCCCTT		
Tailed	Better to use M13-tailed AFC and BRD, analyze via capillary electrophoresis		
AFC_M13	<b>CACGACGTTGTA AACGACT</b> CGTGTTTCGTCGGCGCCGAGATGG		
BDFL_M13	<b>CACGACGTTGTA AACGACT</b> CTGGCCTGCTACCTCAAGAGCAACT		
<b><u>Pre-harvest sprout</u></b>	(Yang, 2007)		
Vp1BF	TGCTCCTTTCCCAATTGG	652	61°C
Vp1BR	ACCCTCCTGCAGCTCATTG	569, 845	Tolerant

**Genotyping Bibliography**

- Beales, J. T. (2007). A Pseudo-Response Regulator is misexpressed in the photoperiod insensitive Ppd-D1a mutant of wheat (*Triticum aestivum* L.),. TAG (115), 721-733.
- de Froidmond, D. (1998). A Co-dominant Marker for the 1BL/1RS Wheat-rye Translocation via Multiplex PCR. J. Cereal Sci (27), 229-232.
- Guttieri, M. S. (2008). Optimized PCR Primer Set for Determining Gluten Strength Qulaity in Soft Wheat Germplasm. PAG poster .
- Lei, Z. (2006). Y-type gene specific markers for enhanced discrimination of high-molecular weight glutenin alleles at the Glu-B1 locus in hexaploid wheat. J Cereal Sci (43), 94-101.
- Ma, W. Z. (2003). Multiplex-PCR typing of high molecular weight glutenin alleles in wheat. Euphytica (134), 51-60.
- McCartney, C. S. (2007). The evaluation of FHB resistance QTLs introgressed into elite Canadian spring wheat germplasm. Mol Breeding (20), 209-221.
- Nakamura, T. P. (2002). Rapid classification of partial waxy wheats using PCR-based markers. Genome (45), 1150-1156.
- Saal, B. a. (1999). Development of simple sequence repeat markers in rye (*Secale cereale* L.). Genome (42), 964-972.
- Sixin Liu, M. O. (2008). Toward positional cloning of FHB1, a major QTL for fusarium head blight resistance in wheat. Third International FHB Symposium .
- Sixin Liu, S. C. (2008). New DNA markers for high molecular weight glutenin subunits in wheat. Theor Appl Genet (118), 177-183.
- Tsilo, Toi J., Yue Jin, and James A. Anderson (2008). Diagnostic Microsatellite Markers for the Detection of Stem Rust Resistance Gene Sr36 in Diverse Genetic Backgrounds of Wheat. Crop Sci. (48), 253-261.
- Wan, Y. Y. (2005). Comparative analysis of the D genome-encoded high-molecular weight subunits of glutenin. TAG (111), 1183-1190.
- Xiaoke Zhang, S. Y. (2006). Distribution of the Rht-B1b, Rht-D1b and Rht8 reduced height genes in autumn-sown Chinese wheats detected by molecular markers. Euphytica (152), 109-116.
- Yang, Y. Z. (2007). Development and validation of a Viviparous-1 STS marker for pre-harvest sprouting tolerance in Chinese wheates. TAG (115), 971-980.
-

Zhang, W. G. (2004). Characterisation and marker development for low molecular weight glutenin genes from Glu-A3 alleles of bread wheat (*Triticum aestivum*). TAG (108), 1409-1419.

Zhang, W. M. (2003). Identification of SNPs and development of allele-specific PR markers for  $\gamma$ -gliadin alleles in *Triticum aestivum*. TAG (107), 130-138.

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