MATERIALS AND METHODS 2017

QUADRUMAT MILLING TESTS – BREEDER SAMPLES

The Soft Wheat Quality Laboratory evaluates thousands of breeder wheat samples yearly. Table 1 summarizes the traits tested and reported to breeders by the SWQL. The SWQL milling methods are described below.

Table 1. Milling and baking measurements and calculations for evaluation of breeder samples

TRAIT	SYMBOL	DESCRIPTION / CALCULATION
Whole Grain Protein	WPRO	Percent protein of whole, untempered grain measured on DA7200 near infrared (NIR) analyzer
Whole Grain Hardness	Hard	Scale of 1-120, soft to hard. Whole, untempered grain measured using Single Kernel Characterization System
Grain Weight	GW	Weight of tempered, whole grain sample
Bran	Bran	Weight of milled product retained by 40-mesh* screen (over 40)
Mids	Mids	Weight of milled product retained by 94-mesh* screen (over 94)
Break Flour	BkFl	Weight of milled product passing through 94-mesh* screen (Grain weight – (bran + mids))
Percent Bran, Mids, Break Flour	%	Expressed as percent of grain weight (Bran Weight/GW) x 100
Total Flour	Flour	Break Flour + Mids
Flour Yield	FY	(Total Flour/GW) x 100
Softness Equivalence	SE	(BkFl/Total Flour) x 100
Flour Moisture	FMOIST	Percent moisture of wheat flour estimated by Unity NIR
Flour Protein	FPRO	% protein of wheat flour by Unity NIR
Cookie Diameter	Cookie Dia	Total diameter of 2 baked cookies (cm)
Cookie Top Grain	Cookie TopG	0-9 visual scale (0 worst, 9 best)
Solvent Retention Capacity Tests	SRC	Percentage of solvent retained by a flour/solvent slurry after centrifugation and draining
Lactic Acid Sodium Carbonate Sucrose Water	LA SC SU WA	((residue wt/ flour wt)-1) x (86/(100 - %FMOIST)) x 100 flour wt = weight of dry flour residue wt = weight of drained, saturated flour

* Mesh size is the number of openings in the SSBC screen per linear inch; smaller particles pass through higher mesh number.

MODIFIED QUADRUMAT MILLING METHOD

Tempering: Prior to milling, wheat grain is estimated for moisture content using a Perten NIR DA7200 whole grain analyzer and tempered to 15% moisture. Grain samples are tempered in glass jars by adding distilled water, sealing with silicon-free, screw-top lids and tumbling on a chain driven roller/conveyor (Lewco) until the water is absorbed, about 30 minutes. Tempered grain samples are kept sealed at room temperature for at least 24 hours prior to milling to allow moisture equilibration throughout the kernel.

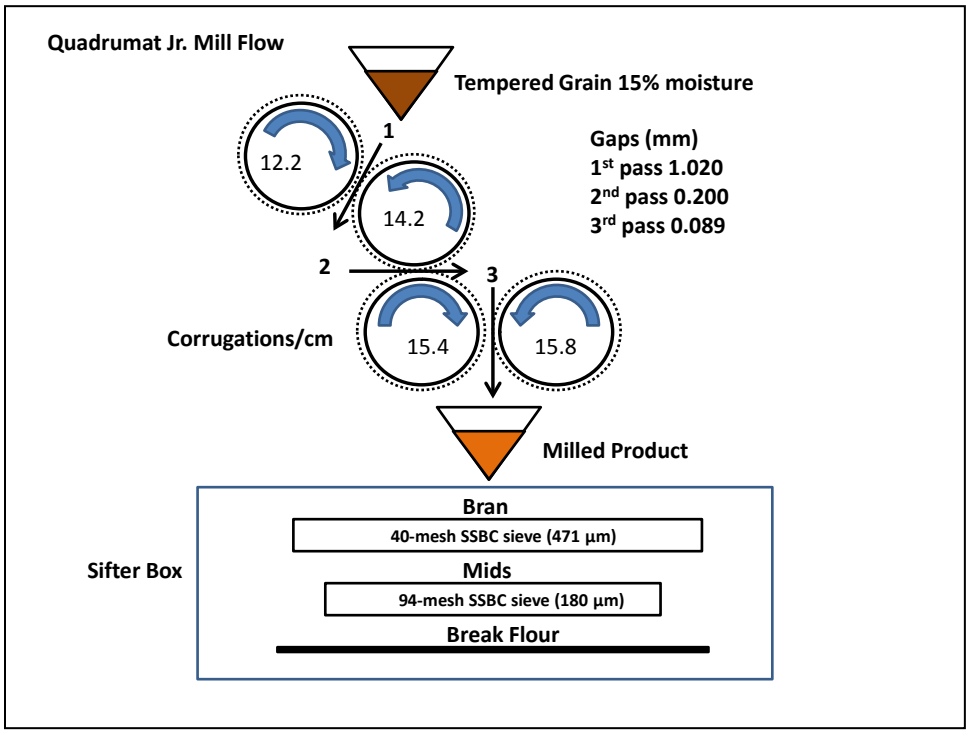
For the ***preliminary*** group samples, *tempered grain* is fed into the Quadrumat break roll unit and passed through three sets of milling rolls, each with increasing corrugations per centimeter and decreasing gaps to decrease particle size sequentially from grain to flour.

Milled product is sifted on a Great Western sifter box through sequential 40- and 94-mesh stainless steel bolting cloth (SSBC) screens, with 471 and 180 micron openings, respectively, to separate the milled product into three fractions: bran, mids and break flour. Bran is recovered above the 40-mesh screen, mids above the 94-mesh screen, and break flour passes through the 94-mesh screen. For ease of handling and accuracy, the bran and mids fractions are weighed as an indirect method for calculating flour yield (grain sample weight less bran as a percent of total grain weight) and softness equivalence (break flour as a percent of total flour).

For the ***intermediate*** group and ***advanced*** group grain samples, middlings are further passed through the Quadrumat reduction roll unit to obtain shorts and reduction flour. The milled fraction is sifted on an 84-mesh screen (213 micron openings) to yield shorts and reduction flour. Break and reduction flours are combined, blended to produce straight grade flour and used for composition, SRCs and cookie baking tests. Bran yield, break flour yield and total flour yield are determined the same ways as described for the preliminary group samples. All samples are milled under controlled temperature and humidity (19-21^{o}C and RH 58-62%). Mill temperature is equilibrated to 33 ± 1.0^{o}C by running the mill empty prior to sample milling.

Bran yield (%) is the percentage of bran retained by a 40-mesh SSBC screen (471 micron opening size) over the grain weight. Break Flour Yield (%) is the percentage by weight of the flour sifted through a 94-mesh SSBC screen (180 micron) over the grain weight. Mids (%) is the percentage middling stock (retained by the 94-mesch screen) over the grain weight. Potential Flour Yield (%) is the percentage by weight of the sum of break flour and middling stock over the grain weight.

Figure 1. Brabender Quadrumat break roll milling unit – adapted from Gaines, et al, 2000.



BREEDING SAMPLES

The SWQL treats samples as ***preliminary, intermediate*** or ***advanced*** group samples. The difference in treatment for each test type is summarized in Table 2.

Preliminary group testing is used for screening early generation selections, *intermediate* testing is used for intermediate generation samples and *advanced* testing is for advanced breeding lines. Milling scores produced for all three sample treatments are determined in the same way. *Intermediate* and *advanced* group testing add SRC and flour protein determinations, and *advanced* group testing includes sugar-snap cookie baking.

Preliminary group testing involves grain characteristics (TW, Grain NIR for protein and kernel hardness) and milling properties for breeders to screen early generation lines. Grain is milled using the Quadrumat break roll unit to obtain bran, middling and break flour. Flour yield and softness equivalence are calculated based on the equations described below in Soft Wheat Quality Laboratory Testing Methods for Quality Traits and summarized in Table 3. No further tests are performed using the break flour.

Intermediate and ***advanced*** group samples are milled using both the break and reduction roll units to produce break and reduction flours. The blend of break flour and reduction flour (straight grade flour) is used for flour quality tests. Grain characteristics and milling properties (TW, Grain NIR for protein and kernel hardness, flour yield and softness equivalence) are determined as for the preliminary groups. In addition, straight grade flour is tested for protein content and solvent retention capacity (SRC) of sodium carbonate and lactic acid. For ***advanced*** group samples, the straight grade flour is used for the sugar-snap cookie baking test.

Table 2. Differential processing of *Preliminary, Intermediate* and *Advanced* testing at SWQL

PROCEDURE	*Preliminary*	*Intermediate*	*Advanced*
Sample Size	80 g		200 g
Test weight	Whole grain		
Milling Method	Break Roll Unit Milling	Break and Reduction Roll Units Milling	
Flour Yield	Mids+Flour/Grain x 100		
Softness Equivalence	(Break Flour/Total Flour) x 100		
Kernel Hardness	Single Kernel Characterization System (SKCS)		
Whole Grain Protein & Moisture	DA7200 NIR		
Flour Test	NO	Straight Grade Flour (blend of break and reduction flours)	
Flour Moisture/Protein Content	NO	YES – Unity NIR	
Solvent Retention Capacity Tests (SRC)	NO	YES	
Sucrose	NO		YES upon request (5-g test)
Lactic Acid	NO	YES (1-g test)	
Water	NO		YES upon request (1-g test)
Sodium Carbonate	NO	YES (1-g test)	
Sugar-snap Cookie Diameter	NO		YES
Sugar-snap Cookie Top Grain	NO		YES

SOFT WHEAT QUALITY LABORATORY TESTING METHODS FOR QUALITY TRAITS

Traits included in the SWQL evaluation of breeding samples, the method used, the purpose of the measurement and measurement units are summarized in Table 31, below. Complete descriptions of the individual SWQL methods follow below.

Table 3. Traits measured at SWQL: methods, purpose and units

TRAIT	METHOD	INDICATES	UNITS
Test Weight	Modified AACC Method 55-10	Grain size, condition, packing efficiency	Estimated Pounds/bushel
Hardness (SKCS)	Perten Single Kernel Characterization System (SKCS) AACC Method 55-31.01	Grain hardness <40 is considered soft wheat	0-120
Whole Grain Protein & Moisture	Near Infra Red (NIR) Perten DA7200	Whole grain Protein & Moisture content	Percent
Falling Number	Perten Falling Number Tester AACC Method 56-81.03	Pre-harvest sprout damage	seconds
Flour Yield	mids + break flour as % of initial grain weight	Flour recovery	Percent
Softness Equivalence	Break flour weight as % of total flour weight (Finney, 1986)	Estimates grain hardness, flour particle size	Percent
Flour Ash	AACC Method 08-01	Inorganic residue after combustion	Percent
Flour Moisture	NIR Unity Spectra-Star	Flour moisture	Percent
Flour Protein		Flour protein content	Percent
Solvent Retention Capacity Profile (SRC)	AACC Method 56-11.02	Solvent affinity	Percent
	Lactic Acid	Gluten strength	
	Sodium Carbonate	Damaged starch	
	Sucrose	Pentosan Content (Arabinoxylans)	
	Water	Overall water affinity	
Sugar-snap Cookie Diameter	Baking Quality of Cookie Flour, Intermediate Method AACC Method 10-52	Cookie spread	Centimeters
Sugar-snap Cookie Top Grain		Visual quality cookie surface	1-10 higher is better

Whole Grain Moisture, Hardness and Protein

Whole grain moisture and protein are estimated using the NIR DA7200 Analyzer (Perten Instruments). Adjustment of calibrations was performed in Wooster, Ohio, for whole grain moisture and protein using values produced on the oven moistures (AACC Method 44-01.01) and nitrogen combustion analysis Rapid NIII Nitrogen Analyzer (Elementar), respectively.

Definitions:

Grain is the cleaned and tempered whole grain used for milling.

Break flour (BkFl) is milled product passing through the 94 mesh screen after a single pass through the Quadrumat break roll unit. Break flour is the smallest fraction of the milled product and has the finest particle size. Break flour weight is approximated by subtracting the weight of bran and mids from the original grain weight.

Mids (also called middlings) is the milled product passing through the 40 mesh screen but retained by the 94 mesh screen after a single pass through the Quadrumat break roll unit.

Bran is the milled product retained by the 40 mesh screen after a single pass through the Quadrumat break roll unit.

Reduction flour is the product passing through an 84 mesh screen after a second, reduction milling of the mids (from break roll unit) through the Quadrumat reduction roll unit.

Straight Grade Flour is a blend of break flour and reduction flour.

Flour Yield

Flour yield (FY) is calculated as the percent total flour weight (break flour + mids) of the sample grain weight (GW) from a single pass through the Quadrumat break roll unit. For calculation of flour yield, the difference between the grain weight (GW) and the bran weight (Bran) is used to estimate total flour (mids + break flour).

$$FY = ((GW-Bran)/GW) \times 100$$

The formula is equivalent to: $(Total\ Flour/GW) \times 100$

Softness Equivalence

Softness Equivalence (SE) is the percentage break flour (BkFl) passing through 94-mesh screen, of the total flour weight (break flour + mids). SE approximates grain softness and particle size of flour produced from a single pass through the Quadrumat break roll unit (*C.W. Brabender Instruments, Inc.*) and is analogous to break flour in a large-scale mill (Finney, 1986). Total flour weight is calculated by subtracting bran weight (remaining over the 40-mesh screen) from initial grain weight. Subtracting the weight of the mids (remaining over the 94-mesh screen) from the total flour gives the weight for break flour.

$$SE = \{(GW - (Bran + Mids))/(GW - Bran)\} \times 100$$

This formula is equivalent to: $(BkFl/Total\ flour) \times 100$

Flour Moisture and Protein

Flour moisture and protein are estimated using the SpectraStar NIR analyzer (Unity Scientific), calibrated yearly for protein by nitrogen combustion analysis using the Rapid NIII Nitrogen Analyzer (Elementar) and for moisture by the oven drying method (AACC method 44-01.01). Units are recorded in percent moisture or protein converted from nitrogen x 5.7 and expressed on a 14% moisture basis.

Solvent Retention Capacity

Solvent Retention Capacity (SRC) assays are performed as described in AACC Method 56-11.02, *Solvent Retention Capacity Profile*. The profile of SRCs in the four solvents (sucrose, lactic acid, sodium carbonate and water) is used to predict milling and baking quality. In general, lower SRCs are preferred for water, sodium carbonate and sucrose solvents (Kweon, Slade, & Levine, 2011).

Breeder samples processed by intermediate and advanced group testing use *straight grade flour* (blend of break and reduction flours) for SRC tests.

With the exception of sucrose, SRCs are performed using 1 gram of flour in glass test tubes with rubber stoppers. Sucrose SRCs are performed with 5 grams of flour in 50 mL disposable screw top centrifuge tubes, because the highly viscous sucrose solution impedes even distribution of solution in 1 gram flour tests, reducing the reliability of the small scale test.

The following descriptions of the biochemistry and correlations of SRCs with milling and baking traits were published in the Soft Wheat Quality Laboratory Annual Report 2011 (Souza, Kweon, & Sturbaum, 2011).

Water SRC is a global measure of the water affinity of the macro-polymers (starch, arabinoxylans, gluten, and gliadins). 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 values are related to the content of arabinoxylans (also known as pentosans), which can strongly affect water absorption in baked products. Sucrose SRC is a good predictor of cookie quality and shows a negative correlation with wire-cut cookie diameter ($r = -0.66$, $p<0.0001$). 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). The 95% target value can be exceeded in flour of high lactic acid SRC.

Sodium carbonate SRC takes advantage of the very alkaline solution to ionize the ends of starch polymers increasing the water binding capacity of the molecule. Sodium carbonate SRC increases as starch damage due to milling increases.

Lactic acid SRC predicts gluten strength of flour. Typical values are below 85% for "weak" protein soft wheat varieties and above 110% for "strong" protein soft wheat varieties. Lactic acid SRC results correlate to the SDS-sedimentation test. The lactic acid SRC is also correlated to flour protein concentration and dependent on genotypes and growing conditions.

Cookie Bakes (Sugar-Snap Cookies)

Two sugar-snap cookies are baked in the SWQL bake laboratory for each sample as described in AACC Method 10-52, *Baking Quality of Cookie Flour*. Cookies are baked exclusively for advanced group samples using straight grade flour (blend of break and reduction flours). Diameter of the two cookies is measured and recorded electronically using a Mitutoyo Absolute Digimatic Caliper. Cookies are graded visually for surface appearance, from worst to best on a scale of 1 to 10. Color is observed for bake quality but not graded.

Falling Number

The falling number test (AACC Method 56-81B) is performed using the Perten Falling Number instrument. A glass tube filled with a suspension of whole grain meal or milled flour is heated in a boiling water jacket to produce gelatinized starch. Immediately after heating, a weighted plunger is released into the suspension, and the travel time of the plunger is measured in seconds (falling number) as it falls from the top to bottom of the glass tube. The higher the viscosity of whole grain meal or flour paste in the glass tube, the longer the travel time of the plunger. The enzyme α-amylase, produced when grain sprouts, hydrolyzes starch molecules and lowers the viscosity of gelatinized starch, resulting in decreased travel time of the plunger (falling number). Alpha-amylase can be measured directly using a kit from Megazyme, International (AACC Method 22-02-01, *Measurement of alpha-Amylase in Plant and Microbial Materials Using the Ceralpha Method*). The SWQL uses a modified micro method of the Megazyme assay.

Flour Ash

Flour Ash is measured according to the AACC method 08-01 and detects residual inorganic materials after combustion. Since inorganic materials are higher in bran than in endosperm, flour ash is an indirect indicator of residual bran in the flour.

GENOTYPING FOR QUALITY TRAITS

DNA markers applied in marker assisted selection and genotyping are included below. Besides in house genotyping, the SWQL sends samples to the Eastern Regional Small Grains Genotyping Laboratory for SNP genotyping.

http://www.ars.usda.gov/Main/docs.htm?docid=19522

Molecular markers and protocols are available at the University of California Davis website:

http://maswheat.ucdavis.edu/

Quality Genotyping - Primer Sequences, Amplification Conditions and References
The molecular markers described below are the most commonly used markers at the SWQL. These are reliable and robust reactions that have been useful in assessing wheat quality. Primer sequences are given 5' to 3'.

High Molecular Weight Glutenins and γ-gliadin

Glu-A1

AxFwd	ATGACTAAGCGGTTGGTTCTT
Ax1 R	ACCTTGCTCCCCTTGTCCTG
Ax2* R	ACCTTGCTCCCCTTGTCTTT

Amplifies at 58°C, 1,200 bp product, present or absent using single forward primer, alternate reverse primers. (Ma et al., 2003), (Liu et al., 2008)

Glu-D1

DxL_151	AGGATTACGCCGATTACGTG
Dx2R ``2+12"	AGTATGAAACCTGCTGCGGAG
Dx5R ``5+10''	AGTATGAAACCTGCTGCGGAC

Amplifies 664 bp product, present or absent using single forward primer, alternate reverse primers, touchdown amplification. (Wan et al., 2005)

Glu-B1

Bx7oe_L1	GCGCGCTCAACTCTTCTAGT
Bx7oe_R1	CCTCCATAGACGACGCACTT

Amplifies at 64°C a 404 bp for wild-type or 447 bp product for over-expressing Bx7. (Lei et al., 2006)

γ-gliadin

GligDF1	AAGCGATTGCCAAGTGATGCG
GligDR1	GTTTGCAACACCAATGACGTA
GligDR2	GCAAGAGTTTGCAACAGCG

Amplifies at 56°C, a 264 bp product for gliadin 1.1 or 270 bp product for gliadin 1.2, using single forward primer, alternate reverse primers. (Zhang et al., 2003)

Translocations and Disease Resistance

1B/1R and 1A/1R – Chromosome 1B or 1A substituted with rye secalin
Tailed Reaction

SCM9_L_M13	CACGACGTTGTAAAACGACTGACAACCCCCTTTCCCTCGT
SCM9_R	TCATCGACGCTAAGGAGGACCC

Amplifies using a labled tailed reaction, 207 bp for 1B/1R or 203 bp for 1A/1R. (de Froidmont, 1998)

2B translocation - Sr 36 stem rust resistance

Stm773-F5	AAACGCCCCAACCACCTCTCTC
Stm773-R5	ATGGTTTGTTGTGTTGTGTGTAGG

Amplifies with 62/55^{o}C touchdown program producing a 162 fragment indicative of the 2B translocation carrying Sr36 or 192 bp for wild type 2B. (Tsilo et al., 2008)

Sucrose Synthase type 2 Sus2

HapH High grain weight associated with upstream 35 bp insertion

Sus2B_7.F	TCCTCGTTCTTTGTTCGTTCT
Sus2B_7.R	CACTTCGTGGTACTTTTCCT

The single 2-step assay replaces HapH and HapL SNP determination amplifying a 358 bp in HapH lines and 322 in HapL, haplotypes indicative of high or low grain weight, respectively. (Jiang et al., 2011).
PCR program uses 7 cycles at 62^{o}C followed by 35 cycles at 55^{o}C annealing.

Pre-harvest sprouting

Vp1BF	TGCTCCTTTCCCAATTGG
Vp1BR	ACCCTCCTGCAGCTCATTG

Amplifies at 62^{o}C a 569 or 845 bp fragment for reported tolerance to preharvest sprouting.
(Yang et al., 2007)

Genotyping References

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