# WET GLUTEN ANALYSIS AND NIRT CALIBRATION
## Hard Red Spring and Hard Red Winter Wheat

In April 2003, GIPSA hosted an ideation meeting in Kansas City, MO to assess needs for rapid field testing of wheat functional qualities and end-use characteristics. The group identified dough strength as a high priority functionality test. Review of a U.S. Wheat Associates global test methods survey identified dough strength tests (besides protein content) that were the most commonly requested by wheat importing countries. Wet gluten ranked as the second most desired test [1]. Wet gluten represents the fraction of the total wheat protein that agglomerates upon hydration leading to dough formation such as bread dough [2].

Since 1998, GIPSA has been collecting Near-Infrared Transmission (NIRT) spectra and wheat functionality reference data, including wet gluten data, as part of its Export Cargo Sampling Project (ECSP). In Fiscal Year (FY) 2003, GIPSA participated in a Rapid Quality Prediction Study (RQPS) with the Agricultural Research Service involving Hard Red Spring wheat (HRS) and Hard Red Winter wheat (HRW). The samples represented U.S. domestic and export markets, pure varieties, and controlled blends of pure varieties. Both the ECSP and RQPS data sets had reference wet gluten results from laboratories other than GIPSA. The ECSP and RQPS data showed a high correlation ($R^2 = 0.94$) between total protein (12% moisture basis) (mb) and flour wet gluten (14% mb) for samples representing all classes except Durum and Hard White wheat. A similar high correlation ($R^2 = 0.95$) was found between flour protein and wet gluten contents [3].

In FY2005, GIPSA selected samples from the available ECSP and RQPS data sets for wet gluten (Glutomatic) analysis by the GIPSA reference laboratory. In addition, samples were selected from the wheat protein quality control program and the moisture calibration survey program for reference wet gluten analyses. A total of 660 samples (representing all wheat classes except Durum) were analyzed with the official NIRT instrument and by the GIPSA wet gluten reference method. These samples were used for calibration development and validation purposes. A subset of 301 HRS and HRW samples was selected as a validation set.

For wet gluten analysis, GIPSA milled the wheat samples using a Quadrumat Junior mill according to the AACC approved method 26-50 [4]. The milled flour samples were then subjected to wet gluten analysis using the Glutomatic 2200 according to AACC approved method 38-12A [5]. GIPSA developed statistical process control (SPC) charts to control milling and Glutomatic method variation. A hard whole-wheat sample for milling, and soft and hard wheat flours for Glutomatic wet gluten analysis were used as standard laboratory method control samples for developing the SPC charts.

The wet gluten reference method, as described in the AACC standards, does not yield meaningful results for wheat samples that do not “agglomerate” properly during automated washing. These samples clog the sieve during the washing step and cause “sample flooding.” For those wheat samples that caused sample flooding, GIPSA repeated the test using 4.2 ml of 2% sodium chloride solution with a 10-minute “rest time” (extra hydration time) before the automated washing step. (The 10-minute rest time is a deviation from the standard AACC International procedure.) Sample flooding occurred with some samples of all classes of wheat,

but it was a more frequent problem with soft wheat classes. The modified procedure successfully resolved the problem (caused proper agglomeration and avoided sample flooding) for all wheat samples that GIPSA encountered during the calibration work.

GIPSA performed a test to assess the repeatability of its wet gluten reference method. Standard deviations were calculated from Glutomatic test results for 10 portions each of a soft wheat flour sample and a hard wheat flour sample. The flour samples were tested "blind" over a period of several days. The standard deviations of the wet gluten reference method repeatability were 0.60 percent wet gluten for the soft wheat sample with 22.8 percent wet gluten (mean) and 0.27 percent wet gluten for the hard wheat sample with 29.51 percent wet gluten (mean). The relative standard deviations for the samples were 2.64 and 0.91, respectively, for the soft and hard wheat flour samples.

GIPSA developed and evaluated both direct wet gluten calibrations and protein-based calibrations. The Partial Least Squares regression method was used to directly calibrate the NIRT spectra to the reference method. The current official whole grain wheat protein data were used with the reference method data to develop the linear regression protein-based calibration. All calibrations were referenced to the GIPSA wet gluten reference laboratory results. The decision to implement a protein-based wet gluten calibration rather than a direct calibration was based on the following considerations:

1. There was no practical difference between the accuracies of the protein-based calibration and the direct wet gluten calibration when compared to the reference method.

2. The global ANN protein calibration (and, therefore the protein-based wet gluten calibration) was much more robust because it was based on a much wider variety of samples from a larger geographic area and more crop years than the direct wet gluten calibration.

3. Implementing a protein-based calibration required no additional stabilization or standardization samples beyond what were currently being used for the wheat protein system.

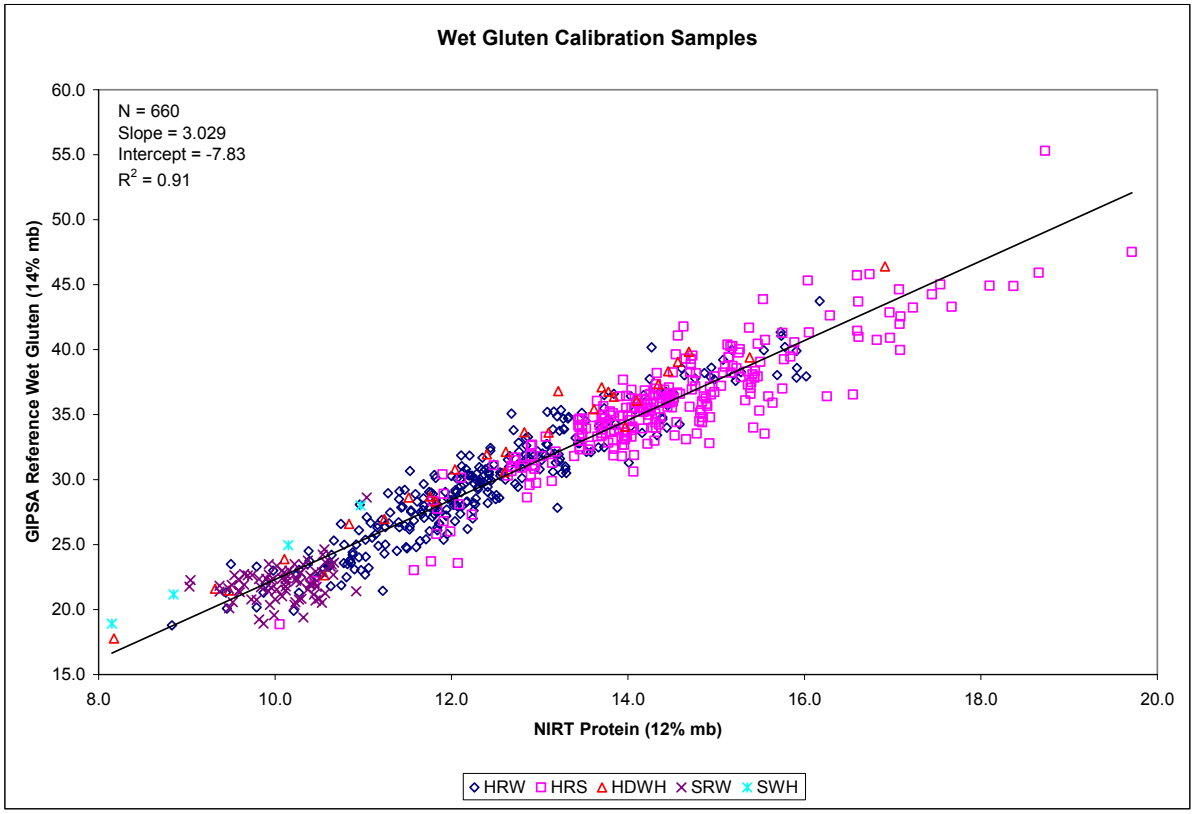
4. There was less inherent variability among instruments for the protein-based calibration than for the direct wet gluten calibration—because of the superior robustness of the ANN-based protein calibration.

5. Wet gluten contents can be calculated directly from official wheat protein results (present or past) by means of the protein-based calibration.

GIPSA plans to implement a NIRT protein-based wet gluten calibration on May 1, 2006, for hard red (HRS and HRW) wheat. The protein-based wet gluten calibration is:

$$\text{NIRT Wet Gluten (14\% mb)} = 3.029 \times \text{NIRT Protein (12\% mb)} - 7.83$$

Data used to calculate the calibration coefficients are shown in the following plot.

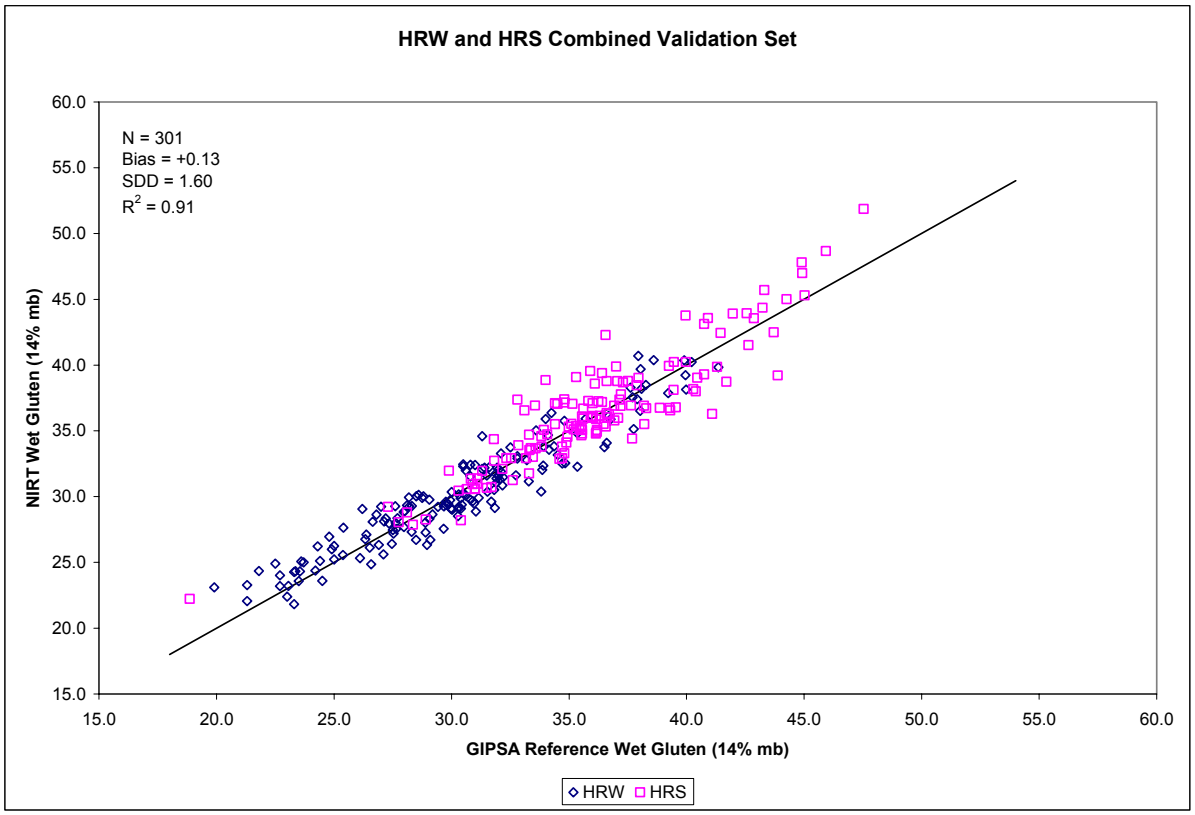



## Wet Gluten Accuracy Estimates

Since official certification of hard red wheat wet gluten is a new service, implementation of this new NIRT calibration does not cause inconsistencies with prior officially certified results.

The following information is provided to allow users of this new service to estimate potential differences between official wet gluten results (based on official NIRT protein) and wet gluten results from laboratories that use the AACC method (Glutomatic). These data may or may not reflect the differences that individual customers will observe for hard red wheat in regional markets when the official wet gluten service is implemented in May 2006.

A set of 301 HRW and HRS samples was tested as a validation set to assess the prediction accuracy of the official wet gluten calibration. The mean difference between the NIRT wet gluten values and the wet gluten values from the AACC reference method was +0.13 percent wet gluten, and the standard deviation of differences between NIRT and AACC method results was 1.60 percent wet gluten. This means that approximately 95 percent of NIRT wet gluten results would be within $\pm$ 3.2 percent wet gluten of the value that would be obtained by GIPSA's wet gluten (Glutomatic) reference method. Other laboratories' wet gluten results (based on AACC methods) may differ from GIPSA's reference wet gluten results. The following graph shows the agreement between the NIRT wet gluten results and Glutomatic results for the validation sample set.




References:


1. Chinnaswamy, R., Kao, C., Norden, T.D., and Johnson A. C. 2005. Role of Rheology in Determining Wheat Gluten Quality. in *Proceedings of the Third International Wheat Quality Conference*, May 22-26, Manhattan, KS.

2. He, H. and Hoseney, R.C. 1990. Gluten, A Theory of How It Controls Bread Making Quality. in *Gluten Proteins*. eds. Bushuk, W. and Tkachuk, R., American Association of Cereal Chemists, St. Paul., MN.

3. Chinnaswamy, R., Kao, C., Norden, T.D., Allvin, B., Perten, J., Brenner, C., and Pierce, R. O. 2005. Evaluation of Wet Gluten Properties of U.S. Export Wheats. in *Proceedings of the Third International Wheat Quality Conference*, May 22-26, Manhattan, KS.

4. AACC Approved Methods. 2000. Brabender Quadrumat Jr. (Quadruplex) Method 26-50. 10th ed. American Association of Cereal Chemists, St. Paul, MN.

5. AACC Approved Methods. 2000. Wet Gluten, Dry Gluten, Water-Binding Capacity, and Gluten Index Method 38-12A. 10th ed. American Association of Cereal Chemists, St. Paul, MN.