Technical Bulletin

CEREALS CANADA

Assessment of Wet Gluten (WG) and Gluten Index (GI) Using the Glutomatic - Best Practices

Using the Glutomatic, the quantity (WG) and quality (GI) of gluten in ground wheat, flour, and/or semolina can be measured.

- Determination of WG content measures gluten forming proteins and does not account for other proteins present in wheat, such as non-gluten (non-functional) forming proteins.
  - This means that it underestimates the total amount of protein present in a sample and therefore should not be used as a proxy for the protein content of a sample.
- GI is a quick method to assess the gluten quality.
  - When other tests are conducted, such as rheological tests, GI helps provide a more comprehensive understanding of quality and the resulting functionality.
- The ratio between WG content to protein content will vary depending on wheat class, environmental conditions during the growing season as well as whether the results for ground wheat, flour or semolina are used in its calculation.

The steps, tables and figures detailed in this document provide useful information on how to minimize errors and reduce variability when completing a WG and/or GI test using the Glutomatic.

For additional information please contact:

Kristina Pizzi, Manager Analytical Services
T: 204-984-6076 / E: kpizzi@cerealscanada.ca

1.1 Sample Preparation

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Sample cleanliness	Sample should be free from dockage and other foreign material.	Using a sample that contains extraneous material.	Extraneous material is considered a contaminant that will affect WG/GI results if not removed from the sample.
Using a representative sample	Test a representative sample. [1,2,3,4,5,6]	Using a sample that is not representative of the lot being tested.	To minimize sampling error, a representative sample amount is required.
Particle size of ground meal (prepared from wheat sample)	Wheat should be ground using a Laboratory Mill LM 3100 or LM 120 with a 0.8 mm sieve.	Grinding wheat with other types of grinders, such as a coffee grinder.	A particle size distribution that conforms to the specifications outlined in Table 1 is required. This helps to ensure precise and reproducible results.

1000-303 Main St, Winnipeg, MB Canada R3C 3G7
T: (204) 942-2166 F: (204) 983-2642 **cerealscanada.ca**

1.1 Sample Preparation, cont.

Cleaning grinder (for testing ground meal samples)	Clean the grinder between each sample.	Grinding samples without cleaning the grinder between each sample.	Residue from the previous sample can result in cross-contamination and affect results.
Moisture content determination of ground meal and flour/semolina	Use a standardized method to determine moisture content of meal and flour/semolina such as: 1) Air-oven[7,8,9] 2) Near Infrared (NIR)	Omitting moisture testing of the meal and flour/semolina.	The moisture content of the sample is needed to correct WG and GI results to a specified moisture basis. In Canada, wheat samples are corrected to 13.5% moisture basis (mb) and flour/semolina samples are corrected to 14% mb.
Cool down of ground meal	Before testing, check that ground meal has cooled to room temperature (22 ± 2°C).	Testing ground meal that is still warm (due to recent grinding).	Weighing samples that are not at room temperature will result in unstable and inaccurate weight readings on the balance.
Balance accuracy	Check that the balance: 1) is accurate by comparing to known standard weights. 2) has a required accuracy of ± 0.01 g or better.	Using a balance that is not routinely checked for accuracy and precision.	Accurate weighing of ground meal or flour/semolina is required to generate repeatable and reproducible WG/GI results.

1.2.1 Glutomatic Setup

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Standardized method or standard operating procedure	The test should be performed according to a standardized method. Some standardized methods are listed below. 1) AACC 38-12.02[10] 2) ICC No. 155[11] 3) ISO 21415-2[12] 4) Manufacturer's instructions[13]	Following an unrecognized or non-standardized method.	The accuracy and precision of the results depend on following a standardized method. Deviating from standardized procedures will lead to erroneous results. To achieve acceptable repeatability and reproducibility, staff must be properly trained. Comparison of results between/within laboratories relies on following standardized procedures.

1.2.1 Glutomatic Setup, cont.

Lubricate the shaft	Add a few drops of distilled water into the hole in the front of the Plexiglas body of the mixing head. This is easily done using a squirt bottle.	Not adding water to the shaft.	Water is needed to lubricate the shaft prior to the first test.
Flush the system with 2.0% NaCl solution	Before testing samples, flush the system with 2.0% NaCl solution by running a wash cycle without a sample.	Running a test without flushing the system first.	It is important to ensure the system is filled with the 2.0% NaCl solution prior to conducting the first test.
Sieves	Sieves must be in good condition. They should not be frayed, torn, warped or clogged with residue (See Figures 1 and 2). New sieves must have the factory finishes removed by washing with ground meal prior to being used for their first use. ▪ Wheat and semolina sieves (two-stage): 88 µm polyester sieve (fine) and 840 µm polyamide sieve (coarse) ▪ Flour sieve (one-stage): 88 µm polyester sieve (fine)	Using damaged/clogged sieves (see Figures 1 and 2).	If sieves are not in good condition they may allow material to flow through the sieve (ripped/torn) and/or cause flooding (clogged).
Kneader hook distance	Distance between kneader hook and sieve should be 0.7 mm.	The distance between the kneader hook and sieve is out of range (too close or too far away).	A kneader hook that is not the correct distance away from the sieve can cause errors in the results as it will not allow for proper preparation and washing of the gluten and therefore affects the accuracy of the results.
Plastic chamber	Plastic chambers should be clean and dry. They should fit smoothly into dispenser head of the Glutomatic and also into the metal sieve holders.	Plastic chambers that are not properly cleaned or in good condition.	Plastic chambers should be clean and dry and in good condition. They should fit properly into the dispenser head of the Glutomatic and the metal sieve holders. Plastic chambers that are in poor condition affect the operation of the test and therefore the accuracy of results.

1.2.1 Glutomatic Setup, cont.

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Sieve holders	Use the correct sieve holder based on the sieve that is being used (See Figure 3). ▪ Fine sieve (88 µm polyester sieve) – goes with the chromed and unmarked sieve holder. ▪ Coarse sieve 840 µm polyamide sieve (coarse) – goes with the chromed marked sieve holder that has a grove or ring.	Using the wrong sieve holder for the sieve size.	The sieve holders are specifically made to be used with the designated sieves. Using the wrong sieve and sieve holder will change the distance between the kneader hook and sieve which may impact the results.
Sodium chloride (NaCl) reagent grade	Use reagent grade NaCl.	Using table salt or iodized salt.	Table salt or iodized salt contains other ingredients which may affect the formation of gluten and therefore the test results.
Preparing the NaCl solution (2.0%)	Dissolve dissolve 20 g NaCl (reagent grade) in distilled water and bring to volume in a volumetric flask (preferably 1000 mL capacity). Prepare a new solution daily.	Not properly preparing a 2.0% NaCl solution. Not preparing fresh NaCl solution daily.	The NaCl solution (2.0%) should be made accurately and fresh daily in order to ensure accurate results.
NaCl solution (2.0%) temperature	The temperature of the 2.0% NaCl solution should be at 22 ± 2°C. If the operating temperature of the lab is too hot, ice cubes made from the 2.0% NaCl solution can be used for cooling and maintaining temperatures.	The temperature range of the 2.0% NaCl solution is out of range (<20°C or >24°C).	Using the 2.0% NaCl solution outside the temperature range can result in less than accurate results.
Calibrate the dispensing pump (Glutomatic 2000)	Calibration should be performed weekly to ensure the Glutomatic is dispensing the appropriate amount of 2.0% NaCl solution during a test (250-280 mL). Calibration should also be performed anytime the volume dispensed is outside of the 250-280 mL range, or there is a large discrepancy between the two washing sides.	Using the Glutomatic when the dispensing amount is out of range (dispensing <250 mL or >280 mL) or not calibrated.	Not calibrating the system may result in less reproducible results which will make it difficult to compared results between repeated tests and labs.

1.2.1 Glutomatic Setup, cont.

Flow rate	The flow rate of the 2.0% NaCl solution should be 50-56 mL/min (250-280 mL over 5 min).	Using the Glutomatic when the dispensing amount is out of range (<50 ml/min or >56 mL/min).	An incorrect flow rate of 2.0% NaCl solution can result in poor repeatability and reproducibility.
NaCl solution (2.0%) dispenser	Dispenser (range 0-10 mL, ±0.1 mL), typically set to 4.8 mL. Ensure the tubing of the dispenser has no air bubbles. Flush the dispenser with 2.0% NaCl solution at the start of testing. This amount may be adjusted for unusual samples (4.2 mL for low gluten samples and 5.2 mL for high gluten samples).	Not using the required amount of 2.0 % NaCl solution (4.8 mL) to wet the sample before starting the test.	Using the wrong amount may impact how the starch and other materials are removed during the washing resulting in incorrect results.

1.2.2 Running the Wet Gluten Test

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Weighing of ground meal or flour/semolina	10.00 ± 0.01 g well mixed ground meal or flour/semolina.	Failure to weigh the proper amount of sample (ground meal or flour/semolina; <9.99 g or >10.01 g).	If the wrong sample weight is used, the dough consistency changes, which can alter gluten recovery and make the wet gluten or gluten index results less reliable.
Assembly of the wash chamber (using the correct sieve and sieve holder)	Use the correct sieve holder based on the sieve that is being used. ▪ Fine sieve (88 µm polyester sieve) - chromed and unmarked ▪ Coarse sieve (840 µm polyamide sieve) - chromed marked with a grove or ring	Using the wrong sieve holder for the sieve size.	The sieve holders are made to be used with the designated sieves. Using the wrong sieve and sieve holder will change the distance between the kneader hook and sieve which may impact the results.
Select the correct washing method; one-stage or two-stage	One-stage method (flour): use the fine sieve (88 µm polyester sieve). Two-stage method (ground meal or semolina): use the fine sieve for the first portion of the wash and then the coarse sieve (840 µm polyamide).	Not choosing the right washing method (one-stage or two-stage) for the material being tested.	The proper washing method (one-stage or two-stage) needs to be used for material that is being tested otherwise results will not be accurate.

1.2.2 Running the Wet Gluten Test

Assemble the wash chamber	Add the fine sieve (88 µm polyester sieve) between plastic chamber and unmarked sieve holder. Centre the sieve over the sieve holder and firmly press plastic chamber over the sieve and twist to lock in place. Ensure the sieve is properly stretched/ taut (no buckling of the sieve material).	Not centering the sieve on the sieve holder. Not ensuring sieve is stretched properly.	If the sieve is not properly centered or not stretched tightly this can impact the accuracy of the results.
Preparation of the washing chamber	Moisten the fine sieve (88 µm polyamide sieve) with distilled water to prevent flour loss.	Using a dry sieve.	Moistening the fine sieve prevents loss of sample when added to the wash chamber.
Adding sample to wash chamber	Add sample to a properly prepared clean sieve that is properly seated in the sieve holder and plastic chamber. Gently swirl to spread sample evenly across the sieve.	Adding the sample and not spreading it out evenly.	Not spreading the sample out evenly will impact how the sample is washed and could lead to inaccurate results.
Dispensing NaCl solution	Add 4.8 mL of 2.0% NaCl to sample by holding the washing chamber on an angle and direct the stream of NaCl solution from the dispenser against the plastic chamber side wall. Shake the test chamber gently to spread solution evenly over the entire sample (see Figures 4 and 5).	Adding the 2.0% NaCl solution directly on the sample and not wetting the entire samples surface (see Figure 5).	Adding the 2.0% NaCl solution directly to the sample in one place may cause the solution to go right through the sample and sieve leading to inaccurate dispensing amounts. It also doesn't allow the entire sample surface to be wetted.

1.2.2 Running the Wet Gluten Test

Removing wet gluten ball	Remove the wet gluten from chamber and kneading hook without tearing it. Ensure no gluten remains in the washing chamber. If the wet gluten is torn in two or more pieces, test should be repeated.	Tearing the wet gluten into multiple pieces when removing from washing chamber.	Using wet gluten that is torn or in multiple pieces reduces the repeatability of the test, leading to inaccurate results.
Two-stage method: transferring between washing chambers	Transfer all loose material from the washing chamber with the fine sieve (88 µm) into the washing chamber with the coarse sieve (840 µm). Hold the washing chamber overtop of the washing chamber with coarse sieve. Using a stream of cold water, allow the material to transfer to the washing chamber with the coarse sieve. A plastic ring can be used to fit the two wash chambers together (see Figure 6).	Not switching to the coarse sieve. Not ensuring all gluten is transferred from the fine sieve to the coarse sieve.	Using an improper sieve and/or improperly transferring the test material will reduce the repeatability of the test and impact the accuracy.
Centrifuge sieve plates (wet gluten) or sieve cassettes (gluten index)	To determine WG only, place each sample of gluten on separate clean sieve plates and place in centrifuge. For GI determination, place each gluten on separate plastic sieve cassette and place in centrifuge (see Figures 7 and 8).	Not using the correct centrifuge sieve plate or sieve cassette. Not balancing the centrifuge.	Use of improper sieve plates or sieve cassettes will impact the results. GI cannot be calculated using the sieve plates.
Centrifuge	Ensure centrifuge and sieve plates/sieve cassettes are clean and don't contain any residue.	Using sieve plates/sieve cassettes with residue in them. Using sieve plates/ sieve cassettes that may have blockages or damage.	Clean centrifuge and sieve plates/sieve cassettes are important for accurate results. If gluten or starch residue (from a previous test) remains on the sieve plates/sieve cassettes it may be included as part of the current sample and impact results. Blocked sieve plates/sieve cassettes means less gluten can pass through during centrifuge and therefore impact results.

1.3 After completion of test

Wash chambers	Disassemble and then rinse sieves and washing chambers with water and dry.	Poor cleaning of wash chambers.	Wash chambers must be free from any residue and dried. Residue and wash chambers that are wet can impact the results.
Clean mixing hook and dispenser head	Remove the dispenser head and clean inside the body and hook. You do not need to dismantle the dispenser head after each test (see Figure 9).	Allowing residue to build up inside the dispenser head and hook.	A build-up of residue inside the dispenser head will impact how the hook turns and therefore impact the results.
Centrifuge sieve plates/ sieve cassettes	Rinse sieve plates/sieve cassettes with water, use a soft brush if necessary to remove any trapped material.	Poor cleaning or not cleaning the sieve plates/ sieve cassettes in between tests or at the end of testing.	Failing to clean the sieve plates/sieve cassettes can cause them to clog which will impact the results.

1.4 End of day

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Glutomatic	Rinse Glutomatic with distilled water to remove traces of the NaCl solution.	Leaving the equipment with NaCl in it once testing is finished.	Rinsing with water prevents salt damage to the equipment.
Container for NaCl solution	Rinse with water at the end of day/testing.	Leaving NaCl solution sitting in the container.	To prevent NaCl build up in the container which could affect the NaCl concentration the next time the container is used.
NaCl solution dispenser	Rinse with water at the end of the day/testing.	Leaving NaCl solution in the dispenser.	To prevent salt damage to the equipment. To prevent NaCl build up in the container which could affect the NaCl concentration the next time the container is used.
Centrifuge sieve plates/ sieve cassettes	Clean with water at the end of day/testing.	Not washing the sieve plates/cassettes.	To prevent salt damage to the equipment.
Centrifuge	Clean the centrifuge bowl at the end of day/testing.	Not cleaning the centrifuge.	To prevent salt damage to the equipment. Cleanliness of the centrifuge may impact results.

Table 1: Particle Size Distribution Specifications of the Wheat Meal for the Glutomatic[11]

Aperture of the sieve	% of sample passing through sieve
710 micrometers	100
500 micrometers	95-100
210 micrometers	80 or less

Table 4: Guidelines for Repeatability/Reproducibility for the FN Test[11]

Two WG results from analysis of the same sample			Test is done in the same lab using the same sample *Is the repeatability within acceptable limits?*	Test is done in different labs using the same sample *Is the reproducibility within acceptable limits?*
Rep 1	Rep 2	Avg		
29.0	30.0	29.5	Yes, acceptable (≤ 1.26%)	Yes, acceptable (≤ 1.37%)
29.0	30.3	29.6	No, outside of acceptable limits (≥ 1.26%)	Yes, acceptable (≤ 1.37%)
29.0	31.0	30.0	No, outside of acceptable limits (≥ 1.26%)	No, not acceptable (≥ 1.37%)

Note: Table above highlights findings from the ICC-Recommendation No. 203, Statistical Analysis of the Results of Collaborative Studies. Guidelines for test acceptability may vary between laboratories. 'Rep' = replicate, repetition of experimental conditions to estimate variability.

Figure 1: Fine sieve 88 micron

Left: sieve in good condition
Right: sieve in poor condition (should not be used)

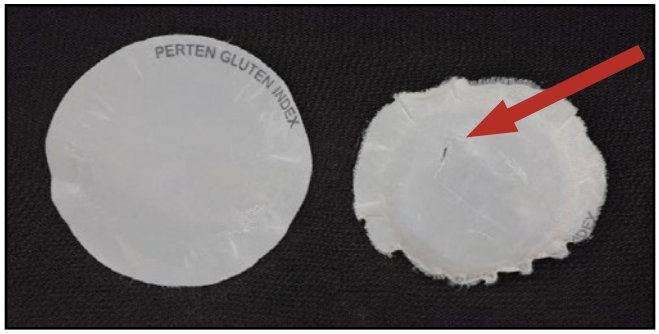


Figure 2: Coarse sieve 840 micron

Left: sieve in good condition
Right: sieve in poor condition (should not be used)

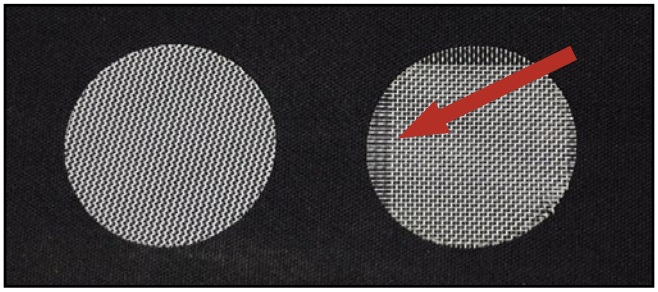


Figure 3: Sieve and sieve holders

Left: Unmarked sieve holder for fine sieve (88 micron)
Right: Marked sieve holder for coarse sieve (840 micron)

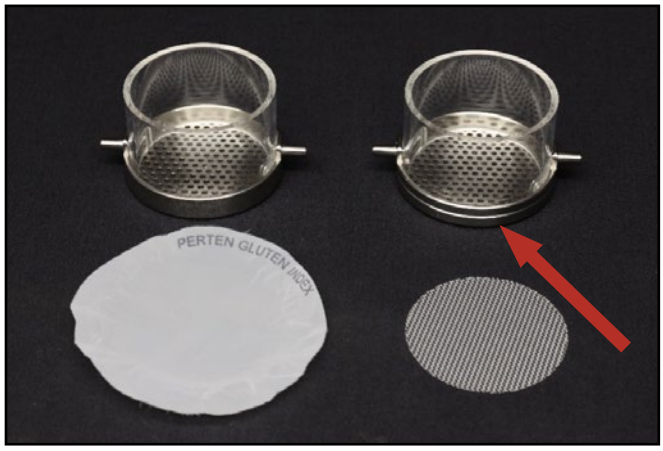


Figure 4: Dispensing NaCl solution



Figure 5: NaCl solution spread evenly over sample (left) and NaCl solution not wetting entire samples surface



Figure 6: Two-stage method. Transferring partly washed-out gluten between chambers. Note the different sieve holders (unmarked on the top and marked with a ring on bottom)



Figure 7: Centrifuge sieve plates (WG only)

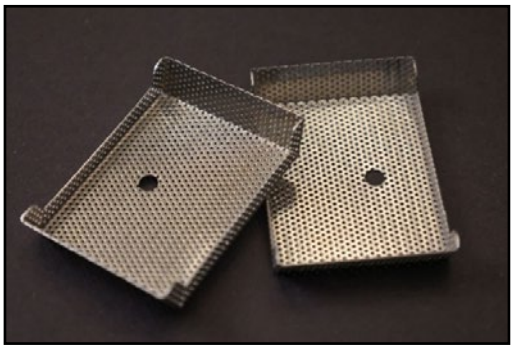


Figure 8: Centrifuge sieve cassettes (GI)



Figure 9: Removing hook and dispenser head



References

1. ICC Standard No. 101/1. (1982). Sampling of Grains.
2. AACC Approved Methods of Analysis, 11th Ed. Method 64-70.02 Manual Sampling of Wheat and Other Whole Grains. Approved Apr 13, 1961. Cereals & Grains Association, St. Paul, MN, U.S.A.
3. AACC Approved Methods of Analysis, 11th Ed. Method 64-71.01 Mechanical Sampling of Dry, Free-Flowing Granular Material. Approved Oct 31, 1971. Cereals & Grains Association, St. Paul, MN, U.S.A.
4. Canadian Grain Commission. (2020). Guide to Taking a Representative Sample. Website. https://grainscanada.gc.ca/en/grain-quality/sampling-grain/guide-taking-representative-sample/05-reducing-composite-sample.html (accessed July 6, 2026).
5. ICC Standard No. 130. (1980). Cereals – Sampling of Milled Products.
6. AACC Approved Methods of Analysis, 11th Ed. Method 64-60.01 Sampling of Flour, Grain Products, and Feed Products in Sacks. Approved Apr 13, 1961. Cereals & Grains Association, St. Paul, MN, U.S.A.
7. ICC Standard No. 110/1. (1976). Determination of the Moisture Content of Cereals and Cereal Products (Practical Method).
8. AACC Approved Methods of Analysis, 11th Ed. Method 44-15.02. Moisture – Air-Oven Methods. Approved Oct 30, 1975. Cereals & Grains Association, St. Paul, MN, U.S.A.
9. International Organization for Standardization. ISO 712:2009. Cereals and cereal products – Determination of moisture content – Reference method.
10. AACC Approved Methods of Analysis, 11th Ed. Method 38-12.02. Wet Gluten, Dry Gluten, Water-Binding Capacity, and Gluten Index. Approved November 8, 2000. Cereals & Grains Association, St. Paul, MN, U.S.A.
11. ICC Standard No. 155. (1994). Determination of Wet Gluten Quantity and Quality (Gluten Index ac. to Perten) of Whole Wheat Meal and Wheat Flour (Triticum aestivum).
12. International Organization for Standardization. ISO 21415-2:2006. Wheat and wheat flour – Gluten content. Part 1: Determination of wet gluten by mechanical means.
13. Perten Glutomatic 2000 System – Users guide