Technical Bulletin

CEREALS CANADA

Falling Number (FN) Test – Best Practices

What is the Falling Number test?

The Falling Number (FN) test indirectly measures alpha-amylase activity, an indication of sprout damage. It can be done on wheat (when ground) or flour/semolina.

- Alpha-amylase, also referred to as α-amylase, is an enzyme which breaks down the starch that is present in grain into smaller units such as sugars.
  - Higher sprout damage = more alpha-amylase = more starch breakdown = lower FN.
  - Less sprout damage = less alpha-amylase = less starch breakdown = higher FN.
- A low FN can negatively impact the quality of various end-products made from wheat.
- Sprout damage, and FN, is influenced by environmental factors during harvest, such as excessive moisture (rain or heavy dew).

The steps, tables and figures detailed in this document provide useful information on how to minimize errors and reduce variability when completing a FN test.

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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 FN results if not removed from the sample.
Sample quantity – whole kernel	A representative sample of wheat is required. [1,2,3,4] A minimum of 250 g is required, 300 g is preferred (if available).	Using <250 g or an unrepresentative wheat sample.	To minimize sampling error due to sprouted kernels, a representative sample of >250 g is required.[5]
Sample quantity – flour/semolina	Test a representative sample. [6,7]	Using a sample that is not representative of the lot being tested.	To minimize sampling error due to sprouted kernels, a representative sample of >250 g is required.[5]
Particle size of ground meal (prepared from a wheat sample)	Wheat should be ground using a Laboratory Mill LM 3100 or LM 120 with a 0.8 mm screen.	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.

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1.1 Sample Preparation, cont.

Cleaning of the grinder	Clean the grinder between each sample.	Grinding samples without cleaning the grinder between each sample.	Residue from grinding the previous sample can result in cross-contamination. This can especially impact results if the previous sample was highly sprouted.
Storage of ground meal	Use an airtight container or sealed bag and store the sample at room temperature (22 ± 2°C) as soon as possible after grinding.	Storing the ground sample in an unsealed container.	The ground sample should be stored in an environment that minimizes moisture changes to ensure greater accuracy when performing the test. Storage at higher temperatures (> 25°C) will accelerate moisture changes.
Moisture content of wheat (whole kernel)	Grind wheat that has a moisture content between 8-16%. Water should be added to wheat when the moisture content is <8% and drying should be done if the wheat moisture content is >16%.	Grinding wheat that has a very low moisture content (<8%) or very high moisture content (>16%).	Grinding efficiency is decreased when wheat with a moisture content >16% is ground. Meal with a moisture content between 8 and 16% can be stored safely for many months.
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[8, 9, 10] 2. Near Infrared (NIR)	Omitting moisture testing of the meal and flour/semolina.	The moisture content of the sample is needed in order to correct as is FN 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.

1.2.1 FN Test Setup

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Standardized method or standard operating procedure	The test should be performed according to a standardized method.[11, 12,13]	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.
Water bath – water	Fill, or top-up, with distilled water or equivalent purity.	Fill, or top-up, with tap water or well water.	Dissolved minerals present in tap or well water can alter the boiling temperature and thereby affect the FN result.

1.2.1 FN Test Setup, cont.

Water bath – water level	Check that the reservoir is full of distilled water to allow for automatic water level control (new FN models) or place beaker under the water level control and fill water bath with distilled water to top edge of the water level control (old FN models). Ensure water bath temperature has reached proper temperature after filling.	Failure to fill, or top-up water bath.	The water level and temperature of the water bath will affect heat transfer efficiency to viscometer tubes during a test (see Table 2).
Check water bath	Periodically check that water is rapidly boiling by pushing the steam flap down with a rod.	Failure to ensure that water in the water bath is boiling.	The temperature of the water bath will affect heat transfer efficiency to viscometer tubes during a test (see Table 2).
Cooling system	Turn on/set-up cold tap water or cooling tower (flow rate 400 mL/min).	Failure to turn on or set up cooling system.	The cooling system prevents evaporation from the water bath and steam buildup on FN visor.
Altitude Correction – altitude >760 m, (when testing flour/semolina) or >610 m (when testing wheat)[12]	1. Ensure that Altitude Correction is "enabled" and the correct sample type is selected or enter altitude in manually (see Figures 1-2). 2. Consult method for equation to manually calculate adjusted FN results.[11, 12, 13]	Failure to correct FN results for altitude of the testing laboratory.	Altitude of the testing laboratory affects the boiling temperature of water and FN results are affected by the boiling temperature of the water bath. Testing in locations at higher altitudes may obtain FN results higher than locations that are at sea level.
Stirrer and rod	Check that stirrer is clean and dry. Ensure the rod is straight and the ring is not worn or chipped (see Figure 3).	Failure to inspect stirrer and using a stirrer/rod that is damaged.	The stirrer should be replaced if it is bent or worn to avoid inaccurate FN results.
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 ± 0.05 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 FN results.

1.2.2 Running the FN Test

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Water for testing	Using distilled water or equivalent purity at room temperature (22 ± 2°C).	Using tap water or well water.	The mineral content and pH of water can affect the activity of alpha-amylase (enzyme present in sprout damaged wheat) thereby affecting FN results.
Dispensing water into viscometer tubes	Check that dispensing equipment is dispensing the proper volume of water (25 ± 0.2 mL). See Figure 4 for examples of acceptable equipment for dispensing water.	Failure to check dispensing accuracy or using dispensing equipment in poor working order.	Accurate dispensing of water is required to ensure the correct volume is used so that repeatable and reproducible FN results are generated.
Mixing and weighing of ground meal or flour/semolina	1. Thoroughly mix ground meal or flour/semolina. 2. Consult a moisture correction table to determine required sample weight -OR- Weigh 7.00 ± 0.05 g and correct FN results after FN test is complete.	Failure to mix sample before weighing and reporting an "as is" FN result without correcting to specific moisture basis -OR- failure to weigh the proper amount of sample (meal or flour/semolina).	Grinding of samples can lead to stratification of ground material. Thorough mixing of the ground material ensures a representative 7 g sample is used for testing. Research has shown greater variation in FN results at lower sample sizes.14 Reporting FN results on an 'as is' basis is not accurate and does not comply with standardized FN methods. FN results need to be corrected for moisture content.
Sequence of addition of water and ground material to viscometer tubes	1. Dispense water into the viscometer tube. 2. Add ground material using a funnel. -OR- 1. Add ground material using a funnel. 2. Dispense water into the viscometer tube.	Failure to ensure proper sample incorporation.	The goal is to create a uniform suspension of water and ground material. The addition of sample into the tube before water can lead to incomplete incorporation after shaking (unhydrated clumps may be present; See Figure 5 left image).
Shaking of viscometer tubes	The Shakematic 1095 is the preferred way to shake the ground material and water compared to manual shaking. If manual shaking is done, proper training is required.	If using a manual shaking method: shaking viscometer tubes more or less times than recommended by the standard method.	Manual shaking can lead to operator fatigue, repetitive strain injuries and can introduce sources of error (for example, if there are differences in shaking technique between lab staff).

1.2.2 Running the FN Test, cont.

Avoid sample loss	After shaking, check that there is not any unincorporated sample (see Figure 5). Remove the stopper, scrape any material on the bottom of the stopper back into the viscometer tube and, with the stirrer, scrape down any material adhering to the sides of the tube.	Failure to ensure all ground material is incorporated before the test is started.	Sample loss and poor sample incorporation are potential sources of error in the FN test.
Timing of shaking and scraping the viscometer tubes	Once the ground material and water are added to the tube, the shaking, scraping and placement of the tubes in the water bath should take 30-60 s.	Taking longer than 60 s to shake and scrape the tubes and place in water bath.	The tubes should be placed in the water bath as quickly as possible to minimize the effect of any α-amylase activity. Proper timing is critical during this step to ensure repeatable and reproducible results.

1.3 After Completion of Test/Clean-up

Step/Parameter	Acceptable Practice	Unacceptable Practice	Why?
Cooling viscometer tubes after test completion	Place tubes in cold water bath.	Neglecting to cool tubes before trying to clean.	Cooling tubes sets the slurry making them easier to clean. Handling hot tubes is a safety risk to staff.
Cleaning viscometer tubes	Insert tubing attached to tap (see Figure 6) to dislodge gelled material or use Perten Spolett 1010 (see Figure 7).	Poor cleaning of tubes leaving residue remaining.	Tubes must be clean and free from any residue. Residue (unclean tube) can affect repeatability and reproducibility of the FN test.
Cleaning stirrer	Ensure stirrer is free of debris.	Poor cleaning of stirrer.	Trapped slurry from a previous test in the neck of the black ebonite part of the stirrer can be a source of error for future tests (see Figure 8).
Strategy for rapid drying of viscometer tubes	Increase speed of tube drying by placing clean, wet tubes in a warm environment and then cooling to room temperature (22 ± 2 oC) before use.	Using wet tubes or using tubes that have not fully cooled to room temperature (22 ± 2 oC).	The use of wet and/or warm tubes can cause erroneous results and greater variability in FN results.

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

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

Table 2: Effect of Water Bath Temperature on FN Values.[15]

Temperature (°C)	Falling Number (s)
99.2	205
98.6	221
97.5	235
96.4	251
95.3	264
94.1	297
93.2	320

Note: Boiling point as measured at Fargo, ND, elevation 274 m.

Table 3: General "Rule of Thumb" for Acceptability Guidelines of Duplicate FN measurements

Two FN results from duplicate FN measurements of the same sample			≤ 10 seconds between duplicates?	Repeat test?
Duplicate 1	Duplicate 2	Avg (Reported result)		
280 s	290 s	285 s	Yes, acceptable	No
280 s	295 s	288 s	No, outside of limits	Yes

Note: Guidelines for test acceptability may vary between laboratories. Generally, as FN increases, difference between duplicates will increase. Duplicate measurement = same sample analyzed at the same time.

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

Two FN results from analysis of the same sample			Test is done in the same lab *Is the repeatability within acceptable limits?*	Test is done in different labs *Is the reproducibility within acceptable limits?*
Rep 1	Rep 2	Avg		
300 s	314 s	307 s	Yes, acceptable (≤ 3.44%)	Yes, acceptable (≤ 5.05%)
300 s	315 s	308 s	No, outside of acceptable limits (≥ 3.44%)	Yes, acceptable (≤ 5.05%)
300 s	322 s	311 s	No, outside of acceptable limits (≥ 3.44%)	Yes, acceptable (≤ 5.05%)
300 s	323 s	312 s	No, outside of acceptable limits (≥ 3.44%)	No, outside of acceptable limits (≥ 5.05%)

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: FN display screen for a sample run showing altitude correction enabled (red circled area)

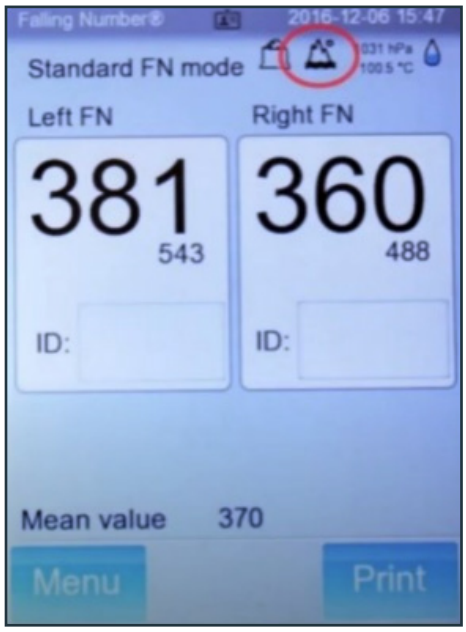


Figure 2: FN display screen showing where to enter altitude manually in local settings menu (red circled area)

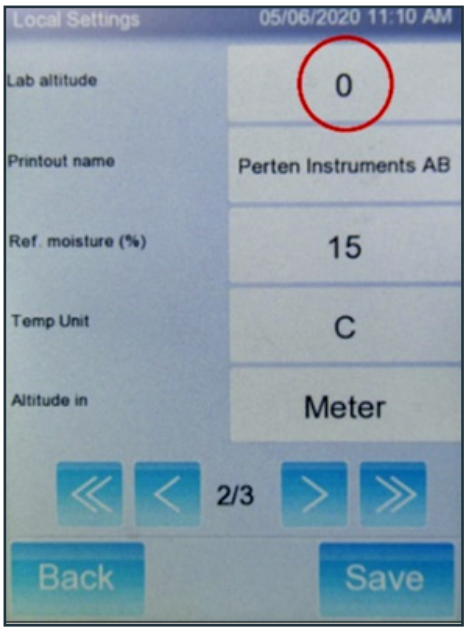


Figure 3: New stirrer (left) and worn stirrer (right)



Figure 4: Examples of acceptable water dispensing equipment.

Top left: Calibrated 25 mL pipette with bulb
Top right: Bottle top dispenser

Bottom: Serological pipette with pipette pump (top right) or Pipet-Aid™ (bottom right)



Figure 5: Examples of poor sample incorporation.

Left: Dry patch of sample on bottom of viscometer tube

Right: Dry layer at top of viscometer tube

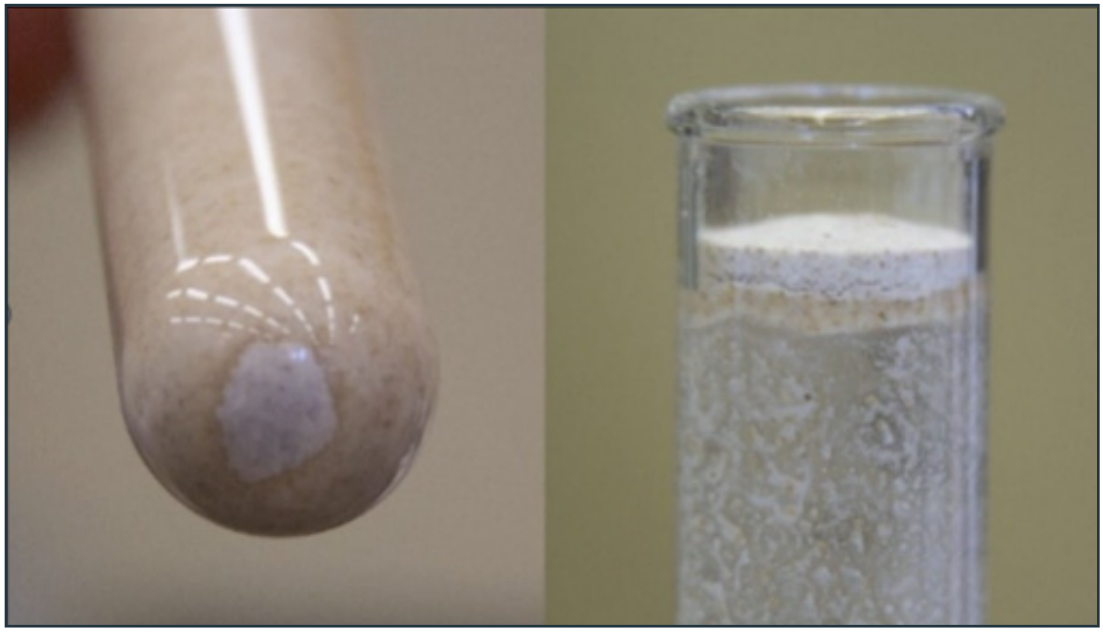


Figure 6: Cleaning procedure: Tubing inserted into cooled viscometer tube to dislodge solid material that has been cooled and set.



Figure 7: Perten Spolett 1010



Figure 8: Inside of black ebonite neck of stirrer containing trapped gelled material from the end of a FN test



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