

# COMMON WHEAT

## <sup>N</sup> Determination of Hardness by Near-Infrared Spectroscopy

Standard NF V03-715

### Determination of common wheat hardness by IR using BIPEA samples

Guidelines for the use of samples



## **1 – Purpose of the document**

This document describes the provisions of Standard NF V03-715:2025 relating to the routine method for the determination of common wheat hardness by near-infrared spectroscopy in the 0–100 range, on ground wheat and whole grains.

## **2 – Principle**

The method is based on near-infrared spectroscopy (NIRS), an indirect and correlative technique that is commonly used to predict the concentration of different components in organic samples.

This method is also highly sensitive to variations in the size and distribution of particles in a ground sample.

For hardness determination, calibration is based on two development methods depending on the sample format (ground or whole grains):

- either on the spectral information of samples selected as representative of the range for ground material, at specific wavelengths (1680 and 2230 nm).
- or on linear or nonlinear regression modeling used to correlate the near-infrared spectrum and hardness using officially approved standardized methods (e.g., multiple linear regression (MLR), artificial neural network regression (ANN), partial least squares regression (PLS)) for whole grains.

## **3 – Analytical method**

### **3.1 General information**

The analytical method is defined as the combination of an NIRS device and a prediction or calibration model.

### **3.2 Equipment**

#### **3.2.1 NIRS Instrument**

Instrument based on measurement by diffuse reflectance or by transmission, covering the wavelength range from 700 nm to 2500 nm, or parts of this range, or the specific wavelengths for hardness: 1680 nm and 2230 nm.

The optical principle may be interferometric (e.g., filters), dispersive (e.g., grating monochromators), or non-thermal (e.g., light-emitting diodes, laser diodes, and lasers). The instrument should be equipped with a system that performs diagnostic tests to evaluate photometric noise and reproducibility, as well as the accuracy and precision of wavelengths/wavenumbers (for scanning spectrophotometers).

The instrument should measure a sufficiently large sample volume or surface area to eliminate any significant influence of inhomogeneity resulting from the chemical composition or physical properties of the test sample. For transmission measurements, to achieve linearity and a maximum signal-to-noise ratio, the signal intensity should be optimized by adjusting the optical path to the common wheat (sample thickness) in accordance with the manufacturer's recommendations.

#### **3.2.2 Laboratory mill**

Laboratory hammer mill, equipped with a 0.8 mm sieve and capable of producing whole grain flour with the particle size specified in 5.1.2.

#### **3.2.3 Sieves**

Laboratory sieves with nominal aperture sizes of 710  $\mu\text{m}$ , 500  $\mu\text{m}$ , and 200  $\mu\text{m}$  (ISO 3310-1).

### **3.3 Prediction models**

Each wheat hardness prediction model is characterized by:

- in the case of using spectral information from selected samples considered representative of the range and ground:
  - the number of reference samples used to develop the prediction model and distributed across the hardness range,
  - the coefficients applied to the specific wavelengths for hardness: 1680 nm and 2230 nm,
  - in the case of linear or nonlinear regression modeling on whole grains,
  - the number of samples used to develop the prediction model (calibration),
  - the hardness range integrated into the model,
  - the expression of the result (without units),
  - the temperature range of the samples,
  - the number and performance of the reference laboratories involved,
  - the stability of the model, expressed as the number of harvests taken into account,
  - the support file of the prediction model, defined by its name and IT designation (e.g., CHECKSUM) ensuring its integrity,
  - the seasonal, geographical, and genetic variabilities covered.

### **3.4 Initial model validation steps in the case of linear or nonlinear regression modelling**

#### **3.4.1 General information**

Since NIRS analysis is an indirect and correlative technique, the results must be validated against a reference method (SKCS or NIRS using spectral information reflected from ground wheat and standardized using referenced samples).

The purpose of validation is to determine the standard error of prediction (SEP or SEP(C)), which expresses the accuracy of routine NIRS results corrected for the mean deviation (bias) between the routine NIRS method and the reference method.

The standard error of prediction between the reference method and the predicted values must be compared with the performance specifications of the prediction model and/or with historical performance data.

#### **3.4.2 Initial set of validation samples**

The initial validation of a prediction model must be conducted in accordance with EN ISO 12099 using independent test sets of wheat samples originating from different geographical areas in France and analyzed using the reference method (SKCS or NIRS using spectral information in reflectance standardized with referenced samples).

The requirements for the set of validation samples are as follows:

- At least 20 representative samples originating from geographical areas in which the model will be used, evenly distributed across the entire hardness range.
- The portion of the range without a reference sample must not exceed 5.
- The same sample analyzed on several devices or several times on the same analytical instrument counts as a single sample.
- Seasonal variations over a period of at least three years, as well as those relating to temperatures, instruments, and reference data must be included in the set.

All this information must be included in the prediction model report.

#### **3.4.3 Updating the prediction model and validation of the new model**

The prediction model must be updated by the person responsible for its development to ensure that new climatic conditions affecting the grains and new varieties introduced onto the market are included. These updates must incorporate the new samples to account for the variability they introduce. The updated

prediction model must be developed in accordance with EN ISO 12099. Validation must be carried out in accordance with the initial validation procedure (3.4) and include at least 20 new samples of the species concerned by the update.

#### **3.4.4 Local validation of the method**

Before use, the method must be validated on a set of independent tests representative of the population of samples to be analyzed. At least 15 samples are required to determine the bias, the standard error of prediction (SEP, see EN ISO 12099; Article “Statistics for performance measurement”) and the slope. The samples shall be subject to two determinations. The arithmetic mean obtained if the repeatability conditions are met (see 7.3) will be used to calculate the statistical criteria.

Bias, or inherent systematic error, as described in EN ISO 12099 (Article “Statistics for performance measurement”), occurs when the predicted results for a specific group of samples or a product show an average offset value when compared to their reference values. This phenomenon can occur with single sample types. The bias (i.e., mean difference between the reference analysis results and the predicted results) may or may not be statistically significant. A confidence limit for bias can be calculated by following the procedure described in EN ISO 12099. When this limit for bias is exceeded, a correction shall be implemented in the instrument software. If the limit for the SEP or the slope is exceeded, validation will not be accepted.

#### **3.5 Initial validation and local validation performance**

The results of initial validation and local validation must at least comply with the specifications indicated below, regardless of the calibration development method (ground or whole grains):

Maximum overall accuracy expressed as SEP<sub>max</sub>:

**SEP<sub>max</sub> = 5 for ground grains and 10 for whole grains**

### **4 – Characteristics of control test samples**

- 1 set of 15 samples of wheat batches, selected on their hardness (14–84)
- Weight: approx. 300g per sample
- Shelf life: 10 years if storage conditions are respected
- Storage conditions: cold room 5±3°C, airtight bag, stable humidity conditions (<70%)

### **5 – Procedure**

#### **5.1 Preparation of the sample for testing**

No specific preparation of the sample is required for whole grain analysis.

For ground wheat, steps 5.1.1 and 5.1.2 must be followed.

##### **5.1.1 Removal of impurities**

If necessary, clean the sample to remove impurities (for example stones, dust, husks, and grains from other cereals). From the laboratory sample, take a representative 250 g test sample in the case of an analysis on ground wheat.

##### **5.1.2 Grinding of grain samples**

The moisture content of grain samples (expressed as a percentage of mass fraction and determined according to ISO 712-1 or EN 15948 or by near-infrared spectroscopy validated according to EN ISO 12099) must be between 9 and 15% before grinding.

Carefully load the laboratory mill (3.2.2) with grains, making sure to avoid overloading or overheating. The mill load may be automatically controlled using an automatic loading device. Grinding should continue for 30 to 40 seconds after the entire sample has entered the mill. Remove any remaining bran particles inside the mill, if these do not represent more than 1% of the quantity of grain taken for grinding.

Thoroughly mix the entire ground product before use.

It is advisable (especially in the case of successive grinding operations) to allow the ground product (flour) to cool for 1 hour before proceeding with the test.

The ground product must comply with the specifications given in Table 1.

**Table 1 – Specifications of the ground product**

<b>Nominal size of the openings</b>	<b>Proportion of ground product passing through the sieve (<math>\mu\text{m}</math>, %)</b>
<b>710</b>	<b>100</b>
<b>500</b>	<b>95 to 100</b>
<b>200</b>	<b>80 or less</b>

Periodically check the particle size distribution of the ground grain using a well-homogenized sample of the ground product (5.1.2). To do this, select the appropriate sieves as specified in Table 1, arranging them in descending order of nominal aperture size, placing sieve aids in each sieve, and placing them over a receiving container.

Weigh a representative sample of 50.0 g and place it on the upper sieve. Sieve horizontally, manually for at least 5 minutes, and until nothing passes through the 710  $\mu\text{m}$  sieve, or mechanically for 10 minutes. Weigh the quantity of product retained on each sieve and the contents of the receiving container. Calculate the percentage of ground material passing through each sieve.

The grinding method used to prepare the ground wheat sample influences the hardness results. Different laboratory mills will produce different particle-size distributions.

To obtain comparable results, the same sample preparation method should be used, or a new prediction model should be developed, or new calibration coefficients should be calculated.

## **5.2 Measurement**

Follow the manufacturer's instructions for the instrument.

## **5.3 Periodic adjustment of the instrument**

As mentioned in EN ISO 12099 (section “Verification of instrument stability”), if several instruments are used within a network, particular attention should be paid to the standardization of the instruments in accordance with the manufacturers’ recommendations.

#### 5.4 Verification of the stability of the instrument

See the article “Verification of the stability of the instrument” in EN ISO 12099 using one or more reference samples.

#### 5.5 Monitoring of method performance

The performance of the method must be monitored against the reference method at a frequency defined by the user to ensure the continued conformity of the model.

This performance check must be conducted on samples selected from among the samples analyzed routinely and according to the reference method, or samples used for the initial calibration of the instrument. It may be necessary to implement a sampling strategy that ensures a balanced distribution of samples across the entire 0-100 range. At least 15 samples are required (to obtain a normal distribution of variance).

For instruments operating within a network and calibrated using a standard reference instrument, it is sufficient to carry out the method performance check on the standard reference instrument.

The adjustment procedure (5.3), in accordance with the requirements of this paragraph, may be used for monitoring method performance with at least 15 samples, in accordance with EN ISO 12099.

### 6. Target values and Uncertainties

Wheat	Target Value	Uncertainty
BT01	14	4
BT02	15	3
BT03	24	4
BT04	33	5
BT05	37	5
BT06	50	5
BT07	47	5
BT09	51	9
BT10	57	7
BT11	62	4
BT13	69	10
BT14	74	11
BT15	84	8
BT16	78	7
BT17	84	8

