SLOVENSKI STANDARD
kSIST FprEN 15948:2014

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Žito - Določanje vlage in beljakovin - Metoda z uporabo bližnje infrardeče spektroskopije v celih zrnih

Cereals - Determination of moisture and protein - Method using Near-Infrared-Spectroscopy in whole kernels

Getreide - Bestimmung der Feuchte und des Proteins - Verfahren der Nahinfrarot-Spektroskopie bei ganzen Körnern

Céréales - Détermination de la teneur en eau et en protéines - Méthode utilisant la spectroscopie dans le proche infrarouge sur des grains entiers

Ta slovenski standard je istoveten z: **FprEN 15948**

ICS:

67.060	Žita, stročnice in proizvodi iz njih	Cereals, pulses and derived products

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Cereals - Determination of moisture and protein - Method using Near-Infrared-Spectroscopy in whole kernels

Céréales - Détermination de la teneur en eau et en protéines - Méthode utilisant la spectroscopie dans le proche infrarouge sur des grains entiers

Getreide - Bestimmung der Feuchte und des Proteins - Verfahren der Nahinfrarot-Spektroskopie bei ganzen Körnern

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Foreword

This document (FprEN 15948:2014) has been prepared by Technical Committee CEN/TC 338 “Cereal and cereal products”, the secretariat of which is held by AFNOR.

This document will supersede EN 15948:2012.

This document is currently submitted to the Unique Acceptance Procedure.

1 Scope

This European Standard defines a routine method for the determination of moisture and protein in whole kernels of barley and wheat using a near-infrared spectrophotometer in the constituent ranges:

a) for wheat:

1) moisture content minimum range from 8 % to 22 %;

2) protein content minimum range from 7 % to 20 %.

b) for barley:

1) moisture content minimum range from 8 % to 22 %;

2) protein content minimum range from 7 % to 16 %.

This European Standard describes the modalities to be implemented by the supplier (5.3 and 5.4) and the user of the method.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN ISO 12099:2010, *Animal feeding stuffs, cereals and milled cereal products - Guidelines for the application of near infrared spectrometry (ISO 12099:2010)*

ISO 5725-2, *Accuracy (trueness and precision) of measurement methods and results - Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN ISO 12099:2010 apply.

4 Principle

The method is based on Near-Infrared (NIR) spectroscopy, an indirect, correlative technique to predict the concentration of various constituents in organic samples. Linear or nonlinear regression modelling is used to relate NIR spectra to moisture or protein concentrations determined by officially approved standard methods (e.g. artificial neural network - ANN, Partial Least Square Regression - PLS).

5 Method of analysis

5.1 General

According to this document, the method of analysis is defined as the association between a NIR instrument and a model of prediction.

5.2 Near Infrared Instrument

Based on diffuse reflectance or transmittance measurement covering the wavelength region of 700 nm–2500 nm or segments of this or at selected wavelengths.

5.3 Prediction models

Each model for the prediction of protein and moisture contents in whole grain of wheat and barley is amongst others defined by:

— the number of samples used for the calibration development;

— the constituent ranges covered in the model for moisture and protein;

— the temperature range of the samples;

— the number and performance of involved reference labs;

— the stability of the model i.e. by number of harvests covered;

— the calibration file defined by its name and its IT name (for example CHECKSUM) insuring its integrity;

— the seasonal, geographic and genetic variations covered.

5.4 Initial validation of the model

5.4.1 General

Since NIR analysis is an indirect, correlative technique, the results shall be validated against chemical analysis reference methods. It is important that the reference methods used are officially approved such as the methods described in the EN ISO standards previously cited (Clause 2). The purpose of validation is to determine the root mean square error of prediction which depends at the same time on the correlation, the bias and the slope.

The root mean square error between chemical analysis methods and predictions shall be compared to calibration performance specifications and/or historical performance.

5.4.2 Initial validation sample set

The initial validation of a calibration model shall be done in accordance with EN ISO 12099 using independent test sets of wheat and barley samples, originating from different countries and analysed by the reference methods given in Clause 2.

Requirements for the validation sample set are:

— at least 200 samples coming from 10 countries (20 representative samples min/country) distributed homogeneously over the entire constituent range;

— the part of the range without any reference sample shall not exceed 0,3 %;

— different scans from one sample shall not be considered as different samples;

— seasonal effects over at least a three year period, temperature effects, instrument variation and the variability of reference data shall be included in the set.

5.4.3 Initial validation performances

The results of the initial validation shall at least fulfil the specifications given in Table 1.

Table 1 — NIR performances for the determination of moisture and protein (see also Annex B)

		Moisture Wheat and barley	Protein Wheat	Protein Barley
Overall accuracy expressed as SEP as constituent % w/w		0,24 %	0,27 %	0,27 %
Constituent concentration in the independent validation data set	Min	8,0 %	7 % d.m.	7 % d.m.
	Max	22 %	20 % d.m.	16 % d.m.

NOTE The minimum performance given in Table 1 includes the variation of reference data as documented by the number of reference labs involved, regional and genetic variations, the number of countries and crop species involved and the robustness over the last five years (see also Annex B).

5.5 Update of calibration model and validation of new model

The prediction model in accordance with this standard shall be updated by the one issuing the calibration model to ensure inclusion of new climatic crop conditions and new varieties introduced on the market. These updates shall be made by keeping the original database with addition of the new samples as needed.

The new prediction model shall be updated according to EN ISO 12099.

Validation shall be made according to the initial validation (5.3) and include at least 20 new samples.

6 Sampling

Sampling is not part of the method specified in this European Standard. A recommended sampling procedure is given in EN ISO 24333 [2].

It is important that the sample analysed in routine is truly representative for the batch and has not been damaged or modified.

7 Procedure

7.1 Preparation of the test sample

No specific sample preparation is required.

7.2 Measurement

Follow the instructions of the instrument manufacturer.

7.3 Local validation of the method

Before use, the method shall be validated on an independent test set that is representative of the sample population to be analysed. For the determination of bias, at least 10 samples are needed; for the determination of Standard Error of Prediction (SEP, see EN ISO 12099:2010, Clause 6.5) at least 20 samples

are needed. Validation shall be carried out for each sample type, constituent/ parameter and temperature (see EN ISO 12099:2010, Clause 5.4).

Bias or inherent systematic error, as described in EN ISO 12099:2010, (Clause 6), is exhibited when the predicted results of a specific sample group or product show a mean offset value when compared to their reference values. This may occur with unique sample types.

The bias (i.e. mean difference between the chemical analysis results and the predicted results) may or may not be statistically significant. Based on the procedure described in EN ISO 12099, a bias confidence limit can be calculated.

When this limit is exceeded, a bias is implemented in the instrument software and the validation process repeated. Refer to the manufacturer instructions and to EN ISO 12099 for procedure.

7.4 Periodical adjustment of the instrument

To ensure its accuracy, each instrument shall be checked at least annually, against the reference method, either directly or through a master instrument.

The execution of this check shall be performed on samples covering a range as wide as possible, taking into account seasonal, geographic and genetic variations.

The number of samples for the adjustment should be sufficient for the statistics used to check the performance. For the determination of the bias, at least 10 samples are needed, for the determination of standard error of prediction (SEP) and for the slope adjustment, at least 20 samples are needed.

7.5 Checking instrument stability

See Clause 9 of EN ISO 12099:2010.

7.6 Follow up of method performance

Performance of the method shall be checked at least annually, against reference methods to secure the constant adequacy of the model with the requirements of this standard (see 5.4.2).

This performance test shall be made on samples selected from the pool of analysed samples. It may be necessary to resort to some sampling strategy to ensure a balanced sample distribution over the entire calibration range and to ensure that samples with a commercially important range are covered. At least 20 samples are needed (to expect a normal distribution of variance).

For instruments operated in a network and adjusted against a master instrument, it is sufficient to run the performance check of the method of this last one.

The adjustment (7.4) respecting the requirements of this clause may be used for the follow-up of the method performance.

It is recommended to participate in an internationally accepted proficiency testing scheme (PTS) that includes NIRS predicted results and results generated by following the standards specified in Clause 2.

8 Calculation and expression of results

The software of the instrument calculates the results for moisture and protein and displays them in % w/w (g/100 g) to two decimal places.

If multiple measurements are made on the same sample, calculate the arithmetic mean.

Express final results to two decimal places.

9 Accuracy and precision of the method

9.1 Accuracy

The accuracy of the prediction model is determined by validation in accordance with EN ISO 12099:2010 and expressed by the Standard Error of Prediction (see Table 1). The Standard Error of Prediction (SEP) is an expression of the bias corrected average difference between predicted and reference values predicted by the model when applied to a set of samples not included in the derivation of the model. The values also include the uncertainty of reference results.

The predicted results will not in more than 5 % of cases deviate more than 1,96 x SEP (as determined in the above paragraph) from the best estimate of the true value.

NOTE As NIR is an indirect method, the typical standard deviation of reproducibility for the used reference methods are given here for comparison:

— Moisture (EN ISO 712) = 0,16 %;

— Protein (EN ISO 20483) = 0,20 %;

— Protein (EN ISO 5983-2) = 0,20 %;

— Protein (CEN ISO/TS 16634-2)= 0,21-0,26 %.

9.2 Precision

9.2.1 General

The precision of the prediction model shall be determined from an interlaboratory test organized according to ISO 5725-2 and at least fulfil the performance criteria of repeatability and reproducibility given below.

Details of an example of an interlaboratory test are summarized in Annex A. The precision data given below are derived from this example.

Figure A.1 and Figure A.2 show that the repeatability and the reproducibility are independent of the concentration. The figures in Annex B show that the dispersion is identical over the validated range (Figure B.1 and Figure B.2). The model can therefore be used in the whole validated range, even though the interlaboratory trial covered a smaller range.

9.2.2 Repeatability

The absolute difference between two independent single test results, obtained using the same method on identical test material in the same laboratory by the same operator using the same equipment within a short interval of time will not in more than 5 % of cases be greater than the repeatability limit r ($r=s_r x2,8$) with:

$r_{protein}$ = 0,42 %

$r_{moisture}$ = 0,15 %

9.2.3 Reproducibility

The absolute difference between two single test results, obtained using the same method on identical test material in different laboratories with different operators using different equipment, will not in more than 5 % of cases be greater than the reproducibility limit R ($R=s_R x2,8$) with:

$R_{protein}$ = 0,45 %

$R_{moisture}$ = 0,25 %

9.2.4 Critical difference

9.2.4.1 General

When the difference between two averaged values obtained from two test results under repeatability or reproducibility conditions is to be assessed, the repeatability or reproducibility limit cannot be used, one shall use the Critical Difference (CD).

9.2.4.2 Comparison of two groups of measurements in one laboratory

The critical difference (CD) between two averaged values obtained from two test results under repeatability conditions is equal to:

$$CD = 2{,}8\, s_r \sqrt{\frac{1}{2n1} + \frac{1}{2n2}} = 2{,}77\, s_r \sqrt{\frac{1}{2}} = 1{,}98\, S_r$$

where

s_r is the standard deviation of repeatability;

$n1$ and $n2$ are the number of test results corresponding to each of the averaged values;

CD_r (protein) = 0,30;

CD_r (moisture) = 0,11.

9.2.4.3 Comparison of two groups of measurements in two laboratories

The critical difference (CD) between two averaged values obtained in two different laboratories from two test results under repeatability conditions is equal to:

$$CD = 2{,}8\sqrt{s_R^2 - s_r^2\left(1 - \frac{1}{2n_1} - \frac{1}{2n_2}\right)} = 2{,}8\sqrt{s_R^2 - 0{,}5 s_r^2}$$

where

s_r is the standard deviation of repeatability;

s_R is the standard deviation of reproducibility;

n_1 and n_2 are the number of test results corresponding to each of the averaged values;

CD_R (protein) = 0,32;

CD_R (moisture) = 0,23.

10 Test Report

The test report shall specify:

a) all information necessary for the complete identification of the sample;

b) the sampling method used (if known);

c) the application model and instrument used with reference to this European Standard;

d) all operating details not specified in this European Standard, or regarded as optional, together with details of any incidents which may have influenced the test result(s);

e) the test result(s) obtained.

Annex A
(informative)

Results of examples of interlaboratory test

A.1 FOSS interlaboratory test

An interlaboratory test, organized by the company FOSS Analytical AB (Sweden) in 2008, involving 20 participants from 12 countries was carried out on 6 wheat and 4 barley samples from the 2007 harvest, containing protein and moisture in various concentrations. The participants were the master labs of European grain networks. The grain networks did also assist in the collection of the samples (Table A.1).

The results obtained were subjected to statistical analysis in accordance with ISO 5725-1 and ISO 5725-2 to calculate the precision data shown in Table A.2 to Table A.5.

Table A.1 — Samples for the interlaboratory study

Sample	Description	Country of origin
B1	Spring barley (2-row, malting barley)	UK
B2	Spring barley (2-row, feed barley)	Denmark
B3	Spring barley (2-row, malting barley)	Denmark
B4	Winter barley (6-row, malting barley)	France
W1	Spring wheat (hard)	Germany
W2	Spring wheat (hard)	France
W3	Winter wheat (hard)	UK
W4	Spring wheat (soft)	Germany
W5	Winter wheat (hard)	Italy
W6	Durum wheat	Italy

Table A.2 — Results of statistical analysis for the determination of the protein content in wheat by the ANN model WB003034

Sample	WG 1	WG 2	WG 3	WG 4	WG 5	WG 6
Number of laboratories	20	20	20	20	20	20
Mean predicted protein content (% d.m.)	16,883	11,789	13,047	10,876	14,985	14,173
Repeatability standard deviation s_r (% P)	0,159[a]	0,113[a]	0,099	0,106	0,087	0,109[a]
Repeatability relative stand. dev. s_r %	0,943	0,958	0,76	0,979	0,583	0,771
Repeatability limit r [r = 2,8 x s_r], %	0,440	0,313	0,274	0,294	0,241	0,302
Reproducibility stand. dev. s_R (% P)	0,159	0,113	0,107	0,143	0,125	0,109
Reproducibility relative stand. dev. s_R %	0,943	0,958	0,819	1,317	0,834	0,771
Reproducibility limit R [R = 2,8 x s_R], %	0,440	0,313	0,296	0,396	0,346	0,302
Best estimate of true protein value (%)[b]	16,88	11,75	13,08	10,86	14,93	14,50
Critical difference (n=2), reference methods	0,31	0,22	0,22	0,34	0,30	0,21
Deviation predicted - true value (%)	0,01	0,04	0,03	0,01	0,06	0,33

[a] $s_r > s_R$, s_R set to be equal s_r.

[b] Average protein value (after elimination of outliers) generated by 17 Master labs of the European grain networks, using Kjeldahl (EN ISO 20483 or EN ISO 5983-2) and Dumas (CEN ISO/TS 16634-2) methods.