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Foodstuffs — Methods for the detection of genetically modified organisms and derived products — Protein based methods

Produits alimentaires — Méthodes pour la détection d'organismes génétiquement modifiés et de produits dérivés — Méthodes basées sur les protéines

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Foreword

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International Standards are drafted in accordance with the rules given in the ISO/IEC Directives, Part 2.

The main task of technical committees is to prepare International Standards. Draft International Standards adopted by the technical committees are circulated to the member bodies for voting. Publication as an International Standard requires approval by at least 75 % of the member bodies casting a vote.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. ISO shall not be held responsible for identifying any or all such patent rights.

ISO 21572 was prepared by the European Committee for Standardization (CEN) in collaboration with Technical Committee ISO/TC 34, *Food products*, in accordance with the Agreement on technical cooperation between ISO and CEN (Vienna Agreement).

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Throughout the text of this document, read "...this European Standard..." to mean "...this International Standard...".

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Foreword

This document (EN ISO 21572:2004) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN, in collaboration with Technical Committee ISO/TC 34 “Agricultural food products”.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by September 2004, and conflicting national standards shall be withdrawn at the latest by September 2004.

The demonstration of the presence of genetically modified proteins can either be qualitative or quantitative. These steps are laid down in this draft European Standard.

Other standards dealing with methods of analysis for the detection of genetically modified organisms and derived products in foodstuffs are the following:

prEN ISO 21568 *Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Sampling (ISO/DIS 21568:2003).*

prEN ISO 21571 *Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products - Nucleic acid extraction (ISO/DIS 21571:2002)*

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prEN ISO 21569 *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Qualitative nucleic acid based methods (ISO/DIS 21569:2002)*

prEN ISO 21570 *Foodstuffs - Methods of analysis for the detection of genetically modified organisms and derived products - Quantitative nucleic acid based methods (ISO/DIS 21570:2003)*

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Further information about definitions and general items involving the steps cited above are collected in:

prEN ISO 24276 *Foodstuffs – Nucleic acid based methods of analysis for the detection of genetically modified organisms and derived products – General requirements and definitions (ISO/DIS 24276:2002)*

Annex A is normative.

This document may touch copyrights and patents: for further information, contact your National Standardisation Institute.

According to the CEN/CENELEC Internal Regulations, the national standards organizations of the following countries are bound to implement this European Standard: Austria, Belgium, Czech Republic, Denmark, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Luxembourg, Malta, Netherlands, Norway, Portugal, Slovakia, Spain, Sweden, Switzerland and the United Kingdom.

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Introduction

Analyses to detect genetically modified organisms (GMOs) and derived products can either be performed to screen, identify or quantify GMOs and their derived products in a given matrix.

For the detection of the transgenic origin of ingredients, the basic principle of a protein-based method is to:

— take a representative sample of the matrix;

— extract the proteins;

— detect and/or quantify the specific protein derived from GMO(s) under study.

As new methods become validated and accepted, they will be annexed to this standard.

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1 Scope

This European Standard provides general guidelines and performance criteria for methods for the detection and/or quantitation of specific proteins derived from genetically modified (GM) plant material in a specified matrix.

These general guidelines address existing antibody based methods. Methods other than those described in annex A may also detect the protein. The same criteria as outlined in this standard generally apply.

2 Normative references

This European Standard incorporates by dated or undated reference, provisions from other publications. These normative references are cited at the appropriate places in the text and the publications are listed hereafter. For dated references, subsequent amendments to or revisions of any of these publications apply to this European Standard only when incorporated in it by amendment or revision. For undated references the latest edition of the publication referred to applies (including amendments).

prEN ISO 21568 *Foodstuffs – Methods of analysis for the detection of genetically modified organisms and derived products – Sampling (ISO/DIS 21568:2003).*

3 Terms and definitions

For the purposes of this European Standard, the following terms and definitions apply.

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3.1 General terms

3.1.1
sample
One or more sampling units taken from a population and intended to provide information of the population.

[ISO 3534-1:1993]

3.1.2
laboratory sample
Sample intended for laboratory inspection or testing.

3.1.3
test sample (test portion)
Sample, as prepared for testing or analysis, the whole quantity being used for analysis or testing at one time.

[ISO 3534-1]

3.1.4
matrix
All components in the sample with the analyte. Each matrix generally has a common name which permits classification.

3.1.5
denaturation of proteins
Physical and/or (bio)chemical treatment which destroys or modifies the structure of the analyte. The denaturation may modify structural, functional, enzymatic or antigenic properties of the protein.

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3.2 Terms relative to antibodies

3.2.1
antibody
Protein (immunoglobulin) produced and secreted by B lymphocytes in response to a molecule recognised as foreign (antigen). The antibody is capable of binding to that specific antigen.

3.2.2
antigen
Substance that is recognised as foreign by the immune system and elicits an immune response.

3.2.3
clone
Population of identical cells derived from a single cell line.

3.2.4
cross-reactivity
Binding of the antibody to substances other than the analyte of primary interest.

3.2.5
monoclonal antibody
Antibody produced from a single hybridoma clone and directed to a single antigen determinant.

3.2.6
polyclonal antibody
Antibody produced by several clones of lymphocytes.

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3.2.7
Specificity of an antibody
Ability of an antibody to specifically bind to an antigen determinant and not to other similar structures on that or other antigens.

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3.3 Terms relative to techniques

3.3.1
conjugate
Material produced by attaching two or more substances together.

NOTE Conjugates of antibodies with fluorochromes (e.g. coloured particles), radiolabelled substances, or enzymes are often used in immunoassays.

3.3.2
Western blotting
Transfer of an antigen (i.e. the protein of interest), following electrophoretic separation, to a binding surface. The antigen may be visualised with a specific radiolabelled or enzyme-conjugated antibody.

3.3.3
ELISA – enzyme linked immunosorbent assay
In vitro assay that combines enzyme-linked antibodies and a substrate to form a coloured reaction product. Depending on the application, this assay can be used for qualitative or quantitative purposes.

3.3.4
test kit
Set of chemicals, materials and instructions for use, packaged together and intended for in vitro measurement for detection of a specified analyte.

3.3.5
dip stick format
Qualitative and rapid assay formats, including lateral flow strips, where an antibody or an analyte is coated to a solid surface.

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3.4 Terms relative to control

3.4.1
reference material
Material or substance one or more of whose property values are sufficiently homogeneous and well established to be used for the calibration of an apparatus, the assessment of a measurement method, or for assigning values to materials.

[ISO Guide 30]

3.4.2
standard
Measured material, measuring instrument, reference material or measuring system intended to define, realise, conserve or reproduce a unit of one or more values of a quantity to serve as a reference or preparation of known characteristics used to standardise the analysis.

3.5 Terms relative to validation

3.5.1
accuracy
Closeness of agreement between a test result and the accepted reference value.

NOTE The term accuracy, when applied to a set of test results involves a combination of random components and a common systematic error or a bias component.

[ISO 3534-1:1993]

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3.5.2
precision
Closeness of agreement between independent test results obtained under stipulated conditions.

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NOTE 1 Precision depends only on the distribution of random errors and does not relate to the true value or the specified value.

NOTE 2 The measure of precision usually is expressed in terms of imprecision and computed as a standard deviation of the test results. Less precision is reflected by a larger standard deviation.

NOTE 3 "Independent test result" means results obtained in a manner not influenced by any previous result on the same or similar test object. Quantitative measures of precision depend critically on the stipulated conditions. Repeatability and reproducibility conditions are particular sets of extreme stipulated conditions.

[ISO 3534-1:1993]

3.5.3
Bias
Estimate of the systematic (consistent) deviation of the measured result from the true result of a given sample.

3.5.4
sensitivity
Capacity to record a small variation in concentration of a substance in the test material.

In this context, sensitivity usually is meant as the smallest quantity or concentration of the analyte that can be reliably distinguished from background.

3.5.5
specificity
Property of a method to respond exclusively to the characteristic or analyte defined in the Codex standard.

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3.5.6
Limit of detection (LOD)
Limit of detection for qualitative methods is the lowest concentration or content of the analyte that can be detected reliably, but not necessarily quantified, as demonstrated by satisfactory collaborative trial or single-laboratory validation [1], [2].

3.5.7
Limit of quantitation (LOQ)
Limit of quantitation of an analytical procedure is the lowest amount or concentration of analyte in a sample which can be quantitatively determined with an acceptable level of precision and accuracy as demonstrated by satisfactory collaborative trial or single-laboratory validation according to ISO 5725, [2], or [3].

3.5.8
Applicability range (range of quantification/linearity/dynamic range)
Upper and lower limits of quantitation as expressed by a set of reference materials (or dilutions).

3.5.9
Repeatability [Reproducibility] limit
Value less than or equal to the absolute difference between two test results, as expected under repeatability [reproducibility] conditions with a probability of 95 %.

NOTE The symbol used is r [R].

[ISO 3534-1]

When examining two single test results obtained under repeatability [reproducibility] conditions, the comparison should be made with the repeatability [reproducibility] limit r [R] = 2,8 s_r [s_R].

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3.5.10
Reproducibility
Precision under reproducibility conditions.

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[ISO 3534-1:1993]

3.5.11
Reproducibility conditions
Conditions where test results are obtained with the same method on identical test items in different laboratories with different operators using different equipment.

[ISO 3534-1:1993]

3.5.12
Repeatability
Precision under repeatability conditions.

[ISO 3534-1:1993]

3.5.13
Repeatability conditions
Conditions where independent results are obtained with the same method on identical test items in the same laboratory by the same operator using the same equipment within short intervals of time.

[ISO 3534-1:1993]

3.5.14
Repeatability [Reproducibility] standard deviation
The standard deviation of test results obtained under repeatability [reproducibility] conditions.

NOTE Repeatability [Reproducibility] standard deviation is a measure of the dispersion of the distribution of test results under repeatability [reproducibility] conditions. Similarly “repeatability [reproducibility] variance” and “repeatability [reproducibility] coefficient of variation” could be defined and used as measures of the dispersion of test results under repeatability [reproducibility] conditions.

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[ISO 3534-1]

3.5.15
Recovery
Ability to measure or recover a known amount of analyte from fortified samples over a range of quantitation.

4 Principle

The target protein is extracted according to the procedure described for that specific matrix, and a specific antibody is used to detect or measure the concentration of the protein in the sample.

5 Reagents

During the analysis, unless otherwise stated, use only reagents of recognised analytical grade and only de-ionised or distilled water or water that has been purified, or equivalent.

Other reagents, such as antibodies, conjugates, substrate, stop solutions and buffer components are method specific. Please refer to the method for specifics regarding reagents such as protein standards or reference materials, antibodies coated to a solid surface or free, controls and samples.

6 Apparatus and equipment

Apparatus and equipment is specified in A.5.

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7 Sampling

Sampling is described in detail in prEN ISO 21568.

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8 Procedure

8.1 General

Storage conditions and shelf-life of antibodies, conjugate, substrate, etc shall be clearly specified by the provider.

For the use of this standard, general requirements of quality assurance for laboratories shall be observed (e.g. concerning calibration of apparatus, double determination, blanks, use of reference materials, preparation of calibration curves, etc). Carefully clean all equipment coming into direct contact with the sample to prevent contamination.

Use appropriate laboratory equipment with low protein binding capacity (e. g. polypropylene tubes) to prevent protein adsorption during the whole procedure.

8.2 Preparation of sample solution

Once a representative sample is obtained, specific sample preparation procedures may be found in annex A.

Grind samples as specified in the method before test portions are taken, if necessary. Powders/flour might have swelling properties and need sometimes to be extracted with double volume of extraction solution.

Laboratory samples containing high amounts of fat may be inhomogeneous and a larger test sample should be extracted. If applicable, instructions may be found in annex A.

Weigh an appropriate amount (as specified in the annex) of a representative analytical sample for analysis to create a test portion for extraction. Add extraction solution and homogenise or mix.

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