SLOVENSKI STANDARD
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Živila - Minimalne zahtevane lastnosti za ugotavljanje glutena z metodo ELIZA

Foodstuffs - Minimum performance requirements for determination of gluten by ELISA

Lebensmittel - Minimale Leistungsanforderungen für die Glutenbestimmung mit ELISA

Produits alimentaires - Exigences de performances minimales pour la détermination du gluten par une méthode ELISA

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Foodstuffs - Minimum performance requirements for determination of gluten by ELISA

Produits alimentaires - Exigences de performances minimales pour la détermination du gluten par une méthode ELISA

Lebensmittel - Minimale Leistungsanforderungen für die Glutenbestimmung mit ELISA

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COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (prEN 17254:2018) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This document is currently submitted to the CEN Enquiry.

Introduction

0.1 Coeliac Disease

Coeliac disease (CD) is a life-long autoimmune disease of the small intestine, primarily affecting genetically susceptible individuals. Its prevalence has been estimated to be 1 % of the population worldwide. CD becomes manifest in a chronic enteropathy, caused by an irreversible intolerance for gluten. Upon ingestion of gluten, the incomplete gastrointestinal digestion of these proteins leads to the appearance of gluten-derived peptides such as 33mer (*LQLQPFPQPQLPYPQPLPYPQPQLPYPQPQPF*). It contains overlapping T-cell epitopes, and its deamidated form is a potent T-cell stimulator. Toxic gluten peptides cause the stimulation of the innate and the adaptive immune system. This leads to histological changes in the small intestine mucosa of coeliac patients, resulting in severe symptoms including chronic diarrhoea, abdominal distension, and malabsorption of nutrients. Coeliac disease, if untreated, is associated with increased morbidity and the only accepted treatment is a strict and lifelong adherence to a gluten free diet, which interrupts the immune response triggered by gluten [1].

0.2 Gluten

The definition of gluten slightly differs depending on the context in which it is used. Gluten in starch industry refers to the protein rich fraction which is separated from starch during the starch production process. The term is therefore applied to different cereals, mainly wheat and corn which are predominantly used for starch production. In the baking industry, gluten refers to a protein rich ingredient which leads to an increase in volume and “fluffiness” of baking products such as bread. In this context, only wheat contains gluten since only the protein rich fraction from this cereal leads to an improvement of the physicochemical properties of baking products. The definition of gluten in the context of coeliac disease refers to the protein fraction which is toxic for CD patients. This definition is based on the Osbourne fractionation of cereal proteins (e.g. from wheat and its crossbred varieties), precisely the proteins insoluble in water and 0,5 mol/l sodium chloride solution.

In this document, the gluten definition according to the Osbourne fractionation is used, similar to the definition stated in the Codex Alimentarius (see 3.1).

Due to thermal processing and enzymatic hydrolysis during food production, gluten in food is often not present in its native form. The above mentioned processing can lead to the denaturation and/or to the fragmentation of gluten proteins into peptides. Gluten peptides have partly different properties in regards to solubility and detectability by immuno-analytical techniques, but are still able to trigger immune reactions in coeliac disease patients [2].

0.3 Regulatory limits

According to Commission Implementing Regulation (EU) No 828/2014 of the European Union, there are two different threshold levels for gluten in foodstuffs.

The term “gluten-free” may be used for food products if the gluten content does not exceed 20 mg/kg. On the other hand, if the gluten content does not exceed 100 mg/kg gluten products may bear the term “very low gluten.

0.4 Measurement of the gluten content

The gluten concentration in food samples can be measured by different test methods. Although the use of mass spectrometry is possible, the most commonly used technique is the enzyme-linked immunosorbent assay (ELISA).

The ELISA uses specific (monoclonal or polyclonal) antibodies that target gluten epitopes. Competitive and Sandwich ELISA are currently used to quantify the gluten level by comparing colour reactions of sample solutions to calibrator solutions.

Reliable analytical methods are required for compliance with national and international regulations in all areas of analysis. Currently, there are no harmonized guidelines available regarding specific requirements on performance of quantitative ELISA for gluten and regarding specific information to be provided by the method developer.

Some guidance is provided by AOAC publications [3, 4].

1 Scope

This document specifies minimum method performance requirements for enzyme-linked immunosorbent assays that quantify non-fragmented or fragmented gluten from wheat (e.g. *Triticum aestivum*), rye, and barley in raw and processed foodstuffs.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15633-1:2009, *Foodstuffs - Detection of food allergens by immunological methods - Part 1: General considerations*

EN 15842:2010, *Foodstuffs - Detection of food allergens - General considerations and validation of methods*

CEN/TR 16338:2012, *Foodstuffs - Detection of food allergens - Template for supplying information about immunological methods and molecular biological methods*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 15633-1:2009 and EN 15842:2010 and the following apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at http://www.electropedia.org/
- ISO Online browsing platform: available at http://www.iso.org/obp

3.1
gluten
protein fraction from wheat, rye, barley, oats or their crossbred varieties [2], [5]

Note 1 to entry: This protein fraction, to which some persons are intolerant, is insoluble in water and 0,5 M sodium chloride solution [5].

Note 2 to entry: According to Commission Implementing Regulation 828/2014 [5], oats contained in foodstuffs for people intolerant to gluten shall have been specially produced, prepared and/or processed in a way to avoid contamination by wheat, rye, barley, or their crossbred varieties and the gluten content of such oats may not exceed 20 mg/kg.

Note 3 to entry: Oats can be tolerated by most but not all people who are intolerant to gluten. Therefore, the allowance of oats that are not contaminated with wheat, rye or barley in foods covered by this standard can be determined at the national level [6],[7].

3.2
prolamin
fraction from gluten soluble in 40 % to 70 % ethanol

Note 1 to entry: Prolamin from wheat is gliadin, from rye is secalin, from barley hordein and from oats avenin.

Note 2 to entry: The prolamin content of gluten is generally taken as 50 % [2].

4 General information on the test system

A description of the method principles according to CEN/TR 16338:2012 shall be given including but not limited to the following parameters:

— **Name of antibody** (if available)

EXAMPLE R5, G12, A1, Alpha 20

— **Target antigen of antibody**

EXAMPLE for monoclonals: epitope or multimer(s) within gluten; for polyclonal antibodies specify substance used for immunization

— **Calibration material**

EXAMPLE Gliadin Standard of the Working Group on Prolamin Analysis and Toxicity [8]

— **Reporting of results**

NOTE According to Codex Alimentarius give the results as mg/kg gluten; Gliadin*2 = mg/kg Gluten

5 Performance requirements

5.1 General

The results shall be provided according to CEN/TR 16338:2012.

5.2 Analytical range

Gluten quantification shall be possible at least within the specified range in all matrices within the scope of the method.

5.3 Limit of Detection (LOD)

Ten sub-samples of each gluten-free matrix shall be extracted using the procedure specified by the assay. Each of the 10 extracts is measured in duplicates using the immunoassay. The LOD is calculated as 3 times the standard deviation of these concentrations by using calibration function by extrapolation [9].

5.4 Limit of Quantification (LOQ)

The LOQ is the lowest concentration in a sample which can be quantitatively determined with acceptable levels of precision expressed as repeatability standard deviation (RSDr) and recovery.

Procedure: At least 10 independent determinations with the method have to be carried out with a sample containing a known amount of gluten in each matrix by using calibration function by extrapolation. Thereof the relative standard deviation and mean recovery should be calculated.

5.5 Recovery

It is strongly recommended to use incurred samples whenever possible. Otherwise spike all matrices within the scope of the method at or around 20 mg/kg gluten. Use characterized spiking materials only (e.g. gliadin standard of the Working Group on Prolamin Analysis and Toxicity for intact gluten; for fragmented gluten a hydrolysed material according [10] can be used).

5.6 Precision: RSDr, RSDR

Use data from collaborative tests for calculation of repeatability and reproducibility following international standards or guidelines (e.g. ISO 5725-1 [11] or AOAC Appendix D: Guidelines for Collaborative Study - Procedures To Validate Characteristics of a Method of Analysis [12]).

5.7 Specificity

Describe the measurand(s) that are targeted by the method: State the accurate name of the species (e.g. Triticum durum) and if available the cultivar.

5.8 Cross-Reactivity

Relevant commodities shall be tested and the results documented. But as a minimum the following commodities shall be tested: Almond flour, Amaranth flour, Arrowroot, Black bean flour, Brown rice flour, Buckwheat flour, Chestnut flour, Coconut flour, Coffee, Corn starch/meal, Dried fruits, Egg powder, Fava bean flour, Flax seed flour/meal, Garfava flour, Green pea flour, Guar gum, Hazelnut flour, Lentil flour, Lima bean flour, Meats, Milk powder, Millet flour, Oat flour[1)], Potato flour/starch, Quinoa flour, Romano bean flour, Sesame flour, Sorghum flour, Soya flour, Spices, Sweet rice flour, Tapioca flour/starch, Tea, White bean flour, White rice flour, Xanthan gum, and Yellow pea flour [3].

Procedure: Assumed cross-reacting substances shall be measured as an undiluted extract using the extraction method provided by the method developer. It is recommended to test this extract also diluted.

5.9 Robustness

Critical parameters and even minor changes thereof that influence the final result of the analysis shall be indicated.

Procedure: Define at minimum one matrix with a gluten concentration near to the threshold of 20 mg/kg, which shall be measured at least 5 times for each experimental condition.

Measure the capacity of the analytical procedure to remain unaffected by small variations in method parameters, like temperature changes (standard incubation/extraction temperature ± 20 %) and changed incubation times (standard incubation time ± 10 %).

A significant influence should be listed if the mean concentration value differs more than 1,96 times the repeatability deviation compared to control conditions.

1) If claimed as analyte do not list here.

5.10 Overview on minimum performance requirements

Table 1 — Minimum performance requirements for non-fragmented gluten

Parameter	Gluten
Analytical Range [mg/kg]	10 to 40
LOD [mg/kg]	5
LOQ [mg/kg]	10
Recovery [%]	70 to 130
RSD_r [%]	15
RSD_R [%]	30
Specificity	Gluten from wheat (e.g. *Triticum aestivum*), rye, and barley
Robustness	Difference between means < 1,96 × SDr

Table 2 — Minimum performance requirements for fragmented gluten

Parameter	Gluten
Analytical Range [mg/kg]	10 to 40
LOD [mg/kg]	5
LOQ [mg/kg]	10
Recovery [%]	60 to 140
RSD_r [%]	15
RSD_R [%]	35
Specificity	Gluten from wheat (e.g. *Triticum aestivum*), rye, and barley
Robustness	Difference between means < 1,96 × SDr