SLOVENSKI STANDARD
SIST EN 17254:2019

01-december-2019

Živila - Minimalne zahteve za ugotavljanje glutena z metodo ELISA

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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67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products

SIST EN 17254:2019 **en,fr,de**

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EUROPEAN STANDARD

NORME EUROPÉENNE

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English Version

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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European foreword

This document (EN 17254:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - horizontal methods”, the secretariat of which is held by DIN.

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of an identical text or by endorsement, at the latest by April 2020, and conflicting national standards shall be withdrawn at the latest by April 2020.

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

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

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Introduction

0.1 Coeliac Disease

Coeliac disease (CD) is an 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. 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. In this document, the gluten definition (see 3.1) according to the Osborne fractionation is used, similar to the definition stated in the Codex Alimentarius.

Gluten in starch industry refers to the protein rich fraction that 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.

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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].

Commission Implementing Regulation 828/2014 [3] states that 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.

0.3 Regulatory limits

Commission Implementing Regulation (EU) No 828/2014 of the European Union states that 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 gluten/kg. On the other hand, if the gluten content does not exceed 100 mg gluten/kg products may bear the term “very low gluten”.

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 [4], [5].

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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 and nucleic acid based methods (e.g. PCR) 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 recommended 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 [6], [7].

0.5 General considerations for the use of this document

For the use of this document the term:

— ‘shall’ indicates a requirement;

— ‘should’ indicates a recommendation;

— ‘may’ indicates a permission; and

— ‘can’ indicates a possibility and/or a capability.

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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.

This document is intended to be used in addition to EN 15842.

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

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

3 Terms and definitions

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

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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 https://www.iso.org/obp

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3.1
gluten
protein fraction from wheat, rye, barley, oats or their crossbred varieties and derivatives thereof, to which some persons are intolerant and which is insoluble in water and 0,5 mol/l sodium chloride solution

Note 1 to entry: Codex Alimentarius has a similar definition [2]

[SOURCE: Regulation (EU) No 828/2014, Article 2 a)[3]]

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.

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4 General information on the test system

A description of the method principles shall be given including but not limited to the following parameters:

— **Name of antibody** (if available);

— **Target antigen of antibody;**

NOTE 1 For monoclonals: epitope or multimer(s) within gluten; for polyclonal antibodies specify substance used for immunization

— **Calibration material;**

— **Sample type and matrices;**

— **Reporting of results;**

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

— **Interferences.**

NOTE 3 A description of the method principles is listed in CEN/TR 16338:2012 [8]

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5 Performance requirements

5.1 General

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All aspects of the performance requirements described in 5.2 to 5.10 should be applied. For method verification purposes refer to EN ISO/IEC 17025 [9].

5.2 Applicability range

Gluten quantification shall be possible at least within the specified range in all matrices within the scope of the method, at least between 10 mg/kg and 40 mg/kg.

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 [10].

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 relative standard deviation of repeatability (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 of repeatability and mean recovery should be calculated.