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BSI Standards Publication

Foodstuffs — Detection of food allergens by molecular biological methods

Part 5: Mustard (Sinapis alba) and soya (Glycine max) — Qualitative detection of a specific DNA sequence in cooked sausages by real-time PCR

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National foreword

This British Standard is the UK implementation of EN 15634-5:2023. It supersedes PD CEN/TS 15634-5:2016, which is withdrawn.

The UK participation in its preparation was entrusted to Technical Committee AW/275, Food analysis - Horizontal methods.

A list of organizations represented on this committee can be obtained on request to its committee manager.

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English Version

Foodstuffs - Detection of food allergens by molecular biological methods - Part 5: Mustard (*Sinapis alba*) and soya (Glycine max) - Qualitative detection of a specific DNA sequence in cooked sausages by real-time PCR

Produits alimentaires - Détection des allergènes alimentaires par des méthodes d'analyse de biologie moléculaire - Partie 5 : Moutarde (*Sinapis alba*) et soja (Glycine max) - Détection qualitative d'une séquence d'ADN spécifique dans des saucisses cuites, par PCR en temps réel

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 5: Senf (*Sinapis alba*) sowie Soja (Glycine max) - Qualitativer Nachweis einer spezifischen DNA-Sequenz in Brühwürsten mittels Real-time PCR

This European Standard was approved by CEN on 16 January 2023.

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European foreword

This document (EN 15634-5:2023) 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of an identical text or by endorsement, at the latest by August 2023, and conflicting national standards shall be withdrawn at the latest by August 2023.

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.

This document supersedes CEN/TS 15634-5:2016.

In comparison with CEN/TS 15634-5:2016, the following technical modifications have been made:

a) the document was converted from a Technical Specification into a European standard;

b) normative references and terms and definitions clause added;

c) PCR controls moved from Clause 3 “Reagents” to Clause 7 “Procedure”;

d) new subclause 7.4.6 “Accept/Reject criteria” added;

e) restructured clauses in alignment with EN 15634-2:2019.

Any feedback and questions on this document should be directed to the users’ national standards body. A complete listing of these bodies can be found on the CEN website.

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Introduction

For the use of this document the term:

— ‘shall’ indicates a requirement;

— ‘should’ indicates a recommendation;

— ‘may’ indicates a permission;

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

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1 Scope

This document specifies a procedure for the qualitative detection of species specific DNA from white mustard (*Sinapis alba*) and soya (*Glycine max*) in cooked sausages using singleplex real-time PCR based on the genes MADS-D (mustard) and lectin (soya).

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 15634-1:2019, *Foodstuffs - Detection of food allergens by molecular biological 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 15842 and EN 15634-1 apply.

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

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

4 Principle

Total DNA from cooked sausages is extracted from the sample using a cetyltrimethylammoniumbromide (CTAB) method. Potential PCR inhibitors are removed from the DNA extracted by purification with solid phase columns and the DNA content is estimated. Real-time PCR is used to detect a 74 base pair (bp) long sequence of the DNA for the MADS-D protein of *Sinapis alba* (NCBI accession no. Y08626[1]) or a 81 bp long sequence from the soya lectin gene. The real-time PCR method involves a fluorescence detection with sequence specific hydrolysis probes, see [1] or EN ISO 21570:2005, C.1 [2].

5 Reagents

5.1 General

The following general conditions for analysis should be followed, unless specified differently. Use only analytical grade reagents suitable for molecular biology. All water shall be free from DNA and nucleases, e.g. double distilled or equivalent (molecular grade). Solutions shall be prepared by dissolving the appropriate reagents in water and autoclaving, unless specified differently.

5.2 Extraction reagents

5.2.1 Chloroform.

5.2.2 Ethanol, volume fraction φ = 70 %.

[1] NCBI-GenBank® is an example of a suitable search tool for free use. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN.