SLOVENSKI STANDARD
SIST EN 15634-5:2023

01-maj-2023

Živila - Določevanje alergenov v živilih z molekularno biološkimi metodami - 5. del: Gorčica (Sinapis alba) in soja (Glycine max) - Kvalitativno določanje specifičnega zaporedja DNK v obarjenih klobasah s PCR v realnem času

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

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

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

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67.120.10	Meso in mesni proizvodi	Meat and meat products

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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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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CEN-CENELEC Management Centre: Rue de la Science 23, B-1040 Brussels

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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.

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.

5.2.3 Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA).

5.2.4 Cetyltrimethylammoniumbromide (CTAB).

5.2.5 Hydrochloric acid, mass fraction w = 37 %.

5.2.6 Isoamyl alcohol.

5.2.7 Isopropanol.

5.2.8 Proteinase K.

5.2.9 Sodium chloride.

5.2.10 Sodium hydroxide solution.

5.2.11 Tris(hydroxymethyl)aminomethane (TRIS).

5.2.12 Chloroform isoamyl alcohol mixture, 24 parts by volume of chloroform (5.2.1) are mixed with one part by volume of isoamyl alcohol (5.2.6).

Similar mixtures commercially available may be used.

5.2.13 CTAB extraction buffer solution, containing

— CTAB (5.2.4), mass concentration ρ = 20 g/l;

— sodium chloride (5.2.9), substance concentration c = 1,4 mol/l;

— TRIS (5.2.11), substance concentration c = 0,1 mol/l;

— Na_2EDTA (5.2.3), substance concentration c = 0,02 mol/l.

The pH is adjusted to 8,0 by adding hydrochloric acid (5.2.5).

5.2.14 Proteinase K solution, ρ = 20 mg/ml.

The freshly produced Proteinase K solution should be stored in the form of aliquots at −20 °C.

5.2.15 TE buffer solution, containing

— TRIS (5.2.11), c = 0,01 mol/l;

— Na_2EDTA (5.2.3), c = 0,00 1 mol/l.

The pH is adjusted to 8,0 by adding hydrochloric acid (5.2.5) or sodium hydroxide solution (5.2.10).

5.2.16 0,2 × TE buffer solution, one part by volume of TE buffer solution (5.2.15) is mixed with four parts water

5.3 DNA purification by means of solid phase extraction

For the DNA purification, different methods may be used.

Several formats are commercially available, among them spin filter columns or plates. Commercially available kits may be used if appropriate. Follow the manufacturer's instructions for this.

5.4 Real-time PCR reagents

5.4.1 PCR master mix (2 ×) for real-time PCR, containing reaction buffer, dNTPs, $MgCl_2$ and Hotstart Taq polymerase, double concentrated.

Ready to use reagents or single components may be used as a PCR master mix, insofar as they provide comparable or better results.

5.4.2 **Oligonucleotides** [1]:

Primers and probes for the real-time PCR are shown in Table 1.

Table 1 — Primers and probes for the real-time PCR

Name	DNA sequence of the oligonucleotide
Soya lectin gene	
Lectin-F	5´– TCC ACC CCC ATC CAC ATT T – 3´
Lectin-R	5´– ggC ATA gAA ggT gAA gTT gAA ggA – 3´
Lectin probe	5´– FAM – AAC Cgg TAg CgT TgC CAg CTT Cg – TAMRA-3´ [a]
Mustard (*Sinapis alba*) MADS D protein	
MADS D-F	5´– TgA AAA CTC TCT TCC CCT CTT Agg – 3´
MADS D-R	5´– ACA AAT gCA CAC AAg ACA gAg ATA TAg A – 3´
MADS D probe	5´– FAM – TAC ATg ATg CTT ACC TCg C – TAMRA – 3´ [a]
[a] FAM: 6-carboxyfluorescein, TAMRA: 6-carboxytetramethylrhodamine; equivalent reporter and/or quencher dyes may be used if they are shown to give comparable or better results.	

6 Apparatus and equipment

6.1 General

In addition to the usual laboratory facilities, the following equipment shall be used.

Due to the high sensitivity of the PCR analytics and the risk of DNA contaminations resulting from it, the use of aerosol protected filter tips in the DNA extraction procedure is obligatory. Plastic and glass materials shall be sterilized and free of DNA before use.

Further general requirements are given in EN ISO 21571.

6.2 DNA extraction

6.2.1 **Suitable reaction vials,** 1,5 ml and 2 ml, DNA-free.

6.2.2 **50 ml centrifuge tubes**, sterile.

6.2.3 **Thermostat or water bath,** preferably with shaker function.

6.2.4 Centrifuge, suitable for centrifuging 50 ml centrifuge tubes at 8 000 g[2].

6.2.5 Centrifuge, suitable for centrifuging 1,5 ml and 2 ml reaction vials at 16 000 g.

6.2.6 Equipment and/or material for grinding the sample, e.g. blender or mill.

6.2.7 UV spectrometer or other detection instruments, suitable for estimating the amount of DNA.

6.3 PCR

6.3.1 Suitable PCR tubes.

6.3.2 Microcentrifuge for PCR tubes.

6.3.3 Real-time PCR equipment, suitable for excitation and for emission measurement of fluorescence-marked oligonucleotides.

7 Procedure

7.1 General

General aspects are described in EN 15634-1 and EN ISO 21571.

7.2 Sample preparation

It should be ensured, that the test sample taken after milling or homogenizing is representative of the laboratory sample.

7.3 Preparation of extracts

7.3.1 DNA extraction with CTAB and DNA purification

It is acceptable to use a commercially available kit instead of the DNA extraction procedure described below, if it is ensured that comparable or better results are obtained.

In parallel to the test samples, an extraction blank control (7.4.3) should be performed adequately.

The analyses should be carried out in accordance with the following scheme:

— Weigh 2 g of the homogenized sample into 50 ml centrifuge tubes (tube A).

— Add 10 ml of CTAB extraction buffer solution (5.2.13).

— Add 30 µl of Proteinase K solution (5.2.14) and mix.

— Incubate and shake for 90 min at 65 °C.

— Centrifuge for 5 min at 6 000 g to 8 000 g at room temperature.

— Place 500 µl of chloroform isoamyl alcohol mixture (5.2.12) in a 2 ml reaction vial (tube B).

— Add 700 µl of supernatant from tube A to tube B and mix thoroughly for 30 s.

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2 $g = 9{,}81\ m \cdot s^{-2}$