SLOVENSKI STANDARD
oSIST prEN 15634-3:2022

01-januar-2022

Živila - Določevanje alergenov v živilih z molekularno biološkimi metodami - 3. del: Lešnik (Corylus avellana) - Kvalitativno določanje specifičnega zaporedje DNK v čokoladi s PCR v realnem času

Foodstuffs - Detection of food allergens by molecular biological methods - Part 3: Hazelnut (Corylus avellana) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 3: Haselnuss (Corylus avellana) - Qualitativer Nachweis einer spezifischen DNA-Sequenz in Schokolade mittels Real-time PCR

Produits alimentaires - Détection des allergènes alimentaires par des méthodes d'analyse de biologie moléculaire - Partie 3: Noisette (Corylus avellana) - Détection qualitative d'une séquence d'ADN spécifique dans du chocolat, par PCR en temps reel

Ta slovenski standard je istoveten z: **prEN 15634-3**

ICS:

07.100.30	Mikrobiologija živil	Food microbiology
67.050	Splošne preskusne in analizne metode za živilske proizvode	General methods of tests and analysis for food products
67.190	Čokolada	Chocolate

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EUROPEAN STANDARD

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ICS 07.100.30; 67.190

Will supersede CEN/TS 15634-3:2016

English Version

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This draft European Standard is submitted to CEN members for enquiry. It has been drawn up by the Technical Committee CEN/TC 275.

If this draft becomes a European Standard, CEN members are bound to comply with the CEN/CENELEC Internal Regulations which stipulate the conditions for giving this European Standard the status of a national standard without any alteration.

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
EUROPÄISCHES KOMITEE FÜR NORMUNG

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Ref. No. prEN 15634-3:2021 E

Contents

European foreword

This document (prEN 15634-3:2021) 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.

This document will supersede CEN/TS 15634-3:2016.

In comparison with the previous edition, 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) added new subclause 7.4.9 “Accept/Reject criteria”;

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

Introduction

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.

1 Scope

This document specifies a method for the detection of hazelnut (Corylus avellana) in chocolate.

Real-time PCR (Polymerase chain reaction) detection of hazelnut is based on an152 bp (base pair) sequence from the corA 1 gene of hazelnut.

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

4 Principle

Total DNA from chocolate 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 hazelnut specific DNA sequence. The real-time PCR method involves a fluorescence detection with a sequence specific hydrolysis probe [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 %.

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 = 0,001 mol/l,

— Na_2EDTA (5.2.3), c = 0,000 1 mol/l.

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

5.3 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 data for this (see also 8.3.1).

5.4 Real-time PCR reagents

5.4.1 PCR master mix, containing reaction buffer, dNTPs, $MgCl_2$ and Hotstart Taq polymerase.

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, 5 µmol each.

5.4.2.1 Hazelnut iF, 5´– TAC AgC ATC ATC gAg ggA ggT C – 3´.

5.4.2.2 Hazelnut iR, 5´– CTC CTC ATT gAT TgA AgC gTT g – 3´.

5.4.2.3 Hazelnut probe, 5´– FAM – AgA Tgg Cgg CAg CCC CTC AT – TAMRA – 3´[1)]

Equivalent reporter dyes 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.

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 14 500 *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.

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1) FAM: 6-carboxyfluorescein, TAMRA: 6-carboxytetramethylrhodamine

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

7 Procedure

7.1 General

General aspects are described in EN 15634-1.

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, the controls listed in 7.4.3, 7.4.6 and 7.4.7 should be performed adequately.

The analyses should be carried out **twice** 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.

— Centrifuge for 15 min at about 14 500 *g* at room temperature.

— Place 500 µl of cold isopropanol (5.2.7) in a 1,5 ml reaction vial (tube C).

— Add 500 µl of supernatant (aqueous phase) from tube B to tube C and mix carefully.

— Incubate for 30 min at room temperature.

— Centrifuge for 15 min at about 14 500 *g* at room temperature.

— Carefully remove and discard the supernatant.

— Fill the reaction vial with 500 µl of ethanol (5.2.2) and mix several times.

— Centrifuge for 5 min at about 14 500 *g* at room temperature.

— Carefully remove and discard the supernatant.

— Dry the extracted DNA.