SLOVENSKI STANDARD
SIST-TS CEN/TS 15634-4:2016

01-maj-2016

Živila - Odkrivanje prisotnosti alergenov v živilih z metodami molekularne biologije - 4. del: Arašidi (Arachis hypogaea) - Kvalitativno odkrivanje specifičnega niza DNK v čokoladi s PCR v realnem času

Foodstuffs - Detection of food allergens by molecular biological methods - Part 4: Peanut (Arachis hypogaea) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 4: Erdnuss (Arachis hypogaea) - Qualitativer Nachweis einer spezifischen DNA-Sequenz in Schokolade mittels Real-time PCR

Produits alimentaires - Détection d’allergènes alimentaires par des méthodes de biologie moléculaire - Partie 4 : Arachide (Arachis hypogaea) - Détection qualitative d’une séquence d’ADN spécifique dans du chocolat, par PCR en temps réel

Ta slovenski standard je istoveten z: **CEN/TS 15634-4:2016**

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

SIST-TS CEN/TS 15634-4:2016 **en**

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 15634-4

March 2016

ICS 07.100.30; 67.190

English Version

Foodstuffs - Detection of food allergens by molecular biological methods - Part 4: Peanut (Arachis hypogaea) - Qualitative detection of a specific DNA sequence in chocolate by real-time PCR

Produits alimentaires - Détection d'allergènes alimentaires par des méthodes de biologie moléculaire - Partie 4 : Arachide (Arachis hypogaea) - Détection qualitative d'une séquence d'ADN spécifique dans du chocolat, par PCR en temps réel

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 4: Erdnuss (Arachis hypogaea) - Qualitativer Nachweis einer spezifischen DNA-Sequenz in Schokolade mittels Real-time PCR

This Technical Specification (CEN/TS) was approved by CEN on 11 February 2016 for provisional application.

The period of validity of this CEN/TS is limited initially to three years. After two years the members of CEN will be requested to submit their comments, particularly on the question whether the CEN/TS can be converted into a European Standard.

CEN members are required to announce the existence of this CEN/TS in the same way as for an EN and to make the CEN/TS available promptly at national level in an appropriate form. It is permissible to keep conflicting national standards in force (in parallel to the CEN/TS) until the final decision about the possible conversion of the CEN/TS into an EN is reached.

CEN members are the national standards bodies of Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, Former Yugoslav Republic of Macedonia, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and United Kingdom.

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

CEN-CENELEC Management Centre: Avenue Marnix 17, B-1000 Brussels

Ref. No. CEN/TS 15634-4:2016 E

Contents

Page

European foreword

This document (CEN/TS 15634-4:2016) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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

EN 15634, Foodstuffs — Detection of food allergens by molecular biological methods, is currently composed with the following parts:

— Part 1: General considerations;

— Part 2: Celery (Apium graveolens) — Qualitative determination of a specific DNA sequence in cooked sausages by real-time PCR [Technical Specification];

— Part 3: Hazelnut (Corylus avellana) — Qualitative detection of a specific DNA sequence in chocolate by real-time PCR [Technical Specification];

— Part 4: Peanut (Arachis hypogaea) — Qualitative detection of a specific DNA sequence in chocolate by real-time PCR [Technical Specification];

— Part 5: Mustard (Sinapis alba) and soya (Glycine max) — Qualitative dectection of a specific DNA sequence in cooked sausages by real-time PCR [Technical Specification].

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

1 Scope

This Technical Specification describes a procedure for the qualitative detection of peanut (*Arachis hypogaea*) in chocolate using real-time PCR based on the gene for the peanut allergen Ara h 2 [4], [5].

2 Principle

The total DNA is extracted from the sample and the DNA content estimated. A sequence specific to peanut from the gene for Ara h 2 is multiplicated using real-time PCR. The amplicon with a length of 86 base pairs (bp) formed in this way is detected by annealing a sequence-specific probe and generating a fluorescence signal [4].

3 Reagents

As a rule, analytical grade chemical reagents suitable for molecular biology shall be used. The water used shall be double distilled or equivalent quality. Solutions should be prepared by dissolving the appropriate reagents in water and autoclaving, unless indicated differently.

3.1 DNA extraction with CTAB

3.1.1 Chloroform.

3.1.2 Ethanol, volume fraction φ = 96 %.

3.1.3 Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA).

3.1.4 Cetyltrimethylammoniumbromide (CTAB).

3.1.5 Hydrochloric acid, mass fraction w = 37 %.

3.1.6 Isoamyl alcohol.

3.1.7 Isopropanol.

3.1.8 Proteinase K.

3.1.9 Sodium chloride.

3.1.10 Sodium hydroxide.

3.1.11 Tris(hydroxymethyl)aminomethane (TRIS).

3.1.12 Chloroform isoamyl alcohol mixture.

Mix 24 parts by volume of chloroform (3.1.1) with one part by volume of isoamyl alcohol (3.1.6).

Commercially available and comparable mixtures can be used.

3.1.13 CTAB extraction buffer solution, containing CTAB (mass concentration ρ = 20 g/l), sodium chloride (substance concentration c = 1,4 mol/l), TRIS (c = 0,1 mol/l), Na_2EDTA (c = 0,02 mol/l). Adjust the pH value with hydrochloric acid to pH = 8,0.

3.1.14 Ethanol solution, φ = 70 %.

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

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

3.1.16 TE buffer solution, containing TRIS (c = 0,001 mol/l) and Na_2-EDTA (c = 0,000 1 mol/l). Adjust the pH value with hydrochloric acid or sodium hydroxide solution to pH = 8,0.

3.2 DNA purification by means of solid phase extraction

Various systems are commercially available for DNA purification by means of solid phase extraction, including spin filter columns or plates or also with vacuum operated systems. Commercially available kits can also be used. Observe the manufacturer's data for this.

3.3 Real-time PCR reagents

3.3.1 PCR master mix[1)]**,** containing reaction buffers, dNTPs, $MgCl_2$ and Hotstart Taq polymerase.

3.3.2 Oligonucleotides, 10 µmol each.

3.3.2.1 Peanut (AR-58 F), gCA gCA gTg ggA ACT CCA Agg AgA CA.

3.3.2.2 Peanut (AR-143 R), gCA TgA gAT gTT gCT CgC Ag.

3.3.2.3 Peanut probe (AR-103 T), FAM – CgA gAg ggC gAA CCT gAg gCC – TAMRA or – BHQ1.

3.3.3 Negative PCR control, conducted with DNA-free water instead of the DNA extract from the sample.

3.3.4 Negative extraction control, performing all steps of the DNA extraction procedure, except addition of the test portion, e.g. by substitution of a corresponding amount of water for the test portion.

3.3.5 Negative process control, sample of the food matrix without target sequence, which passes through all steps of the analytical process (blank sample).

3.3.6 Positive PCR control[2)] reaction containing the target DNA in a specified quantity or number of copies.

3.3.7 Positive process control, sample of the food matrix with known quantity of peanut, which passes through all steps of the analytical process.

3.3.8 External amplification control (inhibition control), control DNA that is added to an aliquot of the extracted nucleic acid in a specified quantity or number of copies and used in a separate reaction to check the influence of co-extracted substances from the sample matrix on the amplification.

4 Apparatus and equipment

General aspects are described in EN ISO 24276 [3].

---

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

2) DNA for the positive PCR control is extracted from phenotypically identified pure peanuts as described in 5.3 and 5.4. DNA mass concentration is determined as described in 5.5.

Plastic and glass materials shall be sterilised and free of DNA before use. In addition, the use of aerosol protected filter tips is obligatory due to the high sensitivity of the PCR analytics and the resultant risk of DNA contamination.

In addition to the usual laboratory facilities, the following equipment is required.

4.1 DNA extraction

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

4.1.2 50 ml centrifuge tubes, sterile.

4.1.3 Thermostat or water bath, preferably with shaker function.

4.1.4 Centrifuge, suitable for centrifuging 50 ml centrifuge tubes at 8 000 *g*[3].

4.1.5 Centrifuge, suitable for centrifuging 1,5 ml and 2 ml reaction vials at 14 500 *g*.

4.1.6 Apparatus and/or material for grinding the sample, e.g. blender or mill.

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

4.2 PCR

4.2.1 Suitable PCR tubes.

4.2.2 Microcentrifuge for PCR tubes.

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

NOTE Laboratories participating in the interlaboratory trial used the following real-time PCR equipment: Rotor Gene 6000™, Stratagene Mx 3005P, ABI PRISM® 7500, ABI PRISM® 7900HT and Roche LightCycler® 1,5. [4]

5 Procedure

5.1 General

General aspects are described in EN ISO 24276 [3].

5.2 Sample preparation

Ensure e.g. by milling or homogenizing, that the test sample is representative of the laboratory sample.

5.3 DNA extraction with CTAB

Measures and work steps to be considered for the DNA extraction are described in EN ISO 21571 [2].

---

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

4) Rotor Gene 6000™, Stratagene Mx 3005P, ABI PRISM® 7500, ABI PRISM® 7900HT and Roche LightCycler® 1.5 are examples of suitable products available commercially. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of these products. Equivalent products may be used if they can be shown to lead to the same results.

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, carry out the controls listed in 3.3.4, 3.3.5 and 3.3.7 adequately.

Prepare every sample **twice** in accordance with the following scheme:

— Weigh 2 g of the sample into 50 ml centrifuge tubes;

— Add 10 ml of CTAB extraction buffer solution (3.1.13);

— Add 30 µl of Proteinase K solution (3.1.15) and mix;

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

— Centrifuge for 5 min at 6 000 *g* to 8 000 *g*;

— Place 500 µl of chloroform isoamyl alcohol mixture (3.1.12) in a 2 ml reaction vial;

— Add 700 µl of supernatant and mix thoroughly for 30 s;

— Centrifuge for 15 min at about 14 500 *g*;

— Place 500 µl of cold isopropanol (3.1.7) in a 1,5 ml reaction vial;

— Add 500 µl of supernatant (aqueous phase) and mix carefully;

— Incubate for 30 min at room temperature;

— Centrifuge for 15 min at about 14 500 *g*;

— Carefully remove and discard the supernatant;

— Fill the reaction vial with 500 µl of ethanol (3.1.2) and swirl the reaction vial several times;

— Centrifuge for 5 min at about 14 500 *g*;

— Carefully remove and discard the supernatant;

— Dry the extracted DNA;

— Dissolve the dried DNA extract in 100 µl of TE buffer solution (3.1.16).

5.4 DNA purification by means of solid phase extraction

Purify the DNA extract according to the instructions given by the respective kit manufacturer.

The DNA extract can be stored cooled (approximately 4 °C) for a short period. If storage times exceed more than one week, the DNA extracts should be stored at temperatures of < – 18 °C.

5.5 Measuring the mass concentration and purity of the extracted DNA

The mass concentration of a DNA aliquot can be determined by means of a UV spectrometer at 260 nm. Calculate the DNA mass concentration as follows:

ρ (DNA) in ng/µl = 50 × optical density × dilution factor of the measured aliquot