PD CEN/TS 15634-4:2016

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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

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National foreword

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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 secretary.

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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.

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Document Sharing Hub

Full document details: PD CEN/TS 15634-4:2016

Contents

Page

European foreword ... 3

1 Scope ... 4

2 Principle ... 4

3 Reagents ... 4
3.1 DNA extraction with CTAB ... 4
3.2 DNA purification by means of solid phase extraction ... 5
3.3 Real-time PCR reagents ... 5

4 Apparatus and equipment ... 5
4.1 DNA extraction ... 6
4.2 PCR ... 6

5 Procedure ... 6
5.1 General ... 6
5.2 Sample preparation ... 6
5.3 DNA extraction with CTAB ... 6
5.4 DNA purification by means of solid phase extraction ... 7
5.5 Measuring the mass concentration and purity of the extracted DNA ... 7
5.6 Real-time PCR ... 8

6 Validation status and performance criteria ... 9
6.1 General ... 9
6.2 Detection ... 10
6.3 Reliability of the method ... 10
6.3.1 Setup of the interlaboratory study ... 10
6.3.2 Results ... 10
6.3.3 Specificity ... 11

7 Test report ... 11

Bibliography ... 13

Documents
Document Sharing Hub
Full document details: PD CEN/TS 15634-4:2016

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.

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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].

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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.

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