TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

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English Version

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

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

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

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EUROPEAN COMMITTEE FOR STANDARDIZATION
COMITÉ EUROPÉEN DE NORMALISATION
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European foreword

This document (CEN/TS 15634-3:2016) 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 hazelnut *(Corylus avellana)* in chocolate. DNA is extracted from the chocolate and a specific DNA sequence for hazelnut detected from the gene for corA 1 [4], [5].

2 Principle

The total DNA is extracted from the sample and the DNA content estimated. A 152 bp long sequence from the gene for corA 1 is multiplicated using real-time PCR. The amplicon formed in this way is detected by annealing a sequence-specific probe and generating a fluorescence signal [4], [5].

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.