PD CEN/TS 15634-3:2016

BSI Standards Publication

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

bsi.

...making excellence a habit.™

National foreword

This Published Document is the UK implementation of CEN/TS 15634-3:2016.

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.

This publication does not purport to include all the necessary provisions of a contract. Users are responsible for its correct application.

Published by BSI Standards Limited 2016

ISBN 978 0 580 90302 1
ICS 07.100.30; 67.190

Compliance with a British Standard cannot confer immunity from legal obligations.

This Published Document was published under the authority of the Standards Policy and Strategy Committee on 30 April 2016.

Amendments/corrigenda issued since publication

Date	Text affected

TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 15634-3

March 2016

ICS 07.100.30; 67.190

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

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 15634-3:2016 E

Contents

Page

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.

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 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.