TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

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English Version

Foodstuffs - Detection of food allergens by molecular biological methods - Part 2: Celery (Apium graveolens) - Qualitative determination of a specific DNA sequence in cooked sausages by real-time PCR

Produits alimentaires - Détection des allergènes alimentaires par des méthodes d'analyse de biologie moléculaire - Partie 2: Céleri (Apium graveolens) - Détermination qualitative d'une séquence d'ADN spécifique dans des saucisses cuites par PCR en temps réel

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 2: Sellerie (Apium graveolens) - Qualitative Bestimmung einer spezifischen DNA-Sequenz in Brühwürsten mittels Real-time-PCR

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EUROPEAN COMMITTEE FOR STANDARDIZATION
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Contents

Page

Foreword

This document (CEN/TS 15634-2:2012) 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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1 Scope

This Technical Specification specifies a method for the qualitative detection of celery (*Apium graveolens*) in emulsion-type sausages (e.g. Frankfurter, Wiener).

Real-time PCR detection of celery is based on an 101 bp (base pair) sequence from the gene of the mannitol dehydrogenase (GenBank Acc. No. AF067082) of celery (*Apium graveolens*).

The method has been validated on emulsion-type sausages (Bavarian “Leberkäse”) spiked with celery. For this purpose meat batter containing mass fractions of 50 % pork meat, 25 % pork fat, 23 % crushed ice and 1,8 % of a mixture of sodium chloride, nitrite, nitrate, phosphates and ascorbates was prepared according to a standard procedure for emulsion-type sausage. The meat batter was spiked with either ground celery seeds or celery root powder to 1000 mg/kg. Lower spiking levels were obtained by diluting with celery-free meat batter. The batter was stuffed into casings and heated at 65 °C for 60 min [2].

2 Principle

Total DNA from emulsion-type sausages are isolated from the sample matrix. DNA is released from the sample matrix using the cetyltrimethylammonium bromide (CTAB) approach. Potential PCR inhibitors are removed from the isolated DNA by purification with solid phase columns. Real-time PCR is used to detect, amplify and quantify a celery specific sequence. The real time PCR method involves a fluorescence approach with a sequence specific hydrolysis probe [1], [2].

3 Reagents

3.1 General

The following general conditions for analysis shall be followed, unless specified differently. Use only analytical grade reagents suitable for molecular biology. Reagents shall be stored in small aliquots to minimise the risk of contamination. 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.

3.2 Extraction reagents

3.2.1 Chloroform, CAS 66-67-3.

3.2.2 Ethanol, volume fraction φ = 70 %, CAS 64-17-5.

3.2.3 Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA), CAS 6381-92-6.

3.2.4 Cetyltrimethylammoniumbromide (CTAB), CAS 57-09-0.

3.2.5 Hydrochloric acid, φ = 37 %, CAS 7647-01-0.

3.2.6 Isoamyl alcohol, CAS 123-51-3.

3.2.7 Isopropanol, CAS 67-63-0.

3.2.8 Proteinase K, EC 3.4.21.64.

3.2.9 Sodium chloride, CAS 7647-14-5.