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BSI Standards Publication

Foodstuffs — Detection of food allergens by molecular biological methods

Part 5: Mustard (Sinapis alba) and soya (Glycine max) — Qualitative detection of a specific DNA sequence in cooked sausages by real-time PCR

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Foodstuffs - Detection of food allergens by molecular biological methods - Part 5: Mustard (Sinapis alba) and soya (Glycine max) - Qualitative detection of a specific DNA sequence in cooked sausages by real-time PCR

Lebensmittel - Nachweis von Lebensmittelallergenen mit molekularbiologischen Verfahren - Teil 5: Senf (Sinapis alba) sowie Soja (Glycine max) - Qualitativer Nachweis einer spezifischen DNA-Sequenz in Brühwürsten mittels Real-time PCR

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European foreword

This document (CEN/TS 15634-5:2016) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

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 detection of a specific DNA sequence in cooked sausages by real-time PCR* [Technical Specification].

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1 Scope

This Technical Specification specifies a procedure for the qualitative detection of species specific DNA from white mustard (*Sinapis alba*) and soya (*Glycine max*) in cooked sausages using singleplex real-time PCR based on the genes MADS-D (mustard) and lectin (soya) [1]. A mustard content of 10 mg/kg or greater and a soya content of 10 mg/kg or greater can be detected with a probability of > 95 %.

2 Principle

The DNA of the sample is extracted and is set to a definite concentration after photometric measurement. A 74 base pair (bp) long sequence of the DNA for the MADS-D protein of *Sinapis alba* (NCBI accession no. Y08626) or a 81 bp long sequence from the soya lectin gene is multiplicated from the sample DNA by real-time PCR. The amplicons formed are detected and verified by annealing a sequence-specific probe and generating a fluorescence signal [2].

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_2-EDTA (c = 0,02 mol/l). Adjust the pH value with hydrochloric acid to 8,0.