



BSI Standards Publication

Foodstuffs — Detection of food allergens by molecular biological methods

Part 2: Celery (*Apium graveolens*) — Detection of a specific
DNA sequence in cooked sausages by real-time PCR

National foreword

This British Standard is the UK implementation of EN 15634-2:2019. It supersedes PD CEN/TS 15634-2:2012, which is withdrawn.

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 2: Celery (*Apium graveolens*) -
Detection 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*) -
Détection 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*) - Nachweis einer spezifischen
DNA-Sequenz in Brühwürsten mittels Real-time-PCR

This European Standard was approved by CEN on 12 August 2019.

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European foreword

This document (EN 15634-2:2019) has been prepared by Technical Committee CEN/TC 275 “Food analysis - Horizontal methods”, the secretariat of which is held by DIN.

This European Standard shall be given the status of a national standard, either by publication of an identical text or by endorsement, at the latest by April 2020, and conflicting national standards shall be withdrawn at the latest by April 2020.

Attention is drawn to the possibility that some of the elements of this document may be the subject of patent rights. CEN shall not be held responsible for identifying any or all such patent rights.

This document supersedes CEN/TS 15634-2:2012.

Significant technical changes between this standard and CEN/TS 15634-2:2012 are as follows:

- a) the document was converted from a Technical Specification into a European Standard;
- b) normative references added (2);
- c) expression of results (8) updated;
- d) requirements regarding the test report added (10)
- e) updated bibliography.

According to the CEN-CENELEC Internal Regulations, the national standards organisations of the following countries are bound to implement this European Standard: Austria, Belgium, Bulgaria, Croatia, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Iceland, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, Netherlands, Norway, Poland, Portugal, Republic of North Macedonia, Romania, Serbia, Slovakia, Slovenia, Spain, Sweden, Switzerland, Turkey and the United Kingdom.

Introduction

For the use of this document the term:

- ‘shall’ indicates a requirement;
- ‘should’ indicates a recommendation;
- ‘may’ indicates a permission; and
- ‘can’ indicates a possibility and/or a capability.

1 Scope

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

Real-time PCR (polymerase chain reaction) detection of celery is based on an 101 bp (base pair) sequence from the gene of the mannitol dehydrogenase (GenBank Acc. No. AF067082¹) 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 [1].

This document is intended to be used in addition to EN 15842 and EN 15634-1.

2 Normative references

The following documents are referred to in the text in such a way that some or all of their content constitutes requirements of this document. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15634-1:2019, *Foodstuffs — Detection of food allergens by molecular biological methods — Part 1: General considerations*

EN 15842, *Foodstuffs - Detection of food allergens - General considerations and validation of methods*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 15842 and EN 15634-1 apply.

ISO and IEC maintain terminological databases for use in standardization at the following addresses:

- IEC Electropedia: available at <http://www.electropedia.org/>
- ISO Online browsing platform: available at <https://www.iso.org/obp>

4 Principle

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

1) NCBI-GeneBank® is an example of a suitable search tool for free use. This information is given for the convenience of users of this document and does not constitute an endorsement by CEN.

5 Reagents

5.1 General

The following general conditions for analysis should be followed, unless specified differently. Use only analytical grade reagents suitable for molecular biology. Reagents should be stored in small aliquots to minimize 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.

5.2 Extraction reagents

5.2.1 Chloroform.

5.2.2 Ethanol, volume fraction $\varphi = 70\%$.

5.2.3 Ethylenediaminetetraacetic acid disodium salt (Na_2EDTA).

5.2.4 Cetyltrimethylammoniumbromide (CTAB).

5.2.5 Hydrochloric acid, $\varphi = 37\%$.

5.2.6 Isoamyl alcohol.

5.2.7 Isopropanol.

5.2.8 Proteinase K.

5.2.9 Sodium chloride.

5.2.10 Sodium hydroxide.

5.2.11 Tris(hydroxymethyl)aminomethane (TRIS).

5.2.12 Chloroform isoamyl alcohol mixture, 24 parts by volume of chloroform (5.2.1) are mixed with one part by volume of isoamyl alcohol (5.2.6).

Similar mixtures commercially available may be used.

5.2.13 CTAB extraction buffer solution, containing:

- CTAB (5.2.4), mass concentration $\rho = 20\text{ g/l}$,
- sodium chloride (5.2.9) (substance concentration $c = 1,4\text{ mol/l}$),
- TRIS (5.2.11), $c = 0,1\text{ mol/l}$,
- Na_2EDTA (5.2.3), $c = 0,02\text{ mol/l}$.

The pH is adjusted to 8,0 by adding hydrochloric acid (5.2.5).

5.2.14 Proteinase K solution, $\rho = 20\text{ mg/ml}$

Store in the form of aliquots at $-20\text{ }^\circ\text{C}$ after dissolving. Do not autoclave.