TECHNICAL SPECIFICATION

SPÉCIFICATION TECHNIQUE

TECHNISCHE SPEZIFIKATION

CEN/TS 15633-3

August 2012

ICS 67.050

English Version

Foodstuffs - Detection of food allergens by immunological methods - Part 3: Quantitative determination of hazelnut with an enzyme immunoassay using polyclonal antibodies and Lowry protein detection

Produits alimentaires - Détection des allergènes par des méthodes immunologiques - Partie3 : Détermination quantitative de la présence de noisette par un immuno-essai enzymatique à l'aide d'anticorps polyclonaux et détection des protéines par la méthode de Lowry

Lebensmittel - Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 3: Quantitative Bestimmung von Haselnuss mit einem Enzym-Immunoassayverfahren unter Verwendung von polyklonalen Antikörpern und Proteindetektion nach Lowry

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Ref. No. CEN/TS 15633-3:2012: E

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Foreword

This document (CEN/TS 15633-3:2012) 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.

This Technical Specification consists of the following parts:

— CEN/TS 15633-1, *Foodstuffs — Detection of food allergens by immunological methods — Part 1: General considerations*

— FprCEN/TS 15633-2, *Foodstuffs — Detection of food allergens by immunological methods — Part 2: Quantitative determination of hazelnut with an enzyme immunoassay using monoclonal antibodies and bicinchoninic acid-protein detection*

— CEN/TS 15633-3, *Foodstuffs — Detection of food allergens by immunological methods — Part 3: Quantitative determination of hazelnut with an enzyme immunoassay using polyclonal antibodies and Lowry protein detection*

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Introduction

The hazelnut (*Corylus avellana)* belongs to a group of foods commonly referred to as tree nuts. Allergic reactions to hazelnut ranging from oral allergy syndrome to severe anaphylaxis have been widely reported. Food labelling legislation requires the presence of hazelnut in food to be declared in numerous nations of the European Union, North America, Asia and Australasia.

IgE binding studies from the sera of sensitized patients have revealed a number of allergens, including both pollen related and non-pollen related allergens [1]. Threshold dose studies have reported provocative doses as low as 1 mg of hazelnut protein [1].

Hazelnut material may occur unintentionally in foods for several reasons. It may be present in contaminated ingredients or cross contamination may occur during food manufacture. Consequently there is a need for sensitive and reliable tests for the detection of hazelnut in food samples.

1 Scope

This Technical Specification specifies an enzyme-linked immunosorbent assay (ELISA)-method for the determination of hazelnut concentration in food samples.

Spiking experiments with diluted ground hazelnut have been used to validate the method's use on food matrices such as mixed grain cereals, dark chocolate (45 % cocoa) and ice cream. The range of the method is 0,5 mg to 5,0 mg hazelnut protein per kg of food sample. As hazelnut kernels typically contain between 12 % to 15 % protein [2], [3], this equates to approximately 3,7 mg to 37 mg hazelnut kernel per kg of food sample. The upper limit of the range of quantitation can be extended, if required, by further dilution of sample extracts.

The method is commercially available[1)] and has been validated in-house by the manufacturer. These data are included in Annex A.2.

The method has been successfully validated by a collaborative study. The study was organized by the Working Group established by the Federal Office of Consumer Protection and Food Safety (BVL) for the execution of § 64 of the German Food and Feed Code (LFGB) for the determination of hazelnut content in dark chocolate. Thirteen German laboratories participated in the collaborative study. These data are included in Annex A.3.

2 Normative references

The following documents, in whole or in part, are normatively referenced in this document and are indispensable for its application. For dated references, only the edition cited applies. For undated references, the latest edition of the referenced document (including any amendments) applies.

EN 15633-1:2008, *Foodstuffs – Detection of food allergens by immunological methods – Part 1: General considerations*

3 Terms and definitions

For the purposes of this document, the terms and definitions given in EN 15633-1 apply.

4 Principle

A direct sandwich ELISA is used for detection of hazelnut protein.

A 13 kDa to 14 kDa protein band common to both raw and roasted hazelnuts was identified using polyacrylamide gel electrophoresis. This protein marker was purified by high performance liquid chromatography and used to raise rabbit anti-hazelnut polyclonal anti-sera. The IgG fraction of this antiserum was purified by affinity chromatography then used to develop a direct sandwich ELISA as outlined below:

1) Soluble hazelnut protein is extracted from a food sample.

2) The extract is then added to micro-titre wells coated with the anti-hazelnut capture antibody. The sample is allowed to react before thorough washing.

1) ELISA Systems Hazelnut Residue ELISA is the trade name of a product supplied by ELISA Systems Pty Ltd, Brisbane, Australia. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN of the product named. Equivalent products may be used if they can be shown to lead to the same results.