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BSI Standards Publication

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

National foreword

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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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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

Produits alimentaires - Détection des allergènes alimentaires par des méthodes d'analyse immunologiques - Partie 2: Détermination quantitative de la présence de noisette par un immuno-essai enzymatique à l'aide d'anticorps monoclonaux et détection des protéines avec l'acide bicinchoninique

Lebensmittel - Nachweis von Lebensmittelallergenen mit immunologischen Verfahren - Teil 2: Quantitative Bestimmung von Haselnuss mit einem Enzym-Immunoassay-Verfahren unter Verwendung von monoklonalen Antikörpern und Proteindetektion mit Bicinchoninsäure

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Foreword

This document (CEN/TS 15633-2:2013) 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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This document consists of the following parts:

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

— CEN/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

Hazelnuts (*Corylus avellana*) have a wide distribution in food industry, especially in chocolate and nougat production. In these cases, the content of hazelnut determines the quality of a product. Hazelnuts are also frequently used in confectionaries, bakery products, biscuits, breakfast cereals and ice-creams.

Unfortunately, hazelnuts are one of the major causes of food allergy. The amount of hazelnut which causes an allergic reaction depends on the sensitivity of the individuals. Even consumption of a few milligrams of hazelnut can induce allergic reactions in highly sensitive allergic consumers. Amounts ranging from 0,7 mg/kg to 100 mg/kg can induce reactions in sensitised individuals [1]. Symptoms of an allergic reaction include local itching of the mouth and throat to severe life-threatening anaphylaxis. Thus deliberately added non-declared hazelnuts in food products are particularly dangerous. Also trace amounts of hazelnuts or nougat, as a result of cross contamination, pose a health risk.

The allergy is caused among other proteins by glycoproteins like corylin, an 18 kDa storage protein contained in the hazelnut, which is similar to the Cor a1-antigen of hazelnut pollen and homologous to the Bet v1 antigen of birch pollen. Corylin is one of the main allergenic proteins beside Cor a8, Cor a9 and Cor a11 as representatives of seed storage and lipid transfer proteins (LTP-proteins). Corylin is differentiated between pollen associated allergy and non-pollen associated allergy.

1 Scope

This Technical Specification specifies an enzyme linked immunsorbent assay (ELISA) method for the determination of hazelnut from food samples. In the ELISA the antibodies bind to hazelnut proteins from the food sample. The result of the ELISA is given in mg hazelnut/kg (ppm) because the calibrators consist of an extract of whole hazelnut.

Matrices like cereals, ice cream, cookies, chocolate, sausage, cottage cheese, yogurt and salad dressing were validated by spiking experiments with a carboxymethylcellulose-suspension containing hazelnut paste [2].

The monoclonal antibodies, raised against the whole aqueous extract of hazelnut, detect proteins with approximate molecular weights of 14 kDa, 18 kDa, and 42 kDa. The antibodies detect the major thermostable allergen Cor a9 (11S storage protein). Both antibodies were evaluated by western blots with partially purified hazelnut extracts and purified allergenic proteins.

The ELISA test method is commercially available[1)]. The performance has been validated by an in house validation performed by the manufacturer. All parameters of interest are indicated.

In addition, the ELISA was successfully validated by a collaborative study in order to determine the interlaboratory reproducibility. This ring trial was organised 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. Fourteen German laboratories participated in this collaborative study.

2 Principles

A direct sandwich ELISA is used for detection of hazelnut. The basis of the test is an antigen-antibody reaction. Two hazelnut specific monoclonal antibodies are used to detect the analyte. The antibodies recognise the hazelnut specific protein Cor a9. A microtiter plate is coated with the capture monoclonal antibody mouse anti Cor a9 antibody. Hazelnut standards provided with the kit or sample extracts are incubated for 10 min. After washing, a detector monoclonal antibody mouse anti Cor a9 antibody, labelled with peroxidase, is added as the enzyme conjugate for further 10 min. The conjugate binds to the hazelnut protein antibody complex on the plate. Any unbound enzyme conjugate is then removed by a washing step. Chromogen/substrate is added to the wells and incubated for 10 min. Bound enzyme converts the chromogen into a blue coloured product. The addition of stop reagent inhibits the enzymatic process and causes a shift of the coloured product to yellow. Absorbance measurement is performed at 450 nm against air. The resulting absorbance values are proportional to the concentration of hazelnut of a sample. The result is expressed as hazelnut in mg/kg. The standard stock solution used is an aqueous hazelnut extract of six different varieties of hazelnut (Hallesche Riesen, Levantiner, Kerassunder, Piemonteser, round Römer, Barcelona Giants). These six varieties, raw and roasted, are representative for the hazelnuts used in food products world-wide by food industry. The standard stock solution is further diluted (see 3.1.2). The extract from the different hazelnuts has a protein content of approx. 9 % protein, measured by the photometric protein determination method according to BCA (Pierce).

1) RIDASCREEN®FAST Hazelnut is the trade name of a product supplied by R-Biopharm AG, Darmstadt, Germany. This information is given for the convenience of users of this Technical Specification and does not constitute an endorsement by CEN-CENELEC of the product named. Equivalent products may be used if they can be shown to lead to the same results.