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Research paper

Sensitive time-resolved fluoroimmunoassay for the detection of hazelnut (*Corylus avellana*) protein traces in food matrices

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Abstract

Allergy to hazelnut is one of the most prevalent causes of severe food-allergic reactions in Norway, as recorded by The Norwegian National Reporting System and Register of Severe Allergic Reactions to Food. In the majority of the reported cases, there is "hidden", unlabelled hazelnut protein in processed foods like chocolate, cookies and cereal mixtures the eliciting agent. For a food survey study performed to evaluate the labelling practices with regards to hazelnut on behalf of the Norwegian Food Safety Authority, a new sensitive time-resolved fluoroimmunoassay (TR-FIA) for the detection of hazelnut protein traces in food matrices was developed and validated. The unique fluorometric properties of the europium-chelates used improved the signal-to-noise ratio because of low matrix interference and led to an enhanced sensitivity. The limit of detection was 0.1 mg/kg and the limit of quantitation was 0.33 mg/kg hazelnut protein. The recovery ranged from 73% to 123% in cookies and cereals, and from 50 to 77% in chocolate. The intra-assay precision was 7% and the inter-assay precision was 19%. Of 100 randomly chosen retail food products in Norway labelled "may contain hazelnut", 36 contained <0.2 mg/kg and seven >10 mg/kg hazelnut protein, demonstrating how differently this precautionary label is used.

Keywords: Europium; Fluoroimmunoassay; Food allergen; Hazelnut; Lanthanide

1. Introduction

Food allergy to hazelnut (*Corylus avellana*) has caused several incidents of severe allergic reactions (Wensing et al., 2002). In Europe, hazelnut has been included into a list of 11 major food allergens and products thereof which have to be mandatorily declared on food products, independently from other labelling policies (European Commission, 2002).

The allergenic proteins identified in hazelnut so far have been found to be thermolabile (Cor a 1, Cor a 2) (Hansen et al., 2003) or thermostabile (Cor a 8, Cor a 9, Cor a 10, Cor a 11) (Pastorello et al., 2002) and seem to differ in their immunogenicity. However, analytical methods for the detection of hazelnut in foods should be able to identify hazelnut proteins in all modifications caused by processing.

Hazelnuts are used in a large variety of confectionery foods like chocolates, nougat, cookies, cereal mixtures, muesli bars and chocolate spreads. Due to manufacturing practices, e.g., shared equipment, or by accidental introduction under the preparation of a meal, cross-contamination of originally hazelnut-free food may occur. Undeclared hazelnut traces represent an acute danger to sensitised persons (Vieths, 2003). In some cases, even

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minute amounts of hazelnut protein are enough to trigger severe allergic reactions. As defined by double-blind placebo-controlled food studies (DBPCFC), the minimum provoking doses in hazelnut-allergic patients range from 1 to 100 mg hazelnut protein (Wensing et al., 2002).

Analytical methods for the detection of hazelnut in foods are requested by manufacturers, authorities and consumer organisations. The first immunochemical assays developed were used to determine the hazelnut content in hazelnut-containing products (Mohr et al., 1983; Klein et al., 1985) and had detection limits > 100 mg hazelnut protein/kg food. In contrary, recent methods are designed for the detection of trace amounts and show considerably improved sensitivities. The LODs for the respective methods, all but the last one reported in amount hazelnut protein/kg food, are 20 mg/kg by rocket immunoelectrophoresis analysis (Malmheden Yman et al., 1994), 5 mg/kg by SDS-electrophoresis/immunoblot with chemoluminescence detection (Scheibe et al., 2001), 10 mg/kg by a Biosensor-based test (Jonsson, 2002), and 1 mg/kg (Koppelman et al., 1999), 0.6 mg/kg (Holzhauser and Vieths, 1999), 1 mg/kg (Blais and Phillippe, 2001), 0.3 mg/kg (Ben Rejeb et al., 2003) and 0.2–1.2 mg whole hazelnut/kg (Kiening et al., 2005) by enzyme-linked immunosorbent assay. With DNA-based polymerase chain reaction (PCR), the LOD was 10 mg hazelnut/kg (Holzhauser et al., 2000) and 4 mg hazelnut/kg with a combined PCR-ELISA (Holzhauser et al., 2002) which is commercially distributed. Currently, four commercial ELISA-kits with nominal LODs of 1.5 or 2.5 mg hazelnut/kg, and 0.5 or 0.8 mg/kg hazelnut protein in food are available.

Time-resolved fluorometry and lanthanide labels have been utilised for immunoassays for some time (Soini and Lovgren, 1987). Several dissociation enhanced lanthanide fluoroimmunoassays (DELFIA) measuring cytokines and plasma proteins for diagnostic reasons have been described (Hemmila, 1988). Generally, the lanthanide ion-chelate which is covalently bound to an antibody is dissociated by pH-shift into a new highly fluorescent chelate after binding to the target protein. Most frequently used is the europium ion (Eu^{3+}) in connection with several different complex chelators. The special fluorescence properties of the lanthanide chelate labels, such as long decay times, large Stokes' shifts between excitation and emission wavelengths, sharp emission peaks and high fluorescence intensities, entail low matrix interferences and allow the development of very sensitive detection methods.

In Norway, hazelnut allergy is responsible for a considerable number of anaphylactic incidents (Løvik et al., 2004). The objectives of this study were to develop and validate a sensitive and specific assay for the detection of hazelnut traces in foods and to survey commercial food products labelled "may contain hazelnut". To reach this aim, we chose lanthanide immunofluorometry as means of detection.

2. Materials and methods

2.1. Hazelnut protein standard and sample preparation

For the preparation of a hazelnut protein standard, raw Mina hazelnuts (Iran) were homogenised with a blade homogenisator (Grindomic GM200, Retsch GmbH, Haan, Germany), suspended in extraction buffer (0.1 M Tris/0.5 M glycine buffer pH 8.7) at a ratio of 1:100, vortexed rigorously for 30 s, extracted at room temperature (RT) for 30 min, centrifuged at 15,000×*g*, 4 °C for 25 min and filtered through glass wool. After determination of the total protein content with the Lowry method (DC protein assay, Bio-Rad, Hercules, CA), the protein standard solution was diluted with phosphate-buffered saline (PBS pH 7.4, Oxoid, Basingstoke, UK) to 2 mg/ml and stored at −20 °C in aliquots.

All food samples were purchased from local stores. They were homogenised and extracted in the extraction buffer at a ratio of 1:5 overnight in a shaking water bath (45 °C) and centrifuged as described above. Extracts not totally cleared by centrifugation were additionally filtered through glass wool. Several hazelnut-free products were used as negative controls and for standard addition in recovery experiments. For the survey study, samples from two different lots of various cereal mixtures (34), cookies (32) and chocolates (34) were analysed in triplicates.

2.2. Antibody purification and europium-chelate labelling

A polyclonal rabbit antiserum, raised against the corylin fraction from native hazelnut protein and immunoabsorbed against a variety of different nut proteins by the manufacturer (no. 45261, Riedel de-Haen, Seelze, Germany), was used in the TR-FIA. The IgG-fraction was isolated by using a HiTrap rProtein A affinity column (no. 17-5070-01, Amersham Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions, and 1 mg of the purified antibody was sent to the Wallac Labelling Service (PerkinElmer Life Science, Turku, Finland) to be labelled with the Eu^{3+}-chelate of N^1-(4-isothiocyanatobenzyl)-diethylenetriamine-N^1,N^2,N^3,N^4-tetrakis(acetic acid) [Eu(III)-N1 ITC]. The labelling reaction was performed at pH 9, 4 °C overnight in a