Journal of Chromatography A, xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Journal of Chromatography A

journal homepage: www.elsevier.com/locate/chroma

Liquid chromatography coupled to tandem mass spectrometry for detecting ten allergens in complex and incurred foodstuffs

M. Planque [a,b], T. Arnould [b], M. Dieu [b], P. Delahaut [a], P. Renard [b], N. Gillard [a,*]

[a] CER Groupe, Health Department, Rue du Point du Jour, 8, 6900 Marloie, Belgium
[b] Laboratory of Biochemistry and Cell Biology (URBC)-Namur Research Institute for Life Sciences (NARILIS), University of Namur, 61, rue de Bruxelles, 5000 Namur, Belgium

ARTICLE INFO

Article history:
Received 14 June 2017
Received in revised form 6 November 2017
Accepted 16 November 2017
Available online xxx

Keywords:
Sensitivity
UHPLC–MS/MS
Multi-matrices
Allergens
Tree nuts
Peanut
Egg
Milk
Soy

ABSTRACT

Food allergy is a considerable heath problem, as undesirable contaminations by allergens during food production are still widespread and may be dangerous for human health. To protect the population, laboratories need to develop reliable analytical methods in order to detect allergens in various food products. Currently, a large majority of allergen-related food recalls concern bakery products. It is therefore essential to detect allergens in unprocessed and processed foodstuffs. In this study, we developed a method for detecting ten allergens in complex (chocolate, ice cream) and processed (cookie, sauce) foodstuffs, based on ultra-high performance liquid chromatography coupled to tandem mass spectrometry (UHPLC–MS/MS). Using a single protocol and considering a signal-to-noise ratio higher than 10 for the most abundant multiple reaction monitoring (MRM) transition, we were able to detect target allergens at 0.5 mg/kg for milk proteins, 2.5 mg/kg for peanut, hazelnut, pistachio, and cashew proteins, 3 mg/kg for egg proteins, and 5 mg/kg for soy, almond, walnut, and pecan proteins. The ability of the method to detect 10 allergens with a single protocol in complex and incurred food products makes it an attractive alternative to the ELISA method for routine laboratories.

© 2017 Published by Elsevier B.V.

1. Introduction

Currently, food contamination with allergens during production remains a challenge for the food industry, especially considering the increasing number of allergic consumers [1]. After an adverse reaction to a food, the allergen must be excluded from the diet of allergic individuals, but involuntary contaminations of the food chain make this avoidance nearly impossible [2]. Allergic patients can be sensitized to one or more proteins and each protein contains several allergenic epitopes that might differ between various individuals. To protect allergic people, various legislative texts (directives 2007/68/EC and 2000/13/EC, regulation 2011/1169/EC) require that consumers be well informed, via the product label, of the presence of 14 allergens when they are incorporated in the recipe [3,4]. These texts, however, take no account of accidental contaminations, which can also cause severe allergic reactions. Hence, the food industry has widely used precautionary allergen labeling (PAL): "may contain...". Yet the undeclared presence of allergens in food products is still the main cause of food recalls in developed countries (42% to 92% depending on the country) [5,6]. The lack of association between PAL and the presence of allergens also leads allergic customers to pay little attention to PAL [7]. To protect the population of allergic people, laboratories need to develop reliable, sensitive methods for the detection and quantification of allergens in food products. An obstacle is the lack of legal recommendations for food allergen thresholds, which complicates the determination of sensitivity thresholds to be achieved and the correct way to express the results of analyses. The European Academy of Allergy and Clinical Immunology (EAACI) and the Allergen Bureau (with its Voluntary Incidental Trace Allergen Labelling (VITAL) system) have established eliciting doses (EDs) for the protection of at least 95% of allergic people [8–10]. Although these referential levels have no regulatory status, laboratories and food authorities use them as indicative thresholds to support decisions, for example in the case of food recalls. According to EAACI/VITAL, the target analytical sensitivity threshold (expressed per kg) should be lower than 0.75 mg for egg proteins, 2.5 mg for milk or tree nut proteins, 5 mg for peanut proteins, 25 mg for soybean proteins, and 50 mg for cashew proteins (portion size: 40 g).

Currently, many methods are available for detecting allergens in foods such as in wine [11–15], chocolate [16–19], and cookies

* Corresponding author at: CER Groupe, Health Department, Rue du Point du Jour, 8, 6900 Marloie, Belgium.
E-mail address: n.gillard@cergroupe.be (N. Gillard).

https://doi.org/10.1016/j.chroma.2017.11.039
0021-9673/© 2017 Published by Elsevier B.V.

[20–23]. Usually, however, the methods developed for the analysis of allergens in a specific food cannot be applied in routine laboratories because of the wide variety of food products to be tested. To overcome this limitation, some laboratories have developed methods for the detection of allergens in several food products [24–26]. A problem with both protein- and DNA-based detection methods for allergens is that the limit of detection or quantification (LOD or LOQ) is mostly determined in spiked matrices (incorporation of extracted proteins after food processing) or solvent instead of incurred matrices (incorporation of raw ingredients before food processing) [27,28]. The determination of LOQs by spiking leads to overestimating method sensitivity [29]. Hence, even though the sensitivities estimated by these methods can be lower than the relevant EAACI/VITAL thresholds (for example, LODs for mass spectrometry of 0.1 mg milk proteins, 0.3 mg egg proteins, and 1 mg soy proteins per kg cookie) [30], such levels of sensitivity might not be achieved with real samples, especially processed foods. Several studies have indeed shown a major decrease in the sensitivity of allergen detection in processed food products [31–33]. It is thus crucial to develop reliable methods for detecting allergens in processed products.

The improvement of sample preparation steps and the high sensitivity of mass spectrometry have allowed impressive progress in terms of detection sensitivity in processed products. Mass spectrometry method must be able to detect specific peptides coming from one or several proteins with a high sensitivity. The target proteins must be abundant, stable to the thermal process and specific for the allergen, but they don't need to be allergenic. A study by Pöpping and collaborators (2012) was the first to analyze 10 allergens simultaneously with high sensitivity in processed bread (60 min–200 °C). These authors used an ultrafiltration purification allowing LODs of 3 mg soluble proteins for almond, 5 for milk and hazelnut, 11 for peanut, 24 for soy, 42 for egg, and 70 for walnut [34]. A recent report describes a size exclusion column (SEC) purification step used before analysis of 5 allergens in incurred cookies (12 min −200 °C) by UHPLC–MS/MS [35]. The LODs, initially expressed in mg whole allergens per kg, were converted to mg proteins per kg by using VITAL conversion factors. They were 2.5 mg per kg for milk, 4.3 for egg, 3.2 for soy, 3.3 for peanut, and 1.1 for hazelnut.

We have previously developed a sensitive method for the detection of four allergens in complex and processed matrices (ice cream, sauce (95 °C–45 min), cookie (180 °C–18 min), and chocolate) [36]. This method uses a solid phase extraction (SPE) purification prior to UHPLC–MS/MS analysis. To the best of our knowledge, it is still the most sensitive method for detecting these allergens in processed matrices, with LOQs (S/N > 10) of 0.5 mg milk proteins, 2.5 mg peanut proteins, 5 mg soy proteins, and 3.4 mg egg proteins per kg food. Here we have extended this approach, applying our method to additional common allergens that must be declared on food labels. The ultimate goal was to develop a sensitive screening method for routine laboratories [36]. Given the prevalence of allergic reactions to tree-nut antigens, we prioritized this allergen category by including six tree nuts [37].

2. Materials and methods

2.1. Materials and reagents

Peanut butter (NIST 2387 22.2% protein), milk powder (NIST1549a 25.64% protein), whole egg (NIST 8445 48% protein), and soy flour (NIST 3234 53.37% protein) were from the National Institute of Standards and Technology (NIST) (Gaithersburg, Maryland, USA). Tree nuts (almonds, cashews, pecan nuts, hazelnuts, walnuts, and pistachios) were purchased from a local store before being finely ground under liquid nitrogen. Tris(hydroxymethyl)aminomethane (TRIS), urea, dimethyl sulfoxide (DMSO), DL-dithiothreitol (DTT), iodoacetamide (IAA), ammonium bicarbonate, and trypsin from bovine pancreas (T8802) were from Sigma-Aldrich (Bornem, Belgium). Acetic acid was from Acros Organics (Geel, Belgium) and hydrochloric acid was obtained from Fisher Chemical (Loughborough, UK). Sep-pak C18 solid phase extraction (SPE) columns (6 cc, 500 mg – WAT043395) were used for peptide purification and enrichment and purchased from Waters (Milford, Massachusetts, USA). Acetonitrile, 2-propanol, methanol (ULC–MS grade), and formic acid were obtained from Biosolve (Valkenswaard, the Netherlands). The labeled peptides TANELNLLI**L** [$^{13}C_6$ ^{15}N]R-OH, FFVAPFPEVFG**K** [$^{13}C_6$ $^{15}N_2$]-OH, GGLEPIN**F** [D_5] QTAADQAR-NH2, and EAFG**V** [D_8] NMQIVR-OH were purchased from Eurogentec (Seraing, Belgium).

2.2. Preparation of non-contaminated matrices

Chocolate, tomato sauce, cookie, and ice cream were selected as targeted food products for the detection of allergens. Cookie dough was prepared by mixing flour (53.4%), sugar (15.2%), oil (16.1%), water (14.8%), NaCl (0.3%), ammonium bisulfate (0.1%), and sodium bicarbonate (0.1%) with a blender. Ice cream was prepared by mixing banana (59.0%), coco milk (29.6%), sugar (11.0%), and lemon juice (0.4%) with a blender. Allergen-free chocolate (containing cacao (45%), sugar (35%), and rice powder (20%)) and tomato sauce (containing mainly tomatoes (75%) and a mixture of onions, carrots, and celery (15%)) were purchased from a local store and finely ground.

2.3. Preparation of incurred materials

Raw food allergens were combined with the flour (cookie), coco milk (ice cream), crushed chocolate, and sauce to obtain a theoretical calculated allergen protein concentration of 20 mg/kg in the milk matrix, 100 mg/kg for peanut and tree nuts, 120 mg/kg for egg, and 200 mg/kg for soybean (n = 3). Crushed chocolate containing allergens was heated in a water bath at 40 °C for 20 min and frozen at −80 °C before being finely ground. After incorporation of allergens, sauce batches were warmed at 95 °C for 45 min. Mechanical grinding (Robot-Coupe, Blixer 4 V.V.) was performed for 3 min to achieve a homogenous distribution of food allergens in ice cream and cookie, before freezing the ice cream at −20 °C. Serial dilutions of contaminated matrices were prepared by mixing non-contaminated and contaminated matrices to reach the following intermediate levels of food allergens: 0, 0.1, 0.5, 1, 2.5, 5, 10, and 20 mg/kg for milk proteins, 0, 0.5, 2.5, 5, 12.5, 25, 50 and 100 mg/kg for tree nut and peanut proteins, 0, 0.6, 3, 6, 15, 30, 60 and 120 mg/kg for egg proteins, and 0, 1, 5, 10, 25, 50 100 and 200 mg/kg for soy proteins. Eight 40-g cookies 7 cm in diameter were prepared per intermediate level of contamination and per independent replicate (n = 3). In each batch, the cookies were baked at 180 °C for 18 min and then finely milled in a blender.

2.4. Preparation of spiked solution

On the basis of NIST protein contents, a milk solution at 0.1 mg/mL was prepared in extraction buffer (200 mM TRIS-HCl pH 9.2, 2 M urea). This solution was used to spike dietary supplements and enzyme samples at 1 mg/kg and 2.5 mg/kg prior to applying the protocol.

2.5. Sample preparation for UHPLC–MS/MS analysis

2.5.1. Extraction and enzymatic digestion protocol

The protocol applied was as described in [36] with slight modifications. Three grams of matrix was weighed into a 50 mL

Download English Version:

https://daneshyari.com/en/article/7609354

Download Persian Version:

https://daneshyari.com/article/7609354

Daneshyari.com