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Review article

Methods of allergen detection based on DNA analysis

Marta Słowianek,* Iwona Majak

Institute of General Food Chemistry, Technical University of Lodz, 90-924, Lodz, Poland

* marta.slowianek@o2.pl

Abstract: *Many allergens, such as hazelnut, peanut, charlock, celery, sesame, lupine, walnut, almond, macadamia nut, hickory, pistachio, wheat gliadins, may be present in food products, however, undeclared or as unintentional additives. Due to the growing number of allergic reactions, it is crucial to have fast, reliable methods of allergen detection in processed food products. This review summarizes the recent methods of allergen detection in food products based on PCR reactions, namely PCR-ELISA, Real-time PCR, PCR-PNA-HPLC, Duplex PCR and Multiplex Real-time PCR, describing their principles, applications, detection limits, drawbacks and advantages.*

Keywords: *PCR allergens, hazelnut, peanut, charlock, celery, sesame lupine, walnut, almond, macadamia nut, hickory, pistachio, wheat*

Introduction

Many allergens, such as hazelnut, peanut, charlock, celery, sesame, lupine, walnut, almond, macadamia nut, hickory, pistachio, wheat gliadins, may be present in food products, however, undeclared or as unintentional additives. Due to the growing number of allergic reactions, it is crucial to have fast, reliable methods of allergen detection in processed food products.

This review summarizes the recent methods of allergen detection in food products based on PCR reactions, namely PCR-ELISA, Real-time PCR, PCR-PNA-HPLC, Duplex PCR and Multiplex Real-time PCR, describing their principles, applications, detection limits, drawbacks and advantages. PCR techniques are promising in the field of allergen detection on the level surpassing immunodetection.

PCR techniques rely on the detection of a defined DNA sequence. The DNA fragment is amplified with DNA polymerase which requires a pair of short primer fragments in order to recognize the required DNA sequence characteristic for a given allergen source.

Methods based on detection of specific DNA sequences

Methods applying detection of coding sequences of allergenic proteins allow direct analysis of allergenic food components. The detection of allergenic protein is performed through amplification of its specific DNA fragment in PCR reaction [1]. The PCR reaction allows obtaining qualitative results pointing to the presence of

sequence coding for given allergen. Semi quantitative determination can be obtained with PCR-ELISA reaction, while precise quantitative determination is granted by Real-Time PCR [2].

The PCR method was successfully applied to the detection of DNA coding for flour gliadins and main allergen of hazelnut. Such determinations are highly sensitive, free from cross-reactions and able to detect amounts of allergen source admixture less than 0.001% [3].

Hazelnut is a potential food allergen present in pastries, confectionery and ice cream. Its undeclared amounts can be present as an unintentional food contamination. Specie-specific amplification of gene fragment - Cor a 1.0401 – an allergen of hazelnut, enables to detect its admixtures in food products in quantity of 0.001% (w/w) [4].

Dovicovicova et al. suggested a specie-specific PCR for celery detection through the amplification of mannitol dehydrgenase gene. The technique is specific towards four celery species with the detection limit ≦ 1.53 ng DNA and 0.1% (w/w) in meat products [5].

Assessing PCR techniques for the detection of allergen DNA the problem of DNA degradation during technological processing of foods should be taken into account. Its reflection is found in research of many scientists who analyze the impact of food processing on the detection levels of allergen DNA. The works of Koeppel et al., who investigated processed oat products (cereals and oatmeals) regarding their wheat gliadin content, are worth of attention.

In the research PCR and ELISA techniques were compared, in which it was demonstrated, that despite the standing out sensitivity of PCR method, only one out of eight samples gave a positive result. However, all samples contained between 1 and 2 mg/100g of gliadin detectable with ELISA technique, which points to high degradation of DNA in processing [6].

The influence of parameters of food processing on transgenic soy and corn identification in bakery products with PCR techniques was also researched by Moser et al. [7] They concluded that the DNA of 0.5% addition of transgenic corn was undetectable in processed product (bread and cake). However, the DNA of soy (0.3%) was detected in white toast bread after each technological step.

Similarly as in the case of ELISA technique, there are several variants and modifications of PCR technique, as: PCR-ELISA, Real-time PCR, PCR–PNA-HPLC, Duplex PCR and Multiplex real-time PCR.

PCR-ELISA

In the PCR-ELISA method the detection of an allergen does not require gel electrophoresis. The amplified DNA fragment is labeled with biotin or digoxygenin, thanks to which it can be easily determined with ELISA test [8, 1]. This combination applies the high specificity of detection based on DNA with the simplicity and low cost of ELISA. Holzhauser et al. demonstrated that PCR-ELISA is an important tool for allergen monitoring. With this method they

determined hazelnut allergens in processed foods at a level below 0.001% (w/w). The high specificity of PCR-ELISA towards hazelnut and high stability of DNA causes that this method is very useful and advisable for monitoring of this allergen in food products [9].

Real-Time PCR

The Real-Time PCR is commonly used for allergen detection in foods as: peanuts, celery, charlock, sesame, lupine, cashew, walnut, pistachios, pecan nuts, almond and macadamia nuts [10]. Development of real-time PCR techniques with Taq-Man probes enables to carry out specific detection of trace amounts of peanuts in food [1]. Analysis conducted with this method allows for detection of undeclared amounts of peanuts in 13% of samples from 0.0001 to 0.0074% (w/w) [12]. Works describing application of real-time PCR with Taq-Man probe for hazelnut [13], pecan nut [14], and walnut [7] in food with detection limit of 0.01% in standard samples of biscuit were published. The research covers DNA isolation by chaotropic extraction of solid phase and secondary PCR with fluorescent Taq-Man probe and primers specific for the gene of hazelnut, pecan nut or walnut. The intrinsic detection limit of this method obtained for hazelnut is 13 pg of hazelnut DNA in confectioner's and bakery products (0.01% hazelnut content) [13], 1 pg of pecan nut DNA (0.01% pecan nut content) [14], for walnut – 0.24 ng DNA (0.01% walnut content). In the case of walnut there were two samples of biscuits detected with an undeclared presence of walnut in the total of 13 samples [7].

Real-Time PCR was also applied for detection of sesame traces in food. Sesame was revealed in all samples with declared sesame content (crackers, sesame biscuits, shortbread) with an exception of sesame oil, however, sesame was not detected in samples which could contain it or where it was not declared. The determination did not give cross-reactions with 17 similar food components [16]. Also, the presence of mannitol dehydrogenase gene was detected in food with this method. The method was tested on over 50 samples of foods with detection limit 0.0005-0.001% (w/w) in standard samples of sausage [17]. Mustorp et al. presented a quantitative and sensitive Real-Time PCR method applying Taq-Man probes able to detect additions of 0.01-0.001% (w/w) of celery and 0.005% of mustard and sesame in food [18].

PCR-PNA-HPLC

PCR–PNA-HPLC technique is a modification of PCR using probes labeled with peptide nucleic acid (PNA) and detection with high performance liquid chromatography (HPLC). The applications of this method for detection of hazelnut in vegetable products allowed determining 5 pg of hazelnut DNA PCR-PNA-HPLC method enables effective detection of potentially hidden allergens even in products with undeclared presence of hazelnut or as possible contamination [19].

Duplex PCR

Reactions of Duplex PCR type was applied to simultaneous detection of hazelnut and peanut in native and processed food with two specific pairs of primers and allowed for detection of less than 50 pg DNA in final product [20]. The method was also used for detection of two species of wheat in pasta dried in high temperature, with detection limit of 0.2% (w/w) [21].

Multiplex real-time PCR

Multiplex real-time PCR allows to amplify several DNA fragments simultaneously by application of several pairs of primers [21]. This method was used to determine DNA of eight allergens at the same time: hazelnut, peanut, celery, soy, egg, milk, almond and sesame. The test exhibits high specificity and sensitivity in the range of 0.01%, lower for egg and milk due to low content of DNA [22].

Advantages and disadvantages of methods based on PCR

Analysis with PCR techniques displays high potential due to its speed, efficiency and simplicity [22]. The technique is reliable, highly specific and sensitive, with noted detection limits less than 10mg/kg of almonds, hazelnuts, soy, milk or peanuts [23]. It allows minimizing the cross-reactivity and avoiding false-positive results by choosing suitable primers differentiating between two closely related DNA sequences [24].

The main disadvantage is DNA degradation during food processing and the dependence of detection limit on the amount and purity of the matrix [24]. What is more, techniques based on PCR do not detect components responsible for allergic reaction, only the specie-specific DNA [25]. Therefore, presence of DNA coding for a protein in food sample does not necessarily signify the presence of the allergen, but only about the origination of the product from a characteristic genus, specie in the case of contamination [8]. The main limitation of methods based on DNA is the fact, that the particular allergenic protein is not directly determined, only the DNA from the given source, and in consequence, there is an possibility of false-negative or false-positive results for the presence of allergens in food [24]. These methods are not recommended for detection of allergens in food products with high protein and low DNA content, e.g. eggs. Consequently, these methods are a good choice where the protein content is low, e.g. in celery [26]. Despite that, PCR techniques are promising in the field of allergen detection on the level surpassing immunodetection.

The advantage of PCR techniques over methods based on protein detection is the efficient extraction of DNA in denaturing conditions, more effective than during protein extraction from food matrices, and DNA stability over geographical and seasonal changes, while protein concentration in food products depends on many factors as genus, specie or growth conditions [1, 26]. PCR techniques rely on the

simple and well defined DNA sequence [24]. Methods basing on PCR are fast and can be set up even in few days, if the analyzed sequence of DNA is known [24].

Processes of food preparation differently influence the DNA, which can be easily degraded during processing, leading to false results [2]. Köppel et al. managed to detect contaminations below 0.1% (w/w) with PCR techniques, while ELISA test was about 10 times less sensitive [6].

Summary

Basing on the literature review it can be concluded that there exists a wide range of research tools presenting different sensitivity for allergen detection in food products. Most frequently used techniques are based on Real-Time PCR. However, there is still a need to enhance reliability of the available analytical methods and develop new standardized methods for food industry in order to ensure safety of the consumers prone to allergic reactions. The new or improved tests have to be faster, more sensitive (lower detection limit), more precise (lower limit of quantitative determination) and more specific for similar allergenic proteins, they should enable to unequivocally identify allergens.

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