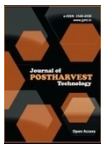

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REVIEWARTICLE

Recent advances in detection of food allergens

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ABSTRACT

Food allergy is an immunological reaction caused as a result of exposure of certain proteins or food allergens when administered in adequate amounts by sensitive people. Eight major food groups causing food allergy consist of cow's milk, tree nut, fish, soy, peanut, wheat, hen's egg and crustacean. Food allergens are the proteins that occur naturally in foods which may be responsible for abnormal immune responses. These allergies are either Immunoglobulin E(IgE)-mediated, non-Immunoglobulin E-mediated, immune complex-mediated or cell-mediated reactions. A number of detection methods used to identify and quantify the possible proteins responsible for allergenic properties exist which includes physicochemical methods such as kjeldahl nitrogen assay, colorimetry, electrophoresis, spectrophotometry, mass spectrometry and polymerase chain reaction methods (real time and conventional) and Immunological methods such as counter electrophoresis, immunoblotting, immunodiffusion, enzyme-linked immunosorbent assays (ELISAs), enzyme-linked immune spot assays (ELISPOTs) , radioimmunoassay to the newer ones i.e. immunodetection methods which include biosensor such as surface plasmon resonance (SPR).This review summarizes the novel detection methods used to identify and quantify these allergens.

Keywords: Food allergy, immunology, immunoglobin, immune system, allergens

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INTRODUCTION

Food Allergy can be considered as one of the emerging issues that can affect the public's health. A food allergy has been defined by the National Institute of Allergy and Infectious Diseases (NIAID) as an "adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food". Such antigens are termed as food allergens and are harmful substances which cause elicitation of an immune reaction (Eyerich et al., 2020). A number of studies suggest a steep rise in the occurrence of food allergy in the world population. The population affected by the allergens are continuously increasing. It has affected 5% of the total population and affecting 8% of children. The FALCPA (Food Allergen Labelling and Consumer Protection Act, 2004) has considered eight food groups responsible for causing allergy from food namely cow's milk, tree nut, crustacean, peanut, fish, wheat, hen's egg and soy (Cho et al., 2020). These adverse effects may be mild such as rashes and itchy sensations to the serious issues including anaphylaxis (Soon, 2018). The effect of food allergens may vary from person to person. Also, the various cooking and processing parameters may contribute to the ability of food to cause possible allergenicity (Siragakis et al., 2013). The only way to prevent the allergic reactions is to avoid the exposure to these allergens by checking the labels of the food showing 'may contain'. Consumers must have the habit of reading the labels on

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making a purchase of food articles in order to reduce the chances of outbreak of food allergies (Soon, 2018) . Diet plays a crucial role in the prevention of food allergies (Hicke-Roberts et al., 2020). Thus, the individuals susceptible to certain food allergies should avoid the exposure to the foods responsible for causing such adverse reactions as these may cause mild to fatal anaphylaxis (Ontiveros et al., 2020). Factors such as consumptions of drugs, antacids or due to enhanced temperature of body or physical exertion are some of the reasons for increasing the risk of food induced allergies (Varshney and Pongracic, 2020).

TYPES OF FOOD ALLERGIES

Broadly, the food allergy is divided into four major types i.e., Type 1, Type2, Type 3 and Type 4. All of these food allergies vary on the type of reactions and symptoms induced.

Table1: Types of food allergies (Mills et al, 2007)

Types	**Type 1**	**Type 2**	**Type 3**	**Type 4**
Other names	Immediate hypersensitivity/ IgE-mediated reactions	Non-IgE mediated	Immune complex mediated reactions	Cell-mediated or delayed hypersensitivity
Reaction time	Occur within minutes or few hours	-	-	Occur after few days
Immune reactant involved	IgE antibodies	IgG and their sub classes	IgE, IgGand IgA antibodies	T-lymphocytes
Mechanism of action	Involves two phases: 1.Sensitization(Occurs on first allergen exposure and stimulates IgE antibodies which combine with the mast cell or basophil and the cell thus formed is called sensitized cell.) 2. Elicitation (On second exposure with the same allergen, the sensitized cells bind with the allergen and degranulation of mast-cell i.e. release of histamine and other	Production of antibodies other than IgE (IgG and their subclasses) takes place. Presence of both IgG4 and IgE indicate the sensitisation in an individual.	Proteins absorbed in body come in contact with antibodies present in body, forming complexes called immune complex and are found to contain IgE, IgG and IgA antibodies.	It involves interaction of sensitized T-lymphocytes with allergens. T-lymphocytes stimulation causes release of cytokines and lymphokines causing inflammation.

	mediators takes place).			
Example of hypersensitivity reactions	Urticaria, hypotension, anaphylaxis, oral allergy syndrome, and angioedema	Celiac disease and enteropathy (caused by protein in food)	Inflammation	Inflammation, Celiac disease and allergic contact dermatitis causing irritant reaction and urticarial

There are different allergens present in different food groups responsible for the allergenic reactions on human consumption. The various allergens along with the component responsible for allergic reactions are provided in the table below:

Table 2: Allergens in different food groups (Boye and Godefroy, 2010)

Allergy	Allergic part	Occur During
Cow's Milk	Casein: α_{s1}, α_{s2}, κ, βand ϒ- casein Whey proteins: Bovine Serum Albumin (BSA), α-lactalbumin, β-lactoglobulin, Immunoglobulins (A, M and G) Lactoferrin Lactoperoxidase Lysozyme	Childhood
Egg	Egg white proteins: Ovomucin, Ovomucoid (Gal d1), Ovalbumin (Gal d2), Ovotransferrin (Gal d3), lysozyme (Gal d4), Egg yolk proteins: Phosvitin,α-livetin (Gal d5), Apovitellenins	Infancy and early childhood
Fish	Parvalbumin (Gad c1)	Adulthood and Children
Crustacean	Tropomyosin	Adulthood
Soy	Major soybean allergens: Soybean glycinin (11S) and β - conglycinin (7S)	Babies and Children
Wheat	Wheat protein: α-, β-, ϒ-, and ω-gliadin, glutenin	Children
Peanut	Classified on the basis of prevalence in population: Major peanut allergens: Ara h1 (vicilin), Ara h2 (conglutin), Ara h3 (glycinin) Minor peanut allergens: Ara h4 (glycinin proteins), Ara h5 (profilin), Ara h6, Ara h7 (conglutin) and Ara h8 (PR-10 protein)	Childhood
Tree Nut	Cashew: Ana o1(vicilin), Ana o2 (legumin), Ana o3 (albumin) Pistachio: Pis v 1, Pis v 2, Pis v 3, Pis v 4, Pis v 5 Walnut: Jug r 1(albumin), Jug r 2 (vicilin), Jug r 3 (lipid transfer protein), Jug r 4 (legumin) Hazelnut: Cor a 1 (pathogenesis-related protein-10), Cor a 2 (profilin), Cor a 8 (lipid transfer protein), Cor a 9 (legumin), Cor a 11 (vicilin) Brazil nut: Ber e 1 (albumin), Ber e 2 (legumin)	Childhood

DETECTION METHODS FOR FOOD PRODUCTS

Milk

Milk is the major allergen responsible for outbreak of food allergy in individuals. The main allergens include caseins, β-Lactoglobulin and α-lactalbumin as they occur in large amounts in cow's milk(Villa et al., 2019).It involves an IgE-mediated reaction eliciting adverse immunological reactions in sensitive individuals due to intake of even trace concentration of milk proteins (Villa et al., 2018).

Detection methods: A number of researches have undergone so far for detecting the presence of allergens in cow's milk employing different techniques. The allergens that occur in milk may be detected employing various methods. However, the more recent ones are highlighted in this review paper.

In a study, Ultrahigh-performance liquid chromatography– tandem mass spectrometry (UPLC-MS/MS) method was employed preceded by digestion using PHMN-Trypsin(trypsin immobilized on hairy polymer-chain hybrid magnetic nanoparticles), which helped reduce the digestion time to 15 minutes and also showed enhanced digestion efficiency. It was developed to detect milk allergens in commercial baked food. Caseins (αs1,αs2, β, and κ-caseins) were used as the allergen proteins. Here, the selection of 2 or 3specific and signature peptides was done in order to achieve higher specificity, stability and mass response intensity. Limits of quantification (LOQ) of the 4proteins in food commodities (baked) were found to be in range of 0.38–0.83μg/g with their recoveries ranging from 65.2% to 86.1% (Qi et al., 2019).

In yet another investigation, a novel electrochemical platform based on poly (aniline-co-anthranilic acid) (PANI/PAA) composite polymer was employed which was coupled with an aptamer for detecting milk allergen (β-lactoglobulin).The method finds many benefits such as application of novel and simple apta sensor. The samples of milk spiked with β-lactoglobulin were analysed for study. It involved the application of aptasensor containing disposable screen-printed cells. The advantages include less cost, involvement of less quantity of reagents, simple to perform, portable, good recovery and easier preparation method. The biosensor was designed in such a manner that poly(aniline-co-anthranilic acid) co-polymer deposition could occur on the surface of graphite screen-printed electrodes followed by optimizing monomers mixture's composition through cyclic voltammetry. Subsequent immobilization of amino-modified aptamer via carboxylic groups of the polymer film were activated by the use of 1-Ethyl-3-(3'- dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxy succinimide (NHS). Performance of apta sensor(sensitivity and reproducibility) was studied in buffered solutions of β-lactoglobulin. The curve for dose response (in case of β-lactoglobulin concentration) was constructed between range of 0.01 and 2 μg/L. LOD was found to be 0.053 μg/L (Lettieri et al., 2019).

Galan-Malo et al. (2019)designed a lateral flow device for detecting β-lactoglobulin and casein that occur in foods. The technique was employed for detecting the allergens in processed foods, rinsing water and ingredients with LOD of 2 ppm for caseins, 1-5 ppm for powdered milk and whey with no cross-reactivity observed among the food commodities.

Another method i.e. surface plasmon resonance (SPR) was employed to detect the allergens in cow's milk using α-casein as the biomarker. It consisted of sensor chip with four sensing arrays (help in sample measurement and control binding of allergen on sensor).The technique was found to be useful in terms of ease of use, high sensitivity, measurements in real time along with use of label-free optical sensor. It involved gold chip for immobilising α-casein-polyclonal antibody using ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDS)/N-hydroxysuccinimide (NHS)coupling procedure. Firstly, optimization of assay performance and sensor were characterised in pure buffer conditions resulting in LOD of 58 ng/mL. The improvement in the sensitivity of assay was performed by employing sandwich assay format along with amplification using nanoparticles(Ashley et al., 2017).This technique (SPR) has also been employed for detecting β-Lg and casein present in milk. It employed the immobilization of antibodies on sensor chip's surface followed by measuring of their binding activity with antigens (Gomaa and Boye, 2015).Also SPR was again used to detect β-lactoglobulin through immobilizing of monoclonal antibodies on the surface of biosensor chip. The shift in resonance angle provided information about the proteins that stick to the surface of antibody. In this technique, the sensor surface was immobilized by monoclonal antibodies which may stick allergens on their surface with high specificity followed by injecting corresponding polyclonal antibodies as the capture antibody for better sensitivity.

Eventually, the sensitivity of the assay was enhanced by employing the polyclonal antibodies in the sandwich assay which showed concentration of βLG to be 5.54 ng/mL. Also, the result showed good linear relation with comparatively lower amounts of protein which indicates good sensitivity (Wu et al., 2015). It also employed determination of the concentrations of different allergens in milk namely lactoferrin, bovine serum albumin (BSA), immunoglobulin G, α-lactalbumin and β-lactoglobulinin raw and processed milk. The six samples were tested in each assay (Nehra et al., 2019).

In another investigation, molecularly imprinted polymer-nanoparticles (nano MIPs) were synthesized showing greater affinity towards α-casein employing solid-phase imprinting. It was then followed by the characterization of nano MIPs and their incorporation into label free SPR based sensor. The advantages of nano MIPs include high selectivity and binding affinity towards α-casein. This sensor showed the detection of α-casein quantitatively(LOD= 0.127 ppm)(Ashley et al., 2018).

WHEAT (*Triticum aestivum*)

Wheat is a common allergy causing grainamong cereals. The main allergens in wheat are gliadins (Li et al, 2020) and glutenins (Boye and Godefroy, 2010).

Detection methods: In a study,2-D western blot electrophoresis was employed for the detection of IgE levels (produced as a result of interaction with wheat allergen) in serum samples. In this experiment, blood samples from 21patients with high wheat-specific immunoglobulin E (sIgE) levels were grouped into 3 patients groups based on their clinical profile followed by their testing via 2-D Western blot. As a result, the standardized wheat protein extracts were separated and then the comparison of sIgE sensitization profiles was conducted. The identification of specific sensitization profiles was performed. After identifying the spots of protein of interest namely Tri a 26, Tri a bA, Tri a 34, Tri a tritin, these were analysed or quantified employing mass spectrometry (UHPLC-MS/MS) (Courtois et al.,2020).

In an investigation, a qualitative method namely immune-histo-fluorescence-quantum dots (IHF-QD) microscopic imaging was employed for detecting the food allergens namely peanut and wheat. In this, two modules of pastry samples (food matrix) were prepared. One model was spiked with peanut and wheat allergenic components and the other was prepared without the addition of allergenic components. Both models were subjected to the detection method of ELISA and IHF-QD microscopic methods in which primary antibodies were labelled at specific wavelengths namely 525, 585, and 655 nm emissions. Results showed that no differences were observed between the ELISA and IHF-QD microscopic methods among experimentally produced pastry samples. In contrast, the differences were observed among commercial samples. It was thus concluded that the highest contrast was found in the application of 585 QD conjugates that may help in measuring and quantifying the presence of peanut and wheat allergens. The advantages of this technique involved reliability for applicability in wider food matrices.

Real Time–Polymerase Chain Reaction (RT-PCR) method was employed for detecting allergens in certain food namely wheat, peanut and buck wheat. In each PCR machine, a reference plasmid was used to provide a cut off for determining the positive results. These reference plasmids consisted of already known copies of the target sequences. In this technique, number of copies of plasmid thus produced were used detection of allergen and gave results showing 10 ppm (w/w) protein in highly processed foods (cooked for >30 min at 122 °C). The advantages of the method involved good sensitivity, specificity and avoid false positives (Miyazaki et al., 2019).

HPLC-MS/MS method was developed for detecting various allergens in meat-based food commodities. Allergens namely wheat, barley, oats, maize, rice and rye proteins were detected. The marker peptides (3-4) were selected in this technique post protein extraction and tryptic digestion. These were quantified using HPLC-MS/MS. LOD were either < 5 or < 10 mg grain protein/kg meat product for every allergen.The advantages of the technique involved good sensitivity, lack of false-positives or -negatives, no greater effect in results obtained after storage and grilling of sausages. In contrast the results were affected on the type of canning process employed (Jira and Münch, 2018).

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) was developed to conduct multiplex detection of 13 food allergens in processed foods and raw materials(Ogura et al.,2019). This technique(LC−MS/MS)was also employed to detect14 different allergens in food via multiple reaction monitoring (MRM). Although, MRM finds many issues during detection as the complex and heterogeneous interferences (that occur in food matrices) participate in the reaction thus affecting the final results. In order to counteract this technique, an open source software package MAtrix-Dependent Interference Correction (MADIC) was developed which had a function of identification of interference along with further employing this technique targeting 14 allergens. As the tests were conducted using this technique and software, it was found that allergen contamination was detected in foods including wheat in grains. The advantages of this technique were good sensitivity and feasibility (Croote et al.,2019).

EGG (*Gallus domesticus*)

Main allergens in hen's egg include ovalbumin (Gal d 2), conalbumin (Gal d 3), ovomucoid (Gal d 1) and lysozyme (Gal d 4).

Detection methods: Numerous studies have been conducted so far to detect allergens in egg employing novel techniques as discussed.

In a study, SDS-PAGE was employed for the detection of ovalbumin, ovomucoid, ovotransferrin and lysozyme in egg white of chicken, layer chicken and duck. On employing this technique, it was found that layers of egg white chicken contained more quantity of allergen protein (such as ovalbumin, ovomucoid, ovotransferrin and lysozyme) than local chicken eggs and ducks. Thus, these are more viable source of allergies (Baharuddin et al.,2020). In a more recent study, HPLC-MS/MS was employed for the quantitative study of allergens of milk, egg and soy in surimi food products. Firstly, a signature peptide was established along with stable isotope-labelled peptide EAFGVNMQI (I, $^{13}C_6$. ^{15}N)VR. The peptide used for egg was GGLEPINFQTAADQAR (egg ovalbumin).Subsequently, extraction of protein and digestion of trypsin were conducted followed by the measurement of selected marker peptide via HPLC-MS/MS. The LOQ for egg allergen in the experiment was calculated to be 0.032 µg/g (Huang et al.,2020).In another study, high resolution mass spectrometry was employed to detect presence of allergen in processed egg matrices (namely fatty rich environment, raw egg, low pH matrix and heated egg). First of all, the potential peptide biomarkers were identified adopting a strategy followed by the detection and quantification by employing Mass Spectrometry. In order to achieve better results, tandem Lys-C/trypsin was employed to enhance the results by increasing the enzymes involved (Gavage et al., 2019).

FISH

Fish allergy may occur due to ingestion, contact or inhalation of fish allergens (Carvalho et al., 2020).The main allergens in fish include parvalbumin, enolases and aldolases and fish gelatine (Kuehn et al., 2014).

Detection methods: Researchers have conducted a great number of investigations in order to develop the already existing techniques in the field of detection of fish allergens.

A novel and highly promising approach for parvalbumin detection namely ELISA method was developed that employed antibody functionalized graphene oxide (GO)and gold nanoparticles (GNPs) for amplification of the detection signal of parvalbumin. In this method, GO and GNPs were modified with monoclonal antibody and secondary antibody respectively to detect the allergen captured by amino-functionalized magnetic beads. This resulted in amplification of the antibody and modified graphene oxide and gold nanoparticles. LOD was 4.29 ng/mL. In case of spiked samples, the recovery rates were found to be between the range89.82% to 115.37%. The method finds many advantages such as novelty and feasibility (Wang et al., 2020).Carrera et al. (2019)employed three methods namely bio-informatics (protein-based), IgE based methods and shotgun proteomics for the detection of fish allergen (parvalbumin). It involves the molecular characterization of B-cell epitomes. Firstly, β-parvalbumins were purified from 15 different fish species using shotgun proteomics followed by combining the identified β-parvalbumins peptide sequences and 98 β-PRVB protein sequences from UniProtKB followed by further aligning and analysing for B-cell epitopes determination via the Kolaskar and Tongaonkar algorithm. ELISA was used in order to predict the highest rated B-cell peptide epitopes by employing the corresponding synthetic peptides and sera from both healthy and fish allergic patients. Result showed that 35 peptides were identified as B-cell epitopes and the top B-cell peptide epitopes (LKLFLQV, ACAHLCK, FAVLVKQ and LFLQNFV) were designated as potential peptide vaccine candidates which may be responsible for immuno therapies.

Chromatography can be employed for the detection of allergens in fish. The most recent study employing liquid chromatography- tandem mass spectrometry and multiple reaction monitoring mass spectrometry(LC-MRM-MS/MS)was employed in order to detect the presence of β-parvalbumin in flounder with anLOD of 0.10μg/g. The method was easy to perform, sensitive, accurate (<13.3%) and precise (RSD<18.35%). It involved use of simple purification (along with heat) with an optimized digestion of trypsin(Sun et al., 2019).A newer technique shows that a technique employing Ion-exchange chromatography along with capillary-zone electrophoresis can be used as one of the methods for detection of parvalbumin in fish with LOD of 0.71μg/mL. In this experiment, the optimization of Ion-exchange chromatography for simple enrichment of allergen crude extract was conducted. Here, the electrophoretic resolution, time of migration and separation efficiency were improved by employing 25 mmol/L borate borax buffer with 15 kV separation voltage maintained at pH 9.2 for capillary zone electrophoresis separation. This technique help achieve results faster (within 2.8 min)on (LOD) along with greater recoveries i.e. 89.6%–104.7% (Fu et al., 2018).

In a yet another study, SDS-PAGE (sodium dodecyl sulphate-polyacrylamide gel electrophoresis), immune blotting, mass spectrometry and Rat basophil leukemia cell line (RBL-2H3) cell model were employed for the detection of parvalbumin extracted from the Japanese flounder (*Paralichthy solivaceus*) followed by development of recombinant parvalbumin from the native forms detected namely PVI, PVII and PVIII. According to the data, it was observed that the only native parvalbumin providing the comparable result with the recombinant form was PVIII, thereby suggesting it can have significant role in detection of fish allergen (Sun et al., 2019).

SOYBEAN (*Glycine maxima*)

Soybean is one of the vegetarian sources containing 40% protein. It has high nutritional value and is found to have good physiological properties. It helps in lowering the bad cholesterol. Glycinin is the major protein responsible for causing allergen

reactions in sensitive people. Soy allergy has affected 0.3-0.4% in adults and 0.8-1.2% in children (Segura-Gil et al., 2018). Seeds of soybean are found to contain many allergenic proteins namely lipoxidase, glycinin and β-glycinin formed by globulins (Nardiello et al.,2019).

Detection methods: In a most recent technique, the highly sensitive and specific primers were developed using DNA-based technique namely Loop-mediated isothermal amplification- Lateral Flow Dipstick (LAMP-LFD) in order to detect presence of soy allergens. In this method, the DNA was amplified using LAMP followed by the detecting the amplified DNA via LFD or gel electrophoresis or fluorescence recording. It was concluded that the LAMP based on ORF160b gene had greater specificity for soybean. LOD was nearly 10 mg/kg of soy allergen in food. The advantages of the technique involved simplicity, robustness, specificity, sensitivity and cost-effectiveness. In a study, a novel LC-MS/MS technique was developed in order to conduct detection of allergens of soy, milk and egg in surimi food products. Three signature peptides were developed for the three allergens. One developed for soy was EAFGVNMQIVR (soy glycinin G2). A fourth stable isotope-labelled peptide namely EAFGVNMQI (I, $^{13}C_6$, ^{15}N) VR was also developed. It was then followed by the extraction of protein and digestion of trypsin. All the marker peptides were then quantified via HPLC-MS/MS. The advantages of the technique involved accuracy and precision. The LOQ was found to be 0.054 µg/g (for soy) (Huang et al., 2020).

More recently, two well-known techniques namely ELISA (Enzyme-Linked Immuno sorbent Assay)and immune blotting were employed for detecting the levels of 8 major allergens present in soybean including Gly m 7 in six genetically modified (GM) soybeans and six non-GM soybeans. In addition, analysis of IgE-reactivity to these soybeans through immune blotting by employing the blood samples from three soybean-allergic patients was also conducted. Observations depicted hardly any significant differences in the amounts of the major allergens in the GM and non-GM samples as well as in the serum of IgE-reactive protein profiles of the patients (via immune blotting) (Matsuo et al., 2020).ELISA technique employing two formats (sandwich and indirect competitive) were developed for detection of β-conglycinin (soybean allergen). It was performed on three food matrices (pasteurized sausages, soy sprouts and baked bread) spiked and incurred model foods with added soy proteins. Results obtained showed that the detection efficiency using sandwich assay was better than the competitive assay for samples subjected to pasteurization and baking process. Also, it was seen that β-conglycinin remained undetected in sterilized plate with any format. So, it was concluded that the heat processing method reduced the detect ability affecting the correct results. The advantages of the technique involved in-house validation, repeatability, reproducibility, robustness, good recovery rates (93.3 and 138.7%) (Segura-Gil et al., 2019).

One of the newer methods namely digital droplet Polymerase Chain Reaction (ddPCR) was developed in order to detect as well as quantify allergen present in soy in various food matrices. This technique involved the formation of a primer or probe system for the purpose of amplification of 140 base pair product of the ndhH gene of the chloroplast DNA. The primer so designed was specific for soy and did not give any reaction with even closely related plant species. The advantages of the method involved in-house validation, good accuracy, reliability, wide applicability to various food matrices including milk, flour, milk, meat products and fatty creams, good rates of recovery (60-100%) and high precision. The LOD and LOQ were found to be 0.16 mg/kg and 0.60 mg/kg respectively (Mayer et al., 2018).

PEANUT (*Arachis hypogea*)

Peanuts are rich source of protein (29%) and essential amino acids like threonine, leucine and lysine. It has high nutritional value but the presence of some proteins has an adverse effect on some sensitive individuals. The main allergen responsible

for adverse effects in sensitive individuals is Ara h 1 (vicilin). Other major allergen includes Ara h 2 (emerging peanut allergen) which may also cause allergenic reactions that may result in urticaria to fatal anaphylaxis. The roasting and processing techniques has no effect on the allergens are they remain in the foods (Koppelman et al., 2006).The best way is to avoid the exposure of inhalation and consumption of foods that may contain traces of peanut or peanut as an ingredient.

Detection methods: Mass spectrometry (MS) was employed for detecting allergens in peanut different food matrices. It included identification of biomarkers (peptides).The marker proteins (>300)were identified and filtered using selection criteria for enhancing the analysis which resulted in 16 peptides. The advantages of this technique were specificity, robustness and sensitivity (Gavage et al., 2019).

In another study, the immunological method i.e. ELISA was employed to find out the appropriate extraction buffer for detecting peanut allergen. Two ELISA kits were used namely Veratox for peanut allergen and peanut ELISA from Morinaga for the detection of peanut residues in heat treated commercial foods. Here, the efficiency of method for detecting allergens was improved by using alternative buffers. Also, recovery rates of peanut from unprocessed samples employing this kit were found to be(46 ± 5)%and(28 ± 2)% via Veratox and Morinaga kits, respectively. In addition, it was also found that the buffers used in the experiment helped in enhancing the recovery rates of the peanut allergen. On employing Veratox kit using buffers namely(Na_2CO_3, pH 9.6) and (0.01 M phosphate buffered saline (PBS) containing 1 M Guanidine hydrochloride (GuHCl)) gave recovery rates of(65 ± 4)%and(77 ± 10)% from unprocessed samples. The performance of these buffers was recorded to be better than the Veratox buffer may it be for high pressure processed, fried or baked samples. Also, the buffer of PBS containing sodium dodecyl sulphate (SDS)and β-mercaptoethanol (β-ME) proved to be a better alternative to the Morinaga buffer to detect the peanut allergen from unprocessed, boiled and fried samples (Jayasena et al., 2019).

A new emerging technology namely near-infrared hyperspectral imaging was investigated for detecting peanut flour in food matrices of wheat flour. The experiment involved the use of a Matched Subspace Detector (MSD) algorithm for detection of peanut adulteration at the pixel scale using the spectrum associated. Following this, a set of simulated data was thus generated and the design which provided best sensitivity was selected. Herein, in total 8 combinations of the two flours i.e. peanut flour and wheat flour were prepared (in the range of 0.02% and 20%)to judge the performances of the algorithm on the basis of results obtained by real hyperspectrum. Finally, the applicability of the observations was judged through the positions and numbers of the detected pixels followed by the validation of MSD algorithm design. It was concluded through the study that the peanut allergen can be detected with a LOD of 0.2% through the application of hyperspectral imaging and an MSD algorithm which is finely-tuned (Laborde et al., 2020).

WALNUTS (Juglans spp.)

Walnuts allergens include Jug r 1, Jug r 2, Jug r 3 and Jug r 4, Jug n 1, and Jug n 2 (Guo et al., 2020).

Detection methods: In a more recent investigation, bioinformatics was employed to find out the linear epitopes of walnut major allergen, Jug r 1. The mapping of these epitopes was performed through overlapping. The identification of the digestion-resistant proteins was conducted through in-vitro simulated gastrointestinal (GI) digestion and High Performance Liquid Chromatography-Mass Spectrometry (HPLC-MS/MS). This method had several advantages i.e. it proved to be an efficient, promising technology providing better results (Guo et al., 2020).

In a study conducted for detection of walnut allergen, Real time Polymerase Chain Reaction (RT-PCR) was employed. This technique showed many advantages including good specificity, sensitivity, relevancy, accuracy and robustness. In this method, the first step was the preparation and validation of novel primer sets on allergen-coding sequences of Jug r 1, Jug r 3, and Jug r 4.This was followed by DNA extraction which was based on CTAB (hexadecyl-trimethyl-ammonium bromide)-phenol-chloroform method. LOD of walnut DNA was2.5 pg. On spiking the food samples, the amplified Jug r 3 primers could be detected up to 100 mg/ kg of raw walnut. LOD and LOQ were found to be 0.01% and 0.05% respectively. It was also found out that high hydrostatic pressure (HHP) caused no interference with the DNA amplification. However, the combined effects of heat and pressure reduced the amplification as well yield of the walnut DNA. The advantages of this method over ELISA included higher sensitivity and specificity (Linacero et al., 2016).

CASHEW NUTS (*Anacardium occidentale*)

Main allergens responsible to cause allergy by consumption of cashew nuts include namely Ana o 1, Ana o 2 and Ana o 3 (He et al., 2020).

Detection methods: Sandwich ELISA assay was employed in a study to detect the presence of cashew allergen namely Ana o 3 (2S albumin)andalso was employed as protein marker. In this study, two sandwich ELISA i.e. CAS-ELISA-2 and Ano3-ELISA-1 were prepared and LODs for them were 0.04 and 0.06 mg protein/ kg respectively. This method finds manyadvantagessuch as high specificity along with negligible cross-reactivity with other allergen namely peanut, pecan, hazelnut, almond and pistachio. In case of model cookies samples, the Ano3–ELISA–1 assay gave 25.2–79.8% protein recovery as compared to 40.2–44.9% with the CAS–ELISA–2 assay. LOQs for both the assays were 40 mg protein/ kg in case of cookies. In contrast, in case of model chocolate, the recovery of proteins was calculated to be (74.0–87.7%) and (62.8–136.2%) for CAS–ELISA–2 and Ano3– ELISA–1 assay. Here, LOQs for both the assays were 4 mg protein/ kg. In other words, tests when performed on model cookies showed lower recovery of the cashew protein and in case of model chocolates showed more cashew protein recovery (Zhao et al., 2018).

PISTACHIO NUTS (*Pistacia vera*)

The main allergens found in pistachio nuts include namely: Pis v 1, Pis v 2, Pis v 3, Pis v 4, Pis v 5 (He et al., 2020).

Detection methods: A loop-mediated isothermal amplification (LAMP) assay was developed in a study. It was designed to target Pis V 1 allergen coding gene. This technique showed many advantages such as robustness, higher specificity for pistachio and no cross-reactivity with peanut, hazelnut, mango, cashew, walnut, almonds, walnut, macadamia nut and soybean. LOD was at least 10 pg of purified pistachio genomic DNA and 10 mg/kg of pistachio nuts in case of binary mixtures. Pistachio nut allergens are majorly detected by PCR (both conventional and real time) techniques. In a study, the two PCR techniques were employed namely SYBR Green and locked nucleic acid probes real time PCR. On comparing both the techniques, it was found that the latter one was more sensitive and better method out of the two. Also, the effects of heat and pressures were also studied via LNA probe real time PCR. It was concluded that on application of high heat and pressure, the amplification of pistachio allergens were reduced. In contrast, the similar effect after boiling was not observed. The tests were conducted on 14 food products and comparative analysis was conducted with commercial ELISA kit (Sanchiz et al., 2017).

HAZELNUTS (Corylus avellana)

The major allergens found in hazel nuts include Cor a 1, Cor a 2, Cora 8, Cor a 9 and Cora 11 (Akkerdaas et al., 2006).

Detection methods: High resonance mass spectrometry (HRMS) was employed for detecting and quantifying the hazelnut allergens. The advantages of the technique were good sensitivity, specificity, universality and of course, robustness. The first step was to select the biomarker peptides followed by their digestion and final analysis via HRMS. Here, in this experiment, 8biomarker peptides were obtained from 3 major hazelnut proteins and selected for detecting hazelnut allergenin processed foods (Van Vlierberghe et al.,2019).

CONCLUSION

Numerous trials are now under progress to conclude the most effective strategies for combating food allergies and tremendous efforts have been put in order to diagnose and manage the food allergies till date. New technologies are being developed for detecting the allergenic partin food commodities even at very low levels. One such technology is multiple-reaction monitoring (MRM) mass spectrometry (MS) coupled in AQUA peptides that detect the allergens at 10 ppb level. Some more recent techniques are underway. Moreover, various guidelines regarding the proper labelling of food allergens are provided to food manufacturers to follow and abide by them in order to safeguard the health of the consumers.

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