A Review of Naturally Occuring Food Allergens and Their Impact on Health

Renu Indhikkattu Chittoor and Harikumaran Thampi
Balakrishnan Saraswathi*

Department of Life Sciences, University of Calicut, Kerala (State), India.
http://dx.doi.org/10.13005/bbra/2965
(Received: 07 December 2021; accepted: 27 January 2022)

Allergic reactions to foods influence a remarkable amount of population and are related with significant wellbeing results. It is one of the most significant issues that have expanding consideration. Current comprehension of the turn of events and utilization of allergenic capability of certain types of food proteins is restricted. In spite of the fact that there is a selection of in vivo models for assessing hypersensitivity, none of the current models has been approved, is prescient, or generally acknowledged with respect to their allergen explicit inhibitors. Hence, there is a proceeded with enthusiasm on the knowledge recovery based on food allergy so as to give more enlightening way to the current research field. In this paper, the current status of purification, characterization, and types of food allergens and their impacts is thoroughly reviewed. The present available methods for the allergen assessment (in view of animal, cell and clinical methodologies) are emphasized.

Keywords: Characterization; Food Allergens; in vivo; in vitro; Mechanism.

Food sensitivity is a most common medical issue of both developed and developing countries causes critical anaphylactic reactions consequently leads to notable morbidity. It affects personal satisfaction and makes a substantial economic burden. The pace of food allergy differs by age, local diet, chemical nature of food and numerous other factors, and different quantities of clinical highlights impacts the common history of food hypersensitivity. Studies conducted in the western countries based on nut hypersensitivity, recommended that the prevalence rates have expanded, virtually duplicating, and outperform 1% in children those who are school aged. The report from Centres for Disease Control and Prevention indicated that, youth food sensitivity raised around 18% with the direct influence of 3.9% of youngsters are happens from 1997 to 2007. In westernized nations, harmful safe reactions to food items influence around 5% of young children and in case of grown-ups is around 3 to 4%, seem to have more predominance.

India is the second most crowded nation, having rate of population nearly 1.3 billion. Recent reports revealed that there are an expected 37.5 million instances of asthma and a rise in predominance of hypersensitive rhinitis. A food specific sensitization study conducted on children aged 7 - 10 years indicated that food allergy appears to be less common when compared with developed nations.
A survey conducted by EuroPrevall - INCO in Indian kids revealed, food hypersensitivity to be 0.14% among youngsters with rate of sensitization was high (around 19.1%) to food allergens. Considering the case of nut hypersensitivity, it was discovered to be low (around 0.03%) in Indian kids, although the sensitization rate was high at 6.3%. Therefore, further examination is expected to explore why predominance of clinically applicable food sensitivity is low in India in spite of high sensitisation rate.

**The function of food related proteins**

Hypersensitive responses to milk, egg, fish, peanut, shellfish, wheat, tree nuts and soy represent most important food sensitivities in the worldwide. Majority food allergens having atomic weight varying from 10 to 70 kDa and are water dissolvable glycoproteins moderately stable to acid, proteases, and temperature.

Furthermore, food preparation, and manner of food ingestion also affect allergenicity. The studies conducted on pea nut indicate that Millard reaction due to high temperature roasting (180 °C) and emulsification due to adjuvant effect are the example which describes how the food preparation methods affect the food allergenicity. The Meta-analysis study conducted on fruits and vegetables accounts that allergic reactions shifted from 0.1 - 1.4% for tree nuts, fruits about 0.1 - 4.3% and for vegetables under 1%. Food initiated hypersensitivity are normally connected with severe reactions. Certain foods like nuts and seafood activates anaphylactic reactions among children and adults. For example: In the case of young children, cow milk and egg are very common culprits. Common food allergies

**Milk allergy**

Milk allergy is common among paediatric population. Carlos & Hania summarizes the dairy animals’ milk sensitivity for its better analysis and risk management. The occurrence of cow’s milk sensitivity in kids living in the created world is roughly 2 to 3%, and lower predominance rate is only observed in breastfed new-born infants (0.5%). The significant allergens seen in bovines milk are belongs to casein fraction of proteins and whey proteins. The two fundamental systems which clarify unfavourably susceptible reaction of cow’s milk hypersensitivity just as other food sources are: IgE intervened and non-IgE interceded.

IgE mediated manifestation includes acute urticaria and angioedema. Non IgE mediated mechanism involves indications on epidermis and the digestive tract. At the level of digestive tract incorporates: bovine’s milk – promptedenterocolitis, bovine’s milk – incite denteropathy and bovine’s milk - initiated proctitis and proctocolitis. The indications may make before multi month mature enough, and as frequently as conceivable inside multi week after the associate of dairy creatures’ milk proteins with their eating routine.

In case of non-IgE mediated response; symptoms are delayed and typically happen in no time and up to 2 h of ingestion. The epidermis is
normally included and followed by gastrointestinal plot and, marginally the respiratory and additionally cardiovascular frameworks. The seriousness related with the responses may fluctuate from mild to moderate, and sometimes leads to critical anaphylaxis. The diagnosis of cow’s milk allergy includes certain routine work-up like skin prick tests (SPTs), direct IgE (sIgE) estimations, and feeding tests. 

Egg allergy
Egg allergy most commonly IgE-mediated, and is seen in childhood. It has been reported that prevalence rate is of 1.3 to 10.1%. This may develops in the main year of life creating, and it is the second most basic reason for food sensitivities in kids. Egg white contains in excess of 20 distinct glycoproteins and proteins. The significant allergens seen in hen’s egg are Ovomucoid (Gal d 1), ovalbumin (Gal d 2), conalbumin (Gal d 3), and lysozyme (Gal d 4). The principle allergen found in egg yolk is Alpha - livetin (Gal d 5). Clinical responses are basically IgE and non - IgE interceded or a combination of the two kinds. The diagnosis of egg sensitivity mainly depending on history taking, in vitro allergen-explicit blood IgE tests, skin prick test, histamine discharge test, and feeding tests. Among this, SPT shows a decent affectability yet a helpless particularity. Currently, oral immunotherapy (OIT) provides better results to egg allergy. It had mild to severe side effects. Additionally, there is a need to develop safer OIT for novel modified food antigens.

Peanut allergy
Peanut allergy affects 1 to 4.5% of children, with severe reactions. reviewed about certain peanut allergens. Bicupin seed storage proteins contain Ara h 1 and Ara h 3. Ara h 2, Ara h 6, and Ara h 7 are seed stockpiling proteins, which are included in the prolamin superfamily. Ara h 5 is said to be an individual from the profilin family.

Diagnostic investigations of nut allergens incorporate skin prick tests, nut explicit IgE (sIgE), and absolute IgE. Thermal processing like boiling, frying, or roasting appears to have impact on event of nut hypersensitivity. Among these cooking methods, boiling of peanuts has lesser prevalence. Recently, researchers have mainly focussed on the deep immunological characterization of nut allergens and the utilization of decontaminated allergens symptomatic field. Kelly Bruton & his colleagues were discussed about the current state of oral immune therapy in the field of peanut allergy.

Tree nut hypersensitivity
Tree nut hypersensitivity predominantly creates by age of 2 years, and the sensitization to allergen exposure naturally increases with age. Most of proteins engaged with tree nut hypersensitivity have a place with protein groups of vicilins, nsLTPs, 2S albumins, and legumins. Of these, dust related tree nut hypersensitivity mediated by Bet v 1 - homologues and profilins. Moreover, oleosins and thaumatin - like proteins were demonstrated to be noteworthy allergens. Andrzej Kuźmiński & his colleague’s reviewed about the different nut allergies. Individual tree nut components and their allergic factors are given in table 2.

The analysis of tree nut sensitivity is performed with detailed clinical history, skin prick testing, serum - explicit IgE, and oral food challenges (OFCs). Dietary management is the only prevention method recommended for tree nut allergy.

Soy allergy
Soy allergy typically happens in the beginning of early stage; with announced pinnacle rate of soy sharpening occur in the age of 2 years. Generally, soy sensitivity is not common as other food hypersensitivities.

International Union of Immunological Societies Allergen Sub - Committee acknowledged birch dust - related proteins, soybean hull proteins (Gly m 1 and Gly m 2), a profiling (Gly m 3), and a PR-10 protein (Gly m 4), are the significant allergenic proteins found in soy. Additionally, certain storage proteins b-conglycinin and glycinin (Gly m 5 and Gly m 6), the thiol protease (Gly m Bd 30 k), the 2S albumin soy protein, and the soybean Kunitz trypsin inhibitor are also have been described. Patients suffering from soy sensitivity present a scope of clinical disorder like IgE - interceded and non - IgE - intervened. Kattan et al., discussed briefly about the clinical manifestation behind soy allergy. The finding of soy sensitivity
is mainly involves by taking the clinical history and allergy testing. Now a days, oral food challenge and oral immunotherapy are the methods used in the field of management of soy allergy.

**Wheat allergy**

Wheat allergy is very common in paediatric population. The prevalence rate of wheat allergy differs based on age and region. Wheat allergens are belonging to the grass family *Poaceae* and contain numerous allergenic proteins, which are partitioned in four classes dependent on the extraction solvents. American chemist T. B. Osborne standardized this classification.

Important wheat allergens responsible for wheat allergy are given below (Table 3):

The clinical sign of wheat hypersensitivity relies upon the course of allergen introduction. Usually, it is liable for IgE - intervened responses, with the event of urticaria, angioedema, bronchial impediment, nausea, stomach torment, or fundamental hypersensitivity. Non –IgE- intervened indication incorporates food protein- incited enterocolitis condition. The prognosis involves SPT and serum IgE tests. The current management of patients suffering from wheat sensitivity is dietary shirking. Recently, feeding tests has been announced for wheat hypersensitivity with positive outcomes, regardless of further investigations are requires for building up the best convention so as to advance resistance in wheat - allergic youngsters.

**Meat allergy**

Meat allergy is varied according to region basis, and is accompanied with local dietary habits and environmental factors. This is mainly occurs during the primary long stretches of life, therefore it is uncommon in grown-ups. The major allergens found in meat are serum albums and immunoglobulin. Important allergens seen in meat are listed below (Table 4).

Several studies shown that activity of this antigenic peptide can be destructed by domestic cooking and some other technological treatments. Certain treatments like warming can adjust or inactivate the epitopic spaces liable for the IgE holding by changing the conformation of the epitopes; or by the denaturation of the three dimensional structure of a protein can create new epitopes and improve the availability of the allergenic determinant to explicit antibodies. Likewise, freeze-drying, homogenization and mechanical cycles are generally employed to meat during the creation of infant nourishments, can altogether diminish the allergic capability of beef.

Cooking methods frequently destroys heat-sensitive allergens. Conversely, Fiocchi et al., shown that a couple of patients will regardless react to cooked nourishments. Apart from this, researchers reported that allergenic potential of the antigen can be tested through cross reactions, and this can be used in the diagnostic field.

**Fruit allergy**

Literature reports confirmed that the prevalence rate of fruit allergy was ~5-8% children and 2 – 3% adults. Mostly 12 - 15 fruits are associated with fruit allergy, commonly market available fruits and vegetables. Fruit allergic reactions are in relation with oral allergy syndrome (OAS) together with pollen - fruit-vegetable syndrome.

**Classification, mechanism of pathogenesis, and clinical features of food allergy**

The epidemic of food sensitivity has expanded over the previous decade. Despite the fact that the innermost processes involved in food related sensitive responses are cleared, yet the ideal molecular events food allergen - instigated hypersensitive showings are not definitely perceived. Recently, researchers evaluated the food allergens mainly through bioinformatics examination, and clinical studies like model tests, cell model creature tests, serological examination, and mimicked gastric assimilation. The studies revealed that using of cell models is more convenient and flexible method instead of using animal models. Yet, in vivo test is the most immediate and instructive strategy to assess the possible sensitivity of the food item. Sampson et al. provide a detailed review of mechanism of food allergy. They described the factors which effects tissue and immune responses to food antigens, the recent findings with respect to the advancement of invulnerable resilience, the role microbiota in the GI tract and the immunologic response of desensitization system.

Food hypersensitivity is generally IgE - mediated type I hypersensitivity, non - IgE-mediated, and mixed type.
IgE mediated

When the food allergens initially enter the body, the initiated cells were emitted interleukin (IL)-4,5,13, and different cytokines which prompt antibody (IgE) creation in B cells. The communication between IgE and the objective cells (granulocyte cells, basophil, and mast cells) leads the body overly sensitive. Again a similar allergen enters the body once more, it communicates with the IgE molecules on the objective cells mediates the spanning response, activating the underlying pathway, brings about the degranulation of target cells, and delivers the mediators like 5-hydroxytryptamine, histamine, and leukotriene. This leads to unfavorably susceptible response. This enhances the permeability of intestinal epithelial cells to the allergenic protein, and accordingly influencing the pathway of specific cytokines, and creating a Th2-type provocative reaction. This is the mechanism behind IgE-mediated food allergy.

Mixed type food allergies

This class of food hypersensitivity is intervened by both IgE-dependent and IgE-free pathways. This is delayed type, and the allergy-associated atopic dermatitis seen after 6–48 hours. This is caused by T helper 2 cells. Apart from this eosinophilic gastrointestinal disorders like eosinophilicosesophagitis (EoE), brought about by the eosinophilic penetration of tissues, further examination is required in this sort of food sensitivity.

Non-IgE-mediated food sensitivity

Mostly Non-IgE-interceded hypersensitivities principally affects digestive tract, as opposed to the skin and respiratory system. At this moment, the framework behind the food sensitivity is very clear and we show it in the figure 2. Major features associated with both type of mechanism are given in the table 6.

Coombs and Gells proposed four types of allergy induction pathways by considering the system of pathogenesis.

It isn’t surely known to what extent the mechanism behind food allergy; hence there is a need to discuss about the extraction and purification of food allergens from various food sources for the detection and diagnosis of its allergenicity. In this communication, the current status of purification and characterization of food allergens and evaluation of food hypersensitivity is briefly reviewed, and the present available methods for the allergen assessment (in light of creature, cell and clinical methodologies) are listed. In addition to this, we presented some related examination on food allergen specific inhibitors, and want to have some motivation in the field of food hypersensitivity.

Plant and animal food allergens

Normally, all food allergens are proteins, yet not all food proteins are allergens. There are in excess of 170 nourishments have been accounted to had hypersensitive responses. Of these, the most widely recognized foods with investigated food sensitivities are peanuts, soyabees, wheat, fish, eggs, crustacea, tree nuts, and dairy animals’ milk. To assess the relative allergenicity of novel proteins, it is fundamental to utilize the regular food allergens as reference proteins like cereals containing gluten, crustaceans, eggs, pea nut, soya, milk etc. The main reason behind the advancement of new food hypersensitivities is to the introduction of new proteins into the eating regimen. Hence, it is needed to elucidate the development and allergenic capability of existing and new food proteins (plants as well as animal sources) for evaluating their safety assessment on clinical studies. Current comprehension of this is deficient and has set number of prescient strategies dependent on in vivo examination of food allergens, and in vitro techniques. Thus, there is a proceeded with enthusiasm for the improvement of appropriate cell models as well as animal models that give an essential way to deal with the appraisal of hypersensitive capability of proteins.

Extraction, purification, and characterization of food allergens

Dietary inclinations for the noteworthy people in an area accept a critical part in the improvement of food hypersensitivity. That is, increased utilization of certain foods may prompt increased sensitization to it, e.g., sensitivity to soy in Japan, nut hypersensitivity in the France, UK, and North America and sesame sensitivity in Israel. Evidences of IgE-mediated hypersensitive responses incited by legumes in Asian and Mediterranean nations have been accounted as legumes are the major source of dietary protein in these nations.
Astwood et al. described different types of extraction methods for allergen proteins from various plant sources. Phosphate buffer of different salt concentrations at pH 7.0 were used for the extraction. With the improvement of molecular biology, genomics, and immunology, numerous allergens and allergenic sources are presently revealed from various sources. Ramkrashan Kasera et al. were purified the kidney bean allergen using anion exchanger Q sepharose column. Its characterization was done with immunochemical methods, and the results showed that the molecular weight of the purified protein was found to be 31 kDa. Pastorello & Tramabaioli reviewed concerning to the field of isolation of allergens from various food sources.

Usually food allergens have been distinguished by immunetests, utilizing serum tests from unfavorably susceptible patients. Enzyme-linked immunosorbent assay (ELISA) was the basic strategy utilized for their detection. Currently, there are mainly three methods accessible for the detection and measurement of food allergen, namely: DNA based techniques, protein based methods and cell based methods. In addition, these methods can likewise be utilized in food sensitivity diagnostics.

- DNA based strategy: Methods for estimating allergen coding genes (e.g.: PCR).
- Protein based strategy: Methods for measuring allergenic protein levels (e.g.: ELISA, Protein microarray, Mass spectrometric methods).
- Cell based strategy (otherwise called basophil activation test (BAT)): Methods for measuring effector cell activation levels.

Literature reports underlined that there is a correlation between quantitative and subjective advancement of allergen extraction and the protein recovery. Hocine et al. constructed a D-optimal plan to advance allergen extraction proficiency at the same time from non-roasted, roasted, non-defatted and defatted pea nut, almond, pistachio flours and hazel nut utilizing three denaturing aqueous buffers at different states of, buffer - to - protein ratio, ionic strength, extraction time duration and temperature. Statistical examination demonstrated that the protein recovery varied by depending on the extraction condition with respect to the samples.

Currently, there is also an improved procedure for the extraction of allergenic proteins in fish was developed. The reports revealed that parvalbumin was the major allergen found in various fish species, cause 95% of patients allergic to fish. Now day, at least 7 different allergens from shellfish have been identified, mostly from crustaceans.

The method of purification and characterization of food allergens is an important criterion in diagnostic field. Commonly, chromatographic techniques are used for their purification like gel-filtration, ion-exchange, and affinity chromatography. Barbara Cases et al. explained with respect to the chromatographic columns, packing materials, buffers, and methods for extract preparation. Clare Mills et al. clarified the analytical tools used in food hypersensitivity for the identification and management of numerous food allergens in the food sources. This approach mainly focussed on the quantitative MS-based method.

Immunoelectrophoresis was the major technique used for the identification of individual allergens from the sample extracts. This can be used to analyze single protein from a complex mixture. The basic disadvantage of this technique is the dependence on rabbit antisera as reagents. Tandem mass spectrometry can also be used for the identification of purified allergen.

Natalia Gasilova and Hubert H Girault summarized the recent advances in food hypersensitivity determination, allergen identification from food items as well as disclosure of the latest allergenic particles via in vitro methods. The review pointed on the new symptomatic strategies under lab advancement includes various aptamer and immune based tests. Furthermore, combination of allergonomic techniques with peptide sequencing by the Edman degradation is the powerful apparatuses for the revelation and study of new allergens.

In vitro studies

Cell models are mainly defined to explain the molecular basis of an illness and to describe the cell pathways included. These can be taken as a tool for recognizable proof of medication targets, and for the screening of inherited, environmental or pharmacological threat factors related with the infections. In line with this, there are various cell models have been introduced to explain the
allergenicity and sensitization processes, including human basophil granulocyte models, mast cell models, and so forth.

Literature reviews revealed that the most ordinarily utilized cell line assigned as RBL-2H3, is commonly considered as mast cell line. Particularly, these cell lines are mainly used for the investigations on binding of IgE to receptors (i.e., FcERI) and further underlying events\(^\text{12,13}\). Mast cells and basophils are the two distinct cell

| Table 1. List of known food allergens and their classification |
|-------------------------------------------------------------|
| **S No.** | **Food source** | **Identified allergens** | **Allergen family** | **Molecular weight (kDa)** |
| 1 | Peanut | Ara h 1 | Cupin superfamily | 64 |
| | | Ara h 2 | Prolamin superfamily | 17 |
| | | Ara h 3 | Cupin superfamily | 60.37 |
| | | Ara h 4 | Cupin superfamily | 60 |
| | | Ara h 5 | Profilin family | 15 |
| | | Ara h 6 | Prolamin superfamily | 15 |
| | | Ara h 7 | Prolamin superfamily | 15 |
| | | Ara h 8 | Bet v 1-related protein family | 17 |
| | | Ara h 9 | Prolamin superfamily | 11 |
| | | Ara h 10 | Olcosin family | 16 |
| | | Ara h 11 | Olcosin family | 14 |
| 2 | Soybean | Gly m 5 | Cupin superfamily | 140–180 |
| | | Gly m 6 | Cupin superfamily | 320–360 |
| | | Gly m Bd 28K | Cupin superfamily | 26 |
| | | Gly m Bd 30K | Papaya proteinase superfamily | 30–34 |
| | | Gly m T1 | Kunitz soybean trypsin inhibitor family | 20 |
| 3 | Egg | Gal d 1 | Kazal-type serine protease inhibitor family | 28 |
| | | Gal d 2 | Serpin serine protease inhibitor family | 44.5 |
| | | Gal d 3 | Transferrin family | 76 |
| | | Gal d 4 | Bet v 1-related protein family | 14.3 |
| 4 | Crustacea | Pen a 1 | Tropomyosin family | 36 |
| 5 | Milk | Bos d 4 | C-type lysozyme/alpha-lactalbumin family | 14.2 |
| | | Bos d 5 | Lipocalin family | 18.3 |
| | | Bos d 8 | 20–30 |
| 6 | Nut | Jug r 1 | Prolamin superfamily | 15–16 |
| | | Jug r 2 | Cupin superfamily | 44 |
| | | Jug r 3 | Prolamin superfamily | 9 |
| | | Jug n 1 | Prolamin superfamily | 19 |
| | | Jug n 2 | Cupin superfamily | 56 |
| | | Ana o 1 | Cupin superfamily | 50 |
| | | Ana o 2 | Cupin superfamily | 55 |
| | | Ana o 3 | Prolamin superfamily | 14 |
| | | 2S albumins | prolamin superfamily | 15 |
| | | NsLTP | prolamin superfamily | 6.038–9.922 |
| | | Vicilins | cupin superfamily | 150–190 |
| | | legumins | cupin superfamily | 150–190 |
| | | Oleosins | oleosin superfamily | ≤15–26 |
| 7 | Wheat | Tri a gliadin | Prolamin superfamily | 30–80 |
| | | Tri a 26 | Prolamin superfamily | 80–120 |
| | | Tri a 36 | Prolamin superfamily | 30–75 |
| 8 | Fish | Parvalbumin | EF hand domain family | 12 |

(Abbreviation used:Ara h - Arachis hypogaea, Gly m - Glycine max, Gal d - Gallus domesticus, Pen a - Peneaus aztecus, Bos d - Bos domesticus, Jug r - Juglansregia, Jug n - Juglansnigra, Ana o – Anacardium occidentale, NsLTP - Non-specific lipid transfer proteins, Tri a - Triticum aestivum)
types that assume vital roles in inception and advancement of type I hypersensitivity responses. Granulocyte basophils represent less than 1 % of the WBC population, while developed mast cells can be seen in tissues and specifically situated at interfaces with the outer environment like skin, lungs, and mucosal surfaces\textsuperscript{114}.

Eccleston and co-workers were found Rat basophilic cells in 1973, when one of the rats was treated with strong cancer-causing agent (2-(a-chlor-b-isopropylamine)ethyl-naphthalene), an uncommon type of granulocytic leukemia marked by a significant peripheral blood basophilia\textsuperscript{115}. It is the usually utilized histamine-releasing cell line in immunological exploration, inflammation, and hypersensitivity. It has been extensively reviewed that the behavior of cells in regard of their reaction to immunological boosts.

RBL-2H3 cells, similar to basophils and mast cells, react with degranulation by cross linking of their IgE-bound Fc\(\varepsilon\)RI by multivalent allergens, with the appearance of a preformed and newly synthesized mediators that brings a powerful immune allergic responses\textsuperscript{116}.EglePassante and Neil

| Tree nut | Allergic component | Protein family | Protein type |
|----------|-------------------|----------------|-------------|
| Hazelnut | Corylus avellana  | Pan-allergens  | PR-10       |
|          | Cor a 1           |                |             |
|          | Cor a 2           |                | Profilin    |
|          | Cor a 8           | Storage proteins | LTP       |
|          | Cor a 9           |                | 11S globulin|
|          | Cor a 11          |                | 7S globulin |
|          | Cor a 14          |                | 2S albumin  |
| Cashew   | Anacardium occidentale | Storage proteins | 7S globulin |
|          | Ana o 1           |                |             |
|          | Ana o 2           |                | 11S globulin|
|          | Ana o 3           |                | 2S albumin  |
| Pistachio| Pistacia vera     | Storage proteins | 2S albumin |
|          | Pis v 1           |                |             |
|          | Pis v 2           |                | 11S globulin|
|          | Pis v 3           |                | 7S globulin |
|          | Pis v 5           |                | 11S globulin|
| Walnut   | Juglans regia     | Storage proteins | 2S albumin |
|          | Jug r 1           |                |             |
|          | Jug r 2           |                | 7S globulin |
|          | Jug r 4           | Pan-allergens  | 11S globulin|
|          | Jug r 3           |                | LTP         |
|          | Jug r 5           |                | Profilin    |
| Pecan    | Carya illinoensis | Storage proteins | 2S albumin |
|          | Car i 1           |                |             |
|          | Car i 2           |                | 7S globulin |
|          | Car i 4           |                | 11S globulin|
| Almond   | Prunus dulcis     | Storage proteins | 11S globulin|
|          | Pru du 6          |                |             |
|          | Pru du 3          | Pan-allergens  | LTP         |
|          | Pru du 4          |                | Profilin    |
| Pine nut | Pinus pinea       | Storage proteins | 2S albumin |
|          | Pin p 1           |                |             |
| Brazil nut| Bertholletia excelsa | Storage proteins | 2S albumin |
|          | Ber e 1           |                |             |
|          | Ber e 2           |                | 11S globulin|

Abbreviation used: LTP, lipid transfer protein.
Table 3. Important wheat allergens

| S. No. | Tri a component | Allergen name          |
|-------|----------------|------------------------|
| 1     | Tri a 12       | Profilin               |
| 2     | Tri a 14       | Lipid Transfer Proteins|
| 3     | Tri a 15       | alpha-Amylase Inhibitors|
| 4     | Tri a 18       | Agglutins              |
| 5     | Tri a 19       | omega-5 gliadins       |
| 6     | Tri a 20       | gamma-gliadins         |
| 7     | Tri a 21       | alpha-beta-gliadins    |
| 8     | Tri a 25       | Thioredoxin            |
| 9     | Tri a 26       | Glutenins              |
| 10    | Tri a 27       | Thioreductase          |
| 11    | Tri a 28       | alpha-amylose Inhibitor|
| 12    | Tri a 29       | alpha-amylose Inhibitor|
| 13    | Tri a 30       | alpha-amylose Inhibitor|
| 14    | Tri a 31       | Triosephosphateisomerases|
| 15    | Tri a 32       | Peroxiredoxines        |
| 16    | Tri a 33       | Trypsin Inhibitors     |
| 17    | Tri a 34       | Glyceraldehyde-3-phosphate dehydrogenases|
| 18    | Tri a 35       | Dehydrins              |
| 19    | Tri a 36       | Glutenins              |
| 20    | Tri a 37       | Thionins               |

Table 4. Common allergens found in meat

| Species         | Allergen component | Molecular weight (kDa) |
|-----------------|--------------------|------------------------|
| Domestic cattle | Bos d 6            | 67                     |
|                 | Bos d 7            | 160                    |
| Chicken         | Gal d 5            | 69                     |
| Dog             | Can f 3            | 69                     |
| Guinea pig      | Cav p 4            | 66                     |
| Domestic horse  | Equ c 3            | 67                     |
| Cat             | Fel d 2            | 69                     |
| Domestic pig    | Sus s 1            | 60                     |

(Abbreviation used: Tri a - Triticum aestivum)
Table 5. List of allergens found in fruits

| S No. | Fruits                          | Allergen                                | Molecular weight (kDa) |
|-------|---------------------------------|-----------------------------------------|------------------------|
| 1     | Apple (Malus domestica)         | TLP (Mal d 2)                           | 23                     |
|       |                                 | Bet v 1 homolog (Mal d 1)               | 17.5                   |
|       |                                 | Profilin (Mal d 4)                      | 14                     |
|       |                                 | LTP (Mal d 3)                           | 9                      |
| 2     | Peach (Prunus persica)          | TLP (Pru p 2)                           | 23                     |
|       |                                 | Bet v 1 homolog (Pru p 1)               | 17.5                   |
|       |                                 | Profilin (Pru p 4)                      | 14                     |
|       |                                 | LTP (Pru p 3)                           | 9                      |
| 3     | Musk melon (Cucumis melo)       | serine protease (Cuc m 1.01)            | 67                     |
|       |                                 | serine protease (Cuc m 1.02)            | 54                     |
|       |                                 | serine protease (Cuc m 1.03)            | 36                     |
|       |                                 | PR-1 protein (Cuc m 3)                  | 16                     |
|       |                                 | Profilin (Cuc m 2)                      | 14                     |
| 4     | Gold kiwi, green kiwi (Actiniadiachinensis, A. deliciosa) | Chitinase (Act d 3) | 43                     |
|       |                                 | Actinidin (Act d 1)                     | 30                     |
|       |                                 | Kiwelling (Act d 5)                     | 28                     |
|       |                                 | TLP (Act d 2)                           | 23                     |
|       |                                 | Cystatin (Act d 4)                      | 11                     |
| 5     | Sweet cherry (Prunus avium)     | TLP (Pruav 2)                           | 23                     |
|       |                                 | Bet v 1 homolog (Pruav 1)               | 17.5                   |
|       |                                 | Profilin (Pruav 4)                      | 14                     |
|       |                                 | LTP (Pruav 3)                           | 9                      |
| 6     | Grape (Vitis vinifera)          | Chitinase, hevein-like (Vit v 5)        | 30                     |
|       |                                 | TLP (Vit v TLP)                         | 23                     |
|       |                                 | Bet v 1 homolog (Vit v 8)               | 17.5                   |
|       |                                 | Profilin (Vit v 4)                      | 14                     |
|       |                                 | LTP (Vit v 1)                           | 9                      |
| 7     | Strawberry (Fragaria ananassa)  | Bet v 6 homolog (isoflavonereductase)   | 35                     |
|       |                                 | Bet v 1 homolog (Fra a 1)               | 17.5                   |
|       |                                 | Profilin (Fra a 4)                      | 14                     |
|       |                                 | LTP (Fra a 3)                           | 9                      |
| 8     | Banana (Musa acuminata)         | 3-1,3-glucanase (Mus a 5)               | 33                     |
|       |                                 | Class I chitinase (Mus a 2)             | 31                     |
|       |                                 | TLP (Mus a 4)                           | 21                     |
| 9     | Custard apple (Annonachermola)  | Class I chitinase (Ann c Chitinase)     | 45                     |
| 10    | Mango (Anacardium occidentale)  | Bet v 1-like (Man i 14kD)               | 14                     |
|       |                                 | Class I chitinase (Man i Chitinase)     | 30-45                  |
| 11    | Pomegranate (Punica granatum)   | PR-4 protein (Barwin family)            | 28                     |
|       |                                 | PR-4 protein                            | 17                     |
|       |                                 | PR-4 protein                            | 16                     |
|       |                                 | LTP (Pun g 1)                           | 9                      |

Abbreviations used: LTP - lipid transfer protein; PR - pathogenesis-related; TLP - thaumatin-like protein.
compared to larger models\textsuperscript{124, 125}. Literature reports suggest that different murine species have the ability to deliver IgE and additionally IgG1 anaphylactic antibodies\textsuperscript{126}. One of the challenges involved in developing murine models of food sensitivity is the propensity of the immune system to form oral tolerance to ingested antigens\textsuperscript{127, 128}. As an option in contrast to mice Brown Norway (BN) had moderate size, and strong IgE antibody responses, therefore, the screening of serum specific antibody reactions inside individual animals can be done effectively. Compared with mice, another advantage of this model is that test antigen without adjuvant can be conveyed day by day over a time of weeks\textsuperscript{129}. Alternatively dogs are spontaneously susceptible to sensitivities, when compared with murine models, and it is a decent animal variety for assessing food hypersensitivity. In any case, it is difficult to utilize dogs as model for safety assessment. Moreover, their maintenance is expensive, predetermined number of strains, more noteworthy inter-animal concerning rodents, and lack of availability of immunological reagents. Similarly, this requires long dosing time for sensitization\textsuperscript{130, 130}. Published reports suggest that there is a momentous advancement with the use of animal models has been made a better comprehension of

| Factors                  | IgE mediated            | Non - IgE mediated         |
|--------------------------|-------------------------|----------------------------|
| Time of exposure to allergen | Minutes to 2 hr         | Several hours to days      |
| Intensity                | Mild to anaphylaxis      | Mild to moderate           |
| Time span                | May continue beyond 1 year of age | Usually resolved by 1 year |
| Detection                | Specific serum IgE, skin prick test (SPT) | Oral challenge |

Fig. 1. Pathogenesis and clinical manifestation of food allergy
(Source: https://www.google.com/url?sa=i&url=https%3A%2F%2Fwww.nature.com%2Fartic les%2Ffmdp201798 &psig=AOvVaw09X1bBRkd02tha1Xb0CAPG&ust=1597914508945000&source=images&cd=vfe&ved =0CAIQjRxqFwoTC Oisw7H1plusCFQA AAAAdAAAAABAx)
the essential mechanisms of food hypersensitivity and the allergen refinement. Considering the current state of food allergy animal model, a single model cannot be satisfactory for all research purposes. The objectives, experiment design, data interpretation are the factors which determine the selection of animal model for experimental purposes. For example, murine models are acceptable for determining the food allergy mechanism, alternatively, swine or dog is acceptable for the studies with a focus on clinical symptoms of allergic disease.

**Food allergy assessment based on clinical trials**

Now days, food allergy assessment have mainly pointed on the better diagnosis and treatment of reactions. Stefano Luccioli reviewed the guidelines for food allergy assessment and its treatment based on clinical aspects. His review suggested that, it is necessary to use a better tool for the management of food allergenicity in order to reduce the risk in human beings.

Similarly, F.Estelle. R. et al. summarize the key points, which are underlying in the anaphylactic management of food allergy. Based on research articles, they validated the clinical strategies involved in food induced anaphylaxis. The proposed review documented that, there is a need to develop a method for allergen specific inhibitors, which will help to reduce the clinical risk.

**Table 7. Disorders associated with food allergy**

| Types                        | Gastrointestinal                                      | Cutaneous                                      | Respiratory                                      |
|------------------------------|--------------------------------------------------------|------------------------------------------------|-------------------------------------------------|
| IgE mediated                 | Oral allergy syndrome, gastrointestinal anaphylaxis    | Urticaria, angioedema, morbilliform rashes and Flushing | Acute rhinoconjunctivitis, bronchospasm (wheezing) |
| Mixed IgE and cell mediated  | Allergic eosinophilic esophagitis, allergic eosinophilic gastroenteritis, food protein induced enterocolitis, food Protein induced proctocolitis, food Protein induced enteropathy syndromes, celiac disease | Atopic dermatitis, Contact dermatitis, dermatitis herpetiformis | Asthma, Food-induced pulmonary hemosiderosis (Heiner syndrome) |

**Fig. 2. System of food sensitivity development**

Antigen → Cells activated → B cell induces specific IgE → IgE interacts with mast cells, basophils, Degranulation → IL 4, IL5, IL13+Cytokines production
of food allergenicity, and hence its management can be done easily\textsuperscript{135}. 

\textit{ThaKarrin Hoffman et al.} studied the applications of molecular tools in food allergy. They validated allergen specific tests associated with food allergy, which will enable easy diagnosis.

- Skin prick testing (SPK)
- Serum IgEtesting
- Basophil activation test
- Atopy patch testing (APT)
- Immunotherapy

### Table 8. Different types of extraction methods used in common food sources

| No. | Food source                  | Method of extraction                                                                                      | References                                      |
|-----|------------------------------|------------------------------------------------------------------------------------------------------------|-------------------------------------------------|
| 1   | Fruits & vegetable sources   | 10 mmol/l potassium phosphate buffer (pH 7.0) + 2\% polyvinylpolypyrrollidone + 2 mmol/l EDTA + 10 mmol/l sodium diethyldithiocarbamate (DIECA) + 3 mmol/l sodium azide (NaN\textsubscript{3}) | Bjorksten et al.\textsuperscript{95}, Vassilopoulou et al.\textsuperscript{96} |
| 2   | Cereals                      | 01 M Ammonium Hydrogen Carbonate (AHC) (pH 7-65)                                                         | Shewry et al.\textsuperscript{97}, Qi et al.\textsuperscript{98}, Sandiford et al.\textsuperscript{99} |
| 3   | Raw & cooked fish            | Phosphate buffered saline (PBS) (pH 8)                                                                   | Crespo et al.\textsuperscript{100}, Pastorello&Trambaioli	extsuperscript{94} |
| 4   | Egg                          | Physiological saline solution                                                                           | Langeland\textsuperscript{101} |
| 5   | Milk                         | Precipitation at pH 4.65 + titration with 1 M HCl + precipitated fraction is to be dissolved in water at pH 7 by slow addition of 1 M NH\textsubscript{4}OH. | Makinen&Sorva\textsuperscript{102}, Pastorello&Trambaioli\textsuperscript{94} |

\textbf{Fig. 3.} General strategies involved in the purification and characterization of allergens from natural sources
Skin prick test (SPK)

Skin prick testing (SPK) widely used in plant food allergens (fresh fruits, vegetables, and nuts). In line with, several studies have been performed on the plant sources. S. Bolhar et al. carried out these test with apple extracts, and the results showed that, this method is highly reproducible and reliable for assessing food allergy. Likewise, Garcia et al. carried out SPKs in peach extracts. Peeters and his coworker’s isolated natural allergens from pea nut are Ara h 1, Ara h 2, Ara h 3, and Ara h 6, and are applied in SPK. Recently, Kollman et al. practiced a purified recombinant Bet v 1, Mal d 1 (birch dust allergen) and birch pollen extract (BPE) in skin prick testing. Literature reviews reported that SPK is an easy and reproducible method for the assessment of plan food allergens.

Serum IgE testing

Serum IgE testing is the currently used, extract based strategy, and this is an in vitro testing for specific IgE antibodies. Research articles shows that this may be a predictive method due to the advanced knowledge and increased applications in molecular biology. Basophil activation tests (BATs)

This test utilizes entire blood to recognize the capacity of allergens to activate basophils. To distinguish the expression of basophil activation markers (CD63, CD203c) after stimulation with allergens, flow cytometry are used. This reflects cell degranulation, which confirms that CD63 is a key biomarker for hypersensitive responses. Furthermore, this test can also be used in immunotherapy to reflect the desensitized condition of the person.

Others

Likewise, cell tests are accessible to get out the presence of patients with antigen specific IgE sera, and their ability to enact basophils. The test is mainly centered on the measurement of cell activation accompanied with determination of histamine or sulfidoleukotriene release. Atopy patch testing is rarely used in clinical studies.

Ru Xin Foong and co - workers led a pilot study to assess the clinical identification of sensitivity in atopic children using a microarray technique in addition with SPK and serum IgE testing. Their findings showed that serum specific IgE testing produces more positive results rather than others. Consequently, the risk of allergic reactions cannot be effectively managed without understanding the associated risk factors. This can be reduced through the confined diets.

Keet et al. proposed another theory that how to forestall food hypersensitivity in most...
ideal manner. They explored the advances in food sensitivity that was distributed in 2017 and beyond. The review highlights the published standardized treatment for food hypersensitivity. Finally, concluding that there was an increased attention to non-IgE-mediated food sensitivities. Impacts of food preparing techniques on food allergens

Currently, food exposed to various kinds of processing treatments like conventional methods to enhance the shelf life of the food, by inactivating the toxins. It has been mentioned that the processing of foods can also alter the stability of allergenic compounds. This is mainly due to the alteration of amino acids or small linear stretch that occurs in the epitopes. Now days, the clinical symptoms associated with the nature of allergenic epitopes and its severity is not well clear. By understanding these features, the targeted allergen can easily be eliminated.

Food processing affects physical, chemical, and biochemical changes of food, which leads to the alteration of different parts including protein and allergenicity of the particular protein epitope. During this process, new epitopes are formed, referred to as 'neoallergens'.

Conventional food processing methods are classified mainly into two types: thermal and non-thermal (figure: 4) Sathe et al., and vanga et al., explained the food processing methods and the effects of allergens during these processes.

Evidently, food processing aids to inactivate or removes the epitopes present on allergens. Based on the quality and acceptability of food products, enzymatic hydrolysis results in unwanted changes in the food structure. Consequently, to develop robust, dependable, sensitive, and exact allergen identification techniques is the crucial thing in the field of food sensitivity.

Modern trends in food allergy

Modern trends in food hypersensitivity are primarily connected with the utilization of new strategies in analysis and detection of food

Fig. 5. Working plan of a biosensor
allergens. Rita c. alves et al. reviewed the latest detection method for food allergens. They mainly focussed on the use of biosensors as tool for food allergen detection. It has been mentioned that immunological tests and the DNA - based measures, mass spectrometry are the classical available methods used for the confirmation of food allergens. Compared to those tools, biosensors have innovative, sensitive, specific, natural friendly, less expensive and quick methods (particularly when computerized as well as on-going investigation). Therefore, this method easily replaces the earlier ones 146.

Various sorts of biosensors are created, and they set for various objectives in food safety assessment. Literature reviews suggest that for the purpose of food allergen detection, three major groups of biosensors are used, namely: optical, electrochemical, and piezoelectric biosensors 147. Immunosensors are the identification element used here. The allergenic proteins are immobilized on the outside of these gadgets, and the coupling action between the particles can be estimated by utilizing transducers. General schematic diagram of biosensors is given in the figure 5146.

Stefanie and colleagues developed the sensitive and specific primers used for the identification of soy allergens (Glycine max) with Loop-mediated isothermal amplification (LAMP). This is simple and rapid detection involved in the DNA - based assays, mainly applicable in the manufacturing of foods. Notably in field - like applications and on - site screening. The study suggests that ORF160b gene was exceptionally explicit for the soybean. Furthermore, LAMP joined with LFD (Lateral Flow Dipstick) -like detection facilitates a simple, highly selective and sensitive recognition of the soybean without the requirement for costly scientific equipment 148.

Overall, additional method validations like to resolve the limit of detection and the performance testing versus established immunoassays for different kind of food matrices are needed for the better detection of allergens. As a next step, the effect of food handling strategies on recognition of allergens and the enhancement of this protocol is need to be validated.

Biomarkers in food allergy

Food allergy is a complex and diverse disease. Therefore it is essential to develop specific and sensitive markers for its proper management. However, many progresses have been made in this way, but further ways are needed for the better comprehension of the reason and system of food sensitivity, giving a remedial methodology focused to the patient.

Currently, biomarkers are used in food allergy, especially on investigations related with allergen immunotherapies (AITs) including epicutaneous immunotherapy (EPIT), sublingual immunotherapy (SLIT), and oral immunotherapy (OIT). The studies associated with reliable biomarkers to foresee treatment results are extremely low in number. Therapeutic benefit of AIT(Investigational allergen immunotherapies) includes different cell types like basophils, T cells, mast cells and B cells. LaKeya C. Hardy and colleagues reviewed biomarkers that are at presently being researched for AIT138.

Numerous endeavours have been created to describe reliable biomarkers in order to recognize specific endotypes and phenotypes in food hypersensitivity. Eventually, sIgE/total IgE ratios, T cell tests, and Specific IgE (sIgE) tests are only a few that have been examined. Muraro & Arasi summarizes the currently using biomarkers in food hypersensitivity149.

Various in vitro biomarkers have been assessed to evaluate clinical viability of allergen immunotherapy. Recognizing singular patient responders and nonresponders are the primary issue behind with this concept. Several intermediate steps like the degree, dose, the circumstance of allergen introduction corresponding to manifestations, and the utilization of rescue medication, all of which may jumble the connection between IgE - Fab and clinical indications and reaction to immunotherapy150. However many progress has been made in the finding and management of food hypersensitivity, the future strategy seeks the underlying immunologic mechanism and tolerance.

Summary and Conclusion

This review summarizes the basic key objectives regarding to food hypersensitivity. It is an anomalous invulnerable reaction to food. The indications of the unfavorably susceptible response may extend from gentle to extreme, and is regularly happens inside minutes to a few hours of presentation. By understanding about particular methods of extraction and optimisation of those
allergens from common food source improves the diagnosis of its allergenicity. The extraction, purification, and characterization of food allergens from various sources are simultaneously reviewed. The improvement of animal models to test different food hypersensitivities has been gainful for more quick and broad examination about the component behind the unfavorably susceptible pathway. Researchers had continued interest on experimental models (animal as well as plant), in order to explain the mechanism of food induced allergy. Therefore, currently available and existing models of food allergy are briefly discussed. Now days, allergen specific inhibition is a great challenge faced by researchers. It is a key problem involved in food allergy. So, there is a need to look at the current techniques for the evaluation of clinical finding of food hypersensitivity. The effect of food handling techniques on the recognition of allergens is likewise significantly affected. Optimisation of this objective is the key element regarding to the future aspect of food allergen detection. The clarification of these issues will help the researchers to design the experiments with various models (both in vivo and in vitro), so as to assist further findings of food allergy.

ACKNOWLEDGEMENTS

The authors are would like to thank to the Faculties, Researchers and Students of the Department of Life Sciences, University of Calicut.

Conflict of interest

The authors have declared no potential conflict of interest.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

1. Faisal M, Vasiljevic T, Donkor ON. A review on methodologies for extraction, identification and quantification of allergenic proteins in prawns. Food Res Int. 2019;121:307-318. doi:10.1016/j.foodres.2019.03.040
2. Sicherer SH, Sampson HA. Food allergy. J Allergy Clin Immunol. 2010;125(2 SUPPL.:S116-S125. doi:10.1016/j.jaci.2009.08.028
3. Savage J, Sicherer S, Wood R. The Natural History of Food Allergy. J Allergy Clin Immunol Pract. 2016;4(2):196-203. doi:10.1016/j.jaip.2015.11.024
4. Sicherer SH, Sampson HA. Peanut allergy: Emerging concepts and approaches for an apparent epidemic. J Allergy Clin Immunol. 2007;120(3):491-503. doi:10.1016/j.jaci.2007.07.015
5. Branum AM, Lukacs SL. Food allergy among United States children: Trends in prevalence and hospitalizations. Food Allergy Overv Child Heal Issues. 2010;(10):31-39
6. Krishna MT, Mahesh PA, Vedanthan P, Moitra S, Mehta V, Christopher DJ. An appraisal of allergic disorders in India and an urgent call for action. World Allergy Organ J. 2020;13(7):100446. doi:10.1016/j.waojou.2020.100446
7. Li J, Ogorodova LM, Mahesh PA, et al. Comparative Study of Food Allergies in Children from China, India, and Russia: The EuroPrevall-INCO Surveys. J Allergy Clin Immunol Pract. 2020;8(4):1349-1358.e16. doi:10.1016/j.jaip.2019.11.042
8. Sicherer SH, Wood RA. Advances in diagnosing peanut allergy. J Allergy Clin Immunol Pract. 2013;1(1):1-13. doi:10.1016/j.jaip.2012.10.004
9. L’Hocine L, Pitre M. Quantitative and qualitative optimization of allergen extraction from peanut and selected tree nuts. Part 1. Screening of optimal extraction conditions using a D-optimal experimental design. Food Chem. 2016;194:780-786. doi:10.1016/j.foodchem.2015.08.031
10. Benhamou AH, Caubet JC, Eigenmann PA, et al. State of the art and new horizons in the diagnosis and management of egg allergy. Allergy Eur J Allergy Clin Immunol. 2010;65(3):283-289. doi:10.1111/j.1398-9995.2009.02251.x
11. Taylor SL. Molluscan Shellfish Allergy. Adv Food Nutr Res. 2008;54(07):139-177. doi:10.1016/S1043-4526(07)50006-4
12. Verhoeckx KCM, Vissers YM, Baumert JL, et al. Food processing and allergenicity. Food Chem Toxicol. 2015;80:223-240. doi:10.1016/j.fct.2015.03.005
13. Weinberger T, Sicherer S. Current perspectives on tree nut allergy: A review. J Asthma Allergy. 2018;11:41-51. doi:10.2147/JAA.S141636
14. Geiselhart S, Hoffmann-Sommergruber K, Dublin M. Tree nut allergens. Mol Immunol. 2018;100:71-81. doi:10.1016/j.molimm.2018.03.011
15. Matsuo H, Dahlström J, Tanaka A, et al. Sensitivity and specificity of recombinant α-5 gliadin-specific IgE measurement for the diagnosis of wheat-dependent exercise-induced anaphylaxis. Allergy Eur J Allergy Clin Immunol. 2008;63(2):233-236. doi:10.1111/
54. Ogawa T, Bando N, Tuji H, Nishikawa K, Kitamura K. á-Subunit of á-Conglycinin, an Allergenic Protein Recognized by IgE Antibodies of Soybean-sensitive Patients with Atopic Dermatitis. *Biosci Biotechnol Biochem*. 1995;59(5):831-833. doi:10.1271/bbb.59.831

55. Beardslee TA, Zeece MG, Sarath G, Markwell JP. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int Arch Allergy Immunol*. 2000;123(4):299-307. doi:10.1159/000035364

56. Helm RM, Cockrell G, Connaughton C, et al. A Soybean G2 Glycinin Allergen. *Int Arch Allergy Immunol*. 2000;123(3):213-219. doi:10.1159/000024446

57. Helm RM, Cockrell G, Herman E, Burks AW, Sampson HA, Bannon GA. Cellular and molecular characterization of a major soybean allergen. *Int Arch Allergy Immunol*. 1998;117(1):29-37. doi:10.1159/000023987

58. Paper O. Identification of IgE-Binding Proteins of Soy. *Pediatrics*. 2001;109(2):219-222.

59. Kattan JD, Cocco RR, Järvinen KM. Milk and Soy Allergies. *J Allergy Clin Immunol*. 2005;116(6):955-965. doi:10.1016/j.jaci.2005.08.016

60. Leickly FE, Kloepfer KM, Slaven JE, Vitalpur G. Peanut Allergy: An Epidemiologic Analysis of a Large Database. *J Pediatr*. 2018;192:223-228. e1. doi:10.1016/j.jpeds.2017.09.026

61. Beyer K, Morrow E, Li XM, et al. Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol*. 2018;141(6):1541-1547.e1. doi:10.1016/j.jaci.2018.04.025

62. Ricci G, Andreozzi L, Cipriani F, Giannetti A, Gallucci M, Caffarelli C. Wheat allergy in children: A comprehensive update. *Med*. 2019;55(7):1-11. doi:10.3390/medicina55070400

63. Beardslee TA, Zeece MG, Sarath G, Markwell JP. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int Arch Allergy Immunol*. 2000;123(4):299-307. doi:10.1159/000035364

64. Helm RM, Cockrell G, Connaughton C, et al. A Soybean G2 Glycinin Allergen. *Int Arch Allergy Immunol*. 2000;123(3):213-219. doi:10.1159/000024446

65. Helm RM, Cockrell G, Herman E, Burks AW, Sampson HA, Bannon GA. Cellular and molecular characterization of a major soybean allergen. *Int Arch Allergy Immunol*. 1998;117(1):29-37. doi:10.1159/000023987

66. Paper O. Identification of IgE-Binding Proteins of Soy. *Pediatrics*. 2001;109(2):219-222.

67. Kattan JD, Cocco RR, Järvinen KM. Milk and Soy Allergies. *J Allergy Clin Immunol*. 2005;116(6):955-965. doi:10.1016/j.jaci.2005.08.016

68. Leickly FE, Kloepfer KM, Slaven JE, Vitalpur G. Peanut Allergy: An Epidemiologic Analysis of a Large Database. *J Pediatr*. 2018;192:223-228. e1. doi:10.1016/j.jpeds.2017.09.026

69. Beyer K, Morrow E, Li XM, et al. Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol*. 2018;141(6):1541-1547.e1. doi:10.1016/j.jaci.2018.04.025

70. Ricci G, Andreozzi L, Cipriani F, Giannetti A, Gallucci M, Caffarelli C. Wheat allergy in children: A comprehensive update. *Med*. 2019;55(7):1-11. doi:10.3390/medicina55070400

71. Beardslee TA, Zeece MG, Sarath G, Markwell JP. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int Arch Allergy Immunol*. 2000;123(4):299-307. doi:10.1159/000035364

72. Helm RM, Cockrell G, Connaughton C, et al. A Soybean G2 Glycinin Allergen. *Int Arch Allergy Immunol*. 2000;123(3):213-219. doi:10.1159/000024446

73. Helm RM, Cockrell G, Herman E, Burks AW, Sampson HA, Bannon GA. Cellular and molecular characterization of a major soybean allergen. *Int Arch Allergy Immunol*. 1998;117(1):29-37. doi:10.1159/000023987

74. Paper O. Identification of IgE-Binding Proteins of Soy. *Pediatrics*. 2001;109(2):219-222.

75. Kattan JD, Cocco RR, Järvinen KM. Milk and Soy Allergies. *J Allergy Clin Immunol*. 2005;116(6):955-965. doi:10.1016/j.jaci.2005.08.016

76. Leickly FE, Kloepfer KM, Slaven JE, Vitalpur G. Peanut Allergy: An Epidemiologic Analysis of a Large Database. *J Pediatr*. 2018;192:223-228. e1. doi:10.1016/j.jpeds.2017.09.026

77. Beyer K, Morrow E, Li XM, et al. Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol*. 2018;141(6):1541-1547.e1. doi:10.1016/j.jaci.2018.04.025

78. Ricci G, Andreozzi L, Cipriani F, Giannetti A, Gallucci M, Caffarelli C. Wheat allergy in children: A comprehensive update. *Med*. 2019;55(7):1-11. doi:10.3390/medicina55070400

79. Beardslee TA, Zeece MG, Sarath G, Markwell JP. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int Arch Allergy Immunol*. 2000;123(4):299-307. doi:10.1159/000035364

80. Helm RM, Cockrell G, Connaughton C, et al. A Soybean G2 Glycinin Allergen. *Int Arch Allergy Immunol*. 2000;123(3):213-219. doi:10.1159/000024446

81. Helm RM, Cockrell G, Herman E, Burks AW, Sampson HA, Bannon GA. Cellular and molecular characterization of a major soybean allergen. *Int Arch Allergy Immunol*. 1998;117(1):29-37. doi:10.1159/000023987

82. Paper O. Identification of IgE-Binding Proteins of Soy. *Pediatrics*. 2001;109(2):219-222.

83. Kattan JD, Cocco RR, Järvinen KM. Milk and Soy Allergies. *J Allergy Clin Immunol*. 2005;116(6):955-965. doi:10.1016/j.jaci.2005.08.016

84. Leickly FE, Kloepfer KM, Slaven JE, Vitalpur G. Peanut Allergy: An Epidemiologic Analysis of a Large Database. *J Pediatr*. 2018;192:223-228. e1. doi:10.1016/j.jpeds.2017.09.026

85. Beyer K, Morrow E, Li XM, et al. Effects of cooking methods on peanut allergenicity. *J Allergy Clin Immunol*. 2018;141(6):1541-1547.e1. doi:10.1016/j.jaci.2018.04.025

86. Ricci G, Andreozzi L, Cipriani F, Giannetti A, Gallucci M, Caffarelli C. Wheat allergy in children: A comprehensive update. *Med*. 2019;55(7):1-11. doi:10.3390/medicina55070400

87. Beardslee TA, Zeece MG, Sarath G, Markwell JP. Soybean glycinin G1 acidic chain shares IgE epitopes with peanut allergen Ara h 3. *Int Arch Allergy Immunol*. 2000;123(4):299-307. doi:10.1159/000035364

88. Helm RM, Cockrell G, Connaughton C, et al. A Soybean G2 Glycinin Allergen. *Int Arch Allergy Immunol*. 2000;123(3):213-219. doi:10.1159/000024446

89. Helm RM, Cockrell G, Herman E, Burks AW, Sampson HA, Bannon GA. Cellular and molecular characterization of a major soybean allergen. *Int Arch Allergy Immunol*. 1998;117(1):29-37. doi:10.1159/000023987

90. Paper O. Identification of IgE-Binding Proteins of Soy. *Pediatrics*. 2001;109(2):219-222.
535.
68. Rekabi M. 2017-Evaluation of a new protocol for wheat desensitization. 2017;9:637-645.
69. Restani P, Ballabio C, Tripodi S, Fiocchi A. Meat allergy. Curr Opin Allergy Clin Immunol. 2009;9(3):265-269. doi:10.1097/ACI.0b013e3282fde3d
70. Wilson JM, Platts-Mills TAE. Meat allergy and allergens. Mol Immunol. 2018;100:107-112. doi:10.1016/j.molimm.2018.03.018
71. Fiocchi A, Restani P, Riva E, et al. Meat allergy: Ii - effects of food processing and enzymatic digestion on the allergenicity of bovine and ovine meats. J Am Coll Nutr. 1995;14(3):245-250. doi:10.1080/07315724.1995.10718503
72. Fiocchi A, Bouygue GR, Sarratud T, Terracciano L, Martelli A, Restani P. Clinical tolerance of processed foods. Ann Allergy, Asthma Immunol. 2004;93(5 SUPPL.):S38-S46. doi:10.1016/S1081-1206(10)61731-0
73. Çelebiöölu E, Akarsu A, Pahiner ÜM. Ige-mediated food allergy throughout life. Turkish J Med Sci. 2021;51(1):49-60. doi:10.3906/sag-2006-95
74. Hassan AKG, Venkatesh YP. An overview of fruit allergens and the causative allergens. Eur J Allergy Clin Immunol. 2015;47(6):180-187.
75. Popescu F-D. Cross-reactivity between aeroallergens and food allergens. World J Methodol. 2015;5(2):31. doi:10.5662/wjm.v5.i2.31
76. Kim JW, Lee JH, Hwang BY, et al. Morin inhibits Fyn kinase in mast cells and IgE-mediated type I hypersensitivity response in vivo. Biochem Pharmacol. 2009;77(9):1506-1512. doi:10.1016/j.bcp.2009.01.019
77. Sampson HA, O’Mahony L, Burks AW, Plaut M, Lack G, Akdis CA. Mechanisms of food allergy. J Allergy Clin Immunol. 2018;141(1):11-19. doi:10.1016/j.jaci.2017.11.005
78. Teng P, Bateman NW, Darcy KM, et al. Guidelines of Care for the Management of Atopic Dermatitis Part 4: Prevention of Disease Flares and Use of Adjunctive Therapies and Approaches. Gynecol Oncol. 2015;136(3):554-561. doi:10.1016/j.ygyno.2014.12.035.Pharmacologic
79. Spergel JM. Nonimmunoglobulin E-mediated immune reactions to foods. Allergy, Asthma Clin Immunol. 2006;2(2):78-85. doi:10.2310/7480.2006.00009
80. Zuo L, Rothenberg ME. Gastrointestinal Eosinophilia. Immunol Allergy Clin North Am. 2007;27(3):443-455. doi:10.1016/j.iac.2007.06.002
81. Simon D, Cianferoni A, Spergel JM, et al. Eosinophilic esophagitis is characterized by a non-IgE-mediated food hypersensitivity. Allergy Eur J Allergy Clin Immunol. 2016;71(5):611-620. doi:10.1111/all.12846
82. Das C Hansen KC and Tyler JK LMS. sN YÄŒEEıÖS HHIS Public Access. Physiol Behav. 2017;176(3):139-148. doi:10.1016/j.physbeh.2017.03.040
83. Cianferoni A. Non–IgE-mediated anaphylaxis. J Allergy Clin Immunol. 2021;147(4):1123-1131. doi:10.1016/j.jaci.2021.02.012
84. Sampson HA. Update on food allergy. J Allergy Clin Immunol. 2004;113(5):805-819. doi:10.1016/j.jaci.2004.03.014
85. Bannon GA. What makes a food protein an allergen? Curr Allergy Asthma Rep. 2004;4(1):43-46. doi:10.1007/s11882-004-0042-0
86. Hefle SL, Nordlee JA, Taylor SL. Allergenic foods. Crit Rev Food Sci Nutr. 1996;36(SUPPL.):37-41. doi:10.1080/10408399609527760
87. Muraro A, Werfel T, Hoffmann-Sommergruber K, et al. EAACI Food Allergy and Anaphylaxis Guidelines: Diagnosis and management of food allergy. Allergy Eur J Allergy Clin Immunol. 2014;69(8):1009-1025. doi:10.1111/all.12429
88. McClain S, Bannon GA. Animal models of food allergy: Opportunities and barriers. Curr Allergy Asthma Rep. 2006;6(2):141-144. doi:10.1007/s11882-006-0052-1
89. Dalal I, Binson I, Reifen R, et al. Food allergy is a matter of geography after all: Sesame as a major cause of severe IgE-mediated food allergic reactions among infants and young children in Israel. Allergy Eur J Allergy Clin Immunol. 2002;57(4):362-365. doi:10.1034/j.1398-9995.2002.1s3412.x
90. Sánchez-Monge R, Pascual CY, Díaz-Perales A, Fernández-Crespo J, Martín-Esteban M, Salcedo G. Isolation and characterization of relevant allergens from boiled lentils. J Allergy Clin Immunol. 2000;106(5):955-961. doi:10.1067/mai.2000.109912
91. Astwood JD, Leach JN, Fuchs RL. Stability of Food Allergens to Digestion in Vitro. Nat Biotechnol. 1996;14(10):1269-1273. doi:10.1038/nbt1096-1269
92. Harrer A, Egger M, Gadermaier G, et al. Characterization of plant food allergens: An overview on physicochemical and immunological techniques. Mol Nutr Food Res. 2010;54(1):93-112. doi:10.1002/mnf.20090096
93. Kasera R, Singh AB, Lavasa S, Nagendra K, Arora N. Purification and Immunobiochemical Characterization of a 31 kDa Cross-Reactive Allergen from Phaseolus vulgaris (Kidney Bean). PLoS One. 2013;8(5). doi:10.1371/journal.pone.0063063
94. Pastorello EA, Tramabiali C. Isolation of food allergens. J Chromatogr B Biomed Sci Appl. 2001;756(1-2):71-84. doi:10.1016/S0378-4347(01)00072-X

95. Björkstén F, Halmepuro L, Hannuksela M, Lahti A. Extraction and Properties of Apple Allergens. Allergy. 1980;35(8):671-677. doi:10.1111/j.1398-9995.1980.tb02020.x

96. Vassilopoulou E V., Zuidmeer L, Akkerdaas J, et al. Optimized techniques for the extraction of grape allergens appropriate for in vivo and in vitro testing and diagnosis. Mol Nutr Food Res. 2007;51(3):360-366. doi:10.1002/mnr.20609194

97. Shewry PR, Napier JA, Tatham AS. Seed storage proteins: Structures and biosynthesis. Plant Cell. 1995;7(7):945-956. doi:10.1105/tpc.7.7.945

98. Qi PF, Wei YM, Yue YW, Yan ZH, Zheng YL. Biochemical and molecular characterization of gliadins. Mol Biol. 2006;40(5):713-723. doi:10.1134/S0026893306050050

99. Sandiford CP, Nieuwenhuijsen MJ, Tee RD, Newman Taylor AJ. Measurement of airborne proteins involved in Bakers’ asthma. Clin Exp Allergy. 1994;24(5):450-456. doi:10.1111/j.1365-2222.1994.tb00933.x

100. Crespo JF, Pascual C, Helm R, et al. Cross reactivity of IgE binding components between boiled Atlantic shrimp and German cockroach. Allergy. 1995;50(11):918-924. doi:10.1111/j.1398-9995.1995.tb02499.x

101. Langeland T. A clinical and immunological study of allergy to hen’s egg white: I. A clinical study of egg allergy. Clin Exp Allergy. 1983;13(4):371-382. doi:10.1111/j.1365-2222.1983.tb02611.x

102. Makinen-Kiljunen S, Sorva R. Bovine ß-lactoglobulin levels in hydrolysed protein formulas for infant feeding. Allergy Clin Immunol. 2010;261(5):737-745. doi:10.1007/978-1-4939-9591-2

103. Cells L, Kulczycki BYA, Isersky C, Metzger H. Quantitative relationships between aggregation of IgE receptors, generation of intracellular signals, and histamine secretion in rat basophilic leukemia (2H3) cells. Enhanced responses with heavy water. J Biol Chem. 1986;261(6):2583-2592.

104. Passante E, Frankish N. The RBL-2H3 cell line: Its provenance and suitability as a model for the mast cell. Inflamm Res. 2009;58(11):737-745. doi:10.1007/s00011-009-0074-y

105. Gibbs BF. Basophils as Key Regulators of Allergic Inflammation and Th2-type Immunity. World Allergy Organ J. 2008;1(7):123-128. doi:10.1097/wox.0b013e31817a76fb

106. Cells L, Kulczycki BYA, Isersky C, Metzger H. (From the Section on Chemical Immunology, Arthritis and Rheumatism Branch, National Institute of Arthritis, Metabolism and Digestive Diseases, National Institutes of Health, Bethesda, Maryland 20014). Published online 1974:600-616.

107. Rifillan AM, Tkaczyk C. Integrated signalling pathways for mast-cell activation. Nat Rev Immunol. 2006;6(3):218-230. doi:10.1038/nri1782

108. Falcone FH, Wan D, Barwary N, Sagie-Eisenberg R. RBL cells as models for in vitro studies of mast cells and basophils. Immunol Rev. 2018;282(1):47-57. doi:10.1111/imr.12628

109. Chen BH, Hung MH, Chen JYF, et al. Anti-allergic activity of grape seed extract (GSE) on RBL-2H3 mast cells. Food Chem. 2012;132(2):968-974. doi:10.1016/j.foodchem.2011.11.079
Bischoff SC, Wedemeyer J, Hermann A, et al. Quantitative assessment of intestinal eosinophils and mast cells in inflammatory bowel disease. Histopathology. 1996;28(1):1-13. doi:10.1046/j.1365-2559.1996.262039.x

Lee EJ, Ji GE, Sung MK. Quercetin and kaempferol suppress immunoglobulin E-mediated allergic inflammation in RBL-2H3 and Caco-2 cells. Inflamm Res. 2010;59(10):847-854. doi:10.1007/s00011-010-0196-2

Castan L, Bøgh KL, Maryniak NZ, et al. Overview of in vivo and ex vivo endpoints in murine food allergy models: Suitable for evaluation of the sensitizing capacity of novel proteins? Allergy Eur J Allergy Clin Immunol. 2020;75(2):289-301. doi:10.1111/all.13943

Gonipeta B, Kim E, Gangur V. Mouse Models of Food Allergy: How Well Do They Simulate the Human Disorder? Crit Rev Food Sci Nutr. 2015;55(3):437-452. doi:10.1080/10408398.2012.657807

Leeman WR, Van Den Berg KJ, Houben GF. Transfer of chemicals from feed to animal products: The use of transfer factors in risk assessment. Food Addit Contam. 2007;24(1):1-13. doi:10.1080/02652030600815512

Aldemir H, Bars R, Herouet-Guicheney C. Marine models for evaluating the allergenicity of novel proteins and foods. Regul Toxicol Pharmacol. 2009;54(3 SUPPL.):S52-S57. doi:10.1016/j.yrtph.2008.11.004

Meeussen EN, Snibson KJ, Hirst SJ, Bischof RJ. Sheep as a model species for the study of human asthma and other respiratory diseases. Drug Discov Today Dis Model. 2009;6(4):101-106. doi:10.1016/j.ddmod.2009.12.002

Helm RM. Food allergy animal models: an overview. Ann N Y Acad Sci. 2002;964:139-150. doi:10.1111/j.1749-6632.2002.tb04139.x

Thang CL, Baurhoo B, Boye JI, Simpson BK, Zhao X. Effects of Lactococcus rhamnosus GG supplementation on cow’s milk allergy in a mouse model. Allergy, Asthma Clin Immunol. 2011;7(1):1-9. doi:10.1186/1710-1492-7-20

Bailón E, Cuetó-Sola M, Utrilla P, et al. A shorter and more specific oral sensitization-based experimental model of food allergy in mice. J Immunol Methods. 2012;381(1-2):41-49. doi:10.1016/j.jim.2012.04.007

Dearman RJ, Kimber I. Animal models of protein allergenicity: Potential benefits, pitfalls and challenges. Clin Exp Allergy. 2009;39(4):458-468. doi:10.1111/j.1365-2222.2008.03194.x

Ladies GS, Knipplings LMJ, Penninks AH, Bannon GA, Goodman RE, Herouet-Guicheney C. Review of animal models designed to predict the potential allergenicity of novel proteins in genetically modified crops. Regul Toxicol Pharmacol. 2010;56(2):212-224. doi:10.1016/j.yrtph.2009.09.018

Oriel RC, Wang J. Diagnosis and Management of Food Allergy. Pediatr Clin North Am. 2019;66(5):941-954. doi:10.1016/j.pcl.2019.06.002

Luccioli S. Food allergy guidelines and assessing allergic reaction risks: A regulatory perspective. Curr Opin Allergy Clin Immunol. 2012;12(3):323-330. doi:10.1097/ACI.0b013e3283535aaf

Simons FER, Arduoso LRF, Bilò MB, et al. 2012 Update: World Allergy Organization Guidelines for the assessment and management of anaphylaxis. Curr Opin Allergy Clin Immunol. 2012;12(4):389-399. doi:10.1097/ACI.0b013e3283557e4

Bolhaar STHP, Van De Weg WE, Van Ree R, et al. In vivo assessment with prick-to-prick testing and double-blind, placebo-controlled food challenge of allergenicity of apple cultivars. J Allergy Clin Immunol. 2005;116(5):1080-1086. doi:10.1016/j.jaci.2005.07.004

García BE, González-Mancebo E, Barber D, et al. Sublingual immunotherapy in peach allergy: Monitoring molecular sensitizations and reactivity to apple fruit and Platanus pollen. J Investig Allergol Clin Immunol. 2010;20(6):514-520.

Kollmann D, Geroldinger-Simic M, Kinaciyan T, et al. Recombinant Mal d 1 is a reliable diagnostic tool for birch pollen allergen-associated apple allergy. J Allergy Clin Immunol. 2013;132(4):1008-1010. doi:10.1016/j.jaci.2013.05.030

Hoffmann-Sommergruber K, Pfeifer S, Rublin M. Applications of Molecular Diagnostic Testing in Food Allergy. Curr Allergy Asthma Rep. 2015;15(9). doi:10.1007/s11882-015-0557-6

Hardy LKC, Smeekens JM, Kulis MD. Biomarkers in Food Allergy Immunotherapy. Curr Allergy Asthma Rep. 2019;19(12):1-7. doi:10.1007/s11882-019-0894-y

Erdmann SM, Sachs B, Schmidt A, et al. In vitro analysis of birch-pollen-associated food allergy by use of recombinant allergens in the basophil activation test. Int Arch Allergy Immunol. 2005;136(3):230-238. doi:10.1159/000083949

Newberry SJ, Riedl MA, Bravata DM, et al. Clinician’S Corner. 2010;30(18).

Foong RX, Roberts G, Fox AT, Toit G. Pilot study: Assessing the clinical diagnosis of allergy in atopic children using a microarray assay in addition to skin prick testing and serum
specific IgE. *Clin Mol Allergy*. 2016;14(1):1-7. doi:10.1186/s12948-016-0046-z

142. Keet CA, Allen KJ. Advances in food allergy in 2017. *J Allergy Clin Immunol*. 2018;142(6):1719-1729. doi:10.1016/j.jaci.2018.10.020

143. Sathe SK, Teuber SS, Roux KH. Effects of food processing on the stability of food allergens. *Biotechnol Adv*. 2005;23(6):423-429. doi:10.1016/j.biotechadv.2005.05.008

144. Thomas K, Herouet-Guicheney C, Ladics G, et al. Evaluating the effect of food processing on the potential human allergenicity of novel proteins: International workshop report. *Food Chem Toxicol*. 2007;45(7):1116-1122. doi:10.1016/j.fct.2006.12.016

145. Vanga SK, Singh A, Raghavan V. Review of conventional and novel food processing methods on food allergens. *Crit Rev Food Sci Nutr*. 2017;57(10):2077-2094. doi:10.1080/10408398.2015.1045965

146. Alves RC, Barroso MF, González-García MB, Oliveira MBPP, Delerue-Matos C. New Trends in Food Allergens Detection: Toward Biosensing Strategies. *Crit Rev Food Sci Nutr*. 2016;56(14):2304-2319. doi:10.1080/10408398.2013.831026

147. Viswanathan S, Radecka H, Radecki J. Electrochemical biosensors for food analysis. *Monatshefte fur Chemie*. 2009;140(8):891-899. doi:10.1007/s00706-009-0143-5

148. Allgöwer SM, Hartmann CA, Holzhauser T. The development of highly specific and sensitive primers for the detection of potentially allergenic soybean (Glycine max) using loop-mediated isothermal amplification combined with lateral flow dipstick (LAMP-LFD). *Foods*. 2020;9(4). doi:10.3390/foods9040423

149. Muraro A, Arasi S. Biomarkers in Food Allergy. *Curr Allergy Asthma Rep*. 2018;18(11). doi:10.1007/s11882-018-0816-4

150. Shamji MH, James LK, Durham SR. Serum Immunologic Markers for Monitoring Allergen-Specific Immunotherapy. *Immunol Allergy Clin North Am*. 2011;31(2):311-323. doi:10.1016/j.iac.2011.03.005