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Recent Advances in Processing for Reducing Dairy and Food Allergenicity

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Abstract More than 160 food materials are identified as allergens. However, 90% of allergic reactions are caused by eight major food allergens. Food Allergen Labeling and Consumer Protection Act (FALCPA) of 2004 came into effect from January 1, 2006. This law requires the identification of ingredients derived from the major food allergens, on the food label. Several research efforts are ongoing to develop hypoallergenic foods. In this article we review recent status of food allergy and studies reporting advanced processing technologies for reducing allergenicity of foods. A brief survey of methods of allergen detection has been also reported as measures to control allergens in food industry.

Keywords Dairy and food allergy, Nonthermal processing, High intensity ultrasound, Nonthermal plasma, Ultraviolet light, High pressure processing

1. Introduction

Food allergy is an adverse reaction to specific food by the immune system [1]. In industrialized countries food allergies affect nearly 2% of the adult population and 8% of children [2]. In the United States alone, it was estimated in 2010 that 30,000 people require emergency treatment, 2000 people hospitalized and 150 people die every year due to food allergies [3]. Food allergy is becoming more and more common and is likely increasing in prevalence which makes it an important public health concern [4]. New inventions of food formulations and novel proteins are likely to cause new cases of food allergy [5].

The symptoms of food allergy include respiratory, gastrointestinal, cutaneous and cardiovascular symptoms and a rare life threatening symptom includes anaphylactic shock. Lack of, or defect in enzymes present in the digestive system lead to food intolerance. One common known example is lactose intolerance, caused due to the absence of lactase enzyme in digestive system. Complete avoidance of the culprit food is the best treatment for both food allergy and intolerance [6].

Food allergens are the components of food (generally a protein) that can cause adverse immunological reactions when consumed. More than 160 foods are identified as allergenic. Approximately 90 percent of all the allergenic reactions to food are caused by eight major foods namely milk, eggs, fish, crustacean, shellfish, tree nuts, peanuts, wheat, and soybeans. The other 10 percent of the allergenic food reactions are caused by remaining foods that are identified as allergens and are less prominent [2].

Currently there is no cure for food allergy and food allergic individuals must completely avoid the sensitive foods [7]. Absolute avoidance of the foods is difficult in the modern days because of the use of major allergens as ingredients in other food products and contamination caused by processing in same facility that handles allergenic foods [8, 9].

Risk assessment and management of food allergy is very difficult because of several reasons: (1) Non-allergic people can consume different food allergens without any risk unlike other chemical and microbiological hazards like toxins and harmful bacteria. (2) Almost all of the major allergenic foods provide nutrition and are part of daily food consumption for survival. (3) The sensitivity to food allergens varies vastly from one patient to other who is allergic to the same food. Hence no two allergenic foods can have similar risk management strategies [10].

2. IgE mediated Allergy

The protein structure is divided into primary, secondary, tertiary and quaternary structure. Amino acid chains connected to each other through peptide bonds form primary structure which is the back bone of the protein structure. The three dimensional organizations of polypeptide chains form secondary structure. Secondary structure of proteins consists of α-helix, β-sheet and random coil. When the linear protein chains with secondary structure segments further folds in

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three dimensional arrangement tertiary structure is formed. Linking of multiple polypeptide chains through non-covalent interaction forms quaternary structure [11].

The allergenicity of a particular protein depends on the functions of the primary structure and conformation of its molecules. Amino acid sequence and side chains are present in the primary structure. Primary structure contains chemical elements that are required for antibody binding and the conformation of the protein structure determines which elements of the primary structure are available for binding. By altering the primary structure or the conformation of the protein, allergenicity can altered [11].

Human body releases immunoglobulin antibodies in response to antigens (proteins) and they bind to the antigens to deliver immune response. The specific amino acid sequence chains in an antigen with which the IgE (immunoglobulin E) binds are called epitopes. Linear epitopes which are a portion of continuous amino acid chains, and conformational epitopes which are formed by three dimensional folding of proteins are the two types of epitopes present in a protein [12].

Disruption or modification of epitopes responsible for IgE binding is often required to alter the allergen reactivity. Linear epitopes can be altered by genetic modification or fragmentation of linear amino acid sequences whereas the conformational epitopes can be destroyed by full or partial denaturation, aggregation induced crosslinking or chemical modification. By altering the IgE binding epitope regions, the antibody does not recognize the allergen and hence allergic reactions are inhibited [12].

3. Processing

Food Processing may alter the structures and availability of epitopes for binding to IgE. Processing may be thermal or nonthermal. The effect of thermal treatment on food allergens has beenwidely researched due to its wide application in food processing [13], [14], [15]. Heat treatment can denature some proteins and thus change the native structure. The conformational epitopes are altered during denaturation due to the modification of secondary and tertiary structure which may reduce the IgE binding. Depending on the type of protein, temperature and extent of heating, heat treatment may induce unfolding which can be reversible or irreversible [14].

Each protein exhibits different resistance to heat treatment. Moreover the response of a food allergen to heat depends on whether the heat is dry or moist [16]. Dry heat Most of the food allergens are resistant to denaturation and can survive the processing treatments like heating and enzymatic degradation [16]. Fruit allergents such as Mal d 1 and Pru av 1 are more heat labile, whereas Gly m 4 of soy are more heat stable [14]. Examples of food allergens that are highly resistant to heat treatment include casein ([18], tropmyosin [19] and ovomucoid [20]. In cow milk, α-casein is the most stable, bovine serum albumin is the most heat labile and β-lactoglobulin is relatively heat stable [1]. Bu et al [21] studied the effect of thermal processing on milk proteins and reported that α-latalbumin and β-lactoglobulin had increased allergenicity from 50 to 90℃ whereas the allergenicity decreased when heated above 90℃. Heating below 90℃ would cause ulfolding of proteins leading to exposure of epitopes for IgE binding whreas heating above 90℃ leads to destruction of epitopes by mailard reaction [22].

Although thermal treatment may reduce allergic reactions of some thermal labile proteins, nonthermal processing offers several advantages over nonthermal processing because of retention of nutrition and natural attributes such as aroma, color, and flavor. Hence the following section describes the effect of nonthermal processing on allergenicity of foods in more details.

4. Nonthermal Processing

Application of nonthermal treatments is widely increasing for different food products due to the benefits of minimal effect on nutritional and organoleptic qualities of foods. Nonthermal treatments are proved to be effective in killing harmful bacteria in different food products with minimum effect on the quality [20]. Recent research on the effect of nonthermal treatments in altering allergenicity of food proteins showed that these treatments can reduce the allergencity of different food allergens [23], [24], [25], [26]. Pulse ultraviolet light, high intensity ultrasound, nonthermal atmospheric plasma, gamma irradiation, genetic modification, physical, chemical and enzymatic processing are some of the nonthermal treatments shown to have the ability to alter the allergenicity of different food allergens [26]. Some of these processes have been described in details in the following sections.

4.1. Enzyme Treatment

Enzymatic treatment hydrolyzes the protein with enzymes and reduces the allergenicity. This treatment destroys the structure of proteins and alters its properties. Development of off flavor and bitterness due to the release of peptides and amino acids makes the enzymatic hydrolyzed product often unacceptable for consumption [11]. By using the thioredoxin enzyme, Del Val et al. [28] removed the disulphide bonds on β-lactoglobulin which became sensitive to pepsin and lost the allergenicity. Expensive equipment and ingredients were the major disadvantages of this treatment.

4.2. Bacterial Culture

Mesophillic and thermophillic strains of bacterial cultures where shown to have reduced the allergenicity of cow milk proteins when tested with in vitro system. Lactic acid bacteria fermentation can reduce the immunoreactivity of sterilized cow milk by 90% while preserving the organoleptic properties of the product [29]. However in vivo tests with allergic patients revealed the existence of allergenicity towards treated proteins.

4.3. Genetic Modification

In recent years genetic modification of foods is the widely discussed topic with much scrutiny. Genetic modification process can be applied to prevent translation of selected allergens using post-transcriptional gene silencing or co-suppresion. Stability of these foods has not been determined and this process might lead to the change in functional and physical properties of foods [30].

4.4. Gamma Irradiation

Application of gamma irradiation to reduce food allergencity is widely researched in recent years [31], [32]. Gamma irradiation was regarded as an effective method to reduce the allergenicity of shrimp and milk allergens [31], [32]. However consumer resistance and need for large investments makes it difficult for application in food industry [33].

4.5. Ultraviolet Light

Researchers used ultraviolet (UV) light as a bactericidal agent since the year 1928 [34]. More recently it is being used in food industry as a sanitizing and disinfecting agent for solid and liquid foods, and food contact surfaces including sanitization of packaging materials. The UV light is divided into four regions, UV-A (wavelength range: 315-400 nm), UV-B (wavelength range: 280-315 nm), UV-C (wavelength range: 200-280 nm) and Vacuum UV (Wavelength range 100-200 nm). Continuous UV and pulsed UV are the two types of UV radiation currently available. Continuous UV has a wavelength range of 200 – 400 nm whereas pulsed UV has a wavelength range of 100-1100 nm [35].

UV light can only penetrate up to a few millimeters through opaque liquids and solid food material and hence these food materials should be placed in thin layers for the treatment to overcome this limitation [35]. It is more effective for surface sterilization and sterilization of highly transparent liquids like water. UV light has sufficient energy to break most of the chemical bonds and can be used to induce changes in chemical compounds, however it cannot effectively penetrate through opaque liquid like milk and hence special considerations should be taken in designing UV pasteurizers for milk, providing UV exposure in thin layers with sufficient turbulence [36].

The effect of UV radiation on food allergenicity varies depending on the amino acid composition and molecular structure of the protein [37]. A 7 fold reduction in IgE binding of peanut allergens in Ci-ELISA analysis was obtained by treating peanut extract and liquid peanut butter with pulsed UV light treatment [38]. UV light exposure of proteins initiates free radical oxidation and lipid oxides, superoxide radicals (SOR). Superoxide radicals can induce protein denaturation and cross linking [39]. Yang et al. [40] reported significant change in the allergenicity of soy proteins after treatment with pulsed UV light. Most recently Tammineedi et al. [41] reported 25% and 27.7% decrease in allergenicity of casein and whey proteins by a 15 minute UV treatment with a continuous UV dose of 3500 mJ/cm^2.

4.6. High Intensity Ultrasound

High-intensity ultrasound is an efficient food processing technology. It is widely used in food industry for different applications such as homogenizing emulsions, deactivating enzymes, enhancing extraction processes, accelerating dehydration, ageing, and ripening processes (Villamiel and Jong, 2000). High intensity ultrasound processing operates under the frequency range of 20 kHz to 100 kHz [42]. The high energy of the mechanical waves leads to formation of sonication bubbles in liquids. These bubbles compress and refract intermittently and collapse at critical bubble sizes. The implosion of bubbles lead to formation of localized high pressure and temperature regions (up to 1000 atm and 5000 ^{o}K, respectively) which may change the conformation of allergens and alter their reactivity [43]. Very little information is available on the effect of high intensity ultrasound treatment on food allergenicity. A recent study on the effect of high intensity ultrasound on shrimp extract and isolated shrimp protein showed a significant decrease in the allergenicity which was confirmed by ELISA and Immunoblot analysis. The shrimp extract and isolated proteins were treated with 30 Hz frequency for 130 to 180 min. After ELISA analysis, IgE binding of isolated shrimp protein was reduced by 81.3-88.5% whereas the IgE binding of shrimp extract was only reduced by 68.9%. [24].

Most recently Choudhary et al. [44] reported a 24% decrease in allergenicity of soy proteins by a 10 minute high intensity ultrasound treatment by a 37kHz ultrasonic processor. The High intensity ultrasound treatment at 500 W power and 20kHz frequency was not effective in reducing the allergenicity of major milk proteins but the ultrasound at 37 kHz reduced allergenicity of soy proteins by altering the secondary structure of proteins. The ultrasound at 37 kHz may be used for future research in reducing allergenicity of other food allergens [44].

4.7. High Hydrostatic Pressure

High Pressure Processing (HPP) is a nonthermal process where food is subjected to elevated pressures (approximately 6,000 atmospheres) to achieve microbial inactivation or to alter the functional properties of food to enhance qualities [45]. Huppertz et al [46] investigated the efficacy of HPP on the denaturation of non-hydrolyzed α-casein and β-lactoglobulin present in milk and whey. Their results showed that α-casein was more pressure resistant than β-lactoglobulin. These two proteins were denatured more readily in milk than in whey. High Pressure treatment was also applied to reduce the allergenicity by improving enzymatic hydrolysis. At 600 MPa pressure, the extent of protein enzymatic hydrolyzation was reported to be higher than at atmospheric pressure [4]. Most recently Shriver [27] studied effect of HPP on shrimp allergens and did not find any reduction in shrimp allergenicity when treated at 600 MPa at 70℃ for 15 min. More systematic study of this

process is required to reach any conclusion on the potential of HPP for reducing food allergenicity. Nooji [26] reported a 42% decrease in allergenicity of wheat proteins subjected to HPP at 75℃ for 15 min.

4.8. Nonthermal Atmospheric Plasma

Recently nonthermal atmospheric plasma (NTAP) has been gaining attention as an alternative microbial inactivation technique in food processing [47], [48], [49], [50]. NTAP treatment requires less power compared to other sterilization techniques and hence this treatment is considered economical and effective in killing microorganisms [48]. Plasma is generated when a significant number of atoms of gas are energized by heat or another energy source which ionizes the atoms and releases electrons. The ionized gas is the fourth state of matter and is called plasma [51]. Nonthermal atmospheric plasmas can be generated using different power sources including alternating current, direct current, pulsed, microwave and radio frequency (RF). Depending on the power source, gas mixture and flow rate, the efficiency of microbial reduction differs [52].

Recent studies showed that nonthermal plasma treatments were effective in reducing the allergenicity of wheat and shrimp proteins [26], [27]. Shriver [27] used NTAP for reducing allergenicity if shrimp protein tropomyosin. The NTAP was generated using a voltage of 30 kV and a frequency of 60 Hz and treatment was carried out for 1, 3, and 5 min at ambient temperature. The levels of IgE binding to tropomyosin were reduced by 76% following NTAP treatment at 5min. Noozi [26] studied effect of NTAP on allergenicity of wheat protein extract. NTAP exposure for 5 min significantly reduced allergenicity of wheat proteins by 37%. Further research on NTAP in combination with heat and/or other nonthermal is required for reducing allergenicity of wheat proteins.

5. Detection of Allergens

Detection of allergenic ingredients in food products has been receiving greater attention over the past few years from both food industry and regulatory agencies [53]. In vivo and in vitro testing are the two types of detection methods used to determine the allergenicity of an antigen. Although in vivo methods provide more accurate results compared to in vitro methods, they are time consuming, costly and have the potency of risk of exposure to human subjects. In vitro methods are inexpensive, quick and do not pose threat to human subjects and hence they are more commonly used [54].

5.1. In-vitro Methods

In vitro methods for determining the presence of allergens include radio-allergosorbent tests (RAST), enzyme-allergos orbent tests (EAST), sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), immunobloting, and enzyme-linked immunosorbent assay (ELISA). RAST test quantifies serum IgE by identifying the antibodies bound to radioisotopes. EAST test is similar to RAST and the enzyme activity is measured which correlates to antibodies attached to enzymes [55]. Of all the several in vitro techniques, immunoassays are most commonly used and in particular the (SDS-PAGE), immunoblot, and (ELISA) are validated by federal agencies for detection of major food allergens like cow milk, egg white, hazelnut, peanut, soybean and wheat proteins [56].

5.2. In-vivo Methods

Skin prick tests (SPT) and oral food challenges (OFC) are the two in vivo methods that are most commonly used. SPT tests are conducted by injecting a small amount of allergen under the skin. The formation of wheal or a circle that is larger than 3 mm confirms the allergenicity of the protein toward the patient [56]. OFC tests require the subjects to consume foods that the patient might be sensitive to. This test can be dangerous for patients and those who are susceptible to anaphylaxis should not participate in this study [56].

5.3. ELISA

Competitive ELISA (Ci-ELISA) and indirect ELISA are the two types of ELISA used for determining the IgE binding activity of allergens. In this method, proteins are absorbed on the surface of the wells of ELISA plate and detected using appropriate antibodies [57]. Western bolt and dot bolt are the two methods of immunoblotting used for allergen analysis. Western blot analysis is conducted by separating the proteins according to their molecular weight with ploy acrylamide gel electrophoresis and transferring them to a membrane (nitrocellulose) to perform antibody detection methods [58]. Dot blot analysis is performed by directly absorbing the sample onto the membrane and analyzing with antibody detection method [59].

5.4. SDS-PAGE

SDS-PAGE analysis is used to determine the presence or absence of allergens and change in the electrophoretic pattern of proteins [60]. Aggregated proteins become too large to penetrate through the wells and protein fragments become too small for the wells to hold. Studies have shown that intramolecular crosslinking of proteins my lead to smeared appearance of bands in gel [61]. This method is quick and inexpensive. However, SDS-PAGE does not determine the IgE binding activity of the allergens [62].

5.5. NMR

Nuclear magnetic resonance (NMR) spectroscopy analysis is widely used to study protein structures, protein folding, modifications of protein structures in solutions and non-covalent protein-protein interactions [63]. NMR has

been used extensively to study milk and milk proteins [64]. NMR has been used to assess the denaturation and to detect structural changes in proteins including transitions between different phases of the conformational mobility of milk proteins [65]. High-resolution 1H-NMR analysis has been demonstrated to be useful for food profiling [66] and access to high-field magnets with increased sensitivity allow the elucidation of components not commonly detected at lower fields [67].

5.6. Mass Spectrometry

Mass spectrometry is one of the most common methods used for identification of proteins and peptides. Since most of the food allergens are proteins, mass spectroscopy is gaining more attention for potential application in detection of food allergens. The use of mass spectroscopic methods for potential detection of peanut allergens using peptide markers were previously reported [68].

5.7. Biosensors

Biosensors are one of the most recent tools being developed for detection of food allergens. Jiang et al [69] developed mast cell-based electrochemical biosensor for quanification of shrimp allergen. The biosensor was a simple label free and sensitive method with detection limit of 0.15 $\mu g\ mL^{-1}$ of shrimp allergen Pen a1 (tropomyosin). Tran et al [70] reported biosensing of peanut allergen in food matrices. The fiber optic surface plasmon resonace biosensor was successfully used to detect the peanut protein Ara h1 in food matrix. Sun et al. [71] also reported a DNA biosensor to detect peanut allergen Ara h1.

Wang et al [72] developed an optical thin-film biosensor chip for simultaneous detection of eight major food allergens. The developed biosensor chip was a sensitive, specific, high-throughput, and ready-to-use analytical tool to detect the presence or confirm the absence of eight food allergens with the absolute detection limit of 0.5 pg of cashew DNA, and the practical detection limit of 0.001%. The detection time of biosensor chip was about 30 min after PCR amplification.

Real time PCR is the emerging detection method for food allergens. Recently real time PCR was used by Herrero et al [73] for detection of fish allergen in food samples. They compared the performance of real time PCR assay with a commercial kit and reported that the real time PCR assay was more sensitive, specific, cheaper and less time-consuming than the commercial kit. Lopez Calleja [74] developed real-time PCR for detecting allergenicity of pistachio in commercial foods. The real time PCR could detect up to 0.1 $mg\ kg^{-1}$ of pistachio content in food samples. The same group [75] also used the PCR assay for detection of almond in commercial food samples. The realtime PCR assay successfully detected upto 0.1 $mg\ kg^{-1}$ almond in 214 commercial food samples. Thus real time PCR is likely to become a popular tool for rapid and sensitive detection of allergens in food samples.

6. Conclusions

With the increasing population of people affected by food allergy, it is important to detect and reduce food allergy. Recent advances in nonthermal processing offers great promise to the food processors to develop hypoallergenic foods. More studies are required in this emerging field of research. Research funding by public and government agencies will boost research activities that will develop processes for reducing and eliminating food allergy. Recent advances in allergen detection technology will provide tools to the food industry to ensure delivery of safe food to consumers.

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