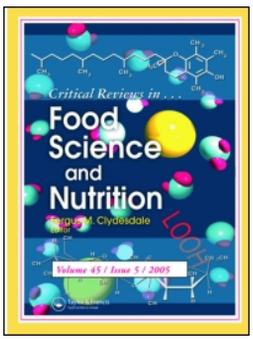

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Review of conventional and novel food processing methods on food allergens

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ABSTRACT

With the turn of this century, novel food processing techniques have become commercially very important because of their profound advantages over the traditional methods. These novel processing methods tend to preserve the characteristic properties of food including their organoleptic and nutritional qualities better when compared with the conventional food processing methods. During the same period of time, there is a clear rise in the populations suffering from food allergies, especially infants and children. Though, this fact is widely attributed to the changing livelihood of population in both developed and developing nations and to the introduction of new food habits with advent of novel foods and new processing techniques, their complete role is still uncertain. Under the circumstance, it is very important to understand the structural changes in the protein as food is processed to comprehend whether the specific processing technique (conventional and novel) is increasing or mitigating the allergenicity. Various modern means are now being employed to understand the conformational changes in the protein which can affect the allergenicity. In this review, the processing effects on protein structure and allergenicity are discussed along with the insinuations of recent studies and techniques for establishing a platform to investigate future pathway to reduce or eliminate allergenicity in the population.

KEYWORDS

Protein structure; microwave processing; food allergenicity; high pressure

1. Introduction

The human race flourished on the face of earth starting millions of years ago and for all these years food has been the primary source of energy. The source of the food can be plants, animal carcasses, or even microbes in many cases. Independent of the source, the main components of food include water, carbohydrates, proteins, lipids, minerals and vitamins along with few minor components such as antioxidants, colorants, flavoring components and any other additives that are added with an intention to impact various qualities and physicochemical properties of food. Depending on the source of food, the above mentioned constituents vary in composition, giving foods their distinct aroma, color, and palatability. The contents also vary depending on various other factors including species and variety of the animal or plant respectively; time of harvesting for plants and slaughtering for animals; nurturing habits and environment during their life. All these factors affect the inherent quantity and quality of the components of food.

Of the aforementioned elements, water content in various foods can vary widely between 3% (dry fruits, milk powder) to 95% (few fruits and vegetables) (Sikorski, 2007)1, 2 and this water content determines various physical and chemical properties of food. In 1957, Scott, W.J. (Scott, 1957) became the first researcher to give the relation between the water content and microbial activity in food. His research suggested that the water activity can be correlated with deterioration of food and hence it in turn influences the microbial activity in food (Scott, 1957; Ayerst, 1969; Rahman, 1995). Thus, the shelf life quality of any food is determined by the amount of water present in it. Apart from the shelf life of the product, the water content also determines the crispiness of the few food products including popcorn, potato chips, and other cereal based food products (Katz and Labuza, 1981; Roudaut et al., 1998) and also plays a primary role in the amino acid—sugar browning reactions (Maillard reaction) (Eichner and Karel, 1972). Moreover, the mobility of the components within a food product is also greatly affected by the water content (Karel, 1985). The water content is also an important factor to consider in various food processes such as fermentation (Oriol et al., 1988; Zaks and Klibanov, 1988; Liu and Tzeng, 1999), baking (Sablani et al., 1998; Thorvaldsson and Skjöldebrand, 1998), and microwave heating (Venkatesh and Raghavan, 2004). Other properties like the thermal conductivity, diffusion, and specific heat also varies depending upon the moisture content in food thus playing an important role in various food processing aspects (Pomeranz, 1985).

Another component in food of high importance to humans are the carbohydrates or sugars, which act as the primary source of energy to the body (Boyle, 1996). The carbohydrates release about 4 kcal of energy for every gram consumed (Lee and Putnam, 1973; Hunt and Stubbs, 1975; Ledikwe et al., 2006). The amount of the sugars present can vary greatly from 1% (few meat products) to 65%–70% (cereal grains) (Pomeranz, 1985; Sikorski, 2007). Apart from being the primary energy source, carbohydrates, most importantly the polysaccharides,

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act as primary structural component in the plant-based foods (Carpita and Gibeaut, 1993). Few of the polysaccharides can also be used as food gums, also called the hydrocolloids, which play a major role in determining physical qualities including texture and stability and various other processing parameters (Eliasson, 2006). Apart from the carbohydrates, lipids also act as an important source of energy. They release about 9 kcal of energy per every gram of fat consumed (Bang et al., 1980; Ledikwe et al., 2006). This is the reason fats are called "the concentrated source of energy" as they tend to release more than double the energy on consumption of the same amount of carbohydrate or protein (Boyle, 1996). Fats also play an important role in delivering the body with vital nutrients including Vitamin A, D, E, and K (Herrmann, 2011).

Proteins are the next major component present in food apart from carbohydrates and lipids. They play a primary role in cell signaling and immune responses within the human body. They are also responsible for the muscle formation in humans and cell wall structure of the plants. In addition to the wide array of functions of protein, they can also act as a source of energy to the body if required, liberating about 4 kcal of energy per every gram of protein (Ledikwe et al., 2006; Phillips and Williams, 2011). Apart from the above, proteins also has wide range of functional, sensorial, and nutritional properties in food. And all of the properties are dependent on the structure of the protein (Singh et al., 2013b).

2. Food proteins

2.1. Structure of the protein

"Amino acids" act as the building blocks of proteins and in total there are about 20 amino acids, which have a common structure as shown in Fig. 1. A molecule of amino acid contains a carboxylic group, primary amine, and another molecule (represented in the Fig. 1 as R). The molecule attached in place of "R" differs, forming various amino acids with different characteristic properties (Table 1) (Phillips and Williams; Rahman, 1995).

The amino acids come together forming a peptide bond as shown in Fig. 2. The peptide bond is formed between the carboxylic group of the first amino acid and amine group of the second amino acid giving out a water molecule (Koshland Jr, 1958; Phillips and Williams; Phillips and Williams, 2011). The new terminals are now available for further bonding with other amino acids. The number of amino acids in each protein molecule can vary from 10 to about 30,000 like in titin molecule (Opitz et al., 2003) which is termed as the largest protein. The linear sequence of the amino acids that join together forming a polypeptide chain gives proteins their primary structure (Singh et al., 2013a). Depending on the amino acids in the primary structure, local sub-structures are formed within the protein molecule like alpha helix and beta sheets giving protein its characteristic secondary structure. A single protein molecule can conform to wide range of structures which can be linear or globular which defines the overall structure of the protein (Phillips and Williams). The stability of the protein can vary depending on the hydrogen bond and disulphide bond formations. Few proteins also have a quaternary structure, where multiple subunits come together to form a complex molecule. Hemoglobin, myoglobin molecules in humans and animals possess a quaternary structure (Klotz et al., 1970; Cleaves, 2011). The hierarchy in structural classification of the proteins into four does not necessarily describe all of the laws, but are made for better understanding of the structural aspects (Gu and Bourne, 2009).

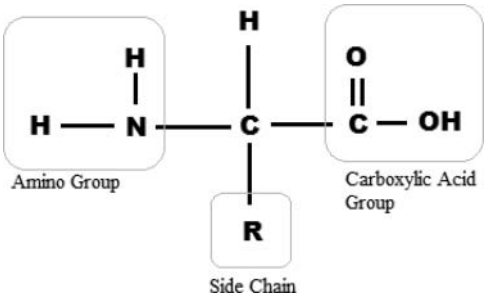


Figure 1. Common structure of amino acid.

2.1.1. Primary structure of protein

The primary structure is defined by the sequence of the amino acids forming the peptide bonds that gives the protein its unique structure. It was in 1920, Fischer and Hofmeister became the first scientists that proposed the linear structure model for proteins (Fruton, 1972). Later, the works of Sanger (Sanger, 1952) on amino acids have provided enough base that proteins follow the linear sequence and moreover, the proteins can be distinguished based on the sequence of amino acids (Gu and Bourne, 2009). As mentioned earlier, the amino acids have an alpha carbon which is bonded by a Hydrogen molecule, amide molecule (acting as N-terminal), and carboxyl molecule (acting as C-terminal). The molecule that forms the fourth bond with the alpha carbon varies which differentiates the amino acids from one another and further, it also confers each amino acid with specific chemical properties (Whitford, 2005; Gu and Bourne, 2009).

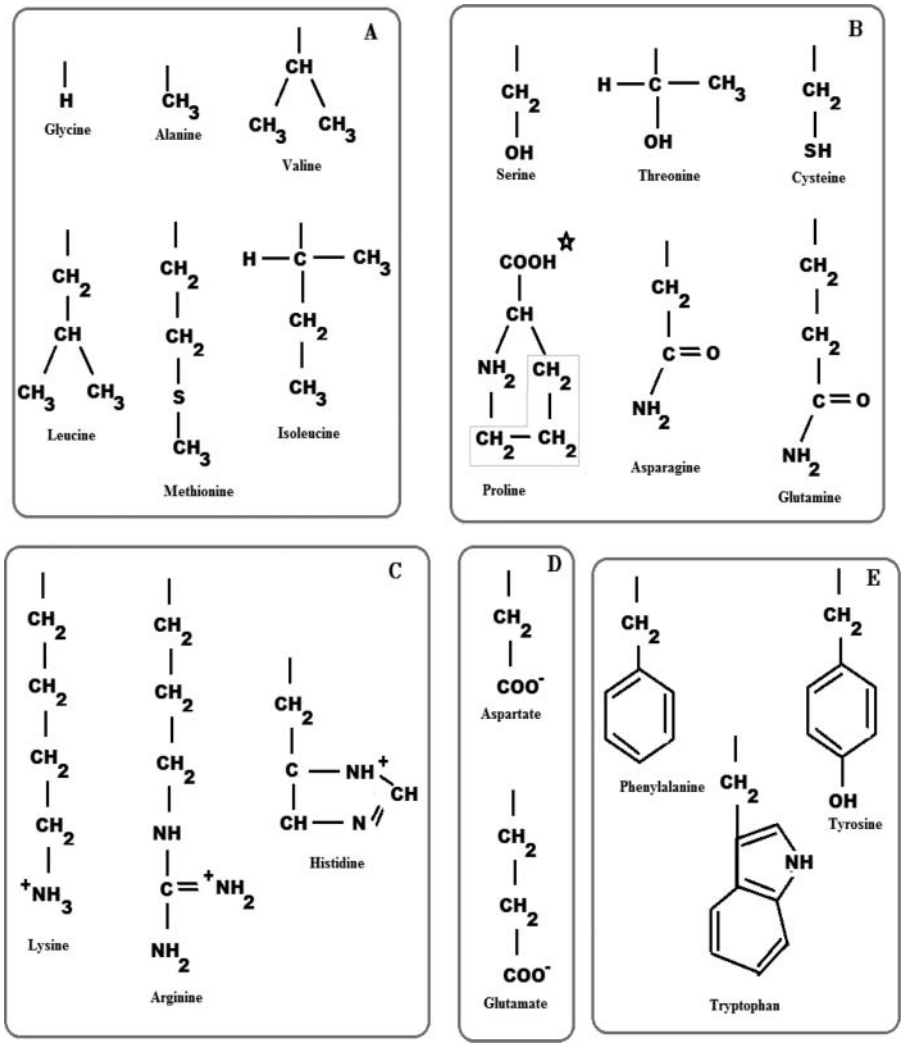
The amino acids in a protein form a covalent bond, which is termed as "peptide bond." The amino acids that form a peptide bond are called "residues" and the atoms that are involved on the formation of peptide bond become the "backbone" of the protein.

2.1.2. Secondary structure

Secondary structures are the three-dimensional ordered local features of biopolymers such as proteins. Protein secondary structures can be classified into four major categories: helices, sheets, loops, and turns. Helices are formed when amino acids in the primary structure of the protein form a winding motif, which is stabilized by formation of parallel hydrogen bonds between backbone atoms along the amino acid sequence. There are three types of helices that commonly occur in a protein: α-helices, π-helices and 3_{10}-helices (3/10 helix). These forms of helices differ in their hydrogen bond coordination, i.e. α-helices are formed when residues i and $i + 4$ form a hydrogen bond, similarly π-helices and 3_{10}-helices are formed when residues i and $i + 3$ and i and $i + 5$ form hydrogen bonds between them, respectively.

β-sheets are the second most common secondary structure observed in proteins. They consist of β-strands that are connected laterally by hydrogen bonds between backbone atoms.

Table 1. List of all amino acids and molecule attached at "R".



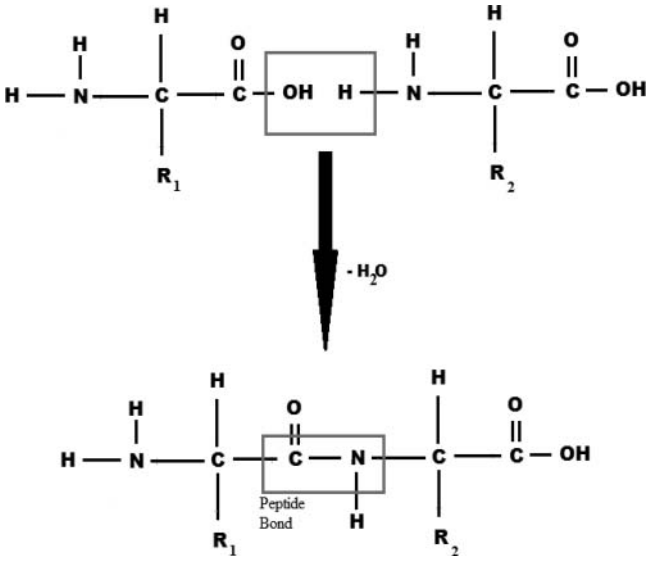


Figure 2. Peptide bond formation.

Two forms of β-sheets are commonly observed: parallel and anti-parallel. Out of these two forms, anti-parallel β-sheets are more stable due to the well-aligned hydrogen bonds.

Loops and turns are one of the most essential secondary structures observed in a protein. They enable proteins to fold and form compact tertiary structures and play a vital role in determining the flexibility of active regions in the protein responsible for enzymatic reactions and interaction with other macromolecules.

2.1.3. Tertiary and quaternary structures

Proteins tertiary structure represents its three-dimensional configuration in space. It has a single polypeptide chain backbone with one or more secondary structures (figure). Interactions and bonds of side chains within a protein determine the formation of tertiary structure; it is also driven by hydrophobic interactions between secondary structures, which is required to minimize the hydrophobic surface area of the protein that is accessible to water and other solvents. The amounts of

hydrophobic residues also determine the stability of the protein tertiary structures.

Quaternary structure of the proteins is formed when a group of proteins interact together and behave in a collective manner. Various bonding interactions including hydrogen bonds, salt bridges and disulphide bonds hold different protein chains into a specific geometry. Two major categories of protein that contain quaternary structures are fibrous and globular proteins. Fibrous proteins include, keratins and collagens which play an important structural role, whereas globular proteins such as insulin, hemoglobin, and most enzymes are involved in regulatory roles.

Protein molecules assume a wide array of functions and participate in large amount of reactions in the body. They are able to perform such distinctive functions only because of their versatility and fidelity in the structures. Thus with such large scope of structural conformations, proteins are able to perform a wide array of functions (Gu and Bourne, 2009; Singh et al., 2013b).

2.2. Proteins in human body—Allergy

Proteins perform diverse biological functions which can vary from oxygen transfer between various cells (transport proteins), Enzymatic functions (enzymatic proteins), acting as substrates for various biological reactions leading to formation of distinct compounds, defense mechanisms in the body against foreign compounds (defense proteins) and as storage proteins (Whitford, 2005). Though each protein is distinct in its structure, and composition leading to that specific structural conformation, they are formed due to the peptide bonds between the amino acids which are common in all the proteins. As mentioned earlier, though there are only 20 amino acids, the size and the diversity in the molecular weights of proteins is vast which also affects the functions performed by a protein. The molecular weights of the proteins are represented in Daltons (Da) where 1 Da = 1 amu i.e. atomic mass unit (Thus, 5500 Da = 5.5 kDa) (Whitford, 2005).

The undesirable reactions that happen to human body on consumption of food can be broadly divided into two categories:

1. Food Intolerance and 2. Food Allergy/Hypersensitivity

2.2.1. Food intolerance

"Food Intolerance" may be defined as the adverse reactions caused within the human body due to the consumption of food within the human body where the immune system has no involvement in their cause (Lopata and Potter, 2000; Lehrer et al., 2002; Andreas, 2009). Irritable bowel syndrome is commonly associated with food intolerances (Alun Jones et al., 1982; Nanda et al., 1989). Various other symptoms can be similar to that caused by food allergies which can be ranging from respiratory, gastrointestinal, and skin to psychological reactions.

2.2.2. Food allergy

Food allergy occurs when a food component, mostly an incompletely digested protein is absorbed in the blood stream and elicits an immune response. During digestion food proteins are broken down into individual components i.e., amino acids, which when absorbed in blood do not elicit any immune response, but an undigested protein or partially digested protein is treated as a foreign particle by the immune system, which then activates its IgE-mediated response to neutralize it (Sampson, 2004; Schmitt et al., 2004; Sicherer and Sampson, 2007). In predisposed allergic individuals, the advent of acute allergic reaction is due to engagement of allergen specific IgE antibodies. These antibodies with their high-affinity receptors are expressed on mast cells and basophils. When allergens interact with an antibody the receptor cells determine the release of mediators. The intrinsic biochemical properties of a food allergen also contribute towards its allergenicity. For example, peanut allergen Ara h 1 is very stable and is resistant to heat and digestive enzyme degradation, it also contains a glycan adduct which acts as a TH2 (T helper cell) adjuvant (Koppelman et al., 1999; Shreffler et al., 2006).

Common symptoms of food allergies include skin rashes, abdominal pain, vomiting, and diarrhea. The most dangerous and sometimes fatal response to food allergies is anaphylaxis, which is a whole body reaction leading to difficulty in breathing and a fall in blood pressure potentially causing a shock. At present once an individual is identified allergic to a food after intensive diagnosis using oral food challenges, skin-prick testing and antibody blood testing a complete avoidance of causing food is the only treatment. A complete avoidance of food sometimes leads to nutritional deficiencies and is one of the major driving force for researchers and industries in the field of food processing to come up with alternate novel food sources which can supplement the avoided food products.

3. Food processing

Processing of food is not a completely modern concept as archeological proofs suggest that the cavemen and hunting-gathering societies cooked their food after the discovery of fire either by direct heating (in the fire) or by boiling in water. Though preservation of food was not their primary target, they observed that cooking food increased its palatability. With the advent of the agricultural societies, people understood the importance of the storage and preservation of their produce. The processing techniques like sun drying, fermentation to produce alcohol and cereal grinding along with bread making have been developed which spread around the world. The development of the trade routes around the world in the medieval age has led to accelerated spread of knowledge regarding food preservation and processing. The scale of processing and operations has accelerated exponentially as the world population grew during the industrial revolution. The development of electricity led to introduction of electrical appliances in the early twentieth century which increased the speed of processing ten folds. Post world-war societies had growth in the confectionary, ready-to-eat foods and convenience foods (Fellows, 2000; Fellows, 2009). With the modernization of the society, the quality of the final food product has gained the importance as the consumers became more health conscious which led to the introduction of novel food processing techniques (Fellows, 2000; Fellows, 2009; Jasim et al., 2009).

Today, food is subjected to a wide range of processing treatments and conditions primarily to improve the sensory attributes and/or shelf life of the product. This is either done by removing or inactivating the toxins and microbes present in food and by altering the properties of various components. The large array of processing techniques also contributes in increasing the diversity of food products.

3.1. Processing effect on allergen stability

The cause of allergic reaction in the body in most cases would be due to a small linear stretch of amino acids or a specific three dimensional structure which is a part of a much larger protein, which are known as "epitopes." A single protein may contain distinct epitopes or just one epitope which repeats itself throughout the structure, but more than one epitope causes the IgE cross-linking. The relationship between the number of epitopes, nature of epitope and the severity of the allergic reaction caused by a certain kind of epitope is still uncertain (Sathe et al., 2005).

The processing of food involves wide array of physical, chemical, and biochemical changes which induce alteration of various components including protein and thus the allergenicity of the specific protein epitope. Depending on the processing the epitopes that are present within the food matrix may be destroyed or new epitopes may be formed which is described as "neoallergen formation" (Sathe et al., 2005; Thomas et al., 2007). These were first reported in 1974 (Spies, 1974) and researchers then believed that the neoallergens might be the cause of allergic reactions in patients who have consumed processed food, but not on consumption of unprocessed food (Sathe et al., 2005). These neoallergens are also observed in wheat flour (Leduc et al., 2003) and pecans (Malanin et al., 1995). Apart from genesis of neoallergens, processing may also cause an increase in the allergenicity of a certain protein or may cause no change in it. As ways for processing the foods are huge and varied, the effect produced by each of them on the specific type of epitope (conformational and linear epitopes) is very important for analyzing the effects on the allergenicity of a certain protein (Davis and Williams, 1998; Davis et al., 2001; Sathe et al., 2005). The food processing methods can be divided as shown in the Figs. 3 and 4 (Sathe et al., 2005; Andreas, 2009; Lopata, 2009).

3.2. Conventional food processing methods

3.2.1. Thermal processing methods

The thermal processing is primarily carried out in food for enhancing the microbial safety, texture, digestibility and detoxification. The heating of food is normally done at two temperature ranges which are pasteurization and sterilization. But the most vital factor that has to be considered is what effect heating has on the allergenicity of the allergic component present in certain foods. It can either mitigate the allergenicity or it can also enhance it by changing the native protein structure as mentioned earlier. Every protein has a native structure which is its most stable form depending on the hydrogen bonds, disulphide bonds, electrostatic, and hydrophobic interactions (Van Holde, 1977; Boye et al., 1997; Davis and Williams, 1998).

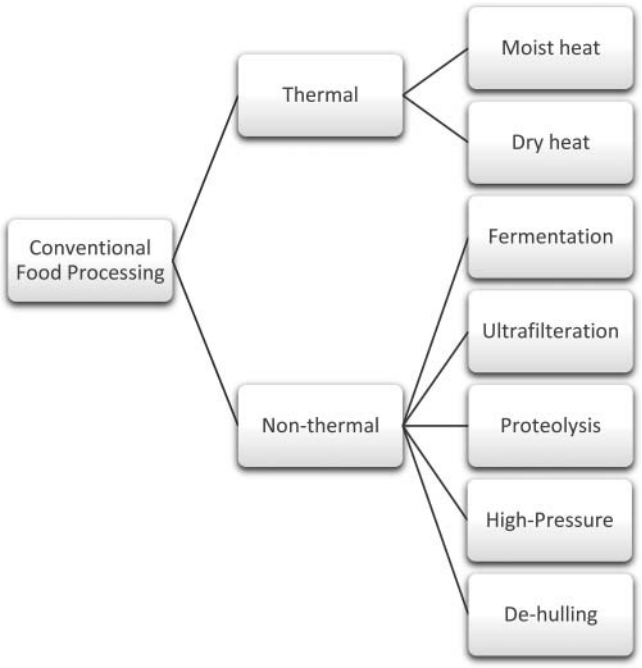


Figure 3. Classification of conventional processing methods.

When the heat energy is applied, the protein slowly starts to lose its native structure and higher is the change when the amount of heat applied increases as shown in Fig. 5.

As indicated, the molecular bonds that hold the protein allergen structures together tend to modify themselves on application of heat, which shatters the finely poised bonds and adjusts the structure accordingly. The hydrophobic groups or units of the protein structure that are normally inward (in the

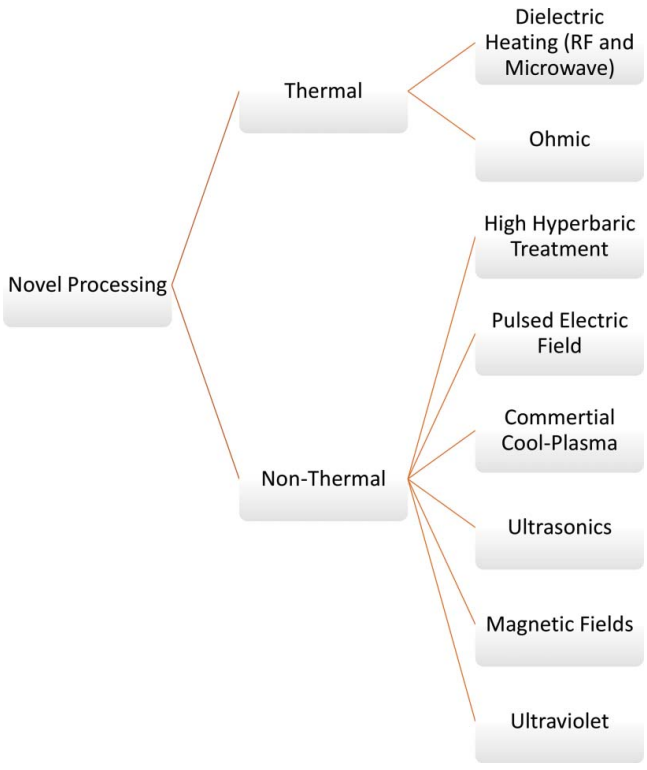


Figure 4. Classification of novel processing method.

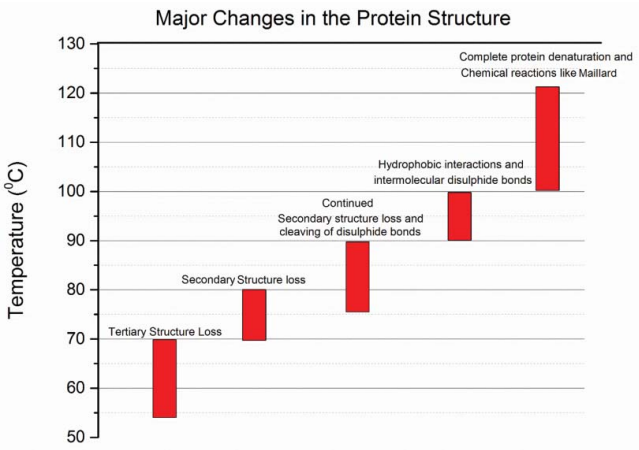


Figure 5. Protein structure profile with temperature.

stable structure), turn in opposite direction (outward) which exposes them to the unfavorable conditions (water present in the protein environment) (Davis and Williams, 1998; Davis et al., 2001). As explained in Fig. 5, the proteins tend to start losing their structure at around 50–60°C depending on their stability, size and molecular weight where loss is in the tertiary structure. And as the temperature is increased, the effect of the heat also grows which leads to a complete denaturation of protein. The temperature can also result in chemical reactions between proteins (amino acids) and other components in the food (Carbohydrates) resulting in undesirable component formation and also discoloration (Example: Maillard reaction) (Davis and Williams, 1998).

Some proteins also possess a unique quality that they tend to fold themselves back to the native structure (not completely) under the absence of external stress primarily due to the interactions between the various amino acids (among themselves) and also between the amino acids and the solvent (Tanford, 1968; Privalov et al., 1989; Boye et al., 1997). But, the changes of protein regaining the original structure are completely dependent on the extent of processing and also on the native protein allergen structure (Tanford, 1968; Thomas et al., 2007). For example, when the protein G-actin was processed to at least 30°C above its denaturation temperature, it retained about 60% of the original helical structures (Smith, 1994). Where as in another case, the molecule of β-lactoglobulin when processed, formed a complex with the α-lactoglobulin which is completely denatured and do not possess any significant similarities with the unprocessed native structures (Baer et al., 1976; Davis and Williams, 1998). It can be said that though the heat treatment results in a modification of the structure by partially unfolding or by completely changing to a random coil, there is always a possibility that the protein folds back to its native structure (at least to a certain extent) which can result in retention of allergenicity in the case of an allergen protein molecule which is the biggest hurdle for researchers to tackle.

3.2.1. (A). Moist heat

To process (heat) the food in presence of water can be considered as "moist heat processing" (e.g.,: boiling, autoclaving) (Table 2). Few protein epitopes (cross-reactive epitopes—2) that show a change in the conformational structure after moist

Table 2. Studies conducted on the moist heat processing.

Processing	Food	Studies conducted
Moist Heating (Boiling, Autoclaving)	Cherry	(Andreas, 2009)
	Apple	(Marzban et al., 2009)
	Kiwi	(Fiocchi et al., 2004)
	Celery	(Jankiewicz et al., 1996; Andreas, 2009)
	Peanut	(Beyer et al., 2001)
	Milk	(Andreas, 2009; Ehn et al., 2004; Sathe et al., 2005)
	Soybean	(Kleine-Tebbe et al., 2002; Mittag et al., 2004)
	Cashew nut	(Venkatachalam et al., 2008)
	Lentil	(Cuadrado et al., 2009)
	Chickpea	(Cuadrado et al., 2009)
	Lupine	(Álvarez-Álvarez et al., 2005)
	Eggs	(Hoffman., 1983)
	Fish	(Bernhisel-Broadbent et al., 1992a; Bernhisel-Broadbent et al., 1992b; Hansen et al., 1994)
	Shrimps	(Ayuso et al., 2002; Daul et al., 1988; Daul et al., 1994; Liu et al., 2010; Naqpal et al., 1989; Reese et al., 1999)

heating have been reported to be present in cherry and the epitopes are homologous to Bet v 1, a major pollen allergen. An another Bet v 1 homologue from apple which is called Mal d 2 also loses its conformational structure when heated to 363K and regeneration of the unfolded structure is minimal when cooled down to 293K (Andreas, 2009; Marzban et al., 2009). Api g 1 also homologous to Bet v 1, is a major celery allergen which was able to retain its allergenicity as it regained most of the initial structure of the molecule (Jankiewicz et al., 1996; Andreas, 2009). Moreover, the thermal stability of the allergen epitopes that belong to the same family also varied widely. For example, consider the Gly m 4 which is also homologous with the Bet v 1 allergen present in soya-bean. It has minimal effects from processing and it tends to retain its allergenicity in lots of high processed soya foods (Kleine-Tebbe et al., 2002; Mittag et al., 2004) suggesting that the environment and the components around the allergen molecule also play a role in the determination of the effects of external stress on the changes occurring in the protein conformational structure. This kind of thermal treatment also showed a reduced IgE reactivity of the Ara h 1 allergen of peanut to be reduced by performing the SDS-Page analysis. They obtained similar results with the Ara h 2 and Ara h 3 allergens in peanuts (Beyer et al., 2001). Also Kiwi fruit sensitivity was shown to be reduced when processed Kiwi (steamed for 5 minutes at 100°C) was consumed (Fiocchi et al., 2004). The sensitivity caused due to the β-lactoglobulin present in milk has also been extensively studied (Ehn et al., 2004; Sathe et al., 2005; Andreas, 2009). It was shown that there was a considerable amount of reduction in the IgE sensitivity of the protein as the treatment temperature increased from 74°C to 90°C.

Autoclaving is also a moist heating technique employed for sterilization purposes and changes in the reactivity of various allergens from autoclaving has been evaluated over the past few years. In a study conducted by Venkatachalam et al., cashew nuts were autoclaved at 121°C for 5, 10 20, & 30 minutes and on testing for reactivity, the processing showed very little effect

on the protein and they remained stable regardless of the processing parameters used (Venkatachalam et al., 2008). Cuadrado et al. (2009) assessed the effect of boiling (60 minutes) and autoclaving for 30 minutes at 1.2 atm and 2.6 atm on lentil and chickpea proteins. They stated that there was a reduction in the immunoreactivity of the allergens present in them, but only under severe processing conditions. It is to be noted that when processed at such extreme conditions, few highly stable reactive proteins were still present in the legumes which could cause sensitivity in patients on consumption rendering no clinical significance. Lupine flour was also autoclaved (at 138°C and 121°C) to evaluate the effect of this heat treatment on the protein. It was reported that t autoclaving at 138°C for 30 minutes completely removed the potent allergens of 23 kDa and 29 kDa previously known. But, at the same time it also generated a new IgE-binding compound with a molecular weight of about 70 kDa (Álvarez-Álvarez et al., 2005).

The effect of soft-boiling (100°C for three minutes) and hard-boiling (100°C for 30 minutes) of eggs was also evaluated. There was a significant decrease in the ovomucoid and ovalbumin present in the egg, but they observed that it can still cause IgE reactivity on testing (Hoffman, 1983). Change in allergenicity of 10 species of fish has been evaluated after boiling. It was observed that on boiling few proteins were denatured and also new higher molecular weight proteins were formed which were absent in raw fish. Though there was a decrease in the IgE binding ability of the boiled fish proteins, it was not completely destroyed (Bernhisel-Broadbent et al., 1992a; Bernhisel-Broadbent et al., 1992b). Another study by Hansen et al. (1994) also reported that the protein from codfish, herring and plaice have a very high stability as the allergens were reactive even after four hours of boiling (Hansen et al., 1994). Various studies have also been conducted on the allergenicity of boiled shrimps, but none of them produced any results of clinical importance because of the stability of the allergens (Daul et al., 1988; Naqpal et al., 1989; Daul et al., 1994; Reese et al., 1999; Ayuso et al., 2002; Liu et al., 2010).

3.2.1. (B). Dry heat

Dry heat processing of food involves minimal interaction with water molecules throughout the processing duration. Millard reaction and the enzymatic browning reactions can occur as a result of this kind of thermal processing. The Millard reaction involves a reaction between the free amino acids of the proteins with the reducing sugars (Mottram et al., 2002) (acetones and ketone) present in food resulting in the formation of aggregates which can affect the allergenicity and the gastric digestibility of the allergen compound or epitope (Thomas et al., 2007). Maleki et al. (2000) studied the effect of roasting on peanuts and has shown that the Millard reactions have increased the IgE binding capacity of allergens Ara h 1 and Ara h 2 by about 90 times compared to the raw peanuts extracts. Thus, in this case, the Millard reaction resulted in a considerable upsurge in the allergenicity. But, the Millard reactions in the case of Pru av 1 which is a major allergen present in cherries have shown a reduction in the allergenicity (Gruber et al., 2004). In another study on the effect of roasting on hazelnuts conducted by Hansen et al. (2003) showed that the allergenicity of the birch pollen related allergens of Cor a 1.04 and Cor a 2 to have decreased considerably.

Table 3. Studies conducted on the dry heat processing.

Processing	Food	Studies conducted
Dry heating (Roasting)	Cherry	(Gruber et al., 2004)
	Peanut	(Maleki et al., 2000)
	Hazelnuts	(Hansen et al., 2003)
	Cashew nuts	(Venkatachalam et al., 2008)
	Almonds	(Venkatachalam et al., 2002)

Dry roasting of few varieties of tree nuts has also been widely evaluated. Cashew nut flour proteins have been evaluated for their allergic activity after dry roasting. It was assessed that the allergic compounds present in the flour were relatively unchanged when heated to temperatures of 140°C (20 minutes and 30 minutes), 175°C (15 minutes and 20 minutes), and 200°C (10 minutes and 15 minutes) (Venkatachalam et al., 2008). In a similar study which was conducted on roasted almonds, which were roasted to a temperature of 137.7°C, 148.8°C, 160°C (20 minutes & 30 minutes); 168.3°C and 176.6°C (8 minutes, 10 minutes, & 12 minutes) also showed a very high heat stability (Venkatachalam et al., 2002) (Table 3).

It is quite clear that the thermal processing which involves the conventional/traditional heating methods have an impact on the protein structure and thus on the allergenicity. But, there are a few cases that resulted in an increased reactivity of the allergen which is not desirable. So a careful analysis has be conducted on the impact of processing on allergenicity of the allergen compounds. Also the effect of the thermal processing on the type of epitope present in the protein is different and thus, it should also be considered (Sathe et al., 2005) as another factor in the evaluation which makes the study of the protein structures a critical factor in achieving the desired results.

3.2.2. Nonthermal processing

Nonthermal processing involves wide variety of processing techniques that do not involve heating the food necessarily to impart a certain change in the product. Currently, there are a large number of processes that fall under this category which induce a change in the conformation structure of the protein (Table 4).

Table 4. Studies conducted on nonthermal conventional processing methods.

Processing	Food	Studies conducted
Proteolysis/ hydrolysis	Rice	(Watanabe et al., 1990)
	Peanut	(Sen et al., 2002; Sathe et al., 2005)
	Soybean	(Herman et al., 2003; Wilson et al., 2005; Thomas et al., 2007)
	Milk	(Cocco et al., 2003; El-Ghaish et al., 2010a; El-Ghaish et al., 2011a; El-Ghaish et al., 2011b; Pescuma et al., 2009; Pescuma et al., 2011)
Ultrafiltration	Peach juice	(Brenna et al., 2000)
	Shrimp	(Nagpal et al., 1989)
	Infant foods	(Besler et al., 2001)
Fermentation	Soy Products	(Herian et al., 1993; Ogawa et al., 2000)
	Milk	(Ehn et al., 2005; Bu et al., 2010; Jedrychowski, 1999)

3.2.2. (A). Proteolysis

Proteolysis or hydrolysis is a reaction that breaks the peptide bond with in the primary structure of the protein between various amino acids, resulting in formation of highly reactive and soluble units of amino acids. This process is also known to produce bitterness which is due to the presence of few specific amino acids formed as a result of hydrolysis; hence not preferred in the processing of foods (Hettiarachchy, 2012). Hydrolysis of proteins can be done either by using acids or enzymes. It was showed that the acid hydrolysis of gluten protein has reduced the foaming ability but had enhanced the emulsifying properties and solubility (Wu et al., 1976). Various enzymes are also used for proteolysis of proteins. This often produces deviations in various protein characteristics which include solubility, foaming property, emulsifying property, and water-binding capacity (Kuehler and Stine, 1974; Wu et al., 1976; Chobert et al., 1988; Panyam and Kilara, 1996; Diniz and Martin, 1997; Qi et al., 1997; Hettiarachchy, 2012).

Thus, we can say that proteolysis potentially disrupts the protein structure which modifies its functional properties and moreover, they can effectively change the structure of both the linear and the conformational epitopes present in food protein. The extent of the processing is highly dependent on the amino acid sequence, secondary structure, and also on the modifications in the structure after the action of a specific enzyme (Thomas et al., 2007). In vitro hydrolysis has been performed on the Ara h 2 protein allergen of the peanut which changed the secondary structure of the molecule drastically. But, in the same study, they observed that this change in the secondary structure has not affected the allergenicity of the Ara h 2 allergen because it was able to retain the linear epitopes within the structure after hydrolysis (Sen et al., 2002; Sathe et al., 2005). Watanabe et al. (1990) reported that when rice was treated with the Actinase enzyme, it decomposed the globulin protein responsible for sensitivity in the patients to a great extent. When the treated rice clinically administered to seven patients who were sensitive to rice, only one showed any allergic reaction. Similar results have been shown with the proteolysis of the soybean (Herman et al., 2003; Wilson et al., 2005; Thomas et al., 2007). In the case of milk, proteolysis is widely associated with the reduction in the number of epitopes which results in the reduction of allergenicity caused due to the milk products particularly infant foods. Protein enzyme treatments are included in production of hypoallergenic infant foods so that the cases of severe allergic reactions can be minimized in infants (Cocco et al., 2003; Pescuma et al., 2009; El-Ghaish et al., 2010a, 2010b; El-Ghaish et al., 2011a, 2011b; Pescuma et al., 2011).

Hence, hydrolysis of proteins can mitigate the allergic properties of few proteins, but further research has to be done in developing specific enzymes that act only on a particular protein causing the allergic reaction in the body. Furthermore, care should be taken such that the proteolysis with enzymes do not result in formation of new epitope centers in treated proteins which could cause a detrimental impact on human health.

3.2.2. (B). Ultrafiltration

Ultrafiltration may be defined as a membrane separation process that is activated by pressures in the range of 2–10 atmospheres resulting in removal of high molecular weight solutes from the solutes having lower molecular weight (Sivasankar, 2002). Ultrafiltration finds wide applications in the food industries involving the protein separations. It is used in the Whey protein separation in the cheese manufacturing industries. It is also widely used in the protein concentrate production for the skim milk for the soft cheese manufacturing (Sivasankar, 2002). Ultrafiltration is also widely used in clarification of various fresh fruit juice products (Kirk et al., 1983; Hsu et al., 1987; Jiraratananon and Chanachai, 1996; Jiraratananon et al., 1997; Vladisavljević et al., 2003; Smith and Charter, 2011). A study was conducted by Brenna et al. (2000) on the peach allergies in juice where they evaluated the effect of ultrafiltration. They have subjected the peach juice to a final ultrafiltration step and they were able to produce a hypoallergenic juice where the ultrafiltration process was able to separate out the epitopes or the proteins containing the epitopes causing allergic reactions. But, they also mentioned that the ultrafiltration step has removed the important components of the juice (like minerals, gums, pectin, sugars) which resulted in a poor quality hypoallergenic juice which lowered sensory attributes (Brenna et al., 2000; Sathe et al., 2005). Moreover, researchers were also able to extract the allergen Sa 1 from the shrimp with the ultrafiltration process in combination with other techniques (Nagpal et al., 1989). Also, ultrafiltration is used in combination with heat denaturation and enzyme proteolysis for production of hypoallergenic infant products (Besler et al., 2001). Olofsson et al. (1981) also said that large peptides that cause allergic reactions can be removed by ultrafiltration process such as fractions of polypeptides having high molecular weights after proteolysis treatment (Adler-Nissen, 1986).

These studies suggest that the ultrafiltration can be effective in separation of allergic compounds from the product when used in combination with other products. But, membranes have to be developed with highly accurate porosity and specificity which could only separate out the specific allergens based on the molecular weight.

3.2.2. (C). Fermentation

The fermentation is a microbial process where the microbes act on food producing enzymes which impart change in the texture, flavor, and various other characteristics such that the final product can be consumed (Bamforth, 2005). Evidences suggest that fermentation was a very old process (Egyptian Civilization) to preserve foods especially milk and fruits which particularly have a very short shelf life when not processed (El-Mansi, 2012). In 1907, Metchnikoff first suggested that the fermented foods are known to have a few health benefits because of the presence of certain kind of microbes (Metchnikoff, 1907). Further research was conducted on fermented foods and it came into light that the presence of Lactic Acid bacteria (LAB) in certain foods and also overall fermentation can promote better health (Elmer et al., 1996; Salminen et al., 1998; Macfarlane and Cummings, 1999; Matsuzaki and Chin, 2000; Anekella and Orsat, 2013).

In todays' society, this process becomes very prominent because of the wide range of products that can be produced from fermenting cereals, legumes, milk and meats which have particularly high content of protein. Thus, changes in the

nutritive content especially in the proteins are a very important aspect during the fermentation which has to be further studied. But, the analyses have indicated that the amount of changes found were minimal (McFeeters, 1988). Studies have also been conducted on assessing the effects of fermentation on the allergens especially soybean products and milk.

Soy products that were analyzed included tofu, miso, tempeh, sprouts, and mold hydrolyzed soy sauce for finding the effects caused by the fermentation process on the allergenicity. It was reported that these products retained the allergic properties, though the binding capacity of the IgE have diminished considerably (Herian et al., 1993). In 2000, techniques like chemical breeding, enzymatic digestion and a physicochemical treatment were used in the development of the hypoallergenic soybean products. They used processing techniques to mitigate the three major allergens in the soybean which are Gly m Bd 60K, Gly m Bd 30K, and Gly m Bd 28K and tested the products using patients allergic to soybean. Their results suggested that about 80% of the patients who are allergic to soybean did not show any adversarial reactions after consumption of the processed hypoallergenic products (Ogawa et al., 2000). In the case of milk, β-lactoglobulin and casein are considered as the most potent allergens (El-Ghaish et al., 2011a). Ehn et al. (Ehn et al., 2005) worked on the effects of fermentation and proteolysis on the β-lactoglobulin, and found that both lactobacilli fermentation and proteolysis with trypsin had reduced the IgE binding capacity. But the reduction observed with proteolysis is higher compared to that of the protein denaturation by acid (Ehn et al., 2005; Sathe et al., 2005). Bu et al. observed that the allergenicity of β-lactoglobulin was reduced when fermentation began, but as the duration of the fermentation process increased, there was no linear relation between the allergenicity reduction and the amount of proteolysis of the protein (Bu et al., 2010). A 99% reduction was observed in the allergenicity of α-lactalbumin and β-lactoglobulin when sterilized cow's milk was treated with meso- and thermophilic strains or mixed cultures when tested with enzyme-linked immunosorbent assay ELISA, but there was hardly any effect when skin-prick tests were conducted (Jedrychowski, 1999).

3.3. Novel food processing methods

3.3.1. Thermal processing methods

Increasing concerns about the environment and the emission of Green House Gases (GHG's) led researchers to think about the alternative methods of manufacturing in various processing industries including food industries around the world. This led to the development of energy efficient and green technologies in food processing industries which are the novel technologies. The materialization of the novel technologies not only saves the energy costs and wastage of the natural resources, but it has been shown that the final quality of the product is well preserved compared to the products produced using conventional techniques (Pereira and Vicente, 2010). Thus, the thermal processing using novel technologies has become popular in recent past with their lower production cost advantages and the ability to maintain better quality of the final product. But, the effects of these processing techniques on the various components are rarely studied especially on the allergens except in processing using microwaves.

3.3.1. (A). Microwave heating

Microwave is an electromagnetic wave within the frequency band of 300 MHz and 300 GHz. These waves find applications in variety of areas which include telecommunications, medical, and other scientific fields (Richardson, 2001). In relation to food industry, microwaves are used for thawing of frozen foods, pasteurization, drying, and pre-cooking (meats like beef). This is primarily due to the advantages that microwave heating provides over other heating methods which include efficient heating, less time to start, easy for mass commercialization and food with better nutritional and sensory qualities (Decareau and Peterson, 1986; Sun, 2005). But, microwaves of all the frequencies cannot be used for the food applications. There are two specific frequencies that are dedicated to the industrial applications (food industry) which are 915 MHz and 2.45 GHz (most commercial ovens) (Tewari, 2007; Doona, 2010).

There are two main principles involved in the microwave heating of food. The first and the most important is the "dipole rotation" and the second one is the "ionic polarization" or "ionic conduction." For the dipole rotation to occur the presence of polar molecules is very important. The most common polar molecules found in food is the water molecule that is randomly oriented all over food. But, in the presence of an electric field (like microwave), the molecule tries to align itself to the orientation of the field appropriately. In a microwave, the field alternates at a very high frequency (2450 MHz) and when the polar molecule is present, after aligning themselves to the field, the resulting interactions between the fast moving (rotating) polar molecules and the other molecules in food produces friction resulting in the heating effect (Oliveira and Franca, 2002; Tewari, 2007; Doona, 2010). The ionic polarization occurs in the presence of high concentrations of ions in the food. Under the influence of the electric field, the ions in food collide with each other and thus dissipate their kinetic energy which is converted to thermal energy on collision (Oliveira and Franca, 2002; Meda et al., 2005; Tewari, 2007; Doona, 2010). In fact, it is said that the temperature of food can be raised by about 10°C per second with the high intensity of heat produced in microwave processing (Lew et al., 2002; Meda et al., 2005).

Thus, the microwave processing generates heat instantly and the amount of heat generated is highly dependent on the local composition and the overall homogeneity of the food product. Moreover, the heat transfer rate within the food relies on the composition (presence of water) and on the geometry of the food. There are a wide variety of changes caused due to the generation of heat in food during microwave processing. Some of them include gas/water vapor generation (baking), starch gelatinization (corn, potatoes, etc.,), protein denaturation (milk & egg), surface browning (Millard Reaction in baking), caramelization (due to sugar dehydration), and enzyme inactivation. They can also effect the textural and organoleptic properties of the food. This suggests that the microwave processing does have an effect on the components of food (Mudgett, 1989; Ponne and Bartels, 1995; Meda et al., 2005; Schubert, 2005). Pomerai et al. (de Pomerai et al., 2003) also studied the effect of the microwaves on the protein conformation without the bulk heating. In this study, they have

reported that microwaves not only have a thermal affect as we have seen, but they also have a nonthermal effect on protein conformation especially in biological proteins (de Pomerai et al., 2003). The surface properties of any protein might change on interaction with the microwaves because of variation in bonds with the water molecules and other ions (Meda et al., 2005). Studies have also been conducted on understanding the effect on microwave processing on the allergen proteins present in food.

The microwave processing effect on three important allergens present in celery has been evaluated by Jankiewicz et al. (1997). The three allergens in celery are Api g 1, celery profilin and multiple allergy bands recognized by the patient's IgE within the mass range of 35 kDa–90 kDa (called Carbohydrate epitopes). Two kinds of microwave treatments have been conducted on the celery; first at 750 W for 10 minutes where the product temperature was around 100°C and the second at 750 W for 30 minutes where the product temperature was also around 100°C. They have reported that the allergic activity of celery has been reduced under thermal processing. The thermal denaturation (at 100°C for 20–30 minutes) have showed no binding of Api g 1 and Profilin allergens and reduced binding in the Carbohydrate epitopes when tested. The Api g 1 showed reduced to no binding with 10 min processing in the microwave at 100°C, whereas profilin had to be processed for 30 minutes to get the same reduction. Carbohydrate epitopes were relatively very stable when processed even for 30 minutes at 100°C. Moreover, they also concluded that further work has to be conducted on the structural changes in the epitopes to understand their modifications under the heat and other external stresses providing more detailed insight into processing effects on them (Jankiewicz et al., 1997).

Gall et al. (1994) evaluated the effect of microwave treatment on the kiwi fruit allergen. They have also reported that the patients with sensitivity towards kiwi fruit allergen have showed high cross reactivity with apple allergen and birch pollen allergen and moderate reactivity towards carrot and avocado. The kiwi fruits were treated in the microwave at four levels of temperature (40°C, 60°C, 80°C, & 90°C) and they reported that the allergenicity of the kiwi fruit decreased with the increasing temperature which was evaluated by skin prick test (SPT). Microwave processing (700 W for 25 minutes) on the soybean was also evaluated. When enzyme-allergosorbent test (EAST) were conducted, nine out of a total of 15 patients showed reactivity towards the microwave processed soybean allergen (Vieths et al., 1995; Besler et al., 2001).

The effect of microwaving on the allergenicity of various nuts has also been evaluated. Wigotzki et al. (Wigotzki et al., 2000) evaluated the IgE binding activity of the hazelnut allergens when processed under microwave and also with conventional heating. They processed the nuts until they reached the temperatures of 155°C or more, but the effect on the allergenicity was minimal. They also mentioned that the allergens of lower molecular weight had higher heat stability (at temperatures up to 190°C). In another study, the effect of microwave processing on almonds has been evaluated. They found that the effect of processing on the proteins was minimal but when treated at extreme temperatures (160°C for 20–30 minutes and then three minutes of microwave heating) changes the allergenicity. But, under normal processing conditions the allergens in almonds are relatively stable to heat treatments (Venkatachalam et al., 2002). Su et al. (2004) also assessed the allergenicity of almonds, cashew nuts and walnuts under microwave processing (500 W for one minute and three minutes) in combination with γ-irradiation (1–25 kGy) and they reported that the allergens were very stable under the processing conditions (Su et al., 2004). Lupine flour was also reported to be responsible for allergies in few patients and hence the effect of microwave treatment on its flour was evaluated. It was reported that treatments extending up to 30 minutes under microwave had almost no effect on the reactivity of the allergens (Table 5) (Álvarez-Álvarez et al., 2005).

Table 5. Studies conducted on microwave processing (novel-thermal) methods.

Processing	Food	Studies conducted
Microwave processing	Kiwi	(Gall et al., 1994)
	Soybean	(Besler et al., 2001; Vieths et al., 1995)
	Hazelnuts	(Wigotzki et al., 2000)
	Cashew nuts	(Su et al., 2004)
	Almonds	(Venkatachalam et al., 2002; Su et al., 2004)
	Walnuts	(Su et al., 2004)
	Lupine	(Álvarez-Álvarez et al., 2005)

3.3.2. Nonthermal processing methods

Novel nonthermal processing methods involve the processing methods that do not heat up the food i.e., the processing is done under sub-lethal temperatures, unless the heat is generated internally (resistive heat). Various nonthermal processes which have been evaluated for processing foods include high hydrostatic pressure processing, gamma-radiation (γ-radiation), pulsed electric field processing, high-intensity ultrasound, and ultraviolet light processing. The increasing pressure for commercialization of these processes makes it important for researchers to study their effect on the various component of the food, especially the allergens. Though not widely studied, researchers are showing keen interest in recent times as their advantages have been accentuated (Table 6).

3.3.2. (A). Gamma-radiation (γ-radiation)

The process of exposing food to an ionizing radiation is called "Irradiation." Though wide range of ionizing radiations is available, the treatment with gamma-radiation is widely employed over the others. These rays are emitted through the radio isotopes Cobalt—60 and Cesium—137 (Farkas, 2006). These are primarily used for increasing storage duration by destroying the surface pathogens present on the food. By varying the dosage of radiation, various other desired effects are achieved which can be extension of shelf life, microbial destruction, and reduction in the losses of produce during storage (Lagunas-Solar, 1995; Farkas, 2006). The process of irradiation is also environmentally safe and is an energy efficient process which adds to its marketability (Lagunas-Solar, 1995; Farkas, 1998). The radiation in the ranges of 2–7 kGy (medium dosage level) can eliminate the surface pathogens in the food products without affecting the organoleptic and nutritional qualities of the food (Farkas, 1998). Researchers also mentioned when irradiation is used in combination with other processing treatments,

Table 6. Studies conducted on nonthermal novel processing methods.

Processing	Food	Studies conducted
Gamma radiation	Shrimp	Byun et al., 2000; Byun et al., 2002; Sinanoglou et al., 2007; Zhenxing et al., 2007a, 2007b)
	Egg	Byun et al., 2002; Kume and Matsuda, 1995; Seo et al., 2007; Seo et al., 2004; Lee et al., 2005)
	Milk	(Lee et al., 2001)
	Cashew nuts	(Su et al., 2004)
	Walnut	(Su et al., 2004)
	Almonds	(Su et al., 2004)
Ultraviolet light	Peanut	(Chung et al., 2008; Yang et al., 2012)
	Soybean	(Yang et al., 2010)
	Shrimp	(Shriver et al., 2011)
	Almonds	(Li et al., 2013)
	Egg	(Manzocco et al., 2012)
	Milk	(Tammineedi et al., 2013)
Ultrasound	Shrimp	(Zhenxing et al., 2006; Li et al., 2006)
	Milk	(Stanic-Vucinic et al., 2012; Tammineedi et al., 2013)
High pressure/high hydrostatic pressure	Milk	(Chicón et al., 2008a; Chicón et al., 2008b; Peñas et al., 2006a; Peñas et al., 2006b; Bonomi et al., 2003; Zeece et al., 2008; López-Expósito et al., 2012; Zhong et al., 2011; Kleber et al., 2007)
	Soybean	(Peñas et al., 2011; Li et al., 2012)
	Shrimp	(Kim et al., 2006)
	Rice	(Kato et al., 2000)
	Almond	(Li et al., 2013)
	Apple	(Houska et al., 2009)

even a low or medium level exposure would actually decrease the chances of the microorganisms to survive in the end product (Lagunas-Solar, 1995; Farkas, 1998; Lado and Yousef, 2002; Farkas, 2006).

With their potential and wide range of applications in disinfection and processing of foods, their effect on the allergenicity of various allergens have also been evaluated, especially in shrimps (Byun et al., 2000; Byun et al., 2002; Sinanoglou et al., 2007; Zhenxing et al., 2007a, 2007b), tree nuts (Su et al., 2004), egg (Kume and Matsuda, 1995; Byun et al., 2002; Seo et al., 2004; Seo et al., 2007), and dairy products (Lee et al., 2001). Byun et al. (2000) studied the effect of gamma radiation on the heat stable proteins present in the Brown shrimp, which were extracted and then irradiated using gamma radiation with dosage levels varying from 0–10 kGy. They reported that a conformational change in the structure of the protein which was deducted by the spectrometric techniques. Also, the IgE binding ability of the heat stable protein allergen was tested using the protein sera from human patients and it was observed that it reduced with the irradiation treatments (Byun et al., 2000). In another study conducted by Zhenxing et al. (2007b) similar results were observed. They also reported the dependency of the irradiation dosage on the allergenicity of the shrimp protein. As the dosage increased from 0 kGy to 5 kGy, the allergenicity increased, but when the dosage of irradiation increased beyond 10 kGy, they observed that the allergenicity of shrimp protein reduced significantly as showed by immunoblotting and ELISA tests (Zhenxing et al., 2007b). These researchers also reported that the combination of thermal processing (boiling) after irradiation using gamma radiation showed better results by further decreasing the immunoreactivity of the shrimp protein (Zhenxing et al., 2007a).

Lee et al. (2001) evaluated the effect of gamma radiation treatments on the milk allergens. They reported a reduction in the solubility of the proteins and also a rise in the turbidity which was caused by the agglomeration of the irradiated protein. All of the results suggest that there is a conformational change in the protein structure due to the irradiation treatment on α-casein and β-lactoglobulin (Lee et al., 2001). The effect of irradiation using gamma radiations on Ovalbumin, which is a major protein allergen present in egg white was also evaluated. It was reported that the gamma radiation was able to impart conformational changes in the protein and furthermore, they also specified that the gamma radiation can be used for allergy control specific to Ovalbumin (Seo et al., 2004; Lee et al., 2005; Seo et al., 2007). Su et al. (2004) studied the impact of gamma irradiation on the almonds, cashew and walnut protein allergens (tree nuts) and they reported that the irradiation alone or in combination of another heat treatments did not affect the allergenicity of protein allergens in any of the specified nuts.

Food irradiation using gamma radiations seems to be an effective alternative processing technique for coping with the increasing allergy cases. Though, the results seem to indicate that the irradiation can result in producing conformational changes in protein structure, further research has to be conducted on studying their clinical relevancy.

3.3.2. (B). Pulsed ultraviolet light (PUV)

With growing concerns regarding the excessive use of chemical preservatives in food for extending the shelf life and protecting food from pathogens, alternative preservation techniques have been developed. UV radiations are already being used widely all round the world for the water and air disinfection treatment and decontamination of surfaces in labs. Recently, its use as a replacement of traditional thermal and chemical treatments for shelf life extension in foods is being investigated. These radiations can be used for pasteurization of fresh juices, postharvest treatments and disinfection of food contact surfaces in processing plants. The wave length of UV rays range from 100 nm to 400 nm. With the increasing demand of using the UV light for various treatments in food industry, the effects of it on the complex food matrix made of distinct components has been investigated widely. Its potential use as an alternative treatment for mitigating allergens in food is also explored (Koutchma, 2009).

Chung et al. (2008) evaluated the effect of PUV treatment on peanut extract and liquid peanut butter and reported a marked reduction in the IgE binding capacity of the allergen present in them. It was observed that the allergen with molecular weight 63 kDa displayed a reduced solubility suggesting a change in its structural conformation. But, on the other hand, the allergen protein of molecular weight 20 kDa showed no change (Chung et al., 2008). Yang et al. (2012) also conducted similar studies on peanut allergens by using PUV. They also reported a reduction in the IgE binding capacity after the treatment of Ara h 1, Ara h 2, and Ara h 3 allergens which are the major peanut allergens. Similar studies were also conducted on the soybean allergen by Yang et al. (2010) where the effect of PUV treatment has been investigated. They also reported a reduction in the soybean allergenicity by up to 50% when treated for about 6 minutes. They concluded mentioning that PUV treatment can

be used in production of lower allergenic soy products, but relevant clinical analysis have to be conducted (Yang et al., 2010). Shriver et al. (2011) also conducted studies to evaluate the IgE binding capacity of the shrimps when treated with PUV. This study also concluded that treating tropomyosin which is the major shrimp allergen with PUV for about 4 minutes reduced its IgE binding capacity. Li et al. (2013) investigated the effect of PUV on the allergenicity of almond protein extracts. The protein extracts of almond were exposed to PUV from 0.5 min to 10 min and their IgE binding capacity was evaluated using the Electrophoresis, Western Blot and ELISA. They reported that PUV to have reduced the IgE binding capacity in the almond extracts better than boiling treatment.

Manzocco et al. (2012) studied the ultraviolet processing on the egg white and they reported no change in the immunoreactivity even though it was sensitive to the UV radiation. The effect of UV light (specifically UV-C) has been evaluated by Tammineedi et al. (2013) on allergenicity of casein and whey proteins (α-lactalbumin and β-lactoglobulin). In this study the major protein allergens extracted from milk were treated for 15 minutes resulting in significant reduction of allergenicity. The electrophoresis tests also revealed that the three protein bands tested had showed a reduced intensity after the treatment with UV radiation.

The use of UV rays for producing hypo allergic food products is possible as most of the work suggests that this treatment had reduced the allergenicity of the allergens. But, clinical tests and in vivo studies have to be conducted for determining the applicability of this technology on a large scale.

3.3.2. (C). Ultrasound treatment

Ultrasound treatments use mechanical waves of frequencies varying between 20 kHz–100 kHz (Feng et al., 2011) which results in agitation and formation of bubbles in the food system due to compression and rarefaction and these bubbles formed collapse eventually when they reach their critical size. When these bubbles blast, the temperatures and pressure can go up to 5000 K and 1000 atm in the local region. These extreme parameters would result in conformational changes in the structure of the protein allergens (Shriver and Yang, 2011). It was also reported by Sonia et al. (Soria and Villamiel, 2010) that physical effects on the components in food can also be caused due to the high shear stress and velocity gradients resulting in micro streams and moreover, the protein modification can also be a result of the radicals generated from water (Soria and Villamiel, 2010; Shriver and Yang, 2011). The work done on the effect of ultrasonic treatment on allergens is very limited to date.

Zhenxing et al. (2006) assessed the effect of high intensity ultrasound on the structures of protein extract from shrimps. He also compared the results with the effect on boiling of shrimp by studying protein by Electrophoresis and ELISA tests. They treated the protein extract with high intensity ultrasonic waves of 30 Hz and 800 W for about 1.5 hours with temperatures ranging from 0°C to 50°C. This study reported that the ultrasonic treatment has reduced the allergenicity of the shrimp protein extract especially the ultrasonic treatment at 50°C. Another study was also conducted which evaluated the effect of ultrasonic treatments on tropomyosin allergen present in the shrimp protein by Li et al. (2006). They also reported a reduction in the IgE reactivity after high intensity ultrasonic treatment of tropomyosin. Moreover, they also observed a rise in the formation of lower molecular weight protein fragments with prolonged treatment times which suggests that the ultrasonic treatment has an effect on the protein conformation (Li et al., 2006).

Stanic-Vucinic et al. (2012) investigated the effect of high intensity unltrasonification on the milk allergenicity especially the β-lactoglobulin. They reported that the ultrasonic treatment has resulted in a very minor changes in the IgE binding capacity of the β-lactoglobulin allergen even though a considerable amount of structural deviations were observed. They concluded that though sonication treatment resulted in conformational changes in the structure of proteins and allergens, there is no significant effect on the allergenicity of the milk proteins (Stanic-Vucinic et al., 2012). Tammineedi et al. (2013) have also reported similar results where they evaluated the effect of high intensity ultrasound treatment on whey proteins and casein which are major milk allergens.

3.3.2. (D). High hydrostatic pressure (HHP)/high pressure (HP)

The use of hydrostatic pressure in the field of food processing was initiated for inactivation of the micro-organisms for preservation of fruits and vegetables (Hite et al., 1914) and milk (Hite, 1899). This led to the expansion of new microbial inactivation technique which was also widely studied for understanding its effectiveness on bacteria and viruses (Basset and Macheboeuf, 1932; Basset and Macheboeuf, 1933; Basset et al., 1933; Basset et al., 1935a; Basset et al., 1935b; Atanasiu et al., 1951; Vignais et al., 1952; Basset et al., 1956). Later on, researchers have observed the potential of the high pressure or high hydrostatic pressure treatments in food processing and evaluated the effect on various food components like enzymes, proteins and various other bio molecules (Morild, 1981; Popper and Knorr, 1990; Knorr, 1993). Researchers understood that the conformational structure can be changed by using pressure as a factor (Rivalain et al., 2010). Moreover, studies conducted by Zhang et al. (Zhang, 1995) and various other researchers revealed that the conformational changes in the protein caused by the high pressure treatment can be different from those changes induced by the heat denaturation and can also result in preservation of few specific structures of the biomolecule (Zhang, 1995; Smeller, 2002; Winter et al., 2005). Thus, the effects of high hydrostatic pressure on allergen proteins have been widely studied especially the milk allergens.

The use of HHP systems in combination with proteolysis treatments have been widely looked on for producing hypoallergenic foods especially whey protein hydrolysates. Chicón et al. (2008a) concluded that the HP treatments (200 MPa to 400 MPa) performed on whey protein isolate resulted in no change of the IgE binding capacity compared to the untreated sample. They mentioned that the HP treatments of 400 MPa had conformational changes in the protein structure and digestibility, but did not affect the allergenicity. In another study conducted by Chicón et al. (2008b) the effect of HP treatment on the enzymatic proteolysis and in turn on the IgE binding capacity were evaluated. Their results show that the high pressure treatment has resulted in a reduction of time required for the proteolysis from about 48 hours (when done at

atmospheric pressure) to within 20 minutes (for trypsin). But, they also reported that the hydrolysates produced through this accelerated process showed IgE reactivity when tested. Thus, it was observed that even though the HP treatment resulted in accelerating the process incredibly, it was unable to completely produce a hypoallergenic product. Further, they also mentioned that it is possible to use HP treatment in accelerated proteolysis treatment with modified parameters which can result in production of hypoallergenic products (Chicón et al., 2008b). Peñas et al. (2006b) further analyzed the relation between the proteolysis and HP treatments. They reported that the combination of treatments can not only accelerate the hydrolysis, but allergenicity can also be reduced depending on the type of enzyme used. The enzymes Corolase PN-L and Neutrase showed a considerable reduction in the IgE binding when treated at 300 MPa for about 15 minutes during proteolysis (Peñas et al., 2006b). They also reported in another study that the enzymes like pepsin, trypsin, and chymotrypsin have a better effect on the allergenicity of β-lactoglobulin when treated under HP (Peñas et al., 2006a) and same results were also obtained by Bonomi et al. (2003). They reported that the reduction in the epitope binding sites might be caused by the unfolding of the hydrophobic core of the β-lactoglobulin protein (Bonomi et al., 2003). Zeece et al. (2008) and López-Expósito et al. (2012) also investigated the effect of HP processing on the β-lactoglobulin using an *in vitro* pepsin digestion and electrophoresis. They reported that the HP treatments have increased the digestibility of proteins when treated at 400 MPa for only about 10 minutes and this treatment has a potential application in manufacturing of hypoallergenic products. Zhong et al. (2011) reported data on the effects of dynamic HP treatments in combinations with temperatures ranging from 70°C to 90°C. Their data suggested that the temperature has enhanced the effect of high pressure processing in reducing the allergenicity when treated above 160 MPa. They also reported that the treatments below the pressure of 80 MPa led to an increase in the allergenicity of the β-lactoglobulin, but it reduced when parameters were above 80 MPa. But, on the contrary, study conducted by Kleber et al. (2007) revealed that HHP treatments at 200 MPa, 400 MPa and 600 MPa at temperatures between 30°C to 68°C (treatment times: 0, 10, and 30 minutes) resulted in an increased allergic responses when tested using ELISA. But, the treatments at temperatures 60°C and 68°C did show a reduction in allergenicity, but it was not very significant.

Apart from the milk allergens, effects of HP treatments have also been evaluated in various other products. Hydrostatic pressure effect on soybean allergens have been evaluated by Peñas et al. (2011) and Li et al. (2012). Peñas et al. (2011) reported that only the HHP process does affect the allergenicity of the soybean. When HHP treated seeds were sprouted, they had a huge reduction in the allergenicity compared to sprouts that were not treated with HHP (prior to sprouting). Thus, this study showed that HHP treatment prior to sprouting would be a great approach in producing hypoallergenic soybean products. Li et al. (2012) reported that the HHP treatments had significant effect on the secondary structures of the soy allergens and this treatment had a great potential in reducing the allergenicity helping in manufacture of hypoallergenic soy based infant formula.

Few other researchers also showed that HHP treatments reduced the allergenicity of the shrimp heat stable proteins (Kim et al., 2006). Kato et al. (2000) worked on HHP treatments on rice and they reported that on treating rice at 300 MPa for about 120 minutes resulted in reduced allergenicity. This was due to the cell damage caused by the high pressure which resulted in solubilization and a better extraction of the allergens into the solvent. But, compatibility of the specific solvent and also the solubility of the allergens in that specific solvent will play a major role in using this method. HHP effect on the almond allergenicity was also studied, but this treatment showed no change in the allergenicity compared to the untreated almonds (Li et al., 2013). Houska et al. (2009) evaluated the effect of HP on apple juice which contains the major allergen Mal d 1. They reported that treatment (parameters followed: 450 MPa–550 MPa for 3–10 minutes) showed no effect on the allergenicity of the molecule.

Thus, the use of HP treatments in production of hypoallergenic foods is being widely evaluated because of the effectiveness in most allergen cases. It has been observed that HP treatments are particularly more effective when used in combination with other processing treatments like proteolysis or heat treatment (boiling). But, it is important to take a note that clinical studies have to be further conducted to analyze the relevance of using these treatments for producing the hypoallergenic foods.

4. Concluding remarks

In conclusion, it is very important for us to characterize the allergies at the molecular level which will help us in understanding them and their reactions with our immune system leading to development of techniques for their mitigation. Most of the food processes have a definite effect on the conformational structure of the protein and thus also on the allergenicity. Few of them have shown a promise for the future in developing the hypoallergenic foods by reduction or by mitigation of the reactivity on processing, but few others showed an increased reactivity and formation of epitope centers resulting in new reactive sites. Thus, further careful evaluation has to be conducted for determining the influence of specific process on the allergens.

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