

Impact of Thermal Processing on Legume Allergens

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Abstract Food induced allergic manifestations are reported from several parts of the world. Food proteins exert their allergenic potential by absorption through the gastrointestinal tract and can even induce life threatening anaphylaxis reactions. Among all food allergens, legume allergens play an important role in induction of allergy because legumes are a major source of protein for vegetarians. Most of the legumes are cooked either by boiling, roasting or frying before consumption, which can be considered a form of thermal treatment. Thermal processing may also include autoclaving, microwave heating, blanching, pasteurization, canning, or steaming. Thermal processing of legumes may reduce, eliminate or enhance the allergenic potential of a respective legume. In most of the cases, minimization of allergenic potential on thermal treatment has generally been reported. Thus, thermal processing can be considered an important tool by indirectly prevent allergenicity in susceptible individuals, thereby reducing treatment costs and reducing industry/office/school absence in case of working population/school going children. The present review attempts to explore various possibilities of reducing or eliminating allergenicity of leguminous food using different methods of thermal processing. Further, this review summarizes different methods of food processing, major legumes and their predominant allergenic proteins, thermal treatment and its relation with antigenicity, effect of thermal processing on legume allergens; also suggests a path that may be taken for future research to reduce the allergenicity using conventional/nonconventional methods.

Keywords Thermal processing · Legume allergens · Neoantigen. IgE-binding proteins

Abbreviations

DIC	Instantaneous controlled pressure-drop (detente instantanee controlee)
SDS-PAGE	Sodium dodecyl sulphate polyacrylamide gel electrophoresis
MW	Molecular weight

Introduction

Food allergy is a serious health concern throughout the world. It affects approximately 8 % children and 3–4 % adults [1–3]. Legumes (family Fabaceae) are one of the common causes of food-related allergic reactions because of their global cultivation and consumption. Legumes are one of the preferred sources of vegetarian proteins throughout the world especially in the United States, United Kingdom, Southeast Asia and Mediterranean countries. Allergenic potential of many leguminous crops like peanuts (*Arachis hypogaea*), soybeans (*Glycine max*), lupin (*Lupinus albus*), lentils (*Lens culinaris*), peas (*Pisum sativum*), and black gram (*Phaseolus mungo*) are well established [4]. Recently, allergenic polypeptides of red gram (*Cajanus cajan*), green gram (*Vigna radiata*), red kidney bean (*Phaseolus vulgaris*) and chickpea (*Cicer arietinum*) have also been identified [5–8]. Therefore, some effective methods are needed to minimize the associated risk with legume allergy as it affects a significant proportion of minor and adult population. To combat the allergenic potential of legumes, several strategies have been reported which includes enzymatic proteolysis, γ -irradiation, genetic transformation, and thermal processing [9–12].

Food processing plays an important role in modifying the allergenicity of food allergens either by [13–16]. It may

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also remove some anti-nutritional factors like polyphenols, amylase inhibitors, protease inhibitors, phytic acid, and oligosaccharides [17–20]. Among the various approaches used for making protein hypoallergenic, thermal treatments are one of the useful methods that can abolish the immuno-reactive proteins or allergens [14, 21]. Apart from reducing allergenicity, thermal treatment methods are mainly used to enhance flavour, texture, digestibility, neutralize toxins, and protect from microbes [22, 23].

Considering the pivotal role of thermal processing in reducing legume allergenicity, this review was intended to assemble the current knowledge and existing lacunae in this regard. Further, it raises a question of great concern that whether thermal processing can be a helpful tool in reducing the allergenicity of legume allergens? Further, this review attempts to summarize the effect of thermal processing on the structural and immunological properties of legume allergens, as well as to select suitable thermal treatment methods (boiling, autoclaving, microwave heating) to reduce/or abrogate the allergenic proteins of leguminous crops.

Different Methods of Food Processing

Processing of food may lead to the transformation of one form of food into another more valuable, safe and nutritious form. Further it may enhance the shelf-life of food as well. Different processing methods (*e.g.*, physical, chemical, or biochemical) may affect the allergenicity of food crop depending on the nature of a particular food [24, 25]. Food processing methods may decrease, enhance or sometimes have no effect on the allergenic potential of food. Further, sometimes processing may lead to formation of entirely new allergens which are known as neoallergens or neoantigens, responsible for enhancing the allergenicity [14–16]. However, complete elimination of allergens from food is not possible through food processing, therefore, a combination of various processing methods can be used together as a better option [26]. Irradiation processing, freezing, drying, fermentation, enzymatic proteolysis based method and thermal treatment are some examples of food processing methods. Irradiation processing involves the exposure of food to x-rays, gamma rays, or electrons to kill microorganisms and improve shelf life [27]. Optimal conditions for microbial growth lie above 10 °C (50 °F). Therefore, freezing of food products is generally done below −9.5 °C (15 °F) to reduce spoilage and inhibit microbial growth. For long-term storage, even lower temperatures can be used [28].

Drying is one of the oldest methods used for preservation of food. Further, drying of food makes storage and transport easy and less expensive as compared to other processing methods [29]. Fermentation of food can lead to the

enhancement of its nutritious property and longevity [30]. As far as the allergenicity of food crops is concerned, enzymatic hydrolysis and heat treatment are commonly used methods to minimize the allergenic potential of food. Furthermore, in enzymatic hydrolysis, food protein is hydrolyzed by the individual and/or sequential action of endoproteases (alcalase) and exoproteases (flavourzyme) to decrease the allergenic potential [31, 32]. Thermal treatment can be the cheapest and easiest way for reducing the allergenicity of food proteins. Further, thermal treatment involves the use of high temperatures that kills microorganisms and inactivates enzymes to ensure the safety of food. Blanching, pasteurization, canning, autoclaving, steaming, boiling, roasting, frying, and microwave heating are some common thermal treatment methods used nowadays [33].

Major Legumes and Their Predominant Allergenic Proteins

Legumes are a rich source of proteins and other nutritious elements including antioxidants. Most of leguminous proteins have been reported to be useful for humans except for few allergenic proteins that can be a problem in susceptible individuals [34]. Present research studies are oriented in order to improve the quality of legume proteins [35]. Unfortunately, higher consumption of any allergenic crop including legumes enhances the probability of initiation of allergenicity in susceptible individuals [36]. Various reports have been published describing the allergenic potential of legumes including peanut, soybean, lentil, lupin, pea, chickpea, mung bean, red gram, red kidney bean, and black gram [4]. Moreover, several allergens have been identified and characterized from aforesaid legumes. Initially, the majority of reports focused on studies related to the allergenicity of peanut and soybean. However, in recent years, the focus has broadened to include allergenicity of protein allergens from other legumes as well (Fig. 1). The pattern of legume allergy prevalence varies in different parts of the world. It has been largely accepted that the different sensitization pattern in a population may be due to the genetic status of the individual, consumption habit and involvement of other factors.

The prevalence of peanut allergy is found frequently in the United Kingdom, France, and North America whereas frequency of soybean allergy incidence is higher in South-east Asia [37]. Further, in Mediterranean and Asian countries, lupin, lentil and chickpea are reported to be the major sources of allergic sensitization [8, 14, 38]. Black gram, red gram and green gram have been reported as major legumes eliciting allergic sensitization in Asian countries including India [39].

Consumption of legumes may provoke mild to severe anaphylactic symptoms in sensitized individuals. Legume

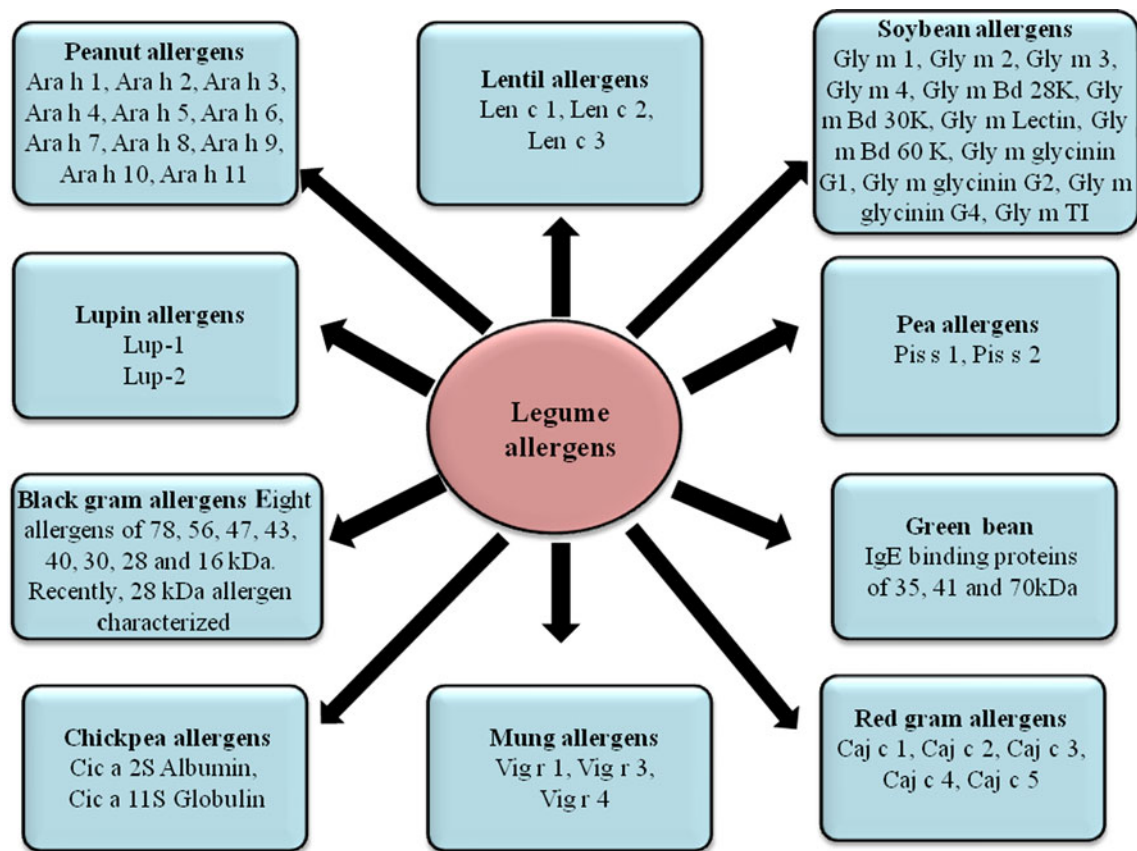


Fig. 1 An outline of allergens from peanut, lentil, soybean, lupin, pea, black gram, green bean, chickpea, mung and red gram with their respective names or molecular weights (as there were no IUIS name of allergens for black gram and green bean)

allergy symptoms are more or less similar to that of other food allergies. Target organs for legume allergy include cutaneous, respiratory, gastrointestinal tract, and cardiovascular system. The frequently observed symptoms include angioedema, vomiting, urticaria, allergic rhinitis, diarrhea, rashes on the skin, swelling of the tongue or throat, and asthma [4, 40, 41]. In severe cases, life-threatening anaphylactic reaction can occur which sometimes may lead to death. Further, legumes have been reported to demonstrate a significant degree of immunological cross-reactivity among themselves [42–44] and also with other known allergens [45–48]. The property of cross-reactivity among different food crops may lead to additional problems in susceptible individuals. Although the allergic manifestations of legume allergens are frequently reported, but till now there is no permanent cure for such complications. Considering this fact, there is a growing interest in the development of pivotal strategies to overcome such allergic problems.

Thermal Treatment and Its Relation with Antigenicity

Most of the legumes are consumed after cooking, therefore, it will be interesting to know what really happens to

legumes allergens after cooking. It will be useful to mention here that allergens are mostly pH resistant, water soluble, resistant to proteolytic enzymes, and temperature. In order to understand the effect of thermal processing on allergenicity of legumes, literature search reveals an enormous amount of details that are summarized in this article. Many factors such as resistance to pepsin in the stomach, solubility, and the ability to be absorbed intact across the intestinal tract are mainly responsible for the allergenicity of a protein, which may be affected by different processing methods [49, 50]. Food processing affects the structural and allergenic properties of allergens by altering its stability and other physicochemical properties [13, 51]. Food matrix may also affect the properties of allergens, which may be involved in enhancing the allergic potential [52].

Three dimensional structure of the majority of proteins is correlated with its functional activity (Fig. 2). Different temperature treatment can lead to variable changes in protein structure. In general, at a temperature of 70–80 °C, loss of secondary structure occurs whereas at 80–90 °C, formation of new bonds and rearrangements of disulfide bonds take place and further, at much higher temperature (90–100 °C) aggregate formation can occur [53]. Moreover, at higher temperature, covalent bond formations

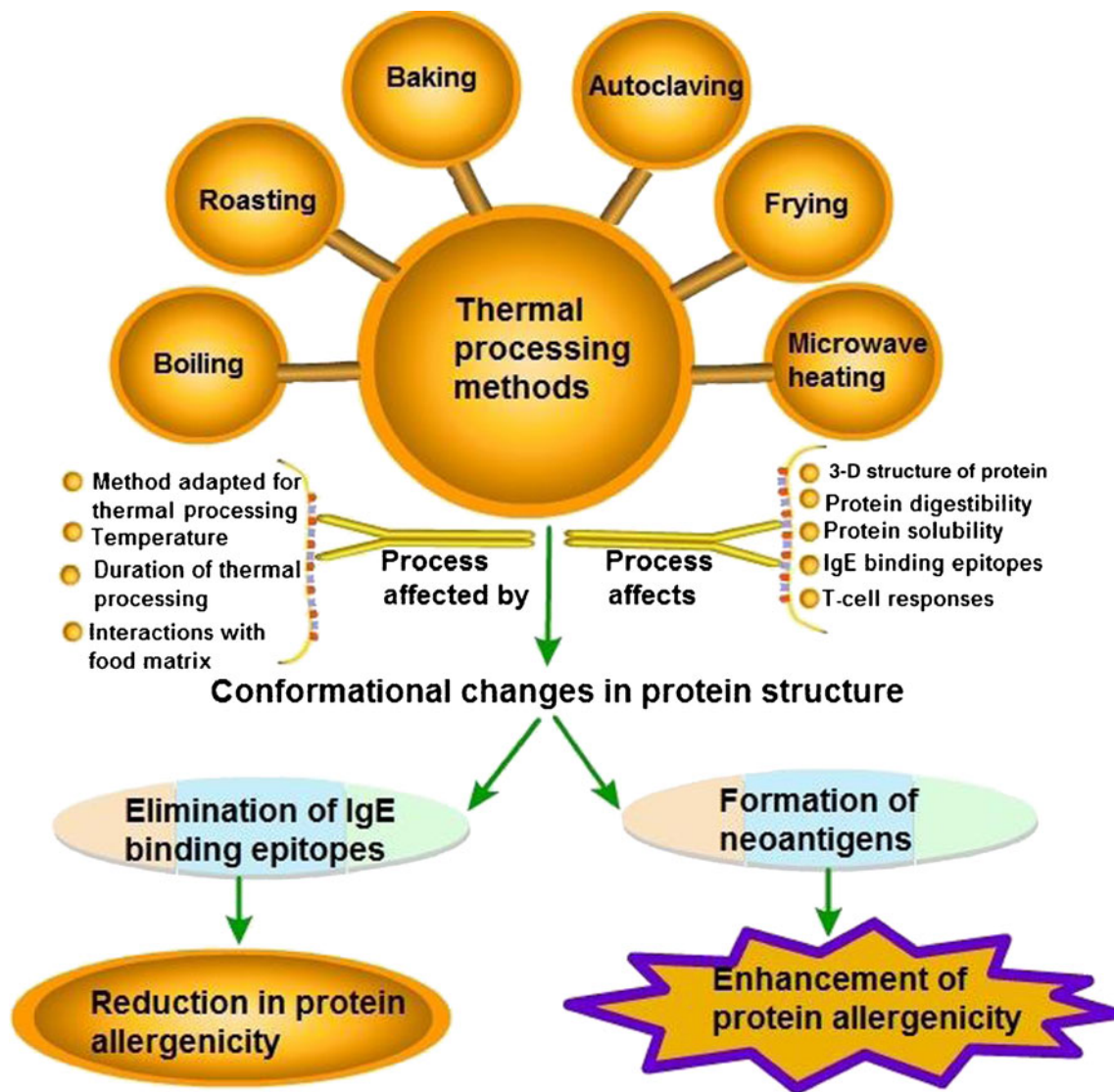


Fig. 2 Effect of thermal processing methods on protein structure. The figure demonstrates different methods of thermal processing, and factors affecting the processing, conformational changes, formation of neoantigens and reduction in allergenicity after thermal processing

between protein lysine residues and other constituents of the food matrix may take place, which lead to the formation of various adducts [54]. Protein digestibility probably increases prior heat treatment [55], it may also increase absorption in the gastrointestinal tract, and because of this, the possibility of allergenic protein to elicit an allergenic response decreases as reported in the case of chickpea, lentil, lupin, black gram, and pea (Table 1). However, in some cases, thermal processing may reduce the digestibility of a particular allergen as reported in the case of peanut viz, Ara h 1 and Ara h 2 [56, 57]. Thermal treatment may also lead to formation of neoantigens that are not present originally [58, 59]. The generation of neoantigens may enhance the allergenic problem in sensitized patients. One of the factors responsible for the formation of neoantigens maybe Maillard reaction, *e.g.*, the interaction of the protein component with sugar residues upon heating,

generating sugar conjugated protein derivatives, that in turn increases the allergenicity of protein [60]. The neoantigens are similar to allergens and may provoke the immune system in the same way (Fig. 3).

The antibodies recognize and interact with IgE-binding epitopes present on allergenic proteins. When allergen comes and cross-link IgE antibodies present on mast cells or basophils, it degranulates the cells, which results in release of allergic mediators like histamine, prostaglandin D2, and cysteinyl leucotriene [61]. Two types of IgE-binding epitopes are possible based on the structural properties, (i) linear, (ii) conformational [62]. In linear epitopes, amino acids are arranged in linear order in polypeptide chains and are generally resistant to heat while, in conformation epitopes, amino acids that are far apart in primary sequence may come together during folding of polypeptide chains but these

Table 1 Effect of thermal processing on allergens from different legumes

Allergen	Reference	MW	Thermal processing methods and their effect on allergenicity			
			Roasting	Boiling	Autoclaving	Microwave heating
Peanut (<i>Arachis hypogaea</i>)						
Ara h 1 allergen	[74]	64	Enhancement in IgE-binding capacity	Decrease in IgE-binding capacity	Decrease in IgE-binding capacity	NDA
Ara h 2 allergen	[69, 71]	17	Enhancement in IgE-binding capacity	Decrease in IgE-binding capacity	Decrease in IgE-binding capacity	NDA
Ara h 3 allergen	[72, 74]	60	NDA	Decrease in IgE-binding capacity	Decrease in IgE-binding capacity	NDA
Soybean (<i>Glycine max</i>)						
Soybean protein extract	[73, 90]	–	NDA	Decrease in IgE-binding capacity	NDA	Decrease in IgE-binding capacity
Lentil (<i>Lens culinaris</i>)						
Lentil protein extract allergens- Len c 1 (48 kDa), Len c 2 (66 kDa), Len c 3 (9 kDa) and other immunoreactive proteins	[14]	–	NDA	Len c 1 allergen is eliminated No effect on other immunoreactive proteins	Len c 1 allergen is eliminated Decrease in other IgE-binding proteins	NDA
Lupin (<i>Lupinus albus</i>)						
Lupin protein extract (protein bands from 19 to 108 kDa, with major IgE-binding proteins at 20 and 34.5 kDa)	[21]	–	NDA	No effect	Decrease in IgE-binding capacity	No effect
Pea (<i>Pisum sativum</i>)						
Pea protein fraction	[86] (albumin fraction) [globulin fraction]	NDA NDA	No effect Decrease in IgE-binding capacity	Decrease in IgE-binding capacity Decrease in IgE-binding capacity	NDA NDA	
French bean (<i>Phaseolus vulgaris</i>)						
French bean protein fraction (identified allergen pha v3 MW 8.8–9)	[95]	–	NDA	32 kDa protein (decrease in IgE-binding capacity) 35 kDa (increase in IgE-binding capacity) 9, 41 and 70 kDa protein (no effect of cooking)	NDA NDA	NDA
Chickpea (<i>Cicer arietinum</i>)						
Chickpea protein fraction (IgE-binding bands with MW between 10 and 70 kDa)	[14]	–	NDA	Decrease in IgE-binding capacity	Decrease in IgE-binding capacity	NDA
Black gram (<i>Phaseolus mungo</i>)						
Black gram protein fraction (eight allergens of 78, 56, 47, 43, 40, 30, 28 and 16 kDa)	[39]	–	Decrease in immunoreactive proteins (six major allergens)	Decrease in immunoreactive proteins (three major allergens)	NDA	NDA

NDA=no data available for mentioned method

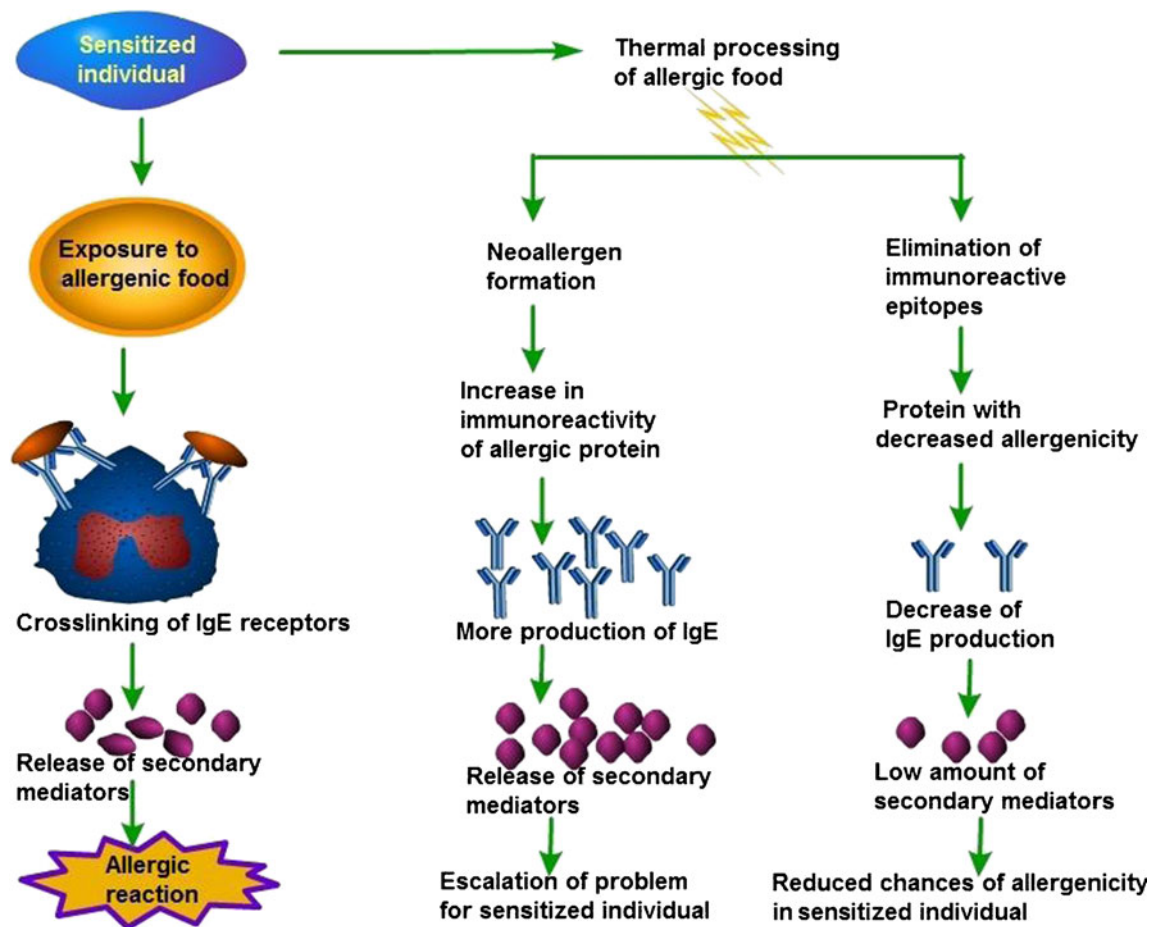


Fig. 3 Immunological aspects associated with thermal processing. Figure demonstrates an outline of allergic manifestation induced by allergens via IgE mediated pathway, effect of neoantigens and reduction in allergic reactions in processed food

bonds may break easily after thermal processing [54, 63]. Therefore, after thermal processing different allergens have different fate which is evident by decreased allergenicity of chickpea, lentil or exacerbated allergenicity in case of Ara h 1 and Ara h 2 allergens of peanut or may have no change on antigenic behaviour as in the case of boiling pea allergens [64]. Considering above facts, it will be interesting to review the current knowledge in this reference.

Thermal Processing and Legume Allergens

Due to the high prevalence of legume allergy, several attempts are being made to minimize the allergenic properties of leguminous proteins (Table 1). Up to some extent, thermal processing seems to be an effective method in reducing the allergenic problem associated with most of legume allergens. It can affect the physical and immunological properties of a protein, can alter the structure, function, solubility, digestibility, IgE-binding epitopes, and T-cell responses. Above all may depend on temperature, duration

of the processing, the kind of heat treatment method used (dry or wet) as well as the interaction with food matrix which is demonstrated in Fig. 2. It is observed that immunoreactivity of protein may be decreased by one thermal treatment method but not another. The duration of thermal treatment and temperature has different effects on antigenicity depending on the protein. In few cases, autoclaving seems to be more effective as compared to boiling. Conversely, microwave heating is more effective than other thermal treatment methods.

Effect of Thermal Treatment on Peanut (*Arachis hypogaea*) Allergens

Peanut is used as a preferred source of protein [65], but perhaps for the same reason is also one of the common causes of allergy in western countries. It affects approximately 0.6–1 % of the U.S. and European populations [66]. Many allergens (Ara h 1–Ara h 11) have been identified from peanut. Major allergens of peanut are the 7S globulin

Ara h 1 (63 kDa) and the 2S albumin Ara h 2 [67, 68]. A study conducted by Mondoulet et al. [69] demonstrated an opposite effect of roasting and boiling of peanut allergens as boiling decreased while roasting enhanced allergenicity potential of Ara h 1 and Ara h 2. Increase in the allergenicity of peanut upon roasting may be due to structural modifications in allergens as Ara h 1 forms trimers upon roasting. Structural changes in Ara h 2 after roasting have also been reported as it increased the IgE-binding capacity, which subsequently resulted into the enhanced allergenicity potential [70, 71]. Boiling and frying of peanut proteins decrease the allergenicity of major peanut allergens Ara h 1, Ara h 2 and Ara h 3 as compared to roasting, which indicates that roasting methods for peanuts employed in the U.S. could be one of the etiological factors for higher incidence of peanut allergy in the U.S. as compared to China where boiling is preferred as cooking method [74].

Autoclaving of peanut allergens (Ara h 1, Ara h 2 and Ara h 3) at 2.56 atm for 30 min decreases its allergenic potential. It has also been reported that pressure treatment unfolds protein and increases digestibility, resulting in the decreased allergenicity of peanut protein [72]. Instantaneous controlled pressure-drop (DIC, detente instantanee controlee) treatment, which combines heat and steam pressure, was used to observe the effect on peanut allergenicity [73]. The DIC treatment of raw and roasted peanut proteins produced no significant difference in SDS-PAGE profile at 3 bar for 1 and 3 min, while the same SDS-PAGE pattern was observed after DIC treatment at 6 bar for 1 min. However, DIC treatment at 6 bar for 3 min resulted into a remarkable decrease in the allergenic proteins. The DIC treatment findings indicate that this method can be used to reduce the overall allergenic potential of the peanut protein extract. Immunoreactivity of peanut proteins following thermal treatment may be due to modification in structure, as well as reactivity of each allergen and also their interactions with the food matrix [74]. In general, the heat processing in this regard has a mixed effect on allergenicity of peanut.

Effect of Thermal Treatment on Black Gram (*Phaseolus mungo*) Allergens

Black gram is mainly consumed in Asian countries including India, Sri Lanka, Thailand, and Indonesia. Black gram is a protein-rich staple food, which contains about 24 % protein. Black gram is used in different ways as a whole seed or after splitting into dal and in culinary preparations. Raw black gram has been found to have 14 IgE-binding proteins of 110, 104, 78, 70, 56, 47, 43, 40, 33, 30, 28, 20, 16 and 12 kDa. A major allergen of 28 kDa from black gram was characterized and, found stable up to 15 min in simulated gastrointestinal fluid. The N-terminus-12 residue sequence

of this allergic protein was 'GRREDDYDNLQL' that showed 87 % homology with Rho-specific inhibitor of transcription termination and 77 % homology with NBS-LRR type disease resistant protein from peanut [75]. Roasting of black gram for 15 min has been found to increase the number of immunoreactive proteins up to 17. Roasting resulted in the formation of some new allergenic proteins, while few original allergenic proteins disappeared. However, boiling black gram for 15 min reduced the number of immunoreactive proteins to 11 allergenic proteins of 56, 47, 43, 30, 28, 25, 23, 20, 18, 12 and 7 kDa. When black gram was boiled for 30 min, only 9 allergenic proteins could be identified and 56 and 28 kDa proteins were not traceable [39]. Further, eight allergens of 78, 56, 47, 43, 40, 30, 28 and 16 kDa have been recognized with pooled human sera out of which six were major allergens in roasted black gram, while three major allergens of 47, 43 and 28 kDa in boiled black gram proteins were identified. It has also been found that protein digestibility of black gram was enhanced as a result of cooking [76]. The probable reason behind the new allergenic proteins in roasted black gram might be either breaking of disulphide bonds, or loss of secondary and tertiary structures. Further studies need to explore a potential reduction in the allergenicity of black gram including the use of thermal processing with other methods.

Effect of Thermal Treatment on Lentil (*Lens culinaris*) Allergens

Lentil is commonly consumed in Mediterranean areas like Italy, Spain, and France. Multiple immunoreactive proteins have been detected in both raw as well as boiled lentil. Two major allergens of lentil as Len c 1 (48–50 kDa) and Len c 2 (66 kDa) have been characterized and isolated from boiled lentils. Two immunoreactive proteins of MW 12–16 kDa and 26 kDa were generated as a result of Len c 1 processing [77–79]. Recently, a third allergen, Len c 3, has also been characterized in lentil [80]. It is also reported that lentil protein upon boiling produces neoallergens in the range of 12–16 kDa, while the intensity of protein bands in the range of 25–45 kDa was found to be reduced. However, major allergen Len c 1 is eliminated after boiling within 30 min, but it does not have any effect on allergenic proteins [14]. Thus, the net effect of boiling on lentil proteins does not seem to be effective in reducing its allergenic potential. This fact is supported by studies where lentil protein boiled for 60 min resulted in the formation of multiple immunoreactive proteins. However, autoclaving (1.2 and 2.6 atm, up to 30 min) significantly decreased the allergenic potential of lentil. Autoclaving at still higher pressure (2.6 atm for 30 min) has an important effect on minimizing the immunoreactive proteins. Five extremely resistant immunoreactive

proteins of MW 16, 18, 21, 30, and 43 kDa were detectable under such condition. Hence, autoclaving may bring out structural modifications in lentil protein, and may result into decreased allergenicity.

Effect of Thermal Treatment on Chickpea (*Cicer arietinum*) Allergens

Chickpea is an important source of protein in several parts of the world especially in developing nations. Immunoblot analysis detected several IgE-binding proteins in chickpea with MW ranged between 10 and 95 kDa. Out of them, seven major chickpea allergenic proteins identified are of MW 95, 70, 55, 45, 35, 26 and 20 kDa [8]. Multiple heat-stable immunoreactive proteins have been found and identified in both raw and boiled chickpea extracts. It has been reported that boiling chickpea extract for 30 min had no effect on immunoreactive proteins. However, boiling for 60 min reduced the immunoreactive proteins to some extent [14]. Autoclaving for 15 min at 1.2 atm pressure was sufficient to reduce the number of allergenic proteins. As autoclaving pressure and time was increased, the number of immunoreactive proteins decreased. Only four immunoreactive proteins could be identified if autoclaving was performed at 2.6 atm for 15 min. If time was increased up to 30 min, only two immunoreactive proteins could be detected. In case of chickpea, DIC treatments produced more or less similar pattern of immunoreactive proteins as observed with autoclaving [73].

Effect of Thermal Treatment on Lupin (*Lupinus albus*) Allergens

Lupin is consumed mainly in the European countries where lupin induced allergic reactions are found frequently in susceptible individuals [38, 81]. Two lupin allergens have been identified and characterized so far; namely Lup-1 (34.5 kDa) and Lup-2 (20 kDa). Lup-1 was identified as conglutin β (7S and vicilin-like protein) whereas Lup-2 corresponds to the conglutin α fraction (11S and legumin-like protein). Like other allergens, these two allergens were also showing sequence homology with other known legume allergens [82]. The effect of boiling (up to 60 min), autoclaving (121 °C, 1.18 atm, up to 20 min and 138 °C, 2.56 atm, up to 30 min), and microwave heating (30 min) on immunoreactive proteins of lupin has been well studied [21]. The protein profiles of raw as well as processed sample (using microwave heating, boiling, and extrusion cooking) were found to be almost similar. The most effective way to reduce allergenic proteins in lupin was autoclaving; autoclaving at 138 °C for 20 min was found to be sufficient to

reduce the number and intensity of immunoreactive proteins. However, a neoallergen of 70 kDa was detectable after autoclaving lupin at 138 °C for 30 min. Allergenicity of lupin protein could be minimized by thermal modifications, and autoclaving seems to be more effective to abolish these immunoreactive proteins. Guillamón et al. [83] used DIC treatment to observe the effect of thermal processing on lupin allergenicity. Lupin cotyledons were subjected to different pressure and time conditions (3, 4.5 and 6 bar for 1, 2 and 3 min, respectively). It was found that with the increase in pressure and time, immunoreactive capacity of allergens decreased. Further, the immunoreactivity of allergens was completely abolished at 6 bar in 3 min treatment group, which suggests that DIC treatment could be a better option to reduce immunoreactive proteins present in lupin responsible for eliciting allergic reactions in susceptible population. Conclusively, thermal treatment is showing a better prospect to combat the allergenic problem associated with lupin, but systematic studies are still required.

Effect of Thermal Treatment on Pea (*Pisum sativum*) Allergens

Being a common legume, pea is consumed throughout the world. It contains a number of immunoreactive proteins but only two major allergens, Pis s 1 (44 kDa) and Pis s 2 (63 kDa), have been characterized so far [84, 85]. Like other legumes, thermal processing modified the structure of pea proteins and has a significant effect on its allergenicity. Albumin fraction possessed more allergenicity provoking potential as compared to the globulin fraction in the case of pea. Albumin does not lose its allergenicity when heated at 60 °C for 30 min or even upon boiling at 100 °C for 5 min while allergenicity of globulin fraction is abolished under these conditions. Like other legumes, autoclaving (120 °C for 15 min) has been found more effective to minimize the allergenicity of pea proteins [86].

Effect of Thermal Treatment on Soybean (*Glycine max*) Allergens

Soybean with more than 200 varieties and approximately 21 known allergenic proteins is consumed throughout the world. Immunoreactive proteins of soybean range between 14 and 78 kDa [87–89]. Major allergens in soybean are P34 (Gly m Bd30K), Gly m 1, Gly m 2, Gly m 3, Gly m 4 and Gly mBd 28 K. Heating soybean extract for 60 min at 100 °C did not cause any decrease in immunoreactive proteins demonstrating that allergenicity remained unchanged in this condition [68]. However, another study demonstrated that boiling (120 min) as well as microwave heating can minimize the

immunoreactive proteins in soybean to a great extent [90]. Thus, it can be inferred that duration of boiling can be important in the modulation of soybean allergenicity. As boiling time was extended, decrease in allergenic proteins was observed. Further, DIC treatment at 3 bar for 1 and 3 min resulted into little modification in immunoreactive proteins present in soybean, while a remarkable decrease in immunoreactive proteins was observed when DIC treatment was done at 6 bar for 3 min [73]. Lakemond et al. [91] studied structural changes in the soybean glycinin following heat treatment. Denaturation of 7S form of glycinin takes place at lower temperature as compared to 11S form. Heat treatment on 7S/11S glycinin forms were found to depend on pH and ionic strength as well. One study with the help of near ultraviolet circular dichroism (near-UV CD) indicated that changes in the tertiary structure occur at lower temperatures as compared to its secondary structure. However, Codina et al. [92] reported that heat treatment can increase the allergenicity of soybean hull as two neoallergens of 15.3 and 10 kDa were formed. During the transport and storage conditions, heat is generated and may enhance the allergenicity of soybean hull. It can be concluded that duration of thermal treatment play a crucial role in reducing the allergenicity of soybean protein. An in-

depth study in regards of the effect of other thermal processing methods on soybean protein is still lacking.

Effect of Thermal Treatment on Green Bean (*Phaseolus vulgaris*) Allergens

The green bean (French bean), is a common bean consumed in different parts of the world. One IgE-binding protein of green bean with a molecular weight of 32 kDa has been identified. Its activity may be completely abolished by heat treatment. Another protein of 35 kDa with allergenic characteristics was detected in a 20 year old patient that provoke a higher response in case of boiled green bean as compared to raw green bean [93]. A study revealed that the patients developed asthma and rhinitis after exposure to raw green beans, but cooked green beans had no such effects. Another study showed two immunoreactive proteins of 41 and 70 kDa both in raw and cooked green bean extract [94]. Asero et al. [93] reported that ingestion of boiled green bean has the ability to provoke allergic potential since one patient showed anaphylactic symptoms after ingestion of boiled green bean. In this case study, one immunoreactive protein of 35 kDa was found to be heat stable. In spite of the

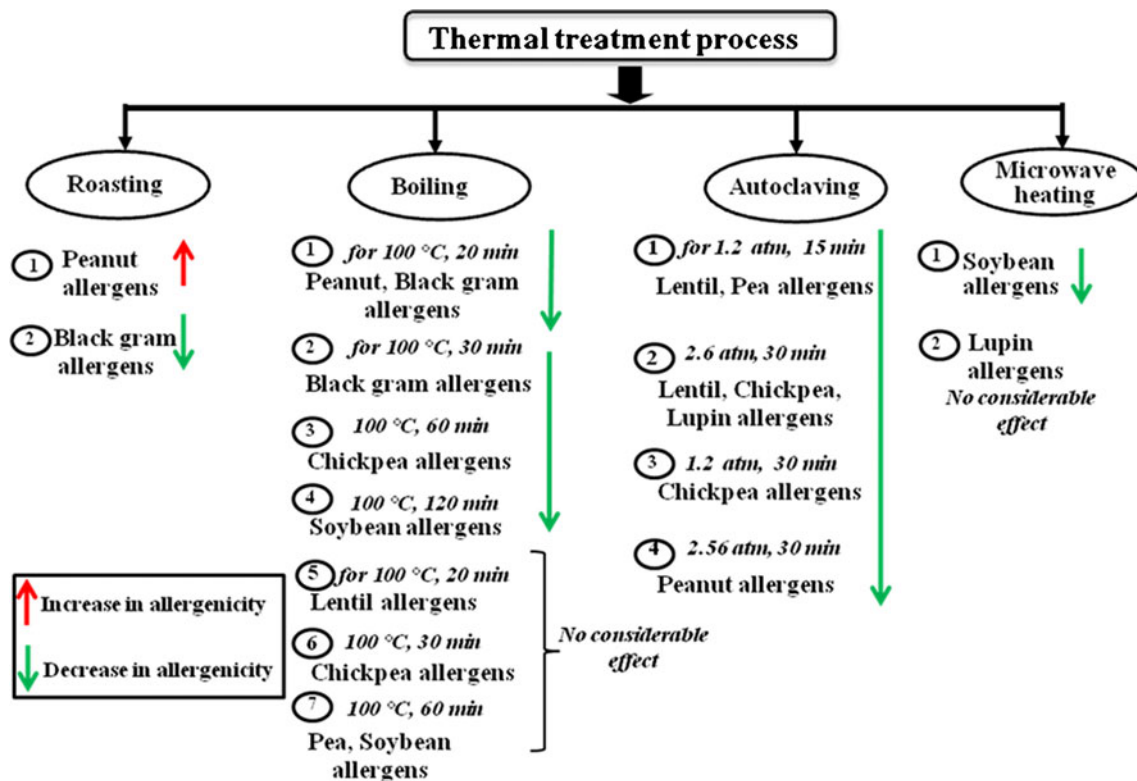


Fig. 4 Different thermal processing methods. An outline of the effect induced by roasting, boiling, autoclaving and microwave heating on different allergens of peanut, lentil, soybean, lupin, pea, black gram,

green bean, chickpea (green arrows and red arrows are indicating reduction and enhancement in allergenic potential of allergens, respectively)

many protein bands present in a cooked green bean extract after electrophoresis, the IgE immunoblotting performed with individual serum showed that all the patients reacted to a unique IgE-binding protein at about 9 kDa [95].

Future Prospective

Besides thermal processing, other currently preventive methods are also available to reduce or eliminate the food induced allergenicity. One such preventive strategy is genetic transformation in which allergens from leguminous crops are removed through RNA interference as reported in the case of soybean and peanut [10, 12]. The allergenicity of soybean major allergen Gly mBd 30 K has also been demonstrated to reduce following protease treatment [11]. Enzymatic hydrolysis and preparation of infant formula based on hydrolyzed proteins may be useful alternative methods for making food hypoallergenic [32, 96]. Tannic acid, an antioxidant, also helps in removing allergens as it binds with proteins forming insoluble complexes as in the case of peanut allergens [9]. Allergenicity of roasted peanut can be reduced by peroxidase treatment [97].

Conclusions

The presence of allergens in leguminous crops have drawn our attention to take preventive measures to save a susceptible consumer group falling sick. Several preventive strategies including food processing, genetic engineering, and traditional breeding are in use since the last decade to minimize the risk factors associated with food allergy. The effect of thermal treatment methods including roasting, boiling, autoclaving, microwave heating and DIC treatment on different legumes has been found promising because in the majority of cases, it reduced the allergenicity induced by consumption of legumes (Fig. 4). However, sometimes there are possibilities that heat treatment may lead to generation of neoallergens in some of the legumes, which need a thorough exploration, thus it is necessary to determine which should be the fate of specific allergens after thermal processing. The studies carried out on peanut, black gram, chickpea, soybean, lentil, lupin, pea and green bean in regard to thermal processing and its effect on their allergens revealed important outcomes. Taken together, thermal processing method is simple, cheap and has effective approaches for large populations of susceptible individuals suffering from allergic manifestations in minimizing the allergenic potential.

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