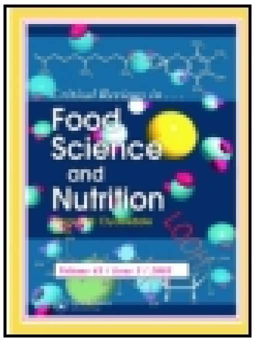

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Processing Effects On Tree Nut Allergens: A Review

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PROCESSING EFECTS ON TREE NUT ALLERGENS: A REVIEW

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Abstract

"Tree nut" is a broad term for classification of nuts that include cashews, almonds, hazelnuts etc. Reports of mild to adverse immune reactions following the consumption of these nuts has been on a rise in recent years. Currently, about 1.2 -- 2% of the world's population suffer from sensitivity to tree nuts. The only solution is complete abstinence from the allergy causing tree nut which is not feasible in most cases due to issues like cross contamination or their presence in the form of hidden ingredients in processed foods. Various studies have shown that food processing can effectively vary the secondary structures of the allergenic protein which in turn influences their functional properties. But, the impact of these processing methods on tree nuts allergens is mixed. This review gives an update on the recent findings on how conventional and novel processing methods influence the tree nut allergens.

Keywords

food allergy; food processing; thermal treatment; immunoreactivity

1. INTRODUCTION

Tree nuts are one seeded dry fruits, whose outer wall becomes harder as the seed matures. Various types of nuts are consumed widely around the world depending on the geographical location and yield. These nuts play a vital role in promoting healthy life style acting as a tasty and nutritious snack. Tree nuts are consumed primarily as whole nuts, either raw or roasted, dressed with salt or chocolate. They are also used as ingredients in wide variety of food products, especially confectionary including chocolates, energy bars and bakery products. The category of tree nuts includes almonds (*Prunus dulcis*), hazelnuts (*Corylus avellana*), cashew (*Anacardium occidentale*), walnuts (*Juglan regia*) and pistachio (*Pistacia vera*) which are widely popular and are produced in large quantities around the world. Brazil nuts (*Bertholletia excels*), macadamia nuts (*Macadamia* spp.), pecan (*Carya illinoinensis*), pine nuts (*Pinus* spp.), betel nuts (*Areca catechu*) and chestnuts (*Castanea* spp.) produced in limited quantities also fall into the category of tree nuts (Alasalvar and Shahidi, 2008).

The huge consumption of tree nuts can be primarily attributed to their nutritional and health benefits. These advantages are not only due to the presence of macro nutrients (carbohydrates, protein and fat) and micro nutrients (minerals and vitamins), but also a wide range of phytochemicals such as phenolic acids and flavonoids (Alasalvar and Shahidi, 2008; Bolling et al., 2010; Chen and Blumberg, 2008). Tree nuts act as a rich source of these phytochemicals which have varied advantages as antioxidants and also possess antimutagenic and anticarcinogenic properties (Kornsteiner et al., 2006; Liu, 2004; Surh, 2003; Takeoka and Dao, 2003). Furthermore, various researchers (Bes-Rastrollo et al., 2009; Jackson and Hu, 2014;

O'Neil et al., 2010) have associated regular consumption of nuts to weight control and higher diet values. Thus, they have become an important part of human diet, which led to a raise in consumption of tree nuts around the world especially in the 21st century (Alasalvar and Shahidi, 2008).

In spite of the widely accepted health benefits mentioned above, they also possess a serious health threat to the population as they are classified to be part of the ‘big 8’. This is a group representing the eight foods responsible for 90% of (Immunoglobulin E) IgE-meditated allergic reactions in the world (Koppelman, 2006). Apart from tree nuts, this group consists of peanut, wheat, milk, egg, soy, fish and shell fish. It was observed that the allergic reactions to tree nuts have been critical when ingested by the sensitive patients, especially infants and children compared to other foods (Alasalvar and Shahidi, 2008; Sicherer et al., 1998; Sicherer and Sampson, 2000). The Table 1 illustrates the list of tree nuts known to cause allergic reactions in sensitive patients.

Adopted from (Alasalvar and Shahidi, 2008; Costa et al., 2014; Crespo et al., 2006; Koppelman, 2006; Masthoff et al., 2013)

1.1 Epidemiology of tree nut allergies

Recent studies suggest that prevalence of food allergies can be higher than generally perceived. This is due to the fact that variations in allergy tests, inconsistencies in defining allergies, and data collected have been obscure though abundant, leading to the discrepancy in reporting allergies around the world. The total population suffering from food allergies can be greater than 10% according to the latest reports by Gray et al and according to this study, about 0.4-1.2% of the population suffers from sensitivity towards tree nuts (Gray et al., 2014). Some estimates claim that the prevalence of tree nut allergies among young children and adults in U.S. was about 0.2% and 0.5% respectively in 2004 (Sampson, 2004). In 2010, researchers reported that at least 0.5% of the total population is suffering from tree nut allergies (Sicherer et al., 2010). The proportion of younger children suffering from tree nut allergies is lower compared to other big 8 allergies like milk (up to 3.5%) and egg (up to 8%). However, it is worth noting that only about 10% of these children outgrow the tree nut allergy compared to milk and egg allergy where > 80% of children outgrow their allergies by the age of 16 (Gray et al., 2014; Sicherer et al., 2010). Tree nut allergy prevalence among the European children was reported to be varying from 0.4 -- 1.4% (Pénard-Morand et al., 2005; Venter et al., 2006) and in Canada about 1.22% of the total population is sensitive to tree nuts (Ben-Shoshan et al., 2010).

Bock et al., (Bock et al., 2001) informed that during the period between 1994-1999, tree nuts were responsible for about 31% of the total fatalities due to food hypersensitivity related anaphylactic shocks. Peanuts caused the majority of fatalities accounting for 63%, and with the addition of tree nuts they rose to over 94% of the total fatalities caused due to allergic reactions pertaining to food. This is one of the key reasons why tree nut allergies are considered to be of a severe concern, though they are not as frequent as egg or milk related allergies.

Van der Valk et al., (van der Valk et al., 2014) pointed out that the cashew nut allergy cases are on a rise which is widely consumed around the world. Moreover, they also mentioned that sensitivity towards cashew is more potent compared to other tree nut allergies as minute amounts can trigger an allergic reaction, especially in the case of children with higher sensitivity. One of the major reasons for the increasing number of people being hypersensitive to cashew nuts is associated to the steep rise in consumption of cashews worldwide. The average age for an allergic reaction to occur against cashew nut for the first time was found to be two years (Rancé et al., 2003).

Overall, though the population's sensitivity to various foods including tree nuts is on a rise, the exact effect of the processing methods in mitigating or aggravating the allergies is largely unknown. This review attempt to bridge this gap by introducing how processing techniques stimulate structural changes in the food protein and provide an update on the effects of processing on various tree nut allergens.

2 FOOD PROCESSING

Food processing is an ancient concept dating back to the Egyptian era, where food was preserved using 'sun drying' and 'fermentation' techniques. Modernization of food processing and preservation was initiated after Nicholas Appert successfully preserved various foods by heat processing them in glass jars which were sealed and placed in hot water. Numerous discoveries and inventions thereafter in food processing and preservation aspects have paved the way food is consumed in years that followed the work of Nicholas Appert (Stumbo, 2013). These processing methods not only helped in enhancing nutritional quality and shelf life, but also increased the variety in food (Dewanto et al., 2002).

Several processing techniques that are being used include thermal, mechanical, chemical and irradiation of food. Out of all the aforementioned methods, thermal treatments are widely followed around the world. Thermal processing can be performed in various ways depending on

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the final product required and these avenues include moist heating (Boiling, Autoclaving), dry heating, dielectric heating (Microwave and Radio frequency processing) and ohmic heating (Vanga et al., 2015d). During thermal processing, heat stresses are inflicted on various components including proteins which are the most sensitive elements in food. These external stresses result in conformational changes in the secondary structures of the protein, which in turn will influence their functional properties (Davis and Williams, 1998; Davis et al., 2001). Thus, change in processing parameters or methods can potentially alleviate or mitigate the allergenicity of a particular epitope present in the protein as this perception assumes allergenicity as an intrinsic property of its structure.

Davis et al. mentioned that the typical unfolding of tertiary structure in a protein occurs between the temperatures 55°C and 70°C which is reversible in most cases. Increasing the temperature from 70°C to 80°C will result in loss of secondary structures and disulphide bond cleavage resulting in collapse of the 3-D structure of protein. As the temperature is further increased to 100°C, new sulphide bonds are formed resulting in the aggregation and random structure formation. The extent of protein denaturation (unfolding) rises with the increasing temperature (>80°C) and time of exposure (Davis and Williams, 1998). The heat stress results in rearrangement of secondary and tertiary structures allowing the protein to adopt fully unfolded and/or random coil structure (Privalov et al., 1989). As a result of this unfolding, the hydrophobic surface of the heat damaged protein is forced to interact with the surrounding water molecules. Because of the strong forces developed as a result of unfavourable interactions of the unfolded protein with water, various protein molecules come together through mutual hydrophobic interactions. This modified structure of aggregated proteins due to denaturation can result in formation of new allergy generating sites or loss of the original allergenicity. The study by Baer et al. reported that the β-lactoglobulin (β-lg) formed heat induced complex structures along with heat denatured α-lactalbumin (α-la) which acts as an example for aggregated protein interaction (Baer et al., 1976). On further increasing the temperature (>100°C), chemical reactions take place in protein which can involve covalent bond formation due to protein interactions with sugars and lipids present in the food matrix (Example: Maillard reaction). These reactions can result in new antigenic sites which could become reactive thus causing allergic reactions to a sensitive patient (Wal, 2003).

Mechanical treatments include use of novel methods like high pressure (HP) (400 -- 600 MPa), ultra-high pressure (UHP) (over 600 MPa) and extrusion techniques to process food materials. These methods have shown a great potential in the last few years where they are extensively used for sterilizing food materials at relatively low temperatures (Smelt, 1998). Johnson et al. evaluated the effects of high pressure on various allergies (Johnson et al., 2010). This study revealed that the HP processing can affect the protein structure to a certain extent when used in combination with high temperature (80°C). The structural changes were studied using the circular dichroism (CD) spectra which revealed deviations in α - helix structure of mal d 3 apple allergen. Jankiewicz et al. studied the effect of high pressure processing on celery and reported structural deviations in the allergic protein, but also stated that allergenicity reduction was very minimal (Jankiewicz et al., 1997). Extrusion processing in foods use shear forces and high temperatures under high pressure at relatively low moisture content. It is a continuous process, which includes operations like transport, mixing and forming used in production of various commercial food products. Extrusion cooking which sometimes is termed as HTST cooking

(High Temperature Short Time) and is perceived as a processing method that can influence the allergenicity caused due to presence of sensitive epitopes in food protein (Camire et al., 1990). Hayakawa et al., reported that twin-screw extrusion has been efficient in mitigating the soy flour allergens, but has shown minimal effect when feed material was soy hull having higher carbohydrate content (Hayakawa et al., 1996). They also reported that twin-screw extrusion cooking which resulted in partial reduction of allergenicity in the case of buckwheat allergens. Effect of extrusion on lupine allergenicity was also evaluated along with boiling and microwave heating. Extrusion had minimal effect on the binding capacity of the allergens in this case (Álvarez-Álvarez et al., 2005).

In various food industries, depending on the final product's application and requirements, proteins contained in raw materials are subjected to modifications which will in turn influences their functional properties. Slight change in pH of the protein can result in a drastic variation in the functional and physicochemical properties. It is well known that asparagine and glutamine amino acids are destroyed under acidic conditions and amino acids like serine are destroyed in alkaline conditions. Furthermore, acid and alkaline hydrolysis can result in peptide bond cleavages and cross linkages which can improve digestibility and thus absorption is made possible in the digestive system (Korhonen et al., 1998). Consequently, chemical modifications of proteins and in turn the allergens may result in reduction of allergenicity (Taylor and Lehrer, 1996). Honma et al., studied the IgE binding capacity of chemically denatured Ovalbumin (Honma et al., 1994). They reported that alkaline treatment using sodium hydroxide at pH 11 destroyed its IgE binding capacity, but use of urea, hydrochloric acid at pH 3 did not affect the IgE binding activity. Further studies have to be conducted for understanding the effects of various chemical processing methods like acylation (addition of acyl group), phosphorylation (addition of phosphate group), succinylation (addition of succinyl group), lipophilization and glycosylation on functional properties of proteins and their allergenicity. Irradiation has been perceived as another potential treatment method in altering the protein structure. Byun et al., studied the effects of gamma irradiation on chicken egg albumin, shrimp tropomyosin and milk β -- lactoglobulin and reported changes in the epitope structure. They reported reduction of various amounts of intact allergens depending on the dosage of gamma radiation (Byun et al., 2002).

Further, researchers have also started looking into the application of electro technologies in processing foods. These include the use of High Electric Voltage (HEV), Pulsed Electric Voltage (PEV) and ohmic heating (Vanga et al., 2015b, 2015c). Singh et al., evaluated the effect of high electric voltage on the wheat gluten protein which showed considerable deviations in the secondary structure of the gluten protein (Singh et al., 2015a; Singh et al., 2015b). This study was significant in understanding the effect of Electro-hydro dynamic (EHD) drying on the protein conformations.

3 PROCESSING EFFECT ON TREE NUTS

3.1 Almond allergy

Almonds are considered to be a rich source of energy due to the presence of fat (monounsaturated fat, MUFA) which can weigh up to 50% of their total weight. Even with such high contents of fat, inclusion of almonds in diet have shown a significant effect on weight loss of a person compared with carbohydrate based low-calorie diet (Wien et al., 2003). Moreover, researchers also found that the glycemic index of almonds is low and they act as a vital source of

Vitamin E, manganese, fibre, protein and various polyphenolic components (Chen et al., 2006). Though almonds may provide wide range of health benefits as stated above, it is to be noted that consumption of almonds can result in allergic reactions in sensitive patients. Sicherer et al., reported in 1999 that about 6% of the total 118 allergic participants reported sensitivity towards almonds (Sicherer et al., 1999). In a five year follow up study, they reported that 22% of the total 145 participants (89 reported tree nut allergies among 145 subjects) were allergic to almonds (Sicherer et al., 2003). Furthermore in 2010, a 11-year follow up study reported that 13.2% of the subjects to be sensitive towards almonds among the total 188 participants (Sicherer et al., 2010). Overall, almonds stand third after walnuts and cashews (among tree nuts) in causing allergic reactions. Despite the statistical relevance and wide consumption of almonds around the world, little work has been done in identification of various almond allergens. Till date, the following are the allergens found to be present in almonds: Pru du 1, Pru du 2S, Pru du3, Pru du 4, Pru du 5 and Pru du 6 (amandin shown in Figure 1) (Masthoff et al., 2013).

The effects of processing methods on almonds have been summarized in Table 2. The first attempt to study the processing effects on almond allergens was made in 1992 (Bargman et al., 1992). They reported that three allergens were detected in almonds with molecular weights of 70 kDa, 45-50 kDa and 15 kDa based on their reactions with the blood sera from allergic patients. Various heat treatments have been performed including blanching and roasting in this study which showed that the IgE reactivity of the 70kDa and 15 kDa allergens reduced but the 45 kDa allergen was stable throughout the processing. Though, the exact reasons for this deviation were not found, this kind thermal stability in few allergens can be due to the presence of disulphide bonds between amino acids as observed in case of soybean trypsin inhibitor (Cabrera-Orozco et al., 2013; Vagadia et al., 2016). Few other studies have also showed that presence of lysine and arginine residues under certain conditions can increase the heat stability (Hudson, 1992). Roux et al., investigated the stability of almond protein called Amandin (also called Almond Major Protein or AMP) which is primarily responsible for the IgE mediated reactions in humans (Roux et al., 2001). ELISA and Western blot methods were used to detect the purified AMP and to test the effectiveness of various commercial processing methods which include blanching, dry roasting (without liquid medium like oil or water) and oil roasting. Though the almonds of both the cultivars Nonpareil and Carmel showed a reduction of about 40% in reactivity when blanched and dry roasted in comparison to unprocessed almonds, it carried no clinical significance (Roux et al., 2001). Venkatachalam et al., also reported the effects of roasting (in oven), autoclaving, blanching and microwave processing on AMP. Blanching was conducted in hot water at a temperature of 100°C for 1 -- 10 min but showed no considerable difference in the reactivity of the protein. Oven was used for roasting the almonds between the temperatures 137°C to 177°C which also showed no considerable effect on the reactivity. Autoclaving and microwave processing methods were also ineffective which showed that AMP was heat stable (Venkatachalam et al., 2002). Su et al., evaluated the effects of γ-irradiation (gamma radiation) on AMP and its clinical relevance using ELISA and Western blot methods. The irradiation processing was used alone and also in combination with various thermal processing methods like roasting, microwave processing and blanching. It was found that γ-irradiation (1-25 kGy) used for processing almonds had no effect on the reactivity of AMP (Su et al., 2004). In continuation, the effects of pulsed ultraviolet light (PUV) and high hydrostatic pressure (HHP) on almond allergenicity have been evaluated (Li et al., 2013). HHP processing showed no effect on the allergens, but PUV when exposed in excess of seven minutes resulted in the reduction of IgE binding (up to 70%). The researchers hypothesized that the reduction in the allergen binding was due to protein cross-linking or fragmentation because of a prominent photo-thermal effect. Non-thermal Plasma (NTP) has also been employed in reducing the allergenicity of almond protein, but was ineffective (Li, 2011). The effects of processing methods have been summarized in Table 2.

It is clear that the major almond protein responsible for eliciting allergic reactions in humans is stable towards thermal processing methods which are normally employed in food industries. Only PUV treatment showed positive results and the researchers predict that this is due to the changes in surface properties of the protein where the epitopes move into the protein and thus reducing the viable binding sites for IgE once entering the human body (Li, 2011; Li et al., 2013).

3.2 Cashew nut allergy

Cashew nut is the seed obtained from the cashew fruit, primarily cultivated in Vietnam, Nigeria and India; and is consumed all over the world. It is rich in various micro and macro nutrients and also contains small quantities of phytochemicals and antioxidants. Though cashews have a promising effect on the human health they are also known for potential allergic reactions in sensitive patients (Shahidi and Tan, 2008b). Sicherer et al., conducted a random telephone survey in the year 1999, in which they found that 6% of the total 118 allergic patients were sensitive to cashew. After five years, a follow up study was conducted by the same group of scientists; here the percentage increased to 25% among the 145 patients who were allergic to at least one nut. In the 11 year follow up study, the patients sensitive to cashew nuts were reduced to 15.5% of the total 188 subjects identified to be allergic to at least one nut. In a study conducted by Rance et al., it was reported that the population among children sensitive to cashew nuts is on the raise and young children with an average age of 2 are at most risk (Rancé et al., 2003). The symptoms experienced on exposure to cashews were also reported to be much severe compared to those caused due to ingestion of other food allergens. Moreover, research has shown that the children are at a higher risk of anaphylaxis when exposed to cashews compared to all the other tree nuts and peanuts (Davoren and Peake, 2005). Ana o 1 (vicilin protein), Ana o 2 (legumin protein) and Ana o 3 (2S albumin protein) are the allergens reported till date to be present in cashew nuts (Masthoff et al., 2013; Robotham et al., 2005; Suzanne et al., 2003; Wang et al., 2002).

The effects of processing methods on cashew nuts has been summarized in Table 3. In a study conducted by Venkatachalam et al. the effects of various processing methods on cashew nut allergens Ana o 1, Ana o 2 and Ana o 3 have been evaluated using rabbit polyclonal antibodies and mouse monoclonal antibodies. The various processing methods include autoclaving, blanching, microwave treatments, roasting, γ-irradiation and pH variations. Roasting showed a slight reduction in the reactivity of the Ana o 1 and Ana o 3. But, Ana o 2 showed an increase in the reactivity especially when treated at higher temperatures (200°C for 15 min) which could be due to a raise in the stability of the protein secondary structure. It is important to take note that the raise in stability after processing is rare phenomenon which is also observed in few peanut allergens especially Ara h 1, Ara h 2/Ara h 6 and Ara h 3. Though the exact reason behind the increased stability is not always easy to predict, there have been attempts where researchers used

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molecular dynamics simulations to understand the structure conformations. Molecular modeling studies conducted on Ara h 6 showed a molecular compaction which resulted in reduction in number of binding sites for water and in turn increased the reactivity as an allergen (Koppelman et al., 2005a; Koppelman et al., 2010; Vanga et al., 2015c). Similar studies can be conducted on Ana o 2 allergen for further understanding the phenomenon of increased stability after thermally processing the allergen. Autoclaving at 121°C for 10 and 30 min showed no effect or little reduction in the immunoreativity (statistically insignificant). Blanching and microwave treatments also had no profound effect on the stability of the cashew allergens. γ-irradiation treatments also showed no significant changes in the reactivity of the protein suggesting that all the allergens have a high heat stability (Venkatachalam et al., 2008). Su et al., also conducted experiments for understanding the effects of γ-irradiation on cashew allergen and found no significant changes in its reactivity. Frying the cashews at high temperature of 191°C for one minute also showed no effect on the stability of cashew protein (Su et al., 2004). Researchers have also tried to evaluate the effects of sulfates on the cashew protein allergenicity. Sulfate containing compounds like Sodium bisulfate, Sodium metabisulface and Sodium sulfate are on the "generally recognized as safe (GRAS)" list mandated by the U.S. Food and Drug Administration (FDA). This means that presence of these components is regarded as safe when they are present with in the specified limits. It was reported that the sodium sulfate treatments have successfully disrupted the secondary structures of the Cashew allergen Ana o 3. But, the clinical relevance is doubtful as complete reduction in immunoreactivity with human serum has not been tested (Mattison et al., 2014).

3.3 Hazelnut allergy

Hazelnut belongs to the *Betulaceae* family, primarily grown in Turkey and Italy (nearly 90% of world production) and is widely consumed in Europe. It contains all of the macro and micro nutrients though there is a slight variation in the composition, aroma and taste depending on the variety. These nuts act as an excellent source for Copper, Vitamin E and Biotin (Cesarettin et al., 2008). In 2000, Ortolani et al., have shown that hazelnuts can also cause allergic reactions in sensitive patients. They reported that out of 86 subjects tested (all subjects in Europe), 78% showed a positive reaction to a double-blind, placebo-controlled food challenge (DBPCFC). In North America, Sicherer et al., reported that only 2.5% of the 118 participants (allergic to tree nuts) have reported sensitivity towards hazelnuts (Sicherer et al., 1999). In a five year follow up study conducted, nearly 17% of the total number of participants reported to be allergic to hazelnuts which is significantly higher compared to the 1999 study (Sicherer et al., 2003). In the 11-year follow up study, 7.5% of the total 188 participants were found to be allergic to hazelnuts (Sicherer et al., 2010). It was also reported that patients sensitive to hazelnuts commonly suffer from cross reactivity to tree pollen allergens (Birch pollen) (Schocker et al., 2000). The various allergens present in hazelnut include Cor a 1 to Cor a 14 (Masthoff et al., 2013). Figure 2 show the secondary structure of Cor a 8 allergen of Hazelnut.

Table 4 outlines the effects of various processing methods on Hazelnuts. Hansen et al., studied the effect of roasting on hazelnuts and evaluated to indicate reduction in allergenicity upon processing them at 140°C for 40 min. But, this reduction is not of clinical significance as 29% of the subjects showed allergic symptoms upon consumption of roasted hazelnuts. Thus, consumption of roasted hazelnuts cannot be recommended as an alternate method for population suffering from sensitivity towards hazelnuts especially Cor a 1.04 and Cor a 2 (Hansen et al., 2003). In 2008, Worm et al. also studied the impact of thermal processed and encapsulated hazelnuts on allergic patients. They reported that that roasting hazelnuts at 144°C (time was not specified) has reduced the reactivity and suggested that this processing method might lower the risk in majority of patients sensitive to hazelnuts. But, they also suggested as in the case of Hansen et al. that roasting failed to eliminate the IgE binding sites completely (Worm et al., 2009). Though there is reduced reactivity due to roasting, the allergens present in hazelnuts are considered to be heat stable and are responsible for causing severe reactions in the sensitive population (Schocker et al., 2000). Wigotzki et al. reported that the effect of microwave processing on the allergenicity of hazelnuts was minimal (Wigotzki et al., 2000). Conventional thermal treatments were also employed where hazelnut meal was processed between temperatures 100°C to 185°C. They reported that the allergens have high stability against heat treatments, especially allergens of lower molecular weight (14 kDa). The same study reported the effects of variety and storage duration showed no influence on the allergenicity of hazelnuts (Wigotzki et al., 2000). Lopez et al. studied the effects of autoclaving and high pressure processing on the hazelnut allergens. In this study, they stated that autoclaving, especially at temperature of 138°C for 15-30 min had a profound effect on the allergens Cor a 1, Cor a 8, Cor a 9 and Cor a 11. The high pressure processing (300-600 Mba) performed on hazelnut allergens showed no reduction in the allergenicity (López et al., 2012). Maillard reaction which is the glycation reaction between the amino acid groups present in protein with reducing sugars was reported to be responsible in reducing the immunoreactivity of Cor a 11 allergen found in hazelnuts. For this study, the Cor a 11 allergen was extracted from the fresh hazelnut flour (Iwan et al., 2011). Cucu et al. also investigated the effects of thermal processing and glycation on the hazelnut allergens. They reported that Cor a 9 and Cor a 1 were stable even after glycation in the presence of glucose at 70°C for two days (Cucu et al., 2011). They also reported that glycation enhanced (in 33% cases) and reduced (in 50% of cases) the reactivity of hazelnut allergens for which the reasons are still unclear (Cucu et al., 2012). Ortiz et al., evaluated the effect of extrusion processing in combination with thermal stress on hazelnuts and reported reduction in the immunoreactivity of few allergens. This study used mouse model and found that allergens in the range 31-47 kDa and 129 kDa have showed a reduction in the allergenicity, but allergens specific to 48-55 kDa and <15 kDa showed no reduction in the reactivity (Ortiz et al., 2014; Ortiz, 2014).

3.4 Walnut allergy

Walnuts belong to the *Juglandaceae* family and are consumed all over the world. They also act as a major ingredient in energy bars and other snack foods. Recent studies have shown that walnuts promote a healthy lifestyle as they have higher quantities of polyphenols when raw and roasted (Vinson and Cai, 2012). They were found to reduce the risk of diabetes and cardio-vascular diseases (Jackson and Hu, 2014; Toshiyuki, 2008). But, walnuts have also been recorded to cause allergic reaction in some sensitive individuals. In 1996 study, 8 patients were found to be allergic to walnut out of the total 103 patients who were diagnosed to be allergic to at least one nut (including peanut) (Ewan, 1996). Sicherer et al., conducted an extensive study over a period of eleven years in estimating the prevalence of tree nut allergies (Sicherer et al., 1999). As a part of this investigation, 118 subjects were studied in total out of which 20% reported to be allergic to walnuts. In the five year follow up, this percentage grew to 35% (in total 145 subjects) making it the most potent tree nut responsible for IgE reactivity in sensitive patients (Sicherer et al., 2003). This percentage reduced to 22% (in total 188 subjects) in the eleven year follow-up study conducted (Sicherer et al., 2010). Despite being reported as a tree nut responsible for highest number of allergic reactions, the work done on walnuts is limited. There are primarily two varieties of walnuts and both of them have different allergens present in them viz., English walnut: Jug r 1, Jug r 2, Jug r 3, Jug r 4, Jug r 5 and Black Walnut: Jug n 1, Jug n 2 (Costa et al., 2014).

Table 5 summarizes the processing effects on Walnuts. Su et al. conducted an extensive study understanding the effects of various thermal processing methods (blanching, microwave treatment, frying, dry roasting and autoclaving) and γ-irradiation (gamma radiation) treatments. They evaluated and found that the allergens present in walnut are highly stable against all the processing methods with the exception of one treatment method. When walnuts were treated at 25 kGy and autoclaved at 121°C at 15 psi for 30 min, they showed a significant reduction in band of 45 kDa (Jug r 2); all the other bands were stable (Su et al., 2004). The impact of roasting was also evaluated in which the walnuts were roasted at a temperature 120°C for 15 min in an oven. This study also showed that that the allergens were highly stable and showed no reduction in reactivity (Doi et al., 2008). Downs (Downs, 2013) also evaluated the effects of roasting on the individual sections of the walnut protein and reported that digestibility of 7S vicilin and 11S legumin had increased which can be associated to secondary structure deviations in the protein and in few cases to a reduction in allergenicity. But, in case of walnuts this might not be true because various other researchers have shown these allergens to be highly stable. However, further studies have to be conducted on the associated changes in the secondary structures to determine its complete effect on the allergenicity of walnut. Vanga et al., 2015 have attempted to evaluate the digestibility of peanut proteins that were roasted in conventional hot air oven, microwave and high voltage electric field (HEF). It showed that the digestibility has improved with processing, but researches like Maleki et al., estimated that roasting has actually increased the allergenicity of Ara h 1 and Ara h 2 major allergens (Maleki et al., 2000; Maleki and Hurlburt, 2004; Vanga et al., 2015b). Sordet et al., illustrated that the Jug r 1 allergen secondary structure is resistant to heat processing (up to 90°C) (Sordet et al., 2009). The influence of high pressure processing and its combination with thermal treatments on walnut have also been evaluated. It was reported that pressure treatment at 256 kPa and 138°C effectively reduced the immunoreactivity of walnut protein. The reduction was significant compared to the effectivity of high pressure treatments (up to 600 MPa) at lower temperatures (Cabanillas et al., 2014).

3.5 Brazil nut allergy

Brazil nuts belong to the *Lecythidaceae* family and is primarily grown in the Amazon rain forest spread over large areas of Brazil, Bolivia and Ecuador in South America. They act as an excellent source for minerals, lipids and phytochemicals (Yang, 2009). The bioactive components like antioxidants that are present in abundance promote health by stimulating the immune system (Shahidi and Tan, 2008a). Recent studies have also shown that regular consumption of brazil nuts can regulate the selenium (Se) levels in humans and reduce the risk of cardiovascular diseases, especially in obese women (Cominetti et al., 2012). In spite of these apparent health benefits, the people suffering from sensitivity towards brazil nuts is on the rise. Random telephone survey conducted by Sicherer et al., showed a varying percentage in the people allergic to brazil nuts over the period of eleven years. The initial survey reported that about 6.7% of the total participants were allergic to brazil nuts (Sicherer et al., 1999). This increased to about 17% in five years and then decreased to about 10% in the 11 year follow up study (Sicherer et al., 2010; Sicherer et al., 2003). The allergic reactions to brazil nuts have been reported to be critical and in few cases life threatening (Ewan, 1996). Moreover, researchers from United Kingdom reported first ever documented case of sexually transmitted allergic reaction to brazil nut proving it to be unique and dangerous (Bansal et al., 2007). The two major allergens present in brazil nuts are: Ber e 1 (shown in Figure 3) and Ber e 2 (Masthoff et al., 2013).

The effects of processing on Brazil nuts have been tabulated in Table 6. Stability of Brazil nut allergen Ber e 1 has been evaluated using *in vitro* gastrointestinal digestion by Moreno et al. It was reported that the allergen was stable and intact even after the gastric and duodenal digestion. The stability of both the raw and roasted (100°C for 20 min) allergens was evaluated and it was evaluated that roasting did not affect the allergic reactions even after extensive digestion (Moreno et al., 2005). The digestion studies using pepsin have been conducted which showed that the denaturation temperature of major protein 2S albumin in brazil nut is higher than 110°C at neutral pH. However, at a lesser pH, the protein was found to denaturize at much lower temperatures (around 82°C). But, the denaturation is reversible as the secondary structure and tertiary structures in brazil nuts are extremely stable, thus making the protein resistant to digestion. Reversible denaturation was reported to be the reason for the structural stability of Brazil nut 2S albumin protein and thus the high stability of Ber e 1 allergen (Koppelman et al., 2005b). It was later reported that pH of 5 -- 7 in combination of temperatures exceeding 110°C can denaturize the Ber e 1 allergen. But, the limitations of thermal processing methods, where major portions of food are under processed would result in allergic reactions upon comsumption (van Boxtel et al., 2008). Van Bilsen et al. determined the structural influence of the brazil nut allergen Ber e 1 in eliciting an allergic reaction. The disulphide bonds present in the 2S albumin protein of brazil nut are reduced by alkaline treatment which resulted in partial denaturation of the protein. This resulted in reduction of the sensitising potential in the rat food allergy model used in the study. However, it failed in eliminating the immunoreactivity of the protein. Thus, the disulphide bond cleavage does not affect the allergenicity (Van Bilsen et al., 2013).

3.6 Pecan nut allergy

Pecan nuts are mostly indigenous to America and the early plantations are known to be 1000 years old. These nuts have served as part of the staple diet for various North American native tribes before the English settled. Currently, USA alone produces more than 80% of the world's pecan supply from thousands of documented cultivars developed over centuries. They are widely used in bakery products, candies and dairy product flavourings (Ronald and Ronald, 2008). Pecans are second most commonly consumed nuts in United States and various reports have suggested that they have an exceptionally positive effect on the human health. Morgan et al., reported that the consumption of pecans at about 60 -- 70 g per day can reduce the Low Density Lipoprotein Cholesterol (LDL-C), there by promoting healthy heart (Morgan and Clayshulte, 2000). They are also a rich source of various phytochemicals, vitamins, minerals and flavonoids which have a wide range of health benefits (Ronald and Ronald, 2008). However, despite the health benefits, pecan nuts have been associated with IgE mediated allergic reactions. Sicherer et

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al., evaluated that about 6% of the total 118 participants of the random telephonic survey have reported to be allergic to pecan nuts which increased to 20% in a period of five years. In the 11 year follow up conducted by the same group of researchers the percentage dropped to 14% (Sicherer et al., 1999; Sicherer et al., 2010; Sicherer et al., 2003). Pecan nuts can be particularly dangerous source of allergic reactions because they develop new epitopes when thermally processed or stored (aged). These new sites with IgE reactivity are called neoallergens and are normally not present in the fresh pecan nuts (Berrens, 1996; Malanin et al., 1995). Moreover, cross reactivity between the allergens of pecan and walnuts have also been documented (Sharma et al., 2011). Car i 1and Car i 4 are the two major allergens found in pecan nuts apart from Car i 2 (Costa et al., 2014; Masthoff et al., 2013).

Table 7 outlines the various processing effects on Pecan nuts. Venkatachalam et al., investigated the effects of thermal processing on the major pecan nut allergens. The treatments used in the evaluation included blanching (100°C for 5 and 10 min), autoclaving (121°C for 5, 15 and 30 min at 15 psi) and Dry roasting (137°C - 175°C). It was concluded that the allergen bands of pecans are relatively stable though there is a slight reduction in the reactivity after exposing the nuts to extreme thermal processing parameters. These parameters might not be viable commercially as the external appearance would be considered and are destroyed affecting the consumer choice (Venkatachalam, 2004; Venkatachalam et al., 2006). Further, the authors predict that the loss of reactivity might be due to protein solubility rather than destruction of the reactive epitopes present in the protein (Masthoff et al., 2013). The influence of boiling and microwave treatment on pecan allergens have also been evaluated (Polenta et al., 2012). It was found that microwave treatment at 600W for 15 min had reduced the detectability of the allergens to 7% in comparison to the control (untreated sample) with 97% detectability (Polenta et al., 2012).

3.7 Pistachio allergy

Pistachio nuts are primarily produced in Iran and United States, but widely consumed all over the world (Navindra et al., 2008). However, they are also attributed to cause acute allergic reactions in people sensitive to the allergens Pis v 1, Pis v 2 and Pis v 3 (Masthoff et al., 2013). Sicherer et al., estimated that about 10% of the total 188 participants evaluated were allergic to pistachios (Sicherer et al., 2010). Only one extensive publication has been found which evaluated the effects of dry roasting and steaming on allergenicity of pistachio protein. The authors of this study have reported that the steaming had reduced the reactivity of the allergens compared to dry roasting methods. They soaked the nuts in a solution containing lemon water (pH 3.2 - 3.2) and Sodium chloride (1.6% w/v) for 12 hours prior to any processing. They hypothesised that the ionic strength of soaking solution in combination with steaming might have altered the secondary structure of the protein resulting in reduced reactivity. They further conducted sensory evaluation of the processed nuts and found no significant difference in various attributes including aroma, color, flavor, taste and overall acceptability. But, this could also be a result of allergens being transferred to water or solution during soaking or steam processing rather than the secondary structure changes. Further analysis using CD spectrometer to evaluate the secondary structure changes can be performed to prove the hypothesis of the authors (Noorbakhsh et al., 2010).

3.8 Other Tree nuts

3.8.1 Chestnut

Chestnut has been cultivated for many centuries in parts of Europe and Asia and was a staple crop and major source of complex carbohydrates before the introduction of potatoes. Currently, China is the largest producer of chestnuts followed by South Korea, Italy and Turkey. It is used as an important ingredient in various sophisticated dishes due to its positive nutritional characteristics. It can be consumed raw, but is usually boiled or roasted to improve the flavor and texture (Korel and Balaban, 2008). However, there have been numerous cases of chestnut allergies around the world. Lee et al., reported that chestnut is the third most prevalent allergy in South Korea (Lee, 2004). Chestnut allergies have been commonly reported in terms of latex-fruit syndrome (Raulf-Heimsoth et al., 2007). There have been various reports of cross reactivity between allergy to chestnuts, latex rubber and various fruits primarily banana, apple, kiwi and pineapple. Cas s 1, Cas s 5 and Cas s 8 are allergens present in chestnuts (Sathe et al., 2005). Lee et al., reported that the allergenicity decreased with boiling and treatment with digestive enzymes (Lee et al., 2005). This might be due to the solubility of the allergens in water rather than secondary structure modifications.

3.8.2 Pine nut allergy

Allergic reactions to pine nuts are relatively uncommon compared to other tree nut allergies (Ponsaille and Bourrier, 2013). Though there have been various reports of allergic reactions, the work done on characterisation and evaluation of allergens in pine nut is very limited. Fleischer et al., estimated that 5% of the subjects in a total of 278 patients suffering from at least one food allergy were sensitive to pine nuts (Fleischer et al., 2005). Patients with primary allergenic reactions to almonds and peanut have also been reported previously cross reacting to Pine nuts (de las Marinas et al., 1998; Ponsaille and Bourrier, 2013). Toomer et al., investigated the effects of digestion (gastric and intestinal) on the allergenicity of pine nuts. They reported that the acid denaturation followed by protease cleavage of allergic proteins has reduced the reactivity in immunoblotting analyses (de las Marinas et al., 1998). But, the application of acidic treatments in food is always in question due to its downsides in terms of nutritional quality and the residues after processing (Gould, 1995).

3.8.3 Coconut allergy

Allergic reactions to coconut are extremely rare and there are very few cases reported in the literature. Moreover, only one patient has been clinically reported to be primarily allergic to coconuts. Two other cases found that these patients were allergic to walnuts and almonds and coconut could be a result of cross-reactivity (Rosado et al., 2002; Tella et al., 2003). Cocosin (as shown in Figure 4) belonging to the protein family cupin was found to be one of the allergens present in coconuts. This allergen was also found to be cross reactive to walnuts and peanuts (Sathe et al., 2005).

3.8.4 Macadamia nut allergy

Macadamia nuts are primarily grown in Australia and are also called as Queensland nuts. Though not widely consumed like other tree nuts and peanuts, there have been reports of patients allergic to macadamia nuts as reported by Pallares (Pallares, 2000), Lerch et al. (Lerch et al., 2005) and Knop et al. (Knop et al., 2010). Sicherer et al., estimated that about 9% of the total

188 participants responding to various allergens are sensitive to macadamia nuts (Sicherer et al., 2010).

4 CONCLUSION

This review provides an update on the impact of various processing methods on tree nut allergies. Though few processing methods seem promising in reducing the IgE reactivity, it is still uncertain to what extent these results are clinically relevant. Various reports have indicated an increase in the cases of food allergies around the world, especially sensitivity to peanuts and tree nuts. The only solution for the allergic patients is complete abstinence from that particular food or ingredient which is not viable in all instances. Under the given circumstances, it is critical to understand the structural changes in the protein due to processing to comprehend whether a specific technique (conventional and novel) is aggravating or mitigating the allergenicity. Work done by Vanga et al., 2015 using bibliometric analysis has also shown a clear difference where a higher research concentration is being emphasized on the immunological aspects rather than understanding the conformational changes in the protein structures (Vanga et al., 2015a). Various modern means like molecular modeling (Vanga et al., 2015c) and genome studies (Di Girolamo et al., 2015) are now being employed to understand the conformational changes in the protein which can affect the allergenicity. The above mentioned studies in combination with analytical techniques like Circular Dichroism (CD) and NMR spectroscopy can help researchers to answer the complex relationship between the between structure and functional properties of the protein.

Conflict of Interest

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The authors report no conflict of interest

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Table 1. Allergic nuts and their Binomial name

Name of the nut	**Scientific name**	**Allergens**
Almond	*Prunus dulcis*	Pru du 1, Pru du 2S, Pru du 3, Pru du 4, Pru du 5 and Pru du 6 (amandin)
Brazil nut	*Bertholletia excelsa*	Ber e 1 and Ber e 2
Cashew	*Anacardium occidentale*	Ana o 1, Ana o 2 and Ana o 3
Chestnut	*Castanea sativa*	Cas s 1, Cas s 5 and Cas s 8
Coconut	*Cocos nucifera*	Cocosin
Hazelnut	*Corylus avellana*	Cor a 1 to Cor a 14

Macadamia nut	*Macadamia intergrifolia*	-
Pecan nut	*Carya illinoinensis*	Car i 1, Car i 2 and Car i 4
Pine nut	*Pinus pinea*	-
Pistachio	*Pistacia vera*	Pis v 1, Pis v 2 and Pis v 3
Walnut	*Juglans regia* *Juglans nigra*	English Walnut: Jug r 1, Jug r 2, Jug r 3, Jug r 4, Jug r 5 Black Walnut: Jug n 1, Jug n 2

Table 2: Summary of processing effects on Almonds

Processing	Effect/Outcome	Studies conducted
Blanching & Roasting	70 kDa -- reduced 45 kDa - Stable	(Bargman et al., 1992)
Blanching, Dry roasting & Oil roasting (Only AMP)	Reduction of reactivity by about 40%. No clinical significance	(Roux et al., 2001)
Blanching, Oven roasting, Autoclaving and Microwave processing (Only AMP)	No considerable change in reactivity. Mostly heat stable	(Venkatachalam et al., 2002)
γ-irradiation (Only AMP)	No effect	(Su et al., 2004)
Non-thermal Plasma	No effect	(Li, 2011)
High Hydrostatic Pressure	No effect	(Li et al., 2013)
Pulsed ultraviolet light	70% reduction in IgE binding. No clinical significance	(Li et al., 2013)

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Table 3: Summary of processing effects on Cashew nut

Processing	Effect/Outcome	Studies conducted
γ-irradiation & Frying	No effect	(Su et al., 2004)
Roasting	Ana o 1, Ana o 3 -- Slight reduction Ana o 2- Increased stability	(Venkatachalam et al., 2008)
Autoclaving, Blanching, Microwave & γ-irradiation	No effect. Highly stable allergens	(Venkatachalam et al., 2008)
Sulfate compounds	Ana o 3 -- Significant effects on secondary structure.	(Mattison et al., 2014)

Table 4: Summary of processing effects on Hazelnut

Processing	**Effect/Outcome**	**Studies conducted**
Conventional treatments & Microwave	No effect. Highly heat stable allergens	(Wigotzki et al., 2000)
Roasting	No clinical significance	(Hansen et al., 2003)
Roasting	Slight reduction in reactivity -- no clinical significance	(Worm et al., 2009)
Maillard reaction	Reduction in reactivity of Cor a 11	(Iwan et al., 2011)
High pressure processing	No effect	(López et al., 2012)
Autoclaving	Effected few allergens	(López et al., 2012)
Glycation	Cor a 9, Cor a 1 -- stable	(Cucu et al., 2011)
Glycation	Enhanced reactivity in few cases and reduced reactivity in few others	(Cucu et al., 2012)
Extrusion	Moderately effective	(Ortiz et al., 2014; Ortiz, 2014)

Table 5: Summary of processing effects on Walnuts

Processing	Effect/Outcome	Studies conducted
Blanching, Microwave treatment, Frying, Dry roasting and Autoclaving	No effect Highly heat stable	(Su et al., 2004)
γ-irradiation	Significant reduction in 45 kDa bands	(Su et al., 2004)
Roasting	No effect	(Doi et al., 2008)
High pressure thermal processing	Moderately effective	(Cabanillas et al., 2014)

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Table 6: Summary of processing effects on Brazil nuts

Processing	Effect/Outcome	Studies conducted
Roasting followed by digestion	No effect. Highly stable secondary structure	(Moreno et al., 2005)
Alkaline treatment	Not effective in eliminating immunoreactivity.	(Van Bilsen et al., 2013)

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Table 7: Summary of processing effects on Pecan nuts

Processing	Effect/Outcome	Studies conducted
Blanching, Autoclaving & Dry roasting	Slightly effective	(Venkatachalam, 2004; Venkatachalam et al., 2006)
Boiling	No effect	(Polenta et al., 2012)
Microwave treatment	Reduced detectability	(Polenta et al., 2012)

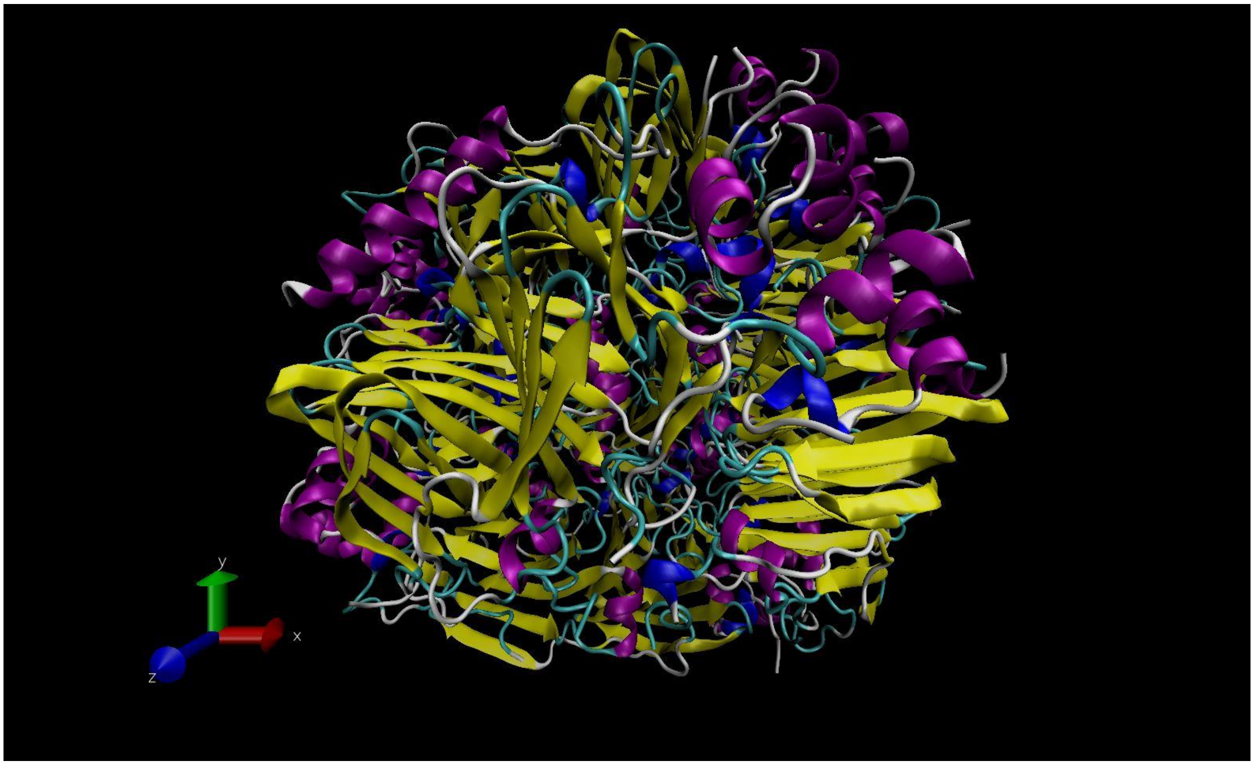

Figure 1. Secondary structures of Amandin allergen in Almond (PDB code: 3FZ3) [α-helix: Purple, 3/10 helix: Blue, π-helix: Red, β-sheets: Yellow, Turns: Cyan, coils: White]

Figure 2. Secondary structures of Cor a 8 allergen in Hazelnut (PDB code: 4XUW) [α-helix: Purple, 3/10 helix: Blue, π-helix: Red, β-sheets: Yellow, Turns: Cyan, coils: White]

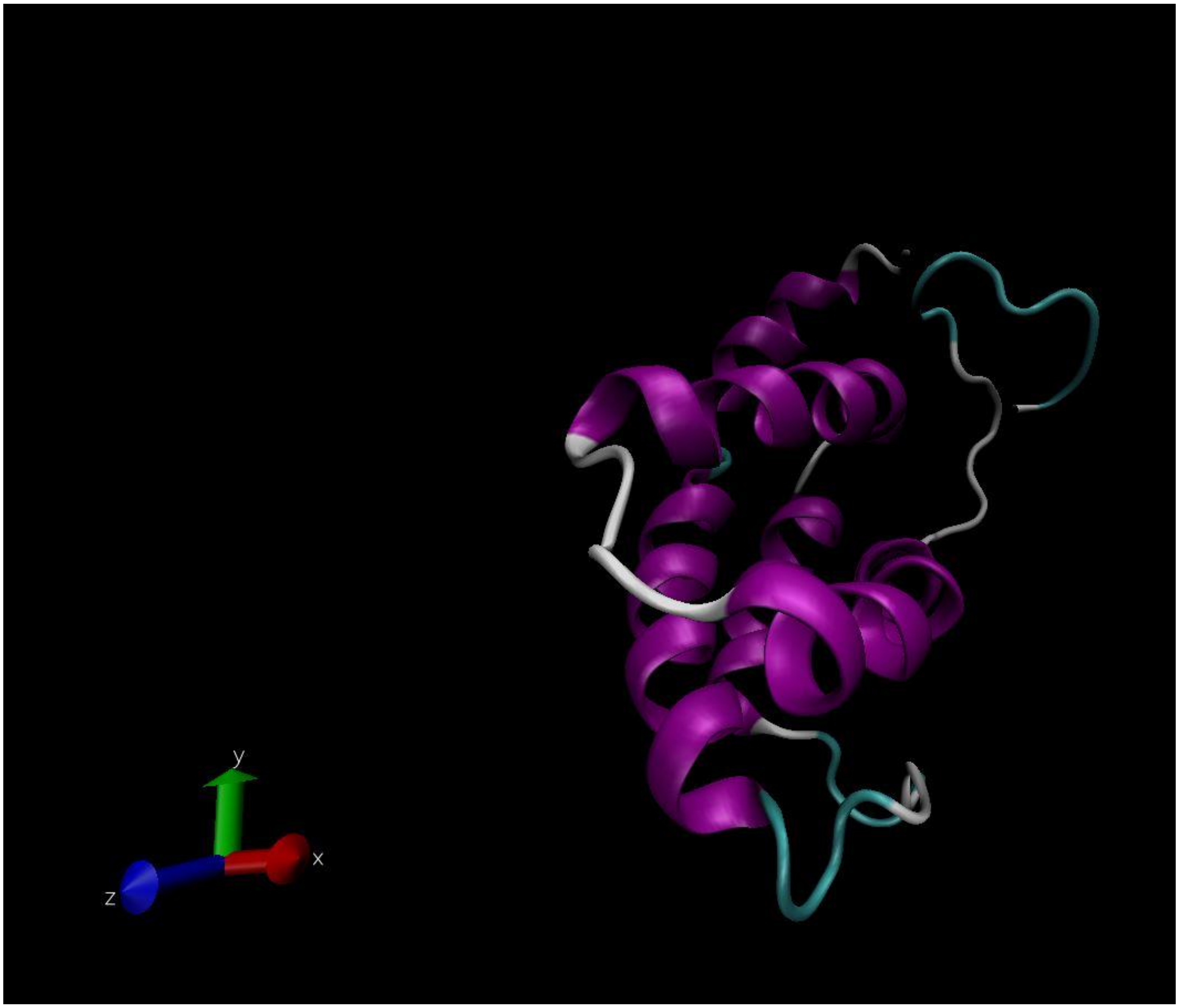

Figure 3. Secondary structures of Ber e 1 allergen in Brazil nut (PDB code: 2LVF) [α-helix: Purple, 3/10 helix: Blue, π-helix: Red, β-sheets: Yellow, Turns: Cyan, coils: White]

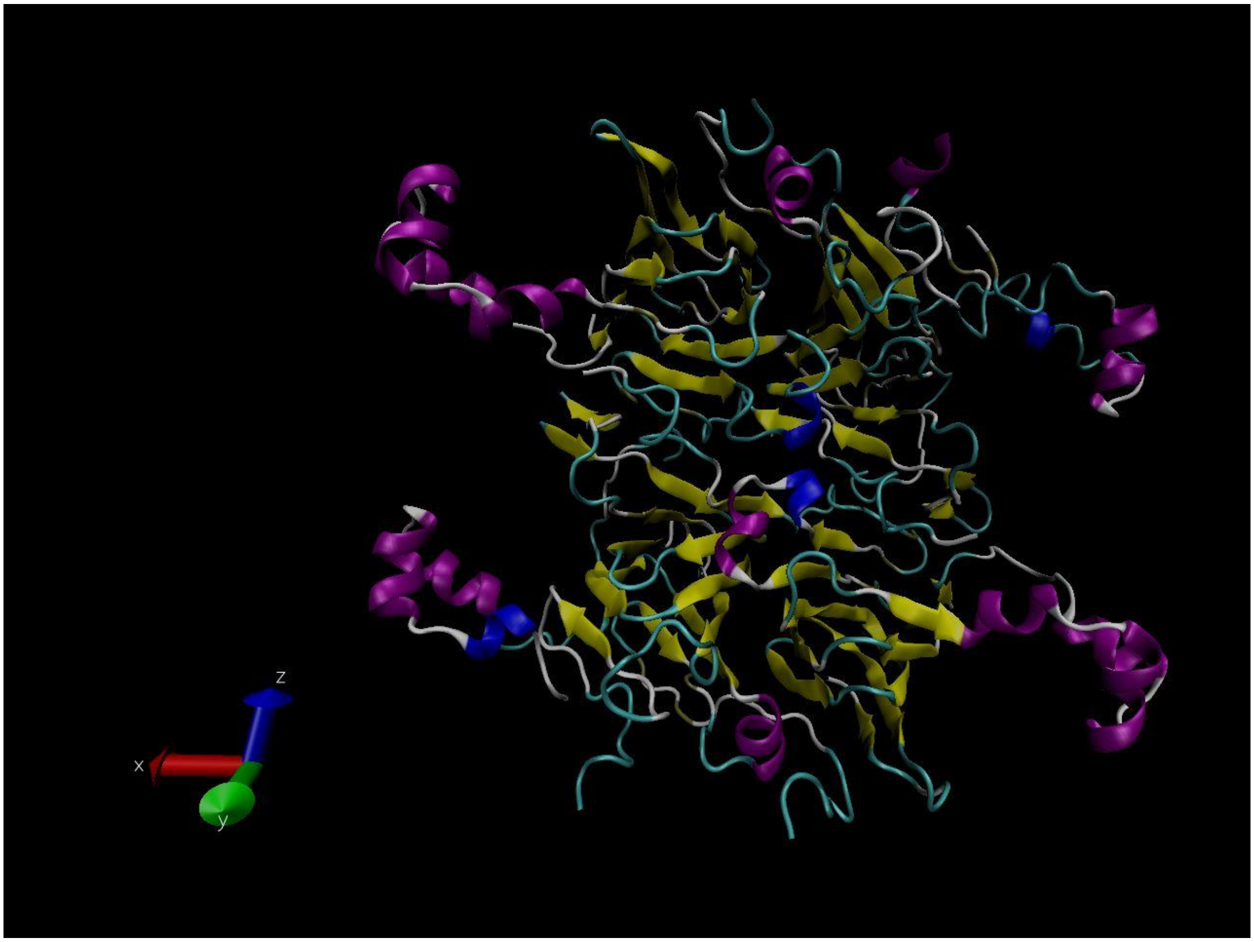

Figure 4. Secondary structures of Cocosin allergen in Coconut (PDB code: 1XGF) [α-helix: Purple, 3/10 helix: Blue, π-helix: Red, β-sheets: Yellow, Turns: Cyan, coils: White]