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Effect of cold plasma treatment on the antigenicity of peanut allergen Ara h 1

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ABSTRACT

The objective of this study was to investigate the effects of cold atmospheric plasma on the antigenicity of protein Ara h 1. Dry, defatted peanut flour (DPF), whole peanut (WP) were subjected to cold atmospheric plasma at voltage of 80 kV for different treatment durations (0, 15, 30, 45 and 60 min). The allergen samples were analyzed using SDS-PAGE, immunoblot and competitive ELISA. Furthermore, the secondary structure was examined using circular dichroism. SDS-PAGE results revealed no change in the protein intensity bands corresponding to Ara h 1 for both DPF and WP. Competitive ELISA of samples showed a reduction in antigenicity up to 43% for DPF and 9.3% for WP. Circular dichroism studies revealed modifications in secondary structure induced by plasma reactive species.

Industrial relevance: Cold plasma has emerged as a novel processing technique. This study provides evidence for reduction of antigenicity of Ara h1 in peanuts using cold plasma. This study also demonstrated the plasma-induced changes in protein structure at high treatment duration. The work described in this research is relevant to the processing of cereal grains and legumes wherein allergenicity is a major concern. This results provide the basis for possible industrial implementation.

1. Introduction

Food allergy is considered a major public health concern in both developing and developed countries. It affects nearly 1–2% of adults and 3–6% of children in developing countries (Jerschow, Lin, Scaperotti, & McGinn, 2014). Food allergy is an allergic reaction or abnormal immunological response that occurs when certain foods are consumed (Chizoba Ekezie, Cheng, & Sun, 2018). Food allergy results from an adverse immunoglobin E (IgE) mediated (type 1) reaction towards antigens, which are mostly proteins (Jerschow et al., 2014; Johnson et al., 2010). The region on an antigen to which an antibody binds is called an epitope, and they are classified into linear and conformational epitopes (Meinlschmidt et al., 2016). Any food is capable of triggering an allergic reaction; however, eight main protein sources are found to trigger 90% of all allergic reaction. These are comprised of milk, eggs, peanuts, tree nuts, crustacean/shellfish, wheat and soy (Meinlschmidt et al., 2016; Pfeifer et al., 2015; Piersma, Gaspari, Hefle, & Koppelman, 2005; Vanga, Singh, & Raghavan, 2017). Of these, peanut allergy is the most prevalent food allergy in United States and in European countries, affecting nearly 1% of the population (Luo et al., 2013; Pfeifer et al., 2015). Some studies also suggest the prevalence to be much higher, with an estimate of 10% of children in the United Kingdom being sensitized to peanuts (Comstock, Maleki, & Teuber, 2016; Luo et al., 2013; Nesbit et al., 2012). Additionally, the incidence of peanut allergy appears to be increasing in the past decade, contributing to a growing global concern, given the severity of allergic reactions. Since, allergies are mainly triggered by IgE, lowering the allergen levels might be an effective way to reduce the allergenic risk (Vissers et al., 2011). Earlier, several thermal methods were used to reduce the allergenicity. However, few allergens were resistant to thermal processing (Cabanillas, Jappe, & Novak, 2018; Comstock et al., 2016; Maleki, Schmitt, Galeano, & Hurlburt, 2014). Generally, food allergens belong to a particular group of protein families. These comprise the cupin family (7S and 11S seed storage proteins) including vicilins and legumins, the prolamines family characterized by 2S albumins and plant-defense proteins including proteases and protease inhibitors (Zhuang & Dreskin, 2013). To date, 16 peanut allergens have been identified of which Ara h 2 and Ara h 6 were most frequently sensitized followed by Ara h 1, Ara h 3 and Ara h 7 (Cabanillas et al., 2018). Out of these five major allergens Ara h 2, Ara h 6 and Ara h 7 belong to the family of the 2S albumin storage proteins with a molecular weight between 14 and 20 kDa. Ara h 6 show 55% amino acid sequence homology to Ara h 2 where Ara h 7 shows 40% (Vissers et al., 2011). Similarly, Ara h 1 and Ara h 3 belong to the cupin family, studies

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indicate that Ara h 3 consists of a set of polypeptides with molecular weights ranging from 14 to 45 kDa (Koppelman et al., 2003). Ara h 1 is a glycosylated, seed-storage protein similar to vicilin in legumes. Ara h 1 has a molecular weight of 63.5 kDa. The subunits of Ara h 1 naturally assemble into trimers (~180 kDa) and aggregate to form multimers (~600–700 kDa) (Nesbit et al., 2012; Vissers et al., 2011). It has been found to have 23 linear epitopes mainly in the areas of the inter-subunit contact which are protected from protease degradation (van Boxtel, van Beers, Koppelman, van den Broek, & Gruppen, 2006). Subsequently, changes in these characteristics may result in changes in allergenicity which include changes in epitope structure, epitope accessibility, and protein digestibility (Besler, Steinhart, & Paschke, 2001). Therefore, there is a need to investigate allergens in their natural form, as particular differences in characteristics may result in incorrect conclusions about their allergenic activity (van Boxtel et al., 2006).

Foods, including peanuts, are subjected to a wide range of processing methods to improve their quality, sensory attributes and shelf-life (Vanga et al., 2017). During processing food proteins undergo modifications like protein-unfolding, aggregation and chemical modification. The changes in structural and chemical properties of the protein itself depend on the type of thermal processing and the operating conditions applied (temperature, pH, and time) (Poms, Capelletti, & Anklam, 2004). For instance, roasting has been shown to increase the allergenicity of Ara h 1 while boiling has been shown to decrease it (Beyer et al., 2001). However, thermal processing methods can provoke losses in organoleptic quality, nutritional profile or even techno-functionality (Chizoba Ekezie et al., 2018). Consequently, researchers are in search for non-thermal technologies to reduce food allergens without affecting the nutritional quality. Different non-thermal processing methods such as pulsed light, pulsed electric field, high pressure processing (Johnson et al., 2010), irradiation (Luo et al., 2013), ultrasound (Li, Lin, Cao, & Jameel, 2006) and cold plasma can induce changes in protein conformation and reduce the allergenicity of certain proteins, while retaining nutritive value of food materials (Chizoba Ekezie et al., 2018). Recently, cold plasma processing has emerged as a novel processing method for improving the quality and shelf-life of food (Misra, Schlüter, Cullen, & Patrick, 2016). Plasma is a partially or wholly ionized state of a gas. Cold plasma generated using a dielectric barrier discharge (DBD) set-up has been widely employed for application of food systems. As a novel technology, plasma oxidation provides several advantages with the production of oxidizing species and radicals (O·, ·OH, N·, HO_2·, N_2^*, N^*, OH^-, O_2^-, O^-, O_2^+, N_2^+, N^+, NO, O^+, O_3 and H_2O_2) and other physical effects including UV light, shock waves and cavitation (Sarangapani et al., 2017). However, very few studies have reported the effects of cold plasma on food allergens (Sarangapani, Patange, Bourke, Keener, & Cullen, 2018). Several authors have investigated the application of non-thermal plasma for microbial decontamination (Ziuzina, Patil, Cullen, Keener, & Bourke, 2014) and its modification of food and packaging surfaces (Thirumdas, Sarangapani, & Annapure, 2015). Further applications include increase in surface hydrophobicity in biscuits (Misra et al., 2014), modification of flour (Sarangapani et al., 2016), dough rheology and mixing properties (Misra et al., 2015; Pal et al., 2016), enhancement of germination in rice grains (Chen et al., 2016), degradation of mycotoxins and pesticides residues (Devi, Thirumdas, Sarangapani, Deshmukh, & Annapure, 2017; Sarangapani, O'Toole, Cullen, & Bourke, 2017). Several authors have reported that plasma reactive species are responsible for such inactivation, and could also modify the structure of allergen proteins (Chauvin, Judée, Yousfi, Vicendo, & Merbahi, 2017). The amount and nature of these reactive species in plasma reactors is dependent on different input process parameters, such as inducer gas composition (O_2, N_2, Ar etc.) gas flow, electrical power applied and electrode shape (Dojčinović et al., 2011). However, the effect of cold plasma on protein structure is not fully understood. The available literature on cold plasma interactions on proteins reveals a change in the protein structure. The loss of secondary structures such as α-helical and ordered β sheet elements occur on the exposure of enzymes PPO and POD to plasma (Surowsky, Fischer, Schlueter, & Knorr, 2013). Similarly, Segat et al. (2014) and Attri et al. (2015) reported plasma induced changes in protein structures. However, very little information is known about its effects on allergens. Early reports by Nooji (2011b) and Shriver, Yang, Chung, and Percival (2011) suggest that cold plasma could possibly reduce the food allergenicity. Recently, Meinlschmidt et al. (2016), reported a profound change in soy immunoreactivty after cold plasma treatment. To the best of our knowledge, there is no report or literature available on the reduction in allergenicity of peanuts particularly Ara h1 by cold plasma. Hence, the objective of this study was to investigate the effects of cold plasma on allergenicity of Ara h 1.

2. Materials and methods

2.1. Materials

Raw peanuts were purchased from a local market (Rongs Asian supermarket, Dublin, Ireland). Peanuts were stored at room temperature. All chemicals used in this study were purchased from Sigma-Aldrich, Dublin Ireland, unless otherwise stated. Peanuts were ground in liquid nitrogen using a mortar and pestle. They were defatted using n-hexane for 5 h at 4 °C.

2.2. Plasma treatment

The experimental apparatus consisted of two aluminum plate electrodes of circular geometry (outer diameter = 158 mm) which were covered with a dielectric material of 2-mm thickness for the ground electrode and 10 mm thick acrylic sheet for the high voltage electrode (Fig. 1). For each experiment, 10 g of each deshelled whole peanut with skin (WP) and defatted peanut flour (DPF) were added to a petri dish separately and 5 mm sample depth was maintained. These petri dishes were placed within a polypropylene container of dimensions 310 mm × 230 mm × 22 mm which acts as a closed reactor and as an additional dielectric barrier. The relative humidity in the box was maintained at 80% by passing the working gas through a water bubbler with specific flow rate and water depth, the resultant humidity was measured with a psychrometer. This container was further sealed inside a high-barrier Cryovac BB3050 film in order to prevent loss of reactive species generated during plasma treatment. The voltage was delivered through a step-up transformer (Phenix Technologies, Inc., California, USA) whose primary winding received input at 230 V, 50 Hz and delivered a high voltage output in the range 0–120 kV and the air was used as discharge gas. Plasma treatment was performed at a voltage of 80 kV and treatment durations (0–60 min) maintained. After processing, containers were stored at room temperature of 16–18 °C for 24 h in line with previous findings that sealed retention time is useful for biocontrol (Ziuzina et al., 2014). This allows contact time of the generated chemical reactive species to be contained with the samples. Control samples were not subjected to plasma-treated.

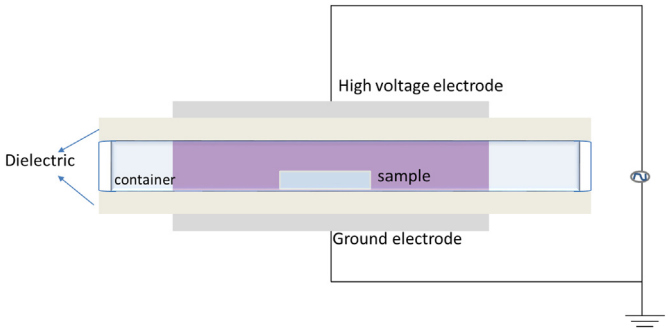


Fig. 1. Schematic diagram of the cold plasma experimental setup.

2.3. Extraction and purification

Extraction and purification of Ara h 1 were performed using the procedures of Maleki et al. (2000) with slight modifications. Briefly, defatted peanut was extracted using extraction buffer (50 mM Tris, 200 mM NaCl, and 1 mM EDTA at pH 8.3) for 2 h at 4 °C. The suspension was centrifuged at 12,000*g* for 15 min. The supernatant was subjected to 100% ammonium sulfate precipitation and protein pellet was collected after centrifugation. The collected protein was resolubilized in buffer at pH 8.3 without NaCl, desalted by dialysis against buffer overnight in the ratio 1:40. This was loaded on to a High Prep Q column and eluted with a linear salt gradient (50–300 mM NaCl). Fractions were collected and Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was run at reducing conditions. Protein concentration was checked at every step using Bradford assay.

2.4. Electrophoresis

SDS-PAGE was performed using 12% hand-cast gels (Laemmli, 1970). Proteins were reduced and denatured using sample buffer. Prior to loading, samples were heated at 100 °C, for10 min. Prestained molecular marker (Fisher Scientific, Dublin, Ireland) were used as a reference. Electrophoresis was performed at a constant voltage of 150 V in Tris glycine buffer. Rapid staining was performed according to Studier (2005). Briefly, gel is suspended in approximately 50 mL of 50% ethanol, 10% acetic acid and 40% water, heated to almost boiling in a microwave oven and rocked on a shaker until the gel shrinks. The liquid is discarded and suspended in 50 mL of 5% ethanol, 7.5% acetic acid and 0.25% coomassie blue in ethanol. The gel is again heated to boiling in a microwave and placed on a rocker. The gels were visualized within 30 min.

2.5. Immunoblotting

SDS-PAGE gels were prepared under reducing conditions, as described in Section 2.4 and the separated proteins were transferred to nitrocellulose membrane at a constant voltage of 20 V for 2 h. The membrane was blocked with 5% skim milk in phosphate-buffer saline containing 2% Tween 20 (PBST) for 2 h at room temperature, followed by overnight incubation with rabbit anti-Ara h 1 (1:5000 in PBST, Indoor Biotechnologies) at 4 °C. Then the membrane was washed thrice using phosphate-buffer saline (PBS), followed by incubation with goat anti-rabbit conjugated with horseradish peroxidase (HRP) (1:5000, Thermo Fischer Scientific, Dublin, Ireland) for 2 h at room temperature. After further washing, images were developed by incubating with an enhanced chemiluminescent luminol substrate for 1 min and observed on C-diGit blot scanner (LI-COR).

2.6. Competitive enzyme linked immune sorbent assay (ELISA)

The IgG-binding abilities of cold plasma-treated WP and DPF Ara h 1 were tested using competitive ELISA. The 96-well microtitre plates were coated with 100 µL of purified native Ara h 1 (0.1 µg/mL) in coating buffer (0.1 M $NaHCO_3$, pH 9.6) and incubated overnight at 4 °C. The wells were washed with phosphate-buffer saline twin 20 (PBST) thrice and later blocked with 3% bovine serum albumin in PBST for 2 h at 37 °C. Samples were mixed with equal volumes of rabbit Anti-Ara h 1 (1:5000) and incubated for 30 min at 37 °C. This mixture was added onto the coated plates and further incubated for 1 h at 37 °C. After further washing, 100 µL of goat anti-rabbit conjugated with HRP (1:4000) were then added to each well, followed by incubation for 1 h at 37 °C for detection of bound immunogen. The plates were further washed, and the colour was developed by adding 100 µL of 3,3′,5,5′-tetramethylbenzidine to each well and incubated for 15 min at room temperature. The reaction was terminated using 100 µL of 1 N hydrochloric acid and the absorbance of processed and unprocessed samples were read at 450 nm with a plate reader.

2.7. Secondary structure determination

Conformational changes in the secondary structure were determined using Far UV (190–240 nm) circular dichroism (CD) spectra. Protein concentration was 0.1 mg/mL and spectra was obtained at room temperature with a JASON Model J-810 spectropolarimeter using a 1-mm path length quartz cell, at a rate of 100 nm/min and bathwidth of 1.0 nm. Cold plasma-treated and native Ara h 1 are desalted using centrifugal filters into Milli-Q water and immediately used in CD measurements. A CD spectrum of Milli-Q water was obtained for background purpose and subtracted from each spectra. The secondary structure composition was calculated by Dichroweb server (program: CDSSTR; reference set: SET 7 optimized for 190–240 nm).

2.8. Statistical analysis

Statistical analysis was performed using SPSS software (IBM statistical analysis Version 19), and the results were statistically analyzed by one-way ANOVA. The significance among the samples was compared at $P < 0.05$ by the least significant difference post-hoc comparison. All the tests were performed in duplicate and the average of the tests are represented.

3. Results and discussion

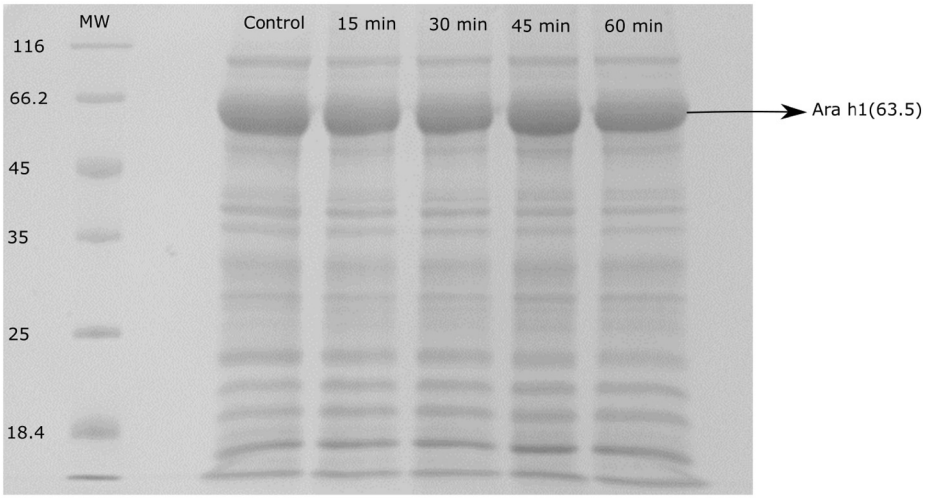
3.1. SDS-PAGE

SDS-PAGE analysis of cold atmospheric plasma-treated WP and DPF under reducing conditions is shown in Fig. 2(a and b). After extraction of the total soluble protein content of plasma-treated DPF and WP, 2 mg/mL of each are loaded on the gels. Analysis demonstrated no notable change in the protein profile of both WP and DPF when compared to control. The band at 63.5 kDa, which corresponds to the major allergen Ara h 1, both WP and DPF showed no change on the gel even with increased treatment time. This suggests that plasma exposure did not have any adverse effect on Ara h 1 and that the total protein degradation was likely to be very small. A similar investigation targeted at the major milk allergen α-casein showed no significant change in the SDS-PAGE band intensity when compared to the untreated (Tammineedi, Choudhary, Perez-Alvarado, & Watson, 2013). Furthermore, studies by Pal et al. (2016) found similar results with no change in the protein profile of plasma-treated rice storage proteins. This could be attributed to changes observed in polysaccharides characteristics. On the contrary, studies on Gly m5, a major soy allergen, which has structural similarity with Ara h 1 showed a decrease in the band intensity after plasma treatment (Meinlschmidt et al., 2016). This decrease in band intensity could be attributed to interactions between plasma active species and protein. Previous studies report that plasma active plasma species such as ozone promotes the oxidation of proteins resulting in disulfide bond formation (Uzun, Ibanoglu, Catal, & Ibanoglu, 2012). However, such effects were not observed in the present study.

3.2. Immunoblotting

The immunoblot of both WP and DPF after cold plasma treatment (15, 30, 45 and 60 min) is shown in Fig. 3. Fig. 3(a) and (b) demonstrated that plasma treatment affected the IgE binding activity of Ara h 1. Band intensity of Ara h 1 decreased with increase in treatment time for both WP and DPF when compared to the control. As shown in Fig. 3 the immunoblot of samples treated at 80 kV showed a decrease in IgE binding reactivity after 30 min exposure (Lane 3). The components of peanuts such as protein and lipids may prevent the IgE binding of Ara h 1 at short doses, however, increase in plasma doses might alter the

a)



b)

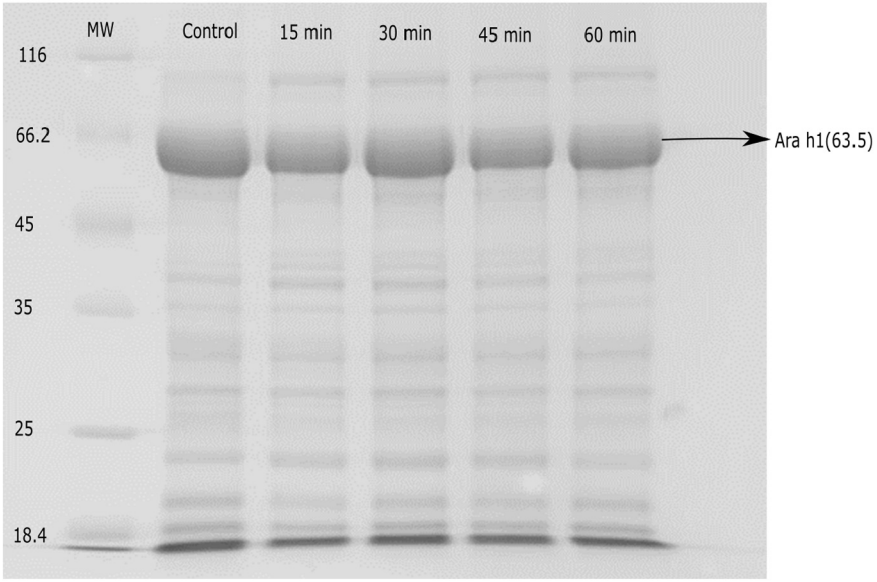


Fig. 2. SDS-Pattern of cold plasma-treated DPF (a) and WP (b) at various treatment duration.

functionality of protein-lipid complex (Lii, Liao, Stobinski, & Tomasik, 2002). In the present study cold plasma treatment of DPF and WP did not appear to cause any intramolecular cross-linking or band smearing when probed with Anti-Ara h 1. It was observed that after 60 min of plasma treatment, the IgE binding patterns of DPF showed low band intensity when compared to WP. Similar reduction in IgE binding was observed in cold plasma-treated wheat protein extracts (Nooji, 2011a). These authors also indicated a decrease in IgE binding depends on treatment time. This reduction is due to the active species such as O·, ·OH, N·, HO_2·, N_2*, N*, OH^-, O_2^-, O^-, O_2^+, N_2^+, N^+, and O^+ generated during plasma treatment could easily penetrate the DPF sample (increased surface area due to grinding) compared to WP (Sarangapani, Danaher, et al., 2017). A decrease in the IgE binding pattern is due to the generation of free radicals during plasma treatment which could disrupt or mask the binding epitope (Sarangapani et al., 2018). Results from this study are in accordance with Zhenxing, Hong, Limin, and Jamil (2007) who reported that components such as lipids in shrimp muscles would help to protect the allergen from damage by free radicals at low irradiation doses. A similar decrease in IgE binding were also observed in cold plasma treatment of shrimp extract (Shriver, 2011).

3.3. Competitive ELISA

In this assay, the antigenicity was measured on the basis of inhibition, the higher the inhibition, the more antigenic Ara h 1. For comparison, the antigenicity of cold plasma-treated Ara h 1 from DPF and WP were investigated. The antigenicity of peanut allergen Ara h 1 after various plasma doses is shown in Fig. 4(a) and (b). The results showed that antigenicity of cold plasma-treated peanuts depends on treatment time. The binding levels of DPF showed a gradual decrease with short plasma treatment doses of 15 and 30 min. However, the binding capacity of peanuts treated for 45 and 60 min showed greater reductions by 20.3% and 44% respectively than the control. Similarly, for WP antigenicity decreased by 8.6% for 45 min and 9.3% for 60 min of plasma treatment compared to the control. These results support the decrease in binding reactivity of Ara h 1 observed on immunoblot. It is already known that cold plasma generates reactive species such reactive oxygen species (ROS) and reactive nitrogen species (RNS), which may

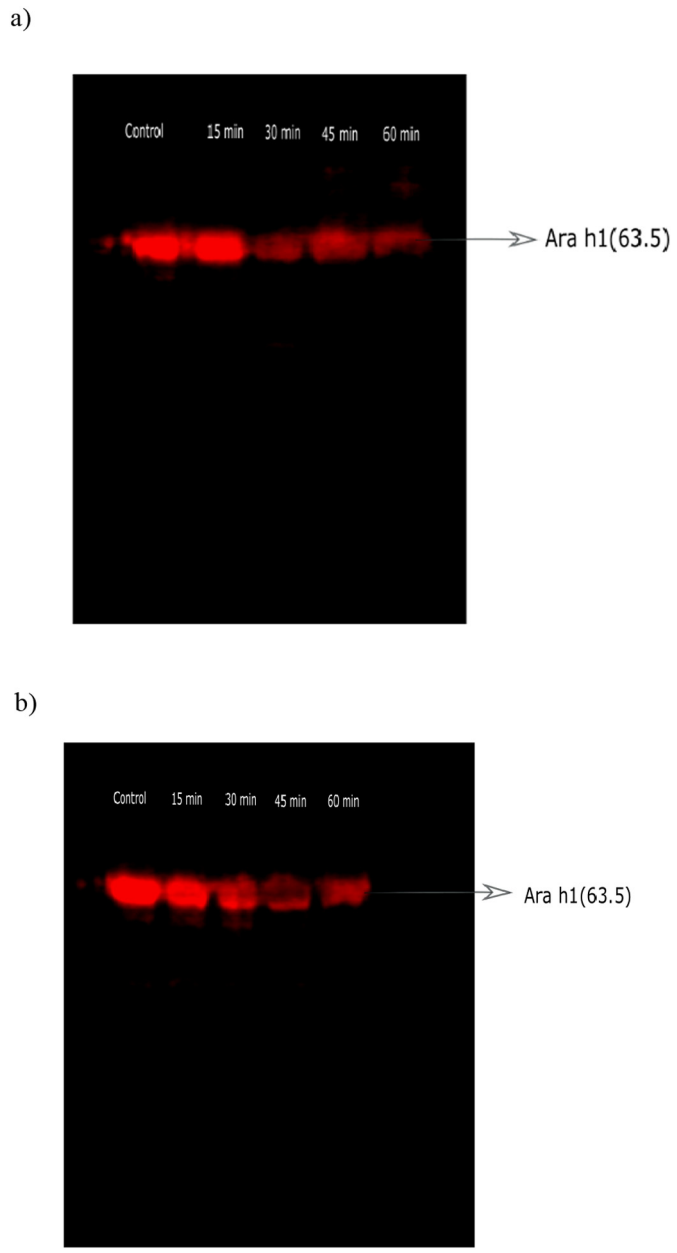


Fig. 3. Immunoblotting of cold plasma-treated Ara h 1 in DPF (a) and WP (b) using anti-Ara h 1 polyclonal antibody.

alter the amino acids, resulting in the change in the binding epitopes (Segat, Misra, Cullen, & Innocente, 2016). Cold plasma treatment may affect the antigenicity by various mechanisms such as protein denaturation, protein fragmentation, induction of protein conformational modifications (Sarangapani et al., 2018). A similar pattern of results was observed in a recent study on cold plasma treatment of the soy allergen Gly m 5, where a reduction in binding activity (91–100%) was reported with direct and remote cold plasma treatment. These authors also reported diminishing of protein in bands due to protein cross-linkage (Meinlschmidt et al., 2016). Similarly, Shriver et al. (2011) used direct cold plasma treatment on shrimp extract and reported the reduction in IgE binding to tropomyosin by up to 76% when compared to the control. In another study, employing similar conditions Nooji (2011b) reported approximately 25% reduction in the IgE binding after 3 min of plasma treatment. Further increase in treatment time to 5 min resulted in 37% reduction. These findings explain that higher exposure to plasma has a profound effect on the IgE binding proteins is in agreement with the present study. On the contrary, no significant decrease was observed in the allergenicity of α-casein and whey solution by indirect atmospheric plasma treatment. However, the mechanism of cold plasma in reduction of allergenicity has not been fully understood (Tammineedi et al., 2013). Hiramoto et al. (2011) has studied the effect of plasma ions on decrease in antigenicity of mite allergens. These authors reported that reduction of allergenic epitopes on the dust mite allergens is due to generation of hydroxyl radicals by plasma ions. Similarly, Wu et al. (2014) successfully demonstrated the efficacy of the cold plasma against aerosolized allergens both lab-prepared and environmental aerosols. Their results revealed that the mechanism of plasma with allergens depends on the form of protein structure. From these results it can be observed the changes in Ara h 1 binding to IgG and increasing plasma doses could be closely related to the changes in the conformational epitope structures of the proteins.

3.4. Secondary structure

The far-UV spectra of Ara h 1 were used to define the extent of secondary structure changes induced by cold plasma at various doses in WP and DPF (Fig. 5). The spectrum of native Ara h 1 exhibited a strong positive peak at 195 nm with a prominent negative peak at 208 nm in both WP and DPF. This type of spectrum is characteristic for a protein with a large number of α-helical structures (Greenfield, 2006). Fig. 5(a) and (b) shows a progressive decrease in the molar ellipticity at different doses in both DPF and WP, and major changes were observed in the 190–210 nm region. Ara h 1 in plasma-treated WP, showed a gradual decrease in the intensity of the positive and negative molar ellipticity at 193–197 nm and 205–210 nm respectively. A similar decline has been observed for plasma-treated DPF at 193–197 nm and 205–210 nm. The CD results from this study revealed that the secondary structure of native (untreated) Ara h 1 is composed of 21% α-helix, 16% turns, 28% of β-strands and 35% of random coil (Fig. 4). All secondary structures for plasma-treated WP samples, exhibited a variation pattern for α-helix, β-strands and turns coils, however, random coil did not display drastic changes (Fig. 4b). The α-helix in DPF treated samples declined after 15 min treatment, however, variations were observed in turn, β-strands and random coils (Fig. 4a). Similar variations in secondary structure has been observed by Meng et al. (2016) in gamma irradiated milk. Surowsky et al. (2013) also observed changes in α-helix and β-strands on exposure to cold plasma treatment of PPO and POD. In another study, Segat et al. (2014) also found loss of α-helix and time dependent decrease β-strands in cold plasma-treated alkaline phosphatase. Attri et al. (2015) reported that the modification in structure depends on the composition of the discharge gas. For instance, when O_2, N_2 and air were used as a discharge major changes were observed in the structure whereas minimal changes were observed with He and Ar plasmas.

The mechanism of action of plasma on the allergenicity of proteins has not been completely understood. However, there are multiple known mechanisms that affect protein structure. The major mechanism by which reduction of immunoreactivity is carried out, is by the modification of conformational as well as linear epitopes. This involves a chemical reaction between the protein and the active chemical species of cold plasma causing alteration in protein conformation. The other mechanisms could involve oxidation of amino acids, cleavage of peptide bonds and formation of protein–protein cross linkages. The chemical modifications are caused by hydroxyl radicals (·OH), superoxide anion radicals (O_2), hydroperoxy radicals (HOO·) and nitric oxide (NO ·) may attack the side chains of amino acids causing loss of its activity (Takai et al., 2014). The plasma active species such as ROS and RNS can cleave the disulfide bonds of the peptide and lead to destruction of binding sites for antibodies. In another study, Pankaj et al. (2014) demonstrated plasma induced changes on the protein based films. These authors suggested that there was formation of more extensive hydrogen bonding between the proteins, leading to the reduction in peptide

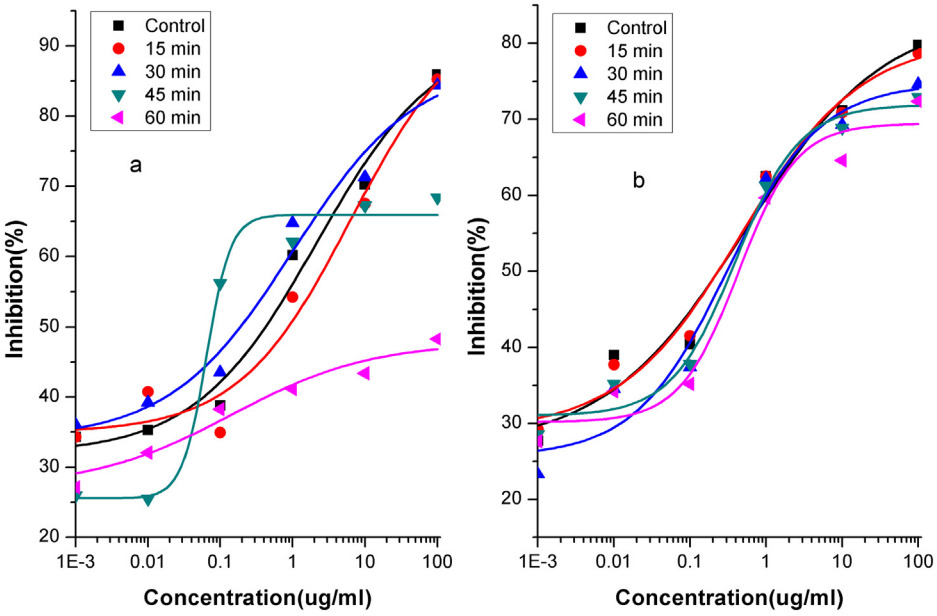


Fig. 4. IgG-binding abilities of cold plasma-treated Ara h 1 in DFP (a) and WP (b) at various treatment times.

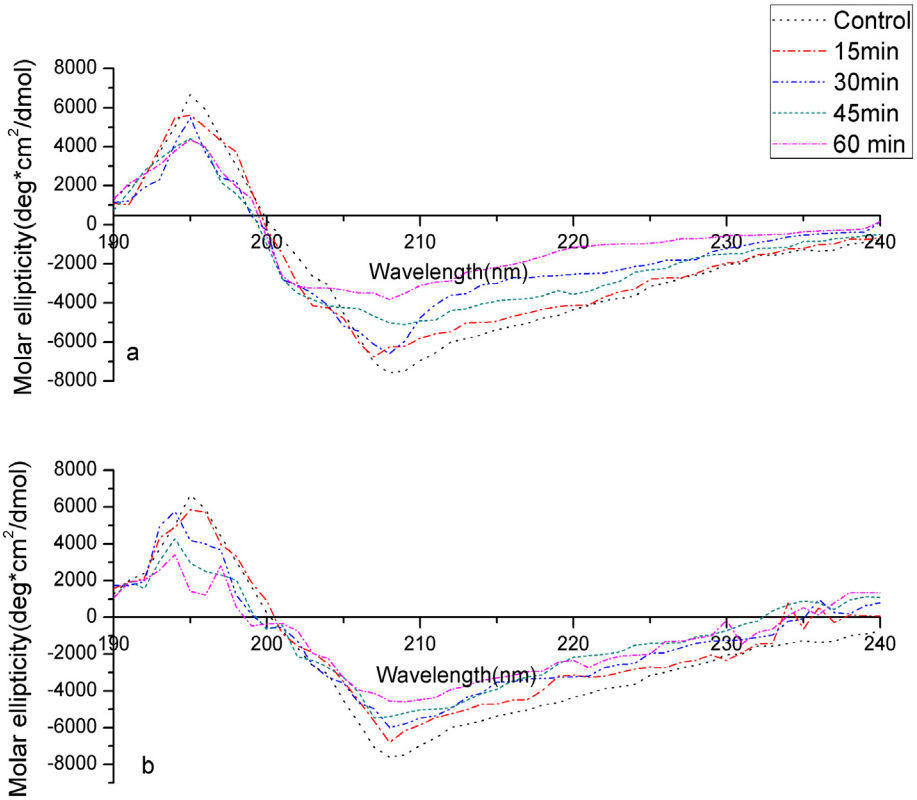


Fig. 5. Circular dichroism of cold plasma-treated Ara h 1 in DPF (a) and WP (b).

linkages and significant effects on protein integrity (Surowsky et al., 2013). These are examples of changes presumably resulting in the destruction of the protein structure.

Besides reduction of allergenicity, cold plasma also improves the techno-functional properties of proteins (Chizoba Ekezie et al., 2018). In a recent study, Bußler, Steins, Ehlbeck, and Schlüter (2015) observed a 113% and 116% increase in the water and fat-holding capacities of pea flour after exposure to atmospheric cold plasma for up to 10 min. The authors successfully demonstrated the incidence of conformational alteration, which can be used to describe the observed effects on the techno-functional properties of ACP-treated pea flour fractions. Segat et al. (2014) studied the effect of ACP treatment on whey protein isolate solutions during treatment times ranging from 1 to 60 min. They observed an increase in surface hydrophobicity and foaming stability of WPI due to protein aggregation. This kind of information gives a better understanding of the fundamental interactions and can be used to design plasma systems with appropriate conditions.

CD spectroscopy results provided the possible inactivation mechanisms, which are most likely be changes in α-helix structure and β-sheet content. It is, however, difficult to imagine a mechanism by which

cold plasma treatment could directly influence the structure of allergenic proteins. From available literature and changes that were observed in this study, it can be suggested that the chemical reactions between protein and reactive species are responsible for alteration. This could involve oxidation of amino acids, cleavage of peptide bonds and formation of protein–protein cross linkages.

4. Conclusion

This study determined the efficacy of cold plasma treatment against antigenicity of Ara h1, the major allergen in peanut. The immunoblot and Competitive-ELISA results revealed that cold plasma treatment decreased the antigenicity of Ara h1 in both WP and DPF. However, there were no significant changes observed in SDS-PAGE. It has been observed that reduction in antigenicity depends on treatment time. Additionally, the results from CD spectroscopy provided important insights of cold plasma against Ara h 1 and possible changes in protein structure and allergenicity. Hence, it can also be assumed that similar change in structural and antigenic properties might occur in other peanut allergens. Thus, cold plasma could be a promising alternative approach to reduce peanut allergenicity. Moreover, future studies should be focused on purified allergen proteins to provide important insights into how processing methods affect the allergen. Furthermore, *in vivo* studies are needed to verify the reduction in allergenicity of cold plasma-treated peanut.

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