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ORIGINAL PAPER

Mitigation of Major Peanut Allergens by Pulsed Ultraviolet Light

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Abstract Peanut allergy represents one of the most severe IgE-mediated reactions with food, but to date, the only effective way to prevent peanut allergy is total avoidance. If allergens could be mitigated during food processing before a product reaches the consumer, this would substantially lessen the food allergy problem. The efficacy of pulsed ultraviolet light (PUV), a novel food processing technology, on reducing peanut allergens, was examined. This study revealed for the first time that PUV was also capable of deactivating Ara h 2, the most potent allergenic protein of peanut. Protein extracts from raw and roasted peanuts were treated for 2, 4, and 6 min and peanut butter slurry was treated for 1, 2, and 3 min in a Xenon Steripulse XL 3000® PUV system. The distance from the central axis of the lamp was varied at 10.8, 14.6, and 18.2 cm. The SDS–PAGE showed a reduction in the protein band intensity for Ara h 1, Ara h 2, and Ara h 3 at the energy levels ranging from 111.6 to 223.2 J/cm^2. Reduction of the protein band intensity for peanut allergens increased with treatment time but decreased with increased distance from the PUV lamp. The ELISA for peanut extracts and peanut butter slurry showed a reduction in IgE binding of up to 12.9- and 6.7-folds, respectively, compared to control.

Keywords Peanut · Peanut butter · Allergen · Ara h 1 · Ara h 2 · Ara h 3 · Pulsed UV light · Non-thermal · IgE binding

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Introduction

Peanut (*Arachis hypogaea* L.) is known to contain the most severe food allergens (Viquez et al. 2003), and peanut allergy is the third most common food allergy in young children and the most common in older children, adolescents, and adults (Perry et al. 2004). It is responsible for about 63–67% of deaths caused by anaphylaxis (Maleki et al. 2003).

Peanut contains eight major intrinsic allergens (Burks et al. 1999; Kleber-Janke et al. 2001), among which Ara h 1 (vicilin), Ara h 2 (conglutin), and Ara h 3 (glycinin) are predominant and responsible for more than 50% of the allergic reactions in sensitive individuals (Konan et al. 2003). Several studies have indicated that more than 90% of individuals with peanut allergy have IgE antibody to Ara h 1 and Ara h 2, while those with IgE antibodies to Ara h 3 varies from 45–95% (Sicherer and Sampson 2007).

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With the increasing prevalence and severity of food allergy, ways including thermal and non-thermal techniques are sought to reduce the allergen potency of foods. Chung and Champagne (2008) reported that adding phenolics to peanut extracts and liquid peanut butter precipitated most of the major peanut allergens, Ara h 1 and Ara h 2, and the complexation was irreversible. They found that IgE binding was reduced approximately 10- to 16-fold. Davis et al. (2001) and Maleki et al. (2003) found that roasting increased the allergen potency, mainly through the introduction of neo-antigens. Davis et al. (2001) found that some patients were allergic to cooked but not raw fish protein, which indicated that thermal treatment had actually increased the IgE response to sensitive individuals. Literature suggests that to a large extent, thermal processing increases the capacity of allergens. Maleki et al. (2003) further reported that although roasting affected the structural as well as other functional characteristics of an allergen, it did not affect its allergenic properties. In a separate study, however, Beyer et al. (2001) found that compared to dry roasting, the IgE binding capacity of the major peanut allergens, Ara h 1, Ara h 2, and Ara h 3, was significantly reduced by frying and boiling.

Astwood and Fuchs (1996) pointed out food allergy threats could be avoided, minimized, or prevented using new and emerging technologies that can better define, detect, and consequently reduce or completely eliminate specific allergens from the food supply. They stated that food allergy cases would be considerably reduced with a combination of technologies such as biotechnology, bioinformatics, and immunodiagnostics. However, despite increasing knowledge in allergens and their interactions with the immune system, there still seem to be few practical ways to achieve the aim of reducing their effects on consumers (Davis et al. 2001).

Some non-thermal processing technologies, such as pulsed ultraviolet light (PUV), power ultrasound, and irradiation, have been shown to be effective on reducing food allergens. Chung et al. (2008) reported a significant reduction in peanut allergens by PUV treatment for both peanut extracts and peanut butter slurry in terms of Ara h 1 and Ara h 3, but not with Ara h 2. Yang et al. (2010) found that PUV was effective in reducing soybean allergens. Also, a study by Lee et al. (2008) showed gamma irradiation was effective on reduction of peanut allergen.

Pulsed ultraviolet light technology uses pulses of an intense broad spectrum which is rich in UV-C light (Gómez-López et al. 2007; Koutchma 2009; Oms-Oliu et al. 2010). Pulsed ultraviolet light is also referred to as pulsed light, high-intensity light, broad-spectrum white light, and pulsed white light. In the PUV light, energy is stored in a capacitor and then released as very short period pulses (Krishnamurthy 2006). The intensity of pulsed light, producing a high-peak intensity, is at least 90,000 times more than that of sun light at sea level (Takeshita et al. 2003). The number of pulses per second typically ranges from 1–20 with a pulse width from 300 to 1,000 ns.

The objective of this study was to study the effect of PUV on the IgE-binding properties of major peanut allergens Ara h 1, Ara h 2, and Ara h 3, especially to examine if Ara h 2, the most potent allergenic protein of peanut (Chu et al. 2008; Dodo et al. 2005), could also be mitigated by PUV.

Materials and Methods

Materials

Peanut protein extracts were prepared from both raw and roasted Valencia peanuts from the Anderson Peanuts Co., AL. Commercial non-hydrogenated peanut butter was purchased from a local store in Huntsville, Alabama. Slurry was prepared using the natural peanut butter (ratio of 1:2 peanut butter to 0.05 M Tris–HCL buffer, pH 8.0).

A pool of human sera from three patients with documented history of peanut allergy and over 95 IU/mL of IgE level was obtained from the PlasmaLab International, Everett, WA and used for the in vitro tests by ELISA.

Pulsed Ultraviolet Light Equipment

A lab-scale, high-intensity PUV system, Steripulse XL-3000® manufactured by the Xenon Corporation, Woburn, MA was used in this study. It consists of a control module, treatment chamber, PUV lamp housing, and a cooling blower.

The treatment chamber is built of stainless steel and has shelves to hold food samples and allows for the change of the sample distance from the lamp. The linear Xenon lamp was placed at the top center of the chamber and protected by lamp housing. The blower aims at dissipating the heat generated by the lamp and also preventing the accumulation of ozone produced during operation. The control module helps modulate the electric current to produce specific pulse width and peak power. It also has the switch and timer to control the duration of the treatment. This unit was operated at a pulse width of 360 μs and a pulse rate of three pulses per second. The lamp of this unit is capable of an output energy of 502 J per pulse or 1,506 J/s, but at different distance from lamp in the treatment chamber, the energy level is varied. Throughout the entire text, an approximate energy level per manufacturer's calibration was given for a particular distance from lamp as reference.

Extraction of Peanut Proteins

Peanuts were roasted at an oven temperature of 178 °C (350°F) for 15 min, following the protocol by Maleki et al. (2003) using a Kenmore electric oven, Model 9119309 (Sears, Hoffman Estates, Illinois). About 10 g of raw peanuts and 10 g of roasted peanut seeds were skin removed and ground in a mortar with a pestle to obtain peanut paste. The peanut sample was defatted for 4 h following the AOAC (1990) official method using hexane. Peanut protein was extracted from both raw and roasted peanuts using 0.05 M, pH 8.0 Tris–HCL buffer. This was followed by agitation of the samples in an environmental shaker for 2 h to obtain a homogenous mixture. The protein extracts were then subjected to first clarification by centrifugation for 5 min at 704×*g* (3,000 rpm) at 4 °C. The supernatant was then separated and subjected to a 2nd step centrifugation for 15 min at 7,826×*g* (10,000 rpm) at 4 °C. The final supernatants (peanut protein extract) were collected from both raw and roasted peanuts which were ready for PUV treatments.

Preparation of Peanut Butter Slurry

Commercial natural peanut butter (50 ml) was mixed with 0.05 M Tris–HCL buffer pH 8 in the ratio of 1:2 *w/v*. Preliminary study showed that Tris–HCL buffer at this concentration delivers maximum protein extraction as compared to NaOH buffer. The mixture was then subjected to stirring for 30 min at room temperature to ensure homogenous mixing.

PUV Treatment of Peanut Samples

Peanut protein extracts from raw and roasted peanuts as well as the peanut butter slurry were PUV treated. Ten milliliters of the liquid sample was placed onto an aluminum dish with a diameter of 7 cm to form a thin layer (<10 mm). The dish was then placed onto the sample rack of the unit for PUV treatment. The sample rack was adjusted to three different positions in order to compare the effect of different light intensities on allergen reduction. Temperature changes before and after treatment were measured using an infrared thermometer model Mini IR Thermometer 42500 (EXTECH Instruments, Waltham, MA). The treatment durations were 2, 4, and 6 min for peanut protein extracts, while for peanut butter slurry, the durations were 1, 2, and 3 min. The treatment times were established based on the preliminary tests conducted as well as the review of literature. After treatment, the samples were centrifuged at 17,608×*g* (15,000 rpm) for 10 min before allergenic properties of the peanut proteins were examined.

SDS–PAGE

The protein profiles of PUV treated and untreated samples were determined by SDS–PAGE. Before running the gels, protein concentration was measured using a SmartSpec™ 3000 Spectrophotometer. Three centrifuge tubes (1.5 ml) were prepared for each sample followed by the addition of 50 μl of protein sample to each tube. This was followed by the addition of 1.5 ml of the Coomassie Plus reagent to each tube and mixed well before incubation for 10 min at room temperature to allow the sample to stabilize. Prior to measurement at 595 nm, the spectrophotometer was zeroed using a cuvette filled with distilled water. The respective concentrations were then determined by the standard curve using the means of three readings for each sample. This was followed by protein profiling by SDS–PAGE for the treated peanut extracts and peanut butter slurry. Before loading, 37.5 μl of each sample was mixed with 12.5 μl of XT buffer ×4. The samples were then placed in a hot bath at 65 °C for 15 min in order to unfold the protein structures. Then, 5 μg/ml of each sample concentration was loaded in the gel. A ×1 Tris glycine buffer was used to run the samples. A 12% polyacrylamide gel and 3% stacking gel were used to separate the proteins. For each gel, 10 μl of the marker was loaded in the first lane. Before loading in the gel, the samples were stained with Coomassie blue to facilitate visualization of the peanut protein bands. The gel was run for one hour at 200 V. Untreated peanut samples were used as the positive control in this procedure.

In Vitro Tests with ciELISA

A competitive inhibition ELISA (ciELISA) with pooled peanut allergy patient sera was conducted to examine the allergens present in each sample following the protocol described by Chung et al. (2005). A high binding plate was coated with the antigen (untreated raw peanut extracts). Prior to coating, the antigen was diluted with 0.1 M sodium bicarbonate, pH 9.6 buffer in the ratio of 1:20 *v/v*. The coated plate was incubated for 2 h at 37 °C. The plate was then washed four times with 100 μl to each well using Tris buffer saline with Tween (TBS/Tw) patting the plate each time on a prepared mat. This was followed by blocking three times using 200 μl of TBS/Tw superblock to each well. Each time the plate was incubated for 5 min at 37 °C followed by washing three times using TBS/Tw and then left to air-dry overnight at 4 °C. Before loading in the plate wells, peanut samples (50 μL; 0.1–100 μg/ml) were mixed with a pool of serum (50 μL). This step was preceded with making serial dilutions of the treated and control samples to obtain concentrations ranging from 0.01 to 10 μg/ml. Before mixing with the peanut samples, the serum was diluted using TBS/Tw superblock in the ratio of 1:30 *v/v*.

The plate was then shaken in the environmental chamber for 45 min at room temperature. After incubation, the liquids were removed from the wells using pipette tips followed by regular washing three times using TBS/Tw. This was followed by addition of goat anti-human IgE horseradish peroxidase conjugate secondary antibody (100 μL), then incubated for 30 min at room temperature. Before adding to the wells, the antibody was diluted with TBS/Tw superblock in the ratio of 1:1,000 *v/v*. After incubation, the plate was washed again four times using TBS/Tw. This was followed by the addition of 100 μL of substrate containing *o*-phenylenediamine dihydrochloride (0.5 μg/ml), dissolved in 10 ml of 0.1 mol L^{-1} citrate buffer, pH 5 mixed with 3.4 μl of 30% hydrogen peroxide. After 5 min incubation at 37 °C, the color changes from clear to golden yellow were observed. The absorbance of the respective samples was immediately read at 450 nm using a U-Quant plate reader (BioTek Instruments, VT) and the means of triplicate measurements were used to plot the corresponding graphs.

Data Analysis

Statistical data analysis was performed using Minitab software (Minitab Inc., version 15). Statistical significance was tested using the general linear model of analysis of variance (ANOVA) at $p<0.05$. In the analysis of statistical significance, ELISA reading was set as the dependent variable and distance from the lamp and time as independent variables. The three samples of raw peanut extract, roasted peanut extract, and peanut butter slurry were used for allergen testing. Comparisons among levels of the two foregoing factors, i.e., distance from lamp and time, were determined using Tukey simultaneous tests with level of significance set at $p<0.05$. To eliminate the chances of experimental errors influencing the outcome of the analysis, a completely randomized block design was used.

Results and Discussion

Moisture Loss during PUV Treatment

After PUV treatment, peanut extracts from raw, roasted, and peanut butter slurry samples were subjected to SDS–PAGE analysis. All samples showed a reduction in volume after PUV treatment. This was similar to the findings by Chung et al. (2008) who reported a sample volumetric reduction of up to 40% due to moisture loss. Moisture loss in the samples was observed to increase with treatment time.

The PUV light contains about 54% UV, 26% visible, and 20% infrared spectra, and the PUV treatment is actually a process of photophysical, photothermal, and photochemical effects (Chung et al. 2008; Krishnamurthy et al. 2008; Yang et al. 2010; Shriver and Yang 2011). The temperature rise and moisture loss observed in this study were possibly due to the photothermal effect caused by the infrared portions of the PUV light spectra at a longer treatment time. It was observed in this study that when the treatment time was short (e.g., up to 30 s), PUV exhibited non-thermal characteristics, as the temperature rise was insignificant; however, a prolonged PUV treatment (e.g., a few min) induced significant temperature rise and moisture loss. In this case, we believe both the non-thermal (mostly due to the UV spectra) and photothermal (mostly from the infrared spectra) effects co-existed and could have exerted combined effect on the samples.

SDS–PAGE for PUV-Treated Raw Peanut Extracts

A SDS–PAGE profile of PUV-treated raw peanut extracts is shown in Fig. 1. All samples were placed at 14.6 cm from the central axis of the lamp, and the treatment time was varied to observe its effect on the three major peanut allergens. After 2 min treatment, the protein band corresponding to Ara h 3 (45 kDa) was not visible, while the bands for Ara h 1 (63 kDa) and Ara h 2 (17–20 kDa) were still visible. However, after 4 and 6 min treatments, the protein bands corresponding to all the three allergens were not visible. This suggests that Ara h 3 can be affected by PUV at a lower treatment time and hence a lower energy level (111.6 J/cm^2), while Ara h 1 and Ara h 2 required longer treatment times and hence higher energy levels to achieve the same effect (223.2 J/cm^2).

It has been known that the structure of Ara h 1 can protect its epitopes from degradation, while all the three dominant epitopes of the Ara h 2 are located on 10 kDa, which is known to be a more resistant fragment (Beyer et

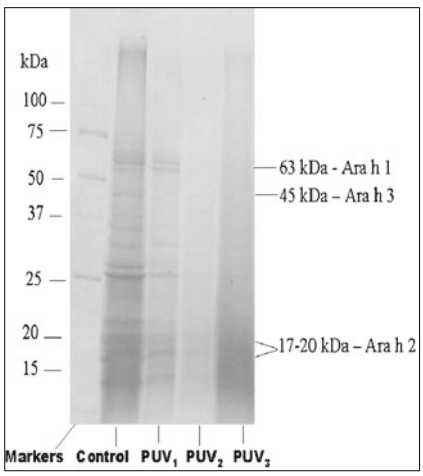

Fig. 1 SDS–PAGE of PUV-treated raw peanut extracts. PUV_1, PUV_2, and PUV_3 represent 2, 4, and 6 min treatments, respectively, at 14.6 cm from lamp

al. 2003). This means that the structures of Ara h 1 and Ara h 2 might protect their epitopes from PUV radiation within a certain energy range and consequently achieve less degradation as compared to Ara h 3. The abovementioned characteristics have been clearly shown in the foregoing SDS–PAGE results, which also showed the capability of PUV on reducing the allergenic potency of Ara h 2.

These results were in line with the study by Sicherer and Sampson (2007) that showed Ara h 1 and Ara h 2 were more stable under certain processing conditions than Ara h 3. In their study on the effect of thermal and enzymatic treatment on major peanut allergens, Ara h 1 and Ara h 2 were found to possess unique physicochemical properties, which rendered them rather stable under thermal processing and enzymatic digestion. Moreover, Gennadios et al. (1998) reported that individual proteins might exhibit different degrees of response to UV radiation depending on the amino acid compositions and molecular structures.

These unique properties may account for the relative stability of Ara h 1 and Ara h 2 under PUV treatment. Generally, all the bands were not visible at 6 min treatment, which produced an energy level of approximately 334.8 J/cm^2. This shows that all the three allergens could be affected by PUV treatment, only that different allergens required different energy levels for the effect to take place. In this study, the result on Ara h 2 was different from that by Chung et al. (2008) who reported that PUV had no effect on Ara h 2 at 4 min treatment and 14.6 cm from lamp (energy level approximately 223.2 J/cm^2).

Disappearance of the allergen protein bands might be due to a change in solubility of peanut proteins and formation of precipitates due to PUV radiation. This explanation has been supported by the findings by Chung et al. (2008) who reported that PUV coagulated the peanut proteins, forming insoluble precipitates, which were then removed during centrifugation. Moreover, a different study by Gennadios et al. (1998) shows that aromatic amino acids in food proteins like phenylalanine and tyrosine can absorb UV light and recombine to form covalent cross-links in proteins, resulting in the formation of larger aggregates. Other forms of radiations like gamma irradiation have also been reported to cause the same effect in foods by forming coagulation (Herian et al. 1993; Byun et al. 2000). For peanut allergens, formation of these insoluble precipitates could limit their intestinal uptake in the body and mask any IgE-biding epitopes, thus changing their allergenic potency. This would consequently reduce the level of allergens available for IgE binding in sensitive individuals.

SDS–PAGE for PUV-Treated Roasted Peanut Extracts

SDS–PAGE of PUV-treated roasted peanut extracts is shown in Fig. 2. The protein bands corresponding to Ara h 1 (63 kDa) and Ara h 2 (17–20 kDa) were still visible even at 4 min PUV treatment. Protein bands corresponding to Ara h 3 were not visible in all the three treatments, which was similar to the results for raw peanut extracts. At 6 min treatment, the bands corresponding to all three allergens were not visible. The reduction of Ara h2 indicated PUV was also capable of mitigating its allergenic potency. These results indicated that Ara h 1 and Ara h 2 from roasted peanut extracts were more resistant to PUV than those from raw peanut extracts. This might be due to the differences in the amount of peanut proteins contained in the samples from raw and roasted peanuts as a result of different amounts of extractable proteins. Several studies have shown that there is higher protein extraction from high-temperature dry-roasted peanuts as compared to raw peanuts. Beyer et al. (2001) showed in their study that dry roasting at 170 °C led to increased extraction of Ara h 1, while Pomés et al. (2003) reported that higher temperatures increased Ara h 1 extraction by 178%.

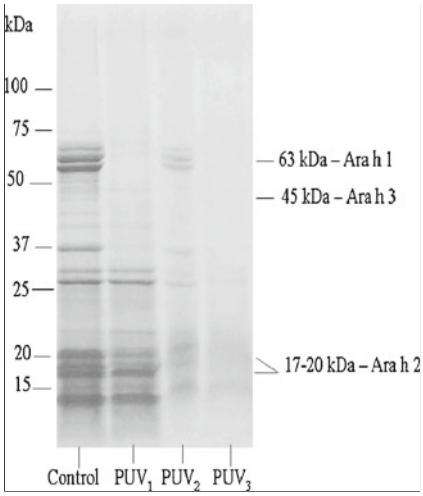

Fig. 2 SDS–PAGE for PUV-treated roasted peanut extracts. PUV_1, PUV_2, and PUV_3 are for 2, 4, and 6 min treatments, respectively, at 14.6 cm from lamp

Moreover, Sicherer and Sampson (2007) reported that extractable Ara h 1 in peanuts roasted for 10 to 15 min was 22-fold higher compared to raw peanuts. Since the same concentrations of protein as raw extracts were loaded, this suggests that there were more allergens in the roasted than raw peanut extracts. Also, this might be the reason for the fact that in roasted peanut samples, Ara h 1 and Ara h 2 bands were still visible even after 4 min PUV treatment as compared with those from raw peanuts.

The increased allergen potency in roasted peanuts was also explained by Maleki et al. (2000) as coming from chemical modifications of the protein structure due to the Maillard reaction. They stated that IgE recognition sites in roasted peanuts differed from those of raw peanuts. Beyer et al. (2001) hypothesized that the differences between

roasted and raw peanuts were due to the fact that heat treatment increased the allergen potency by exposing more binding sites which were otherwise unavailable. They further suggested that these chemical modifications at higher temperatures might result in new IgE-binding epitopes which might result in increased allergen capacity. Breiteneder and Mills (2005) added that peanut proteins became more stable after thermal treatment and glycation reactions cross-link individual protein molecules, increasing their allergenic potential. Moreover, other studies had attributed these changes under thermal treatment to the size of the protein, stressing that different food allergens with different sizes behaved differently under thermal processing conditions. For example, Bargman et al. (1992) reported that roasting and blanching reduced the IgE-binding capacity of larger almond allergens (70 kDa) but had no effect on smaller allergens (45–50 kDa). Previous studies have linked almond allergens to Ara h 2, since both shared common IgE-binding epitopes (de Leon et al. 2007). Ara h 2 is a member of conglutin family of seed storage proteins that are known to contribute to allergenic reactions of almonds.

SDS–PAGE for PUV-Treated Peanut Butter Slurry

Figure 3 shows the SDS–PAGE result for PUV-treated peanut butter slurry. All the samples were positioned at 14.6 cm from lamp. The results showed a decrease in the protein band intensity for all the three major allergens after 3 min of PUV exposure as compared to control. However, there was almost no reduction in protein band intensity for samples which were subjected to 1 min treatment, but a slight reduction was observed for the samples treated for 2 min. At 3 min treatment, the Ara h 1, Ara h 2, and Ara h 3 bands were barely visible. These results indicated that the level of Ara h 1, Ara h 2, and Ara h 3 in the peanut butter slurry was reduced with PUV treatment, and the reduction increased with treatment time.

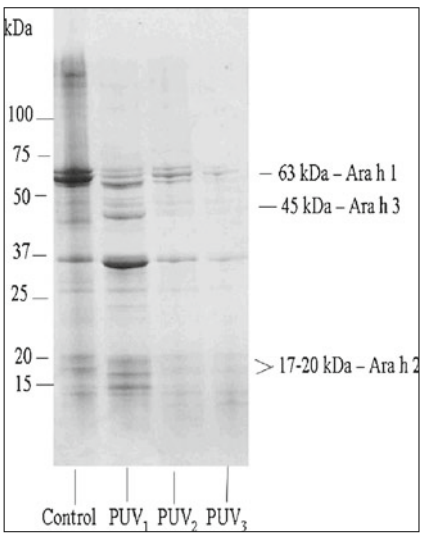

Fig. 3 SDS–PAGE for PUV-treated peanut butter slurry. PUV_1, PUV_2, and PUV_3 represent 1, 2, and 3 min PUV treatment, respectively, at 14.6 cm from lamp

Chung et al. (2008) reported no reduction for Ara h 2 of peanut butter slurry after PUV treatment, but this study has shown that even the relatively stable Ara h 2 reduced its potency after 3 min PUV treatment in peanut butter slurry.

ELISA for PUV-Treated Raw Peanut Extracts

In the ELISA tests, the absorbance values at 450 nm on the U-Quant plate reader for the PUV-treated raw peanut extracts are presented in Table 1. The higher the protein concentration in the sample, the lower the absorbance, and vice versa. From Table 1, it can be seen that the absorbance values of all treated samples were significantly different from the control. The values were significantly different as well among one another at $p<0.05$, as evident from the lower case preceding superscripts in Table 1.

After conversions of the absorbance values in respect to control, the IgE-binding capacity of PUV-treated raw peanut extracts is presented in Fig. 4. The gap between the control and the PUV-treated curves indicated the level of reduction in IgE binding. The bigger the gap was, the more reduction in IgE binding was found.

IC_{50}, defined as the concentration of the inhibitor (protein) required to inhibit IgE binding by 50%, is normally used to explain the reduction in IgE binding. The higher the IC_{50} value, the higher the reduction in IgE binding. Results showed that the PUV-treated raw peanut extract had lower IgE-binding capacity as compared to the control, with the capacity decreasing with increased treatment time. At 2, 4, and 6 min treatments, the reduction in IgE binding decreased with increased distance from the PUV lamp. For example, at 6 min treatment, sample positioned at 10.8, 14.6, and 18.2 cm had IC_{50} values of 9, 4, and 2 μg/ml, respectively.

In terms of energy levels, the sample positioned at 10.8 cm from the lamp and exposed to 6 min treatment received approximately 388.8 J/cm^2, while at the same treatment time, the sample positioned at 18.2 cm received only about 205.2 J/cm^2.

ANOVA results showed that there were significant interactions between treatment time and distance ($p<0.05$) at all selected treatment conditions. The significant interactions between the two factors indicated that for the raw peanut extracts, the effect of treatment time on reduction of peanut protein concentrations as well as IgE binding were dependent on the distance from the lamp.

Allergens are known to contain multiple IgE binding sites or epitopes. Several studies had suggested that the

Table 1 The ELISA absorbance values at 450 nm for PUV treated raw peanut extracts

Distance (cm)	Time (min)		
	6	4	2
10.8	A,a 1.8037±0.0012a	B,b 1.6927±0.0025a	C,c 1.5610±0.0017a
14.6	A,d 1.6727±0.0025b	B,e 1.5170±0.0017b	C,f 1.3610±0.0017b
18.2	A,g 1.5393±0.0011c	B,h 1.3837±0.0011c	C,j 1.2417±0.0028c
Control[a]	0.9125±0.0041		

Means of triplicate measurements ± standard deviation; values not followed by the same letter in the same column are significantly different from each other at $p<0.05$; values not preceded by the same upper case letter in the same row are significantly different from each other at $p<0.05$; values not preceded by the same lower case letter in rows and columns are significantly different from each other $p<0.05$

[a] Control is significantly different from all other values

reduction in IgE binding might be due to conformational changes of the protein structure which might mask or destroy the protein epitopes, making them unavailable for IgE binding (Herian et al. 1993; Lee et al. 2001). In this study, masking and destruction of the allergen epitopes might have taken place and seemed to increase with increased energy levels. Similarly, Gennadios et al. (1998) reported that aggregation of soy protein increased with UV light dose.

IC_{50} values were calculated from Fig. 4 and used to quantify the IgE reduction for PUV-treated raw peanut extracts. For raw peanut extracts, the maximum reduction in IgE binding (i.e., 12.9-fold) was achieved after 6 min treatment at 10.8 cm from lamp with an energy level of approximately 388.8 J/cm^2. At this treatment condition, the IC_{50} value for PUV-treated sample was 9 μg/ml, while that of control was 0.7 μg/ml. Fold reduction is obtained by dividing the IC_{50} value of the treated sample with that of the control. This means that the IgE binding of PUV-treated sample was 13-fold lower than that of control. The second largest reduction was by 4 min treatment at 10.8 cm from the lamp, which had 7.9-fold reduction in IgE binding. The lowest reduction was obtained for the sample treated at 2 min and placed at 18.2 cm from lamp. The energy at this treatment condition was approximately 68.4 J/cm^2. At this treatment condition, the IC_{50} values for PUV-treated sample and control were 1.2 and 0.7 μg/ml, respectively, and the IgE binding of PUV-treated sample was only 1.7-fold lower than that of control.

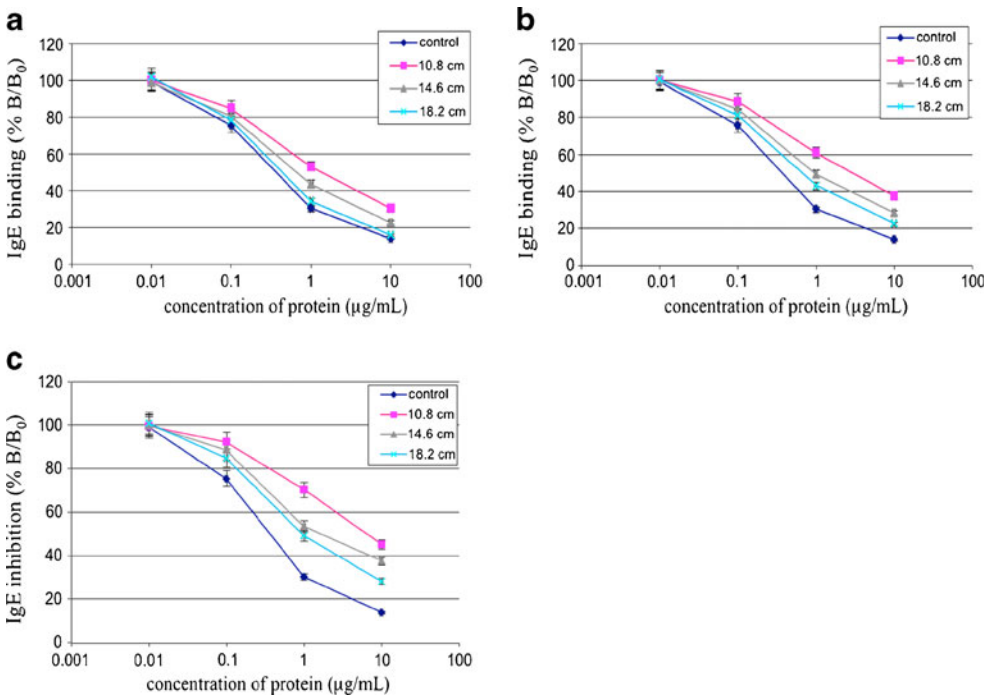

Fig. 4 IgE-binding capacity of PUV-treated raw peanut extracts after **a** 2 min, **b** 4 min, and **c** 6 min PUV exposure. B/B_0 on *Y*-axis is the absorbance of the sample containing both IgE and peanut protein over that of the control sample containing IgE only. The *error bars* indicate standard deviations based on triplicate measurements

ELISA for PUV-Treated Roasted Peanut Extracts

The ELISA absorbance values of PUV-treated samples were significantly different from that of control and also different from one another, as shown in Table 2, except for the two conditions, i.e., 18.2 cm distance from lamp, 4 min treatment and 14.6 cm distance, 2 min treatment, which were not significantly different from each other.

Similarly, absorbance data were converted into the reduction in IgE binding of the PUV-treated roasted peanut samples at 2, 4, and 6 min, as presented in Fig. 5.

Results showed that reduction in IgE binding increased with reduced distance from the PUV lamp. For example, At 6 min treatment, samples at 10.8, 14.6, and 18.2 cm from the lamp had IC_{50} values of 7, 4, and 1.2 μg/ml, respectively.

Similar to raw peanut extracts, there were significant interactions between treatment time and distance at selected treatment conditions ($p<0.05$). That is to say, the effect of treatment time on the reduction of peanut proteins and IgE binding was dependent on distance from the lamp.

IC_{50} values and the fold reduction calculations showed similar trend as raw peanut. The maximum reduction was achieved at 6 min and 10.8 cm from lamp. At this treatment condition, the IC_{50} values for the sample and control were 7.0 and 0.6 μg/ml, respectively, signifying that the IgE binding for PUV-treated sample was about 11.7-fold lower than that of control. The lowest reduction in IgE binding was observed for the sample at 18.2 cm from lamp for 2 min of exposure. The corresponding IC_{50} values were 0.7 and 0.6 μg/ml, respectively, with the IgE binding of the PUV-treated sample being only 1.2-fold lower than that of control.

ELISA for PUV-Treated Peanut Butter Slurry

In comparison to control, the two samples, i.e., those treated for 1 min at both 14.6 and 18.2 cm, were not significantly different in the ELISA absorbance values at $p<0.05$ (Table 3). When comparing the differences in the absorbance values of PUV-treated samples for 1, 2, and 3 min, positioned at 10.8, 14.6, and 18.2 cm from lamp, it was found that some samples were significantly different from one another, while some others were not different (Table 3).

IgE-binding capacity of the PUV-treated peanut butter slurry analyzed using ELISA at 1, 2, and 3 min treatments is presented in Fig. 6. The IgE binding values are means of triplicate measurements. Results showed that the reduction in IgE binding increased with treatment time and decreased with distance from the lamp. For example, at 3 min treatment, the IC_{50} values for samples positioned at 10.8, 14.6, and 18.2 cm were 4, 3, and 1 μg/ml, respectively.

For peanut butter slurry, there were no significant interactions between the treatment time and distance from the lamp ($p<0.05$). This means the two factors were independent of each other in terms of their effects on the reduction of peanut proteins and IgE binding. Peanut butter slurry contained much less moisture as compared to the extracts. This means that exposure time had significant importance only when the moisture was adequate. This was because when moisture was evaporated, any increase in treatment time would have little additional effect on the sample, as most of the light energy could not easily penetrate through the sample. Increased treatment time beyond this point would only lead to localized heating on the surface of the sample due to PUV's infrared spectra. Therefore, in this case, the efficacy of treatment time would not depend on the position at which the sample was placed.

The observed differences in the results between the extracts and peanut butter slurry could also be attributed to the differences in treatment time, namely, the latter was treated for a shorter time (1 to 3 min) as opposed to a longer time (2 to 6 min) for the former. Longer treatment times were not practically possible for peanut butter slurry due to the problem of sample dry-out. Apart from being highly viscous, peanut butter slurry also contained oil and

Table 2 The ELISA absorbance values for PUV-treated roasted peanut extracts

Distance (cm)	Time (min)		
	6	4	2
10.8	A,a 1.5843±0.0011a	B,b 1.4210±0.0017a	C,c 1.3027±0.0025a
14.6	A,d 1.3593±0.0011b	B,e 1.2587±0.0011b	C,f 1.1717±0.0028b
18.2	A,g 1.2443±0.0011c	B,fh 1.1737±0.0011c	C,j 1.0727±0.0025c
Control[a]	1.0067±0.0032		

Means of triplicate measurements ± standard deviation; values not followed by the same letter in the same column are significantly different from each other at $p<0.05$; values not preceded by the same upper case letter in the same row are significantly different from each other at $p<0.05$; values not preceded by the same lower case letter in rows and columns are significantly different from each other $p<0.05$

[a] Control is significantly different from all other values

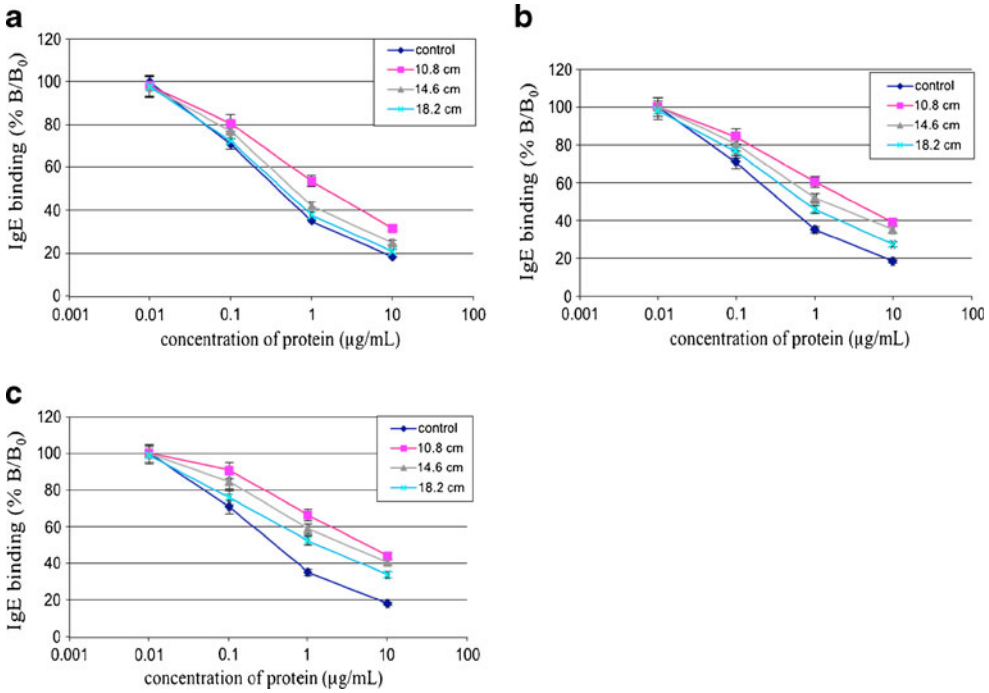

Fig. 5 IgE-binding capacity of PUV-treated roasted peanut extracts after **a** 2 min, **b** 4 min, and **c** 6 min PUV exposure. B/B_0 on *Y*-axis is the absorbance of the sample containing both IgE and peanut protein over that of the control sample containing IgE only. The *error bars* indicate standard deviations based on triplicate measurements

particulates which might limit PUV penetration into the sample.

Again, the IC50 values derived from Fig. 6 were used to calculate the fold reduction. The maximum reduction was achieved for the sample treated for 3 min at 10.8 cm from lamp. The maximum available PUV energy at this location was approximately 194.4 J/cm^2. The IC_{50} values were 4 and 0.6 μg/ml for the treated sample and control, respectively. Therefore, the IgE binding of PUV-treated peanut butter slurry was 6.7-fold lower than that of control. The sample subjected to 1 min treatment at 18.2 cm from lamp had an IgE binding 1.2-fold lower than that of control.

Comparing among the raw, roasted, and peanut butter slurry samples at 2 min PUV treatment and 10.8 cm from lamp, the raw peanut extracts had the highest reduction in IgE binding followed by roasted peanut extracts and peanut butter slurry, i.e., 3.5, 2.5, and 2 μg/ml, respectively. The difference in IgE binding reduction could be attributed to the difference in sample composition. Peanut butter slurry contained peanut particulates and a considerable amount of peanut oil. It has been reported that oil can minimize the efficacy of PUV by absorbing the radiation and in doing so minimizing the effective radiation dosage needed for reducing allergens (Gómez-López et al. 2007). Also, it was reported that particulates might hinder the efficient penetration of PUV rays through the liquid sample due to the shadow effect (Elmnasser et al. 2007).

Table 3 The ELISA absorbance values of PUV-treated peanut butter slurry

Distance (cm)	Time (min)		
	3	2	1
10.8	A,a 1.1557±0.0051a	B,b 1.0700±0.0529a	B,bc 1.0157±0.0445a
14.6	A,ab 1.1033±0.0028a	B,bc 1.0033±0.0404a	B,cf 0.9508±0.0112a
18.2	A,bg 1.0400±0.0200c	B,gc 0.9408±0.0088c	B,f 0.8990±0.0052c
Control[a]	0.8842±0.0142		

Means of triplicate measurements ± standard deviation; values not followed by the same letter in the same column are significantly different from each other at $p<0.05$; values not preceded by the same upper case letter in the same row are significantly different from each other at $p<0.05$; values not preceded by the same lower case letter in rows and columns are significantly different from each other $p<0.05$

[a] Control is significantly different from other values except for 1 min treatment at 14.6 and 18.2 cm, respectively

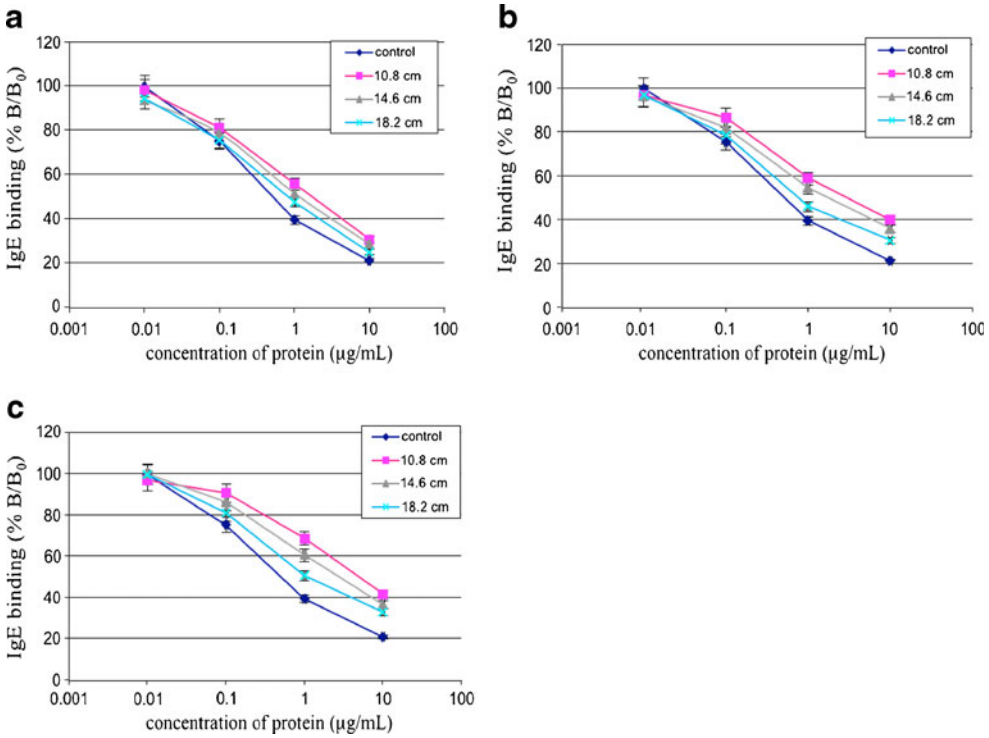

Fig. 6 IgE-binding capacity for PUV-treated peanut butter slurry after **a** 1 min, **b** 2 min and **c** 3 min PUV exposure. B/B_0 on *Y*-axis is the absorbance of the sample containing both IgE and peanut protein over that of the control sample containing IgE only

Conclusions

Results from this study showed that pulsed ultraviolet light had a significant impact on allergen reduction in peanut extracts and peanut butter slurry. In addition to reducing Ara h 1 and Ara h 3 the same way as literature reported, this study also revealed for the first time the effectiveness of pulsed ultraviolet light on reducing the reactivity of Ara h 2, the most potent allergenic protein of peanut, and showed a significant reduction in IgE binding at the treatment conditions tested in this study. Reduction in IgE binding increased with treatment time and decreased with increased distance from the PUV lamp. Both the factors of treatment time and distance from lamp had a major effect on the level of reduction on IgE binding. There was a significant interaction between the two factors for the peanut extracts ($p<0.05$). However, for peanut butter slurry, the factors had no significant interaction at $p<0.05$, meaning that the effect of one factor in reduction of IgE binding was independent of the other.

Future Work

There is a need to further understand the underlying mechanism for the PUV effects on peanut proteins in order to develop an optimal process which would maximize the mitigation of peanut allergens. The current conditions used in this study could only reduce peanut allergens to a significant level but not yet completely eliminated them. Also, the effect of PUV treatment on the quality and nutritional attributes of peanut samples needs to be examined, especially when PUV treatment could cause proteins to cross-link, aggregate, and form precipitates as discovered in this study and by Chung et al. (2008) and Yang et al. (2010). The PUV system used in this study was not allergen-specific, as all the proteins in the extracts and slurry were affected. Using filters to target only allergenic proteins during the PUV treatment should be investigated to minimize adverse effects on other proteins. Moreover, using purified proteins instead of the protein extracts may help PUV treatments target on specific allergens.

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