

# Effect of Pulsed Ultraviolet Light and High Hydrostatic Pressure on the Antigenicity of Almond Protein Extracts

Yiqiao Li · Wade Yang · Si-Yin Chung · Haiqiang Chen ·  
Mu Ye · Arthur A. Teixeira · Jesse F. Gregory ·  
Bruce A. Welt · Sandra Shriver

Received: 23 June 2011 / Accepted: 1 August 2011 / Published online: 11 August 2011  
© Springer Science+Business Media, LLC 2011

**Abstract** The efficacy of pulsed ultraviolet light (PUV) and high hydrostatic pressure (HHP) on the IgE binding to the almond extracts was studied using sodium dodecyl sulfate polyacrylamide-gel electrophoresis, Western blot, and enzyme-linked immunosorbent assay (ELISA) probed with human plasma containing IgE antibodies to almond allergens and a polyclonal antibody against almond major protein. Crude almond protein extracts were treated with PUV (3 pulses/s, 10 cm from lamp) for 0.5, 1, 2, 3, 4, 6, 7, and 10 min. In comparison, boiling treatments were also carried out. The HHP treatments were conducted at 600 MPa for 5, 15, and 30 min at three temperatures of 4 °C, 21 °C, and 70 °C. Western blots and indirect ELISA demonstrated a reduction in the levels of allergens and IgE

binding in PUV-treated extracts at 7 min, which was found to be the optimal time for PUV exposure. Boiling was not as effective as PUV in reducing the overall IgE-binding of the almond extracts. Unlike PUV, HHP did not affect the allergen levels and IgE binding under the conditions tested.

**Keywords** Almond · Allergen · Pulsed UV light · High pressure · IgE binding

## Introduction

According to the UN Food and Agriculture Organization (FAO 2011), global production of almonds is around 1.7

Y. Li · A. A. Teixeira · B. A. Welt  
Department of Agriculture and Biological Engineering,  
University of Florida,  
207 Frazier Rogers Hall,  
Gainesville, FL 32611, USA

Y. Li  
e-mail: yiqiaoli@ufl.edu

A. A. Teixeira  
e-mail: atex@ufl.edu

B. A. Welt  
e-mail: bwelt@ufl.edu

W. Yang (✉) · J. F. Gregory · S. Shriver  
Department of Food Science and Human Nutrition,  
University of Florida,  
359 FSHN Bldg. Newell Drive,  
Gainesville, FL 32611, USA  
e-mail: wade.yang@ufl.edu

J. F. Gregory  
e-mail: jfgy@ufl.edu

S. Shriver  
e-mail: sandrakshriver@ufl.edu

S.-Y. Chung  
USDA-ARS Southern Regional Research Center,  
1100 Robert E. Lee Blvd.,  
New Orleans, LA 70124, USA  
e-mail: siyin.chung@ars.usda.gov

H. Chen · M. Ye  
Department of Animal and Food Sciences,  
University of Delaware,  
020 Townsend Hall,  
Newark, DE 19716, USA

H. Chen  
e-mail: haiqiang@udel.edu

M. Ye  
e-mail: yemu@udel.edu

million metric tons at an average growth of 5% annually from 1993 to date, with a low throughput of 1 million tons in 1995 and a peak of around 1.9 million tons in 2002. In 2009, the US production increased to 734,000 tons, of which 631,000 tons were exported, according to the 2009 statistics of California Almond Board (2010).

Almonds are globally popular and are often used as a snack food and ingredients in food products (Venkatachalam et al. 2002). In tree nut production, almonds rank the first in the world. Almond is a highly nutritious food and a natural source of various nutrients (Sathe and Sze 1997). In spite of their popularity and health benefits, tree nuts like almond can induce acute generalized allergy symptoms and even anaphylactic shock. Unlike some food allergies that are temporary and only affect young children, allergies to tree nuts are often lifelong and can be life-threatening (Roux et al. 2001).

In almond, its major protein, amandin or AMP, is an allergenic protein as recognized by almond-allergic patients (Roux et al. 2001). AMP is an oligomeric protein containing prunin monomers. It is a legumin seed storage protein belonging to 11 S globulin families which belong to cupin superfamily. Almond allergen proteins are located in the 12, 20–22, 25, 30, 37, 42–46, 48–50, 62, 65, and 70 kDa regions (Bargman et al. 1992; Scheibe et al. 2001).

There have been several approaches tested so far for reducing almond allergens, including microwave heating (Venkatachalam et al. 2002), thermal processing (Shriver and Yang 2011), chemical processing (Acosta et al. 1999), and gamma irradiation (Su et al. 2004) with partial alterations or no reduction in almond allergens.

Pulsed ultraviolet light, an intermittent intense broad spectrum, is also referred to as pulsed light and high intensity light. In the PUV technology, the electrical energy is stored in a capacitor and released as short-period (several nanoseconds), intermittent pulses. An inert gas within the PUV lamp is ionized by the electrical energy and produces broad spectra of light containing wavelengths from near-infrared to ultraviolet. Studies have shown that pulses can be approximately 90,000 times more intense than the sunlight at the sea level (Shriver et al. 2011). Previous studies have shown reduction of peanut allergens (Chung et al. 2008; Yang et al. 2011) and soybean allergens (Yang et al. 2010) by PUV treatment.

Pulsed UV light is normally regarded as nonthermal when the time of exposure is short (e.g., seconds), and the product temperature rise is insignificant (Krishnamurthy et al. 2010). However, recent discovery (Krishnamurthy et al. 2009; Yang et al. 2011) shows that extended exposure (e.g., minutes) with PUV, which contains approximately 54% UV, 25% visible, and 20% infrared light (Oms-Oliu et al. 2010; Shriver et al. 2011), can induce significant photothermal

effect. As a result, considerable instantaneous temperature rise and moisture loss can occur to the sample (Chung et al. 2008; Mwakatage 2008; Yang et al. 2010; Nooji 2011; Shriver et al. 2011). Greenberg (1979), Fiedorowicz et al. (2001), and Kramer et al. (1991) also found that prolonged UV light treatment caused formation of insoluble complex in food, depolymerization of starch, peroxidation of unsaturated fatty acid, carbohydrate crosslinking, protein crosslinking, and protein fragmentation.

High hydrostatic pressure treatment (HHP), a non-thermal food processing technology, achieves food preservation by applying a hydrostatic pressure ranging from 100 to over 800 MPa to foods placed in a pressure vessel, which is filled with transmitting fluid compressed by a pump or pressure intensifier (Spilimbergo et al. 2002; Norton and Sun 2008). The subsequent high pressure has the ability to break non-covalent bonds, including hydrogen, ionic, and hydrophobic bonds (Hoover et al. 1989). Soybean seeds treated with HHP grew sprouts with a significant reduction in antigenicity but no reduction in nutritional value (Peñas et al. 2011). In eggs treated with HHP (600 MPa) at 70 °C, the allergen reactivity was reduced by 8.9-fold, because the treatment caused a change in the structure of the egg allergens (Hildebrandt et al. 2010). Conversely, HHP with and without heat supplement was reported to increase the reactivity of major milk allergen ( $\beta$ -lactoglobulin; Kleber et al. 2007).

The objectives of this study were to determine the effectiveness of PUV and HHP treatments for reducing almond allergen and the optimal treatment condition for reducing the IgE-binding of almond extracts.

## Materials and Methods

### Materials

#### *Sera*

Plasmas containing almond-specific human IgE antibodies were pooled from three donors with history of almond allergy (PlasmaLab International, Everett, USA). Rabbit anti-AMP antisera were obtained from the Florida State University, Tallahassee, FL.

#### *ELISA, SDS-PAGE, and Western Blot Reagents*

Tris buffered saline (TBS), phosphate buffered saline (PBS), *o*-phenyldiamine dihydrochloride (OPD), StartingBlock TBS/T-20 blocking buffer, Gelcode Blue stain reagent, bicinonchonic protein assay kit, 2-mercaptoethanol, and Tween-20 were obtained from Pierce Chemical Company (Rockford, IL). Precast Tris glycine minigels (4–20%) and

reagents for sodium dodecyl sulfate polyacrylamide-gel electrophoresis (SDS-PAGE) were obtained from BioRad Laboratories (Hercules, CA). Costar 96-well enzyme immunoassay plates were purchased from Corning Incorporated (Corning, NY) and were used for enzyme-linked immunosorbent assay (ELISA). Immobilon P blotting membrane was obtained from Millipore Corporation (Bedford, MA). Horseradish peroxidase-labeled goat anti-rabbit IgG and goat anti-human IgE were purchased from Invitrogen (Carlsbad, CA). SuperSignal West Pico Chemiluminescent Substrate (Pierce) was purchased from Thermo Scientific (Rockford, IL).

### Equipment

A Xenon Steripulse XL-3000 batch PUV system (Xenon Corporation, Woburn, MA) was used for the PUV treatments. In the Xenon PUV unit, the distance between the sample and the quartz window of the PUV lamp can be changed by moving the sample rack up or down. The total energy at 14.6 cm distance in a similar unit was determined to be 0.92 J/cm<sup>2</sup>/s (Krishnamurthy 2006). In this study, a distance of 10 cm from the sample to the quartz window was used. Since the distance from quartz window to lamp is 10.5 cm, the total distance from the lamp to the sample was 20.5 cm.

A handheld infrared thermometer (Omega OS423-LS, OMEGA, Stamford, CT) was used for measuring the temperature of the samples immediately after PUV treatment. BioRad electrophoresis and semi-dry transfer equipment were used for SDS-PAGE and Western blotting. A Spectramax 340 (Molecular Devices, Sunnyvale, CA) was used to read the 96-well plates. Laboratory-scale high-pressure unit (model Avure PT-1; Avure Technologies, Kent, WA) monitored with DASyLab<sup>®</sup> 7.0 software (DASYTEC USA, Bedford, NH) was used for HHP treatment.

### Almond Protein Extract Preparation

Finely grounded almond flour (meal) from blanched whole almonds (Bob's Red Mill, USA) was purchased from the local market. The flour contained 6 g of protein per 28 g of flour and was defatted by multiple extractions with cold acetone (meal to acetone ratio of 1:5 w/v) with constant magnetic stirring. After each extraction, the slurry was filtered through Whatman filter paper #4, and the residue was used for the next extraction. Residue from final extraction and filtration step was dried in a fume hood and collected for treatment. The residue was then extracted with 0.1 M sodium phosphate buffer pH 7.0 (flour/buffer 1:20 w/v) for 30 min at 25 °C with gentle shaking to obtain a protein extract. The samples

were then centrifuged at 15,000×g for 20 min, and the supernatant was stored at −20 °C for use in HHP and PUV experiments.

### Protein Determination

Protein concentrations of the untreated and PUV- and boiling-treated samples were measured by Bradford assay. Protein concentration was determined using 10 µl of sample and 300 µl of Coomassie Plus Reagent in a 96-well plate at room temperature. The plate was read at 595 nm with a plate reader; diluted bovine serum albumin was used as standard. For boiling and PUV treatment, a volume of 10 ml of the untreated sample at a concentration of 5 mg/mL was used. After the PUV treatment, some moisture was lost due to the photothermal effect of the PUV treatment. Prior to protein determination, the volume of PUV-treated and boiled samples was adjusted to the initial volume (10 mL) in order to maintain the same level of total protein concentration including soluble and insoluble proteins. The adjusted samples were also used for SDS-PAGE, Western blot, and ELISA tests.

### Pulsed Ultraviolet Light and Boiling Treatment of Almond Extracts

Almond extracts, 10 mL each, placed in an aluminum dish 7 cm in diameter, was treated with PUV at a preset time. Although the sample surface temperature before and after PUV treatment was recorded using the foregoing handheld infrared thermometer, the instantaneous temperature of the sample during PUV treatment could be higher than the recorded temperature because there was a few seconds delay in opening the door of the chamber before measuring and recording the sample temperature.

For boiling treatment, the almond extracts (10 mL each) in capped centrifuge tube were placed in a boiling water bath (100 °C) for 0.5, 1, 4, 6, and 7 min. The purpose of heating the sample was to determine if boiling treatment affected almond allergens in the same way as PUV treatment.

After treatments with PUV and boiling, the volume of treated samples was adjusted to the initial volume (10 mL) prior to analyses.

### High Hydrostatic Pressure Treatment of Almond Extracts

Almond extract (1.5 ml) was transferred to a sterile polypropylene pouch (Fisher Scientific, Fair Lawn, NJ), heat-sealed, and enclosed in a secondary pouch for HHP treatment. Treatment was carried out using a laboratory-scale high-pressure unit (model Avure PT-1; Avure Tech-

nologies, Kent, WA) monitored with DASyLab<sup>®</sup> 7.0 software (DASYTEC USA, Bedford, NH). The experiments were conducted at 600 MPa for 5, 15, and 30 min at 4 °C, 21 °C, and 70 °C, respectively, using water as the hydrostatic medium. The pressure was increased at a rate of approximately 22 MPa/s, and pressure release was almost immediate. Pressurization time reported in this study did not include the time needed to bring the pressure up for treatment or the time following pressure release. Three independent trials were conducted.

### SDS-PAGE

Following PUV-treatment, sample extracts with equal amount of proteins in Laemlli sample buffer containing 0.05% 2-mercaptoethanol were heated in a water bath (100 °C) for 10 min, and then loaded onto the wells of 4–20% Tris glycine gel in a BioRad Mini-Protean tank apparatus. Electrophoresis was performed for 1.5 h at 150 V. The gel was then stained with a GelCode Blue solution and destained with distilled water.

### Western Blot

After electrophoresis, the gel was removed from the cassette, equilibrated in transfer buffer for 1 h, and then transferred onto Immobilon P blotting membrane using a semi-dry transfer apparatus at 15 V for 30 min. The unbound sites on the membrane were blocked using StartingBlock for 15–30 min at room temperature with gentle rocking. The membrane was then incubated with a pooled patient plasma diluted in PBS/Tw (1:80) or anti-AMP polyclonal antibody in PBS/Tw (1:40) at 37 °C while rocking for 50 min. The membrane was washed twice for 10 min while rocking. The blots were incubated at room temperature for 1 h with a mouse anti-human IgE–HRP (Invitrogen, Carlsbad, CA) diluted in PBS/Tw at 1:1,000 or a goat anti-rabbit IgG–HRP (1:2,500). The blot was then washed twice for 30 min, each time with gentle shaking. All washes were with 1× TBS/0.05% Tween 20. Finally, a SuperSignal West Pico Chemiluminescent substrate was used for detection.

### Indirect ELISA

Costar Enzyme Immunoassay polystyrene 96-well plates were coated for 2 h at 37 °C with control, boiled, PUV-, and HHP-treated almond extracts diluted in PBS to a concentration of 20 µg/ml (100 µl per well). Following washing, the plates were blocked with StartingBlock (200 µl per well) at 37 °C for 1.5 h. Human plasma containing IgE antibodies specific for almond allergens

was diluted in PBS/Tw (1:4) and added to each well (100 µl). After incubation at 37 °C for 1 h, the plate was washed three times with TBS/Tw and then incubated with a monoclonal mouse anti-human IgE–HRP (1:1,000) at 37 °C for 1 h (100 µl per well). After incubation and a wash, a substrate solution (pH 5.5) containing OPD (0.5 mg/mL) and 0.03% hydrogen peroxide was added (100 µl per well). The color reaction was stopped at 15–30 min with 2.5 N sulfuric acid (100 µl per well). Absorbance was read on Spectramax 340<sup>384</sup> Spectrophotometer at 490 nm.

### Statistical Analysis

Statistical analysis was conducted using one-way analysis of variance (ANOVA) with SAS 9.0 (Cary, NC) software. Differences were considered at  $\alpha=0.05$ . In addition, least significant difference test was conducted on treatment ANOVA results to see if there were any differences between the treatment time and treatment type.

## Results and Discussion

### Effects of PUV and Boiling Treatments on Almond Proteins/Allergens in Extracts

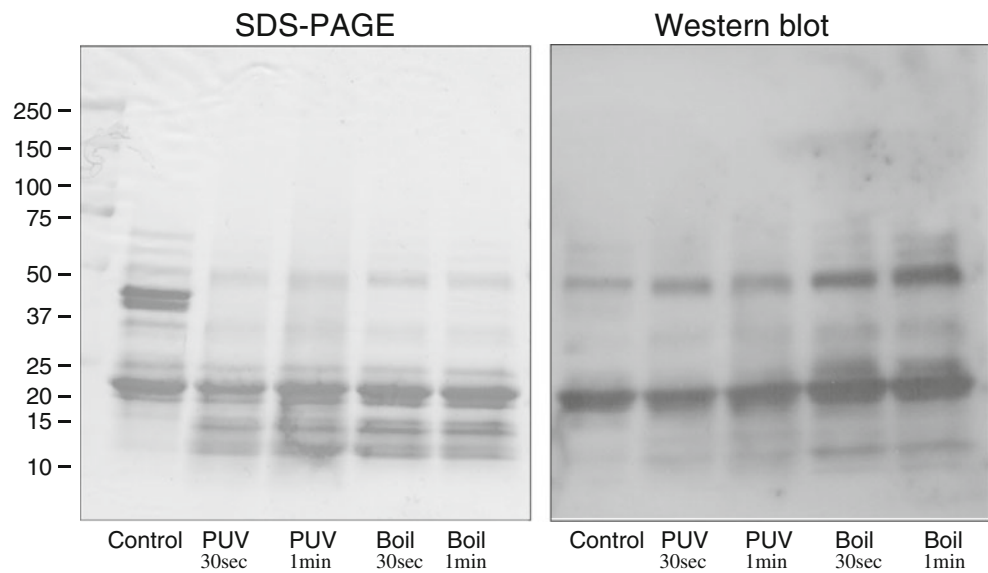
#### Treatment Time—30 s to 4 min

From the SDS-PAGE result on Fig. 1, the control almond sample contained proteins from around 20 to 70 kDa, with higher concentrations at 22 and 38 kDa.

After 30-s and 1-min PUV or boiling treatment, the heat-stable proteins (22–25 and 48 kDa) remained almost unchanged, but the heat-labile proteins (38 and ~70 kDa) were reduced, causing the appearance of bands between 12 and 20 kDa.

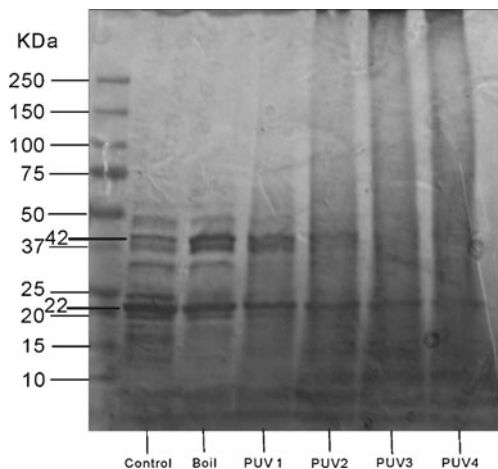
From the Western blot result on Fig. 1, the two major allergenic proteins at 22 and 48 kDa were detected. As compared with control, PUV treatment for 30 s and 1 min did not change the immunoreactivity of the two major almond allergens, but the 30-s and 1-min boiling treatments appeared to increase the reactivity slightly, as evidenced from the darker and thicker bands on the last two lanes. In addition, the two bands (12–14 kDa), which were reported to be minor allergens (Pasini et al. 2000; Poltronieri et al. 2002) and appeared as dark bands in SDS-PAGE after 30-s and 1-min PUV treatments, were markedly reduced in the blots. This indicates that the minor allergens were not fully recognized by the IgE antibody after PUV treatment. It is possible that the allergens might have undergone some conformational changes following PUV treatment, which caused their IgE-binding sites to become unavailable.

**Fig. 1** SDS-PAGE (*left*) and Western blot (*right*) of almond extracts treated with PUV and boiling for a short time (30 s and 1 min). Western blot was probed with pooled plasma from almond-allergic individuals and a goat anti-human IgE–HRP conjugate



The current finding that major almond allergens (22 and 48 kDa) were not affected by short PUV exposure (30 s and 1 min) indicated that they were stable under PUV and were comparable to other almond proteins that remain stable under different food processing treatments (Su et al. 2004; Venkatachalam et al. 2002). Unlike almond allergens, egg and milk allergens have been found reduced substantially following 30 s to 1 min PUV treatments (Anugu et al. 2009; 2010).

Additionally, 2-, 3-, and 4-min treatments were performed. Figure 2 shows the SDS-PAGE of almond extracts resulting from 4-min boiling and 1-, 2-, 3-, and 4-min PUV exposure. The profile of 1-min boiling was not shown because it was similar to that of 4-min boiling. It can be seen that, compared with 1-min PUV, the band pattern and intensity for the major allergens (22 kDa and above) were unchanged for 4-min boiling. However,

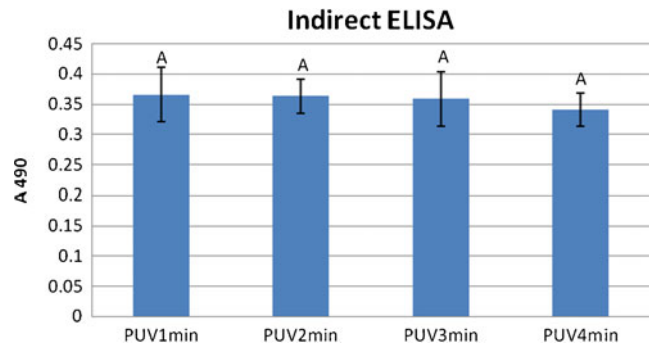


**Fig. 2** SDS-PAGE of almond extracts treated with boiling for 4 min and with PUV for 1, 2, 3, and 4 min. Data for 1-min boiling were not shown because they were similar to 4 min

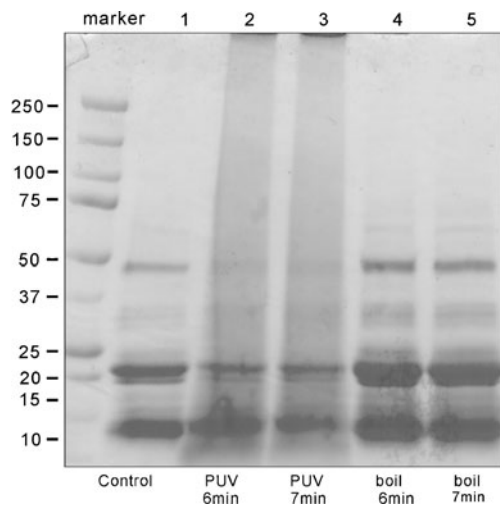
for 2-, 3-, and 4-min PUV treatments, the band intensity decreased accordingly. Further investigations on the IgE binding of these PUV-treated extracts in an ELISA probed with pooled plasma from almond-allergic individuals indicated that, despite the difference in band density between the PUV treatments (1–4 min) in SDS-PAGE, there were no significant changes in IgE binding between 1- and 4-min PUV exposure (Fig. 3), suggesting the immunological stability of almond allergens in the extracts under PUV treatment for up to 4 min.

*Treatment Time—6 to 7 min*

Since there was no significant difference in IgE binding between treatments at 1 and 4 min, samples were further tested at two other exposure times for PUV and boiling—6 and 7 min—and Fig. 4 shows their SDS-PAGE profiles. The pattern of the protein bands for the boiling-treated extracts was similar to that of the control, except for slightly increased intensity. This indicated that 6- and 7-min



**Fig. 3** IgE binding of almond extracts treated with PUV for 1, 2, 3, and 4 min in ELISA probed with human plasma from almond-allergic individuals. Values are mean of triplicates with standard error bars. Values with different letters are significantly different ( $\alpha=0.05$ )



**Fig. 4** SDS-PAGE profile of almond extracts boiled- and PUV-treated for 6 and 7 min. Molecular weights shown are in kDa

boiling did not seem to change much the almond proteins in terms of molecular weight or structure. For the 6- and 7-min PUV samples, heat-labile protein bands between 25 and 50 kDa were hardly detected, compared with the control. Also, the intensity of the two heat-stable allergenic proteins (12 and 20–22 kDa) was reduced, especially for the 7-min PUV treatment. All this (protein reduction) was due to the combination of PUV's photothermal and nonthermal effects, which probably caused the allergenic proteins to disintegrate or cross-link with other proteins/peptides to form proteins of larger molecular weight, thus resulting in the appearance of smears seen on lanes 2 and 3 in Fig. 4.

Almond proteins were clearly shown to be affected by the PUV illumination at this time range (6–7 min; Fig. 4). The smears exhibited on lanes 2 and 3 indicated that the cross-links or aggregates were obviously formed and not able to run through the gel smoothly. Figure 4 also indicated that the energy introduced into the extracts by boiling alone did not change the proteins the way PUV did, and the reason for the PUV effect was because extended PUV exposure entailed a combined consequence of photothermal, photophysical, and photochemical effects (Yang et al. 2011). Furthermore, it was observed during the experiments that the PUV treatment changed the color of almond extracts from a transparent-white color to light-yellow color and showed much more aggregation than the boiled sample.

Bargman et al. (1992) reported that the proteins from 45 to 50 kDa were stable and reactive after dry-roasting heat treatment. Conversely, in this study, proteins from 45–50 kDa were reduced instead by PUV as shown in Fig. 4.

Western blots with both human plasma from almond-allergic individuals and polyclonal antibodies against

almond proteins for almond extracts treated with PUV and boiling for 6 and 7 min are shown in Fig. 5. Figure 5a shows that IgE binding of 20–22 kDa band of the boiled samples (lanes 4 and 5) was almost the same as that of control. This indicated that boiling treatment did not reduce the IgE reactivity of almond allergens.

However, IgE binding of the PUV-treated almond extract was shown to be reduced (lanes 2 and 3), and the reduction appeared to be much higher at 7 min PUV than at 6 min PUV. Treatments with polyclonal anti-AMP antibody gave the same result as with IgE antibody. This suggests that the allergenic proteins at 12, 22, and 48 kDa were basically changed in terms of their levels and probably epitopes after 6 and 7 min PUV illumination, causing a reduction in IgE binding.

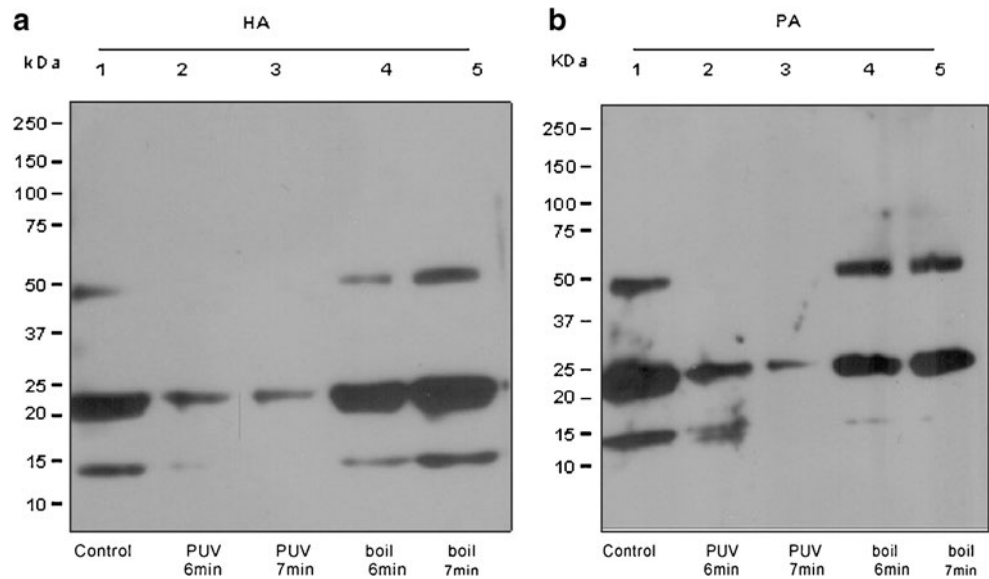
Furthermore, aggregates above 250 kDa were detected in Western blot probed with IgE. As shown in Fig. 6, dark bands corresponding to aggregates (>250 kDa) due to PUV treatment for 7 min were seen. These bands were much darker and more obvious when the PUV treatment time was extended to 10 min. This demonstrates that PUV treatment did promote and cause the formation of aggregates. As mentioned before, aggregates and smears were formed possibly because of the cross-linking between almond proteins (peptides) as induced by PUV, which was then accompanied by a pronounced decrease in the intensity of the bands corresponding to the 22-kDa allergen.

The result from Western blot (Fig. 6) indicates that IgE antibodies bound to the aggregates or cross-linked proteins in the PUV-treated sample. Bargman et al. (1992) reported similar results due to heating.

Although the almond cross-links had an affinity for IgE antibodies in Western blot (Fig. 6), the overall IgE binding of the PUV-treated almond extract was reduced. This was demonstrated by the ELISA data (Fig. 7), which showed a 76% reduction in the absorbance value (representing IgE binding) with the 7-min PUV-treated extract, as compared with control. The overall IgE binding of the PUV-treated almond extract could have been reduced even more if it was not because of the cross-links that contributed to part of the overall IgE binding. Another possible explanation for the reduction in overall IgE binding is that the cross-links and the reduced allergens were less allergenic than the original allergens in the untreated extract.

In addition, the overall IgE-binding of 7-min boiling-treated extract was compared with that of the control and PUV-treatments. Data (Fig. 7) showed that boiling caused a 40% reduction in the absorbance value (IgE binding) compared with control. This was not so obvious in Western blot (Fig. 5a), indicating that some unknown components in the boiled extracts could play a role in the reduction of IgE binding in ELISA. Compared with PUV,

**Fig. 5** Western blots of treated almond extracts probed with a pooled plasma (1:80; HA) and polyclonal anti-AMP antibody (1:40; PA). Dilutions of anti-human IgE-HRP and anti-rabbit IgG-HRP were 1:1,000 and 1:2,500, respectively



boiling exhibited a higher IgE-binding than PUV, in agreement with the results from blots.

In summary, PUV was capable of reducing the overall IgE-binding of almond extracts to a great extent and was more effective than boiling in this regard.

*Effect of High Hydrostatic Pressure on Almond Proteins/Allergens in Extracts*

Almond extracts were also subjected to treatments with HHP at various times (5, 15, and 30 min) and different temperatures (4 °C, 21 °C, and 70 °C) to determine if HHP has an effect. Results from SDS-PAGE and blots (Fig. 8)

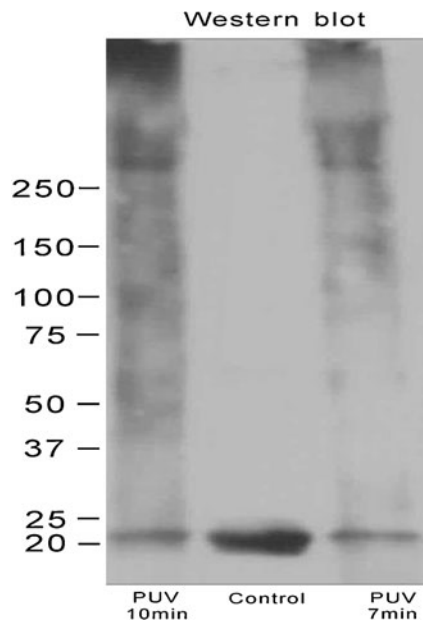
show that there was no difference in the protein profile between all the HHP treatments. This indicates that HHP was not able to reduce the almond allergens or their immunoreactivity under the conditions tested.

However, in one study (Messens et al. 1997), HHP has been demonstrated to alter the reactivity of some food allergens through modification of their structures. Further investigation with ELISA in our study (Fig. 9) showed that HHP treatments did not significantly change the IgE binding, compared with the control.

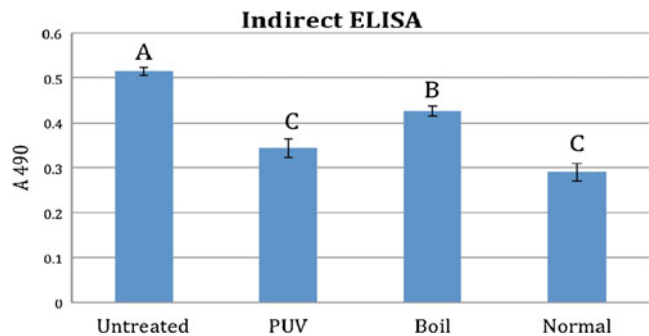
In summary, HHP had no effect on the almond allergens in the extract under the conditions tested.

*Factors to be Considered Using PUV*

It should be noted that, during PUV treatment, factors such as changes in sample temperature and volume may occur. Therefore, before and after PUV treatments of almond extracts, the sample weight was measured using a digital

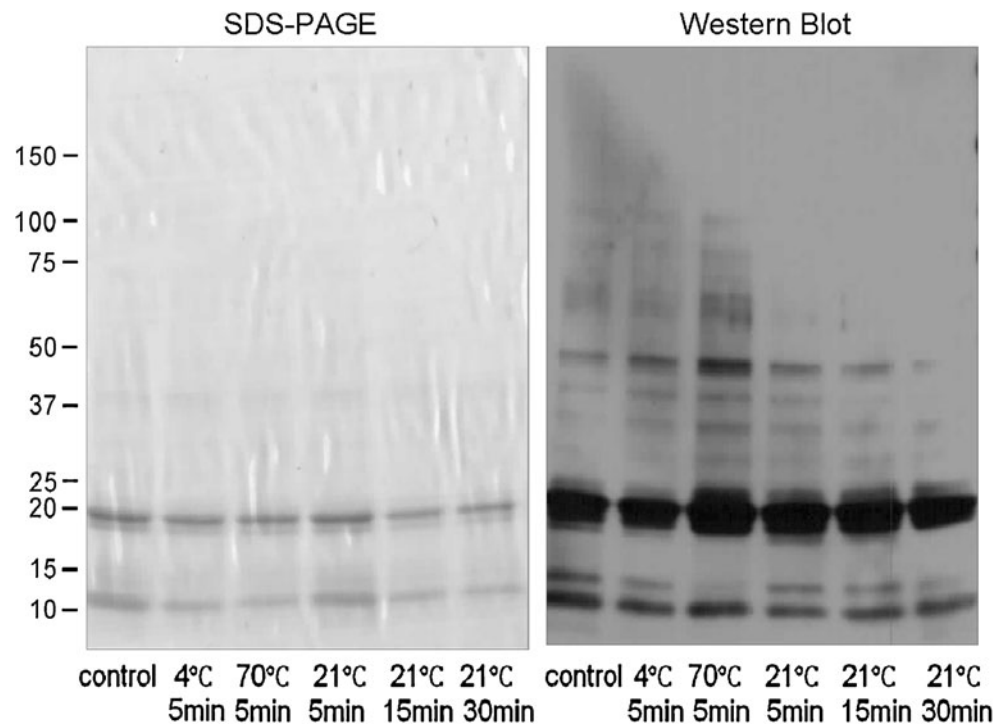


**Fig. 6** Western blot profile of almond extracts treated with PUV for 10 min, compared with 7 min. Molecular weights shown are in kDa



**Fig. 7** Indirect ELISA for untreated, 7-min PUV-treated and 7-min boiled almond extracts using human plasma containing IgE antibodies against almond and negative control (*normal*). The values show the mean of six replicates with standard deviation bars. Values with different letters are significantly different ( $\alpha=0.05$ )

**Fig. 8** SDS-PAGE and Western blot of HHP-treated almond extracts probed with plasma (1:80) from almond-allergic individuals and a goat anti-human IgE HRP (1:1,000). Molecular weights shown are in kDa



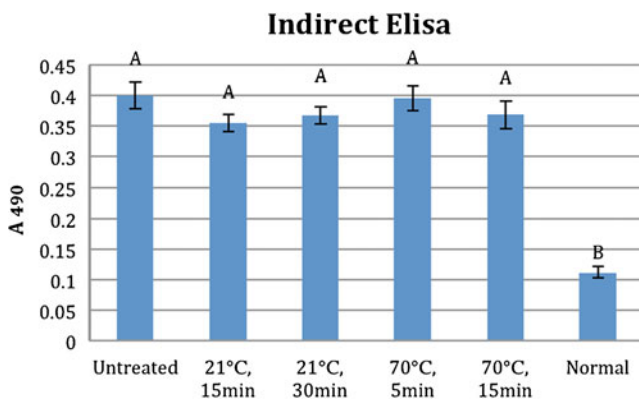
balance to determine moisture loss. After PUV treatments, temperatures of each sample were measured using an infrared thermometer. Also, the volume of each treated sample was adjusted (due to moisture loss) to the initial sample volume (10 mL) before any analyses (e.g., SDS-PAGE, Western blots, and ELISA) were performed. The results for water loss and temperature increase after PUV treatments are shown in Table 1.

The temperatures read from 60.9 °C to 115.0 °C after 1 to 7 min PUV treatment. It was noted that there was a few seconds delay in temperature measurement, which was the time spent to open the door of the treatment chamber, so the instantaneous temperature during the PUV treatment could

be higher. After PUV treatments, all almond samples exhibited changes in volume (mass) as a result of water evaporation. The volumes (masses) were reduced by 5.7–58% after 1 to 7 min PUV treatment.

It should be noted that, despite the temperature rise, PUV is not considered a heat treatment but rather a treatment with high energy produced by a combination of photochemical, photothermal, and photophysical effects. Such energy is instantaneous and ultimately converted into heat, which is different from the normal heating process.

With this high energy from PUV, tests in this laboratory showed that even a dry wood stick in the chamber of the PUV equipment was ignited after 8 min exposure at 10 cm from lamp. The distance and pulse rate were the same as in the conditions for the almond extracts. Because the



**Fig. 9** Indirect ELISA for HHP-treated and untreated almond extracts using human plasma containing IgE antibodies against almond. The negative control (*normal*) is also shown. The values are the mean of nine replicates with standard deviation bars. Values with different letters are significantly different ( $\alpha=0.05$ )

**Table 1** Changes in temperature and mass of almond extracts as affected by PUV

PUV treatment time	Water loss (%)	Temperature after treatment
30 s	3.1	50.5±1.5
1 min	5.7	60.9±4.5
2 min	10.2	75.9±1.2
3 min	17.5	80.5±0.7
4 min	24.9	83.1±2.8
6 min	38.9	111.7±1.5
7 min	58.5	115.0±0.8
8 min	Some area dried out	Ignited a wood stick

Data are mean±standard deviation ( $n=3$ )



temperature needed to auto-ignite wood is at least 204 °C or above (Babrauskas 2001), so the instantaneous temperature of the sample during the PUV treatment for the 8 min duration could also be 204 °C or above. In this PUV unit, the energy intensity is about 0.12 J/cm<sup>2</sup> per pulse when the distance from lamp to the sample is 20.5 cm, according to Krishnamurthy (2006) and Yang et al. (2011).

## Conclusions

Following PUV treatment, the almond extract exhibited a reduction in the levels of allergens and IgE binding, as demonstrated by SDS-PAGE, Western blots, and ELISA. Results indicate that PUV was more effective than HHP in almond allergen reduction. A 7-min time appeared to be the optimal condition for PUV treatment while a short-time (1–4 min) PUV treatment was not effective in allergen or IgE-binding reduction. The HHP treatment had little effect on the allergens under the conditions tested. The mechanism behind the allergen and IgE-binding reduction was likely that PUV induced protein cross-linking and/or fragmentation due to its photothermal, photochemical, or photophysical effect, with photothermal effect being more prominent.

For future studies, one focus can be placed on PUV treatment of whole almond kernels, and the PUV-treated products will undergo a sensory test and nutritional analysis to ensure the retention of nutrients and quality. In addition, the wavelength region of PUV that played an important role in altering the almond protein structures can be investigated. Also, in vivo studies will be needed to verify the validity of the current in vitro studies.

**Acknowledgments** The authors would like to thank Dr. Ken Roux and Leanna Willison in Florida State University, Tallahassee, FL, USA for their assistance in this study.

## References

- Acosta, M. R., Roux, K. H., Teuber, S. S., & Sathe, S. K. (1999). Production and characterization of rabbit polyclonal antibodies to almond (*Prunus dulcis* L.) major storage protein. *Journal of Agricultural and Food Chemistry*, 47(10), 4053–4059.
- Anugu, A., Yang, W., Shriver, S. K., Chung, S.-Y. & Percival, S. S. (2010). Efficacy of pulsed ultraviolet light on reducing the allergenicity of isolated egg proteins. IFT Abstract, 2010 Institute of Food Technology Annual Meeting, Chicago, IL.
- Anugu, A., Yang, W. & Krishnamurthy, K. (2009). Efficacy of pulsed ultraviolet light for reduction of allergenicity in isolated milk proteins. IFT Abstract, 2009 Institute of Food Technology Annual Meeting, Anaheim, CA.
- Babrauskas, V. (2001). Ignition of wood: A review of the state of the art. In *Interflam 2001* (pp. 71–88). London: Interscience Communications Ltd.
- Bargman, T., Rupnow, J., & Taylor, S. (1992). IgE binding proteins in almonds (*Prunus amygdalus*): Identification by immunoblotting with sera from almond allergic adults. *Journal of Food Science*, 57(3), 717–720.
- California Almond Board. (2010). Almond Board of California official website: <http://www.almondboard.com/Growers/Pages/Default.aspx>. Accessed on July 10, 2010.
- Chung, S. Y., Yang, W., & Krishnamurthy, K. (2008). Effects of pulsed UV-light on peanut allergens in extracts and liquid peanut butter. *Journal of Food Science*, 73(5), C400–C404.
- FAO. (2011). FAOSTAT Website: <http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567#ancor>. Accessed on February 15, 2011.
- Fiedorowicz, M., Tomasik, P., & Lii, C. Y. (2001). Degradation of starch by polarised light. *Carbohydrate Polymer*, 45(1), 79–87.
- Greenberg, J. R. (1979). Ultraviolet light-induced crosslinking of mRNA to proteins. *Nucleic Acids Research*, 6(2), 715–732.
- Hildebrandt, S., Schütte, L., Stoyanov, S., Hammer, G., Steinhart, H., & Paschke, A. (2010). In vitro determination of the allergenic potential of egg white in processed meat. *The Journal of Allergy*, 2010. doi:10.1155/2010/238573.
- Hoover, D. G., Metrick, C., Papineau, A. M., Farkas, D. F., & Knorr, D. (1989). Biological effects of high hydrostatic pressure on food microorganisms. *Food Technology*, 43(3), 99–107.
- Kleber, N., Maier, S., & Hinrichs, J. (2007). Antigenic response of bovine beta-lactoglobulin influenced by ultra-high pressure treatment and temperature. *Innovative Food Science and Emerging Technologies*, 8(1), 39–45.
- Kramer, G. F., Norman, H. A., Krizek, D. T., & Mirecki, R. M. (1991). Influence of UV-B radiation on polyamines, lipid peroxidation and membrane lipids in cucumber. *Phytochemistry*, 30(7), 2101–2108.
- Krishnamurthy, K. (2006). Decontamination of milk and water by pulsed UV-light and infrared heating. Ph.D. dissertation, The Pennsylvania State University.
- Krishnamurthy, K., Demirci, A., Irudayaraj, J., & Yang, W. (2009). Chapter 11. UV pasteurization of food materials. In J. M. Irudayaraj & S. Jun (Eds.), *Food processing operations modeling: Design and analysis* (2nd ed., pp. 281–299). Boca Raton, FL, USA: CRC. ISBN 978-1-4200-5553-5.
- Krishnamurthy, K., Tewari, J. C., Irudayaraj, J., & Demirci, A. (2010). Microscopic and spectroscopic evaluation of inactivation of *Staphylococcus aureus* by pulsed UV light and infrared heating. *Food and Bioprocess Technology*, 3(1), 93–104.
- Messens, W., Van Camp, J., & Huyghebaert, A. (1997). The use of high pressure to modify the functionality of food proteins. *Trends in Food Science and Technology*, 8(4), 107–112.
- Mwakatage, N.R. (2008). Efficacy of pulsed UV light treatment on removal of peanut allergens. M.S. thesis, Department of Food and Animal Sciences, Alabama A&M University, Normal, AL, USA 35762.
- Nooji, J. (2011). Reduction of wheat allergen potency by pulsed ultraviolet light, high hydrostatic pressure and nonthermal plasma. M.S. thesis, Department of Food Science and Human Nutrition, University of Florida, Gainesville, FL, USA 32611.
- Norton, T., & Sun, D.-W. (2008). Recent advances in the use of high pressure as an effective processing technique in the food industry. *Food and Bioprocess Technology*, 1(1), 2–34.
- Oms-Oliu, G., Martín-Belloso, O., & Soliva-Fortuny, R. (2010). Pulsed light treatments for food preservation. A review. *Food and Bioprocess Technology*, 3(1), 13–23.
- Pasini, G., Simonato, B., Giannattasio, M., Gemignani, C., & Curioni, A. (2000). IgE binding to almond proteins in two CAP-*FEIA*-negative patients with allergic symptoms to almond as compared to three CAP-*FEIA*-false-positive subjects. *Allergy*, 55(10), 955–958.

- Peñas, E., Gomez, R., Frias, J., Baeza, M. L., & Vidal-Valverde, C. (2011). High hydrostatic pressure effects on immunoreactivity and nutritional quality of soybean products. *Food Chemistry*, *124*(2), 423–429.
- Poltronieri, P., Cappello, M. S., Dohmae, N., Conti, A., Fortunato, D., Pastorello, E. A., et al. (2002). Identification and characterisation of the IgE-binding proteins 2S albumin and conglutin gamma in almond (*Prunus dulcis*) seeds. *International Archives of Allergy and Immunology*, *128*(2), 97–104.
- Roux, K. H., Teuber, S. S., Robotham, J. M., & Sathe, S. K. (2001). Detection and stability of the major almond allergen in foods. *Journal of Agricultural and Food Chemistry*, *49*(5), 2131–2136.
- Sathe, S. K., & Sze, K. W. C. (1997). Thermal aggregation of almond protein isolate. *Food Chemistry*, *59*(1), 95–99.
- Scheibe, B., Weiss, W., Ruff, F., Przybilla, B., & Gorg, A. (2001). Detection of trace amounts of hidden allergens: Hazelnut and almond proteins in chocolate. *Journal of Chromatography. B, Biomedical Sciences and Applications*, *756*(1–2), 229–237.
- Shriver, S. K., & Yang, W. (2011). Thermal and nonthermal methods for allergen control. *Food Engineering Reviews*, *3*(1), 26–43.
- Shriver, S., Yang, W., Chung, S.-Y., & Percival, S. (2011). Pulsed ultraviolet light reduces immunoglobulin E binding to Atlantic white shrimp (*Litopenaeus setiferus*) extract. *International Journal of Environmental Research and Public Health*, *8*, 2569–2583.
- Spilimbergo, S., Elvassore, N., & Bertucco, A. (2002). Microbial inactivation by high-pressure. *Journal of Supercritical Fluids*, *22*(1), 55–63.
- Su, M., Venkatachalam, M., Teuber, S. S., Roux, K. H., & Sathe, S. K. (2004). Impact of  $\gamma$ -irradiation and thermal processing on the antigenicity of almond, cashew nut and walnut proteins. *Journal of the Science of Food and Agriculture*, *84*(10), 1119–1125.
- Venkatachalam, M., Teuber, S. S., Roux, K. H., & Sathe, S. K. (2002). Effects of roasting, blanching, autoclaving, and microwave heating on antigenicity of almond (*Prunus dulcis* L.) proteins. *Journal of Agricultural and Food Chemistry*, *50*(12), 3544–3548.
- Yang, W., Chung, S. Y., Ajayi, O., Krishnamurthy, K., Konan, K., & Goodrich-Schneider, R. (2010). Use of pulsed ultraviolet light to reduce the allergenic potency of soybean extracts. *International Journal of Food Engineering*, *6*(3), 1–2.
- Yang, W., Mwakatage, N. R., Goodrich-Schneider, R., Krishnamurthy, K., & Rababah, T. M. (2011). Mitigation of major peanut allergens by pulsed ultraviolet light. *Food and Bioprocess Technology*. doi:10.1007/s11947-011-0615-6.