Use of Pulsed Ultraviolet Light to Reduce the Allergenic Potency of Soybean Extracts

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Use of Pulsed Ultraviolet Light to Reduce the Allergenic Potency of Soybean Extracts*

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Abstract

Pulsed ultraviolet light (PUV), a non-thermal food processing technology, is reported to be able to inactivate enzymes and reduce allergen levels from peanut extracts. The objective of this study was to determine if PUV would reduce the allergen levels and allergenic potency of soy extracts. Soy extracts were treated with PUV at various times (2, 4 and 6 min), centrifuged, and analyzed by SDS-PAGE and an indirect ELISA for IgE binding or allergenic potency. Results showed that PUV treatment led to an increase in sample temperature/weight loss but a decrease in the levels of soy allergens (i.e., glycinin and β-conglycinin) as shown in SDS-PAGE. Allergens were reduced probably through aggregation which increased with treatment time. IgE binding was reduced as well in the following order: 20%, 44% and 50% reductions in absorbance values at 2, 4, 6 min, respectively (the latter two were not significantly different (p < 0.05%) from each other). It was concluded that PUV was capable of reducing the allergenic potency of soy extracts, and that the optimal PUV treatment time was 4 min. Clinical data is still needed before PUV can find an application in the development of less allergenic soybean beverages and products.

KEYWORDS: soybean, allergens, pulsed ultraviolet light, PUV, IgE

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Introduction

Soy consumption has increased in recent years due to the awareness of its nutritional and health benefits (Franke et al., 2008, Friedman & Brandon, 2001). However, the number of incidents of soy allergy are also on the rise (Cordle, 2004; Zuidmeer et al., 2008). This is not surprising because soybean has been recognized as one of the “big 8” food allergens (i.e., milk, egg, peanut, tree nut, soy, wheat, fish, and shellfish) (Sicherer and Sampson, 2006). To date, more than 20 allergens in the molecular weight range of 7-71 kDa have been identified in soybeans (Amnuaycheewa & de Mejia, 2009, L’Hocine & Boye, 2007). However, only a few of these proteins are responsible for the majority of allergenic responses (Amnuaycheewa & de Mejia, 2009, Holzhauser et al., 2009). Soy allergens that are commonly known and responsible for most of the allergic reactions are glycginin (11 S) (12-45 kDa) and β-conglycinin (7 S) (50-76 kDa), which are hexameric and trimer proteins, respectively, with several subunits (Amnuaycheewa & de Mejia, 2009, Holzhauser et al., 2009, Krishnan et al., 2009).

To combat soybean allergy, a number of approaches with aims to change the allergen structures, remove the allergens or desensitize the soy-allergic individuals with allergens have been reported. These approaches include thermal processing (Amnuaycheewa & de Mejia, 2009, Blaschek et al., 2005, van de Lagemaat et al., 2007), digestion with enzymes (Lee et al., 2007, Moreno, 2007), fermentation (Kobayashi, 2005, Song et al., 2008), genetic engineering (Holding & Larkins, 2008, Singh & Bhalla, 2008), and oral food challenge (Nowak-Wegrzyn et al., 2009).

While thermal processing is known to have a reducing effect on the allergenicity of soybeans (Amnuaycheewa & de Mejia, 2009, van de Lagemaat et al., 2007), little is known about the effect of pulsed ultraviolet light (PUV) on soybean allergens. PUV is a non-thermal food processing and preservation technology with polychromatic radiation in the wavelength range of 100 to 1100 nm. Because of its high instantaneous energy, PUV has been successfully used to kill microorganisms in foods (Hierro et al., 2009, Krishnamurthy et al., 2004). PUV generates energy that is converted to heat when absorbed by proteins, and has been shown to induce aggregations among peanut allergens, and reduce the allergenic capacity of peanut extracts and liquid peanut butter (Chung et al., 2008). Since peanut and soybean are members of the legume family and share several common antigenic sites (Herrero et al., 2009, Peeters et al., 2007), we postulated that PUV would have the same reducing effect on the allergenicity of soy proteins as that on peanuts. The objective of this study was to determine if PUV would reduce the allergen levels and allergenic potency of soy extracts.
Materials and Methods

Materials. Raw soybean seeds (Hutcheson commercial soybean variety) were purchased from the local market. Tris buffered saline, 96-well microtiter plates, tetramethylbenzidine (TMB), Tween 20, and goat anti-human IgE peroxidase conjugate were obtained from Sigma Chemical Co (St. Louis, MO). Tris-glycine gels (10-20%), gel electrophoresis apparatus (BioRad Mini Protean), and reagents were from BioRad (Hercules, CA). Superblock blocking buffer, GelCode Blue Stain Reagent, and Coomassie Plus Bradford assay were purchased from the Pierce Chemical Company (Rockford, IL). Plasmas from two patients with documented soy allergy were purchased from PlasmaLab International (Everett, WA). Gen 5 plate reader was purchased from BioTek Instruments, Inc. (Winooski, VT). For PUV treatments, a Xenon RS-3000C PUV sterilizer (Xenon Corp., Woburn, MA) (Fig. 1) was used.

Figure 1. Xenon Steripulse XL-3000 sterilization system.

Preparation of soy extracts. Raw soybean kernels were de-hulled, ground, and defatted in hexane (1:4 w/v). Soy extracts were prepared by stirring the resultant defatted meals (1 g) in 10 ml 0.05 M Tris buffer (pH 8.0) for 2 h at room temperature, followed by centrifuging at 12,000 g for 20 min. Protein concentration in the soy extracts thus obtained were determined, using the Coomassie assay.

PUV treatment of soy extracts. PUV was operated at three pulses per second using a Xenon PUV system. Samples were treated with PUV at 13.2 cm from the light source for 2, 4, and 6 min (three replications each) with energy levels approximately at 117.6, 235.2, and 352.8 J/cm², respectively. Sample
weights were measured before and after treatment. Immediately after PUV treatments, surface temperature of the samples \((n = 2)\) was measured using an infrared thermometer. Little fluctuations in the temperature were observed between two determinations, thus confirming the reproducibility of the treatments. Samples were then centrifuged, and supernatants were retrieved for determination of protein concentration, using the Coomassie Plus Bradford assay prior to SDS-PAGE and enzyme-linked immunosorbent assay (ELISA).

_SDS-PAGE of PUV-treated soy extracts._ SDS-PAGE was performed according to the method of Chung et al. (2008), using the BioRad Mini Protean system and 10-20\% Tris-glycine gel. Gels were stained, using Gelcode Blue reagent and de-stained with water. Protein bands in de-stained gels were scanned using a Kodak ID 3.6 Scientific Image System. The reproducibility of SDS-PAGE was confirmed by matching the gel patterns from two determinations.

_Determination of IgE binding of PUV-treated soy extracts._ Immunoglobulin E (IgE) binding or allergenic potency was determined in an indirect ELISA with some modification of the method of Chung et al. (2003). A pooled plasma \((1:20)\) \((100 \, \mu l/well)\) from individuals allergic to soybean was added to a microtiter plate coated with PUV-treated and untreated soy extracts \((10 \, \mu g/ml)\) in triplicates, and incubated for 1 h. A normal plasma was used as control. After incubation and wash with TBS/Tween 20, a goat anti-human IgE peroxidase conjugate \((1:1000)\) was added and incubated for 1 h. The plate was washed and a TMB substrate solution containing 0.03\% hydrogen peroxide was added. The absorbance was read at 620 nm using a Gen 5 plate reader.

_Statistical analysis._ ELISA data \((n = 3)\) were analyzed with SAS Version 9 (SAS Institute Inc., Cary, NC) and Student’s t-test was employed to compare the means. Probability \(p < 0.05\) indicated statistically significant differences.

**Results and Discussions**

_Effect of PUV on sample weight and temperature._ Though pulsed UV light technology is a non-thermal process, prolonged exposure to pulsed UV light increases the energy absorbed by the samples. Some of the energy absorbed by the sample is converted into heat energy and thus the temperature of the sample increases. This temperature increase also leads to the evaporation of water present in the soybean extract and thus results in sample weight loss. The weight loss and temperature increase are shown in Table 1.

As expected, longer treatment time resulted in increased sample weight loss. For instance, weight losses of 9.5\%, 31.9\%, and 48.5\% were observed with PUV treatments at 2, 4, and 6 min, respectively. Similarly, the temperature increased with treatment time. For instance, increases of 49 \(^\circ C\), 53 \(^\circ C\), and 61\(^\circ C\)
Table 1. Sample weight loss and temperature increase due to pulsed UV light treatment.

<table>
<thead>
<tr>
<th>Treatment time (min)</th>
<th>Weight loss (%)</th>
<th>Increase in temperature (°C)</th>
<th>Final temperature* (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>9.5</td>
<td>49</td>
<td>73</td>
</tr>
<tr>
<td>4</td>
<td>31.9</td>
<td>53</td>
<td>76</td>
</tr>
<tr>
<td>6</td>
<td>48.5</td>
<td>61</td>
<td>85</td>
</tr>
</tbody>
</table>

*Mean of two determinations

were observed, respectively, with 2-, 4-, and 6-min PUV treatments. The maximum treatment time in this study was limited to no longer than 6 min, or else the sample would become opaque. After treatment and centrifugation, the treated samples were reevaluated for their protein concentrations.

**SDS-PAGE of PUV-treated soy extracts.** SDS-PAGE profiles of soy proteins labeled as glycinin (11S) (50-72 kDa) and β-conglycinin (7S) (12-34 kDa) are shown in Figure 2. These proteins are known as major allergenic proteins in soybeans as described in two recent studies (Amnuaycheewa & de Mejia, 2009, Krishnan et al., 2009). Based on these studies, it appears that almost all proteins in soybean are allergens. However, Holzhauser et al. (2009) has reported that glycinin and β-conglycinin are the only potential diagnostic markers for severe allergic reactions to soy in Europe.

When exposed to PUV radiation, some of the glycinin (50-72 kDa) and β-conglycinin (12-34 kDa) in the soy extract precipitated. This was evidenced by the decrease in protein bands with treatment time (2, 4 and 6 min) in SDS-PAGE (Figure 2). At 2-min after PUV exposure, a slight reduction in protein bands was seen compared to the control. As the treatment time increased to 4 min, more reduction in protein bands was observed. In this case, protein bands corresponding to 14-34 kDa (glycinin) and 50 kDa (β-conglycinin) were markedly reduced. By contrast, bands corresponding to 45 kDa (glycinin) and 68-75 kDa (β-conglycinin) were only slightly reduced, indicating that these proteins were more stable than others to PUV treatment. Heat may be a factor contributing to this difference in the stability of soy proteins of various sizes. Indeed, although PUV is a non-thermal process, it generates energy that is converted to heat when absorbed by the sample. As a result, proteins in the sample may or may not be stable. In one study (Herrero et al., 2009), heat has been shown to contribute to the unfolding of protein which may lead to aggregation. In the current study, the observed final temperatures for PUV-treated samples at 2-, 4-, 6-min were, respectively, 73 °C, 76 °C and 85 °C (Table 1).
Figure 2. SDS-PAGE of PUV-treated and untreated raw extracts of soybean, *glycine max*. M = protein standard. Samples were treated at the time indicated (0, 2, 4, and 6 min).

Although β-conglycinin denatures at 74 °C (German et al., 1982, Renkema, et al., 2000), several studies indicated that β-conglycinin remained soluble at that temperature because it formed soluble complexes with glycinin, thus stabilizing and preventing the precipitation of β-conglycinin (German et al., 1982, Yamagishi et al., 1983). This phenomenon may explain why some of the proteins described above were still seen and stable to PUV-treatment. Another possibility is that the secondary structure of some of the proteins did not change, thus making the proteins remain soluble. Herrero et al. (2009) has reported that the secondary structure of soy proteins did not change when heated at various temperatures. On the other hand, heating soymilk is reported to result in a reduction of profilin (Gly m3, 14 kDa) (Amnuaycheewa & de Mejia, 2009). This is in agreement with our finding of a 14-kDa protein disappearing at 4-min (Figure 2). In addition to the 14-kDa protein, proteins of 28-34 kDa, 50 kDa, 85-150 kDa were precipitated or reduced by PUV. This probably was due to the exposure of hydrophobic and aliphatic side chains which could lead to protein-protein interactions or aggregations (Herrero et al., 2009). This phenomenon (protein disappearance or
precipitation) was also observed in peanut extracts and liquid peanut butter treated with PUV (Chung et al., 2008).

Besides the disappearance of protein bands, a band of larger molecular weight or macro-aggregate (between 150 and 250 kDa) was observed at 4-min of treatment (Figure 2). According to Mills et al. (2001), soluble macro-aggregates are formed due to heating at a certain protein concentration. For instance, with β-conglycinin at a concentration higher than 0.4% (w/v), soluble macro-aggregates were formed, following heating to 100 °C (Mills et al., 2001). Similar results were observed by Nagano et al. (1992) who detected soluble macro-aggregates while heating a 10% 7S globulin solution. In this study, macro-aggregate was formed probably due to a concentration change caused by moisture loss during PUV treatment.

Additionally, more proteins disappeared at 6 min than at 4 min after PUV treatment (Figure 2). This was because more insoluble aggregates were formed at 6 min, which probably was due to higher energy/heat causing more protein-protein interactions (Herrero et al., 2009). The result suggests that a 6-min or longer PUV-exposure time was not ideal for the sample size tested in this study. This assumption was supported by further observation that the sample was gelatinized after a 10-min PUV exposure. Therefore, it was recommended that the PUV treatment time be limited to 4 min. A 6-min PUV treatment was not preferred because a large amount of proteins were lost during the process, and as a result, a loss of soy flavors and its nutritional value was anticipated.

Allergenic capacity or IgE binding of soy extracts as affected by PUV. Since PUV reduced levels of some of the soluble allergens in the soy extracts (Figure 2), the allergenic capacity or IgE binding of the PUV-treated extracts possibly would decline. To verify our postulation, IgE binding of treated and untreated extracts at various times (0-6 min) were analyzed in ELISA. Data showed that PUV treatment at different times led to a reduction in IgE binding (Figure 3). Approximately 20%, 44% and 50% reductions in absorbance values at 2, 4, and 6 min, respectively, were observed (the latter two were not significantly different (p < 0.05) from each other). Analyses with a normal serum showed no difference between the treated and untreated. This indicates that the data obtained by the pooled serum from soybean-allergic individuals was not due to non-specific binding. Also, the data correlated with the reduction in soy allergens shown in Figure 2.

Although a lower IgE binding was observed at 4 min (compared to 2 min) (Figure 3), the reduction in IgE binding was not as extensive as anticipated. This suggests that proteins that remained soluble after 4-min PUV treatment still possessed some IgE-binding activity. One of these soluble proteins was probably the aggregates (~250 kDa) that formed after PUV treatment (Figure 2). These aggregates possibly originated from the allergenic soy proteins (glycinin and β-
conglycinin) with some of their IgE-binding sites masked by the aggregation effect. Chung et al. (2005) has demonstrated that masking of IgE-binding sites due to cross-linking between peanut allergens was responsible for the reduction of allergenic capacity of peanut extracts treated with polyphenol oxidase which induced the cross-linking reaction.

Figure 3. IgE binding (expressed as A_{620}) of PUV-treated and untreated raw soy extracts in indirect ELISA. A pool of plasma from soybean-allergic patients and a normal plasma were each incubated in a plate coated with equal amounts of proteins (10 μg/ml) from PUV-treated and untreated samples. Goat anti-human IgE peroxidase conjugate, and a colored substrate were used as the detecting agents. Data represents mean of three determinations. Data with same letters are not statistically different from each other (p < 0.05).

Additionally, IgE binding from PUV treatment at 6-min appeared to be not significantly different from that at 4 min (p < 0.05) despite the former having less allergenic proteins in the extract (Figure 2). One possible reason for this phenomenon is that other soy components might exist with the ability to bind to IgE antibodies. For instance, soy components such as 2S globulins and low molecular-weight proteins (7-20 kDa) in soy lecithin and whey have been shown to bind to IgE antibodies (Burks et al., 1988, Gu et al., 2001). When these protein components were heated, their IgE-binding capacity increased (Burks et al., 1988). Such phenomenon (increase of IgE binding) possibly occurred with the PUV-treated extract at 6 min because more heat (generated by PUV) was
absorbed by the sample at 6 min, which had the highest temperature (Table 1). All this possibly explains why IgE binding at 6-min was not so much different from that at 4-min.

Another possible reason for the little change in IgE binding at 6 min (compared to 4 min) is probably because other chemical components such as Maillard reaction adducts (MRAs) were involved. MRAs, which are protein-bound byproducts from reactions between reducing sugars and proteins during heating, have been linked to the enhancement of IgE binding to allergens from peanuts that have been roasted (Chung & Champagne, 1999, Chung & Champagne, 2001, Maleki et al., 2000). As soybean contains various carbohydrates (Bainy et al., 2008), MRAs were likely to occur during PUV treatment, especially at 6-min which had the highest temperature (Table 1), and the end result was an increase of IgE-binding which compensated the loss due to a reduction of soy allergens. In one study (van de Lagemaat et al., 2007), MRAs have been shown to reduce rather than enhance the antigenicity of soy proteins. This is contradictory to our assumption (i.e., MRAs enhanced allergenicity) probably because of variations in experimental conditions and crops. For instance, some studies showed that MRAs were linked to an increase in IgE binding of roasted peanuts (Chung & Champagne, 2001, Maleki et al., 2000, Nakamura et al., 2005), while others showed a decrease (Taheri-Kafrani et al., 2009) or no difference in IgE binding (Mondoulet et al., 2005). In summary, soy components such as whey proteins (7-20 kDa) and protein-bound MRAs were thought to be factors that contributed to IgE-binding at 6 min with no difference from that at 4-min.

Overall, the data suggests that 4 min was the optimal PUV-treatment time to reduce the allergenic potency of the soy extract. The 6-min treatment time was not preferred because most of the proteins disappeared, resulting in a loss of proteins, flavors and nutritional value. Compared to the conventional heat treatment (90 °C) for 5 h (van de Lagemaat et al., 2007), PUV required only 4 min to reduce the allergenic potency of the soy extract. This difference in treatment time indicates the efficiency of PUV over conventional heating method in reducing the allergenic potency of soy extracts.

Potential applications. If proven by clinical studies, the research may lead to the development of less allergenic soy drinks or products. However, there still remain several major questions and concerns, most prominently regarding whether the less allergenic soy drinks or products will be safe and effective in people with soy allergy. Therefore, this would be not ready for general use for many years until the allergy problem is better understood.
Conclusions

PUV treatments (2, 4, and 6 min) led to an increase in sample temperature and a decrease in the levels of soy allergens (i.e., glycinin and β-conglycinin) in soy extracts. Allergens were reduced the most at 4-6 min probably through aggregation, and aggregates thus formed could be removed by centrifugation. IgE binding or allergenic potency was reduced as well, as shown by a 44% reduction in absorbance value in ELISA at 4 min. The 4-min time was considered the optimal because a longer treatment time such as 6 min resulted in no difference in IgE binding but a greater loss of proteins and flavors. The finding indicated that PUV was capable of reducing the allergenic potency of soy extracts. If confirmed by clinical studies, PUV may have an application in the development of possibly less allergenic soy beverages and products.

References


