

Control of Airborne and Liquid-borne Fungal and Pet Allergens Using Microwave Irradiation

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In this study, the dog, cat allergens (Can f 1 and Fel d 1) and fungal allergens (Alt a 1 and Asp f 1) were aerosolized and exposed to the microwave irradiation (2450 MHz) at different output powers for up to 2 min. The allergen bioaerosols were collected by a BioSampler, and analyzed using enzyme-linked immunosorbent assay (ELISA). Control and microwave-irradiated Asp f 1 allergens were also tested with IgEs in human blood sera samples.

For airborne Asp f 1 and Alt a 1 allergens, the allergenicity was shown to decrease about 50% when exposed to microwave irradiation at 385 and 119 W and relatively no change at 700 W. For airborne Can f 1 allergen, the allergenicity was shown to increase about 70% when exposed to the irradiation at 385 W, but remained relatively unchanged at 700 and 119 W. In contrast, airborne Fel d 1 allergen was observed to lose allergenicity completely at 700 W, and retained about 40% and 80% at 385 and 119 W, respectively. Radioallergosorbent (RAST) tests showed that changes detected in IgE levels in human blood sera mixtures were not statistically significant for the control and microwave-irradiated waterborne Asp f 1 allergens. This study implies that although certain allergenicity reductions were observed for some allergens in certain cases, particular care should be taken when the microwave irradiation is used to disinfect food, water, and air because of its complex effects.

Keywords microwave irradiation, airborne allergens, waterborne allergens, allergenicity, IgE

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INTRODUCTION

Asthma problems are common all over the world and continue to be one of the major human allergic diseases.⁽¹⁾ For example, in 2008 there were 19,462,632 adults and 5,058,373 children with self-reported asthma in the United States alone.⁽²⁾ Among other environmental stimuli, exposure

to allergens is shown to play important roles in development of such illness. For those allergens, cat (Fel d 1), dog (Can f 1), and fungal allergens such as Asp f 1 and Alt a 1 were widely investigated in allergic diseases.^(3–11) It was shown that the sensitization to cat and dog allergens was strongly associated with asthma.⁽³⁾

In a recent study, it was demonstrated that a patient's asthma control improved after a monthly washing regimen of the walls contaminated with mold.⁽¹²⁾ In another study, it was shown that there was a link between the *Alternaria* sensitization and allergic rhinitis, which is possibly attributable to the deposition of *Alternaria* in the upper airways.⁽¹³⁾ While extensive data regarding allergen levels in dust samples exist, various levels of such allergens both naturally occurring and/or resuspended from the dust were also found in the air.^(14–19) Such evidence in both sensitization and development of allergic diseases such as asthma and rhinitis as a result of fungal and pet allergens exposure are increasing.^(3,20–25) Accordingly, to protect humans from the exposure, it is important to develop allergen control technology.

Over the past decades, a variety of measures were investigated to reduce the allergen levels. Traditionally, chemical treatment is applied. A study was conducted to chemically treat carpets using tannic acid, a protein-denaturing agent, which resulted in significant reductions of allergen levels including Der p 1, Der f 1, and Fel d 1.⁽²⁶⁾ Similar results were obtained in another study using a solution containing tannic acid and an acaricide to spray the indoor environment.⁽²⁷⁾ Benzyl benzoate, another popular chemical, was also applied to controlling the exposure to indoor allergens.^(28,29)

However, despite their success, the chemical treatments introduce additional environmental pollutants. In the meantime, there are some physical measures, such as washing bedding using cold or hot water, covering mattresses and pillows with allergen-impermeable covers, or vacuuming regularly. These have been shown to be effective in reducing allergen levels.^(30–33) Apart from the methods mentioned above, the high efficiency particulate air (HEPA) cleaner was also utilized to control indoor airborne allergens with significant reduction of aeroallergen levels.⁽³⁴⁾ However, these physical treatments

only remove aeroallergens from the air, but do not degrade and permanently diminish them.

Microwave irradiation is widely applied to treating biological agents in food and water and on surfaces.^(35–37) In a study, microwave irradiation was also applied to treating the food allergen gliadin under different combinations of exposure time (1, 2, 3, and 5 min) and different output power (70, 200, and 500 W).⁽³⁸⁾ They have shown that a significant increase in reactivity, almost 210% over the untreated control sample, was observed for gliadins exposed to the energy dose of 40 kJ.⁽³⁸⁾ But, gliadins treated with higher energy doses showed a drop in an immune response independent of monoclonal or polyclonal antibodies used in ELISA.⁽³⁸⁾

In another study, it was indicated that continuous-wave radio-frequency with conditions (2450 MHz, 7 h/day, 30 days, 0.16 W/kg whole-body specific absorption rates) had no influence on several immune and degenerative parameters or on prenatal development.⁽³⁹⁾ However, some studies showed that that microwave radiation from mobile phones significantly increased allergen-specific IgE production.^(40–42) These investigations of microwave irradiation are limited to only surface-borne allergen contents or immunological responses.

Recently, microwave irradiation was used to directly inactivate airborne bacteria and fungi,^(43,44) and the results indicated that microwave irradiation of less than 2 min at power level of 700 W achieved up to 70% of inactivation of airborne hardy species of *Bacillus subtilis* var niger and more than 90% for *Pseudomonas fluorescens* and *Aspergillus versicolor*.⁽⁴³⁾ Scanning electron images showed that microwave irradiation resulted in significant alternations and damages to the microbial cells.⁽⁴³⁾ In addition, microwave irradiation coupled with filtration was also applied to inactivating airborne viruses and bacteria, and significant inactivations were observed.^(45,46)

However, to the best of knowledge, microwave irradiation has not been utilized in treating airborne allergens. In this study, Asp f 1, Alt a 1, Can f 1, and Fel d 1 allergens were aerosolized and exposed to microwave irradiation (2450 MHz) at three different output powers (700, 385, and 119 W) for up to 2 min. For comparison, waterborne allergens were also exposed to the microwave irradiation at similar power levels for 3 min, and further tested with human blood sera samples. The effects of the microwave irradiation on the allergenicity of allergens and the reaction of treated allergens with IgE in human sera samples were investigated using both enzyme linked immunosorbent assay (ELISA) and a RAST method. The information provided here might help guide the development of microwave-based technology in minimizing allergen exposures.

MATERIALS AND METHODS

Allergen Sampler

In this study, we used the BioSampler (SKC Inc., Eighty-Four, Pa.) to sample airborne allergens. The BioSampler, collecting bioaerosols directly into liquids through both impaction and centrifugation forces, has been widely used as a standard

liquid sampler for bioaerosol sampling. In this study, 20 ml deionized (DI) water (Millipore) and a sampling flow rate of 12.5 L/min (an optimal flow rate) (SKC, Inc.) were used for the BioSampler in airborne allergen sampling. The flow rate for the sampler was calibrated using a mini-Buck calibrator (A.P. Buck, Inc., Orlando, Fla.). For the BioSampler, it is suggested that the sampling time be limited to 15–30 min, above which the collection efficiency decreases.⁽⁴⁷⁾ Accordingly, 20 min sampling time was used in this study.

Allergens Used

In this study, cat allergens (Fel d 1), dog allergens (Can f 1), *Alternaria alternata* allergens (Alt a 1), and *Aspergillus fumigatus* allergens (Asp f 1) were used for the experiments. Fel d 1, which is the primary allergen associated with cats can be detected in environments where cats are even not present in certain environments such as schools and public transportation.^(48–52) Dog allergen Can f 1 was also found universally present in US homes, even those without pets because of the transportability of these allergens on clothing.⁽⁵³⁾

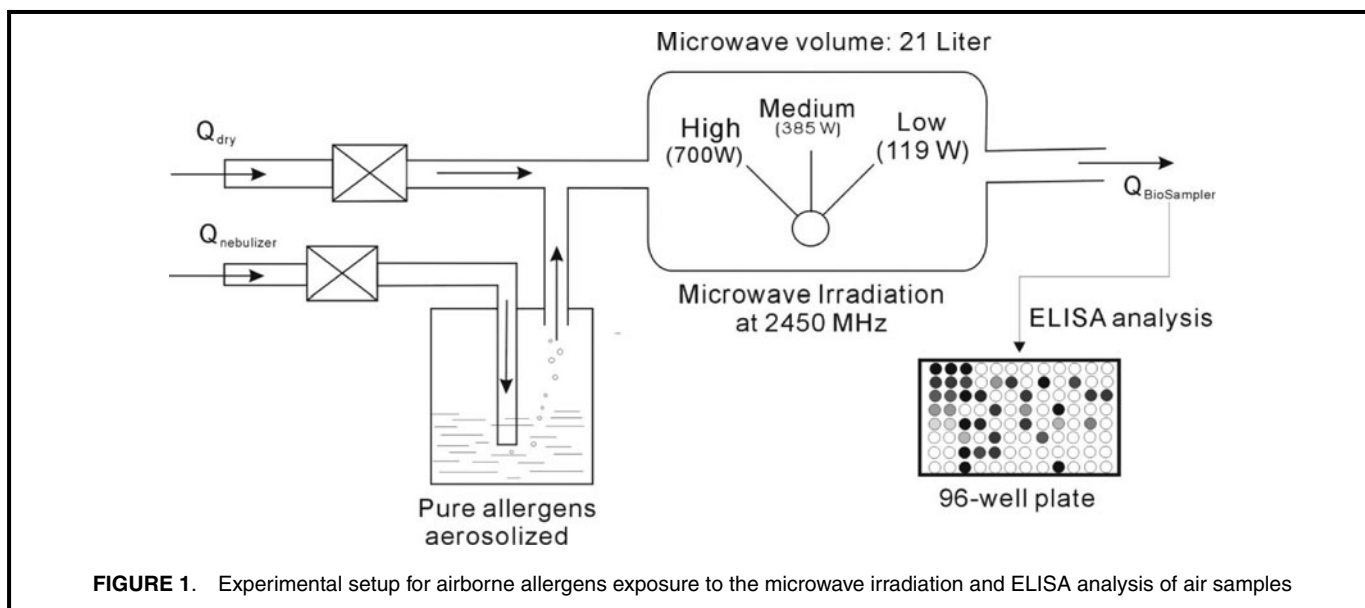
Aspergillus and *Alternaria* are two of the most frequently occurring fungal genera that are recognized as being associated with allergy.^(25,54) Asp f 1, a major 18-kDa *Aspergillus fumigatus* allergen, a member of the mitogillin family of cytotoxins, is thought to be a virulence factor for *A. fumigatus*.⁽⁷⁾ Alt a 1 and Asp f 1 have been described in the literature as the major allergens of *A. alternata* and *A. fumigatus*, respectively, and more than 80% of patients sensitized to the fungi were diagnosed to have IgE antibody to these allergens.^(7,11,55,56) In this study, for these two fungal allergens, standards from the ELISA kit (Indoor Biotechnologies Inc., Charlottesville, Virginia), were used for the experiments.

Experimental Setup and Procedures

Exposure of airborne allergens to microwave irradiation

The schematic representation of the experimental setup for exposing airborne allergens (Fel d 1, Can f 1, Asp f 1, and Alt a 1) to microwave irradiation is shown in Figure 1. As observed in the figure, the experimental setup is composed of three major parts: an aerosol generator (Collison nebulizer, BGI Inc., Waltham, Mass.), a modified microwave oven, and a bioaerosol collector (BioSampler, SKC Inc.). A commercial microwave oven (Midea Inc., Foshan, Guangdong Province, China) was modified to operate with one bioaerosol inlet and one sampling outlet of 1.5 cm in diameter. As described in our previous study,⁽⁴³⁾ the inlet and outlet were drilled from the thin back of the microwave oven.

Before the experiments, the allergen standards (25–40 μ l) from the ELISA kits were pipetted into the aerosolization glass vessel (Collison Nebulizer) containing about 20 ml sterilized DI water (Millipore). Similar to our previous work,⁽⁴³⁾ the allergens suspended in water were aerosolized by the Collison Nebulizer which was operated at a flow rate of $Q_{\text{neb}} = 2.5$ L/min. The resulting allergen aerosols were dried and diluted by an additional pure N_2 airflow, Q_{dry} , at about 13 L/min. The allergen aerosol flow was further drawn into the modified



microwave exposure chamber, where airborne allergens were exposed to the microwave irradiation (2450 MHz) at different output powers (700, 385, and 119 W) that are pre-set on the modified microwave oven used.

In this study, the exposed and control allergen aerosols were continuously collected by a BioSampler operated at the standard flow rate of 12.5 L/min for 20 min to ensure enough air sample while avoiding the re-aerosolization problems and system instability. The microwave oven has a total volume of 21 L; therefore the airborne allergens exposure time was approximately 1.7 min. The collected airborne allergen samples were individually poured into 50-mL Corning tubes and stored at 4°C for subsequent allergenicity analyses using ELISA.

For each test allergen, the experiments with both control and exposed were independently repeated three times, and the temperatures inside the exposure chamber were measured by a meter. In our work, for medium (385W) and low power (119 W) levels, the measured temperatures were 80°C and 40°C, respectively. For high power, the meter unit was damaged due to the high heat from the microwave irradiation; thus the actual value was not obtained.

However, we estimate that the temperature should be above 100°C. And the humidity level was measured at about 38% inside the biosafety hood in which the experiments were conducted. However, the relative humidity level inside the microwave exposure chamber should be slightly higher than this value because the allergen particles were being aerosolized with water droplets. The air flow inside the microwave exposure chamber is not homogenous; thus the exposure time for a particular allergen could be very different from another.

In this work, we continuously sampled aerosolized allergens for 20 min and thus we believe that our method would be a good estimate for the exposure time of allergen particles, on average, under certain power conditions tested. Although filtration can be used together with the microwave irradiation

to achieve high efficiency, such an effort would subject allergen particles to high temperature treatment, which could shadow its non-thermal effect. Accordingly, in this work a filter was not used.

Exposure of waterborne allergens to microwave irradiation and allergen reaction with IgE in human blood sera

In this work, waterborne allergens were also exposed to microwave irradiation at three different power levels (119, 385, and 700 W). Before the exposure, 100 µl of pure allergen standards of 2 ng/ml (diluted) from the ELISA kits (Asp f 1, Alt a 1, and Fel d 1) were pipetted into the wells of a 96-well plate, and the wells were then covered with a parafilm and exposed to the microwave irradiation at three indicated power levels for 3 min. In addition, an allergen mixture of Can f 1, Asp f 1, Fel d 1, and Alt a 1 with a concentration of 2 ng/ml for each test allergen was also exposed to the microwave irradiation at three different power levels following a similar procedure already described. The allergen samples were further analyzed using standard the ELISA method. In this research, a field emission scanning electron microscope (FE-SEM, Amray 1910FE) was used to investigate the effects of microwave irradiation on allergens.

In addition, the control and microwave-irradiated pure Asp f 1 allergen samples were also tested with IgE in human blood sera samples (obtained from Jiangsu Centers for Diseases Prevention and Control, Nanjing, China) which were sensitized to Asp f 1 allergens. For the tests, 75 µl control or high-power microwave irradiated Asp f 1 allergens (0.2 ng/ml) at 700W for 3 min were incubated with 25 µl human blood sera (pre-diluted 5 times) for 1 hr. The mixtures of human blood sera and allergen samples were analyzed using a RAST method.

Analysis of allergen samples using ELISA and RAST methods

For the airborne allergen samples collected by the BioSampler, a sandwich ELISA kit (Indoor Biotechnologies, Charlottesville, Va.,) was used to quantify dog allergens (Can f 1), cat allergens (Fel d 1), *A. alternata* allergens (Alt a 1), and *Aspergillus fumigatus* allergens (Asp f 1) in the air samples collected by the BioSampler.

A standard allergen dilution series (200 to 0.5 ng/ml) was prepared in PBS-T containing 0.05% Tween 20 for each set of allergen tests. volumes of standards of 100 μ L with doubling dilutions (from the ELISA kit) in duplicate, allergen samples in triplicate, and negative controls (PBS-T containing 0.05% Tween 20) were pipetted into pre-designated wells in a 96-well microplate (NUNC, Maxisorp Cert., Rochester, N.Y.) and the manufacturer's instructions were followed for each step of the assay.

The microplate was finally placed inside a spectrophotometer, SpectraMax (Molecular Devices, Inc., Sunnyvale, Calif.), and the optical density was measured using an endpoint assay at 405 nm after 30 min color development. Sample concentrations were determined by a 4-parameter standard curves (Sigmoid Function) of optical density versus standard concentrations ($r^2 > 99\%$). The standard curve was generated by software based on the optical densities and concentrations of the standards of doubling dilutions. The allergen standards were provided with the analysis kits. The effect of the microwave irradiation, η , was measured using the following equation:

$$\eta = \frac{\text{Exposed/control allergenicity}}{C_{\text{allergen_irradiated}}/C_{\text{allergen_control}} \times 100\%}, \quad (1)$$

where $C_{\text{allergen_control}}$ is the allergen concentration in the air sample without the microwave irradiation, and $C_{\text{allergen_exposed}}$ is the allergen concentration in the air sample with the microwave irradiation.

For the mixture of blood sera and allergen (both control and microwave-irradiated) samples, Human Allergen Specific IgE ELISA Blood Assay from ALerCHEK, Inc. (Portland, Me.) was used for the analysis following the manufacturer's instructions. Human IgE levels were measured using Asp f 1 antigen coated strip in the mixture through use of monoclonal Anti-human IgE HRP conjugate and TMB/peroxide substrate (ColorburstTM Blue II). The optical density of the final solution was read using the SpectraMax (Molecular Devices) at 450 nm, and IgE levels were normalized for the mixture of human blood sera and allergen samples (control and microwave-irradiated).

STATISTICAL ANALYSIS

The experimental data were analyzed statistically by a paired t-test via SigmaPlot version 10.0 software package, and one-way ANOVA tests. P-values less than 0.05 indicated a statistically significant difference.

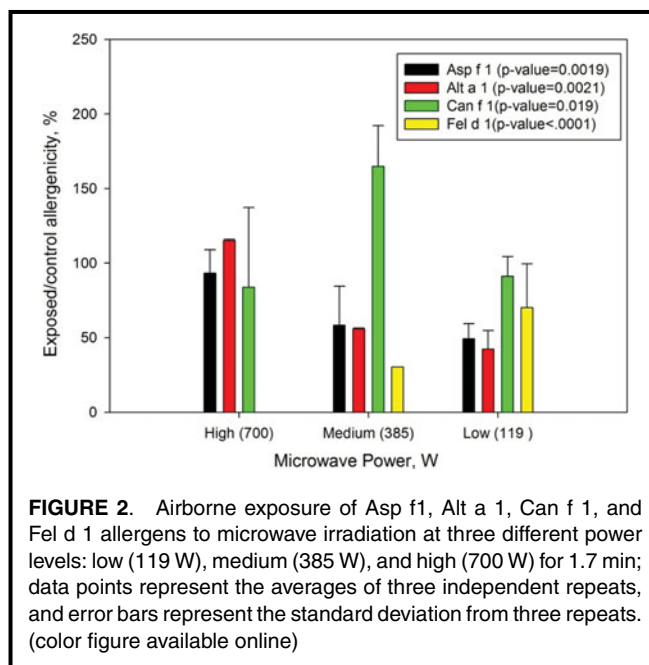


FIGURE 2. Airborne exposure of Asp f 1, Alt a 1, Can f 1, and Fel d 1 allergens to microwave irradiation at three different power levels: low (119 W), medium (385 W), and high (700 W) for 1.7 min; data points represent the averages of three independent repeats, and error bars represent the standard deviation from three repeats. (color figure available online)

RESULTS AND DISCUSSION

The effects of microwave irradiation at different power levels (119, 385, and 700W) on the allergenicity of both airborne and waterborne allergens were investigated in this study. Figure 2 shows the allergenicity change of airborne allergens Can f 1, Fel d 1, Asp f 1, and Alt a 1 when treated by the microwave irradiation at three different output powers (700, 385, and 119 W) for about 1.7 min. As observed in the figure, there were statistically significant effects of power levels of the microwave irradiation on the allergenicity regardless of the allergen aerosols tested (all p-values < 0.05). For fungal allergen aerosols (Asp f 1 and Alt a 1), it seems that the allergenicity of the allergen aerosols decreased about 50% when medium and low microwave power levels (385 and 119 W) were applied, which corresponded to 80°C and 40°C in temperature.

For medium and low power levels, the allergenicity seemed to be at similar levels. At high power level, the allergenicity of the Asp f 1 and Alt a 1 allergens appeared to not be affected. In contrast, pet allergens such as Can f 1 and Fel d 1 followed completely different patterns as observed in Figure 2. For dog allergen Can f 1, at medium power level (385 W), the allergenicity increased more than 50%, while at high and low power levels the allergenicity remained relatively unchanged. However, for cat allergen Fel d 1, the allergenicity decreased with increasing power levels as shown in the figure. At high power (700 W), Fel d 1 allergens completely lost their allergenicity, while at medium and low levels the allergenicity decreased from 25% to 75%. These results indicated that different types of airborne allergens responded differently to the microwave irradiation at different power levels. In addition to the temperature difference for different power levels, non-thermal effects should also play a role.

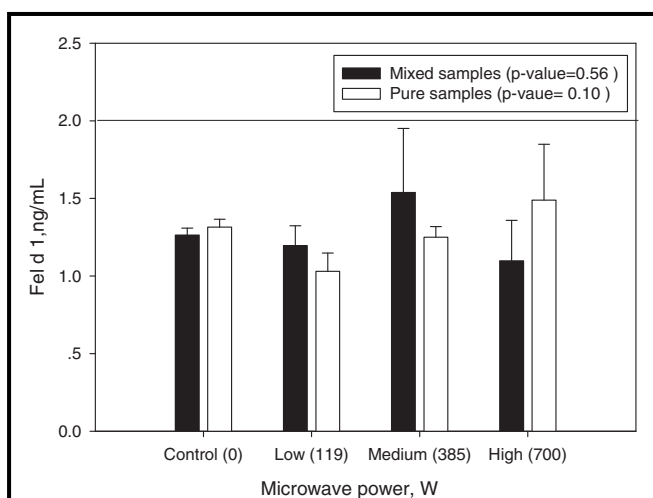


FIGURE 3. Waterborne exposure of pure Fel d 1 allergens (2.0 ng/ml) which were mixed with Alt a 1, Asp f 1, Can f 1 with the same concentration to microwave irradiation at three different power levels: low (119 W), medium (385 W), and high (700 W) for 3 min; data points represent the averages of three independent repeats, and error bars represent the standard deviation from three repeats.

In addition to the airborne exposure of the allergens, waterborne exposures to the microwave irradiation at three different power levels were also performed as a comparison. Figure 3 shows the waterborne exposure of pure Fel d 1 allergens and those mixed with Alt a 1, Asp f 1, and Can f 1 at three different power levels for 3 min—low (119 W), medium (385 W), and high (700 W). As observed in the figure, exposure of Fel d 1 allergens to the microwave irradiation did not result in allergenicity loss regardless of whether they were pure or mixed samples (p -value = 0.56 and 0.10). It seems there is a discrepancy between the labeled allergen concentration from the ELISA kit and that measured using the ELISA method here. Nonetheless, there seems to be little cross-reaction observed between Fel d 1 allergen and other allergens such as Asp f 1, Can f 1, and Alt a 1 in a waterborne state—as observed in the figure—when no microwave exposure was applied.

Comparison of the results shown in Figures 2 and 3 suggested that waterborne Fel d 1 allergens responded differently to the microwave irradiation than they did to the airborne ones. Similar to the airborne exposure experiments, both thermal and non-thermal effects could have occurred.

However, the situation was different for waterborne fungal allergens. Figure 4 shows waterborne exposure of pure Alt a 1 allergens and those mixed with Asp f 1, Fel d 1, and Can f 1 to microwave irradiation at three different power levels for 3 min: low (119 W), medium (385 W), and high (700 W). As observed in the figure, there were statistically significant effects of power levels of the microwave irradiation on the allergenicity of the Alt a 1 allergens regardless of pure and mixed samples (p -values < 0.05). In general, the allergenicity decreased with increasing power levels of microwave irradiation. At a lower power level (119W), the allergenicity of Alt a 1 remained

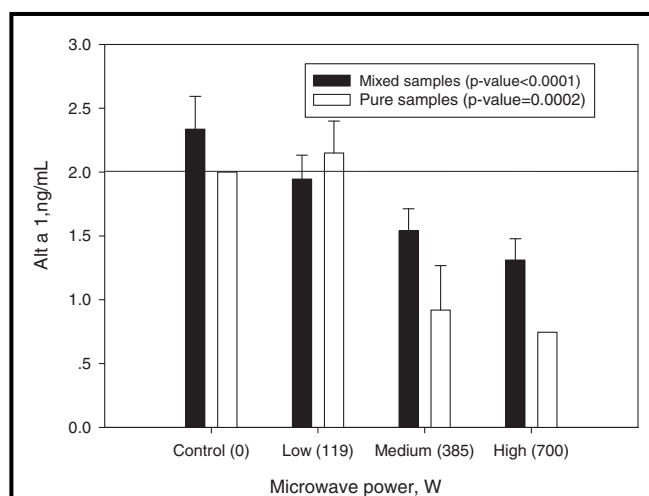


FIGURE 4. Waterborne exposure of pure Alt a 1 allergens (2.0 ng/ml) which were mixed with Asp f 1, Fel d 1, Can f 1 with the same concentration to microwave irradiation at three different power levels: low (119 W), medium (385 W), and high (700 W) for 3 min; data points represent the averages of three independent repeats, and error bars represent the standard deviation from three repeats.

relatively unchanged, while for high and medium power levels, the Alt a 1 allergens lost more than 50% of allergenicity after 3 min exposure. As observed from the figure, it seems there were some enhancements for Alt a 1 allergens by other allergens for both control and exposed experiments, especially pronounced for high and medium levels.

Results for waterborne Asp f 1 allergens exposure are shown in Figure 5. Similar to waterborne Alt a 1 allergens, the allergenicity of Asp f 1 allergens decreased with increasing

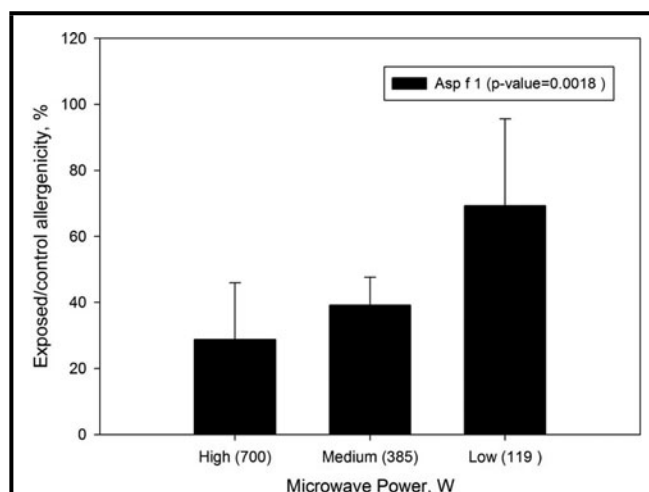


FIGURE 5. Waterborne exposure of pure Asp f 1 to microwave irradiation at three different power levels: low (119 W), medium (385 W), and high (700 W) for 3 min; data points represent the averages of three independent repeats, and error bars represent the standard deviation from three repeats.

Liquid-borne Asp f 1 exposure to microwave irradiation

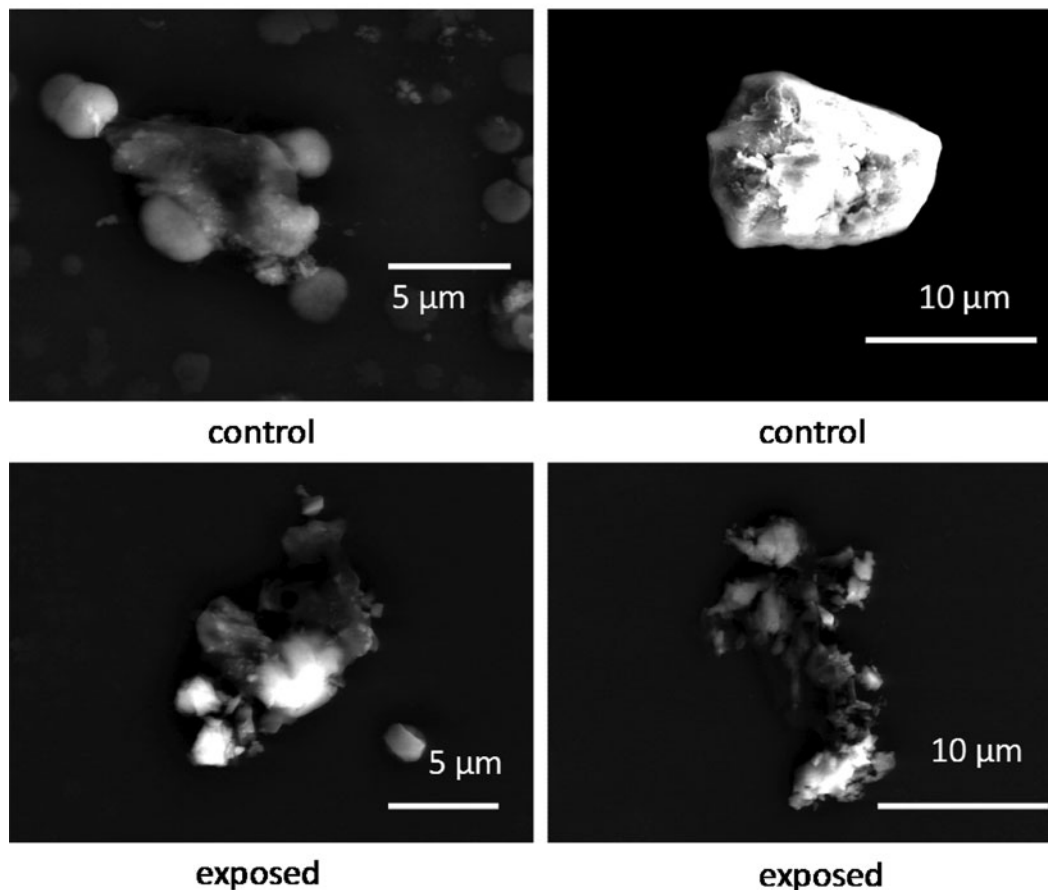


FIGURE 6. Scanning electron microscopy (SEM) images of control and microwave-irradiated Asp f 1 allergens at the power level of 700 W for 3 min.

power levels of the microwave irradiation as observed in the figure. At medium and high power levels (385 and 700 W), the Asp f 1 allergens lost more than 50% of their allergenicity, while the allergens lost about 30% of allergenicity at lower power (119W). Compared to the airborne exposure of Asp f 1 allergens, at low power levels (119 and 385 W) instead resulted in more reduction of the allergenicity than did the high power level (700W).

ANOVA analysis also indicated that there was a statistically significant effect of power levels on the allergenicity of water-borne Asp f 1 allergens (p -value = 0.0018). Figure 6 shows the SEM images of control and microwave-irradiated Asp f 1 allergens. As observed from the figure, it seems that microwave irradiation resulted in damage to the allergens, however, the exact mechanisms remain to be investigated.

Figure 7 shows the results of human blood sera tests with control and high power microwave-irradiated Asp f 1 allergens. IgE level tended to be lower in the mixture after the blood sera sample was mixed with Asp f 1 allergens without the microwave irradiation compared to those of the mixture with microwave-irradiated Asp f 1 allergens at high power (700W)

for 3 min. Those allergens without microwave irradiation could react with IgEs in blood sera, which correspondingly reduced the levels of free IgEs in the mixture.

In contrast, those microwave-irradiated Asp f 1 allergens would not be able to react with IgEs in human blood sera, thus the free IgE levels remained higher as measured using a radioallergosorbent (RAST) test. However, high variations were observed among three human blood sera sample tests as shown in Figure 7, and paired t-test showed the differences between the control and exposed experiments were not statistically significant (p -value = 0.168). This might be attributed to the sensitivity of the RAST method or because IgEs could react with multiple sites present on Asp f 1 allergens.

An allergen is defined as an antigen that induces IgE antibody synthesis in atopic patients in response to the allergen, leading to release of histamine and other pharmacological mediators of immediate hypersensitivity from mast cells and basophils.⁽⁵⁷⁾ Allergic diseases contribute major human diseases and cause significant human and economic costs^(1,2) Thus, it is imperative to develop allergen control technology. This study focused on the use of microwave irradiation at

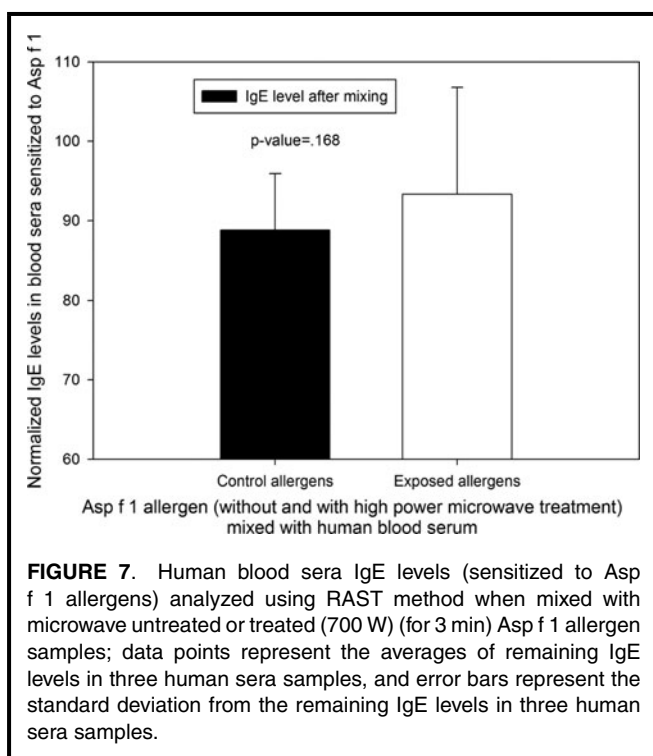


FIGURE 7. Human blood sera IgE levels (sensitized to Asp f 1 allergens) analyzed using RAST method when mixed with microwave untreated or treated (700 W) (for 3 min) Asp f 1 allergen samples; data points represent the averages of remaining IgE levels in three human sera samples, and error bars represent the standard deviation from the remaining IgE levels in three human sera samples.

different power levels to control both fungal and pet allergens commonly found in indoor environments. In general, we have shown that the microwave irradiation could affect the allergenicity of both fungal and pet allergens, and the actual changes depended on the allergen types, power levels, and allergen-residing medium (waterborne or airborne).

For fungal allergens, the allergenicity changes for both allergens (Asp f 1 and Alt a 1) followed similar patterns. However, it seems that the fungal allergens in airborne and waterborne responded very differently to the microwave exposure. This might be due to the difference in allergen-residing medium (water and air) and other unknown reaction mechanisms.

Among other fungal allergens, Asp f 1 from *A. fumigatus* is extensively studied⁽⁵⁷⁾ and it was shown that the majority of the Asp f 1 specific T-cell clones isolated from PBMCs of ABPA responded to two peptide fragments of Asp f 1 representing amino acid residues 46–65 and 106–125.^(58,59) While for Alt a 1, it was found that the allergen is the most frequently recognized allergen demonstrating IgE binding in more than 80% of asthmatic patients with allergies to *Alternaria*. Alt a 1 is a heat-stable dimer of 28 kd, which dissociates into 14.5-kd and 16-kd sub-units under reducing conditions.^(60,61) It was shown that Alt a 1 protein with two sub-units of 15 kDa each, interact with IgE antibody only when present in unreduced form, indicating the presence of conformational epitopes specific for IgE antibodies to Alt a 1.⁽⁵⁷⁾

However, information about the effects of microwave irradiation on fungal allergens is rather limited. The observed microwave effects could be attributed to the conformational or chemical changes to the epitopes present on the allergens.

Modification of the epitopes by the microwave irradiation is unknown. Previously, we have demonstrated lethal effects of microwave irradiation on the culturability of both airborne and liquid-borne bacterial and fungal species, and scanning electron images and transmission electron images showed that there were damages to both membranes and inner components of the exposed microbial cells. However, the actual mechanisms for the inactivation (thermal or non-thermal effects) remain debatable.

In this study, use of microwave-irradiated fungal allergen Asp f 1 at higher power was shown not to lead to a statistically significant increase in free IgE levels when mixed with human blood sera samples (p-value = 0.168). In contrast, the allergenicity of the Asp f 1 allergen was found to decrease with increasing power levels for 3 min exposure. The discrepancy could be due to the possibility that IgE could bind to multiple sites on allergens that were not altered upon microwave irradiation. Another possible reason, as mentioned before, could be the sensitivity of the RAST method, in which such allergenicity change could not be detected.

Nonetheless, in some studies the microwave irradiation was observed to induce immunological changes. One study demonstrated that the microwave radiation from cellular phones enhanced allergic responses in patients with atopic eczema dermatitis syndrome (AEDS), but did not do so for normal subjects.⁽⁴⁰⁾ Another study, demonstrated that the microwave irradiation could result in latex-specific IgE production but not for Japanese cedar pollen (JCP)-specific IgE productions in patients with AEDS.⁽⁴¹⁾ However, the exact mechanisms remain unknown.

In this study, for pet allergens such as cat allergens (Fel d 1) and dog allergens (Can f 1), we observed completely different allergenicity patterns upon microwave irradiation. For airborne Can f 1 allergens, microwave irradiation at medium power level (385 W) for 3 min (total 69 kJ) resulted in an increase of allergenicity by as much as 70%, while for medium and low power levels, the allergenicity remained relatively unchanged.

There was a similar finding by Leszczynska et al. (2003) when microwave irradiation was applied to control gliadin and wheat flour.⁽³⁸⁾ The same study found a significant increase in reactivity, almost 210% over the untreated control sample, for gliadins exposed to the energy dose of 40 kJ (medium power), while the immunoreactivity underwent a drop independent of monoclonal or polyclonal antibodies used in ELISA.⁽³⁸⁾ Leszczynska et al. also confirmed such an increase of immunoreactivity by immunoblotting assay with the use of sera of patients susceptible to wheat flour allergy.⁽³⁸⁾ They have hypothesized that conformational and chemical changes in gliadin structure produced by the microwave irradiation could have important consequence on their immunoreactivity.⁽³⁸⁾ Dog allergen Can f 1 was shown to be the major dog allergen, and its sequence encodes 26 amino acid signal peptide followed by a 148 amino acid protein.⁽⁶²⁾ The microwave irradiation might have had very complex interaction with the amino acid protein and the molecular structure of Can f 1 allergens.

In contrast, the allergenicity of airborne cat allergen Fel d 1 was found to decrease with increasing power levels in this study. At high level (700 W for 3 min), airborne Fel d 1 allergens have lost their allergenicity completely. However, for waterborne Fel d 1 allergens their allergenicity remained relatively unchanged regardless of the power levels applied compared to the control. It was shown that Fel d 1 is an all-helical protein and consists of eight helices, having a three-dimensional molecular structure.⁽⁶³⁾ The interaction of microwave irradiation with this three-dimensional structured protein in airborne or waterborne state could be very complex, and the exact mechanisms remained unclear.

CONCLUSIONS

Although exposure to airborne allergens via Inhalation plays a major role in allergenic diseases, adequate airborne allergen control technology is still lacking. Here, microwave irradiation was investigated for possible control of airborne allergens including major fungal (Asp f 1 and Alt a 1) and pet (Can f 1 and Fel d 1) allergens present in the environment. The results from this study indicated that the allergenicity of both airborne and waterborne allergens, when exposed to microwave irradiation, depended on the microwave output power, allergen types, and allergen-residing medium.

Microwave inactivation of airborne biological contents offers a simple, cost-effective, and non-invasive decontamination method compared to currently available air cleaning technologies.⁽⁴³⁾ Here, certain allergenicity reductions were observed for some allergens in certain cases, but particular care should be taken when the microwave irradiation was used to disinfect food, water, and air because of its complex effects.

The microwave power density used in our study was about 900 mW/cm², which is about 100 times that of a safe exposure limit 10 mW/cm² commonly described for non-thermal effect to occur. Accordingly, the developed technique might be only applicable for treating mechanically ventilated air, and cannot be applied to irradiate room air when occupants are present with the tested power density. In the future, more human blood sera sensitized to these allergens should be tested for possible clinical effects of the allergens treated by the microwave irradiation.

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