Bioscience, Biotechnology, and Biochemistry, 2021, Vol. 85, No. 12, 2442-2449

https:/doi.org/10.1093/bbb/zbab171
Advance access publication date: 7 October 2021
REGULAR PAPER

REGULAR PAPER

Preparation of hypoallergenic ovalbumin by high-temperature water treatment

Kazunobu Okon,[1] Tadashi Yoshida,[1,*] Makoto Hattori,[1] Hiroshi Matsuda,[2] and Mitsumasa Osada[3]

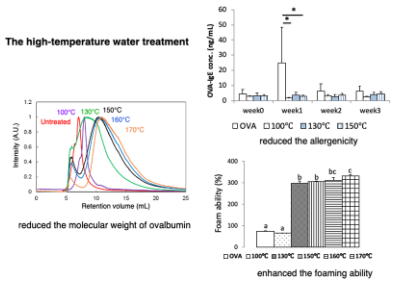
[1]Department of Applied Biological Science, Tokyo University of Agriculture and Technology, Tokyo, Japan; [2]Division of Animal Life Science, Tokyo University of Agriculture and Technology, Tokyo, Japan; and [3]Department of Chemistry and Materials, Faculty of Textile Science and Technology, Shinshu University, Ueda, Nagano, Japan

*Correspondence: Tadashi Yoshida, tyoshi@cc.tuat.ac.jp

ABSTRACT

The high-temperature water treatment is one of the methods used to reduce the molecular weight of proteins. In this study, in order to establish a practical method for preparing hypoallergenic materials using the high-temperature water treatment, we investigated the effects of processing temperature on the antigenicity and allergenicity of a food allergen. Additionally, the foaming ability of the samples was also evaluated as a function desired in the food industry. We used ovalbumin as a model allergen. As a result, although there was no significant difference among the samples treated with different processing temperatures, all the antigens treated with high-temperature water showed a decrease in antigenicity and allergenicity. In addition, when ovalbumin was treated at a temperature of 130 °C or higher, there was a significant improvement in foaming properties. These findings indicate that high-temperature water treatment is a potential strategy for preparing practical hypoallergenic materials.

Graphical Abstract



HTW treatment is a promising method for preparing practical materials with low allergenicity and high functionality.

Keywords: allergenicity, antigenicity, foam ability, HTW, IgE

Abbreviations: HTW: high-temperature water; OVA: ovalbumin; TCR: T cell receptor

Received: 7 June 2021; **Accepted:** 28 September 2021

Following a large-scale epidemiological survey in Japan, the prevalence of food allergies is estimated to be 5%-10% in infants, 5% in young children, and 4.5% in schoolchildren (Ebisawa, Ito and Fujiswa 2020). Hen's eggs, cow's milk, and wheat were all identified within the top 10 antigens. The most frequent causative food was an egg (occupies 39% of causative foods), while an egg allergy is the most common food allergy in childhood.

Although allergic diseases are not life-threatening in many cases, persistent symptoms and constant avoidance of antigens can affect the quality of life (QOL) of patients, which can have a significant negative impact on social activities (Ebisawa, Ito and Fujiswa 2020). Food allergies often develop immediately after birth, and they regularly accompany other allergic diseases throughout life, such as bronchial asthma and allergic rhinitis. Currently, the number of allergic patients is increasing rapidly, and urgent measures are required.

Previously, the standard therapy for an egg allergy is the strict avoidance of eggs (Martorell *et al.* 2013). However, the most common food allergens, including egg, are ubiquitous, so complete avoidance can generally be difficult with unintentional ingestions commonly occurring (Fleischer *et al.* 2012). Furthermore, strict antigen avoidance prevents adequate nutrition and growth throughout childhood. According to current Japanese guidelines, the patients are instructed to take in lower amounts or hypoallergenic forms of allergens (Ebisawa, Ito and Fujiswa 2020), even though such measure cannot exclude the risk of anaphylactic reactions. Therefore, the development of food materials with reduced allergenicity is in strong demand. In that case, it is important for the materials not to induce anaphylactic reactions and also not to be an allergen itself, in other words, not to have a novel allergenicity.

Acid decomposition and enzymatic treatment are widely used as the methods for reducing the molecular weight of proteins. However, these methods require some appropriate pretreatments. In addition, production of by-products is often a big problem of the methods. Therefore, a simpler method without producing any by-products is strongly required. High-temperature water (HTW) treatment is a method currently used to reduce the molecular weight of proteins, depending on the reaction temperature and time (Aida, Oshima and Smith 2017; Koh *et al.* 2019). Generally, HTW treatment accompanies high-pressure during treatment. In the previous study, it was shown that high hydrostatic pressure (HHP) treatment altered the allergen conformation, which then changed the immunoreactivity in foods. For example, the allergenicity in foods, such as rice, fish, milk, and apple was decreased by HHP treatment (Kato *et al.* 2000; Järvinen *et al.* 2001; Kleber, Maier and Hinrichs 2007; Buckow, Weiss and Knorr 2009; Liu and Xue 2010). HTW treatment, which is a type of HHP combined with heat treatment, was able to diminish the immunoreactivity more efficiently than HHP treatment in some cases, for example β-lactoglobulin in milk, whey proteins in soybeans, Ara h 2 in peanuts and walnut protein (Peñas *et al.* 2006; Long *et al.* 2016; Yang *et al.* 2017). Therefore, HTW treatment, the method that combines heating and pressurization, is more reliable to diminish allergenicity of various proteins. It is also a quite simple method that does not require the high-performance pumps that are used for the simple pressurization method. As described above, it is important for hypoallergenic materials not to induce allergy by itself (allergenicity) as well as not to be recognized by antibody that had been already induced by native allergen (antigenicity). However, the allergenicity of hypoallergenic materials has rarely been investigated. Particularly, neither the antigenicity nor allergenicity of egg proteins treated with HTW was investigated.

Moreover, it has not also been examined if the materials produced by HTW treatment are practically useful. Therefore, we aimed to establish a method for preparing a practically useful hypoallergenic egg protein by HTW treatment, focusing on antigenicity, allergenicity and foaming property. We used a murine model for food allergy to evaluate allergenicity and the emergence of a novel allergenicity of the hypoallergenic protein.

Materials and methods

Mice

Ovalbumin (OVA)-specific T cell receptor (TCR)-transgenic DO11.10 mice were obtained from Jackson Laboratory (Bar Harbor, ME, USA). The T cells of these mice recognize OVA 323-339 restricted to I-A^d. The mice produce IgE antibodies following oral ingestion of OVA. The TCR-transgenic mice were bred at the Tokyo University of Agriculture and Technology. All mice were maintained on irradiated food and autoclaved distilled water in our animal facility. Their offspring were used for experiments at 7-9 weeks of age. All mice were maintained and used in accordance with the guidelines for the care and use of experimental animals of Tokyo University of Agriculture and Technology. All the animal procedures were approved by animal ethics committee of Tokyo University of Agriculture and Technology (30-06, April 19th, 2019).

Preparation of antigens treated with HTW

The experiments were conducted in an 800 mL autoclave batch reactor fabricated by Toyo Koatsu Inc. (Hiroshima, Japan). The reactor has instrumentation for measuring temperature and pressure. The pressures in the reactor at 100, 130, 150, 160, and 170 °C were 0.1, 0.3, 0.5, 0.6, and 0.8 MPa, respectively. An experiment began with loading 25 g of albumin from egg (012-09885; FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) and 225 g of distilled and deionized water (225 mL at room temperature) into the reactor. The reactor was heated for 3 h, which includes a warmup time of approximately 30 min. After 3 h of treatment time, the reactor was cooled by turning off the heater. The cooling time below 50 °C was approximately 2 h. The samples were used for the following experiments after lyophilization, except for molecular weight analysis.

Treatment at lower temperature was also conducted. 10% OVA solution was added to 50 mL tube and heated in a hot water bath at 80 °C for 3 h. The sample was used for the following experiments after lyophilization.

Measurement of molecular weight of OVA samples

A high-performance (HP) size exclusion chromatography (SEC) system with ultraviolet (UV), refractive index (RI) and light-scattering detection (Malvern Panalytical, Ltd., Malvern, UK) was employed to determine the molecular mass distribution of the OVA samples. The eluent was 1/15 molar (M) pH 7.0 phosphate buffer system containing 2 M Urea and 0.1 M Na_2SO_4. The samples after HTW treatment were diluted with the elution at a concentration of 1.5 mg/mL. The sample solutions and the eluent were filtered through 0.45 µm polytetrafluoroethylene disposable membrane filters prior to SEC analysis. The Shodex PROTEIN KW802.5 (8.0 mm ID × 300 mm, Showa Denko K.K., Tokyo, Japan) and PROTEIN KW-G columns were used at a flow rate of

0.5 mL/min. The temperature of the columns was maintained at 30 °C and the injection volume was 100 µL. A 670 nm incident light source was used for light scattering detection at 7° and 90°. Data acquisition and processing to calculate the weight-average molecular mass was performed with OmniSEC software (Malvern Panalytical, Ltd.).

SDS-PAGE

SDS-polyacrylamide gel electrophoresis for separating the HTW-treated OVA samples was performed according to the method of Laemmli (1970), using a discontinuous gel comprising a 4% stacking gel, 15% separating gel. Low molecular weight calibration kit (GE healthcare) was used as the molecular weight marker. OVA samples treated with HTW were diluted to a final protein concentration of 1 mg/mL in sample buffer. The gel was stained for 1 h using the rapid Coomassie Brilliant Blue R-250, and photographed after destaining.

Oral administration of antigens and preparation of sera

OVA or treated OVA samples were mixed with CE-2 at a rate of 10% and given freely to DO11.10 mice. Blood was collected weekly from the tail artery to prepare the sera. The sera were used for an antibody production assay.

ELISA

For measurement of the antigenicity of HTW-treated samples, the competitive ELISA for anti-OVA-IgM antibody was conducted using a pooled serum of OVA-immunized mice. OVA or OVA treated with HTW were prepared to various concentrations with PBS-T, mixed with the serum and shaken overnight. Meanwhile, Maxisorp immunoplates (Nunc, Roskilde, Denmark) were coated with 0.01% OVA, washed, and blocked. Following these procedures, all the samples prepared as above were added to the plates. Bound IgM antibody was detected using biotinylated antimouse IgM (BD Biosciences), before incubating with alkaline phosphatase–streptavidin (Invitrogen). The substrate (*p*-nitrophenol phosphate) was added after washing the wells, and the absorbance was determined at 405 nm.

To determine the amount of antigen-specific IgE antibody in the sera, noncompetitive ELISA was performed. Maxisorp immunoplates were coated with 0.01% OVA or OVA treated with HTW at 100 °C, and the samples and standards were added after washing and blocking the plates. A pooled serum sample was prepared by mixing a small portion of each serum obtained from mice immunized with OVA and was used as the standard for OVA-specific IgE antibody. The concentration of anti-OVA IgE antibody in the pooled serum was measured by an OVA-specific mouse IgE ELISA kit (Cayman Chemical, MI, USA). Bound IgE antibody was detected by using biotinylated antimouse IgE (BD Biosciences), before incubating with alkaline phosphatase–streptavidin. The substrate (*p*-nitrophenol phosphate) was added after washing the wells, and the absorbance was determined at 405 nm.

Foaming property

Foaming property was measured by the method of Huang *et al.* (1997) with small modification. OVA or OVA treated with HTW were dissolved to 1% w/v with 0.1 M phosphate buffer (pH7.0). A 10 mL volume of the solution was added to a 50 mL tube and mixed with a whisk for 15 s to prepare the foams. The volume of bubbles was recorded over time.

The foaming ability was defined as the ratio of the foam volume after 2 min of foaming against the original volume of the solution. The foaming stability was defined as the ratio of the foam volume after 30 min of mixing against the starting volume of the solution.

Measurement of coloring of OVA samples

OVA or OVA treated with HTW were dissolved to 1% w/v with distilled water. After centrifuging the samples (4 °C, 10 000 *g*, 5 min), the absorbance of the supernatant was determined at 450 nm.

Statistical analysis

The OVA-specific IgE antibody concentration was measured in the sera of 5 mice per group. Statistical significance was analyzed by the Steel–Dwass test. The foaming properties and coloring of OVA samples treated with HTW were conducted at least in triplicate, and analyzed by the Tukey–Kramer test. A *p*-value of less than 0.05 was considered statistically significant.

Ethics approval

All the animal procedures were approved by animal ethics committee of Tokyo University of Agriculture and Technology (30-06, April 19, 2019).

Results

The HTW treatment at a temperature of 150 °C or higher decomposed OVA, while treatment at under 130 °C hardly decomposed it

To confirm the decomposition of OVA by HTW treatment, the average molecular weight was measured using size exclusion chromatography equipped with a light scattering detector (Figure 1a). The average molecular weight of untreated OVA was 40 000. The average molecular weights of OVA treated by HTW at 100, 130, 150, 160, and 170 °C were 37 000, 34 000, 4800, 4700, and 3500, respectively. In the samples treated at 100 and 130 °C, the molecular weight did not decrease remarkably from the native OVA, suggesting that the decomposition did not proceed sufficiently. Conversely, it is suggested that the decomposition proceeded in the OVA samples treated at temperature of 150 °C or higher temperature. As a result of SDS-PAGE (Figure 1b), the sample treated at 100 °C showed a band similar to that of the native OVA. In the sample treated at 130 °C, the staining was spread and any specific bands were not observed. These results were consistent with Figure 1a. Meanwhile, at 150 °C or higher temperature, the staining bands were vague and shifted to low molecular weight. This result was also consistent with Figure 1a.

HTW-treated OVA has reduced antigenicity

We next performed the competitive ELISA method to confirm the antigenicity of OVA samples treated with HTW (Figure 2). Comparing the IC_{50} value, a decrease in antigenicity was confirmed in all treated samples. However, no differences were identified among the samples treated at different temperature.

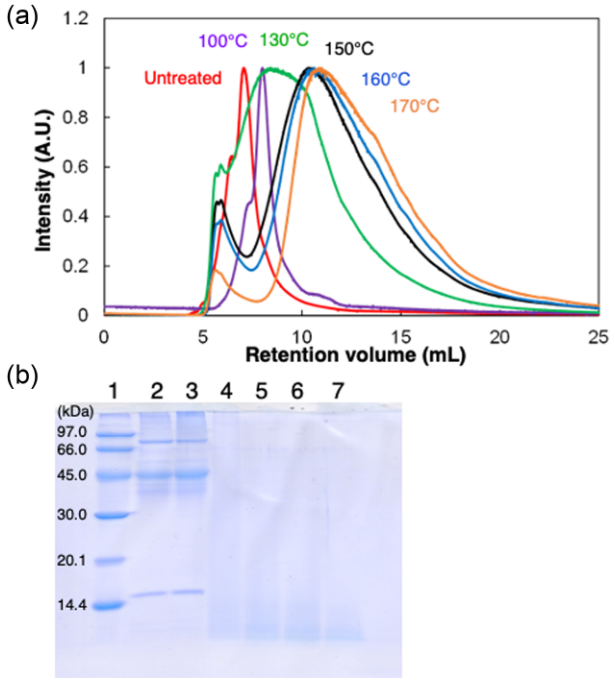


Figure 1. Effects of HTW treatment on the molecular weight distribution. (a) The relationship between the elution volume and elution pattern obtained by UV detector at a wavelength of 210 nm. (b) SDS-PAGE was performed for separating the HTW treated OVA. Lane (1) molecular weight marker, (2) untreated OVA, (3) HTW treatment at 100 °C, (4) at 130 °C, (5) at 150 °C, (6) at 160 °C, and (7) at 170 °C.

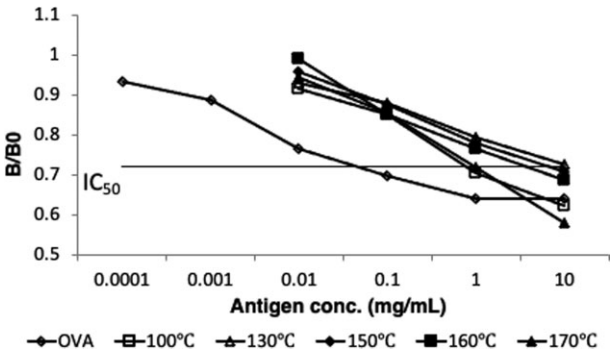


Figure 2. Effects of HTW treatment on antigenicity. Antigenicity was measured by the competitive ELISA method. Each HTW-treated OVA sample was mixed with the serum of DO11.10 mice immunized with OVA as a competing antigen. These samples were added to OVA-coated plates and OVA-specific IgM antibodies bound to the coated antigen were measured. The IC_{50} value shows a concentration indicating that the binding of the antibody was inhibited by 50%.

Oral administration of HTW-treated OVA did not induce OVA-specific IgE production in DO11.10 mice

DO11.10 mice on a diet containing 10% OVA presented an increase in the OVA-specific IgE antibody concentration (Figure 3a), indicating that an allergy was induced. Then, IgE antibody production in the mice was subsequently inhibited because of the induction of oral desensitization. In contrast, a remarkable reduction in antibody production was confirmed in mice fed with the antigens treated with HTW regardless of the treatment temperature (Figure 3a). We further checked IgE production specific to the administered antigen using the sera from mice fed OVA treated with HTW at 100 °C. The result showed that the administration of OVA treated with HTW at 100 °C did not induce native OVA-specific IgE and also did not induce treated-OVA-specific IgE (Figure 3b). These data indicate that the allergenicity is reduced by the HTW-treatment without generating any novel allergenicity.

In addition, we checked OVA-specific IgE production in mice fed with OVA heated at 80 °C. OVA-specific IgE production in mice given OVA treated with HTW at 100 °C was significantly suppressed compared to untreated OVA, while that in mice fed with OVA heated at 80 °C did not significantly change although a tendency to decrease was observed (Figure 3c).

Foaming properties of OVA treated with HTW at temperature higher than 130 °C were significantly increased

We used a foamer to whisk the HTW-treated OVA solution (1% w/v) for the measurement of foam ability. The samples treated at temperature of 130 °C or higher showed a significant increase in the volume of foams compared to those of the untreated OVA (Figure 4a), while the foams prepared from the OVA sample treated at 100 °C did not change. The foam volumes 30 min after the foam preparation were also significantly higher in the foams of the OVA samples treated at 130 °C or higher than the native OVA samples (Figure 4b and c).

The HTW-treated OVA color altered as the processing temperature was increased

The color of the HTW-treated OVA powders was brown. We confirmed the degree of coloration using the HTW-treated OVA solution (1% w/v). The color of the HTW-treated OVA became darker as the treatment temperature increased (Figure 5).

Discussion

The aim of this study was to create practical food materials with hypo-allergenicity using the HTW treatment. Our results demonstrated that we successfully prepared food materials with low antigenicity, low allergenicity and high foaming properties following HTW treatment under certain conditions.

HTW treatment is a method employed to reduce the molecular weight of various proteins, while the degree of decomposition can be controlled by the processing temperature. The results of the molecular weight analysis showed that the OVA samples treated at 100 and 130 °C had almost the same molecular weight as the native OVA. The results of SDS-PAGE suggested that the sample treated at 100 °C was hardly decomposed, while the sample treated at 130 °C had a broad range of molecular weight even though the average molecular weight of it was similar with the native OVA as described above. Contrastingly, OVA treated at 150 °C or higher had lower molecular weights, indicating that HTW treatment must occur at temperature of 150 °C or higher in order to complete the effective decomposition of OVA.

Our results illustrate that following treatment at 100 and 130 °C, the antigenicity of OVA reduces by a similar level to those treated at higher temperatures, although decomposition was hardly observed in the samples. The HTW treatment would have denatured OVA and caused changes in the conformation. Actually, the solubility appeared lower in the samples. It is suggested that the three-dimensional structure of the samples changed during the denaturation, the hydrophobic amino acids buried inside were exposed, and the molecules were associated through the hydrophobic residues, resulting in aggregation. Therefore, HTW treatment at 100 and 130 °C would have resulted in

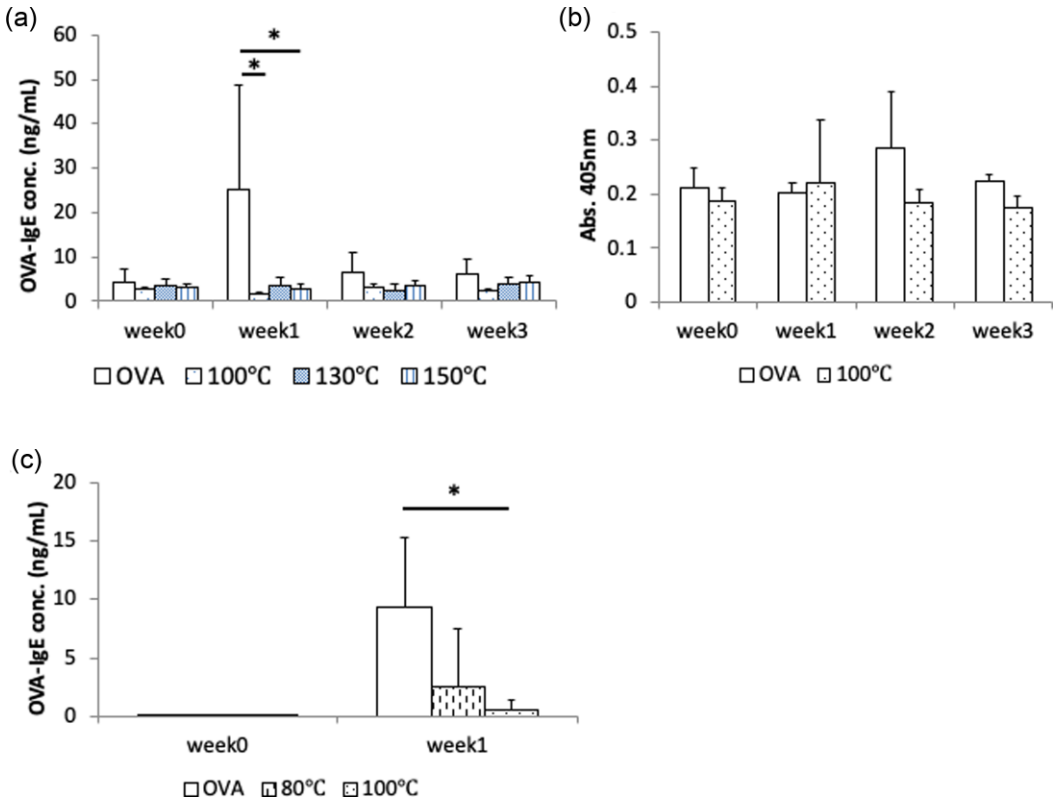


Figure 3. Effects of HTW treatment on allergenicity. DO11.10 mice were given diets containing OVA treated with HTW or untreated OVA for 3 weeks ($n = 5$/group). (a) The amount of OVA-specific IgE in the sera from mice given OVA treated with HTW was measured by ELISA. (b) The amount of OVA treated with HTW at 100 °C specific IgE in the sera from mice fed the antigen or native OVA. (c) The amount of OVA-specific IgE in the sera from mice given OVA heated at 80 °C or treated with HTW at 100 °C. Symbol "*" indicates statistical significance by the Steel–Dwass test.

significant structural changes, which reduced antigenicity due to changes in the structure of the epitope. On the other hand, it is further suggested that the antigenicity decreased due to the disappearance of the epitopes following decomposition in the samples treated at the higher temperatures. In this study, the most decomposed samples had an average molecular weight of about 4000 Da, although the epitopes of OVA have been previously reported as I53D60, V77R84, S103E108, G127T136, E275V280, G301F306, I323A332, and A375S384 in mouse (Mine and Yang. 2007). Our data demonstrate that the degradation into 4000 Da peptides is enough to invalidate these epitopes.

In addition to the antigenicity, we examined the allergenicity of the HTW-treated samples. A diminished OVA-specific IgE antibody production was confirmed in mice given the HTW-treated antigens. This is the first study to evaluate the allergenicity of HTW-treated antigens using a food allergy model. It is important for practical hypoallergenic food materials not to induce allergy as well as not to be recognized as antigens. In addition, our results demonstrated that HTW treatment did not generate any novel allergenicity (novel epitopes). The emergence of novel epitopes cannot be evaluated without using a food allergy model. The IgE antibody is produced by B cells and antigen recognition by B cell receptors (BCR) is needed to produce these antibodies. Therefore, the low allergenicity of HTW-treated OVA samples is considered to be achieved partly because of their reduced antigenicity. In addition, some of the OVA samples treated with HTW were observed to decrease in solubility due to aggregation, and it is suggested that the decrease in solubility reduces its absorption. This property might also contribute to the decrease in allergenicity.

Alternatively, it has been reported that the high-pressure treatment of egg white protein increases the sensitivity to enzymatic hydrolysis (Iametti *et al.* 1998, 1999; Van der Plancken *et al.* 2004, 2005a, 2005b, 2007). In this present study, we suggested that the structure changed remarkably following treatment at 100 and 130 °C, making the antigens more susceptible to digestion although the decomposition progressed slightly during the treatment. Therefore, it cannot be denied that digestion occurred more rapidly in those samples at the intestine than native protein and small peptides were generated capable of reducing allergenicity.

From our all findings, it is suggested that the reduction of allergenicity was not limited at some specific epitopes. In addition, any novel allergenicity was not generated by HTW treatment. The epitopes of OVA have been previously reported as L38T49, D95A102, E191V200, V243E248, and G251N260 in human (Mine and Rupa. 2003). Although the position of these epitopes was slightly different with mouse, this method can be applied to produce hypoallergenic materials for human.

Foaming property was measured as a functional property which is important in many cases when using OVA as a food material. Our results demonstrate that the foaming property is improved by HTW treatment at 130 °C or higher temperature. This result indicates that degradation of OVA enhances the foaming property. However, molecular weight analysis indicates that there is only a partial decomposition of OVA when treated at 130 °C. On the other hand, the range of molecular weight was spread, indicating that small fragments were generated. Such peptides would contribute to the improvement of foam ability. In addition, some structural change of the protein might be

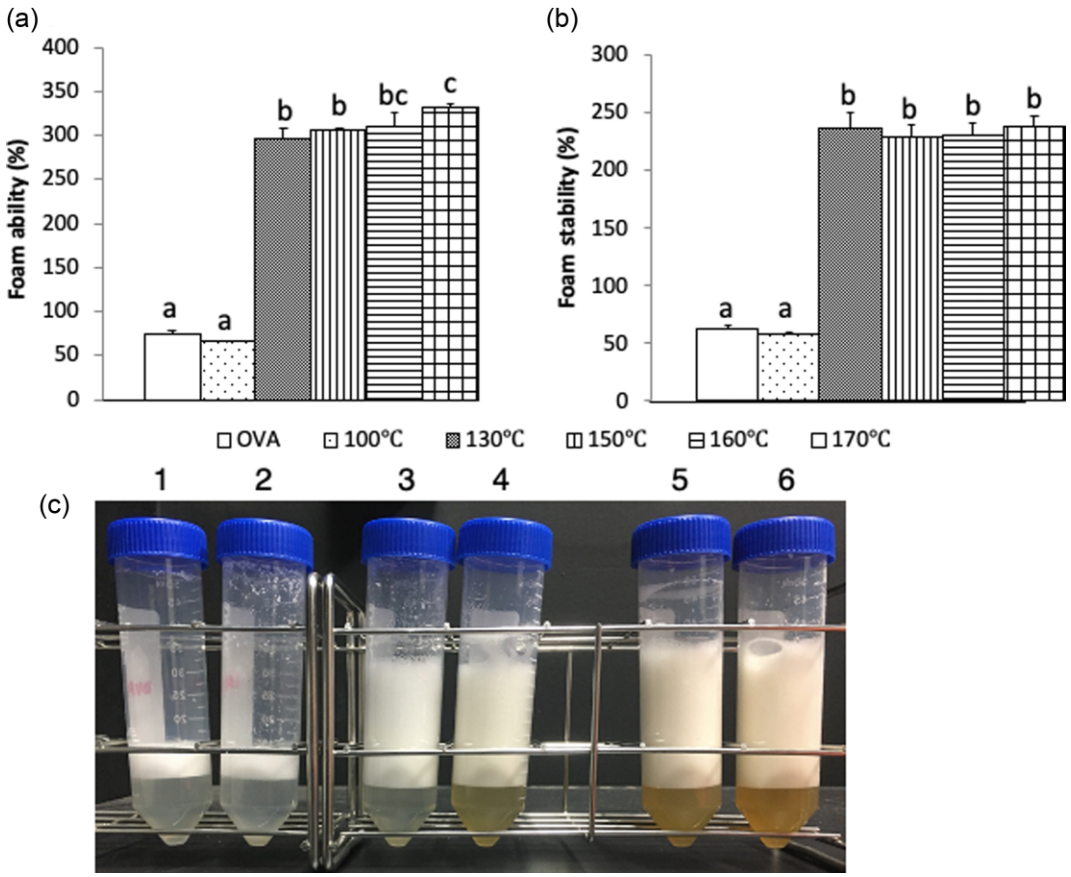


Figure 4. Effects of HTW treatment on foaming properties. Foams were prepared by mixing for 15 s with a whisk ($n = 3$/group). (a) Foam ability was shown by comparing the amount of foams 2 min after preparation against the amount of original solution. (b) Foam stability was shown by comparing the amount of foams 30 min after preparation against the amount of original solution. (c) Photos of the foams 30 min after preparation of (1) untreated OVA, (2) HTW treatment at 100 °C, (3) at 130 °C, (4) at 150 °C, (5) at 160 °C, and (6) at 170 °C were taken. Different characters indicate statistical significance calculated by the Tukey–Kramer test.

involved in the improvement. Indeed, Hagolle *et al.* (2000) also observed an increase in foam ability after preheating an OVA and lysozyme solution. Furthermore, Cabanillas *et al.* (2014) reported that protein flexibility increased after the walnut protein was autoclaved. Taken in conjunction with these data, it is considered that the significantly improved foaming property observed in this study was partly due to the increased flexibility of the protein, which occurred following denaturation during the treatment at 130 °C. Conversely, the treatment at 100 °C did not enhance the foaming property, which is likely because, the effects of the improvement in flexibility, alongside the decrease in hydrophobicity by the treatment, offset each other in the OVA sample treated at 100 °C.

Heat treatment is useful because it does not require any pretreatments which are sometimes required for enzyme treatment and any other reagents, in addition to producing no by-product. For example, the reduction of OVA was required for effective digestion with trypsin, and the OVA samples prepared by acid treatment included a lot of salt generated by neutralization done for stopping the reaction. It has been reported that treatment by heating alone also changed the structure of the proteins and reduces antigenicity to some extent. Indeed, Watanabe *et al.* (2014) reported that partial heat treatment of egg white could reduce the allergenicity of OVA. However, Stănciuc *et al.* (2016) reported that 90% of the antigenicity remained and only partial unfolding was confirmed in the tertiary structure after treating OVA at 100 °C for 20 min. These results suggest that heat treatment alone can induce only partial structural changes of proteins, which would be insufficient to reduce antigenicity in some cases. Indeed, this present study suggested that the allergenicity of OVA could not be sufficiently reduced by heat treatment at 80 °C. In addition, high-pressure treatment was also reported to change the immunoreactivity of proteins by changing the structure without any pretreatments and by-products. A previous study reported that an increase in turbidity, surface hydrophobicity, exposed SH content and susceptibility to enzymatic hydrolysis could be observed after treatment at least 400 MPa in egg whites (Hoppe *et al.* 2013). It follows, therefore, that in order to alter the structure of the protein sufficiently to diminish allergenicity only by high-pressure treatment, a considerably high pressure should be required. However, our present study showed that the allergenicity of OVA is completely reduced by combining the heat treatment and the high-pressure treatment. The method used in this study can reduce allergenicity with moderate pressure, being considered highly practical. Cabanillas *et al.* (2014) reported that autoclave treatment could reduce immunoreactivity in walnut proteins more efficiently than high-pressure treatment, which is consistent with the present results. Furthermore, HTW treatment can be applied to various proteins by only controlling the treatment temperature. HTW is a method that can obtain the same or better effects compared with heat treatment and high-pressure treatment without any special facilities, and can be applied for the production of various low allergenic materials.

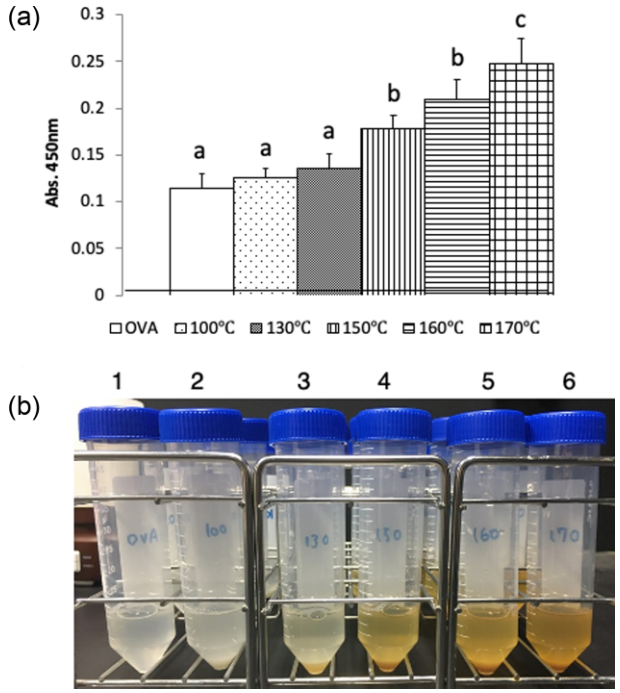


Figure 5. Effect of HTW treatment on coloring. OVA treated with HTW were dissolved in water to 1%. (a) The solution was centrifuged, and the absorbance of the supernatants was measured at 450 nm. (b) Photos of (1) untreated OVA, (2) HTW treatment at 100 °C, (3) at 130 °C, (4) at 150 °C, (5) at 160 °C, and (6) at 170 °C were taken. Different characters indicate statistical significance calculated by the Tukey–Kramer test.

The OVA samples prepared in this study by the HTW treatment at higher than 130 °C were confirmed to achieve decreased allergenicity and improved foaming property simultaneously. However, the coloration of the samples progressed depending on the treatment temperature. A previous study reported that treatment of egg white at 121 °C caused the Maillard reaction (Watanabe *et al.* 2014). It is not clear whether the coloring observed in this study was due to the Maillard reaction but it could be, because OVA reagent used in this study must contain contaminated saccharides to some extent. Considering practical use, it is certain that the coloring is more likely to occur because materials with a lower purity than the reagent used in this study should be used in the industry. The dark color of food ingredients is generally undesired. Therefore, it is considered that there is an optimum treatment temperature to produce practically useful low allergenic OVA, which would be between 100 and 130 °C. Bitterness is also a negative attribute associated with the majority of food protein hydrolysates. OVA treated between 100 and 130 °C will be hardly decomposed, and will not generate bitter peptides. Therefore, HTW treatment under a certain condition will provide a potential strategy for preparing practical hypoallergenic materials.

Data availability

The data underlying this article are available in the article and also from the corresponding author upon request.

Author contribution

T.Y. and M.O. assisted in conceptualization, data curation, and writing manuscript; K.O. and M.H. assisted in data curation and writing manuscript; and H.M. assisted in conceptualization, writing manuscript, and funding acquisition.

Funding

This work was supported, in part, by grants from JSPS (KAKENHI Grant Number: 16H06383, H.M.). The funding bodies had no involvement in the study design, collection, analysis and interpretation of data, writing of the manuscript, or decision to publish the work.

Disclosure statement

No potential conflict of interest was reported by the authors.

References

Aida MT, Oshima M, Smith LR, Jr. Controlled conversion of proteins into high-molecular-weight peptides without additives with high-temperature water and fast heating rates. *Sustainable Chem Eng* 2017;**5**:7709-15.

Buckow R, Weiss U, Knorr D. Inactivation kinetics of apple polyphenol oxidase in different pressure–temperature domains. *Innov Food Sci Emerg Technol* 2009;**10**:441-8.

Cabanillas B, Maleki SJ, Rodríguez J *et al.* Allergenic properties and differential response of walnut subjected to processing treatments. *Food Chem* 2014;**157**:141-7.

Ebisawa M, Ito K, Fujisawa T. Japanese guidelines for food allergy 2020. *Allergol Int* 2020;**69**:370-86.

Fleischer DM, Perry TT, Atkins D *et al.* Allergic reactions to foods in preschool-aged children in a prospective observational food allergy study. *Pediatrics* 2012;**130**:e25-32.

Hagolle N, Relkin P, Popineau Y *et al.* Study of the stability of egg white protein-based foams: effect of heating protein solution. *J Sci Food Agric* 2000;**80**:1245-52.

Hoppe A, Jung S, Patnaik A *et al.* Effect of high-pressure treatment on egg white protein digestibility and peptide products. *Innov Food Sci Emerg Technol* 2013;**17**:54-62.

Huang XL, Catignani GL, Swaisgood HE. Micro-scale method for determining foaming properties of protein. *J Food Sci* 1997;**62**:1028-60.

Iametti S, Donnizzelli E, Pittia P *et al.* Characterization of high-pressure-treated egg albumen. *J Agric Food Chem* 1999;**47**:3611-6.

Iametti S, Donnizzelli E, Vecchio G *et al.* Macroscopic and structural consequences of high pressure treatment of ovalbumin solutions. *J Agric Food Chem* 1998;**46**:3521-7.

Järvinen K, Chatchatee P, Bardina L *et al.* IgE and IgG binding epitopes on lactoglobulin in cow's milk allergy. *Int Arch Allergy Immunol* 2001;**126**:111-8.

Kato T, Katayama E, Matsubara S *et al.* Release of allergenic proteins from rice grains induced by high hydrostatic pressure. *J Agric Food Chem* 2000;**48**:3124-9.

Kleber N, Maier S, Hinrichs J. Antigenic response of bovine β-lactoglobulin influenced by ultra-high pressure treatment and temperature. *Innov Food Sci Emerg Technol* 2007;**8**:39-45.

Koh BB, Lee EJ *et al.* Characterization of bovine serum albumin hydrolysates prepared by subcritical water processing. *Food Chem* 2019;**278**:203-7.

Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970;**227**:680-5.

Liu R, Xue W. High-pressure treatment with silver carp (*Hypophthalmichthys molitrix*) protein and its allergic analysis. *High Press Res* 2010;**30**:438-42.

Long FY, Yang X, Wang SJ *et al*. Effects of combined high pressure and thermal treatment on the allergenic potential of peanut in a mouse model of allergy. *Innov Food Sci Emerg Technol* 2016;**35**:135-8.

Martorell A, Alonso E, Boné J *et al*. Position document: IgE-mediated allergy to egg protein. *Allergol Immunopathol* 2013;**41**:320-36.

Mine Y, Rupa P. Fine mapping and structural analysis of immunodominant IgE allergenic epitopes in chicken egg ovalbumin. *Protein Eng Des Select* 2003;**16**:747-52.

Mine Y, Yang M. Epitope characterization of ovalbumin in BALB/c mice using different entry routes. *Biochim Biophys Acta* 2007;**1774**:200-12.

Peñas E, Préstamo G, Polo F *et al*. Enzymatic proteolysis, under high pressure of soybean whey: analysis of peptides and the allergen Gly m 1 in the hydrolysates. *Food Chem* 2006;**99**:569-73.

Stănciuc N, Banu I, Turturică M *et al*. pH and heat induced structural changes of chicken ovalbumin in relation with antigenic properties. *Int J Biol Macromol* 2016;**93**:572-81.

Van der Plancken I, Delattre MI, Van Loey A *et al*. Kinetic study on the changes in the susceptibility of egg white proteins to enzymatic hydrolysis induced by heat and high hydrostatic pressure pretreatment. *J Agric Food Chem* 2004;**52**:5621-6.

Van der Plancken I, Van Loey A, Hendrickx M. Combined effect of high pressure and temperature on selected properties of egg white proteins. *Innov Food Sci Emerg Technol* 2005a;**6**:11-20.

Van der Plancken I, Van Loey A, Hendrickx M. Changes in sulfhydryl content of egg white proteins due to heat and pressure treatment. *J Agric Food Chem* 2005b;**53**:5726-33.

Van der Plancken I, Van Loey A, Hendrickx M. Kinetic study on the combined effect of high pressure and temperature on the physico-chemical properties of egg white proteins. *J Food Eng* 2007;**78**:206-16.

Watanabe H, Toda M, Sekido H *et al*. Heat treatment of egg white controls allergic symptoms and induces oral tolerance to ovalbumin in a murine model of food allergy. *Mol Nutr Food Res* 2014;**58**:394-404.

Yang X, Sun J, Tao J *et al*. The allergenic potential of walnuts treated with high pressure and heat in a mouse model of allergy. *Innov Food Sci Emerg Technol* 2017;**39**:165-70.