Preparation of hypoallergenic ovalbumin by high-temperature water treatment

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Running title: HTW treatment for preparing hypoallergenic OVA

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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.

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

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

40 **Introduction**

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

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

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

64 Acid decomposition and enzymatic treatment are widely used as the methods for

65 reducing the molecular weight of proteins. However, these methods require some
66 appropriate pretreatments. In addition, production of by-products is often a big problem
67 of the methods. Therefore, a simpler method without producing any by-products is
68 strongly required. High-temperature water (HTW) treatment is a method currently used
69 to reduce the molecular weight of proteins, depending on the reaction temperature and
70 time (Aida, Oshima and Smith 2017; Koh et al. 2019). Generally, HTW treatment
71 accompanies high-pressure during treatment. In the previous study, it was shown that high
72 hydrostatic pressure (HHP) treatment altered the allergen conformation, which then
73 changed the immunoreactivity in foods. For example, the allergenicity in foods, such as
74 rice, fish, milk and apple was decreased by HHP treatment (Kato et al. 2000; Liu and Xue
75 2010; Järvinen et al. 2001; Kleber, Maier and Hinrichs 2007; Buckow, Weiss and Knorr
76 2009). HTW treatment, which is a type of HHP combined with heat treatment, was able
77 to diminish the immunoreactivity more efficiently than HHP treatment in some cases, for
78 example β-lactoglobulin in milk, whey proteins in soybeans, Ara h 2 in peanuts and
79 walnut protein (Peñas et al. 2006; Long et al. 2016; Yang et al. 2017). Therefore, HTW
80 treatment, the method that combines heating and pressurization, is more reliable to
81 diminish allergenicity of various proteins. It is also a quite simple method that does not
82 require the high-performance pumps that are used for the simple pressurization method.
83 As described above, it is important for hypoallergenic materials not to induce allergy by
84 itself (allergenicity) as well as not to be recognized by antibody that had been already
85 induced by native allergen (antigenicity). However, the allergenicity of hypoallergenic
86 materials has rarely been investigated. Particularly, neither the antigenicity nor
87 allergenicity of egg proteins treated with HTW is investigated.

88 Moreover, it has not also been examined if the materials produced by HTW
89 treatment are practically useful. Therefore, we aimed to establish a method for preparing

90 a practically useful hypoallergenic egg protein by HTW treatment, focusing on
91 antigenicity, allergenicity and foaming property. We used a murine model for food allergy
92 to evaluate allergenicity and the emergence of a novel allergenicity of the hypoallergenic
93 protein.
94

95 **Materials and methods**

96 **Mice**

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

107

108 **Preparation of antigens treated with HTW**

109 The experiments were conducted in an 800 mL autoclave batch reactor fabricated by Toyo
110 Koatsu Inc. (Hiroshima, Japan). The reactor has instrumentation for measuring
111 temperature and pressure. An experiment began with loading 25 grams of albumin from
112 egg (012-09885; FUJIFILM Wako Pure Chemical Corp., Osaka, Japan) and 225 grams of
113 distilled and deionized water (225 mL at room temperature) into the reactor. The reactor
114 was heated for 3 hours, which includes a warmup time of approximately 30 min. After 3
115 hours of treatment time, the reactor was cooled by turning off the heater. The cooling time
116 below 50°C was approximately 2 hours. The samples were used for the following
117 experiments after lyophilization, except for molecular weight analysis.

118 Treatment at lower temperature was also conducted. 10% OVA solution was added
119 to 50 mL tube and heated in a hot water bath at 80°C for 3 hours. The sample was used

120 for the following experiments after lyophilization.

121

122 **Measurement of molecular weight of OVA samples**

123 A high-performance (HP) size exclusion chromatography (SEC) system with ultraviolet
124 (UV), refractive index (RI) and light-scattering detection (Malvern Panalytical, Ltd.,
125 Malvern, U.K.) was employed to determine the molecular mass distribution of the OVA
126 samples. The eluent was 1/15 molar (M) pH 7.0 phosphate buffer system containing 2 M
127 Urea and 0.1 M Na_2SO_4. The samples after HTW treatment were diluted with the elution
128 at a concentration of 1.5 mg/mL. The sample solutions and the eluent were filtered
129 through 0.45 μm polytetrafluoroethylene disposable membrane filters prior to SEC
130 analysis. The Shodex PROTEIN KW802.5 (8.0 mm I.D. × 300 mm, Showa Denko K.K.,
131 Tokyo, Japan) and PROTEIN KW-G columns were used at a flow rate of 0.5 mL/min.
132 The temperature of the columns was maintained at 30°C and the injection volume was
133 100 μL. A 670 nm incident light source was used for light scattering detection at 7° and
134 90°. Data acquisition and processing to calculate the weight-average molecular mass was
135 performed with OmniSEC software (Malvern Panalytical, Ltd.).

136

137 **SDS-PAGE**

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

145

146 **Oral administration of antigens and preparation of sera**

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

150

151 **ELISA**

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

161 To determine a measurement of the amount of antigen-specific IgE antibody in the
162 sera, non-competitive ELISA was performed. Maxisorp immunoplates were coated with
163 0.01% OVA or OVA treated with HTW at 100°C and the samples and standards were
164 added after washing and blocking of the plates. A pooled serum sample was prepared by
165 mixing a small portion of each serum obtained from mice immunized with OVA and was
166 used as the standard for OVA-specific IgE antibody. The concentration of anti-OVA IgE
167 antibody in the pooled serum was measured by an OVA-specific mouse IgE ELISA kit
168 (Cayman Chemical, MI, USA). Bound IgE antibody was detected by using biotinylated
169 anti-mouse IgE (BD Biosciences), before incubating with alkaline phosphatase-

170 streptavidin. The substrate (*p*-nitrophenol phosphate) was added and the absorbance was
171 determined at 405 nm.
172
173 **Foaming property**
174 Foaming property was measured by the method of Huang et al. (1997) with small
175 modification. OVA or OVA treated with HTW were dissolved to 1% w/v with 0.1 M
176 phosphate buffer (pH7.0). A 10 mL volume of the solution was added to a 50 mL tube
177 and mixed with a former for 15 seconds to prepare the foams. The volume of bubbles was
178 recorded over time.
179 The foaming ability was defined as the ratio of the foam volume after two minutes
180 of foaming against the original volume of the solution. The foaming stability was defined
181 as the ratio of the foam volume after 30 minutes of mixing against the starting volume of
182 the solution.
183
184 **Measurement of coloring of OVA samples**
185 OVA or OVA treated with HTW were dissolved to 1% w/v with distilled water. After
186 centrifuging the samples (4°C, 10000 g, 5 minutes), the absorbance of the supernatant
187 was determined at 450 nm.
188
189 **Statistical analysis**
190 The OVA-specific IgE antibody concentration was measured in the sera of five mice per
191 group. Statistical significance was analyzed by the Steel-Dwass test. The foaming
192 properties and coloring of OVA samples treated with HTW were conducted at least in
193 triplicate, and analyzed by the Tukey–Kramer test. A *p*-value of less than 0.05 was
194 considered statistically significant.

195

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 40000. The average molecular weights of OVA treated by HTW at 100°C, 130°C, 150°C, 160°C, and 170°C were 37000, 34000, 4800, 4700, and 3500, respectively. In the samples treated at 100°C 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.

Oral administration of HTW-treated OVA did not induce OVA-specific IgE

221 **production in DO11.10 mice**

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

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

239

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

242 We used a foamer to whisk the HTW-treated OVA solution (1% w/v) for the measurement
243 of foam ability. The samples treated at temperature of 130°C or higher showed a
244 significant increase in the volume of foams compared to those of the untreated OVA
245 (Figure 4A). Moreover, the foams prepared from the OVA sample treated at 100°C did

246 not change. The foam volumes 30 minutes after the foam preparation were also
247 significantly higher in the foams of the OVA samples treated at 130°C or higher than the
248 native OVA samples (Figure 4B and C).
249

250 **The HTW-treated OVA color alters as the processing temperature is increased**

251 It was observed that the HTW-treated OVA powder was colored brown. We confirmed
252 the degree of coloration using the HTW-treated OVA solution (1% w/v). The color of the
253 HTW-treated OVA became darker as the treatment temperature increased (Figure 5).
254

255 **Discussion**

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

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

270 Our results illustrate that following treatment at 100°C and 130°C, the antigenicity
271 of OVA reduces by a similar level to those treated at higher temperatures, although
272 decomposition was hardly observed in the samples. The HTW treatment would have
273 denatured OVA and caused changes in the conformation. Actually, the solubility appeared
274 lower in the sample. It is suggested that the three-dimensional structure of the samples
275 changed during the denaturation, the hydrophobic amino acids buried inside were
276 exposed, and the molecules were associated through the hydrophobic residues, resulting
277 in aggregation. Therefore, HTW treatment at 100°C and 130°C would have resulted in
278 significant structural changes, which reduced antigenicity due to changes in the structure
279 of the epitope. On the other hand, it is further suggested that the antigenicity decreased

280 due to the disappearance of the epitopes following decomposition in the samples treated
281 at the higher temperatures. In this study, the most decomposed samples had an average
282 molecular weight of about 4000 Da, although the epitopes of OVA have been previously
283 reported as I53D60, V77R84, S103E108, G127T136, E275V280, G301F306, I323A332,
284 A375S384 in mouse (Mine and Yang. 2007). Our data demonstrate that the degradation
285 into 4000 Da peptides is enough to invalidate these epitopes.

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

300 Alternatively, it has been reported that the high-pressure treatment of egg white
301 protein increases the sensitivity to enzymatic hydrolysis (Iametti et al. 1998, 1999; Van
302 der Plancken et al. 2004, 2005a, 2005b, 2007). In this present study, we suggested that
303 the structure changes remarkably following treatment at 100°C and 130°C, making the
304 antigens more susceptible to digestion although the decomposition progressed slightly

305 during the treatment. Therefore, it cannot be denied that digestion occurred more rapidly
306 in those samples at the intestine than native protein and generated small peptides capable
307 of reducing allergenicity.

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

314 Foaming property was measured as a functional property which is important in
315 many cases when using OVA as a food material. Our results demonstrate that the foaming
316 property is improved by HTW treatment at 130°C or higher temperature. This result
317 indicates that degradation of OVA enhances the foaming property. However, molecular
318 weight analysis indicates that there is only a partial decomposition of OVA when treated
319 at 130°C. On the other hand, the range of molecular weight was spread, indicating that
320 small fragments were generated. Such peptides would contribute to the improvement of
321 foam ability. In addition, some structural change of the protein might be involved in the
322 improvement. Indeed, Hagolle et al. (2000) also observed an increase in foam ability after
323 preheating an OVA and lysozyme solution. Furthermore, Cabanillas et al. (2014) reported
324 that protein flexibility increased after the walnut protein was autoclaved. Taken in
325 conjunction with these data, it is considered that the significantly improved foaming
326 property observed was partly due to the increased flexibility of the protein, which
327 occurred following denaturation during the treatment at 130°C. Conversely, the treatment
328 at 100°C did not enhance the foaming property, which is likely because, the effects of the
329 improvement in flexibility, alongside the decrease in hydrophobicity by the treatment,

330 offset each other in the OVA sample treated at 100°C.

331 Heat treatment is useful because it does not require any pretreatments which are
332 sometimes required for enzyme treatment and any other reagents, in addition to not
333 producing any by-products. For example, the reduction of OVA was required for effective
334 digestion with trypsin, and the OVA samples prepared by acid treatment included a lot of
335 salt generated by neutralization done for stopping the reaction. It has been reported that
336 treatment by heating alone also changed the structure of the proteins and reduces
337 antigenicity to some extent. Indeed Watanabe et al. (2014) reported that partial heat
338 treatment of egg white could reduce the allergenicity of OVA. However, Stănciuc et al.
339 (2016) reported that 90% of the antigenicity remained and only partial unfolding was
340 confirmed in the tertiary structure after treating OVA at 100°C for 20 minutes. These
341 results suggest that heat treatment alone can induce partial structural changes of proteins,
342 which would be insufficient to reduce antigenicity in some cases. Indeed, this present
343 study suggested that the allergenicity of OVA could not be sufficiently reduced by heat
344 treatment at 80°C. In addition, high-pressure treatment was also reported to change the
345 immunoreactivity of proteins by changing the structure without any pretreatments and
346 by-products. A previous study reported that an increase in turbidity, surface
347 hydrophobicity, exposed SH content and susceptibility to enzymatic hydrolysis could be
348 observed after treatment at least 400 MPa in egg whites (Hoppe et al. 2013). It follows,
349 therefore, that in order to alter the structure of the protein sufficiently to diminish
350 allergenicity only high-pressure treatment, a considerably high pressure should be
351 required. However, our present study showed that the allergenicity of OVA is completely
352 reduced by combining the heat treatment and the high-pressure treatment. The method
353 used in this study can reduce allergenicity with moderate pressure, and is considered
354 highly practical. Cabanillas et. al. (2014) reported that autoclave treatment could reduce

355 immunoreactivity in walnut proteins more efficiently than high-pressure treatment, which
356 is consistent with the present results. Furthermore, HTW treatment can be applied to
357 various proteins by only controlling the treatment temperature. HTW is a method that can
358 obtain the same or better effects compared with heat treatment and high-pressure
359 treatment without any special facilities, and can be applied for the production of various
360 low allergenic materials.
361 The OVA samples prepared in this study by the HTW treatment at higher than
362 130°C were confirmed to achieve decreased allergenicity and improved foaming property
363 simultaneously. However, the coloration of the samples progressed depending on the
364 treatment temperature. A previous study reported that treatment of egg white at 121°C
365 causes the Maillard reaction (Watanabe et al. 2014). It is not clear whether the coloring
366 observed in this study was due to the Maillard reaction but it could be, because OVA
367 reagent used in this study must contain contaminated saccharides to some extent.
368 Considering practical use, it is certain that the coloring is more likely to occur because
369 materials with a lower purity than the reagent used in this study should be used in the
370 industry. The dark color of food ingredients is generally undesired. Therefore, it is
371 considered that there is an optimum treatment temperature for the production of
372 practically useful low allergenic OVA, which is between 100-130°C. Bitterness is also a
373 negative attribute associated with the majority of food protein hydrolysates. OVA treated
374 between 100-130°C will be hardly decomposed, and will not generate bitter peptides.
375 Therefore, HTW treatment under a certain conditions will provide a potential strategy for
376 preparing practical hypoallergenic materials.
377

378 **Ethics approval**

379 All the animal procedures were approved by animal ethics committee of Tokyo University

of Agriculture and Technology (30-06, April 19th, 2019).

Data availability

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

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author contributions

TY, MO: Conceptualization, Data curation, Writing manuscript, KO, MH: Data curation, Writing manuscript, HM: Conceptualization, Writing manuscript, Funding acquisition.

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, Vecchio G *et al*. Macroscopic and structural consequences of highpressure treatment of ovalbumin solutions. *J Agric Food Chem* 1998;46:3521–7.

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

Järvinen K, Chatchatee P, Bardina L *et al*. IgE and IgG binding epitopes on lactoglobulin

424 in cow's milk allergy. *Int Arch Allergy Immunol* 2001;126:111–8.
425 Kato T, Katayama E, Matsubara S *et al*. Release of allergenic proteins from rice grains
426 induced by high hydrostatic pressure. *J Agric Food Chem* 2000; 48:3124–9.
427 Kleber N, Maier S, Hinrichs J. Antigenic response of bovine β-lactoglobulin influenced
428 by ultra-high pressure treatment and temperature. *Innov Food Sci Emerg Technol*
429 2007;8:39–45.
430 Koh BB, Lee EJ *et al*. Characterization of bovine serum albumin hydrolysates prepared
431 by subcritical water processing. *Food Chem* 2019;278:203-7.
432 Leammli UK. Cleavage of structural proteins during the assembly of the head of
433 bacteriophage T4. *Nature* 1970;227:680-5.
434 Liu R, Xue W. High-pressure treatment with silver carp (Hypophthalmichthys molitrix)
435 protein and its allergic analysis. *High Press Res* 2010;30:438–42.
436 Long FY, Yang X, Wang SJ *et al*. Effects of combined high pressure and thermal treatment
437 on the allergenic potential of peanut in a mouse model of allergy. *Innov Food Sci*
438 *Emerg Technol* 2016;35:135–8.
439 Martorell A, Alonso E, Boné J *et al*. Position document: IgE-mediated allergy to egg
440 protein. *Allergol Immunopathol* 2013;41:320-36.
441 Mine Y, Rupa P. Fine mapping and structural analysis of immunodominant IgE allergenic
442 epitopes in chicken egg ovalbumin. *Protein Eng Des Select* 2003;16:747–52.
443 Mine Y, Yang M. Epitope characterization of ovalbumin in BALB/c mice using different
444 entry routes. *Biochim Biophys Acta* 2007;1774:200-12.
445 Peñas E, Préstamo G, Polo F *et al*. 2006. Enzymatic proteolysis, under high pressure of
446 soybean whey: Analysis of peptides and the allergen Gly m 1 in the hydrolysates.
447 *Food Chem* 2006;99:569–73.
448 Stănciuc N, Banu I, Turturică M *et al*. pH and heat induced structural changes of chicken

449 ovalbumin in relation with antigenic properties. *Int J Biol Macromole* 2016;93:572–
450 81.
451 Van der Plancken I, Delattre MI, Van Loey A *et al*. Kinetic study on the changes in the
452 susceptibility of egg white proteins to enzymatic hydrolysis induced by heat and high
453 hydrostatic pressure pretreatment. *J Agric Food Chem* 2004;52:5621–6.
454 Van der Plancken I, Van Loey A, Hendrickx M. Combined effect of high pressure and
455 temperature on selected properties of egg white proteins. *Innov Food Sci Emerg*
456 *Technol* 2005a;6:11–20.
457 Van der Plancken I, Van Loey A, Hendrickx M. Changes in sulfhydryl content of egg
458 white proteins due to heat and pressure treatment. *J Agric Food Chem*
459 2005b;53:5726–33.
460 Van der Plancken I, Van Loey A, Hendrickx M. Kinetic study on the combined effect of
461 high pressure and temperature on the physico-chemical properties of egg white
462 proteins. *J Food Eng* 2007;78:206–16.
463 Watanabe H, Toda M, Sekido H *et al*. Heat treatment of egg white controls allergic
464 symptoms and induces oral tolerance to ovalbumin in a murine model of food allergy.
465 *Mol Nutr Food Res* 2014;58:394–404.
466 Yang X, Sun J, Tao J *et al*. The allergenic potential of walnuts treated with high pressure
467 and heat in a mouse model of allergy. *Innov Food Sci Emerg Technol* 2017;39:165–
468 70.
469

470 **Legends to figures**

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

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477

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

484

485 **Figure 3.** Effects of HTW treatment on allergenicity. DO11.10 mice were given diets
486 containing OVA treated with HTW or untreated OVA for 3 weeks (n = 5/group). (A) The
487 amount of OVA-specific IgE in the sera from mice given OVA treated with HTW was
488 measured by ELISA. (B) The amount of OVA treated with HTW at 100°C-specific IgE
489 in the sera from mice fed the antigen. (C) The amount of OVA-specific IgE in the sera
490 from mice given OVA treated with HTW or heated at 80°C and 100°C. * indicates
491 statistical significance by the Steel-Swass test.

492

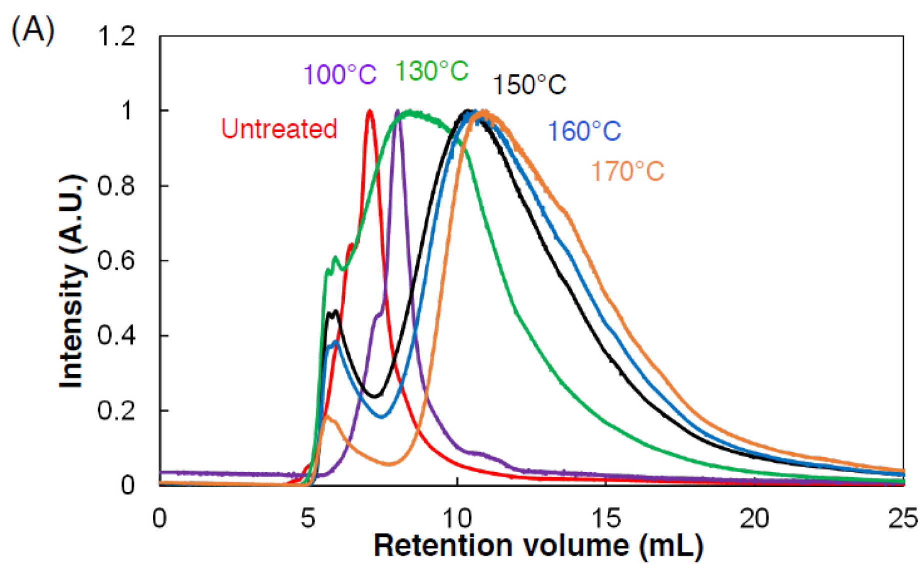
493 **Figure 4.** Effects of HTW treatment on foaming properties. Foams were prepared by
494 mixing for 15 seconds with a foamer (n = 3 / group). (A) Foam ability was shown by

comparing the amount of foams two minutes after preparation against the amount pre-mixing. (B) Foam stability was shown by comparing the amount of foams 30 minutes after preparation against the amount of the solution before mixing. (C) Photos of foams 30 minutes after preparation of (1) untreated OVA, (2) HTW treatment at 100°C, (3) at 130°C, (4) at 150°C, (5) at 160°C, (6) at 170°C were taken. Different characters indicate statistical significance calculated by the Tukey-Kramer test.

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 450nm. (B) Photos of (1) untreated OVA, (2) HTW treatment at 100°C, (3) at 130°C, (4) at 150°C, (5) at 160°C, (6) at 170°C were taken. Different characters indicate statistical significance calculated by the Tukey-Kramer test.

Caption for the graphical abstract

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



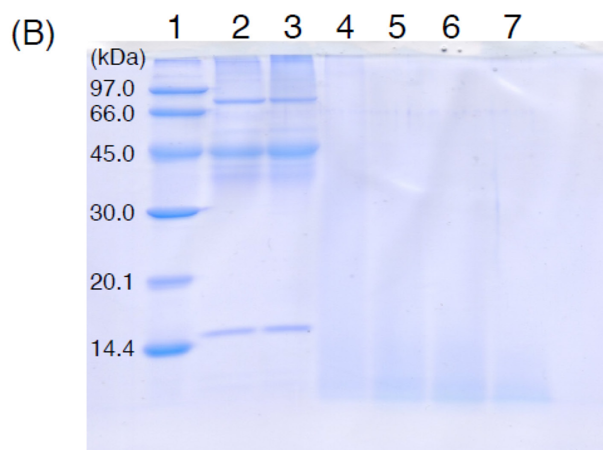


Figure 1

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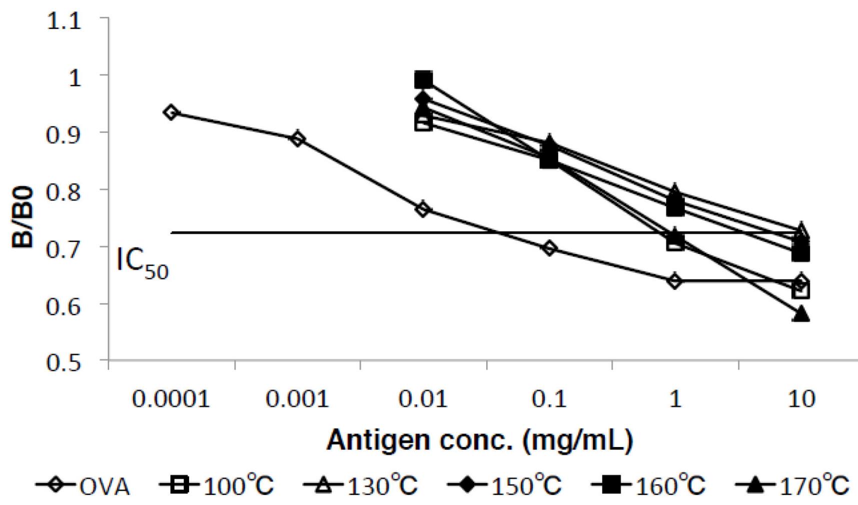

Figure 2

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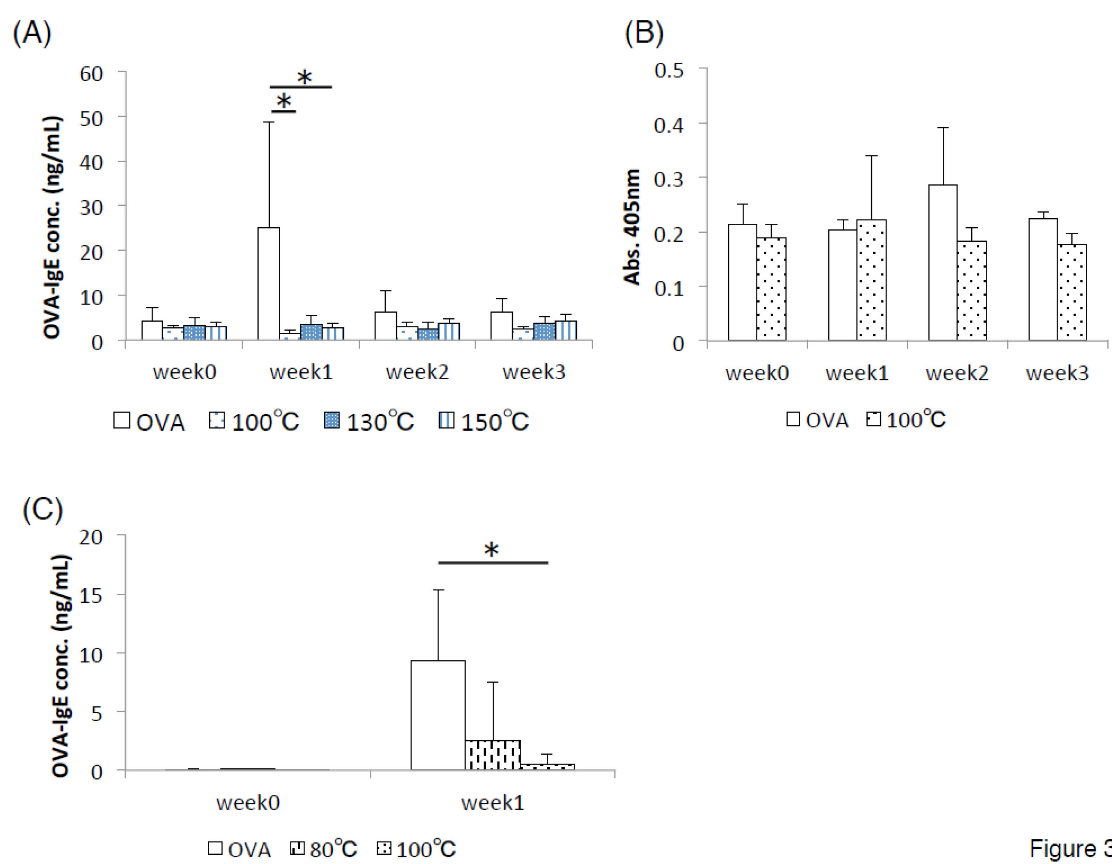

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Figure 3

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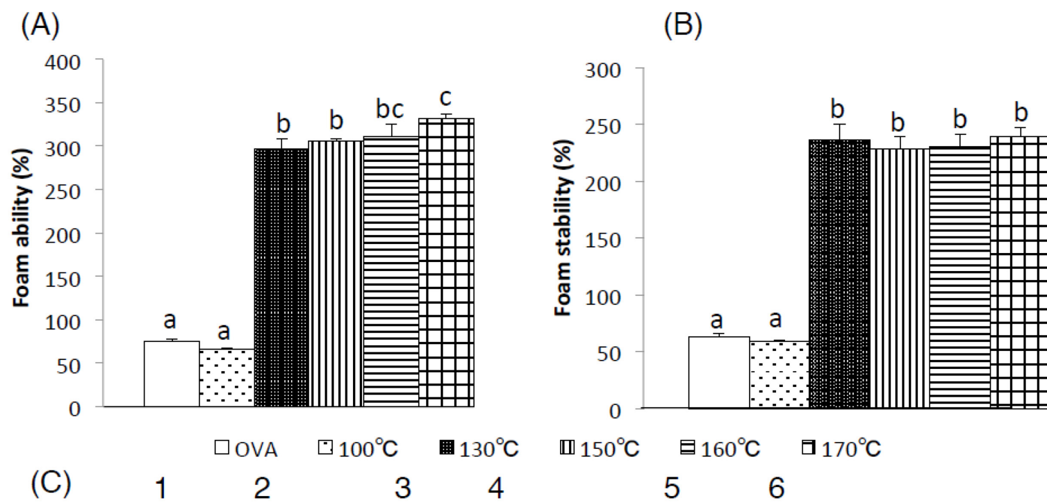

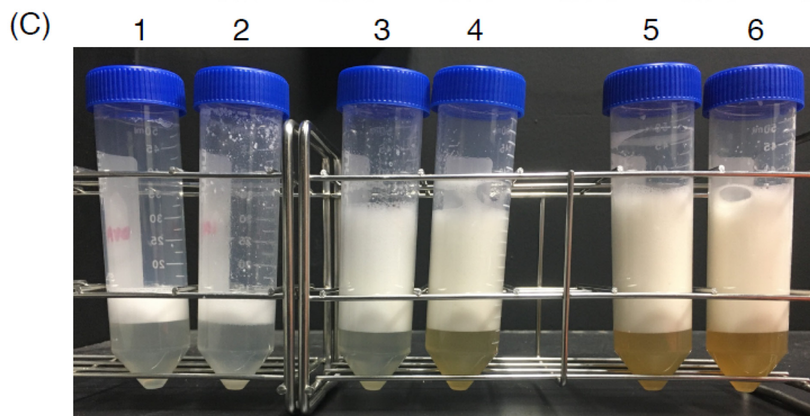

Figure 4

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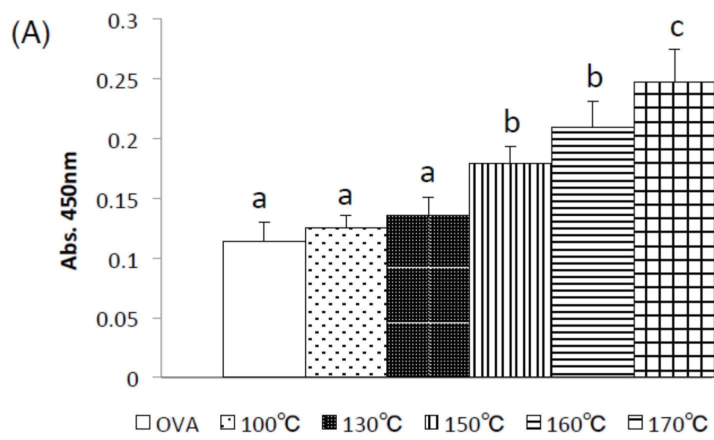

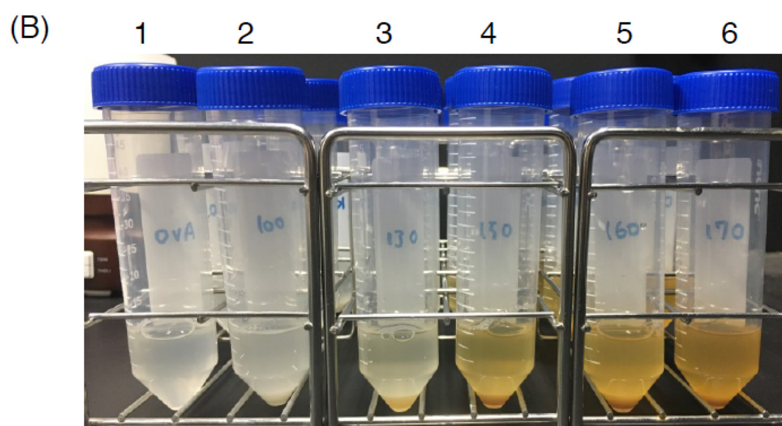

Figure 5

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