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Variation in the Allergenicity of Scrambled, Boiled, Short-Baked and Long-Baked Egg White Proteins

Purevsan Gantulga [1,2] **Jeongmin Lee** [3] **Kyunguk Jeong** [1] **Se-Ah Jeon** [1] **and Sooyoung Lee** [1]

[1]Department of Pediatrics, Ajou University School of Medicine, Suwon, Korea
[2]Department of Medical Sciences, Graduate School of Medicine, Ajou University, Suwon, Korea
[3]Department of Pediatrics, Yonsei University Wonju College of Medicine, Wonju, Korea

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Address for Correspondence:
Sooyoung Lee, MD, PhD
Department of Pediatrics, Ajou Unicersity School of Medicine, 164 World cup-ro, Yeongtong-gu, Suwon 16499, Republic of Korea.
Email: jsjs87@ajou.ac.kr

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ORCID iDs
Purevsan Gantulga
https://orcid.org/0000-0003-3562-270X
Jeongmin Lee
https://orcid.org/0000-0002-1672-7445
Kyunguk Jeong
https://orcid.org/0000-0002-1261-4507
Se-Ah Jeon
https://orcid.org/0000-0002-8955-7440
Sooyoung Lee
https://orcid.org/0000-0003-1734-4101

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The authors have no potential conflicts of interest to disclose.

ABSTRACT

Background: Hen's egg white (HEW) is the most common cause of food allergy in children which induces mild to fatal reactions. The consultation for a proper restriction is important in HEW allergy. We aimed to identify the changes in HEW allergenicity using diverse cooking methods commonly used in Korean dishes.
Methods: Crude extract of raw and 4 types of cooked HEW extracts were produced and used for sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE), enzyme-linked immunosorbent assay (ELISA), and ELISA inhibition assays using 45 serum samples from HEW allergic and tolerant children. Extracts were prepared; scrambled without oil for 20–30 seconds in frying pan without oil, boiled at 100°C for 15 minutes, short-baked at 180°C for 20 minutes, and long-baked at 45°C for 12 hours with a gradual increase in temperature up to 110°C for additional 12 hours, respectively.
Results: In SDS-PAGE, the intensity of bands of 50–54 kDa decreased by boiling and baking. All bands almost disappeared in long-baked eggs. The intensity of the ovalbumin (OVA) immunoglobulin E (IgE) bands did not change after scrambling; however, an evident decrease was observed in boiled egg white (EW). In contrast, ovomucoid (OM) IgE bands were darker and wider after scrambling and boiling. The IgE binding reactivity to all EW allergens were weakened in short-baked EW and considerably diminished in long-baked EW. In individual ELISA analysis using $OVA^{+}OM^{+}$ serum samples, the median of specific IgE optical density values was 0.435 in raw EW, 0.476 in scrambled EW, and 0.487 in boiled EW. Conversely, it was significantly decreased in short-baked (0.406) and long-baked EW (0.012). Significant inhibition was observed by four inhibitors such as raw, scrambled, boiled and short-baked HEW, but there was no significant inhibition by long-baked HEW (IC_{50} > 100 mg/mL).
Conclusion: We identified minimally reduced allergenicity in scrambled EW and extensively decreased allergenicity in long-baked EW comparing to boiled and short-baked EW as well as raw EW. By applying the results of this study, we would be able to provide safer dietary guidence with higher quality to egg allergic children.

Keywords: Egg White Proteins; Food Allergy; Cooking; Allergenicity

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Author Contributions

Conceptualization: Lee S. Data curation: Gantulga P, Lee J. Formal analysis: Jeong K. Methodology: Gantulga P, Lee J, Jeon SA, Lee S. Writing - original draft: Gantulga P. Writing - review & editing: Lee S, Jeong K.

INTRODUCTION

Hen's egg (HE) is a food that is widely consumed worldwide and is one of the most common causes of food allergy in children. Allergy to HE is believed to affect 0.3–2% of young children, with its prevalence varying depending on race, ethnicity, and other factors. In recent decades, allergy to HE has exhibited an increasing trend.[1-3] HE is reportedly the leading cause of allergic reactions in infants (34.3%) and preschoolers (15.4%) in Korea.[4] Typically, egg allergy develops early in life and can diminish with age, with most children achieving tolerance by the time they reach preschool age.[5,6] However, recent studies have suggested that the resolution of hen's egg white (HEW) allergy is delayed and persists longer than predicted into adolescence.[7,8] HEW allergy symptoms vary from mild urticaria to life-threatening anaphylaxis. However, there is a lack of pharmacological treatment for food allergy, and the most effective management is an elimination diet and careful planning to avoid accidental ingestion of causative food.[9] Nevertheless, a strict elimination diet can lead to malnutrition and impaired growth, further affecting the quality of life of children with food allergies.[10]

Most HE allergens, including Gal d 1 (ovomucoid, OM), Gal d 2 (ovalbumin, OVA), Gal d 3 (ovotransferrin or conalbumin, OT), and Gal d 4 (lysozyme, LYZ), are present in egg whites (EW).[11] Various cooking methods, including thermal and non-thermal food processing, can affect HEW allergens by altering their protein conformation.[12] Therefore, heat treatment, especially cooking, which is the most common food processing method in daily life, can differentially alter the allergenicity of HEW proteins based on their duration and temperature. Notably, the allergenicity of OVA, the most abundant protein in HEW, as well as OT and LYZ, decreases significantly following heat treatment. Conversely, OM is a heat-stable and highly allergenic protein in HEW.[13-15] Nonetheless, over 70% of children with HE allergy can reportedly tolerate extensively cooked eggs and cooked egg-containing food products; additionally, consuming extensively or adequately baked HEW may induce tolerance in children with allergies following careful evaluation by supervised oral food challenge (OFC).[16,17]

Therefore, we aimed to identify the changes in HEW allergenicity using diverse cooking methods commonly used in Korean dishes and applied the results to help provide appropriate dietary consulting for developing tolerance acquisition in children with HEW allergy.

METHODS

Patient serum samples

We collected clinical data from patients with clinical symptoms of HEW allergy and elevated EW-specific immunoglobulin E (IgE) via ImmunoCAP (Thermo Fisher Scientific, Upsala, Sweden) from January 2019 to March 2021 using a retrospective electronic medical records review. We used three groups of serum samples from patients based on the sensitization patterns to HEW major allergen-specific IgEs: OVA- and OM-specific IgE-positive (OVA^+OM^+), OVA-specific IgE-positive but OM-specific IgE-negative (OVA^+OM^-), and OVA- and OM-specific IgE-negative (OVA^-OM^-) samples. To minimize analytical bias, we used serum samples with mid-strong EW-specific IgE-positive in OVA^+OM^+ and OVA^+OM^- patient groups.

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Preparation of crude EW extracts

HEs were purchased from a local market, and a 200 mL of raw EW, and 100 g of scrambled, boiled, and long-baked EW, and 96 g of short-baked EW samples were prepared immediately for the production of the crude extract. Cooked EW samples were prepared, scrambled without oil for 20–30 seconds in a frying pan, boiled at 100°C for 15 minutes, and short-baked at 180°C for 20 minutes. A long-baked egg, the very unique cooking method in the world, was purchased from the market. It undergoes processing at 45°C for 12 hours, with a gradual increase in temperature up to 110°C for an additional 12 hours (**Table 1**). All EW samples were separated from the yolk, washed twice with distilled water, and shredded using a hand mixer to make a smooth paste. Samples were added to 1× phosphate-buffered saline (PBS, pH 7.4) solution at 1:5 w/v and stirred overnight at 4°C. Extracts were then centrifuged at 14,000 rpm for 1 hour at 4°C. The extracts were defatted five times (duration was 10 minutes) using cold petroleum ether (4°C) and dialyzed against 1× PBS solution in a dialysis membrane bag (cutoff pore size: 3.5 kDa) to remove small-sized molecules. This step was repeated several times daily for 2 days at 4°C. The extracts were then filtered through a vacuum filter (pore size: 0.45-μm) and freeze-dried. Protein concentration was determined using the Bradford protein assay. The extracts were stored at −20°C until use.

Sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE)

HEW proteins were mixed in 2× Tris-Glycine SDS-PAGE sample buffer (Koma Biotech, Seoul, Korea) and heated for 10 minutes at 100°C in a heating block. Subsequently, 25 μL (5 μg) of each sample and 10 μL of protein marker (LC5925, SeaBlue® Plus2 Pre-stained Standard 1×; Thermo Fisher Scientific) were loaded in each well (4–20% Tris-Glycine gel; Koma Biotech) and separated at 120 V for 2 hours. Gels were stained using Coomassie brilliant blue or used for immunoblotting.[18]

Immunoblotting

After electrophoresis, the separated proteins in the gel were transferred onto a nitrocellulose membrane (Millipore Co., Bedford, MA, USA), blocked with blocking buffer (3% bovine serum albumin in 1× PBS) overnight at 4°C, and incubated at room temperature (RT) for 4 hours with $OVA^{+}OM^{+}$-, $OVA^{+}OM^{-}$-, and $OVA^{-}OM^{-}$-pooled serum samples diluted to 1:20 in blocking buffer. For detecting an IgE-binding band, samples were incubated with biotinylated anti-human IgE (Vector Laboratories, Burlingame, CA, USA) diluted to 1:1,000 for 1 hour and then incubated with streptavidin-alkaline phosphatase (BD PharMingen, San Diego, CA, USA) diluted to 1:1,000 in blocking buffer for 15 minutes at RT. The membranes were developed using BCIP/NBT substrate (SIGMA FAST™ 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium; Sigma-Aldrich, St. Louis, MO, USA), and the development was terminated by adding distilled water. In all steps, the membranes were washed thrice with 0.05% Tween-20 in PBS (PBST, pH 7.0).[19]

Table 1. Hen's egg white extracts with various cooking methods

Egg white samples	Temperature	Cooking time and method
Raw	-	-
Scrambled	Not checked	20–30 sec in frying fan without oil
Boiled	100°C	15 min in boiling water
Short-baked	180°C	20 min in oven
Long-baked[a]	45–110°C	12 hr at 45°C, then slowly increasing temperature till 110 for 12 hr

[a]Obtained from market.

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Individual enzyme-linked immunosorbent assays (ELISAs)

For ELISA, 96-well microplates were coated with 100 μL (5 μg/mL) of raw, scrambled, boiled, short-baked, and long-baked EW samples in coating buffer (0.1 M carbohydrate/bicarbonate, pH 9.5, Sigma C3041) and incubated overnight at 4°C. Microplate wells were washed five times with 0.05% PBST and incubated for 2 hours at RT with 200 μL of blocking buffer (10% fetal bovine serum in 1× PBS) for reducing non-specific binding. Fifty microlitres of serum samples (OVA^+OM^+, OVA^+OM^-, OVA^-OM^-) diluted to 1:10 was added to the wells and incubated for 1 hour at RT. After washing with 0.05% PBST, plates were incubated with 100 μL of working detectors (horserum dish peroxidase and biotinylated anti-human IgE diluted to 1:1,000 in blocking buffer) for 1 hour at RT. The wells were then reacted with 100 μL of 3,3′,5,5′-tetramethylbenzidine (TMB; BD PharMingen) substrate solution for 10 minutes at RT in the dark. The reaction was quenched using 50 μL of 2N H_2SO_4. Optical density (OD) was measured at 450 nm using a microplate reader (iMark™ Microplate Absorbance Reader; Bio-Rad, Hercules, CA, USA).

EW inhibition ELISA

Inhibition ELISA was performed with the pooled serum samples of 15 OVA^+OM^+ patients. The 96-well microplates were coated with 100 μL (5 μg/mL) of the raw EW sample in coating buffer and incubated overnight at 4°C. The plates were washed five times with 0.05% PBST and blocked with 200 μL of blocking buffer for 2 hours at RT. Subsequently, 100 μL of the equally mixed antibody-antigen mixture containing pooled serum samples diluted to 1:10 in blocking buffer and serially diluted inhibitors (0, 0.1, 1, 10, and 100 μg/mL) were added to the wells and incubated for 1 hour at RT. The raw, scrambled, boiled, short-baked, and long-baked EWs and fetal bovine serum were utilized as inhibitors. After washing microplate wells with 0.05% PBST, 100 μL of working detectors (horserum dish peroxidase and biotinylated anti-human IgE diluted to 1:1,000 in blocking buffer) were added to wells and incubated at RT for 1 hour. For developing the assay, wells were reacted with 100 μL of TMB (BD PharMingen) substrate solution. The reaction was quenched using 2N H_2SO_4, and OD was measured at 450 nm using the microplate reader. The percentage of inhibition was calculated for each inhibitor using the following formula:

$$\% \text{ Inhibition} = \frac{\text{Uninhibited OD Value} - \text{Inhibited OD Value}}{\text{Uninhibited OD Value}} \times 100$$

Statistical analysis

All statistical analyses were performed using GraphPad Prism v.9.2 (GraphPad Software, San Diego, CA, USA). A χ^2 test was used to compare categorical variables, and the Kruskal–Wallis test was used to compare continuous variables. One-way analysis of variance followed by Tukey's multiple comparisons test was used to compare raw and diverse cooking EW in positive groups. A P value < 0.05 was considered statistically significant.

Ethics statement

All study participants provided written informed consent for obtaining and using serum samples for the experiment, and the study design was approved by the Institutional Review Boards at Ajou University Hospital (AJIRB-MED-MDB-18-111).

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RESULTS

Specific IgE levels in the tested serum samples

In OVA^+OM^+ and OVA^+OM^- patient groups, the median concentrations of EW-specific IgEs were 10.5 (range, 5.62–33.8) kU/L and 10.6 (range, 5.61–32.7) kU/L, OVA-specific IgEs were 7.46 (range, 2.07–25.2) kU/L and 8.71 (range, 7.02–40.8) kU/L, and OM-specific IgEs were 5.71 (range, 2.71–29.7) kU/L and < 0.1 kU/L, respectively. All EW-, OVA-, and OM-specific IgE concentrations were < 0.1 in the OVA^-OM^- group, which served as a negative control (**Table 2**).

SDS-PAGE

SDS-PAGE profiles of the raw and four differently cooked HEW extracts are displayed in **Fig. 1**. Most protein bands of raw EW were observed at 98 kDa (corresponding to OT), 50–54 kDa

Table 2. Total IgE and specific IgE levels to whole EW, OVA, and OM in study groups

Characteristics	OVA^+OM^+ (n = 15)	OVA^+OM^- (n = 15)	OVA^-OM^- (n = 15)	*P* value
Total IgE, kU_A/L	177 (15–721)	63 (18–617)	16 (4–97)	< 0.001
EW specific IgE, kU_A/L	10.5 (5.62–33.8)	10.6 (5.61–32.7)	< 0.1	0.019
OVA specific IgE, kU_A/L	7.46 (2.07–25.2)	8.71 (7.02–40.8)	< 0.1	0.002
OM specific IgE, kU_A/L	5.71 (2.74–29.7)	< 0.1	< 0.1	NA

Data are expressed by median and range.
IgE = immunoglobulin E, OVA = ovalbumin, OM = ovomucoid, EW = egg white, NA = not analyzed.

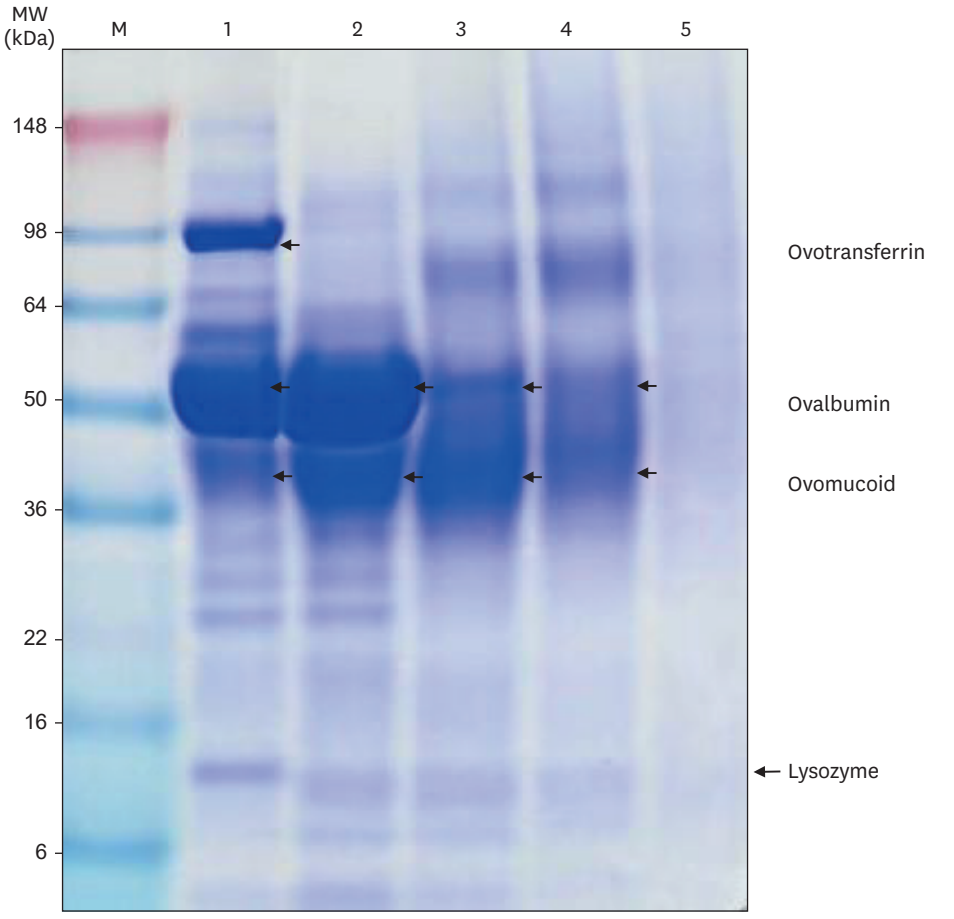

Fig. 1. SDS-PAGE profiles of raw and 4 different cooked hen's egg white proteins. M-marker: 1, Raw EW; 2, Scrambled EW; 3, Boiled EW; 4, Short-baked EW; and 5, Long-baked EW.
SDS-PAGE = sodium dodecyl-sulfate polyacrylamide gel electrophoresis, EW = egg white.

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(corresponding to OVA), 36–48 kDa (corresponding to OM), and 14 kDa (corresponding to LYZ). The intensities of the 98 and 14 kDa bands were substantially weakened by the four cooking methods. The intensity of the 50–54 kDa band decreased with boiling and short-baking. In contrast, the 36–48 kDa band intensified with scrambling and boiling, whereas it was substantially reduced by short-baking. Furthermore, all protein bands almost disappeared in long-baked EW.

Immunoblotting

Using pooled serum samples from three groups of patients, immunoblotting was performed to evaluate the IgE binding reactivity of raw and four types of cooked EW extracts in individual major patients (**Fig. 2**). In the IgE immunoblot using $OVA^{+}OM^{+}$ serum samples (**Fig. 2A**), IgE strongly bound to all major EW allergens in raw EW. The binding bands corresponding to OT diminished after all kinds of cooking methods. The intensity of the OVA IgE bands did not change after scrambling; however, an evident decrease was observed in boiled EW. In contrast, OM IgE bands were darker and wider after scrambling and boiling. The IgE binding reactivity to all EW allergens weakened in short-baked EW and considerably diminished in long-baked EW.

In the IgE immunoblot using $OVA^{+}OM^{-}$-pooled serum samples (**Fig. 2B**), the IgE–OVA band was evident in raw and scrambled EW, whereas it substantially decreased in boiled EW and disappeared in baked EW extract. No definite IgE binding band was observed in the immunoblot of $OVA^{-}OM^{-}$-pooled serum samples (**Fig. 2C**).

Individual ELISA

In individual ELISA analysis using $OVA^{+}OM^{+}$ serum samples (**Fig. 3A**), the median of specific IgE OD values was 0.435 (range, 0.317–1.996) in raw EW, 0.476 (range, 0.225–1.854) in scrambled EW, and 0.487 (range, 0.201–1.842) in boiled EW. Conversely, it was significantly decreased in short-baked (0.406; range, 0.233–1.726) and long-baked EW (0.012; range, −0.006–1.125; $P < 0.05$). In ELISA with $OVA^{+}OM^{-}$ serum samples (**Fig. 3B**), the medians of specific IgE OD values were considerably decreased in all types of cooked EWs and were

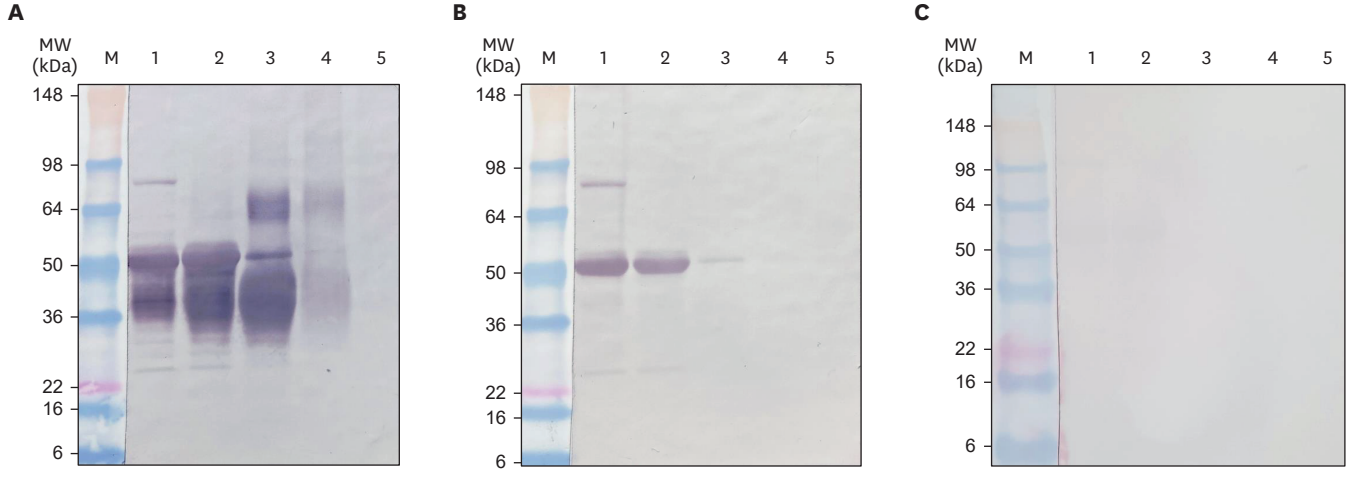

Fig. 2. Immunoblot analysis of raw and 4 different cooked hen's egg white proteins with pooled serum samples of patients. (**A**) $OVA^{+}OM^{+}$-pooled serum samples. (**B**) $OVA^{+}OM^{-}$-pooled serum samples. (**C**) $OVA^{-}OM^{-}$-pooled serum samples. M-marker: 1, Raw EW; 2, Scrambled EW; 3, Boiled EW; 4, Short-baked EW; 5, Long-baked EW.
OVA = ovalbumin, OM = ovomucoid, EW = egg white.

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significantly lower than that of raw EW (0.059; range, 0.018–0.732; all $P < 0.05$). The specific IgE reactivity was negative in raw and cooked EWs of $OVA^{-}OM^{-}$ control serum samples (**Fig. 3C**).

IgE ELISA inhibition analysis of raw EW extract

An IgE ELISA inhibition analysis of raw HEW extracts was conducted using $OVA^{+}OM^{+}$ pooled serum samples (**Fig. 4**). Significant inhibition was observed using four inhibitors: raw HEW (half-maximal inhibitory concentration [IC_{50}] = 0.24 μg/mL), scrambled HEW (IC_{50} < 0.1 μg/mL), boiled HEW (IC_{50} < 0.1 μg/mL), and short-baked HEW (IC_{50} = 0.38 μg/mL). However, we observed no significant inhibition by long-baked HEW (IC_{50} > 100 μg/mL).

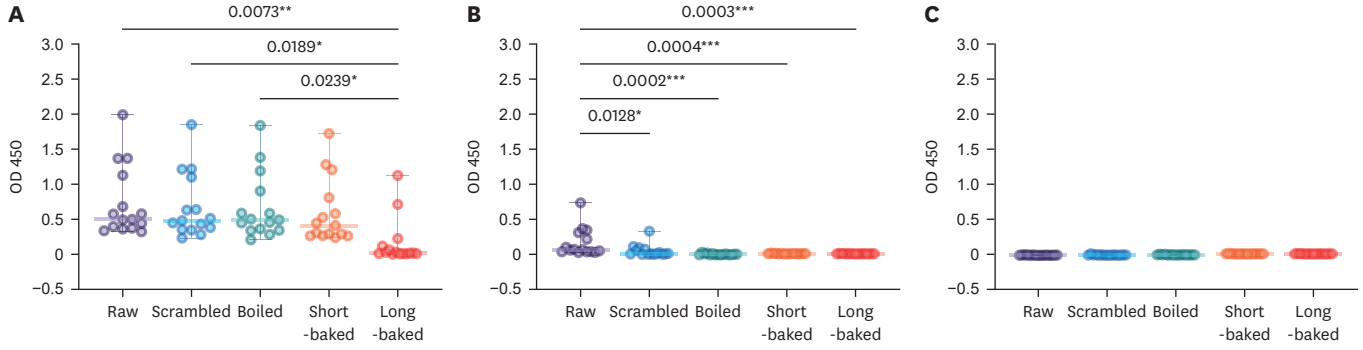
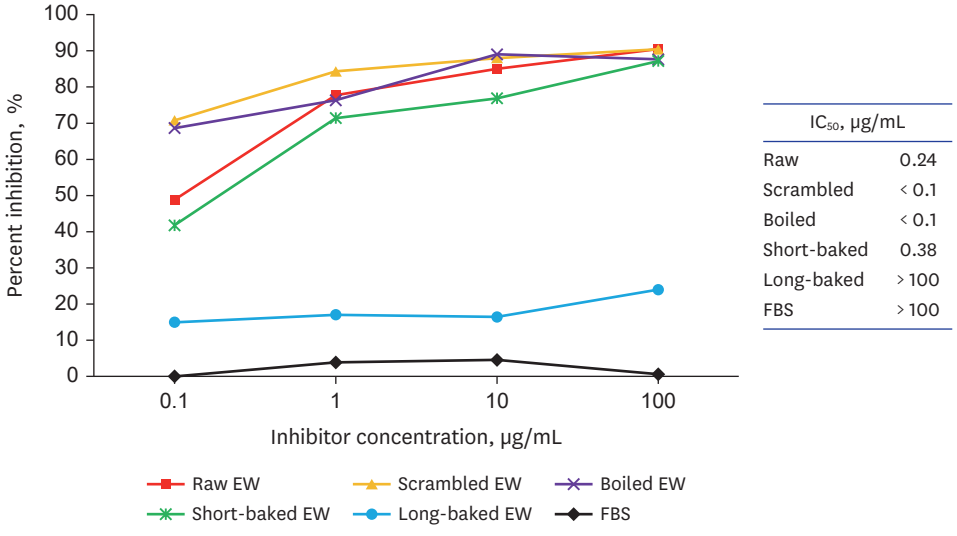


Fig. 3. Individual ELISA of raw and 4 different cooked hen's egg white crude extracts with individual serum samples of patients. (**A**) $OVA^{+}OM^{+}$ serum samples of 15 patients. (**B**) $OVA^{+}OM^{-}$ serum samples of 15 patients. (**C**) $OVA^{-}OM^{-}$ serum samples of 15 patients. Results are expressed by median and range of OD 450 value.
ELISA = enzyme-linked immunosorbent assay, OVA = ovalbumin, OM = ovomucoid, OD = optical density.
$^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.



IC_{50}, μg/mL	
Raw	0.24
Scrambled	< 0.1
Boiled	< 0.1
Short-baked	0.38
Long-baked	> 100
FBS	> 100

Fig. 4. Egg white inhibition ELISA of raw and 4 different cooked hen's egg white crude extract with $OVA^{+}OM^{+}$-pooled serum samples of patients. Inhibitors: Raw EW, Scrambled EW, Boiled EW, Short-Baked EW, Long-baked EW and FBS.
ELISA = enzyme-linked immunosorbent assay, OVA = ovalbumin, OM = ovomucoid, EW = egg white, FBS = fetal bovine serum, IC_{50} = half-maximal inhibitory concentration.

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DISCUSSION

In this study, we investigated the change in allergenicity to HEW in three groups of pooled patient serum samples (OVA^+OM^+, OVA^+OM^-, and OVA^-OM^-) using four different cooking methods commonly used in Korea. Consequently, we observed that mild heat treatment, such as scrambling, did not significantly reduce the allergenicity of OM and OVA; on the other hands the long-time baking treatment, a unique cooking method in Korea, extensively reduced HEW allergenicity. The findings of this study provide practical information on a food ladder for tolerance induction and dietary education in children with egg allergies.

Heat treatment is a common food processing method widely used in homes or industries and affects food proteins in various ways. Several studies have evaluated the effect of food processing on the allergenicity of major HEW proteins.[12-15,20-24] The most abundant protein in HEW, OVA, is heat labile, and its allergenicity can considerably be reduced by boiling for 15 minutes.[14] Despite the smaller proportion of OM in HEW, it is the most heat-stable and clinically relevant allergen in HEW that contributes to the severity and persistence of HEW allergy.[15,25] Therefore, most recent studies have mainly targeted weakening OM allergenicity for managing HE allergy. In this study, we demonstrated that the intensity of the OM band was slightly enhanced by scrambling and boiling, whereas it was substantially reduced by short-baking of the whole egg and almost disappeared after long baking. In a previous Korean study, Shin et al.[22] demonstrated the influence of time and temperature on HEW allergens, suggesting that the intensity of OVA bands significantly decreased after 30 minutes of boiling and baking at 170°C for 20 minutes, whereas OM band was not decreased by all studied cooking methods. These results were similar to our findings in boiled and 20 minutes baked (short-baked) egg white. However, different from Shin's study, we evaluated IgE binding allergenicity in 2 groups of patients who have different components specific IgE profiles, such as OVA^+OM^+ vs. OVA^+OM^- groups. Furthermore, we confirmed remarkably reduced OVA and OM allergenicity by long-baking, and this finding is the novelty of our study.

Avoiding foods containing HEW is crucial for managing HE allergy; however, overly strict elimination diets may harm nutrition and quality of life.[9,10] Furthermore, some children with HEW allergy can tolerate baked or extensively heated eggs.[16] However, most patients with HEW allergy avoid all HEW-containing dishes and products out of fear of clinical reactions, miseducation, or misunderstanding. Furthermore, tolerance to baked eggs is a clinical indicator of a favorable tendency toward resolution of HEW allergy, and consuming tolerable baked or extensively-heated HEW can accelerate immunological tolerance and clinical reactivity to HEW.[17,26,27] Therefore, people must be educated about consuming tolerable forms of cooked or processed HEW under the supervision of a doctor to alleviate disease persistence or lower the threshold triggering symptoms.

Using the "food ladder" approach, a stepwise reintroduction of adequately-to-less processed allergy-inducing foods could be used for patients with HEW allergy. Several studies using baked eggs with matrix incorporation indicated lower allergenicity of baked eggs. HEW baking with wheat can decrease OM antigenic activity,[20] and heating with wheat gluten can decrease OM antigenicity compared to milk casein.[21] IgE antibody binding is decreased in eggs baked with wheat.[13] Contrary to previous reports, we used raw-to-adequately baked whole EW extracts in this study, without matrix incorporation. Individual ELISA and immunoblotting revealed that long-baked EW was substantially less allergenic than boiled and short-baked EWs. Therefore, long-baked EW might be recommended as the bottom

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of the EW ladder for tolerance induction and enhancing the quality of life, particularly in patients with severe HEW allergy.

Uncommon food processing methods, such as high pressure, ultraviolet radiation, ultrasound, gamma radiation, enzymatic hydrolysis, and acid treatment, affect HEW allergenicity.[12,23,24] However, for general meal preparation, recommending these kinds of processed HEWs for dietary consultation for children with EW allergies is difficult. Therefore, the findings of our study are valuable in real-world HEW allergy management and in promoting a better quality of life for children with HEW allergies.

Furthermore, individuals tend to outgrow HEW allergy at different ages.[5-8] According to one study, sensitization to Gal d 1 (OM) or several egg allergen components (Gal d 1, 2, 3, and 4) increases the risk of developing persistent HEW allergy.[25] Different from previous studies in Korea and other countries, in this study, we used $OVA^{+}OM^{+}$ and $OVA^{+}OM^{-}$ groups of patient serum samples to examine the changes in IgE binding capability in response to various cooking methods. Immunoblotting and individual ELISA revealed some differences between pooled serum samples from $OVA^{+}OM^{+}$ and $OVA^{+}OM^{-}$ patient groups. ELISA revealed that $OVA^{+}OM^{+}$ serum samples reacted strongly to raw, scrambled, boiled, and short-baked HEW, whereas $OVA^{+}OM^{-}$ serum samples reacted less strongly. In the immunoblotting analysis using $OVA^{+}OM^{-}$ pooled serum samples, we observed IgE binding bands of OVA in raw and scrambled HEW but a decreased reaction to boiled HEW. These results demonstrate that children who are not sensitized to OM could consume well-cooked HEW, such as boiled and short-baked egg, and these are unique findings in our study.

The limitations of our study are that we selected patients based on their clinical history and sensitization profiles rather than their OFC results, and we did not confirm the reduced allergenicity in vivo. However, to minimize diagnostic inaccuracy, we utilized experienced food allergists to select patients through detailed history collection. Despite this limitation, our study provides valuable insights into the effect of heat treatment on HEW allergenicity using four different cooking methods that are commonly used on a daily basis. Further research on reduced HEW allergenicity using OFC results could be conducted in the future, which will be of remarkable value in dietary guidance for patients that are allergic to eggs.

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