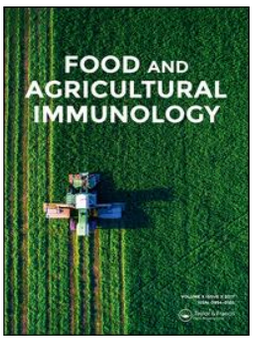

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Effects of heating, autoclaving and ultra-high pressure on the solubility, immunoreactivity and structure of major allergens in egg

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ABSTRACT

Ovotransferrin (OVT) and ovomucoid (OVM) were treated, respectively by different food processing conditions. The protein recovery and immunoreactivity of OVT significantly decreased during heat treatment ($P < .05$), and aggregates appeared after dry heating at 150°C or 200°C or boiling at 100°C. OVM appeared more stable than OVT under the same treatment. Greatly reduced solubility occurred only after dry heating at 200°C. The immunoreactivity increased after dry heating at 100°C or 150°C or moist heating (60°C, boiling). Autoclave sterilization had a strong effect on the characteristics of both OVT and OVM. Ultra-high pressure had very little effect on OVT and OVM, except at 600 MPa in the moist treatment. Results showed that temperature synergistic processing with water and high pressure had the greatest impact on the characteristics, which could be a potential strategy for mitigating the negative effects of allergens in egg.

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Egg allergens; protein recovery; immunoreactivity; primary structure; food processing

Introduction

Eggs compose one of the eight main categories of food allergens and are widely used in the food industry (Acero-Lopez, Ullah, Offengenden, Jung, & Wu, 2012). Approximately 0.5–2.5% of children worldwide are allergic to eggs, and two-thirds of children suffer from severe atopic dermatitis caused by egg hypersensitivity (Anet et al., 1985). Therefore, eggs are considered the second most common food allergen for infants, with milk being the most common (Bush & Hefle, 1996).

Most egg allergens are mainly contained in egg whites (Dubois et al., 2015). Ovotransferrin (OVT), which accounts for 12% of the total egg white protein (Fu, Maks, & Banaszewski, 2010; Herman, Helm, Jung, & Kinney, 2003), is an iron-binding glycoprotein that contains 2.63% carbohydrate (Hirose, 2000). Ovomucoid (OVM) is present at the highest concentration and exhibits the strongest allergenicity in egg whites; OVM contains 20–25% sugar, which makes it is fairly stable under treatment

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with trypsin, and is therefore also known to be a trypsin inhibitor (Kume & Matsuda, 1995).

Because of the threat that allergens pose to human health, many scientists have been working on strategies to reduce allergenicity, including physical methods, such as heat processing (Lasekan & Nayak, 2016), irritation (Lemon-Mulé et al., 2008; Liu et al., 2017), and ultra-high pressure (Liang, Xu, Pan, Ge, & Zong, 2015); chemical methods, such as the Maillard reaction (Li, Jiang, You, Luo, & Feng, 2014; Li, Offengenden, Fentabil, Gänzle, & Wu, 2013), and enzymatic hydrolysis (Mine, 1995), and biological methods, such as traditional fermentation (Moon & Song, 2001), or gene silencing (Kovacs-Nolan, Phillips, & Mine, 2005; Nicolai & Durand, 2013). These methods have diverse effects on allergens based on different principles, even leading to the formation of new allergenic compounds via the Maillard reaction (Rahaman, Vasiljevic, & Ramchandran, 2016; Yu, Goktepe, & Ahmedna, 2013). Among all of these strategies, thermal processing has been verified to be a promising tool that can lead to the destruction of the secondary and tertiary structures of proteins, along with disulfide bond cleavage and new disulfide bond formation, thereby masking protein epitomes and reducing the allergenicity of the allergen (Rao et al., 2016). Therefore, many studies have investigated the thermal effects on common allergens, primarily the allergens in peanut (Rao et al., 2016), soybean protein (Li, Zhu, Zhou, Peng, & Guo, 2016), milk (Sampson, 2004; Xu, Shi, Yao, Jiang, & Luo, 2015), wheat, and almond (Taheri-Kafrani et al., 2009), as well as egg OVT (Fu et al., 2010; Tong, Gao, Chen, & Li, 2012), at different temperatures. Due to their complex conformational structure, different allergens exhibit different changes in their characteristics. Regarding the allergens in egg, Tong et al. investigated the effect of the mild temperature on hen OVT. The OVT structure unfolded when heated to 55–60°C, and the secondary structure disappeared when heating to 70–80°C. Conversely, the allergenicity of OVT was enhanced under these conditions as the protein structure expanded and disulfide bonds fractured (Williams, Elleman, Kingston, Wilkins, & Kuhn, 1982).

Ultra-high-pressure treatment, which can reduce the molecule spacing and change the electronic and crystal structures, while maintaining the original nutritional value, colour, and flavour of the food (Rastogi, Raghavarao, Balasubramaniam, Niranjan, & Knorr, 2007), has also been used to treat allergens. Liang found a positive effect on pineapple juice, with a reduction in allergenicity that correlated with increasing pressure and a maximum reduction of approximately 20% under 500 MPa (Liang et al., 2015). A greater reduction of the major peanut allergens and IgE-binding capacity was induced when using a high-pressure treatment at 500 MPa combined with polyphenol oxidase (Chung, Houska, & Reed, 2013). Acero-Lopez et al. (2012) reported that OVT treated with high pressure and different pH levels resulted in changes in the sulfhydryl groups, surface hydrophobicity, enthalpy, and intrinsic fluorescence, along with partial aggregation. Our previous study also showed that high-pressure treatment at 500 MPa with water significantly reduced the solubility and immunoreactivity of almond proteins (Zhang, Zhang, Sheng, Wang, & Fu, 2016).

The present study aimed to systematically investigate the effects of general food processing, including heat, autoclave sterilization, and ultra-high pressure, on the solubility, primary structure, and immunoreactivity of OVT and OVM proteins, specifically concerning the combined effect of temperature, water and high pressure.

Materials and methods

Dry heat treatment

OVT (20 mg) or OVM (20 mg) powder (both from Sigma-Aldrich, St. Louis, MO, USA) was weighed into glass tubes or crucibles and heated in an oil bath (Yuhua, He'nan, China) at 100°C or 150°C or in a muffle furnace (Zhonghuan, Tianjin, China) at 200°C for 10 min.

Moist heat treatment

OVT (20 mg) or OVM (20 mg) powder was weighed into tubes, dissolved in 2 mL of PBS, and then heated in an oil bath at 60°C or boiled for 10 min, respectively.

Autoclave sterilization

OVT (20 mg) or OVM (20 mg) powder was weighed into 15-mL centrifuge tubes and autoclaved in an HVA-110 autoclave (Hirayama, Kasukabe, Saitama-ken, Japan) at 121°C and 0.15 MPa for 10 min. The same quantity of each protein was also dissolved in 2 mL of PBS as a moist sample and subjected to autoclave treatment as described above.

High-pressure treatment

OVT (20 mg) or OVM (20 mg) powder was weighed into plastic pouches. Half of the samples were dissolved in 2 mL of PBS and then hot-sealed. The samples were then subjected to high-pressure treatment in an HPP.L2-600/0.6 high-pressure system (Huatai, Tianjin, China) at 400, 500, or 600 MPa of hydrostatic pressure and 20°C for 10 min.

Protein extraction of the treated samples

After treatment, 2 mL of PBS was added to the solid sample, which was then mixed on a shaker (30 rpm/min) for 5 min. Then, the sample was centrifuged at 10,000*g* for 10 min, and the supernatant was removed for further use.

Determination of the protein recovery

The protein concentration in the supernatant was determined using a bicinchoninic acid (BCA) Protein Assay Kit (Solarbio Scientific Co., Beijing, China), according to the manufacturer's instructions. The protein recovery was calculated as the ratio of the protein concentration in the treated samples to those in the control sample.

Determination of the immunoreactivity of OVT and OVM by indirect competitive enzyme-linked immunosorbent assay (IC-ELISA)

IC-ELISA was used to evaluate the immunoreactivity changes in the target proteins. The detailed procedure of IC-ELISA for OVT or OVM is as follows.

OVT (0.03 μg) or OVM (0.05 μg) in 100 μL of coating buffer (50 mM carbonate buffer, pH 9.6) was added to each well of a 96-well plate (Thermo Scientific, Rochester, MN,

USA), which was then incubated at 4°C for 12–16 h. The plate was then washed with washing buffer (0.05% Tween 20 in PBS solution, pH 7.4, 200 µL/well) for three times. Two hundred microlitres of blocking buffer (0.5% skimmed milk powder in PBS, pH 7.4) was added to each well and incubated at 37°C for 1 h to block the unbound sites. After three washes with washing buffer, 50 µL of PBS was added into the control wells, and 100 µL of PBS was added into the blank wells. Fifty microlitres of serially diluted OVT or OVM samples (quantified by BCA assay kit after treatment) was added into each well (triplicate wells for each diluted sample) except the blank well, followed by 50 µL of diluted rabbit anti-OVT (1:10,000 dilution) or rabbit anti-OVM (1:40,000 dilution) antibodies (generated in-house) in PBS, and then incubated for 1 h at 37°C. After four washes, 100 µL of HRP-labelled goat anti-rabbit IgG (Promega, Madison, WI, USA) diluted at 1:10,000 with PBS was added, and the mixture was incubated at 37°C for 30 min. After three washes, 100 µL of a TMB substrate solution (1.25 mM 3,3′,5,5′-tetramethylbenzidine-1.6 mM hydrogen peroxide in acetate buffer, pH 5.0) was added to each well, and the mixture was incubated for 15–30 min. Finally, 50 µL of a stop solution (1.25 mol L^{-1} H_2SO_4) was added into each well.

A microplate reader (Thermo Scientific) recorded the absorbance at 450 nm (test wavelength) and 650 nm (reference wavelength). Inhibition curves were established by graphing the inhibition values versus the concentrations of the tested samples. The inhibition ratio was calculated using the following equation:

$$\%\text{inhibition} = \frac{A_0 - A_2}{A_0 - A_1} \times 100$$

where A_0 is the absorbance of the control sample, A_2 is the absorbance of the tested sample, and A_1 is the absorbance of the blank well. The protein concentration that gives 50% inhibition (IC_{50}) was obtained from the OVT or OVM inhibition curve.

Electrophoresis

The protein profiles were analysed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The supernatants of the treated proteins were diluted to 1 mg/mL and boiled in gel loading buffer (Bio-Rad Laboratories, Richmond, CA, USA) for 5 min. Ten microlitres of the boiled samples were then loaded onto the pre-prepared gel (stacking gel was 5% acrylamide, separating gel was 12% acrylamide; Bio-Rad Laboratories, Richmond, CA, USA) with an electrophoresis buffer (0.025 M Tris/HCl buffer containing 0.1% SDS and 0.192 M glycine). The electrophoresis voltage was constant at 80 V. The gel was stained and destained according to Zhang (2016).

Experimental design and statistical analysis

All the treatments were carried out in three independent experiments. For each trial, the protein extracts were analysed using the BCA assay and immunoassay in triplicate wells. Analysis of variance between the normalized mean values was carried out using Duncan's test in IBM SPSS (version rel. 19.0, 2010) with a significance level of $P < .05$.

Statement of human and animal rights

No experiments were related to human or animal rights.

Results and discussion

OVT and OVM were subjected to various conditions that usually occur during home cooking or industrial food manufacturing, including thermal and non-thermal processing. Water was also considered under the same conditions to investigate whether it had any effect on the proteins in question. Water and pressure treatment were also combined with thermal treatment to investigate any potential synergistic effects. The solubility, immunoreactivity, and protein structure were evaluated by determining the protein recovery, immunoassay IC_{50} and SDS-PAGE profile.

Effect of processing on the solubility of OVT protein

The solubility of a protein is an important indicator of whether the protein is denatured or coagulated. Changes in protein quantity were used to assess changes in the solubility of the treated OVT and OVM proteins. The results were normalized and calculated as the ratio of the values of treated samples to those of untreated samples to avoid data fluctuations between different experiments.

The changes in the solubility of the processed OVT proteins are shown in Table 1. After dry heating at 100°C, the relative recovery of OVT significantly decreased ($P < .05$)

Table 1. Effect of processing on the solubility of OVT and OVM.

	OVT		OVM	
Food processing	Protein concentration (mg L^{-1}, mean ± SD)	Relative protein recovery (mean ± SD)	Protein concentration (mg L^{-1}, mean ± SD)	Relative protein recovery (mean ± SD)
Unprocessed	10.0 ± 0.136	1.00^{a}	9.99 ± 0.0912	1.00^{a}
Dry heat (°C)				
100	8.71 ± 0.0792	0.87 ± 0.0473^{b}	8.29 ± 0.137	0.830 ± 0.0503^{bc}
150	7.41 ± 0.169	0.74 ± 0.0351^{c}	7.79 ± 0.159	0.779 ± 0.0656^{cd}
200	0.30 ± 0.0846	0.03 ± 0.0115^{d}	0.40 ± 0.0823	0.04 ± 0.0058^{e}
Unprocessed	9.97 ± 0.128	–	10.0 ± 0.0932	–
Moist heat (°C)				
60	8.37 ± 0.0833	0.84 ± 0.0709^{b}	8.72 ± 0.125	0.872 ± 0.0379^{b}
100	0.30 ± 0.119	0.03 ± 0.0153^{d}	8.12 ± 0.1004	0.812 ± 0.0451b^{c}
Unprocessed	10.2 ± 0.153	–	9.91 ± 0.118	–
Autoclaving				
Dry	0.20 ± 0.0916	0.02 ± 0.0057^{d}	7.04 ± 0.0157	0.717 ± 0.0404^{cd}
Moist	0.05 ± 0.113	0.005 ± 0.0058^{e}	6.64 ± 0.0138	0.676 ± 0.0950^{d}
Unprocessed	9.82 ± 0.0527	1.00^{a}	10.00 ± 0.0815	1.00^{a}
Dry pressure (MPa)				
400	8.84 ± 0.0812	0.90 ± 0.056^{a}	10.30 ± 0.0923	1.03 ± 0.157^{a}
500	8.84 ± 0.0935	0.90 ± 0.084^{a}	9.80 ± 0.138	0.98 ± 0.134^{a}
600	8.54 ± 0.103	0.87 ± 0.119^{a}	10.00 ± 0.119	1.00 ± 0.136^{a}
Moist pressure (MPa)				
400	8.54 ± 0.0792	0.87 ± 0.079^{a}	10.50 ± 0.123	1.05 ± 0.146^{a}
500	8.94 ± 0.0986	0.91 ± 0.106^{a}	10.10 ± 0.194	1.01 ± 0.115^{a}
600	7.46 ± 0.132	0.76 ± 0.073^{b}	9.80 ± 0.0934	0.98 ± 0.122^{a}

Note: "–": not applicable.

Relative protein recovery was analysed by one-way ANOVA (Duncan). Values with the same letters in the same column are not significantly different. Lowercase letters represent a $P = .05$ level of significance.

compared with the control. The differences between the protein samples processed at 150°C and 200°C were also significant ($P < .05$); when heated at 200°C, almost all protein was precipitated, and the protein recovery was very low, indicating that heat has a strong effect on OVT.

The protein recovery of OVT under moist heat at 60°C was similar to that observed after the dry heat treatment at 100°C. After the boiling water treatment, the relative protein recovery significantly decreased ($P < .05$), similar to the result obtained after dry heat treatment at 200°C, showing that moist heat has a greater effect on OVT than dry heat.

Autoclaving also exerted a strong effect on the solubility of OVT proteins (Table 1). The treated proteins were mostly precipitated and difficult to re-dissolve in PBS. To investigate the precipitated protein, an 8 M urea solution was added, but all the precipitates did not re-dissolve and likely formed higher molecular aggregates via inter-molecular cross-linking. Compared with the moist heat treatment, autoclaving caused a dramatic change in the protein properties, which may have been due to the combined effect of pressure and temperature.

The effect of high pressure on the solubility of OVT protein is also shown in Table 1. The protein recovery of OVT from the different pressure treatments was not significantly different from that in the control group ($P > .05$), except in the treatment with 600 MPa and moist heat, where a small amount of protein coagulated and could not be re-dissolved in PBS. Therefore, ultra-high pressure had a limited effect on the solubility of OVT.

Effect of processing on the solubility of OVM protein

Under dry heat treatment, the trend of the OVM protein recovery was similar to that of OVT; under dry heat at 200°C, the protein precipitated, and very low recovery was obtained (Table 1). However, in the moist heat and autoclave treatments, the protein recovery of OVM decreased significantly compared with that of the unprocessed sample ($P < .05$; Table 1). However, there was no significant difference within the results of these treatments, and higher recoveries were obtained compared with the recoveries of OVT, indicating that OVM is more stable than OVT in moist heat and autoclave treatments.

In the high-pressure treatment, neither dry nor moist pressure could significantly change the solubility of OVM ($P > .05$; Table 1), indicating that OVM is stable under high pressure.

The relative protein recovery of OVM was always higher than that of OVT; therefore, OVM is more stable than OVT during heat treatment, regardless of the application of dry or moist heat or concurrent autoclaving or ultra-high-pressure treatments.

Effect of processing on the immunoreactivity of OVT

IC-ELISA was used to evaluate the immunoreactivity of the allergens. The change in immunoreactivity after various treatments can be estimated from the IC_{50} determined from ELISA based on the specific reaction between the OVT or OVM allergen and their cognate antibodies. Structural alteration of the target proteins can change the immunoreactivity, with an increased IC_{50} representing reduced immunoreactivity. To avoid data

fluctuations between different experiments, relative IC_{50} values were calculated as the ratio of the IC_{50} of the processed protein to those of unprocessed samples.

The relative IC_{50} (Table 2) of OVT was not significantly affected by dry heating at 100°C ($P > .05$) but obviously increased when heated to 150°C ($P < .05$), indicating that the immunoreactivity of OVT was significantly reduced. Spatial structural changes in OVT under these conditions may result in some epitopes being destroyed. When heated to 200°C, the IC_{50} was undetectable, indicating no identified epitopes, which may due to the protein aggregation besides the destroyed epitopes.

After exposure to moist heat at 60°C or 100°C, the IC_{50} of OVT significantly increased ($P < .05$) to an even greater extent than that in the dry heat treatment, indicating that moist heat has a greater effect on immunoreactivity than dry heat. In the autoclaved (121°C, 0.15 MPa) samples, whether treated as a solid or in PBS solution, the protein completely precipitated and could not be re-dissolved in PBS; therefore, no IC_{50} values could be determined. These results also suggest a synergistic influence of the temperature and water on immunoreactivity, resulting in greater attenuation of immunoreactivity than that observed with heat alone.

In the high-pressure treatment, no obvious differences in the IC_{50} values of the processed and unprocessed samples were observed, regardless of whether moist or dry pressure was applied ($P > .05$). These results are inconsistent with those obtained for OVT solubility, which decreased with increasing pressure, even at 600 MPa of moist pressure. High pressure likely did not damage the epitopes of the OVT that remained in the aqueous solution, further verifying that ultra-high pressure has a limited effect on the characteristics of OVT (without considering the undissolved OVT).

Table 2. Effect of food processing on the immunoreactivity of OVT and OVM.

	OVT		OVM	
Food processing	IC50 (mg L^{-1}, mean ± SD)	Relative IC50 (mean ± SD)	IC50 (mg L^{-1}, mean ± SD)	Relative IC50 (mean ± SD)
Unprocessed	0.37 ± 0.163	1.00^{d}	1.11 ± 0.131	1.00^{c}
Dry heat (°C)				
100	0.41 ± 0.139	1.11 ± 0.162^{cd}	0.78 ± 0.147	0.703 ± 0.0800^{de}
150	0.47 ± 0.0972	1.28 ± 0.159^{c}	0.37 ± 0.103	0.333 ± 0.0628^{f}
200	n.d.	–	3.42 ± 0.0812	3.08 ± 0.530^{b}
Unprocessed	0.35 ± 0.0891	–	1.28 ± 0.0859	–
Moist heat (°C)				
60	0.49 ± 0.112	1.40 ± 0.0451^{bc}	1.04 ± 0.168	0.813 ± 0.0361^{cd}
100	1.00 ± 0.125	2.86 ± 0.161^{a}	0.81 ± 0.141	0.633 ± 0.0451^{e}
Unprocessed	0.35 ± 0.0844	–	1.28 ± 0.169	–
Autoclaving				
Dry	n.d.	–	0.56 ± 0.173	0.437 ± 0.0503^{f}
Moist	n.d.	–	23.85 ± 0.126	18.6 ± 1.11^{a}
Unprocessed	0.32 ± 0.131	1.00^{a}	1.35 ± 0.182	1.00^{a}
Dry pressure (MPa)				
400	0.31 ± 0.0719	0.96 ± 0.132^{a}	1.35 ± 0.0819	1.00 ± 0.132^{a}
500	0.31 ± 0.0976	0.98 ± 0.120^{a}	1.19 ± 0.112	0.88 ± 0.110^{a}
600	0.31 ± 0.157	0.98 ± 0.121^{a}	1.12 ± 0.107	0.83 ± 0.128^{a}
Moist pressure (MPa)				
400	0.32 ± 0.135	1.00 ± 0.097^{a}	1.30 ± 0.0935	0.96 ± 0.153^{a}
500	0.35 ± 0.0932	1.08 ± 0.137^{a}	1.27 ± 0.0138	0.94 ± 0.117^{a}
600	0.31 ± 0.0812	0.97 ± 0.155^{a}	1.05 ± 0.169	0.78 ± 0.111^{b}

Note: n.d.: no result could be detected; "–": not applicable.

Relative IC_{50} values were analysed by one-way ANOVA (Duncan). Values with the same letters in the same column are not significantly different. Lowercase letters represent a $P = .05$ level of significance.

Effect of processing on the immunoreactivity of OVM

Processed OVM produced unexpected results with regards to immunoreactivity (Table 2). The IC_{50} values of OVM obviously decreased after exposure to dry heat at 100°C and 150°C and moist heat at 100°C ($P < .05$), indicating that the immunoreactivity of OVM had significantly increased. Because OVM contain linear epitopes that, when heated within a certain temperature range, become exposed as the spatial structure of OVM decomposes, thus increasing the binding sites for specific antibodies and leading to the observed enhancement in immunoreactivity. Upon heating at 200°C, the immunoreactivity significantly decreased ($P < .05$) possibly due to the destruction of the primary structure of OVM, including the linear epitopes.

After autoclaving (121°C, 0.15 MPa), different samples showed opposing immunoreactivities (Table 2). The IC_{50} values of solid OVM significantly decreased ($P < .05$), whereas the IC_{50} of OVM in PBS solution significantly increased ($P < .05$), indicating that water plays an important role in the denaturation of OVM protein. Serving as an energy transfer agent, water increases the effect of heat and pressure on the protein, leading to significant changes in the protein structure and thus immunoreactivity.

The immunoreactivity of OVM under high-pressure processing was similar to that observed for OVT, except in the treatment consisting of 600 MPa of moist pressure. In this treatment, the IC_{50} values of OVT remained unchanged, indicating unchanged epitopes, whereas those of OVM decreased (Table 2), indicating increased immunoreactivity. The inconsistent changes in protein solubility under the same processing conditions further verified that OVM possesses linear epitopes. With the synergistic treatment of extremely high pressure and water, the spatial structure of OVM was destroyed, the linear epitopes were exposed, and the immunoreactivity was enhanced.

SDS-PAGE analysis of OVT after the different treatments

The structures of OVT and OVM after the different treatments were evaluated by SDS-PAGE. SDS and β-ME destroy the secondary and tertiary structures of the protein, respectively; therefore, the SDS-PAGE profile reveals changes in the primary structure of proteins.

Figure 1 shows the protein/peptide profiles of OVT following different treatments. Most bands remained unchanged (lanes 1, 2, 4, and 9–14) compared with the untreated sample (lane 15), indicating that, the structure of OVT was not destroyed by treatment with dry heat at 100°C, moist heat at 60°C, or high pressure at 400–600 MPa. Higher molecular weight bands appeared after dry heat treatment at 150°C and 200°C and boiling (lanes 2, 3, and 5); in particular, more protein was observed with dry heating at 200°C, and aggregation was observed with boiling, verifying the hypothesis that protein tends to form aggregates under high temperature. The solubility of the autoclaved proteins was very low, so the extraction had to be concentrated to achieve the same content as in the other samples. However, no band was observed. It was supposed due to the small molecular weight protein or the soluble aggregated proteins, which cannot be invisible in the normal SAS-PAGE.

Based on the unchanged primary structure, in combination with the solubility and decreased immunoreactivity after treatment at 100°C, 150°C, and moist 60°C, it may be

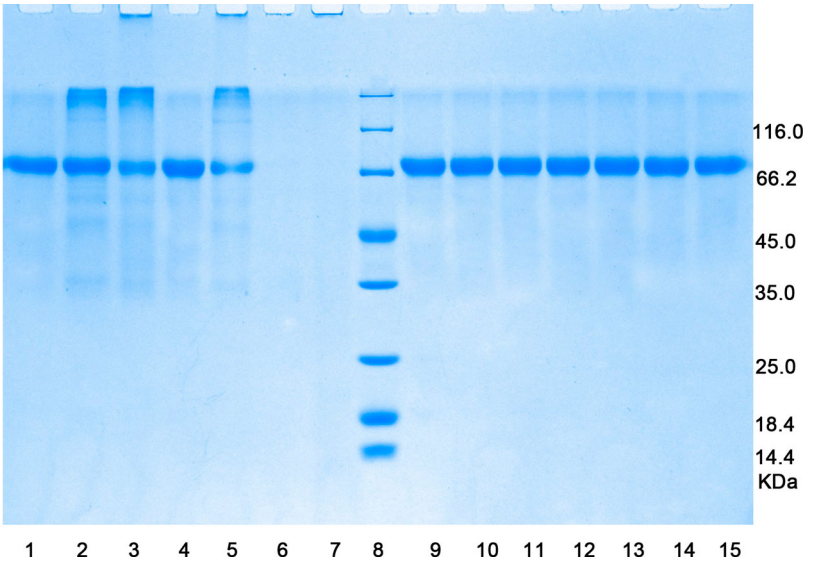

Figure 1. SDS-PAGE patterns of OVT after different treatments. Lane 1, Dry heat at 100°C; 2, dry heat at 150°C; 3, dry heat at 200°C; 4, moist heat at 60°C; 5, moist heat at 100°C; 6, dry autoclave sterilization; 7, moist autoclave sterilization; 8, marker; 9, dry high-pressure processing at 400 MPa; 10, moist high-pressure processing at 400 MPa; 11, dry high-pressure processing at 500 MPa; 12, moist high-pressure processing at 500 MPa; 13, dry high-pressure processing at 600 MPa; 14, moist high-pressure processing at 600 MPa; 15, untreated sample.

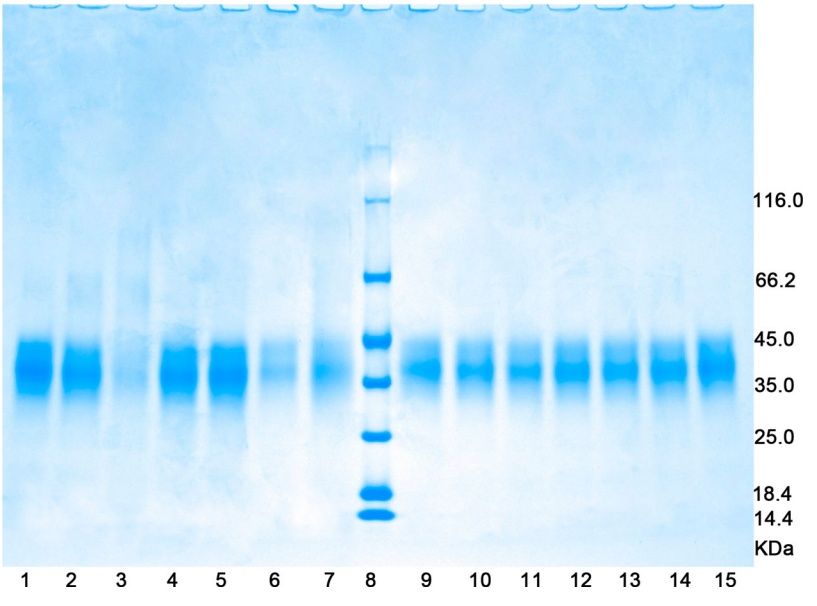

Figure 2. SDS-PAGE patterns of OVM after different treatments. Lane 1, Dry heat at 100°C; 2, dry heat at 150°C; 3, dry heat at 200°C; 4, moist heat at 60°C; 5, moist heat at 100°C; 6, dry autoclave sterilization; 7, moist autoclave sterilization; 8, marker; 9, dry high-pressure processing at 400 MPa; 10, moist high-pressure processing at 400 MPa; 11, dry high-pressure processing at 500 MPa; 12, moist high-pressure processing at 500 MPa; 13, dry high-pressure processing at 600 MPa; 14, moist high-pressure processing at 600 MPa; 15, untreated sample.

concluded that the conformational epitopes of OVT had been destroyed, making it impossible to bind with specific antibodies.

SDS-PAGE analysis of OVM after different treatments

Figure 2 shows the SDS-PAGE profiles of OVM after the different treatments. The profiles of OVM determined after dry heating at 200°C (lane 3) and under dry or moist autoclave sterilization (lanes 6 and 7) were obviously different from that of the unprocessed sample (lane 15), which indicates that these treatments can partially or totally destroy the primary structure of OVM protein.

As observed with OVT in the ultra-high-pressure treatments, no significant changes in the SDS-PAGE profiles of OVM were observed after the high-pressure treatment (lanes 9–14), showing that the primary structure of OVM was stable under ultra-high pressure.

Conclusion

Heating, autoclaving, and ultra-high-pressure treatments had different effects on the characteristics of OVT and OVM allergens in egg. Temperature was the key factor that significantly contributed to the changes in immunoreactivity of OVT and OVM proteins, which were induced by the structural alteration. Autoclave sterilization had a substantial effect on the solubility, immunoreactivity, and structure of both OVT and OVM, whereas high pressure exerted a moderate effect that did not alter the primary structures of OVT or OVM, nor the solubility or immunoreactivity significantly. Based on all the determined characteristics of OVT and OVM after these various treatments, OVM was more stable than OVT under the same treatment.

The responses of the solubility, immunoreactivity, and structure of the tested allergens were consistent under the same processing conditions. The protein structure determined the solubility and immunoreactivity of these allergens. Any processing that alters the structure of the protein could alter the immunoreactivity and solubility. Therefore, synergistic processing with temperature, water, and high pressure had the greatest effect on the characteristics of OVT and OVM. This information could be used to mitigate the negative effects of allergens in egg.

Disclosure statement

No potential conflict of interest was reported by the authors.

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