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Analysis of epitopes and structural responses in egg allergen Gal d 1 using bioinformatic tools and molecular dynamics simulation

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ABSTRACT

Egg allergy is a growing concern worldwide. Investigating the allergenic and molecular characteristics of egg allergen Ovomucoid (Gal d 1) can enhance our understanding of egg allergies. In this study, the B-cell linear epitopes of Gal d 1 were predicted by using different bioinformatic tools, based on the primary sequence properties of Gal d 1, and obtained 10 potential B-cell linear epitopes. Meanwhile, we conducted molecular dynamics (MD) simulations to apply thermal and an oscillating electric field treatments to Gal d 1 in order to comprehend the structural alterations of Gal d 1 intuitively. The results indicated that Gal d 1 was a thermally stable protein, while the secondary structure and surface characteristics of Gal d 1 were obviously influenced by the combination of thermal stress and electric field, which finally resulted in conformational changes. This study provided a new way to understand the development of hypoallergenic egg products.

1. Introduction

Food allergies (FAs) are defined as an adverse health effect arising from a specific immune response that occurs reproducibly on exposure to a given food (Sicherer, 2011). This definition includes both Immunoglobulin E (IgE)-mediated FAs and non-IgE-mediated FAs. And a large proportion FAs are IgE-mediated, which mainly be caused by egg, peanut, milk, fish, tree nuts, sesame, crustacean, and cereal (Zeng et al., 2024). FAs have become a condition of growing concern around the world, which almost affected people of patients of all ages (Sampath et al., 2021; Warren et al., 2020). The prevalence rates and healthcare utilization of FAs seemingly increasing over the past few decades across much of the globe. As reported, FAs influenced approximately 8 % of children and 3 %–10 % of adults globally (Messina and Venter, 2020). Hence, FA is a significant food safety and public health concern in both developed and developing nations. Eggs, as one of the best dietary sources of high-grade protein, contain several nutrients that help prevent chronic disease, such as selenium, vitamin D, lutein, and vitamin (Breškić Ćurić et al., 2023; Myers and Ruxton, 2023). Among FAs, egg allergy (EA) has become a common condition and affected up to 9 % of children worldwide on average (Tan et al., 2017). It was proved that incidence of EA ranked as the second leading FA among young children in Europe Spolidoro et al. (2023). Luo et al. (2019) additionally documented that nearly 10 % of children in southern region of China were allergenic to eggs. Gal d 1, which makes up 11 % (w: w) of the proteins in egg whites, is believed to be the dominant allergen in egg (Ma et al., 2020). A study reported that the Gal d 1 was identified as the primary and most allergenic protein among patients (Palosuo et al., 2018), which is typically linked to the increased likelihood of systemic and or severe reactions (Ansotegui et al., 2020).

Currently, the most efficacious method to avert EA is to abstain from consuming eggs and its products. But eggs are unavoidable in food processing application and daily life. Therefore, the attention has shifted towards food processing techniques aimed at diminishing the allergenicity of eggs. Wen et al. (2022) indicated that superheated steam reduced allergenicity of the Gal d 1 due to modifications of functional groups and amino acids as well as alteration of protein structure. Xia et al. (2024) discovered that the IgE/IgG binding capacities of the Gal d 1 were reduced after the Maillard reaction with maltose. Nevertheless, the allergenicity of protein is subject to variation based on factors such

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as the biochemical properties of protein, matrix, application conditions, processing technique, and individual sensitivity of each patient. Jiménez-Saiz et al. (2011) reported that glycation with glucose enhanced IgE-binding capacity of the Gal d 1 to sera of patients, due to the formation of new epitope. Different food processing environments may cause food products to show varying levels of allergenicity (Jiaqi & Yanjun, 2023). Therefore, it is crucial to select reasonable food processing methods to reduce the allergenicity of the Gal d 1. Recent years, there has been a surge of interest in the application of MD simulation technology to simulate food processes. And the MD provided us with novel insights into the effects of temperature, ionic strength, solvent composition, pH, and electric field exposure on the denaturation of proteins in a variety of foods (Smith et al., 2022). Compared with traditional structural analysis techniques of protein, MD simulation not only focus on the structural changes of target protein after the end of food processing, but also the trend of structural modifications during food processing (Singh et al., 2018). Vanga et al. (2019) simulated the alterations in the structural properties of soybean allergen Gly m 4 by using MD simulations under the temperature and pressure deviations, which provided theoretical foundation for exploring the changes of soybean allergen epitope. Zhang et al. (2023) analyzed the structural changes of peanut protein induced after cross-links by MD simulation. In addition to improving experimental design efficiency and reducing costs and time, MD simulation can also facilitate ongoing learning and improvement. Hence, MD simulation not only offers a novel way to explore the details of structural changes that can aid in comprehending the characteristics and structural changes of protein, but also provides a basis for screening the suitable food processing to reduce the allergenicity of allergens. Meanwhile, the prediction of B cell linear epitopes of allergenic proteins by bioinformatics has become the main method to research B cell epitopes. Huang et al. (2021) predicted the antigenic epitopes of the major egg yolk allergens by bioinformatics method, and finally predicted 6 B cell epitopes of the Gal d 5 and 4 B cell epitopes of the Gal d 6. This method not only saves the time and experiment cost, but also has high efficiency.

In the present study, we explored the alteration of structure and potential influence on epitopes of the Gal d 1 b y combining MD simulation and bioinformatic tools. We firstly predicted the B cell linear epitopes of the Gal d 1 b y bioinformatic tools. Subsequently, we used MD simulations to evaluate the structural alterations of the Gal d 1 induced by temperature and electric field variations. The root mean square deviation (RMSD), root mean square fluctuations (RMSF), solvent-accessible surface areas (SASAs), and the B-factor were measured. This study provided a further and innovative understanding in the structural changes and potential effects on epitopes of the Gal d 1 allergen as a result of processing, which had a potential application in the reduction of food allergenicity of egg.

2. Materials and methods

2.1. Bioinformatics predict epitopes

The complete amino acid sequence of Gal d 1 (GenBank: P01005.1) was obtained from the NCBI database. The signal peptide prediction analysis was analyzed by SignalP 6.0 Server. To predict B cell linear epitopes of Gal d 1, the complete sequences of Gal d 1 was analyzed by using five immunoinformatic tools based on computational approaches including Immunomedicine Group, ABCpred, BCEpred, BepiPred-3.0 Server and DNAStar. The Immunomedicine Group and the ABCpred server predicted the B-cell linear epitopes were followed method of Kolaskar and Tongaonkar (1990), and Saha and Raghava (2006) respectively. The identification of uninterrupted B-cell linear epitopes in BCEpred server was determined by physico-chemical properties of sequences (Saha and Raghava, 2004). Bepipred server predicted the B-cell linear epitopes by using neural networks trained on state-of-the-art protein language embeddings (Clifford et al., 2022). In the DNAStar Protean software, epitope prediction was informed by four critical characteristics of amino acid sequences: hydrophilicity, flexibility, accessibility, and antigenicity. Peptide exhibited suitable hydrophilicity, elevated flexibility, enhanced surface exposure, and a significant antigenic index were identified as linear epitopes using the DNAStar system. On the other hand, Immunomedicine Group, BepiPred-3.0, ABCpred, and BCEpred identify B cell linear epitopes by considering the combination of physicochemical properties of amino acids, including the hydrophilicity, secondary structure, flexibility, exposed surface, accessibility, polarity, turns, and antigenic propensity. Finally, the data from the five bioinformatic tools were integrated, and allergenic epitopes identified by at least 3 of these tools as potential epitopes were regarded as candidates.

2.2. MD simulations

Based on prior researches, MD simulations were conducted utilizing Groningen Machine for Chemical Simulations (GROMACS) software (version 2021.5, Stockholm Center for Biomembrane Research, Stockholm, Sweden) based on the classical MD algorithm. Gal d 1, one of the major allergens in eggs, was the subject of the simulations. The structure of Gal d 1 was predicted by AlphaFold and was numbered AF-P01005-F1. OPLS-AA (Optimized Potentials for Liquid Simulations-All Atom, OPLS-AA) force field is adopted. The PDB file was verified to confirm that all the required atoms were present. The topology file is generated by the pdb2gmx module. The protein was solvated with water model SPC216. And the ions were added according to the charge on the protein to ensure that the electroneutral system was used for simulation. The structure was then relaxed through the energy minimization (EM) process which carried out by the steepest descent method (50000 steps with a maximum force of 1000 kJ/mol/nm).

The system was subjected to two stages of equilibration: a 400 ps (ps) simulation under conditions of constant particle number, volume, and temperature (NVT), followed by a simulation under conditions of constant particle number, pressure, and temperature (NPT). Following the completion of the two equilibration stages, a 100 ns (ns) MD simulation was executed for the purpose of gathering data. Throughout the MD simulations, the Berendsen thermostat was employed to regulate the target temperatures, and the Parrinello-Rahman barostat was utilized to maintain the pressure at a level of 1 bar. The temperatures were capped at a maximum of 298 K (25 °C), 313 K (40 °C), 333 K (60 °C), 353 K (80 °C), and 373 K (100 °C) to simulate the thermal processing, and were named as 298 K, 313 K, 333 K, 353 K, and 373 K respectively. In addition, an external oscillating electric field, with a strength of 0.5 V/nm, was introduced to the systems at various temperatures to mimic the microwave processing. These systems were designated as 298 K + E, 313 K + E, 333 K + E, 353 K + E, and 373 K + E respectively. Visual Molecular Dynamics (VMD) software (version 1.9.3, University of Illinois, Urbana-Champaign, USA) and PyMOL software (version 2.5.2, Schrödinger, New York, NY, USA) were used to compare the differences of structure between the Gal d 1 at various temperatures and electric fields.

2.3. Measurement of RMSD

In the field of structural biology, arguably the most common metric for comparing structures is the RMSD of atomic positions, which is typically calculated after applying a least-squares superposition to account for rotational and translational differences (Kuzmanic and Zagrovic, 2010). Recently, Zhang et al. (2022) analyze and predicted the structural changes of whey proteins under different temperatures by RMSD. The subsequent formula was applied to determine the RMSD of the egg protein Gal d 1:

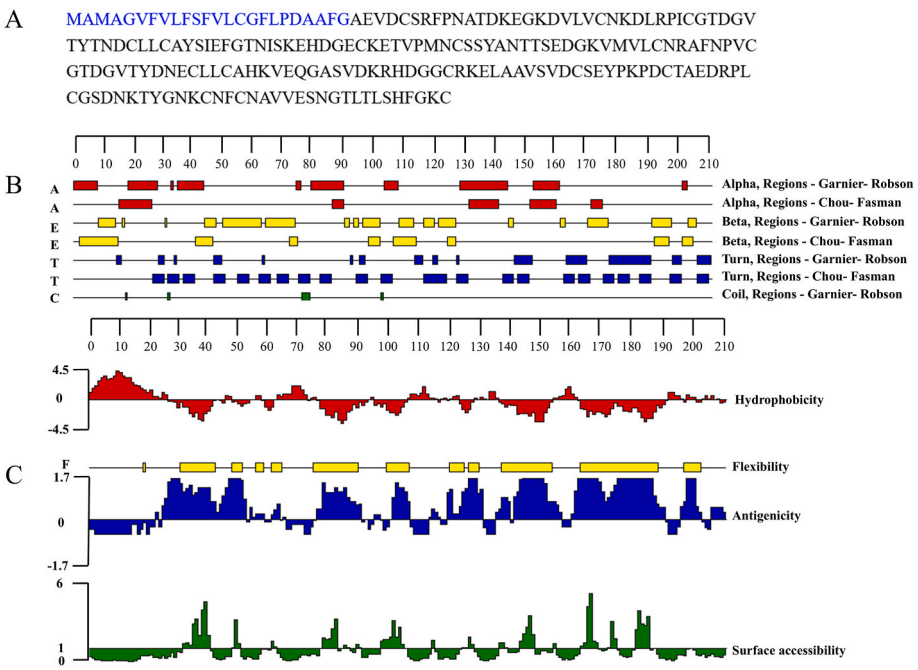

Fig. 1. Prediction of B-cell linear epitopes of Gal d 1 using bioinformatic tools.
(A) Prediction of Gal d 1 signal peptide using the SignalP-6.0 server, the blue letters were the predicted signal peptide sequence. (B) Prediction of secondary structures of Gal d 1 using the DNAStar Protean system. (C) Prediction of primary sequence of Gal d 1 using the DNAStar Protean system.

$$RMSD = \sqrt{\frac{1}{N}\sum_{i=1}^{N}\left|r_{final}\ (i) - r_{initial}\ (i)\right|^2} \quad (1)$$

Where $r_{final}\ (i)$ represents the final position of an atom i, $r_{initial}\ (i)$ denotes the initial coordinate of the atom i, and N stands for total count of atoms.

2.4. Analysis of RMSF

The RMSF of each system was calculated to understand the displacement and stability of each atom or residue in the simulation trajectory (da Fonseca et al., 2024). During the MD simulations, the RMSF of Gal d 1 was quantified using the subsequent formula:

$$RMSF_i = \sqrt{\frac{1}{T}\sum_{t_i=1}^{T}\left|r_i\ (t_i) - r_i^{ref}\right|^2} \quad (2)$$

Where T denotes the time and r_i^{ref} represents the reference position of atom i.

2.5. Measurement of SASAs

The SASAs of a molecule were aeras which were available to interact with solvents and molecules (Vagadia et al., 2016). The SASAs were commonly utilized to assess the interactions between a protein and solvents, which can provide insights into the protein's characteristics and functions. In this study, the built-in commands of the GROMACS software were employed to determine the alteration of SASAs. The subsequent formula was applied to compute the SASA of the protein Gal d 1:

$$SASA = A = \sum\left(R \Big/ \sqrt{R^2 - Z_i^2} \times D \times L_i\right) \quad (3)$$

Here, A represents the surface area, R is the atomic radius, L_i denotes the length of the arc drawn on a given segment i, and Z_i signifies the perpendicular distance of segment i from the center of the sphere.

2.6. Measurement of B-factor

The B-factor, as known as the temperature factor, quantified the degree of atomic position uncertainty and reflected the spread of atomic electron density within a crystal lattice (Sun et al., 2019). The following equation was used to calculate the B-factor:

$$B-factor = RMSF^2 \times \frac{8}{3}\pi^2 \quad (4)$$

2.7. Structure alteration of Gal d 1

The timeline tool plug-in of VMD software was used to evaluate the secondary structure changes of egg allergen Gal d 1, and the PyMOL software was used to observe the conformational changes of Gal d 1.

2.8. Statistical analysis

The data derived from MD simulations under external thermal and electric field conditions were statistically analyzed using one-way analysis of variance (ANOVA) subsequently subjected to Duncan's multiple range test at a significance level of $P < 0.05$. This analysis was conducted with the SPSS 22.0 analytical software (SPSS Inc., Chicago, USA). The RMSD, and RMSF were computed and graphically represented in 2D using GraphPad (version 8.0, San Diego, California, USA). Each MD simulation and experimental trial was conducted three times to ensure the reproducibility and accuracy of the results.

3. Results and discussion

3.1. Prediction of primary sequence and secondary structure of Gal d 1

As shown in Fig. 1A, the signal peptide region of Gal d 1 located from amino acid (AA) 1–24 which predicted by SignalP-6.0 server, demonstrated a high probability of 99.93 %. Signal peptide regions typically have a lower likelihood of being linear epitopes due to their hydrophobic nature (Upadhyay et al., 2020). As described in Fig. 1B, the Chou-Fasman and Garnier-Robson methods indicated a prevalence of

Table 1
B-cell linear epitopes of Gal d 1 predicted by bioinformatic tools.

Gal d1	Tools	Positions
	Immunomedicine Group	4-21, 23–31, 40–48, 50–56, 61–72, 90–96, 105–121, 126–144, 151–164, 187-198
	ABCpred	24-39, 30–45, 37–52, 54–69, 65–70, 76–91, 87–102, 98–113, 106–121, 117–132, 130–145, 136–151, 157–172, 167–182, 176–191, 182–197, 189-204
	BCEpred	4-20, 27–33, 40–50, 61–68, 105–113, 118–124, 127–133, 158–166, 204-210
	Bepipred	30-45, 47–52, 72–77, 79–83, 87–91, 95–110, 112–117, 134–142, 144–148, 152–161, 163–169, 171–175, 186–190, 203-209
	DNAStar	25-53, 55–64, 76–91, 95–97, 101–107, 113–117, 119–131, 135–157, 160–191, 196–203, 206-210

Table 2
Summary of B-cell linear epitopes of Gal d 1.

Rank	Amino acid sequence	Position
G-1	VDCSRFP	27–33
G-2	GKDVLVCNKDLRP	40–52
G-3	YTND	61–64
G-4	TSEDGKVMVL	101–110
G-5	NRAFNPVCGT	112–121
G-6	DNECLL	127–132
G-7	HKVEQGASVD	135–144
G-8	AVSVDCSEYP	157–166
G-9	NKCN	187–190
G-10	HFGK	206–209

α-helix, β-sheet, and β-turn structures with a minor presence of random coil structures in Gal d 1, which suggested Gal d 1 had a compact structure. In addition, the region of AA 1–24 exhibited hydrophobicity consistent with the signal peptide predictions (Fig. 1C). The high flexibility and surface accessibility regions of Gal d 1 were widespread, which were highly matched with areas of significant antigenicity. The overlapping regions were easy to interact with antibodies, and the probability of linear epitopes was high (Zhao and Li, 2010). The implication of these results is that the Gal d 1 had dense structure and high allergenic potential.

3.2. Prediction of linear epitopes of Gal d 1

Bioinformatic tools predicted the B-cell linear epitopes of Gal d 1 were shown in Table 1. Potential B-cell linear epitopes which were obtained from different bioinformatics tools relied on distinct screening and calculation methods had differences.

Subsequently, the outcomes of the five bioinformatic tools were integrated. The 4 or more bioinformatic tools overlapping regions were screened for Gal d 1 candidate B-cell linear epitopes with AA number ≥3. These candidate epitopes, named as G-1 to G-10 were, respectively (Table 2).

As shown in Fig. 2, the potential B-cell linear epitopes predicting by bioinformatic tools were located in three dimensional models, which included cartoon models and surface models. In cartoon models (Fig. 2A–B), G-1, G-6, G-7, G-8, and G-9 were positioned at both α-helix and random coil structure, G-2, G-5, and G-10 were located at β-sheet and random coil structure, G-3 was lied at α-helix, β-sheet, and random coil structure at the same time, G-4 was located at β-sheet and β-turn structure. Besides, the surface models of Gal d 1 suggested that most potential B-cell linear epitopes were partially positioned on the molecular surface at least, except for G-3. This indicated the potential B-cell linear epitopes were more likely to interact with the antibody. With the development of bioinformatics and the accumulation of allergen knowledge, the accuracy of bioinformatic tools for predicting epitopes

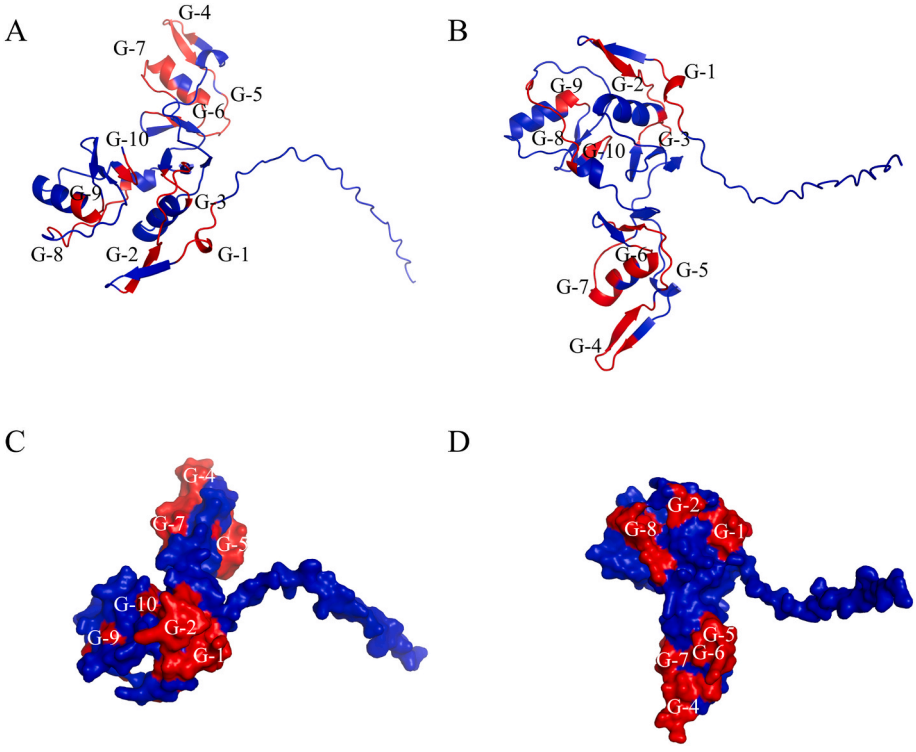

Fig. 2. The spatial location of potential B-cell linear epitopes. (A–B) The cartoon model of Gal d 1. (C–D) The surface model of Gal d 1. The red parts were regions of potential epitopes (G-1 to G-10), the blue parts were regions of common amino acids.

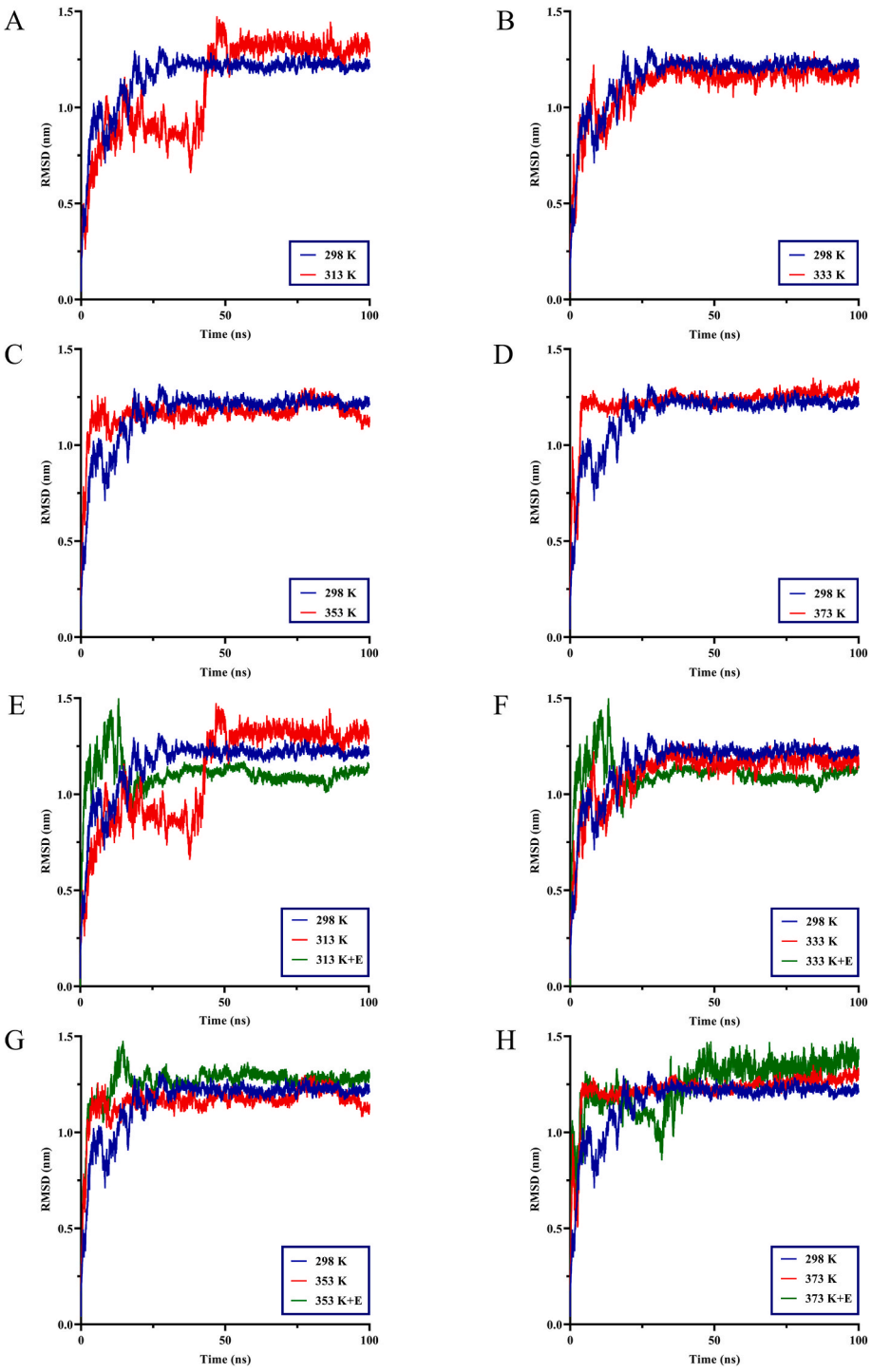

Fig. 3. The variations of RMSD in egg allergen Gal d 1 under thermal processing (A–D) and microwave processing (E–H).

of allergens is increasingly reliable. Fu et al. (2018) applied the multiple bioinformatic tools predicted potential allergenic epitopes of tropomyosin and arginine kinase in Chinese shrimp and also identified the critical amino acids in allergenic epitopes, which provided new targets for immunotherapy of shrimp allergy.

3.3. Analysis of RMSD of Gal d 1

As shown in Fig. 3A–D, the changes of RMSD of Gal d 1 were not obvious when simulated under thermal processing at 313 K, 333 K, and 353 K compared to at 298 K, while the values of RMSD at 373 K were higher than those at 298 K slightly, especially after 75 ns. These results indicated that Gal d 1 produced a slight atomic deviation at 373 K, and no significant change at other relatively lower temperature simulations. This may be due to the presence of disulfide bonds in Gal d 1, causing the Gal d 1 to be thermally stable (Hoffman, 1983). Stănciuc et al. (2018) also proved that there was no significantly change of the antigenicity of Gal d 1 after heating at 353 K and 373 K for 20 min respectively. In addition, the RMSD values were increased obviously when combined with 0.5 V/nm oscillating electric field (Fig. 3G and H). And the tendency of RMSD under 373 K + E was continued to increase compared with 353 K + E. The above results indicated that thermal processing

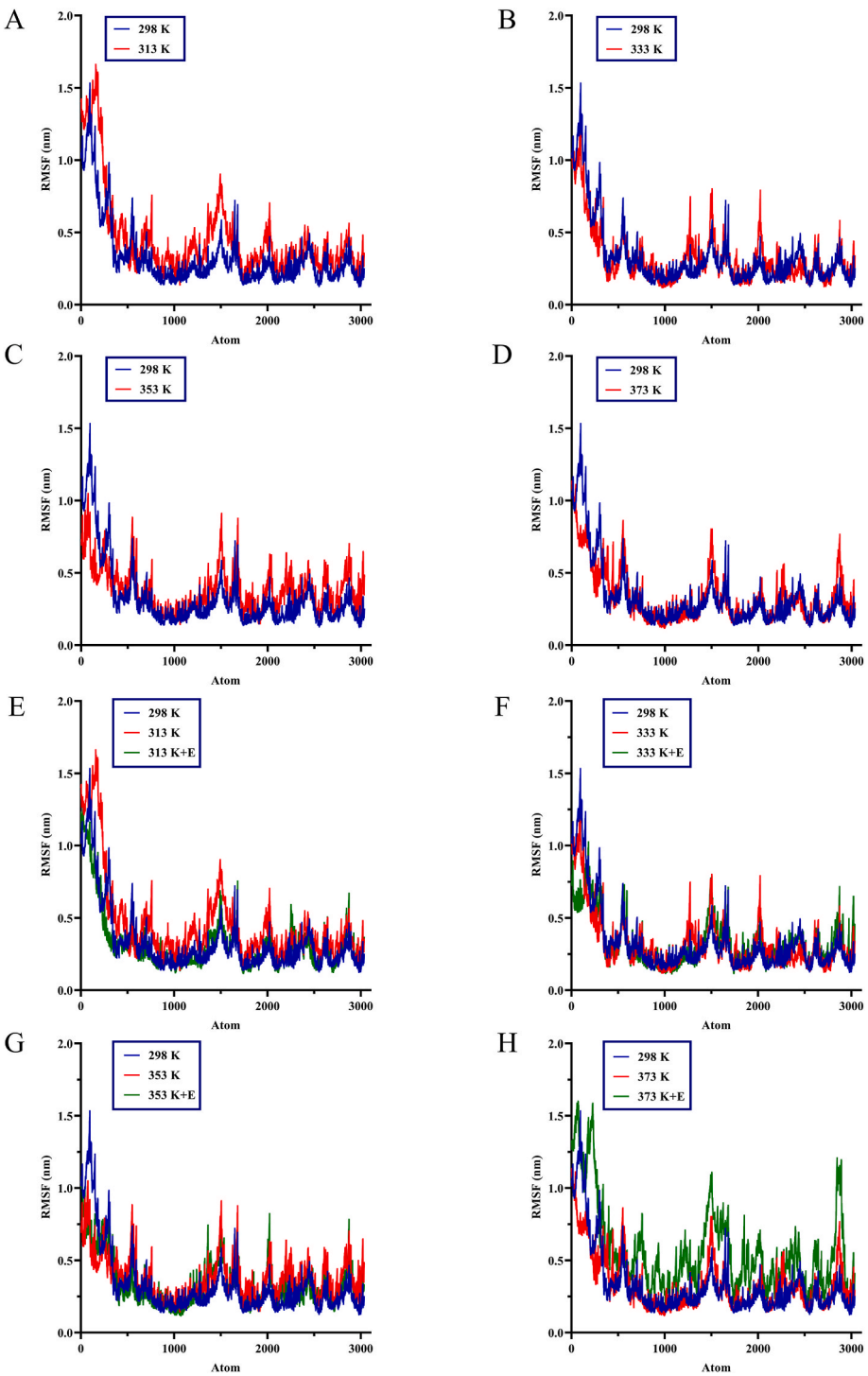

Fig. 4. The variations of RMSF in egg allergen Gal d 1 atoms under thermal processing (A–D) and microwave processing (E–H).

alone induced slight atomic deviation of egg allergen Gal d 1, while relatively high temperature combined with oscillating electric field processing resulted in obvious deviation. These ongoing escalations in the RMSD of Gal d 1 at higher temperature settings might stem from the aggregate impacts of thermal and electric field stresses, as indicated by Wang et al. (2020).

3.4. Analysis of RMSF of Gal d 1

The atomic fluctuation of Gal d 1 was measured in Fig. 4A–D. The values of RMSF of Gal d 1 atoms were increased to varying degrees. The RMSF of Gal d 1 peaked at atoms 160, 1494, and 2022 when at 313 K, while the peaks of value were appeared nearly at atoms 1273, 1505, and 2022 at 333 K. With the increase of temperature, the RMSF of Gal d 1 showed peaks at atoms 1506 at 353 K, and there were more low peaks in atoms 2000–3000. The RMSF of Gal d 1 showed obvious peaks around 1494, 2276 and 2873 atoms. Besides, the RMSF values changed to vary degree with oscillating electric field (Fig. 4E–H), especially the RMSF of 373 K + E shown significant fluctuation. This suggested that adding electric field stress might efficiently increase the mobility of atoms in a protein molecule, potentially allowing for structural alteration of the protein at specific temperatures.

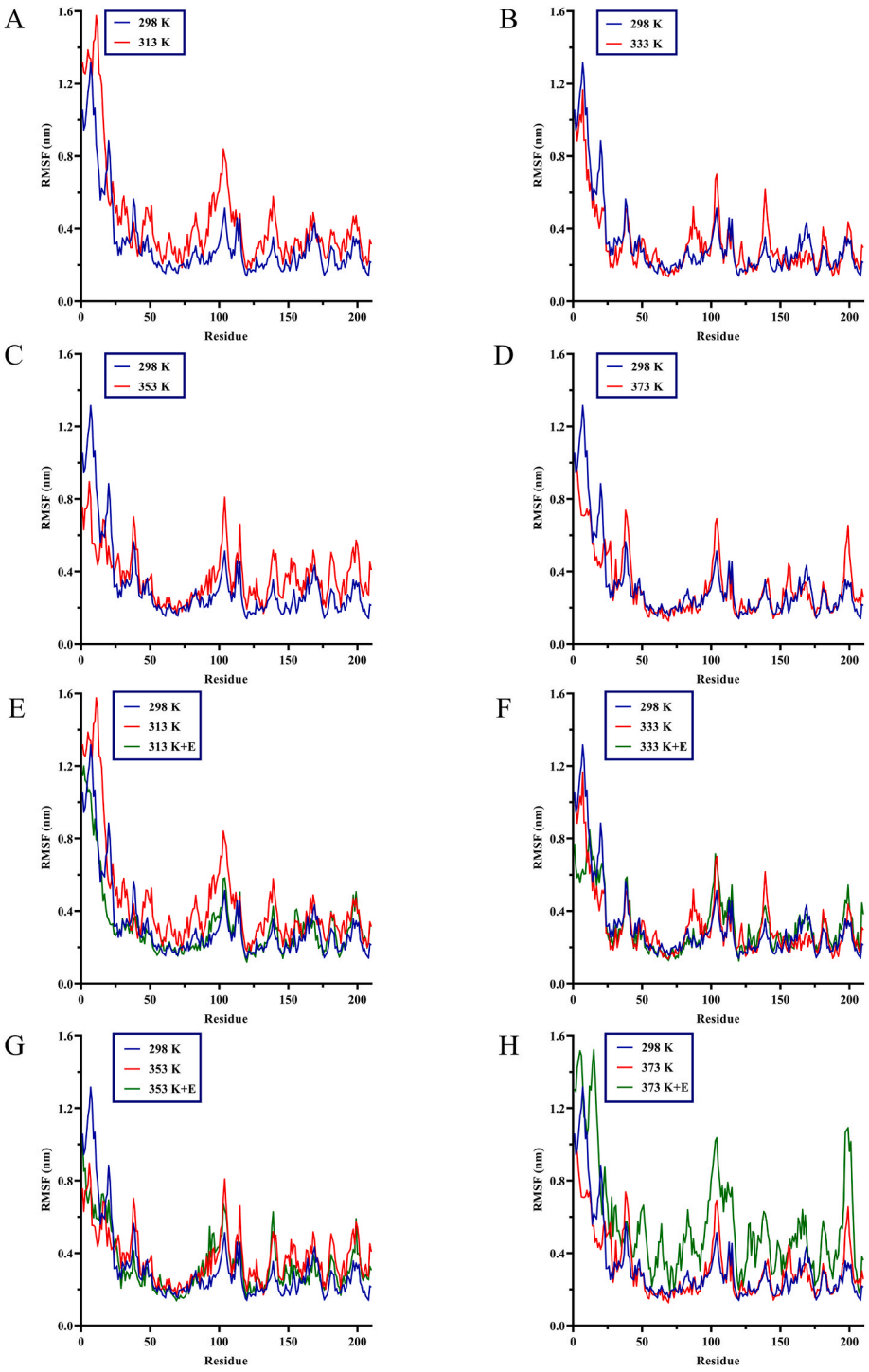

Fig. 5. The variations of RMSF in egg allergen Gal d 1 amino acids under thermal processing (A–D) and microwave processing (E–H).

As described in Fig. 5, the fluctuation trend of amino acid residues of Gal d 1 was roughly the same as that of atoms. Specifically, 3 sharp peaks were observed around the amino acids 11 (S), 103 (E), and 139 (Q) under 313 K thermal treatment compared to the control (298 K). Several sharp peaks were found under 333 K thermal simulation at amino acids 87 (K), 104 (D) and 139 (Q). As the temperature further increased, more sharp peaks formed during the 353 K thermal simulation compared to the others, which were observed at amino acids 8 (K), 104 (D), 115 (F), and 139 (Q). Meanwhile, the peaks of RMSF value under 373 K were detected at amino acids 38 (K), 104 (D) and 199 (N). What is noteworthy is that the amino acids under 373 K + E were fluctuated obviously when compared with 373 K, as reflected in amino acid sequences of 28 (D), 31 (R), 51 (R), 64 (D), 83 (D), 104 (D), 128 (N), 130 (C), 139 (Q), 166 (P), 181 (D), and 199 (N). These amino acids were influenced by microwave processing which can be attributed to structural changes or shift of the amino acids or atoms. Zhao et al. (2022) also demonstrated that the corresponding amino acid position had higher RMSF values of sarcoplasmic calcium-binding protein from shrimp, indicating that the structure was unfolded. Furthermore, not all predicted B-cell linear epitopes exhibit elevated RMSF values, a phenomenon that may be attributed to their structural location. Stable structural regions (e.g., α-helix) are predominantly with unobvious RMSF fluctuations due to

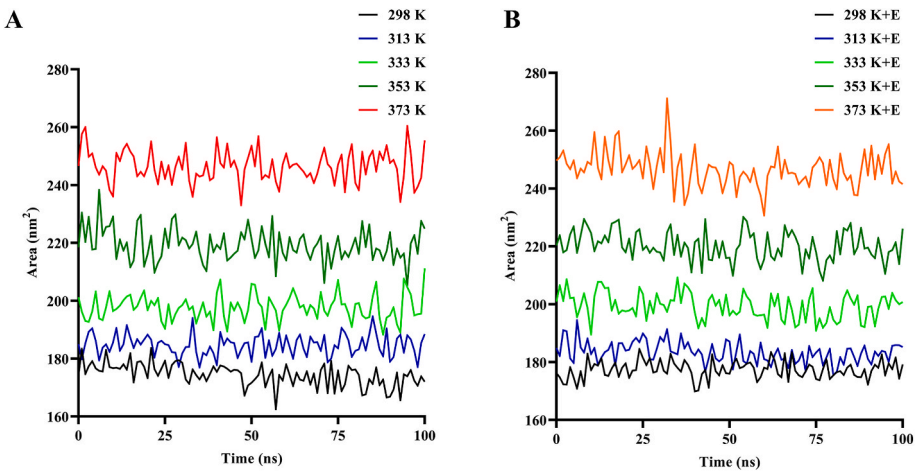

Fig. 6. The change of SASAs of egg allergen Gal d 1 amino acids under thermal processing (A) and microwave processing (B).

their inherent structural stability.

3.5. Analysis of SASAs of Gal d 1

The SASAs of Gal d 1 exhibited an upward trend as the temperature was raised (Fig. 6). Under the thermal processing simulations, the average SASAs were increased by 5.9 %, 13.34 %, 25.71 %, and 41.30 % respectively, when compared with 298 K. The tendency of SASAs change of microwave processing simulations was consistent with thermal processing, specifically, the average SASAs were expanded by 1.64 %, 5.13 %, 14.30 %, 26.22 %, and 41.39 % respectively. The SASAs increased under higher temperatures could be due to the collapse of the hydrophobic core. The functional and physicochemical attributes of proteins are related to their surface characteristics, the alterations of SASAs could lead to deviations in conformational structure which further affect the conformational epitopes, thereby influencing allergenicity of Gal d 1.

3.6. Analysis of B-factor of Gal d 1

The B factor was commonly indicated the motility and flexibility of atoms in proteins or other molecules (Xie et al., 2014), the higher the B-factor, the more unstable the conformation of the corresponding site, and the B-factor of egg allergen Gal d 1 was shown in Fig. 7. The unstable regions of Gal d 1 increased with the increasing temperature. Except for the signal peptide areas, the B-factor values of amino acids 101 (T)- 106 (K)were exceeded 1000 (Fig. 7D and E), which meant thermal processing affected this region obviously. In comparison with thermal processing, microwave processing significantly increased the values of B-factor in this region (Fig. 7I and J). In addition, the B-factor values of amino acids 196 (V)- 201 (T) were also exceeded 1000 indicating that microwave relaxed the structure of Gal d 1 more effectively than thermal processing alone.

3.7. Analysis of structural change of Gal d 1

The VMD timeline tool was applied to analyze the secondary structure alterations of the egg allergen Gal d 1 under various external stress conditions. As depicted in Fig. 8, the *Y*-axis values correspond to the protein's residue numbers, while the *X*-axis values indicate the simulation time or frames. Each distinct colored band corresponds to the residues in the secondary structure of Gal d 1 under different simulation conditions, and the results revealed that obvious disruptions were observed between residues 121–127 (frame 77–95) and a large number of 3–10 helices were appeared in residues 151–163 (frame 2–101) when the temperature increased from 298 K to 353 K (Fig. 8D). A higher amount of production of the sheet structures were observed in residues 193–210 due to the intensification of secondary structure fractures when the temperature was raised further (Fig. 8E). Meanwhile, the secondary structure changes of Gal d 1 simulated by microwave processing were shown in Fig. 7F–J, Gal 1 residues 133–145 (frame 32–101) and 199–210 (frame 77–101) produced more β-turn structures when Gal d 1 under 353 K + E treatment. While the β-turn structures presented in residues 199–210 (frame 77–101) originally were replaced by β-sheet structures. The above prediction results indicated that microwave processing has more potential to change the secondary structure of Gal d 1 than thermal processing. The structural changes of Gal d 1 was demonstrated by PyMOL software. As shown in Fig. 9, thermal processing and microwave processing changed the structure of Gal d 1, especially microwave processing. The change of conformational structure may result in the shielding of linear epitopes or the destruction of conformational epitopes, thereby reducing the allergenicity of allergen. Sindher et al. (2018) also discovered that the antigenicity of Gal d 1 was diminished following treatment at 100 °C, which was attributed to alterations in the exposure of linear epitopes or the positioning of conformational epitopes. Microwave processing might produce hypoallergenic foods with the desired functional properties. A study also demonstrated that microwave processing was used to process egg allergens to reduce the allergenicity (Liu et al., 2023). However, the specific effect of microwave processing on the allergenicity of Gal d 1 needs further research.

In addition, amino acids 101–106 were significantly increased the values of B-factor after thermal and microwave processing simulations, the average B-factor value of amino acids 101–106 was 472.70 ± 136.76 under 298 K treatment, while 884.98 ± 290.16 under 373 K, 2298.99 ± 447.95 under 373 K + E. Meanwhile, the alteration of secondary structure under 373 K + E also suggested that numerous random coil structures were generated around amino acid 103 (G) compared with other conditions, replacing the β-turn structures (Fig. 7J). These results indicated that amino acids 101–106 were affected obviously by thermal and microwave processing, especially microwave processing. And the secondary structure at amino acids 101–106 were loosen or even destroyed. Combining the results of bioinformation predictions, a

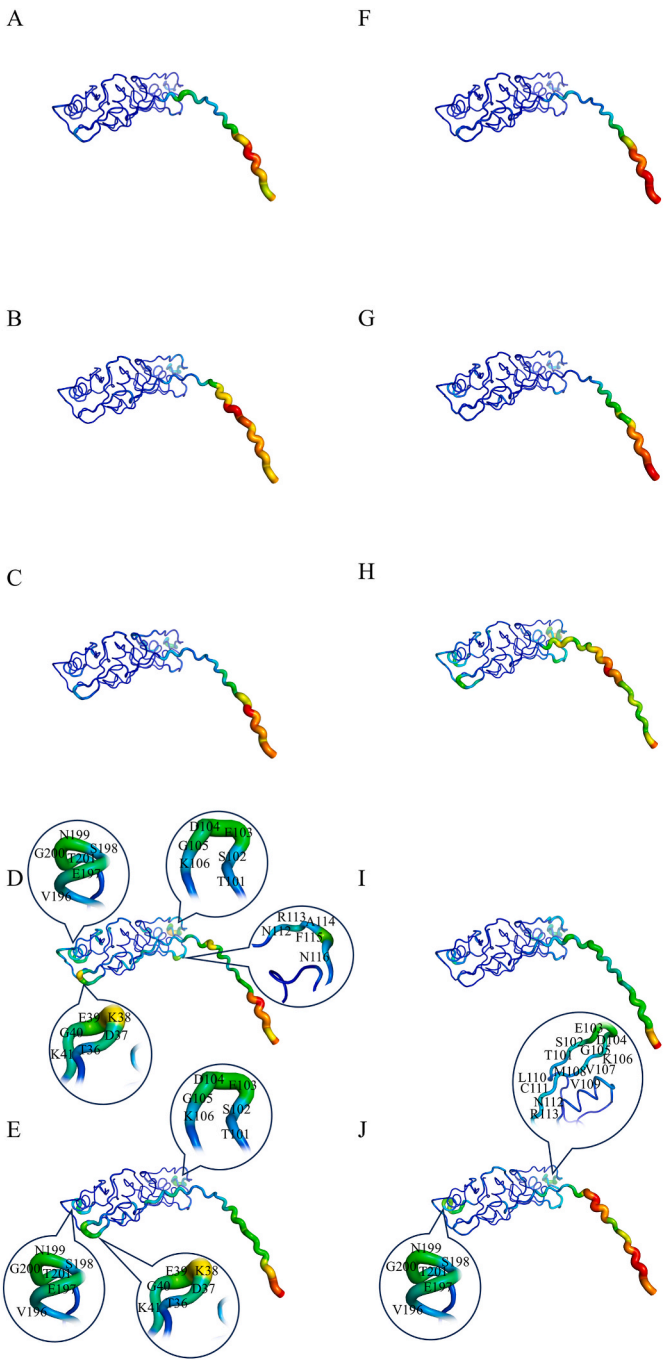

Fig. 7. The B-factor of Gal d 1 under thermal processing (A–E) and microwave processing (F–J). Blue regions were stable relatively, green regions were moderate, red regions were flexible.

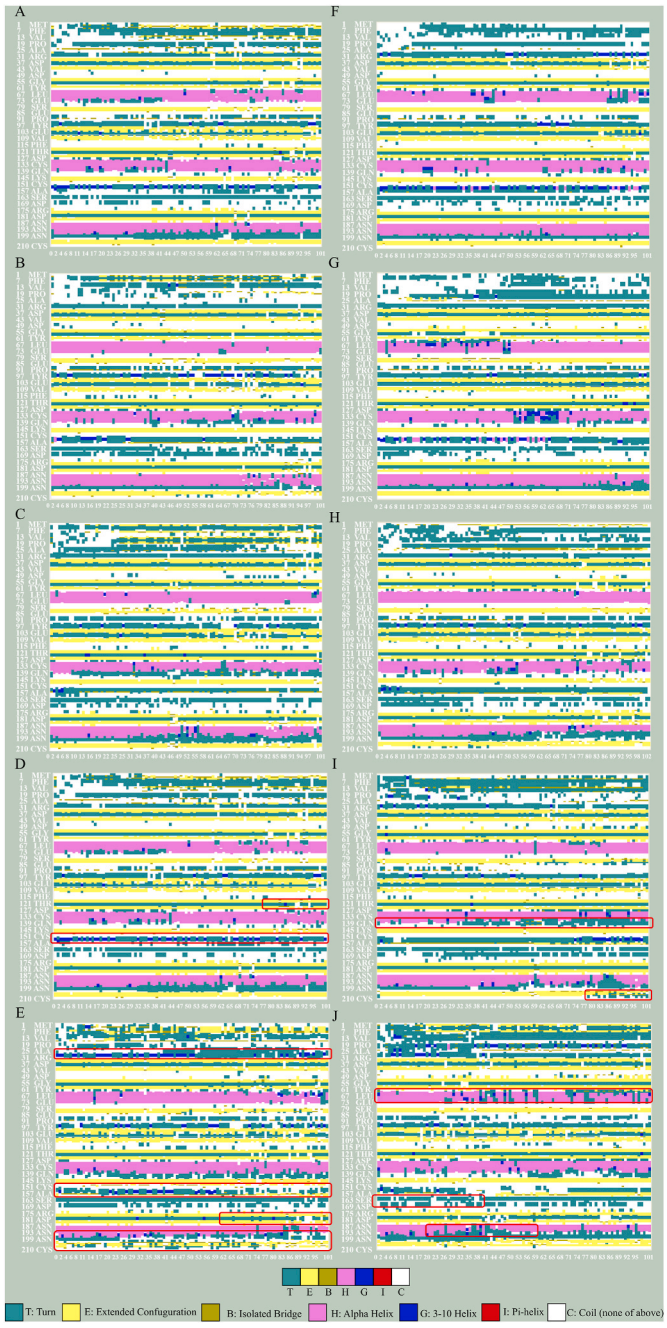

Fig. 8. The secondary structure of Gal d 1 under thermal processing (A–E) and microwave processing (F–J).

coincidental phenomenon was discovered. The G-4 as the potential B-cell linear epitope contained exactly amino acids 101–106, which manifested that thermal and microwave processing might affect the G-4 B-cell linear epitope or change the secondary structure to reduce the allergenicity of egg allergen Gal d 1, especially microwave processing. These results indicated that molecular dynamics simulation combined with bioinformatic prediction of antigenic epitopes can be a potential method for screening hypoallergenic food processing methods, and provide directions for the analysis of structure and epitope mechanisms.

4. Conclusions

In this study, 10 potential B-cell linear epitopes were predicted by bioinformatic tools, and then the results derived from simulations under thermal and electric field stress indicated that egg allergen Gal d 1 possessed thermal stability. However, the significantly changes of RMSF values and secondary structure were started when added the electric field, which indicated that microwave processing influenced the Gal d 1 more obviously. Besides, the G-4 potential B-cell linear epitope may affect by thermal processing with high temperature and microwave processing. Bioinformatics approaches combining with molecular dynamics simulation technology provided a novel method to outline an efficient and exhaustive method for evaluating epitopes and allergenicity, thereby laying the groundwork for a deeper comprehension of the

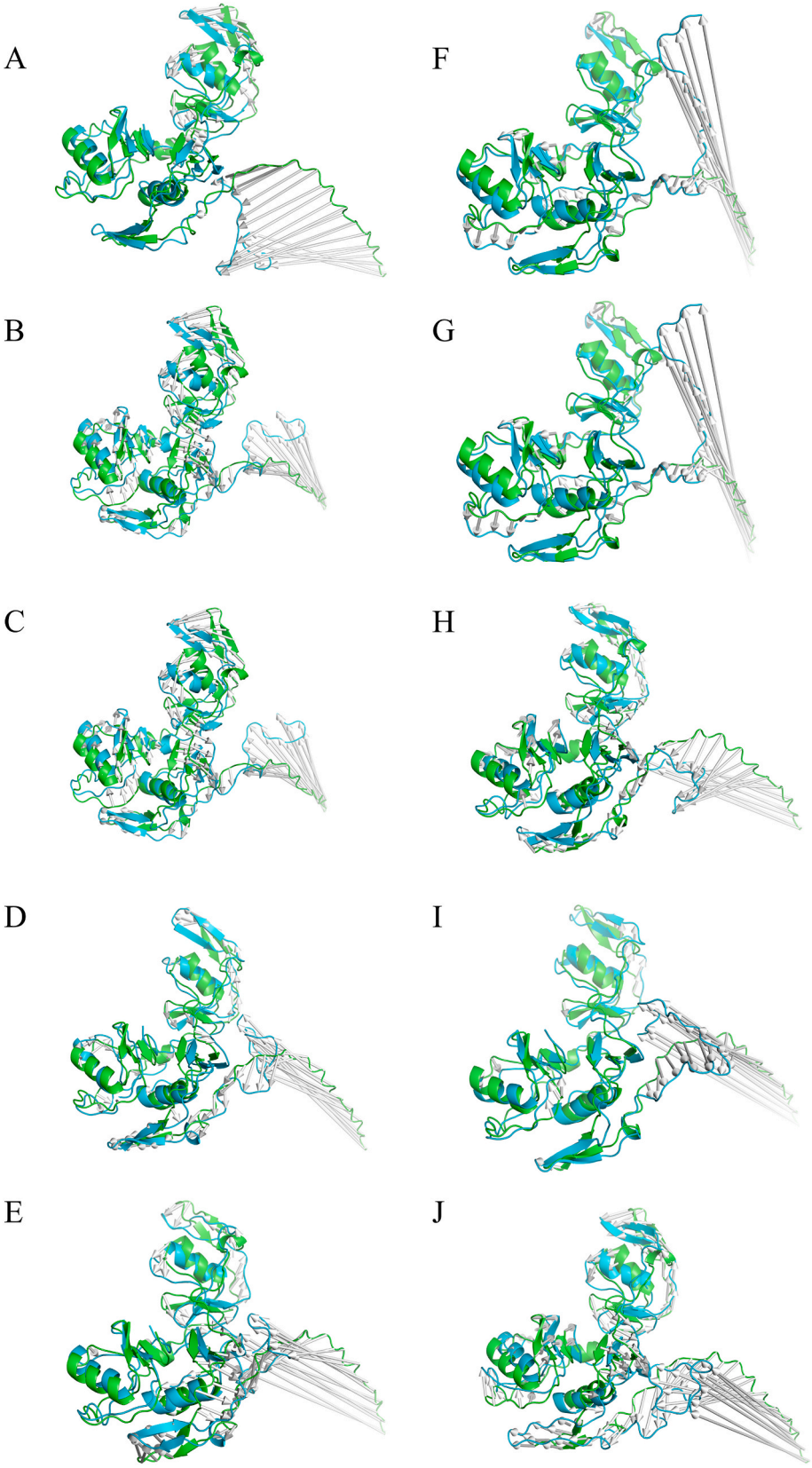

Fig. 9. The structural changes of Gal d 1 under thermal processing (A–E) and microwave processing (F–J). Green models were initial cartoon structures, blue models were final structures, white arrows were direction of migration.

biological mechanisms and the fundamentals of food allergies. However, the limitation of this study is necessity for experimental validation, which will be the focus of our subsequent investigations to confirm these computational predictions. The future research will concentrate on screening and validating emerging food processing methods that can reduce the allergenicity of protein allergens.

CRediT authorship contribution statement

Tao Wang: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Lili Zhang:** Formal analysis, Data curation. **Vijaya Raghavan:** Writing – review & editing. **Yang Liu:** Writing – review & editing. **Jin Wang:** Writing – review & editing, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

Data will be made available on request.

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