



Gastrointestinal digestion products of shrimp (*Penaeus vannamei*) proteins retain an allergenic potential

Yao Liu^a, Songyi Lin^{a,b}, Kexin Liu^a, Shan Wang^a, Na Sun^{a,b,*}

^a National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China

^b Collaborative Innovation Center of Seafood Deep Processing, Dalian Polytechnic University, Dalian 116034, PR China

ARTICLE INFO

Keywords:

Penaeus vannamei
Food allergy
Simulated digestion
BALB/c mice model

ABSTRACT

The allergenic potential of shrimp (*Penaeus vannamei*) proteins during gastrointestinal (GI) digestion is currently unclear. In this study, the role of gastric, intestinal, and GI digestion on the sensitizing and eliciting capacity of shrimp proteins was investigated using a BALB/c mice model. Gastric digestion products of shrimp proteins produced lower specific IgE/IgG₁, histamine and mMCP-1 levels and significantly reduced the proportion of degranulated mast cells as compared to the shrimp protein extract; furthermore, no histopathological changes were observed in the lung, spleen and jejunum. Conversely, their GI digestion products caused high specific IgE/IgG₁ levels, mast cells degranulation and severe allergic symptoms in the mice, similar to those in the shrimp protein extract group. These results may be due to the masking or exposure of shrimp epitopes during digestion. Consequently, the GI digestion products of shrimp proteins retained an allergenic potential, although gastric digestion products showed a lowering effect.

1. Introduction

Penaeus vannamei (synonym *Litopenaeus vannamei*) is one of the most cultivated shrimp species in the world (Córdova-Murueta, Navarrete-Del-Toro, & García-Carreo, 2017). Its protein content is higher than 22%, the fat content is only 0.73%, and it is rich in unsaturated fatty acids and minerals (Soon, 2019; Zhang et al., 2021). However, *Penaeus vannamei* could cause allergic reactions and result in severe allergic symptoms, including urticarial (hives), abdominal pain, diarrhea, dermatitis, and bronchospasm (asthma). More severe allergic reactions could manifest as breathing difficulties, shock, and even life-threatening (but comparatively rare) and these allergic symptoms might continue a lifetime (Toomer, Do, Fu, & Williams, 2015; Sheu, Yu, Lien, & Lee, 2020; Ostblom, Wickman, Hage, & Lilja, 2008; Ayuso, 2011).

Food intake was the main way of anaphylaxis in humans. The Food and Agriculture Organization of the United Nations (FAO), the European Food Safety Authority (EFSA), the World Health Organization (WHO),

and the Codex Alimentarius Commission (CAC) have adopted *in vitro* simulated digestion as a significant step of allergen risk assessment. In 2003, the CAC confirmed that the stability of pepsin proteolysis is a key parameter in the evaluation of protein allergy (Codex, 2003). Simultaneously, the researchers discovered that the assessment of pepsin stability cannot be parallel to *in vivo* digestion conditions (acid denaturation, pepsin, pancreatin), and consequently protein resistance to the gastrointestinal (GI) digestive system was widely used (Toomer, Do, Fu, & Williams, 2015). However, recent studies have found that proteins unstable to digestive enzymes still had the sensitizing capacity (Sun et al., 2022). Digestion potentially affects the allergenicity of proteins by hydrolyzing the conformational and linear epitopes (Bavaro et al., 2018; Gazme, Rezaei, & Udenigwe, 2020). Changes in conformational epitopes include unfolding and aggregation of proteins. These changes may disrupt the conformational epitopes or generate new ones, and may also make the allergens more sensitive or resistant to gastric and GI enzymes (Sun et al., 2022). Meanwhile, linear epitopes are

Abbreviations: GI, Gastrointestinal; FAO, Food and Agriculture Organization of the United Nations; EFSA, European Food Safety Authority; WHO, World Health Organization; CAC, Codex Alimentarius Commission; mMCP-1, Mouse mast cell protease-1; SDS-PAGE, Sodium dodecyl sulfate-polyacrylamide gel electrophoresis; SGF, Simulated gastric fluid; SIF, Simulated intestinal fluid; SPE, Shrimp protein extract; SGD, Simulated gastric digests; SID, Simulated intestinal digests; SGID, Simulated gastrointestinal digests; TCA, Trichloroacetic acid; ELISA, Enzyme-linked immunosorbent assay; PBS, Phosphate buffer saline; BSA, Bovine serum albumin; H&E, Hematoxylin and eosin; SEM, Standard error of mean; TM, Tropomyosin; MHC, Myosin heavy chain; AK, Arginine kinase; MLC, Myosin light chain.

* Corresponding author at: No. 1 Qinggongyuan, Ganjingzi District, National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, PR China.

E-mail address: sunna1215@126.com (N. Sun).

<https://doi.org/10.1016/j.foodres.2022.111916>

Received 8 April 2022; Received in revised form 27 July 2022; Accepted 7 September 2022

Available online 13 September 2022

0963-9969/© 2022 Elsevier Ltd. All rights reserved.

thought to be more important for food allergens. The change of linear epitopes may be the root cause of allergenic changes in allergens after digestion (Liu, Lin, & Sun., 2022). Digestion may expose new amino acids (Liu et al., 2019). Thus, digestion of food allergens in the stomach and intestine might reduce or increase their sensitizing capacities. Tong et al. (2020) reported that the levels of specific immunoglobulins (IgE, IgG, IgG1, IgG2a), mouse mast cell protease-1 (mMCP-1) and histamine in serum of digested egg custard-treated mice were higher than those of the control mice, indicating that digested egg custard had the sensitizing and eliciting capacity. Meanwhile, Korte, Bräcker, & Brockmeyer (2017) found that samples from the digestion of peanut allergens were capable to lead to the production of specific IgE in rats. Therefore, it is necessary to study the potential allergenicity of proteins or peptides produced after digestion of food allergens.

Most previous studies focused on the effect of gastric digestion on the IgE binding potential of allergens, but there were few studies on the role of gastric, intestinal and GI digestion on the sensitizing and eliciting capacity of food proteins. In most studies, purified shrimp allergens were digested (Xu et al., 2020; Zhang, Xiao, Zhang, & Zhou, 2019). The primary immunoreactive protein in most species of shrimp is tropomyosin (TM); however, the effects of other shrimp allergens and whole shrimp proteins on the digestive stability and the allergenicity of the digestive products are unknown. This study here aims to systematically characterize the potential allergenicity of protein by conducting a comprehensive investigation through the digestion of allergens in shrimp.

A BALB/c mice model can reflect the actual situation that food proteins trigger allergic reactions *in vivo*, and is the most direct method to evaluate the allergenic potential of food. Consequently, the BALB/c mice model has been widely utilized to evaluate the sensitizing and eliciting capacity of food proteins. In this research, female BALB/c mice were sensitized with protein extract and its simulated digests on Day 0, 7, 14, and 21, respectively and then challenged with different test proteins on Day 33. The digestive stability of shrimp proteins was assessed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and peptide yield. Meanwhile, the sensitizing and eliciting capacity of shrimp was tested by anaphylaxis, body temperature, serum specific IgE/IgG1 binding ability, plasma histamine and mMCP-1 levels, pathological examination of lung, spleen and jejunum, and degranulation level of mast cells.

2. Materials and methods

2.1. Materials

Shrimp (*Penaeus vannamei*) was supplied from the Shahekou seafood market (Dalian, China). Pepsin and pancreatin were bought from Sigma Aldrich (St Louis, MO, USA). Toluidine blue solution was bought from Solarbio Science & Technology Co., Ltd (Beijing, China). SDS-PAGE Gel Preparation Kit was bought from Solarbio Science & Technology Co., Ltd (Beijing, China). Pre-stained protein maker was purchased from Bio-Rad (California, USA).

2.2. Whole protein extraction

Whole protein was extracted from boiled shrimp by the method of alkali extraction and acid precipitation (Gao et al., 2019; Yi, Van Boekel, & Lakemond, 2017), and some modifications were made. Shrimps were boiled in boiling water for 5 min. The skin and head of the whole shrimp were removed to obtain the boiled shrimp meat. Then the shrimp meat was stirred at pH 11.0 for 8 h and the precipitate was collected. After 8 h, the precipitate was stirred at pH 5.5 for 1.5 h and the supernatant was collected. The whole experiment was completed at 4 °C. Finally, the shrimp protein extract (SPE) was lyophilized and stored at -80 °C until use.

2.3. *In vitro* digestion

After lyophilized, the SPE sample was digested *in vitro* as described by Minekus et al. (2014), simulated gastric fluid (SGF) and simulated intestinal fluid (SIF) were prepared.

Gastric phase: the SPE sample was mixed with SGF at 37 °C for 30 min. Subsequently, the mixture was adjusted to pH 3.0 and the porcine pepsin (2,000 U/mL of final enzymatic activity) was added to initiate the gastric digestion for 2 h at 37 °C. Samples from simulated gastric digestion were collected at different times (0, 2, 5, 10, 30, 60, 90, and 120 min). Then, the simulated gastric digestion was terminated by increasing the pH value to 7.0. The simulated gastric digests (SGD) for 120 min were lyophilized and all the samples were stored at -80 °C.

Intestinal phase: the SPE sample was mixed with SIF at 37 °C for 30 min. The sample was adjusted to pH 7.0 and the pancreatin (100 U/mL of final enzymatic activity) was added to initiate the intestinal digestion for 2 h at 37 °C. Samples from simulated intestinal digestion were collected at different times (0, 2, 5, 10, 30, 60, 90, and 120 min). Then, the simulated intestinal digestion samples were taken and the enzyme was inactivated at 100 °C for 5 min. The simulated intestinal digests (SID) for 120 min were lyophilized and all the samples were stored at -80 °C.

GI phase: the SGD sample was added to SIF with an equal volume. Subsequently, the sample was adjusted to pH 7.0 and pancreatin (100 U/mL of final enzymatic activity) was added to initiate the GI digestion for 2 h at 37 °C. Samples from simulated GI digestion were collected at different times (0, 2, 5, 10, 30, 60, 90, and 120 min). Then, the simulated GI digestion samples were taken and the enzyme was inactivated at 100 °C for 5 min. The simulated gastrointestinal digests (SGID) for 120 min were lyophilized and all the samples were stored at -80 °C.

2.4. SDS-PAGE

The sample buffer (with β -mercaptoethanol) was added to samples with four volumes and digested/undigested sample (1 mg/mL of protein) was boiled at 100 °C for 5 min. 10 μ L of cooled mixture or 3 μ L of molecular standard marker (10–250 kDa) were loaded into each lane of the gel and electrophoresis was performed using a Mini vertical electrophoresis apparatus (Beygene, Beijing, China). Proteins were separated for 30 min at 80 V and 150 min at 100 V. After the proteins reached the bottom, the gel was stained for 2 h with Coomassie Brilliant Blue and decolorized using a decolorizing solution (with ethanol and acetic acid) until the bands became clear in a shaking incubator at the room temperature. Images were captured using a gel imager (Bio-Rad, California, USA).

2.5. Trichloroacetic acid (TCA)-soluble peptide index

The peptide yield was determined according to the method described by Zhou et al. (2012), with some modifications. Briefly, 0.5 mL of SGD, SID and SGID were mixed with 0.5 mL of TCA (20%). Then the mixture was incubated at room temperature for 20 min and centrifuged at 12000 rpm for 10 min. The peptide present in the supernatant was TCA-soluble peptide. Finally, TCA-soluble peptide was determined using a Lowry Protein Assay Kit (Solarbio, Beijing, China).

2.6. Animal treatment design

Female BALB/c mice aged three to four weeks were bought from Changsheng Biotechnology Co., Ltd (Benxi, Liaoning, China). All female BALB/c mice were maintained under a temperature of 22 ± 2 °C, a humidity of $50 \pm 15\%$, and a 12 h/12 h dark/light cycle with free access to water and shrimp-free chow. All mice were acclimated for one week before commencing the experiments. All experiment designs were approved by University of Dalian Polytechnic Animal Care Committee (protocol number: DLPU2021015).

In order to avoid the secondary digestion of the digestion products of shrimp protein extract in BALB/c mice, the methods of Misra et al. (2011), Kumar, Vijaykrishnaraj, Kurrey, Shinde, & Prabhasankar (2019) and Morafo et al. (2003) were referred to sensitize the mice by intraperitoneal injection. Mice were randomly distributed in six groups ($n = 10$) and sensitized by intraperitoneal injection with 100 μg of SPE, SGD, SID and SGID plus 100 μL of aluminum hydroxide (alum, Thermo Scientific, Cramlington, UK) in 100 μL of saline per mice, and other two groups were injected with 200 μL of saline and 100 μL of alum in 100 μL of saline, respectively. The mice were injected once a week during 4 consecutive weeks. Then, serum samples were collected from the retro-orbital plexus at 30 d and 32 d. On the 33 d, BALB/c mice were challenged with 500 μg of different test proteins. In addition, access to food was prevented 1 d before the challenge. Fig. 2 (A) summarizes the detailed experimental design. Moreover, food intake and water intake of mice were recorded weekly.

2.7. Measurement of anaphylactic score and temperature

Anaphylactic scores were monitored after protein injection. Mice were examined for 20–30 min after challenge using a scoring system assessed by Li et al. (2000): 0, no reaction; 1, scratching and rubbing around the nose and head; 2, swelling around the eyes, puffer hair, diarrhea; 3, no activity; 4, wheezing, labored respiration; and 5, death.

Handheld thermometer was used to measure the ear temperature in the administered mice and their ear temperature was recorded before challenge and after 20–30 min of challenge (Freidl et al., 2020).

2.8. Measurement of specific IgE and IgG₁ levels

Measurement of specific IgE and IgG₁ levels was referenced to Zhao et al. (2022). The serum of the mice was centrifuged at 4000 rpm for 10 min at 4 °C. The levels of specific IgE and IgG₁ were determined by indirect enzyme-linked immunosorbent assay (ELISA). SPE and its SGD, SID and SGID samples were diluted to 10 $\mu\text{g}/\text{mL}$ with 0.05 M sodium carbonate buffer (pH 9.6). The SPE, SGD, SID and SGID samples were coated into a 96-well ELISA plate overnight at 4 °C, respectively. The 96-well ELISA plate was washed for three times with PBS-0.1% BSA-0.1% Tween 20 and the plate was blocked with PBS-1% BSA (150 μL per well) for 2 h at 37 °C. Subsequently, the 96-well ELISA plate was washed for three times and 100 μL of the test serum with dilutions of 1:10 was added to the wells and incubated for 2 h at 37 °C. Afterwards, the plate was washed for five to six times. 100 μL of rat monoclonal anti-mouse IgE epsilon chain (Biotin) antibody (1:500) (Abcam, England) was added to each well for 1 h at 37 °C and washed for five times. Then 100 μL of streptavidin (HRP) (1:500) (Abcam, England) was added to each well and plates were incubated at 37 °C for 1 h. In contrast to specific IgE determination, 100 μL of anti-mouse IgG₁ (HRP) pre-adsorbed antibody (Abcam, England) with dilutions of 1:4000 was added to the wells for 1 h at 37 °C and washed. Next, 100 μL of TMB Chromogen Solution for ELISA was added to the wells for 15 min at 37 °C. Finally, 50 μL of 2 M H₂SO₄ was added to each well to stop the reaction and the samples were measured at 450 nm with a Microplate Reader (Infinite™ M200, TECANE, Switzerland).

2.9. Measurement of histamine and mouse mast cell protease-1 (mMCP-1)

Plasma samples were collected in anticoagulant tube (with EDTA-K₂) by intraocular canthal vein puncture after 20 min of challenge and plasma was obtained by centrifugation at 4000 rpm and 4 °C for 10 min. The plasma samples were kept at -80 °C. Histamine and mMCP-1 were determined by a Mouse HIS ELISA Kit (Jianglai, Shanghai, China) and Mouse Mast Cell Protease 1 ELISA Kit (Jianglai, Shanghai, China) according to the manufacturer's instructions. The absorbance was measured at 450 nm with a Microplate Reader.

2.10. Pathological examination of lung, spleen and jejunum

On day 33, the fresh lung, spleen and jejunum were removed after euthanasia. The lung, spleen and jejunum tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sliced into 5 μm thick slices of flakes using a slicer (Xu, Liang, Li, Jiang, & Lin, 2020).

Subsequently, the lung, spleen and jejunum were stained with hematoxylin and eosin (H&E) to observe the histopathology. The spleen was stained with toluidine blue for the mast cells studies. A total of fifteen fields (toluidine blue) were randomly observed using the microscope and the ratio of degranulated to intact mast cells was counted (Choi et al., 2019).

2.11. Statistical analysis

All the quantitative results were presented as means $\pm$ standard error of mean (SEM), and were subjected to a one-way of analysis of variance using SPSS 18.0 software (IBM, Chicago, IL, USA). Differences at p -values < 0.05 were considered statistically significant. Charts were drawn using Origin 9.0 software (OriginLab Inc., Northampton, USA).

3. Results and discussion

3.1. Electrophoretic analysis of simulated gastric, intestinal, and GI digestion products of shrimp proteins

To evaluate the effect of simulated gastric, intestinal, and GI digestion on the stability of shrimp proteins and define how the digestion affected the allergenic properties of shrimp proteins, the kinetics of different digestion products were analyzed by SDS-PAGE (Fig. 1). As previously mentioned, the allergenicity of shrimp is dominated by the key allergenic proteins as shown in lane 1 of Fig. 1 (A) and (B). The known molecular weights of allergenic proteins are listed as follows: myosin heavy chain (MHC) (approximately 200 kDa), actinin (approximately 100 kDa), actin (approximately 42 kDa), arginine kinase (AK) (approximately 40 kDa), TM (approximately 36 kDa), troponin (approximately 34 kDa), myosin light chain (MLC) (approximately 17.5 kDa) (Toomer, Do, Fu, & Williams, 2015; Zhao et al., 2022; Zhang, Fang, Hao, & Zhang, 2018). Among all the bands, there were several clear bands corresponding to pepsin, trypsin, and chymotrypsin as shown in lane pepsin and pancreatin of Fig. 1. The pepsin and pancreatin were stable during digestion *in vitro*.

During gastric digestion, as shown in Fig. 1 (A), the allergenic proteins of shrimp were degraded from 2 min, with a loss of high molecular weight proteins, which was accompanied by the appearance of stable polypeptide fragments (8, new proteins, 25–34 kDa and 9, new low molecular weight proteins, <17.5 kDa). Subsequently, the new proteins and new low molecular weight proteins were also rapidly digested, while only two lighter bands remained after gastric digestion for 120 min (band 10 and band 11).

Gastric digests of shrimp proteins were further subjected to the simulated intestinal digestion for 120 min. As shown in Fig. 1 (C), the two stable gastric digestion bands were disappeared after only 2 min, indicating that bands 10 and 11 were rapidly digested by trypsin and chymotrypsin. Intestinal digestion resulted in rapid degradation of any remaining soluble protein, as previously described by other researchers (Rao et al., 2020). The gradual emergence of newly formed low molecular weight peptides was the result of enzymatic hydrolysis by pancreatin enzymes (Fig. 1 (C)). These low molecular weight protein bands could not be seen in the SDS-PAGE gels.

Moreover, shrimp proteins were also subjected to a separate intestinal digestion as shown in Fig. 1 (B). The allergenic proteins were rapidly degraded from 2 min, and created a series of new bands under 17.5 kDa (band 9). But the low molecular weight proteins (9, <17.5 kDa) rapidly disappeared at 10 min.

The proteins that are unstable to digestive enzymes still had the

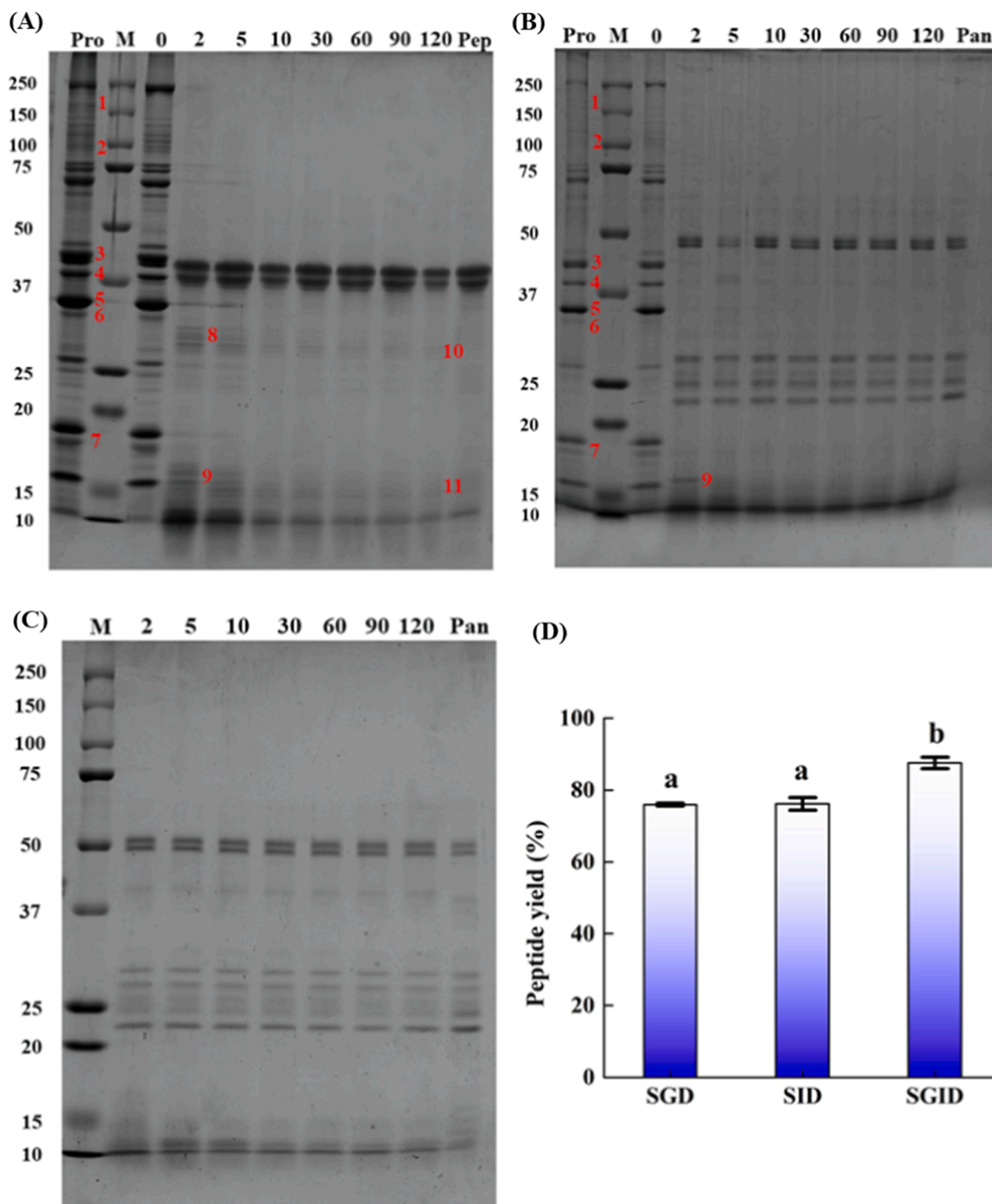


Fig. 1. SDS-PAGE analysis of simulated gastric (A), intestinal (B) and GI digestion (C) of shrimp proteins. Lanes: M, molecular weight marker; Pro, shrimp protein extract (SPE); Pep, pepsin; Pan, pancreatin. The numbers on the top line indicate the digestion times in minutes. Reducing electrophoretic analysis remarked several protein bands: 1, myosin heavy chain (MHC) (approximately 200 kDa); 2, actinin (approximately 100 kDa); 3, actin (approximately 42 kDa); 4, arginine kinase (AK) (approximately 40 kDa); 5, tropomyosin (TM) (approximately 36 kDa); 6, troponin (approximately 34 kDa); 7, myosin light chain (MLC) (approximately 17.5 kDa); 8, new proteins (25–34 kDa) ; 9, new low molecular weight proteins (<17.5 kDa); 10, 11, stable gastric digestion bands. (D) Peptide yield of simulated gastric, intestinal, and GI digestion of shrimp proteins.

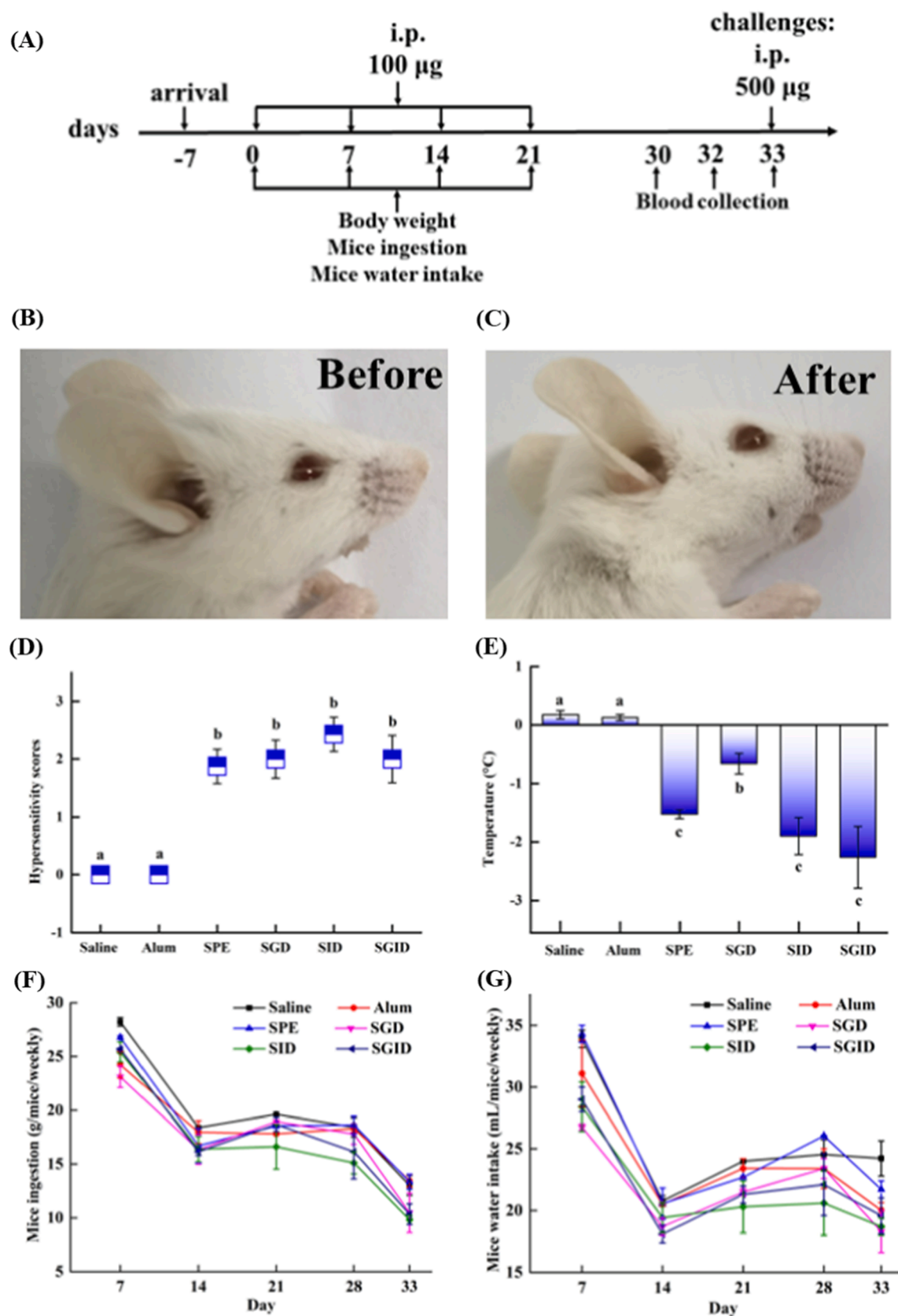


Fig. 2. Systemic anaphylaxis responses after challenge. (A) Experimental design of food allergy model. (B-C) Representative reaction of sensitized mice before and after challenge of protein. Note the swelling of the eyes and nose in the sensitized mouse. (D) Anaphylactic score was measured after 20-30 min of challenge. (E) Ear temperature was measured on day 33 before and after 20-30 min of challenge. (F-G) Food intake and water intake were measured once a week. Bars represent means $\pm$ SEM.

potential allergenicity, which is due to the IgE-binding capacity of small-sized peptides released by digestion (Prodic et al., 2018). Therefore, the content of short digestion-resistant peptides released by digestion might be related to the changes of allergenicity. To directly reflect the yield of small peptides, the yield in SGD, SID and SGID of TCA-soluble peptide were measured. As shown in Fig. 1 (D), the yield of TCA-soluble peptide in the SGD, SID and SGID were 75.93%, 76.15% and 87.57%, respectively. The results showed that the pepsin, trypsin and α -chymotrypsin made the shrimp proteins hydrolyzed into more small-sized peptides when compared to the gastric digestion and intestinal digestion. These newly small-sized peptides might affect the IgE-binding capacity.

Guo et al. (2021) and Ahmed et al. (2020) mentioned that intact TM was detected during the whole GI digestion. However, Klueber et al. (2020) found that the TM was rapidly degraded after digestion and the digestion products still had IgE-binding ability. Consequently, different conditions have a significant influence on the digestibility of food allergens, such as the activity of the enzyme, pH and the origin of the allergen. In addition, the proteins resistant to the digestive enzymes and unstable to digestive enzymes has the sensitizing and eliciting capability (Bøgh & Madsen, 2016). The findings demonstrated that the intact allergens of shrimp proteins were lost and a large number of small-sized peptides were produced during gastric, intestinal, and GI digestion. Moreover, due to the production of these small-sized peptides, the immunodominant allergen epitopes in the proteins might be disrupted, preserved or exposed. After GI digestion, the conformational structure was expanded so that a large number of hydrophobic regions were exposed. As compared to the gastric and intestinal digestion groups, GI digestion group contained more small-sized peptides, which might expose more immunodominant allergen epitopes. Therefore, the sensitizing and eliciting capacity of shrimp protein extract and its digestion products was further evaluated using a BALB/c mice model.

3.2. Allergic responses of shrimp protein extract or its digestion products

BALB/c mice were intraperitoneally administered with SPE, SGD, SID and SGID once a week for 4 consecutive weeks concomitantly with alum as an adjuvant. Subsequently, mice were challenged with different test proteins, as the schematic protocol shown in the Fig. 2 (A). The anaphylactic scores and ear temperature were monitored after 20–30 min of challenge. Puffiness around the eyes and nose was observed after SPE, SGD, SID and SGID administration to mice, as shown in Fig. 2 (C). SPE-, SGD-, SID- and SGID-challenged mice showed obvious illustrations of anaphylactic symptoms; however, no significant differences in hypersensitivity scores were observed among these four groups ($p > 0.05$). The body temperature of mice dropped below the normal range by 0.7 °C after being challenged with SGD ($p < 0.05$) and the body temperature dropped by 1.5–2.3 °C after being challenged with SPE, SID and SGID ($p < 0.05$). Meanwhile, the higher drop in body temperature observed in SPE-, SID- and SGID-challenged groups signifies the more serious systemic anaphylaxis in the mice. However, unchanged hypersensitivity scores and constant ear temperature were evident after saline administration to mice (Fig. 2 (D) and 2 (E)). The mice treated with alum displayed comparable hypersensitivity scores and ear temperature to those in the saline-treated group. It was indicated that anaphylactic scores and body temperature of mice were not affected by saline and alum. No signs of anaphylactic symptoms were observed in the mice of the saline- or alum-treated group and unchanged physical activity were evident in the two groups (Fig. 2 (B)).

Food ingestion and water intake of the mice were recorded once a week during the experiment. It could be seen from Fig. 2 (F) and 2 (G) that food ingestion and water intake obviously decreased on the whole.

Taking together, the challenge of SPE, SGD, SID and SGID samples could cause allergic symptoms, a loss of temperature and suppression of water intake and appetite. Additionally, SPE, SID and SGID showed significantly serious anaphylactic reaction in the mice.

3.3. Specific IgE and IgG₁ levels of shrimp protein extract or its digestion products

It is thought that allergic reactions in BALB/c mice might be induced by multiple mechanisms, among which serum-specific IgE antibodies might play an important role in mediating type I allergic reactions (Meng, Li, Gao, & Chen, 2016). The levels of specific IgE in serum were determined using an indirect ELISA. Among the four sensitized groups, the SPE-sensitized group had the highest specific IgE levels (SPE-, SGD-, SID- and SGID-sensitized groups). As shown in Fig. 3 (A), the levels of specific IgE in the SGD-, SID- and SGID-sensitized groups were about 48.57%, 49.72% and 47.56% of those in SPE-sensitized groups, respectively ($p < 0.05$). Little amounts of specific IgE were measured in the serum of the saline- and alum-treated groups.

To determine the allergenicity of shrimp proteins, the levels of specific IgG₁ in serum were determined (Fig. 3 (B)). The change of serum IgG₁ level in the SPE-, SGD-, SID- and SGID-sensitized groups was similar to that of the IgE level. The results of Fig. 3 (B) showed that specific IgG₁ levels in the SGD-sensitized group were lower than those in the SPE-sensitized group ($p < 0.05$). Moreover, the levels of specific IgG₁ in the SID- and SGID-sensitized groups were significantly higher than those in the SGD-sensitized group ($p < 0.05$), but not significantly different from those in the SPE-sensitized group ($p > 0.05$). The specific IgG₁ levels in the saline- and alum-treated groups were significantly lower than those in the SPE-, SGD-, SID- and SGID-sensitized groups. These results revealed that gastric and intestinal digestion could decrease the binding capacity of *Penaeus vannamei* with specific IgE and IgG₁ and that GI digestion could still cause the rise of specific IgE and IgG₁.

Gómez et al. (2015) showed that the protein profile (SDS-PAGE) of boiled shrimp was not significantly altered, but the high-molecular-weight proteins could bind more IgE (Immunoblotting). The results showed that the specific IgE-binding capacity of boiled shrimp (SPE) was higher, which may be due to protein aggregation occurred or some allergen epitopes were more easily obtained after boiling (Gómez et al. 2015; Carnés et al., 2007), so the levels of specific IgE were higher in the SPE group as compared to the SGD, SID and SGID groups. After simulated gastric digestion, the binding ability of proteins to IgE and IgG₁ was lower and its allergenicity was significantly decreased. Similarly, Guo, Li, & Lin (2009) also found that the specific IgE-binding ability of Pen a 1, the main shrimp allergen, decreased after simulated gastric digestion. The change of allergenicity might be due to the disruption of the immunodominant allergen epitopes in shrimp proteins by pepsin. However, after simulated GI digestion, trypsin and chymotrypsin might break the structure of simulated gastric digests, altering their secondary and/or tertiary structures and their ability to bind to specific IgE and IgG₁, resulting in the subsequent production of new allergens (Luo et al., 2020; Pfeifer et al., 2015; Prodic et al., 2018; Rao et al., 2020; Bøgh & Madsen, 2016). In addition, after trypsin and chymotrypsin were digested alone, the IgE and IgG₁ binding abilities of shrimp proteins also decreased, but an immune response remained (Toomer, Do, Fu, & Williams, 2015). In other words, the GI digestion products had a higher allergenicity as compared to the gastric and intestinal digestion products because digestion can lead to catastrophic destruction of protein structures. More immunodominant allergen epitopes might be retained or exposed in the GI digestion products, while those in gastric and intestine might be destroyed by digestive enzymes. Therefore, after certain GI digestion, protein structure alterations would cause higher epitope accessibility. It can promote the antigen–antibody interaction and enhancing IgE/IgG binding, thus led to stronger IgE/IgG reactivity. Since the levels of specific IgE and IgG do not directly equate to the allergenicity, functional tests *in vivo*, such as the levels of histamine and m MCP-1 in the plasma, are necessary (Pelletier et al., 2021).

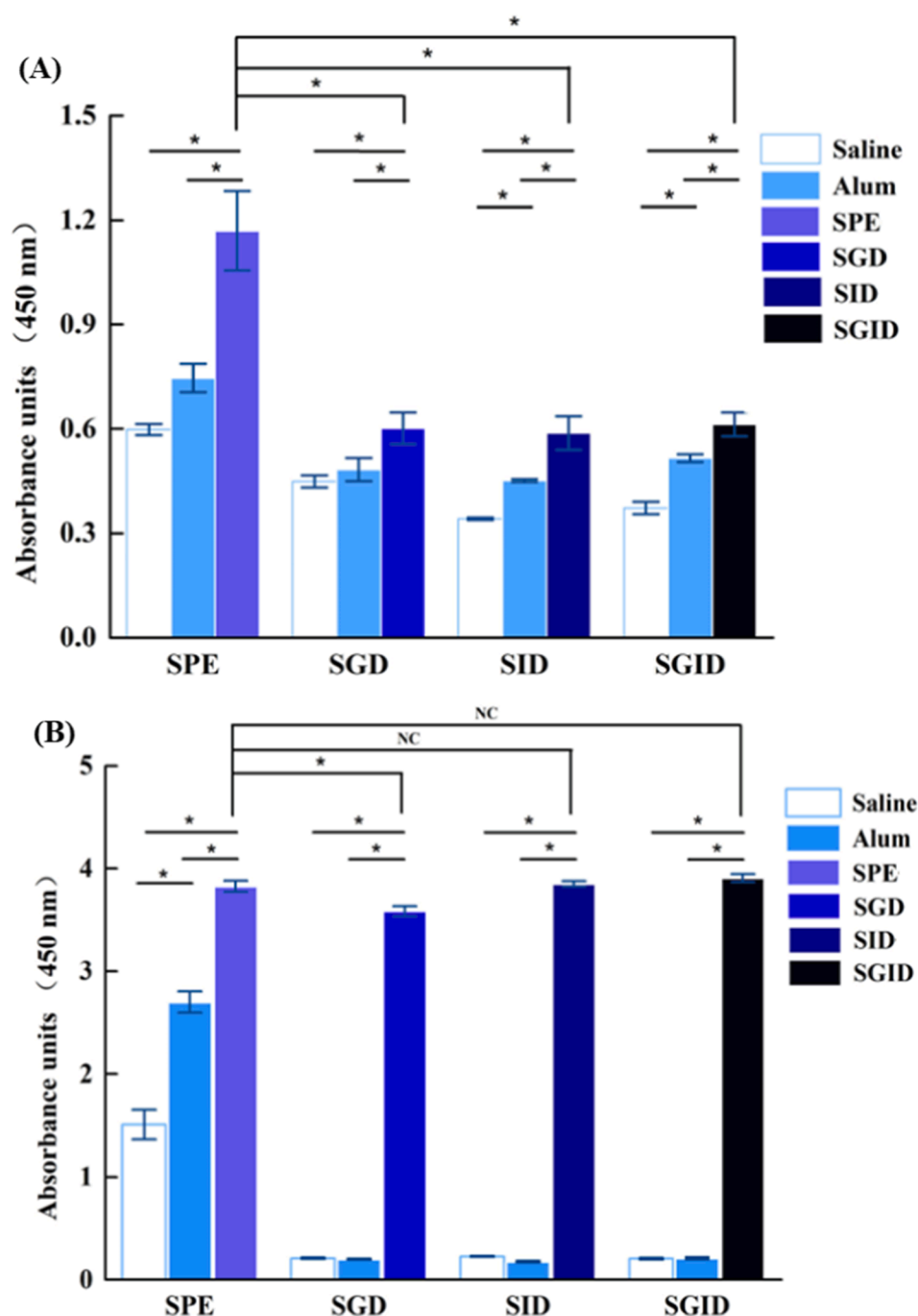


Fig. 3. Anaphylaxis in the BALB/c mice sensitized with SPE and its SGD, SID and SGID samples. (A) The levels of specific IgE. (B) The levels of specific IgG₁. The levels of specific IgE and IgG₁ were measured by ELISA with HRP-conjugated goat antimouse IgE and IgG₁ antibodies. Bars represent means $\pm$ SEM.

3.4. Histamine and mMCP-1 levels of shrimp protein extract or its digestion products

To monitor the mast cell activation and degranulation, the levels of histamine and mMCP-1 in the plasma were measured (He et al., 2021). On the 33 d, plasma samples were extracted after challenge, and centrifuged at 4000 rpm for 10 min. Then, the plasma was collected and kept at -80 °C until analysis. Fig. 4 illustrates the levels of histamine and mMCP-1 in the plasma. As shown in Fig. 4 (A), the levels of plasma histamine in SPE-, SGD-, SID- and SGID-challenged groups were 29.53 ng/mL, 29.04 ng/mL, 29.16 ng/mL and 29.55 ng/mL, respectively. Meanwhile, the levels of plasma histamine in saline- and alum-treated groups were 28.82 ng/mL and 28.89 ng/mL and no significant

differences were found between two groups ($p > 0.05$). Especially for SPE- and SGID-challenged groups, the levels of plasma histamine increased significantly compared with that of the alum-treated group ($p < 0.05$). In addition, as compared with the SPE-challenged group, the levels of plasma histamine in the SGD- and SID-challenged group were decreased. Although the SGID-challenged group showed the growth at the levels of plasma histamine, it showed no significant difference with the SPE- and SGID-challenged group ($p > 0.05$).

As shown in Fig. 4 (B), the similar results were observed in the levels of plasma mMCP-1. The levels of mMCP-1 were higher in SPE-, SGD-, SID- and SGID-challenged groups. However, although the levels of mMCP-1 in the alum-treated group were remarkably higher than that those in the saline-treated group ($p < 0.05$), both groups were at a lower

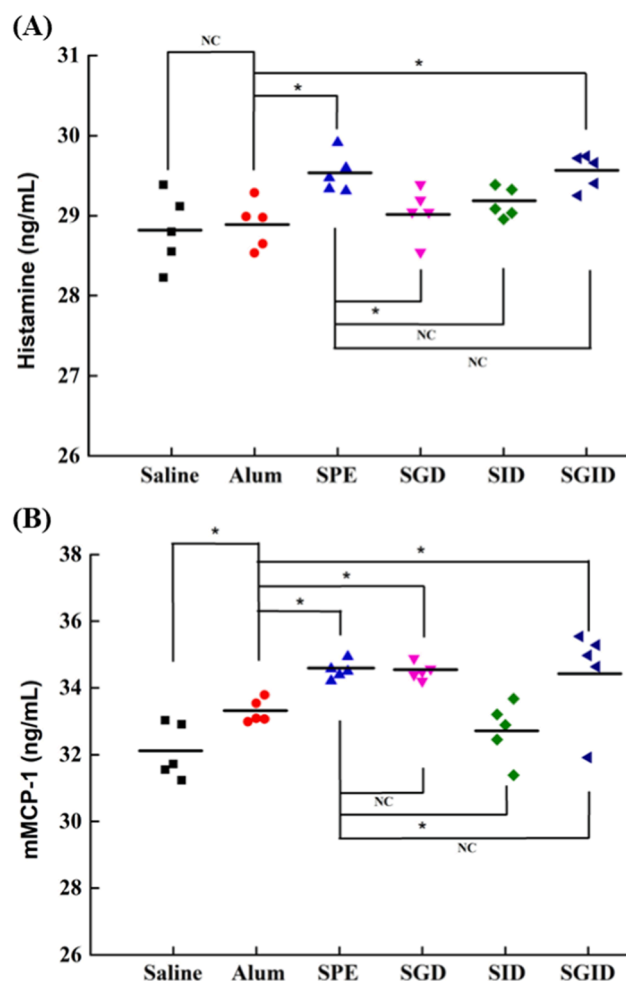


Fig. 4. Mast cell degranulation induced by SPE and its SGD, SID and SGID samples. The levels of histamine (A) and mMCP-1 (B) in 20-30 min after challenged the BALB/c mice. The levels of histamine and mMCP-1 were determined by a commercial ELISA kit according to the manufacturer's instructions. Splashes represent individual mice ($n = 5$).

level. As compared to the alum-treated group, the levels of mMCP-1 in the SPE-challenged group increased to 34.52 ng/mL ($p < 0.05$). Meanwhile, the levels of mMCP-1 in the SGD- and SGID-challenged group decreased to 34.50 ng/mL and 34.47 ng/mL, it showed a slight but no significant decrease ($p > 0.05$). However, in comparison with the SPE-challenged group, the SID-challenged group did not cause the higher mMCP-1 levels (32.72 ng/mL) ($p < 0.05$).

Since the histamine and mMCP-1 in the plasma were released from the activated mast cells or basophils after secondary exposure to allergens, the increase in allergic mediators indicated severe anaphylactic symptoms (He et al., 2021). Compared to the saline- and alum-treated groups, the levels of histamine and mMCP-1 were higher in the SPE- and SGID-challenged groups, which would trigger and aggravate the anaphylactic reactions. In contrast, the levels of plasma histamine and mMCP-1 in the SGD- and SID-challenged groups were lower than those in the SPE- and SGID-challenged groups. The results suggested that the digestive patterns of gastric, intestinal and GI digestion were different due to the different protease cleavage sites, and the sensitizing and eliciting capacity of SPE-, SGD-, SID- and SGID-challenged groups were different. The specific cleavage of peptide bonds by pepsin, trypsin and α -chymotrypsin made the proteins more easily hydrolyzed into small fragments, thus reducing the allergenicity of *Penaeus vannamei*. In addition, simulated GI digestion could further improve the digestibility of shrimp proteins, exposing more cleavage sites and making the SGD

more susceptible to proteolytic attack. Thus, simulated GI digestion induces the exposure of hidden allergen epitopes in SGD, resulting in changes in antigenicity and allergic responses.

3.5. The degranulation of mast cells after challenge with shrimp protein extract or its digestion products

Mast cells are involved in allergic reactions through the activation of IgE antibodies (Msallam et al., 2020), and there may be significant differences between different groups. Therefore, to characterize the effects of simulated GI digestion of shrimps on spleen mast cells of BALB/c mice, the spleen sections were stained with toluidine blue solution, and the spleen mast cells were observed under the microscope (Kumar et al., 2019). For each field, the total, intact, and degranulated mast cells were calculated under the microscope. The cells were considered degranulated if the cells outline appeared to be disrupted. Furthermore, intact mast cells demonstrate deep blue (orthochromatic) under the microscope. As shown in Fig. 5 (A), a large number of mast cells and degranulated mast cells could be observed in the spleen of SPE-challenged mice. In contrast, a small number of degranulated mast cells were detected in the spleen of SGD- and SID-challenged mice and SGD and SID administration significantly reduced the proportion of degranulated

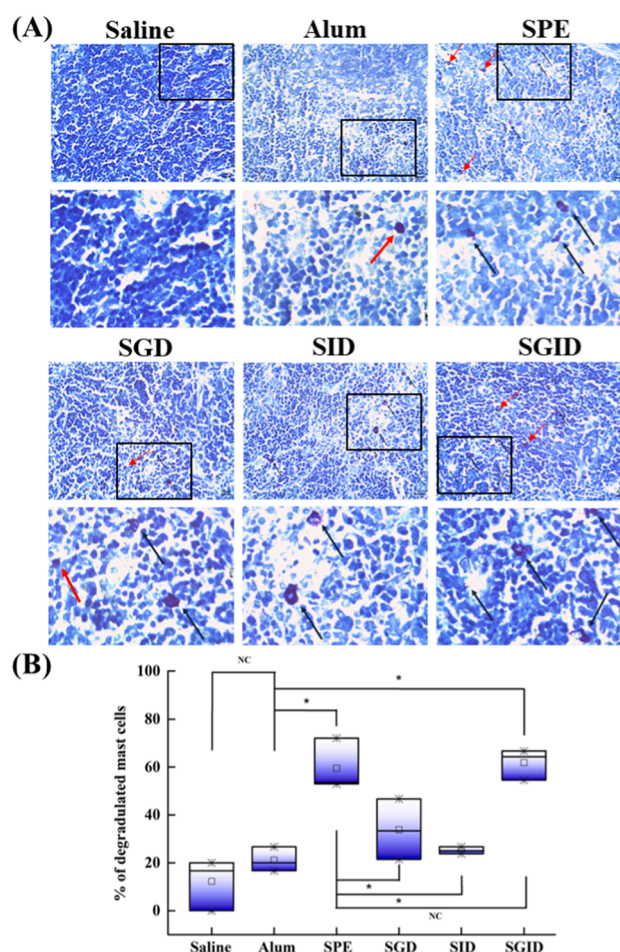


Fig. 5. Toluidine blue staining of spleen in SPE and its SGD, SID and SGID samples challenged mice. Mast cells were stained (A) and the percentage of degranulated mast cells in different groups (B). There were less degranulated mast cells in saline-treated mice and alum-treated mice, while SPE-challenged mice, SGD-challenged mice, SID-challenged mice and SGID-challenged mice showed degranulated mast cells. Black arrows indicate degranulated mast cells and red arrows indicate intact mast cells. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

mast cells when compared to the SPE-challenged mice. However, of note, the SGID-challenged mice demonstrated an effective augmentation effect on degranulated mast cells of the spleen. In contrast, saline- and alum-treated mice had intact mast cells in the spleen and the mast cells were less degranulated (Fig. 5 (A)). Meanwhile, the count of

degranulated mast cells was found to be parallel to the histological examination of the toluidine blue stained spleen tissue (Fig. 5 (B)).

The results demonstrated that the changes of the proteins altered the mast cell activation by changing the degranulation of mast cells. We found that gastric and intestinal digestion of proteins acted as a

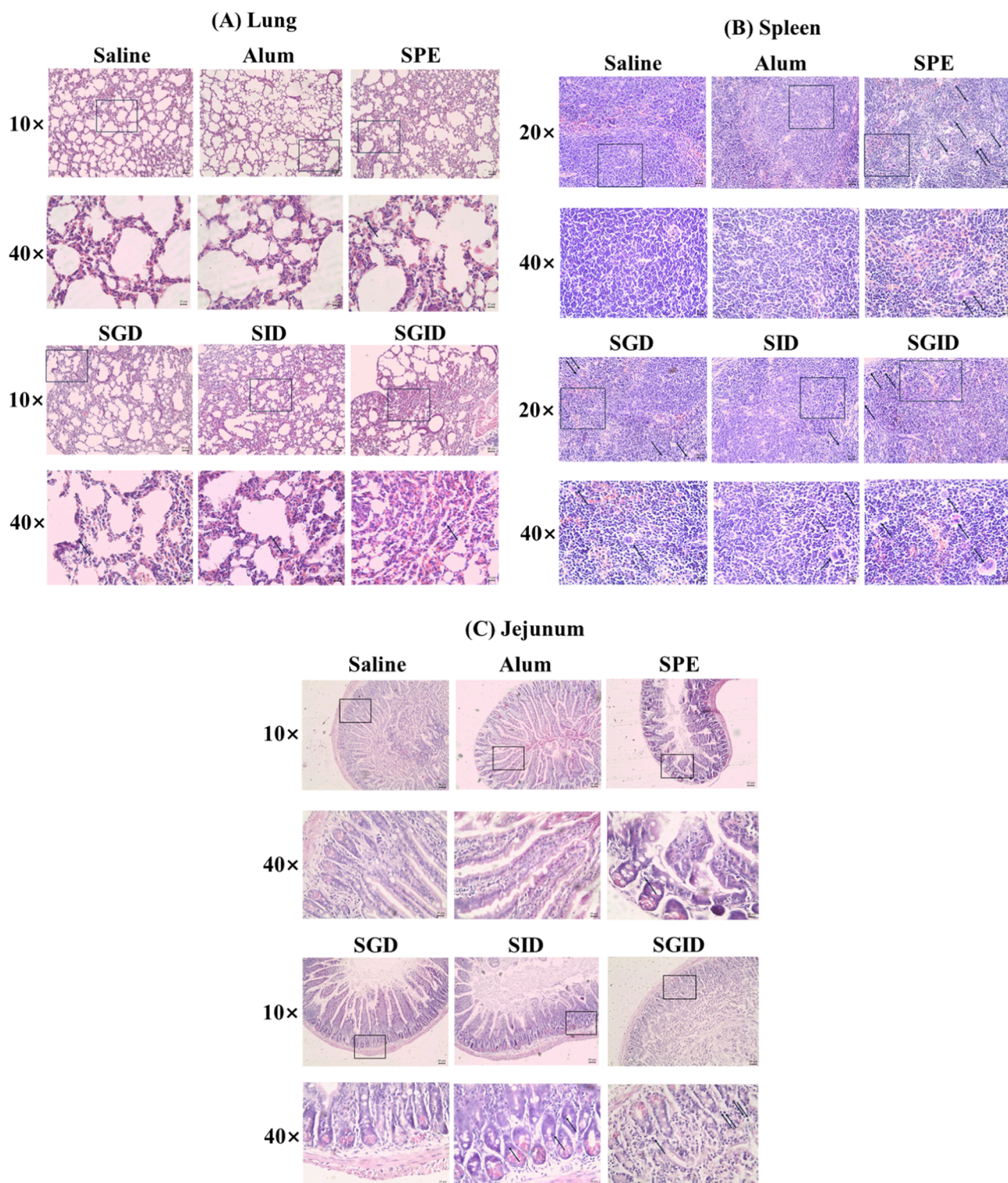


Fig. 6. Histopathological examination of lung, spleen and jejunum of saline, alum and sensitized mice. (A) Representative images of lung stained with H&E and photographed at 10 × and 40 × magnifications. (B) Representative images of spleen stained with H&E and photographed at 20 × and 40 × magnifications. (C) Representative images of jejunum stained with H&E and photographed at 10 × and 40 × magnifications. There were no identifiable histopathological changes in saline-treated mice and alum-treated mice, while SPE-challenged mice, SGD-challenged mice, SID-challenged mice and SGID-challenged mice showed significant allergic symptoms. Black arrows indicate inflammatory cells and megakaryocytes.

suppressor in degranulated mast cells. However, the simulated GI digestion of proteins promoted the degranulation of mast cells again. Therefore, SGID presented a comparable allergenic potential to SPE, whereas SGD and SID administration significantly reduced the allergenicity in the mice. These results suggested that the change of allergenicity in shrimp protein digestion products might be related to the change of allergen epitopes in the products.

3.6. Histopathological evaluation of lung, spleen and jejunum after challenge with shrimp protein extract or its digestion products

The immune response was further characterized by observing the sections of lung, spleen and jejunum after H&E staining (Fig. 6). As shown in Fig. 6 (A), lung sections of SPE-, SGD-, SID- and SGID-challenged mice showed hyperplasia of bronchial lining epithelial and thickening of alveolar septa. Meanwhile, inflammatory cell infiltration was observed in the SPE-, SGD-, SID- and SGID-challenged mice. However, the lung sections of saline- and alum-treated mice showed significantly different properties when compared to the SPE-, SGD-, SID- and SGID-challenged mice. No obvious inflammatory cell infiltration and neat alveolar septa were observed in the lung of saline- and alum-treated mice in Fig. 6 (A).

The results of spleen sections in mice were similar to the lung sections. Hyperplastic lymphoid and large-size megakaryocytes were observed in spleen sections of SPE-challenged mice (Fig. 6 (B)). However, the inflammatory cells and megakaryocytes in the SGD- and SID-challenged mice were significantly fewer than that of the SPE-challenged mice. After the SGID treatments, the inflammatory cells and megakaryocytes were significantly increased. However, in Fig. 6 (B), saline- and alum-treated mice retained the normal structure of spleen. The results showed that the anaphylactic levels were increased by SPE and SGID administration and decreased after SGD and SID administration in the BALB/c mice model.

The pathological changes of jejunum could be observed in Fig. 6 (C). The inflammatory cell infiltration was observed in the jejunum of SPE-, SID- and SGID-challenged mice (black arrows indicate inflammatory cells). However, the jejunum of SGD-challenged mice was free of inflammatory cell infiltration. Furthermore, the destruction of the mucosal lining was observed in the jejunum of SPE- and SGID-challenged mice and the mucosal lining of jejunum was undamaged, the structure was normal and the villi was not deformed after SGD and SID administration. At the same time, the jejunum of saline- and alum-treated mice had neatly arranged villi, intact mucosa with no inflammation cell infiltration as shown in Fig. 6 (C). Histopathological analysis of the mice showed that the jejunum of the saline- and alum-treated mice was healthy intestinal tissue, whereas the jejunum destruction was more severe in the SPE- and SGID-challenged mice. Similar pathological lesions to the lung, spleen and jejunum in the mice as shown in Fig. 6 have been observed previously in studies with other allergenic foods (Kumar, Sharma, Gupta, Verma, & Dwivedi, 2018; Verma et al., 2012).

4. Conclusion

In this study, the gastric, intestinal and GI digests of *Penaeus vannamei* were studied using a BALB/c mice model to systematically characterize the allergenicity of shrimp proteins. Results indicated that a large quantity of low molecular weight proteins were produced after simulated GI digestion. Moreover, our research demonstrated that SPE and SGID could cause allergic symptoms, a drop in temperature, and suppression of water intake and appetite. In addition, SPE- and SGID-sensitized mice had higher specific IgE and IgG₁ levels than SGD-sensitized mice in serum. Similarly, the levels of histamine and mMCP-1 in plasma of the SPE and SGID-challenged mice were higher when compared to the SGD-challenged mice. Likewise, a higher number of degranulated mast cells and evident histopathological changes were observed in SPE- and SGID-challenged mice. This study suggests that the

allergenicity of shrimp proteins reduced significantly after gastric digestion and increased again after GI digestion. These changes are related to the low molecular weight proteins released by digestion. After simulated digestion, the pepsin, trypsin and chymotrypsin might alter the protein structure of *Penaeus vannamei*, while disrupting, preserving, or exposing the immunodominant allergen epitopes, thereby changing the sensitizing and eliciting capacity of *Penaeus vannamei*.

CRediT authorship contribution statement

Yao Liu: Investigation, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. **Songyi Lin:** Conceptualization, Writing – review & editing. **Kexin Liu:** Resources, Investigation, Writing – review & editing. **Shan Wang:** Resources, Writing – review & editing. **Na Sun:** Conceptualization, Methodology, Formal analysis, Supervision, Writing – review & editing, 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.

Acknowledgments

This work was financially supported by the National Natural Science Foundation of China (No. 32022067).

References

- Ayuso, R. (2011). Update on the diagnosis and treatment of shellfish allergy. *Current Allergy and Asthma Reports*, 11(4), 309–316.
- Bavaro, S. L., Stasio, L., Mamone, G., De Angelis, E., Nocerino, R., Berni Canani, R., ... Monaci, L. (2018). Effect of thermal/pressure processing and simulated human digestion on the immunoreactivity of extractable peanut allergens. *Food Research International*, 109, 126–137.
- Bøgh, K. L., & Madsen, C. B. (2016). Food Allergens: Is there a correlation between stability to digestion and allergenicity? *Critical Reviews in Food Science and Nutrition*, 56(9), 1545–1567.
- Carnés, J., Ferrer, Á., Huertas, Á. J., Andreu, C., Larramendi, C. H., & Fernández-Caldas, E. (2007). The use of raw or boiled crustacean extracts for the diagnosis of seafood allergic individuals. *Annals of Allergy, Asthma & Immunology*, 98(4), 349–354.
- Choi, J. E., Werbel, T., Wang, Z., Wu, C. C., Yaksh, T. L., & Di Nardo, A. (2019). Botulinum toxin blocks mast cells and prevents rosacea like inflammation. *Journal of Dermatological Science*, 93(1), 58–64.
- Codex Alimentarius Commission. (2003). Alinorm 03/34: Joint FAO/WHO Food Standard Programme, Codex Alimentarius Commission, Twenty-Fifth Session, Rome, Italy, 30 June–5 July, 2003.
- Córdova-Murueta, J. H., Navarrete-Del-Toro, M. A., & García-Carreo, F. L. (2017). Advances in the study of activity additivity of supplemented proteases to improve digestion of feed protein by *Penaeus vannamei*. *Aquaculture Nutrition*, 23(2), 414–421.
- Freidl, R., Gstöttner, A., Baranyi, U., Swoboda, I., Stolz, F., Focke-Tejkl, M., ... Linhart, B. (2020). Resistance of parvalbumin to gastrointestinal digestion is required for profound and long-lasting prophylactic oral tolerance. *Allergy*, 75(2), 326–335.
- Gámez, C., Zafra, M. P., Sanz, V., Mazzeo, C., Ibáñez, M. D., Sastre, J., & del Pozo, V. (2015). Simulated gastrointestinal digestion reduces the allergic reactivity of shrimp extract proteins and tropomyosin. *Food Chemistry*, 173, 475–481.
- Gao, C., Wang, F., Yuan, L., Liu, J., Sun, D., & Li, X. (2019). Physicochemical property, antioxidant activity, and cytoprotective effect of the germinated soybean proteins. *Food Science & Nutrition*, 7(1), 120–131.
- Gazme, B., Rezaei, K., & Udenigwe, C. (2020). Effect of enzyme immobilization and *in vitro* digestion on the immune-reactivity and sequence of IgE epitopes in egg white proteins. *Food & Function*, 11(7), 6632–6642.
- Guo, Y., Li, Z., & Lin, H. (2009). The effect of simulated gastrointestinal digestion on shrimp *Penaeus vannamei* allergenicity. *Chinese Journal of Oceanology and Limnology*, 27(4), 703–707.
- Guo, Y., Luo, C., Xu, L., Lin, H., Li, Z., Pavase, T. R., ... Chen, Y. (2021). Comparison of digestibility and potential allergenicity of raw shrimp (*Litopenaeus vannamei*) extracts in static and dynamic digestion systems. *Food Chemistry*, 345, Article 128831.
- He, S., Zhao, J., Zhang, Y., Zhu, Y., Li, X., Cao, X., ... Sun, H. (2021). Effects of low-pH treatment on the allergenicity reduction of black turtle bean (*Phaseolus vulgaris* L.) lectin and its mechanism. *Journal of Agricultural and Food Chemistry*, 69(4), 1379–1390.

- Klueber, J., Costa, J., Randow, S., Codreanu-Morel, F., Verhoeckx, K., Bindsløv-Jensen, C., ... Kuehn, A. (2020). Homologous tropomyosins from vertebrate and invertebrate: Recombinant calibrator proteins in functional biological assays for tropomyosin allergenicity assessment of novel animal foods. *Clinical & Experimental Allergy*, 50(1), 105–116.
- Korte, R., Bräcker, J., & Brockmeyer, J. (2017). Gastrointestinal digestion of hazelnut allergens on molecular level: Elucidation of degradation kinetics and resistant immunoreactive peptides using mass spectrometry. *Molecular Nutrition & Food Research*, 61(10), 1700130.
- Kumar, B. V. M., Vijaykrishnaraj, M., Kurrey, N. K., Shinde, V. S., & Prabhasankar, P. (2019). Prolyl endopeptidase-degraded low immunoreactive wheat flour attenuates immune responses in Caco-2 intestinal cells and gluten-sensitized BALB/c mice. *Food and Chemical Toxicology*, 129, 466–475.
- Kumar, S., Sharma, A., Gupta, R. K., Verma, A. K., & Dwivedi, P. D. (2018). Allergenicity assessment of *Buchanania lanzan* protein extract in Balb/c mice. *International Immunopharmacology*, 63, 170–182.
- Li, X. M., Serebrisky, D., Lee, S. Y., Huang, C. K., Bardina, L., Schofield, B., ... Sampson, H. (2000). A murine model of peanut anaphylaxis: T- and B-cell responses to a major peanut allergen mimic human responses. *The Journal of allergy and clinical immunology*, 106, 150–158.
- Liu, M., Liu, S. H., Han, T. J., Xia, F., Li, M. S., Weng, W. Y., ... Liu, G. M. (2019). Effects of thermal processing on digestion stability and immunoreactivity of the *Litopenaeus vannamei* matrix. *Food & Function*, 10(9), 5374–5385.
- Liu, Q. Z., Lin, S. Y., & Sun, N. (2022). How does food matrix components affect food allergies, food allergens and the detection of food allergens? A systematic review. *Trends in Food Science & Technology*, 1–11.
- Kumar, S., Khan, S., Verma, A. K., & Dwivedi, P. D. (2019). Identification and characterization of major IgE binding of purified allergenic protein (11 kDa) from *Buchanania lanzan*. *Food Research International*, 125, 108640.1–108640.10.
- Luo, C., Guo, Y., Li, Z., Ahmed, I., Pramod, S. N., Gao, X., Lv, L., & Lin, H. (2020). Lipid emulsion enhances fish allergen parvalbumin's resistance to *in vitro* digestion and IgG/IgE binding capacity. *Food Chemistry*, 302, 125333.1–125333.8.
- Meng, X., Li, X., Gao, J., & Chen, H. (2016). Characterization of the potential allergenicity of irradiated bovine α -lactalbumin in a BALB/c mouse model. *Food and Chemical Toxicology*, 97, 402–410.
- Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., ... Brodtkorb, A. (2014). A standardised static *in vitro* digestion method suitable for food - an international consensus. *Food & Function*, 5(6), 1113–1124.
- Misra, A., Kumar, R., Mishra, V., Chaudhari, B. P., Raisuddin, S., Das, M., & Dwivedi, P. D. (2011). Potential allergens of green gram (*Vigna radiata* L. Millsp) identified as members of cupin superfamily and seed albumin. *Clinical & Experimental Allergy*, 41(8), 1157–1168.
- Morafo, V., Srivastava, K., Huang, C. K., Kleiner, G., Lee, S. Y., & Sampson, H. A. (2003). Genetic susceptibility to food allergy is linked to differential TH2-TH1 responses in C3H/HeJ and BALB/c mice. *Journal of Allergy and Clinical Immunology*, 111(5), 1122–1128.
- Msallam, R., Balla, J., Rathore Abhay, P. S., Kared, H., Malleret, B., Saron Wilfried, A. A., Liu, Z., Hang Jing, W., Dutertre Charles, A., Larbi, A., Chan Jerry, K. Y., St. John Ashley, L., & Ginhoux, F. (2020). Fetal mast cells mediate postnatal allergic responses dependent on maternal IgE. *Science*, 370(6519), 941–950.
- Ostblom, E., Wickman, M., Hage, M. V., & Lilja, G. (2008). Reported symptoms of food hypersensitivity to common foods in 4-year-old children. *Acta Paediatrica*, 97(1), 85–90.
- Pelletier, B., Perrin, A., Assoun, N., Plaquet, C., Oreal, N., Gaulme, L., ... Hervé, P. L. (2021). Epicutaneous immunotherapy protects cashew-sensitized mice from anaphylaxis. *Allergy*, 76(4), 1213–1222.
- Pfeifer, S., Bublin, M., Dubiela, P., Hummel, K., Wortmann, J., Hofer, G., ... Hoffmann-Sommergruber, K. (2015). Cor a 14, the allergenic 2S albumin from hazelnut, is highly thermostable and resistant to gastrointestinal digestion. *Molecular Nutrition & Food Research*, 59(10), 2077–2086.
- Prodic, I., Stanic-Vucinic, D., Apostolovic, D., Mihailovic, J., Radibratovic, M., Radosavljevic, J., ... Cirkovic Velickovic, T. (2018). Influence of peanut matrix on stability of allergens in gastric-simulated digesta: 2S albumins are main contributors to the IgE reactivity of short digestion-resistant peptides. *Clinical & Experimental Allergy*, 48(6), 731–740.
- Rao, H., Baricevic-Jones, I., Bernard, H., Smith, F., Sayers, R., Balasundaram, A., ... Mills, E. (2020). The effect of the food matrix on the *in vitro* bio-accessibility and IgE reactivity of peanut allergens. *Molecular Nutrition & Food Research*, 64, 1901093.
- Sheu, S. C., Yu, M. T., Lien, Y. Y., & Lee, M. S. (2020). Development of a specific isothermal nucleic acid amplification for the rapid and sensitive detection of shrimp allergens in processed food. *Food Chemistry*, 332, Article 127389.
- Soon, J. M. (2019). Food allergen knowledge, attitude and practices among UK consumers: A structural modelling approach. *Food Research International*, 120, 375–381.
- Sun, N., Liu, Y., Liu, K. X., Wang, S., Liu, Q. Z., & Lin, S. Y. (2022). Gastrointestinal fate of food allergens and its relationship with allergenicity. *Comprehensive Reviews in Food Science and Food Safety*, 1–29.
- Tong, P., Xiong, L., Gao, J., Li, X., Wu, Z., Yang, A., ... Chen, H. (2020). Influence of heat treatment and egg matrix on the physicochemical and allergenic properties of egg custard. *Journal of Food Science*, 85(3), 789–799.
- Toomer, O. T., Do, A. B., Fu, T. J., & Williams, K. M. (2015). Digestibility and immunoreactivity of shrimp extracts using an *in vitro* digestibility model with pepsin and pancreatin. *Journal of Food Science*, 80(7), T1633–T1639.
- Verma, A. K., Kumar, S., Tripathi, A., Chaudhari, B. P., Das, M., & Dwivedi, P. D. (2012). Chickpea (*Cicer arietinum*) proteins induce allergic responses in nasobronchial allergic patients and BALB/c mice. *Toxicology Letters*, 210(1), 24–33.
- Xu, L. L., Zhang, H. W., Zhang, X. M., Lin, H., Guo, Y. M., Yu, C., ... Li, Z. X. (2020). Natural shrimp (*Litopenaeus vannamei*) tropomyosin shows higher allergic properties than recombinant ones as compared through SWATH-MS-Based proteomics and immunological response. *Journal of Agricultural and Food Chemistry*, 68(41), 11553–11567.
- Xu, X., Liang, R., Li, D., Jiang, C., & Lin, S. (2020). Evaluation of sea cucumber peptides-assisted memory activity and acetylation modification in hippocampus of test mice based on scopalamine-induced experimental animal model of memory disorder. *Journal of Functional Foods*, 68, Article 103909.
- Yi, L., Van Boekel, M. A. J. S., & Lakemond, C. M. M. (2017). Extracting *Tenebrio molitor* protein while preventing browning: Effect of pH and NaCl on protein yield. *Journal of Insects as Food and Feed*, 3(1), 21–31.
- Zhang, B., Fang, C. D., Hao, G. J., & Zhang, Y. Y. (2018). Effect of kappa-carrageenan oligosaccharides on myofibrillar protein oxidation in peeled shrimp (*Litopenaeus vannamei*) during long-term frozen storage. *Food Chemistry*, 245, 254–261.
- Zhang, H., Liu, M., Shao, R., Zhang, J. J., Zuo, R. T., Tan, B. P., ... Wan, M. (2021). The effects of different lipid sources on the growth, intestinal health, and lipid metabolism of the pacific white shrimp (*Litopenaeus vannamei*). *Aquaculture*, 543(2), Article 737655.
- Zhang, Z., Xiao, H., Zhang, X., & Zhou, P. (2019). Conformation, allergenicity and human cell allergy sensitization of tropomyosin from *Exopalaemon modestus*: Effects of deglycosylation and Maillard reaction. *Food Chemistry*, 276, 520–527.
- Zhao, J. L., Li, Y. H., Xu, L. L., Ji, Y., Zeng, J. H., Timira, V., ... Li, Z. X. (2022). Insight into IgG/IgE binding ability, *in vitro* digestibility and structural changes of shrimp (*Litopenaeus vannamei*) soluble extracts with thermal processing. *Food Chemistry*, 381, Article 132177.
- Zhou, D. Y., Qin, L., Zhu, B. W., Li, D. M., Yang, J. F., Dong, X. P., & Murata, Y. (2012). Optimisation of hydrolysis of purple sea urchin (*Strongylocentrotus nudus*) gonad by response surface methodology and evaluation of *in vitro* antioxidant activity of the hydrolysate. *Journal of the Science of Food and Agriculture*, 92(8), 1694–1701.
- Ahmed, I., Lin, H., Xu, L., Li, S., Costa, J., Mafra, I., Chen, G., Gao, X., & li, Z. (2020). Immunomodulatory effect of laccase/caffeic acid and transglutaminase in alleviating shrimp tropomyosin (Met e 1) allergenicity. *Journal of Agricultural and Food Chemistry*, 68(29), 7765–7778.