

Cite this: *Food Funct.*, 2021, **12**, 5351

Immunostimulatory and allergenic properties of emulsified and non-emulsified digestion products of parvalbumin (*Scophthalmus maximus*) in RBL-2H3 cells and BALB/c mouse models

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In the present study, the influence of lipid emulsion on the allergenicity of digestion products of fish parvalbumin (PV) was investigated, which was initially subjected to simulated gastric/intestinal digestion both under emulsified and non-emulsified conditions. The release of β -hexosaminidase (β -hex), histamine (His), tryptase (TPS), interleukin 4 (IL-4), and IL-13 in RBL cells was decreased by 79.32, 26.19, 41.67, 53.95 and 54.40%, respectively, following stimulation with the gastric digestion products of PV. Whereas, lipid emulsified digestion products of PV (e-PV) significantly enhanced the release of active mediators and cytokines. The digestion products of emulsified PV at 180 min resulted in a higher release of β -hex (197.60%), His (12.18%), TPS (38.85%), IL-4 (48.19%) and IL-13 (59.40%), as compared to that of PV. However, no obvious differences in the release of active substances and cytokines were noted between intestinal digestion products of PV and intestinal digestion products of emulsified PV. In the mouse model studies, digested PV products reduced the anaphylactic scores, whereas e-PV manifested a higher level of allergic symptoms. Moreover, mice treated with 50% e-PV had significantly higher levels of specific IgE (32.56%), total IgE (16.67%) and total IgG1 (5.15%) than those treated with 50% PV. Mice treated with 50% e-PV had significantly higher levels of His (8.50%) and TPS (10.07%) compared with mice treated with 50% PV. Lipid emulsions altered the digestibility of PV in gastrointestinal digestion and enhanced the allergenicity of PV digestion products at the cellular levels, subsequently posing a higher risk of allergic reactions in susceptible individuals.

Received 24th February 2021,

Accepted 17th April 2021

DOI: 10.1039/d1fo00575h

rsc.li/food-function

1. Introduction

Food allergy refers to the exposure of susceptible individuals to specific harmful food proteins or dietary antigens, resulting in abnormal or exaggerated immune reactions.^{1,2} It is becoming a serious global health concern with an estimated prevalence of 5–8% in adults and 2–4% in children.³ The clinical symptoms of food allergies include gastrointestinal symptoms such as nausea, diarrhea, vomiting, skin symptoms (eczema, itching and urticaria), and neurological symptoms (headache and dizziness). In some instances, patients may suffer a

sudden drop in blood pressure, loss of consciousness, poor breathing, anaphylactic shock and even death.⁴ Food allergies are divided into two categories based on the pathogenesis, which include IgE-mediated and non-IgE-mediated. More than 90% of food allergies are mediated by IgE and are considered to be an important aspect of food safety issues.⁵ According to the report of the Food and Agriculture Organization of the United Nations, more than 90% of allergic reactions in the world are caused by eight types of foods.^{6–8} Fish is a rich source of nutrients and an important part of human diet, however, it can also cause severe allergic reactions. PV is the major allergen of fish, with a molecular weight of about 10–12 kDa. These small molecular weight muscle proteins belong to the calcium binding protein family and are resistant to enzymatic hydrolysis and heating.⁹

It is generally accepted that the amino acid fragments at the IgE binding site of the native or denatured proteins, which are resistant to gastrointestinal digestion fluids in the body can still maintain their sensitization potential for eliciting food-induced allergy. Because the mechanism of food allergic

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reactions occurring due to drugs and dietary foods are different: the sensitized protein must be processed by the human digestive system (low acid, bile salt, protease hydrolysis, *etc.*) to reach the intestinal mucosa and following absorption can elicit an immune reaction. Food allergens (or fragments thereof) must pass through the gastrointestinal (GI) mucosa to interact with the immune system, which is an important criterion for allergens to cause allergic reactions in sensitized individuals.^{10,11} Therefore, the stability of protein to gastrointestinal digestion is considered to be an important indicator for evaluating food allergenicity.

However, any changes in food processing or processing methods related to protein digestion may lead to changes in hydrolysis patterns and efficiencies of protein, which in turn affect the allergenicity of the food allergen.¹² In this regard, the interaction between allergens and other food ingredients is seriously ignored in the allergenicity assessment.^{11,13} Proteins, sugars, and lipid in the food matrix may affect the hydrolysis of allergens by digestive enzymes and affect the way allergens are presented to the immune system, thereby affecting their potential allergenicity.^{14,15}

Compared to the sugar and protein components, lipids are more likely to affect the digestion of allergens and immune system uptake due to their hydrophobicity, which in turn affects the allergenicity of allergens. Lipids may be bound to proteins by covalent binding or by non-covalent bonds such as hydrophobic interaction and electrostatic interaction.¹⁶ Research studies have revealed that the interaction of food allergens with lipids can largely alter the ability of allergens to reach active immune sampling sites in intestinal lymphoid tissues. Lipids can change the degradation mode and efficiency of allergens in the gastrointestinal tract, promote allergens to pass through the intestinal epithelial barrier, or both, thereby affecting the potential immunogenicity of allergens. Additionally, recent studies have found that certain dietary lipids can act like an adjuvant, and when ingested with specific allergens can trigger initial sensitization and subsequently enhance the allergen-specific immune response.¹⁷

Although the anti-digestibility of parvalbumin and the allergenicity of intact proteins, as well as, the digestion stability of the parvalbumin under emulsified conditions have been reported in the previous study.¹⁸ In the present study, the allergenic and immunostimulatory potential of the digestion products of PV under emulsified conditions were evaluated in order to assess the effect of lipid emulsion on fish PV allergenicity. The effect of dietary lipids on allergenicity of protein allergens may need more attention with regard to allergy risk assessment.

2. Materials and methods

2.1. Materials and chemicals

Turbot (*Scophthalmus maximus*) was procured from the local market of Qingdao and PV was purified from turbot as

described earlier.¹⁸ Mice serum for RBL cell assays was obtained from the same lab. Trypsin (from porcine pancreas), pepsin (from porcine gastric mucosa), 3,3',5,5'-tetramethylbenzidine (TMB) and compound 48–80 were acquired from Sigma Chemical Co. (St Louis, MO, USA). Bovine serum albumin (BSA) and α -chymotrypsin (from bovine pancreas) were purchased from Solarbio Co. (Beijing, China). HRP-labelled goat anti-mouse IgE antibody was bought from Southern Biotech Co. (Birmingham, AL, USA), while Imject Alum Adjuvant was purchased from Thermo Fisher Scientific Inc. (Waltham, MA, USA). *N*-Hexadecane was purchased from Macklin Co. (Shanghai, China). Fetal bovine serum (FBS), penicillin–streptomycin and advanced Dulbecco's modified Eagle's medium (DMEM) were purchased from Gibco Life Technologies (New York, USA). The ELISA kits for total IgE (t-IgE), total IgG1 (t-IgG1), histamine (His), tryptase (TPS), IL-4 and IL-13 were acquired from Jinsui Biotech Co. (Shanghai, China). Other reagents used in this study were of analytical grade and pure.

2.2. BALB/c mice

Specific pathogen-free (SPF) female BALB/c mice (6–8 weeks old) were purchased in Qingdao (Daren Fortune Animal Technology Co., China). The animals were housed under SPF conditions with controlled temperature ($23 \pm 2^\circ\text{C}$) and relative humidity ($55 \pm 5\%$) and maintained a 12/12 h light/dark cycle. All the mice were given food lacking fish protein and given access to tap water. The experiments were carried out according to the guidelines and experimental protocols set by the Ethical Committee of Experimental Animal Care at the Ocean University of China (No: SPXK20150001).

2.3. Simulated gastric and intestinal digestion of PV and emulsified PV

Detailed *in vitro* simulated intestinal and gastric digestion models had been described in the early research.¹⁸ Briefly, the ratio of pepsin to substrate was 0.0007 : 1 (w/w) and 1.20 U per mg protein in SGF/ESGF (simulated gastric fluid/emulsified simulated gastric fluid). While, 40 U per mg trypsin was used in a 1 : 1 (w/w) ratio of trypsin to protein, and the ratio of α -chymotrypsin was 0.0004 : 1 w/w (0.5 U mg^{-1}) in SIF/ESIF (simulated intestinal fluid/emulsified simulated intestinal fluid).

During the gastro-intestinal digestion, the digestion products of PV were withdrawn at 0, 1, 5, 15, 30, 60, 90 and 180 min, which were then examined *via* Tris-Tricine-SDS-PAGE, following the method of Yuan *et al.*¹⁹ The protein marker having the molecular weight of 3.3–31 kDa was used for determining the molecular weight of the digestion products of PV.

2.4. Allergenicity assessment of digestion products in RBL-2H3 cell model

2.4.1. Cell culture. The basophilic leukaemia cell line RBL-2H3 was obtained from the Chinese Academy of Sciences.

Briefly, the cells were cultured in DMEM with the supplementation of FBS (10%), penicillin (100 U mL^{-1}) and streptomycin ($100 \text{ } \mu\text{g mL}^{-1}$). Besides, the cells were kept in an incubator maintaining a 5% CO_2 /95% air atmosphere at 37°C .²⁰

2.4.2. Active mediator and cytokine release. After treatment with trypsin, RBL-2H3 cells (2×10^5 cells per mL) were seeded in a 96-well microtiter plate. Following this, the cells were passively sensitized for 12 h by adding mouse sera. Afterwards, the cells were stimulated with $100 \text{ } \mu\text{L}$ of 0.01 mg mL^{-1} digestion products of PV for 1 h at 37°C . The concentration of PV was optimized by titrations in DMEM to elicit higher release of β -hexosaminidase (β -hex). Moreover, Tyrode's solution was set as the blank control and 1% Triton X-100 (diluted with Tyrode's solution) was set as the full release group. 0.2 mg mL^{-1} BSA (diluted with Tyrode's solution) and 0.2 mg mL^{-1} compound 48–80 were set as negative and positive control, respectively. After incubation, the supernatants were collected and were used to measure the content of active mediators, like β -hex, His, TPS and cytokines, like interleukin 13 (IL-13) and IL-4. The release of β -hex was determined according to the method depicted earlier.^{21,22} Other mediators and cytokines were analysed using ELISA assay kits in accordance with the manufacturer's instructions.

2.5. Mouse allergy model

The mouse model of allergy was developed according to previously described methods.^{23,24} Mice were distributed into eight groups, which were Positive, Negative, 0% PV, 50% PV, 100% PV, 0% e-PV, 50% e-PV and 100% e-PV. The murine model of food allergy involved two sensitizations, one stimulation and one challenge.

Briefly, during the initial sensitization, the mice were intraperitoneally (i.p.) injected with PV or e-PV ($10 \text{ } \mu\text{g}$) absorbed in 2 mg mL^{-1} Imject Alum Adjuvant (1:1, v:v) on day 1, 7, and 14. Afterwards, the mice were stimulated by i.p. injection on day 22, 25, 28, 31, 34, 37, and 40, and each mouse was injected with $100 \text{ } \mu\text{g}$ of digestion products of PV or e-PV per injection. Subsequently, in the second sensitization stage, the mice were i.p. injected with PV or e-PV ($40 \text{ } \mu\text{g}$) absorbed in 2 mg mL^{-1} Imject Alum Adjuvant (1:1, v:v) on day 50, 57, 64, and 71. Finally, the mice were challenged by i.p. injection on day 75, and each mouse was injected with $500 \text{ } \mu\text{g}$ of digestion products of PV or e-PV per injection, whereas Negative and Positive control groups were injected with equal volume of PBS or OVA solution.

2.6. Anaphylactic symptoms score

The clinical anaphylactic symptoms of BALB/c mice were assessed using the allergy symptom scoring system, as reported by Xu *et al.*²⁵ Half an hour after the challenge, scores were given by five experienced panelists in a blinded way. The scores were as follows: 0, no symptoms; 1, scratching and rubbing around the nose and mouth; 2, puffiness and scratching around eyes/nose and mouth; 3, laboured respiration, wheezing, cyanosis around the mouth and tail; 4, no movement after prodding; and 5, death. The total scores are based on the average scores for individual clinical signs.

2.7. Histological analysis of spleen and duodenum

Spleen and duodenum sections from each mice group were isolated immediately after sacrifice, which were fixed with 4% paraformaldehyde. Afterwards, the samples were dehydrated with a graded series of ethanol, and embedded in paraffin wax. Then samples were cut into appropriate sizes and subjected to toluidine blue (TB) staining. The final samples were examined using an inverted research system microscope (Olympus Corporation, Tokyo, Japan).

2.8. Serum levels of s-IgE, t-IgE, t-IgG1, histamine, MCP-1 and tryptase

PV specific IgE (s-IgE) content was examined by indirect ELISA using mice serum (1:10 dilution with blocking buffer, v/v) as primary antibody and HRP-labelled goat anti-mouse IgE antibody (1:5000 dilution with blocking buffer, v/v) as secondary antibody. The time of incubation for the first and second antibodies was set at 90 minutes. Afterwards, TMB was added and incubated for 20 min at 37°C . Following incubation, 2 M sulfuric acid was added to stop the reaction and the absorbance was recorded with a microplate reader (CMax Plus, Molecular Devices, LLC., San Jose, CA, USA) at 450 nm. The concentrations of t-IgG1, t-IgE, His, TPS and IL-4 in mice serum were evaluated using commercially available sandwich ELISA kits.

2.9. Statistical analysis

Each experiment was performed in triplicate and expressed as mean $\pm$ standard error. Significant differences ($p < 0.05$) were recorded by one-way analysis of variance followed by *post hoc* multiple comparisons by means of Tukey's test.

3. Results

3.1. Effect of SGF/ESGF treated PV on the RBL mediator release assay

RBL cells were employed to analyze the potential allergenicity of digestion products by monitoring the release of active mediators (β -hex, His and TPS) and cytokines (IL-4 and IL-13). The detailed identification of PV, PV digestion products, including SDS-PAGE profiles and IgG/IgE binding capacity analysis had been reported in our previous research.¹⁸ As shown in Fig. 1, the digestion products of PV induced a lower release of active mediators and cytokines. The contents of β -hex, His and TPS, which were stimulated by gastric digestion products of PV (180 min) decreased by 79.32%, 26.19% and 41.67%, respectively, compared with that stimulated by intact PV. The release of IL-4 and IL-13 also displayed a similar reduction patterns, which were reduced by 53.95% and 54.40% (Fig. 1).

Lipid emulsion improved the ability of PV's gastric digestion products to stimulate RBL cells to significantly ($p < 0.01$) release active mediators and cytokines. After 180 min of gastric digestion, PV treated with ESGF had a higher release of β -hex, His and TPS, which was increased by 197.60%, 12.18%, and 38.85%, respectively (Fig. 2). The digestion products of emulsified PV at 180 min showed a higher release of IL-4

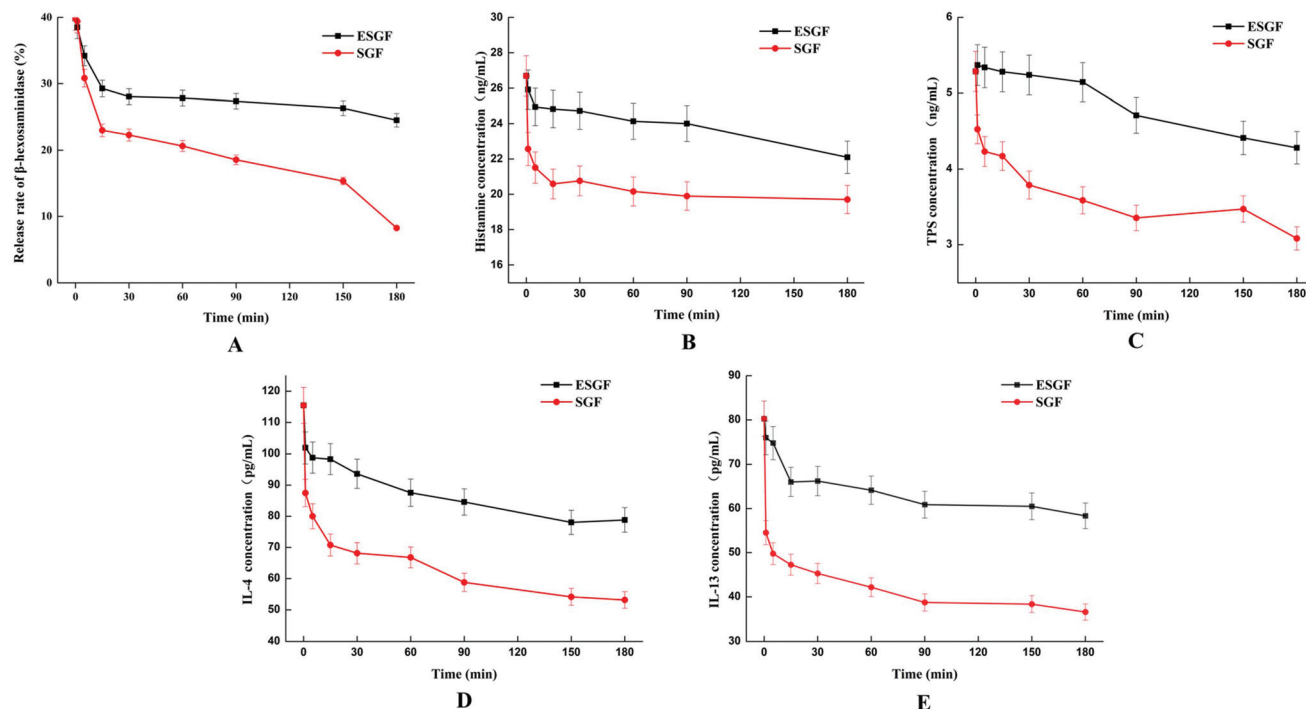


Fig. 1 Release of mediators and cytokines from RBL cells following stimulation with emulsified and non-emulsified digestion products of PV in SGF/ESGF. (A) β -Hexosaminidase, (B) histamine, (C) tryptase, (D) IL-4, and (E) IL-13.

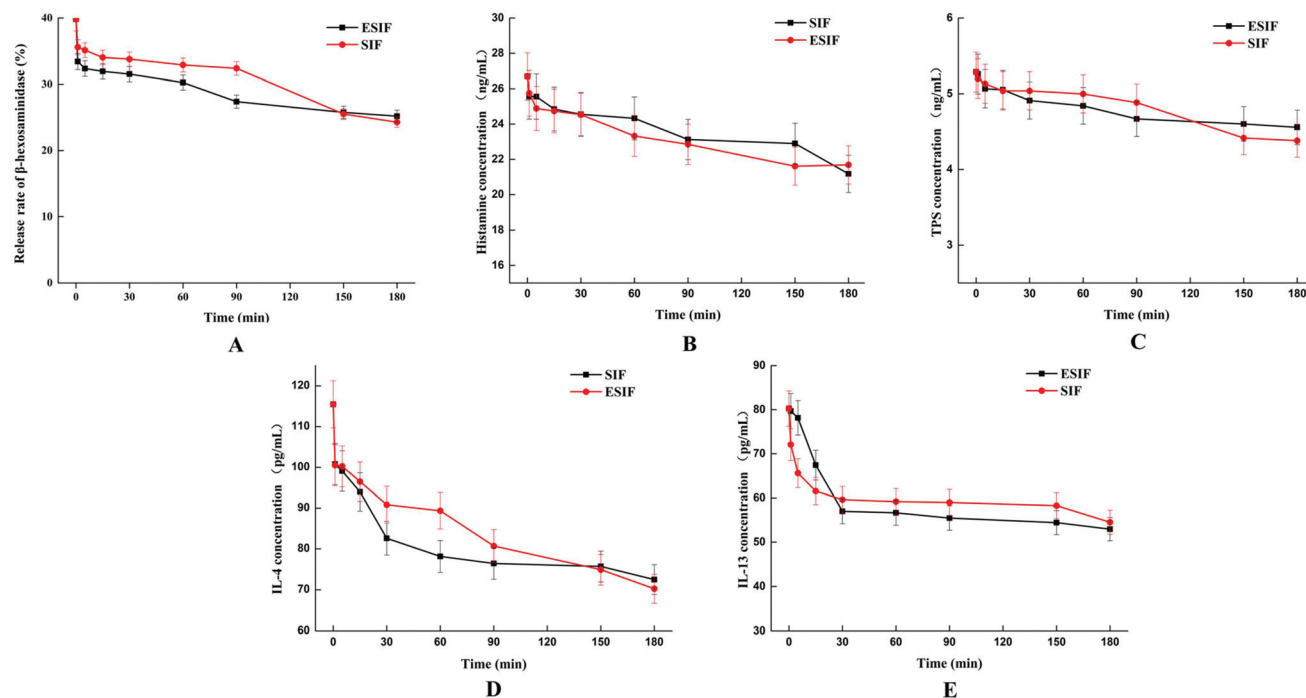


Fig. 2 Release of mediators and cytokines from RBL cells following stimulation with emulsified and non-emulsified digestion products of PV in SIF/ESIF. (A) β -Hexosaminidase, (B) histamine, (C) tryptase, (D) IL-4, and (E) IL-13.

(48.19%) and IL-13 (59.40%), as compared to that of PV at 180 min (Fig. 1).

3.2. Effect of SIF/ESIF treated PV on the RBL mediator release assay

Intestinal digestion products of PV exhibited a lower release of active mediators and cytokines, in the absence or presence of the lipid emulsion. Compared with the native PV at 0 min, the release of β -hex, His and TPS were reduced by 39.0%, 20.66% and 17.13%, respectively, upon stimulation with PV's intestinal digestion products (Fig. 2). In addition, the concentrations of IL-4 and IL-13 were reduced by 37.22% and 32.08%, respectively (Fig. 2).

With respect to the release of active mediators and cytokines, no significant difference between the intestinal digestion products of PV and the intestinal digestion products of emulsified PV was observed. After 180 min of intestinal digestion, PV treated with ESIF resulted in a high release of β -hex (3.73%), His (2.41%) and TPS (4.03%), compared with that of

PV in SIF (Fig. 2). In addition, IL-4 and IL-13 concentrations were reduced by 3.08% and 2.87%, respectively.

3.3. Lipid emulsion enhanced anaphylaxis symptoms

The effects of the digestion products of PV were studied in a murine model of food allergy following the experiment protocol presented in Fig. 3A and B. The clinical allergy signs revealed by different groups of mice are shown in Fig. 3C. Animals in the Positive group developed statistically significant ($p < 0.05$) clinical scores (3.67 ± 0.82) compared with those in the Negative group (0.17 ± 7.41). There were no significant effects of 0% PV (3.00 ± 0.63) and 0% e-PV (3.17 ± 0.41) treatments on the intensity of clinical allergy symptoms. Treatment with digested PV (50% PV, 100% PV) significantly ($p < 0.05$) reduced the allergy symptoms compared with 0% PV. 50% e-PV (2.67 ± 0.52) treated mice have a significantly ($p < 0.05$) higher level of anaphylactic scores compared with 50% PV (2.00 ± 0.63) treated mice. Also, a significant difference ($p <$

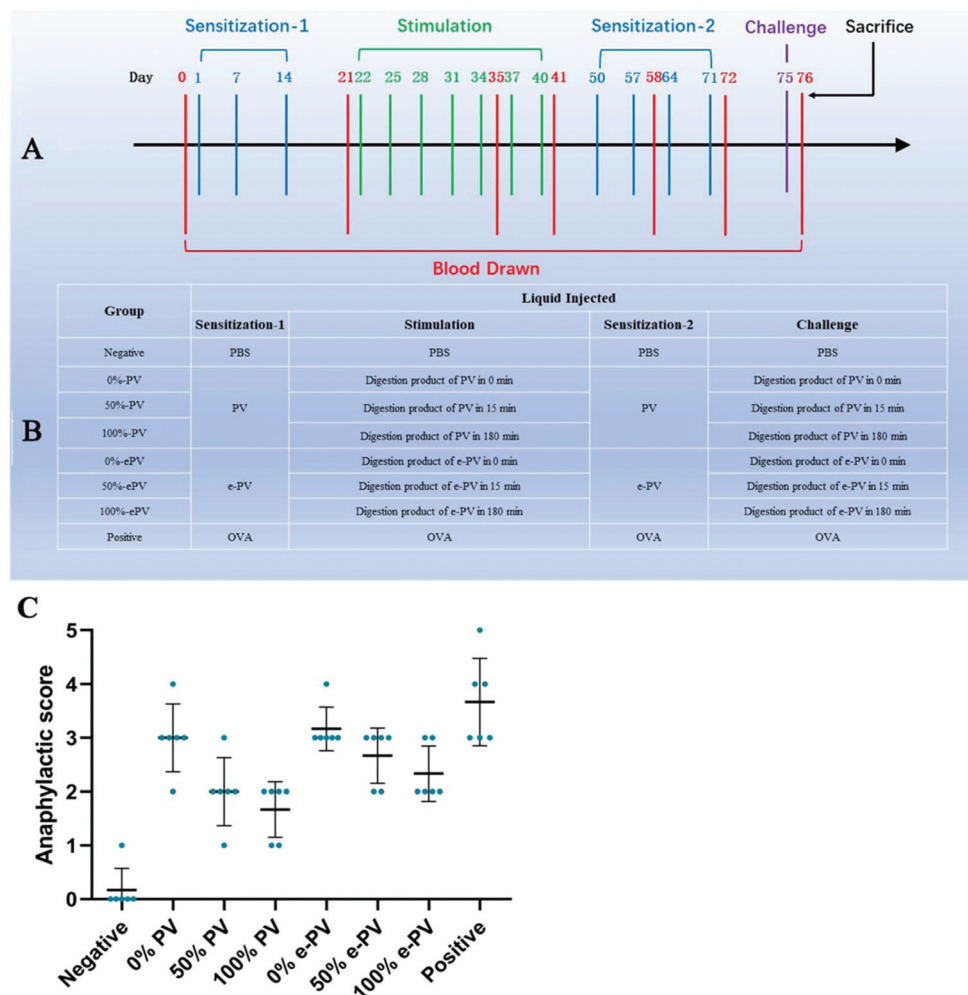


Fig. 3 Effects of emulsified and non-emulsified digestion products of PV on the PV-induced mouse food allergy model. Detailed protocol of the mouse experiment. (A) Immune cycle of the mouse experiment; (B) immune protocol of the mouse experiment; (C) anaphylaxis score of mice after the final challenge. Data are expressed as mean $\pm$ SD ($n = 6$).

0.05) was found when it comes to 100% PV (1.67 ± 0.52) and 100% e-PV (2.33 ± 0.52).

3.4. Histological analysis of the spleen and duodenum

The results of the histopathological analysis *via* TB staining of spleen and duodenum tissue are presented in Fig. 4. Proliferative infiltration of mast cells was observed in the spleen of Positive and PV allergic groups (0% PV, 50% PV, 100% PV, 0% e-PV, 50% e-PV, and 100% e-PV), which was not found in the Negative. In the Positive and PV allergic groups, damaged epithelial tissue of the intestinal villi was observed, and the lamina propria became thicker. However, no obvious inflammatory symptoms were observed in the Negative: the small intestinal villus epithelium was intact and the thickness of the lamina propria remained normal. Also, in the Positive and PV allergic groups, the aggregation of mast cells was witnessed in the lamina propria of the duo-

denum. Moreover, the cell membranes of some mast cells were ruptured and the cells were irregular and diffused in shape.

3.5. Effect of lipid emulsified digestion products of PV on s-IgE, t-IgE and t-IgG1

The contents of PV s-IgE, t-IgE and t-IgG1 increased significantly ($p < 0.01$) in comparison with the Negative control (Fig. 5). Moreover, the levels of s-IgE, t-IgE and t-IgG1 were significantly ($p < 0.01$) reduced following treatment with 100% PV compared to treatment with 0% PV. A similar significant ($p < 0.05$) reduction was found in 100% e-PV compared with 0% e-PV. However, no significant ($p > 0.05$) difference was found between treatment with 0% PV and treatment with 0% e-PV. However, mice treated with 50% e-PV had a significantly ($p < 0.05$) higher levels of s-IgE (32.56%), t-IgE (16.67%) and t-IgG1 (5.15%), compared with mice treated with 50% PV. A similar

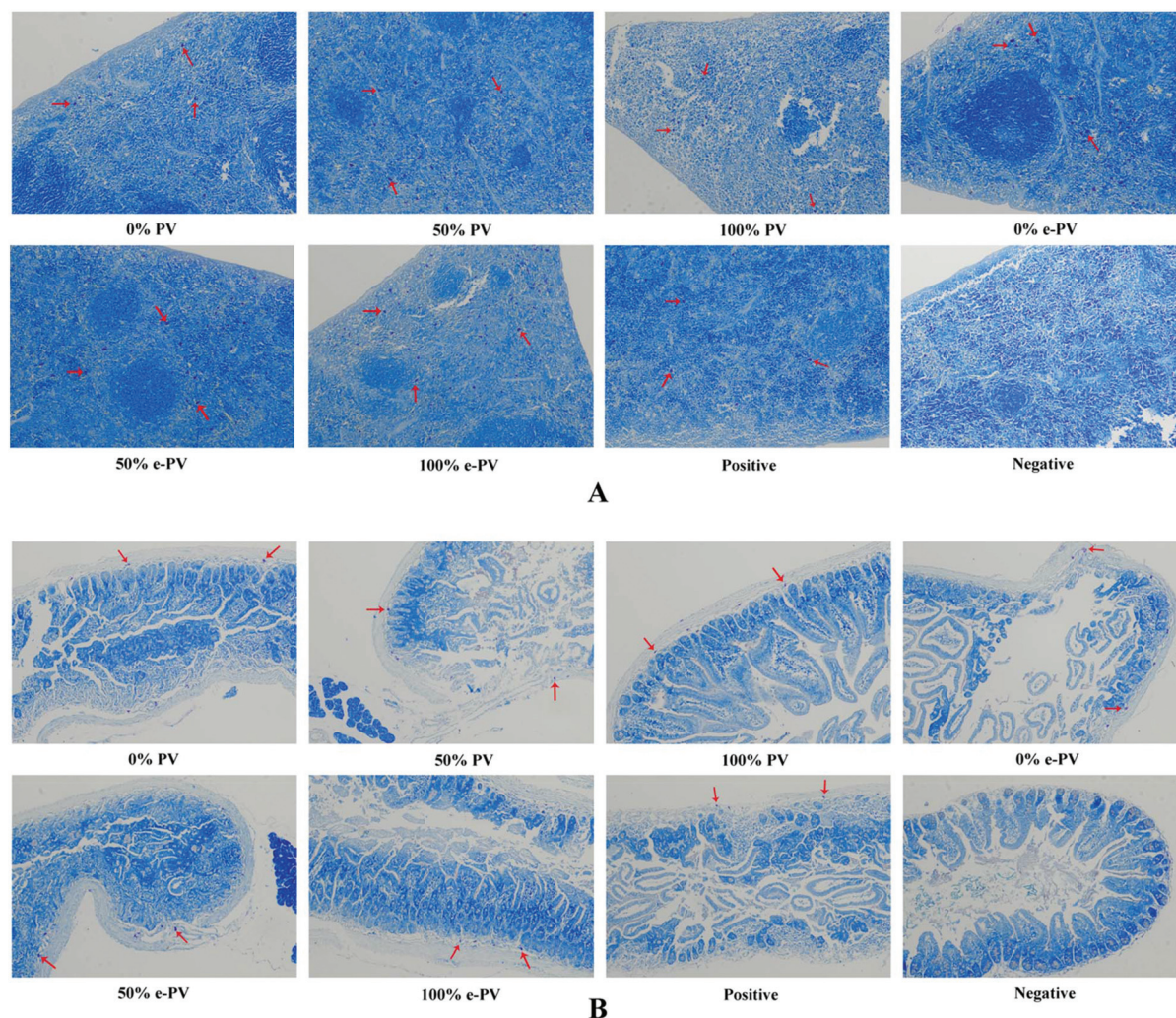


Fig. 4 Effect of emulsified and non-emulsified digestion products of PV on the histopathological examination of duodenal and splenic tissue of mice. Splenic (A) and duodenal (B) samples were sectioned at 5 μ m thickness, stained with toluidine blue, photographed at 100 $\times$ magnification using a 3D HISTECH Panoramic 250. The red arrow stands for mast cells.

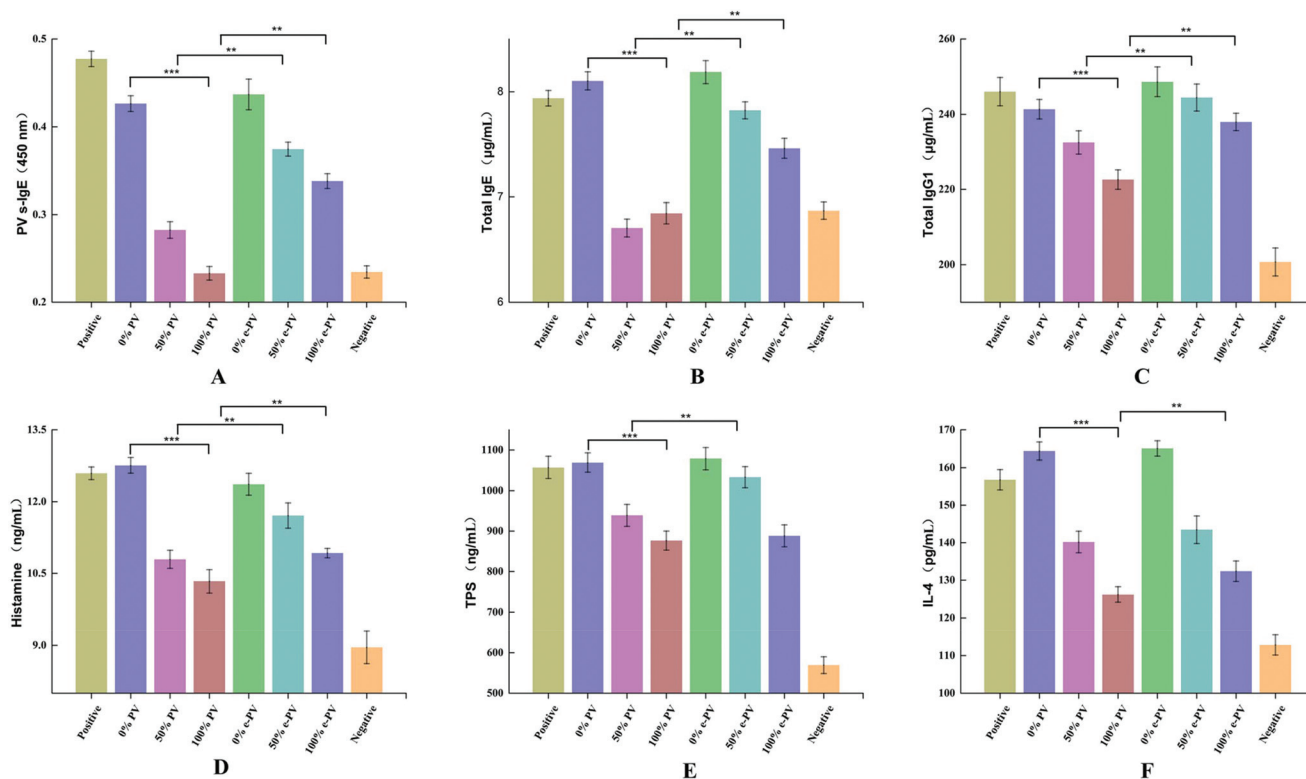


Fig. 5 Effect of emulsified and non-emulsified digestion products of PV on the serum levels of antibodies, active media and cytokines. (A) PV s-IgE; (B) t-IgE; (C) t-IgG1; (D) histamine; (E) TPS; and (F) IL-4. Data are expressed as means $\pm$ SD ($n = 6$). * represents significance at $p < 0.1$, ** represents significance at $p < 0.05$ and *** represents significance at $p < 0.01$.

significant ($p < 0.05$) increase was found in 100% e-PV compared with 100% PV.

3.6. Effects of lipid emulsified digestion products of PV histamine, tryptase and IL-4

His, TPS and IL-4 concentrations were evaluated in order to measure the degranulation of mast cells. Levels of His, TPS and IL-4 increased significantly ($p < 0.01$) in the Positive, 0% PV and 0% e-PV when compared with the Negative. His, TPS and IL-4 concentrations were significantly ($p < 0.01$) reduced in 100% PV or 100% ePV, compared with 0% PV or 0% e-PV (Fig. 5). Whereas, no obvious difference was observed in 0% e-PV treated group compared with 0% PV. However, mice treated with 50% e-PV had a significantly ($p < 0.05$) higher levels of His (8.50%) and TPS (10.07%), compared with mice treated with 50% PV. A similar significant ($p < 0.05$) increase was found in 100% e-PV compared with 100% PV.

4. Discussion

Food matrices such as sugars, proteins and lipids may affect the digestibility and subsequent immunostimulatory properties. As shown in our previous study, a lipid emulsion enhanced PV's resistance to gastric digestion, leaving PV in the

stomach for an extended period of time. Moreover, the gastric digestion products of emulsified PV have higher IgG and IgE binding capacities.¹⁸

RBL-2H3 cells, which can express large amounts of high-affinity IgE receptors (FcεRI), are a typical IgE-mediated type I allergic cell model. After being sensitized with allergic serum and stimulated with allergens, RBL cells can release various active mediators and cytokines, such as β-hex, His, TPS, IL-13 and IL-4,^{1,26–29} which are supposed to cause clinical allergic reactions.

The present study evaluated the allergenic and immune-stimulatory properties of digestion products of emulsified PV, assessed using a RBL-2H3 cell model and an allergic mouse model. Although ELISA and immunoblot were used to determine the IgG/IgE binding capacity of PV's digestion products in previous studies,¹⁸ these methods cannot determine whether digested allergens will cause IgE cross-linking on FcεRI receptors, which are expressed on the surface of mast cell membranes. RBL cells express a large number of high IgE affinity receptors on the surface of cell membranes, which are commonly used to evaluate type I allergy.^{26,30,31} It can be inferred from the present study that the digestion process has affected the allergic potential of PV. After 180 min of gastric or intestinal digestion, the concentrations of active mediators and cytokines released by RBL cells were substantially

reduced, following stimulation with PV's digestion products. These results are in accordance with our previous findings that PV digestion products maintained excessive IgG binding potential, for the reason that digestion products of PV still retained a large number of antigen-binding epitopes for IgG/IgE.¹⁸ However, it should be noted that the digestion products of PV still have high allergenic potential (about 20.68–82.87%). The result also indicated that PV proteins may still have a high sensitization risk after gastrointestinal digestion.

It was also observed that lipid emulsion in the digestion environment had an effect on the allergenicity of PV's digestion products. Compared with SGF-treated PV, ESGF-treated PV exhibited a higher release of active mediators and cytokines. Lv *et al.*³¹ also found that acrolein treatment can lead to structural changes of allergens and decrease the secretion of active mediators such as β -hex. As shown by the previous research, lipid emulsion can delay the pepsinolysis of PV and retain the immuno-reactive fragment of PV in the GIT for a longer time, which delays enzyme hydrolysis to IgE-binding epitopes.¹⁸ The preserved binding epitopes are capable of binding with IgE on the mast cells, which might result in a higher degranulation level. Therefore, dietary lipids may have led PV to retain a higher sensitization potential after gastric digestion.

However, the above differences were not observed in SIF or ESIF treated PV. The contents of active mediators and cytokine released by SIF and ESIF-treated PV were relatively the same, and no obvious differences were seen. These results are consistent with the previous study, which showed the same digestion kinetics and IgE binding patterns for SIF or ESIF treated PV.¹⁸ So the lipid emulsion did not change the immune characteristics of PV in intestinal digestion.

BALB/c mice were also used as an allergy model to assess the immunogenicity of the digestion products of PV. BALB/c mice are inbred high IgE response strains, which are commonly used as an animal model in allergy research.²³ The IgE content is an important criterion for determining the severity of IgE-mediated type 1 hypersensitivity.^{32,33} According to the concentration of s-IgE in the mice serum, the severity of clinical allergic reactions of PV sensitized mice is lower than that reported by Yang *et al.*³⁴ Compared with mice treated with native PV, the PV s-IgE, t-IgE and t-IgG1 levels of 100% PV mice were significantly decreased. Even the contents of PV s-IgE and t-IgE, stimulated by PV's gastric digestion products (180 min), were close to those of the Negative. This indicates that after gastrointestinal digestion, PV's potential to induce sensitization may be greatly reduced. Reduced sensitization also suggests that the protein structure of PV, especially the epitopes, may be destroyed during digestion. Studies have found that the destruction of allergen's IgE epitopes can change its immunological characteristics, thereby reducing its ability to stimulate clinical allergic symptoms in mice.^{31,35,36}

No significant difference was observed in the concentrations of s-IgE, t-IgE and t-IgG1 between 0% PV and 0% e-PV, which indicates that the lipid has no effect on the allergic properties of intact PV (before digestion). These results vary from the studies of covalent modification of allergens by tyrosinase

and laccase, which were reported previously by Ahmed *et al.*¹ and Tian *et al.*³⁷ The way of interaction of lipid and PV in this study is *via* non-covalent bonds, such as hydrophobic interactions and electrostatic binding. Moreover, the secondary and tertiary structures of the proteins have been little affected, as reported by Burnett *et al.*¹⁶

As shown in the RBL cell degranulation assay, no difference was observed when it comes to the allergenicity of digestion products in SIF or ESIF. So in the present study, the immune characteristics of digestion products in SGF or ESGF were investigated. As it was depicted in the previous study, lipid emulsion enhanced PV's resistance to gastric digestion and it also improved the ability of PV's gastric digestion products for stimulating the mast cells to degranulate at the cellular level. The present study showed that PV's gastric digestion products can retain more immunostimulatory properties under emulsified conditions. Mice treated with 50% e-PV showed severe allergic symptoms, and have a higher level of s-IgE, t-IgE and t-IgG1. Moreover, 50% e-PV sensitized mice had increased propensity to stimulate the release of active mediators and cytokines. Structural changes of allergens led to the exposure or destruction of epitopes, which led to lower severity of clinical allergic reactions in BALB/c mice.^{26,36,38,39} Lipid emulsion enhanced PV's resistance to gastric digestion, making it easier for the food allergens to reach the intestinal epithelial cells, thus migrating to the mast cells, thereby causing a higher risk of allergic reactions.

5. Conclusion

In conclusion, this study showed that gastric digestion can significantly reduce the ability of PV to stimulate individuals to produce allergy-related serum antibodies, active mediators and cytokines, and reduce the sensitivity of PV at the individual level. However, there is still a certain sensitization of PV gastric digestion products. The presence of lipid had no effect on the allergenicity of intact PV. However, the ability of PV gastric digestion products of PV to stimulate mice to produce serum antibodies, active mediators, and cytokines was increased. Lipids can enhance the allergenicity of PV by reducing the digestion stability of PV during peptic digestion. Lipids introduced during food processing and in daily diets may escalate the potential allergy risk in allergy-prone people.

Author contributions

Chen Luo: conceptualization, methodology, investigation, software, data curation and writing – original draft. Ishfaq Ahmed: software, analysis, writing – review & editing. Guanzhi Chen: resources. Lirui Sun: investigation, software. Wenjie Li: visualization. Tushar Ramesh Pavase: data curation. Zhenxing Li: conceptualization, supervision, project administration and funding acquisition.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors would like to express their sincere thanks to the National Natural Science Foundation of China (31871719) for supporting the research, and extend their thanks to the Food Safety Laboratory, College of Food Science and Engineering, Ocean University of China for providing the research facilities.

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