



Cite this: DOI: 10.1039/c8fo01167b

Reduction of the histamine content and immunoreactivity of parvalbumin in *Decapterus maruadsi* by a Maillard reaction combined with pressure treatment

Huang Yang,[†] Juan Min,[†] Xin-Yu Han,[†] Xiao-Yan Li, Jia-Wei Hu, Hong Liu, Min-Jie Cao and Guang-Ming Liu *

The aim of this study was to develop an effective method for decreasing the content of histamine and the immunoreactivity of parvalbumin in *Decapterus maruadsi*. As demonstrated by reverse phase high performance liquid chromatography, no effect on histamine content was found when fish were treated by boiling (100 °C), ultrasonication, ultraviolet irradiation, pressure treatment (121 °C, 0.12 MPa). However, the histamine content was reduced by 73.55% when the Maillard reaction was combined with pressure treatment (MPT). Further, the allergenicity of parvalbumin was retained after boiling, ultrasonication and ultraviolet irradiation, but was effectively decreased when fish were treated by MPT. Animal experimental results showed lower levels of IgE, IgG1 and IgG2a and contents of serum histamine when measured in a group of MPT sensitized mice. These results showed that the MPT is an effective method for simultaneously reducing the histamine content and the immunoreactivity of parvalbumin from *Decapterus maruadsi*.

Received 11th June 2018,
Accepted 25th August 2018

DOI: 10.1039/c8fo01167b

rsc.li/food-function

1. Introduction

Blue scad (*Decapterus maruadsi*) is a highly histamine-rich marine fish belonging to the mackerel family. As an important economic seafood in China, its production reached 600 913 tons in 2016.¹ The nutritional value of blue scad is high, and the contents of protein and fat are 18.50% and 3.40%, respectively, and include 18 kinds of amino acid.^{2,3} However, safety incidents caused by eating fish are common. Histamine fish poisoning, also known as 'scombroid poisoning', occurs commonly worldwide. Histamine is a typical biogenic amine, and it is mainly formed by decarboxylation of histidine or by amination and transamination of aldehydes and ketones, as result of bacterial action during storage.⁴ A level of 50 mg kg⁻¹ of histamine has been suggested by the Food and Drug Administration (FDA) as a histamine poisoning limiting value in fish.⁵ Histamine is widely found in fish and processed aquatic products, and it is one of the most powerful biologi-

cally active compounds as it exerts many responses within the body, including anaphylaxis and immunoreactions.^{6,7} Histamine poisoning can lead to allergy-like food poisoning, with symptoms such as difficulty in breathing, hives, rash, vomiting, fever, facial swelling and hypertension.^{8,9}

It is equally important to note that aquatic products may not only contain histamine, which causes poisoning, but also parvalbumin (PV), which causes allergic reactions in humans. Elsayed and colleagues first identified PV (called Gad c I) from cod (*Gadus callarias*) as a fish allergen in 1971.¹⁰ It was reported that 92.5%¹¹ of individuals who are allergic to fish have serum-specific IgE against PV. PV represents the major allergen of fish, and it is a Ca²⁺-binding protein with a molecular mass of about 12.5 kDa and an acidic isoelectric point.¹² In general, PV can be divided into α -PV and β -PV, and it is β -PV that mostly causes the allergic reaction in fish allergy sufferers.¹³ It is notable that the symptoms associated with fish histamine poisoning are similar to those of an allergic reaction. This is in fact the link between histamine poisoning and food allergy because the ingestion of exogenous histamine in spoiled fish and the degranulated histamine produced during an allergic reaction share the same pathogenetic mechanism.¹⁴ Such histamine-induced reactions are often misdiagnosed as IgE-mediated fish allergy. So it is necessary to prevent both scombroid fish poisoning and food allergy due to fishes at the same time.

College of Food and Biological Engineering, Xiamen Key Laboratory of Marine Functional Food, Fujian Provincial Engineering Technology Research Center of Marine Functional Food, Fujian Collaborative Innovation Center for Exploitation and Utilization of Marine Biological Resources, Jimei University, 43 Yindou Road, Xiamen, 361021 Fujian, P.R. China. E-mail: gmlu@jmu.edu.cn; Fax: +86-592-6180470; Tel: +86-592-6180378

[†]These authors contributed equally.

However, biogenic amines have been reported to be heat stable compounds.¹⁵ Most research has therefore focused on the prevention of histamine formation in stored fish, by means such as temperature control, modified atmosphere packaging, irradiation, addition of preservatives and so on.¹⁶ Pressure treatment (PT) does have an effect on the structure and antigenicity of food allergens,¹⁷ high pressure (more than 100 MPa) is high in cost and energy consumption in industrial application, however the condition of canning is a very mature production technology that to increase the pressure and temperature (121 °C, 0.12 MPa). Histamine is difficult to eliminate by pressure once it has formed. In another way, the Maillard reaction (MR) seems to be a more effective way of removing histamine. The glucose/histamine Maillard reaction has been proposed for histamine control,¹⁸ and histamine can be almost eliminated under appropriate conditions. Moreover, the IgE binding properties of recombinant silver carp PV were partly weakened after the glucose/histamine Maillard reaction because of its effect on PV's structure and epitopes.¹⁹ However, there have been no reports of an effective way to control the histamine content and eliminate allergenicity during food processing and storage in industrial production. And since previous research was based on different reaction conditions, combining the Maillard reaction with pressure treatment was worth considering.

The following study compared the differences in reducing the content of histamine and the immunoreactivity of parvalbumin upon boiling treatment (BT), ultrasonication (US), ultraviolet irradiation (UV), PT and MR. In addition, a cell model was constructed to verify the effects of different food processing methods on the rat mast cell line RBL-2H3. Serum and animal experiments were designed to ensure the effects of the Maillard reaction combined with pressure treatment (MPT) could reduce the histamine content and the allergenicity of PV in blue scad at the same time. The method MPT reduced the risk of scombroid fish poisoning and food allergy, and also provided a technical theoretical foundation for the development of low allergy foods.

2. Materials and methods

2.1 Materials

Frozen blue scad (*Decapterus maruadsi*) was purchased from the market of Jimei, Xiamen. Fish without heads, tails and viscera were cut into uniform blocks, and the fish were mashed as a control sample reserve. Each sample contains equal amount of histamine or PV and those samples were stored at −30 °C until further use. Standard histamine and dansyl chloride (Dns-Cl) were purchased from Sigma-Aldrich (St Louis, MO, USA). Fish allergic serum and normal human negative serum were provided by the local Ethics Committee of the First Affiliated Hospital of Xiamen University (XFH, Xiamen, Fujian, China). This study was approved by the XFH Institutional Review Board (XFH2017-EAN014). The patients had a histories of fish anaphylaxis with clear fish-exposure-

related symptoms. Their specific IgE antibodies to fish was quantified *in vitro* with ImmunoCAP (Phadia AB, Uppsala, Sweden) in Table 1. All sera were stored at −30 °C until analysis. Goat anti-rabbit IgG antibody and goat anti-human IgE antibody were provided by Kirkegaard and Perry Laboratories (Gaithersburg, MD, USA). Alum adjuvant was provided by Pierce Biotechnology Inc (Rockford, IL, USA). Unless otherwise indicated, all other chemicals and solvents used were of analytical and chromatographic grade, respectively.

2.2 Different processing methods

Various methods were used to evaluate their effects on reducing the histamine and PV contents of blue scad.

Boiling treatment: Samples of the blue scad that had been mashed were boiled at 100 °C for 180 min.

Ultrasonic treatment: Samples were placed in the ultrasonicator (200 W) for 5 h.

Ultraviolet treatment: Samples were placed under an ultraviolet lamp for 5 h, with wavelength and power settings of 302 nm and 15 W, respectively.

Pressure treatment: Samples were placed in the autoclave sterilizer (121 °C, 0.12 MPa) for 20 min as the condition of canning.

Maillard reaction treatment: The reaction conditions of Maillard reaction referred to the method of Zhao *et al.*¹⁹, and it has been perfected. Samples and 4% glucose solution (v/v, 1 : 1) were mixed, and then placed in a water bath at 100 °C for 30 min (pH 7.0).

Maillard reaction combined with pressure treatment: Samples and 4% glucose solution (v/v, 1 : 1) were mixed. Then the samples were placed in the autoclave sterilizer (121 °C, 0.12 MPa) for 20 min.

Table 1 Specific IgE levels in patients in relation to the fish

No.	ID	Age (years)	Sex	Specific IgE level (kU L ⁻¹)	Symptom
1	839758	25	M	0.16	Cough
2	856386	38	F	0.10	Influenza
3	422384	5	M	0.47	Lung shadows
4	598870	45	M	0.70	Bronchial asthma
5	556766	21	M	1.10	Bronchial asthma
6	437753	35	F	1.10	Edema
7	568825	66	F	1.50	Bronchial asthma
8	307351	51	F	1.80	Allergic purpura
9	492982	4	M	2.20	Cough
10	319293	3	M	2.20	Congenital asthma
11	349416	75	F	2.80	Edema
12	413340	5	F	3.60	Bronchitis
13	483373	14	M	5.60	Edema
14	484581	1	M	7.60	Cough variant asthma
15	494031	1	F	9.60	Edema
16	383250	2	M	10.00	Edema
17	480821	10	M	78.00	Allergic purpura

F: Female, M: male, A serum with the specific IgE > 0.35 (kU L⁻¹) is defined as positive.

2.3 Detection of the histamine content

Histamine was quantified using reverse phase high performance liquid chromatography (RP-HPLC) after pre-column derivatization with Dns-Cl.²⁰ A 5 g portion of each fish sample (BT, US, UV, PT, MR, MPT) after homogenization was mixed with 15 mL of trichloroacetic acid (5%, w/v, TCA) in a 50 mL centrifuge tube. Then the homogenate was centrifuged at 10 000g for 15 min at 4 °C. The supernatant was decanted into a 25 mL volumetric flask. Next, 10 mL TCA (5%) was added to the sediment and the mixture was centrifuged as before. The supernatants were combined in a 25 mL volumetric flask and diluted to the mark with 5% TCA.

An aliquot of 300 µL of standard histamine solution (and each extract) was filtered through a water filter membrane. Forty microliter of sodium hydroxide (2 M) and 600 µL of Dns-Cl solution (10 mg mL⁻¹ in acetone) were gently added to the 2 mL centrifuge tube. The mixture was incubated in the dark at 40 °C for 20 min. After the reaction, the sample was mixed with 100 µL ammonia at room temperature for 30 min. Finally, the volume of the reaction mixture was adjusted to 1.5 mL with acetonitrile. The solution was filtered through a 0.22 µm organic filter membrane prior to RT-HPLC analysis.

Samples were injected into an RP-HPLC to analyze the histamine concentration.²¹ The RP-HPLC system consisted of an Agilent 1260 HPLC unit, a pump, a standard automatic sampler and a variable-wavelength detector from Agilent (Palo Alto, CA, USA). The Zorbax Extend-C18 column (5 µm, 4.6 mm I.D. × 150 mm; Agilent) was used as the analytical chromatography column. An aliquot of 20 µL was injected into the solvent delivery system. The mobile phase of the isocratic elution system was a mixture of water (solvent A) and acetonitrile (solvent B). The flow rate was 1.0 mL min⁻¹ and the temperature was set at 35 °C. The derivatized histamine was detected at 254 nm. The solvent A/solvent B ratio was held constant at 30 : 70 (v/v) for 7 min. All experiments were performed in duplicate three times at least.

2.4 Detection of the immunoreactivity of parvalbumin

The crude sample for the detection of fish PV was prepared using a previously published method.²² A 5 g portion of each fish sample (untreated, BT, US, UV, PT, MR, MPT) was mixed with 15 mL ultrapure water. After homogenization, each homogenate was centrifuged at 12 000g at 4 °C for 20 min. The supernatants contained the crude protein that was filtered through four layers of silk cloth and stored at 4 °C. The PV in blue scud was purified by heat treatment and DEAE-Sepharose chromatography by the method of Liu *et al.*²³

SDS-PAGE and western blot were conducted according to a previous method with minor modifications.²⁴ All experiments were duplicated, and the variation among three assays was always less than 5%. Analysis of the IgG-/IgE-binding activity by western blotting/dot blotting was carried out as previously described,¹⁹ with minor modifications. Briefly, proteins in the polyacrylamide gels were electrophoretically transferred onto a nitrocellulose (NC) membrane. Then, the NC membranes were

blocked with 5% skim milk and incubated with rabbit-PV IgG antibodies or human serum (1 : 4 dilution). The immunoassay was carried out using ECL after incubation with HRP-anti-mouse IgG antibody (1 : 20 000 dilution) or HRP-anti-human IgE antibody (1 : 2000 dilution).

2.5 Rat basophilic leukemia cell assay

The rat basophilic leukemia mast cell line (RBL-2H3) was obtained from the American Type Culture Collection (Bethesda, MD, USA) and grown in essential medium with 10% fetal bovine serum and 1% MEM/EBSS at 37 °C. The final cell count was 1 × 10⁶ cell per mL.

The release rate of β-hexosaminidase from RBL-2H3 cells was measured in a model of an IgE-mediated mast cell allergic reaction.²⁵ Sensitized mouse sera (mouse blood from a group sensitized to PV collected on day 15) were stimulated by PV or PV treated by different processing methods (500 ng mL⁻¹) for 6 h or 15 min at 37 °C, respectively. The cell supernatant was used to measure the release levels of β-hexosaminidase and histamine using an ELISA kit from IBL (Hamburg, Germany).

2.6 Detection of the histamine content and the immunoreactivity of PV *in vivo*

Female Balb/c mice were purchased from Slaccas Lab Animal Ltd (Shanghai, China) at 6–8 weeks old. Mice were housed in a specific pathogen-free barrier room (room temperature 23 ± 2 °C, relative humidity 50–70%, 12 h/12 h light/dark cycle). Food and water were available *ad libitum*. Experiments were performed in conformity with the laws and regulations for treatment of live animals of Jimei University, SCXK 2012-0005.

Fish samples that contained a low content of histamine were designated FLH; fish samples that contained a high content of histamine were designated FHH. Each group of mice received a different sample, including PBS, FLH, FHH, or FLH, FHH treated by MPT, *via* intragastric gavage (3 mg per mouse, *n* = 6). Blood was obtained after 30 min. Histamine levels in the sera were determined using commercially available ELISA kits from IBL following the manufacturer's instructions.

Another batch of mice were exposed to 150 µL of purified PV (150 µg per mouse, *n* = 6) with alum adjuvant by intraperitoneal (i.p.) injection on days 0 and 14. Mouse blood was collected on day 15 after immunization. The sera from individual mice were stored at –30 °C until analysis. On days 21 and 28, mice received 2 mg PV or MPT-treated parvalbumin (MPT-PV) *via* intragastric gavage (3 mg per mouse, *n* = 6). The samples were dialysed against 10 mM PBS (pH 7.0) after the MRT to remove the remaining reducing sugar. Rectal temperatures were measured for 30 min after the challenge on day 28. Blood was obtained on day 29, and the allergen-specific antibody levels were determined. Also, histamine in the serum was determined.

B/T cells were isolated from the spleens of mice on day 29 and stained for CD3 and CD19. Cells were cultured in the presence of 25 µg mL⁻¹ anti-CD3-APC and 25 µg mL⁻¹ anti-CD19 for 30 min, and analyzed by flow cytometry. All flow cytometry experiments were performed using the Guava easyCyte 6-2L system and GuavaSoft 3.1.1 software.

2.7 Statistical analysis

The reported results were obtained using the software SPSS statistics 17.0 from IBM (Armonk, New York, USA). The differences among samples at different measurement points were determined by one-way analysis of variance (ANOVA) for independent samples at a probability level of 0.05 ($P < 0.05$). The collection of fish samples spanned many seasons with good reproducibility and the experiments were performed in triplicate and the data are expressed as average $\pm$ standard deviation.

3. Results

3.1 Effects of different processing methods on the histamine content

The results for chromatograms of histamine showed that the elution time of histamine was 5.5 min, with a standard peak shape. The linear relationship between peak area and histamine content was good when the histamine content was in the range of 1–500 $\mu\text{g mL}^{-1}$ (Fig. 1A).

First, we investigated the histamine content resulting from different processing methods (Fig. 1B). Samples that were treated by BT, US, UV and PT showed an insignificant effect on the level of histamine compared with the untreated sample ($P > 0.05$). But the histamine content was reduced significantly by the Maillard reaction ($P < 0.01$).

In fact, the histamine content decreased significantly as the Maillard reaction proceeded. When the Maillard reaction was combined with pressure treatment, the amount of histamine was reduced by 73.55%, and its content was 27.45 mg kg^{-1} . These results suggested that an appropriate glucose concentration is critical for the activation of the Maillard reaction.

3.2 Effects of different processing methods on the IgG/IgE-binding activities of parvalbumin

The fish proteins extracted from blue scad after the different processes were analyzed by SDS-PAGE. PV appeared as a band corresponding to a molecular mass of 12.50 kDa (Fig. 2A), and

did not change significantly after US or UV treatment. Fig. 2A shows that the migration of objective protein bands was obvious in the experimental groups treated by BT and MR, and the protein bands became diffuse for the experimental groups of PT and MPT.

Whether processing time shifts caused by different processing methods affected the IgG/IgE binding activity of PV was then determined. The IgG-binding activity of PV was evaluated with western blotting using anti-fish PV rabbit antibody. The IgG-binding capacities were essentially identical to that of the untreated protein for samples treated by BT, US and UV. Meanwhile, the Maillard reaction was effective in reducing the IgG-binding capacity. However, the maximum effect on the IgG-binding capacity of PV was caused by the PT and MPT processes, which both decreased binding. Fig. 2C shows the PV binding capacity of IgE from the pooled sera (Table 1) of fish allergic patients. When PV was processed by PT or MPT, the inhibitory effect on IgE binding was very obvious, whereas the other experimental groups still displayed substantial immunoreactivity.

3.3 Effects of processing on the degranulation of RBL-2H3 cells

The effects of PV allergenicity after the different processing methods on the degranulation of RBL-2H3 cells are shown in Fig. 2D. The β -hexosaminidase release rate of the negative control (PBS) was 13.24%, and the β -hexosaminidase release rate of the positive control (raw fish) was 45.46%. The β -hexosaminidase release rates were 42.50%, 45.39% and 44.26% ($P > 0.05$) when the fish were processed by BT, US and UV methods, respectively. In contrast, the β -hexosaminidase release rates were 17.10%, 28.33% and 15.67% ($P < 0.01$) when the fish were processed by PT, MR and MPT, respectively.

3.4 The toxicity of Maillard-reacted histamine *in vivo*

The toxicity potential of Maillard-reacted histamine was tested in a histamine experimental protocol (Fig. 3A). The histamine contents in the fish extracts of FLH, FHH and their Maillard-

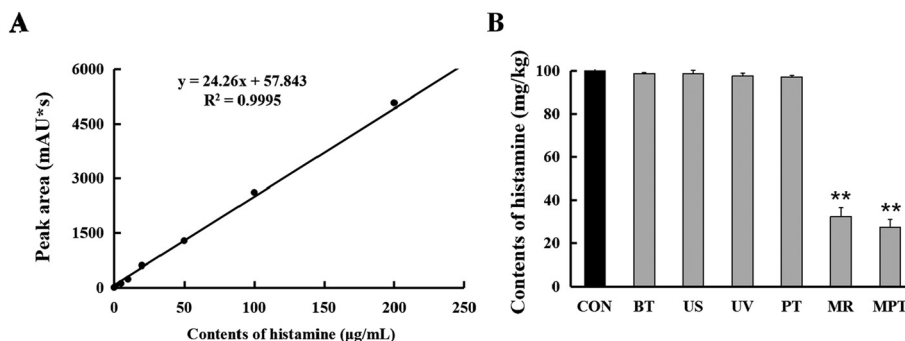


Fig. 1 The effects of different processing methods in reducing histamine by RP-HPLC. A, A linear relationship between peak area and histamine content was demonstrated when the content of histamine was 1–500 $\mu\text{g mL}^{-1}$. B, The histamine concentration in a sample was determined using reverse phase high performance liquid chromatograph (RP-HPLC). CON: negative control; BT: boiling treatment; US: ultrasonic; UV: ultraviolet; PT: pressure treatment; MR: Maillard reaction; MPT: Maillard reaction combined with pressure treatment. * $P < 0.05$, ** $P < 0.01$. The data represent the mean $\pm$ SD of triplicate determinations.

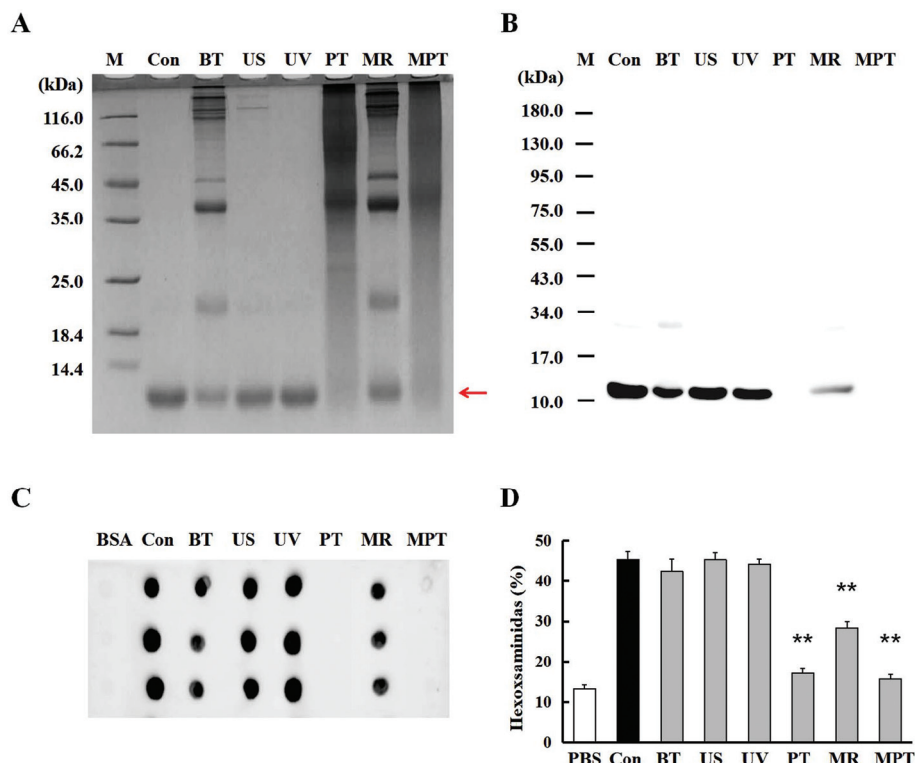


Fig. 2 The effects of different processing on reducing PV. A–B, SDS-PAGE or western blot of the effects of different processing methods in reducing PV. Using the rabbit anti-PV IgG antibody. C, Dot blot showing the effects of different processing methods in reducing PV. Patient serum pool was used as the antibody. D, The effects of different processing methods on stimulation of rat basophil (RBL-2H3) release by β -hexosaminidase. β -Hexosaminidase release rate was calculated as $(A_1 - A_2)/[(A_1 - A_2) + (A_3 - A_4)]$, in which A is the absorbance of each well, A1 is the absorbance of the supernatant well, A2 is the absorbance of a blank of supernatant, A3 is the absorbance of cell lysate, A4 is the absorbance of a blank of cell lysate. Con: negative control; BT: boiling treatment; US: ultrasonic; UV: ultraviolet; PT: pressure treatment; MR: Maillard reaction; MPT: Maillard reaction combined with pressure treatment. * $P < 0.05$, ** $P < 0.01$. The data represent the mean $\pm$ SD of triplicate determinations.

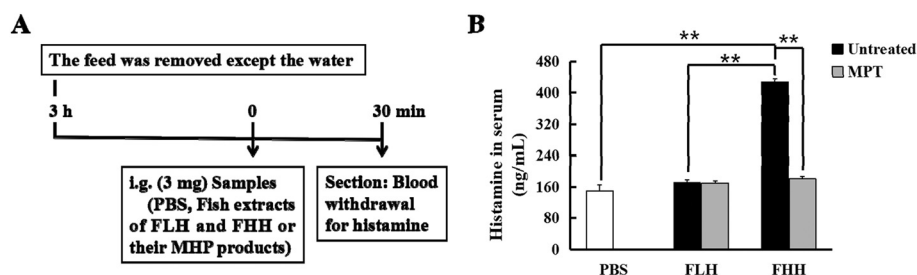


Fig. 3 The effect of the Maillard product in the mouse histamine poisoning experiment. A, Histamine poisoning experimental protocol. Six- to eight-week-old female mice were used. Each group of mice received a different sample, including PBS, and the fish contained a low content of histamine (FLH) or a high content of histamine (FHH). FLH or FHH were treated by MPT *via* intragastric gavage (3 mg per mouse, $n = 6$), and the blood was obtained after 30 min. B, The levels of histamine in the sera were measured. * $P < 0.05$, ** $P < 0.01$. The data represent the mean $\pm$ SD of triplicate determinations.

reacted products were 14.01, 150.51, 6.08 and 11.99 mg kg⁻¹, respectively (determined by RP-HPLC). These results suggested that the level of histamine (Fig. 3B) was significantly higher in the group of mice fed with spoiled fish extracts, and the histamine came back to a normal level after the Maillard reaction. It was also noted that mice had diarrhea only after feeding with spoiled fish extracts, but there were no significant differences in body temperature or body weight.

3.5 Allergic responses after intragastric exposure to Maillard-reacted parvalbumin

The sensitizing and allergenic potential of food allergens is commonly tested in different animal models of food allergy.^{26–28} Therefore, a challenge animal model of fish allergy was used to test the allergenicity of MR-PV *in vivo* (Fig. 4A). First, the rectal temperature is commonly used to

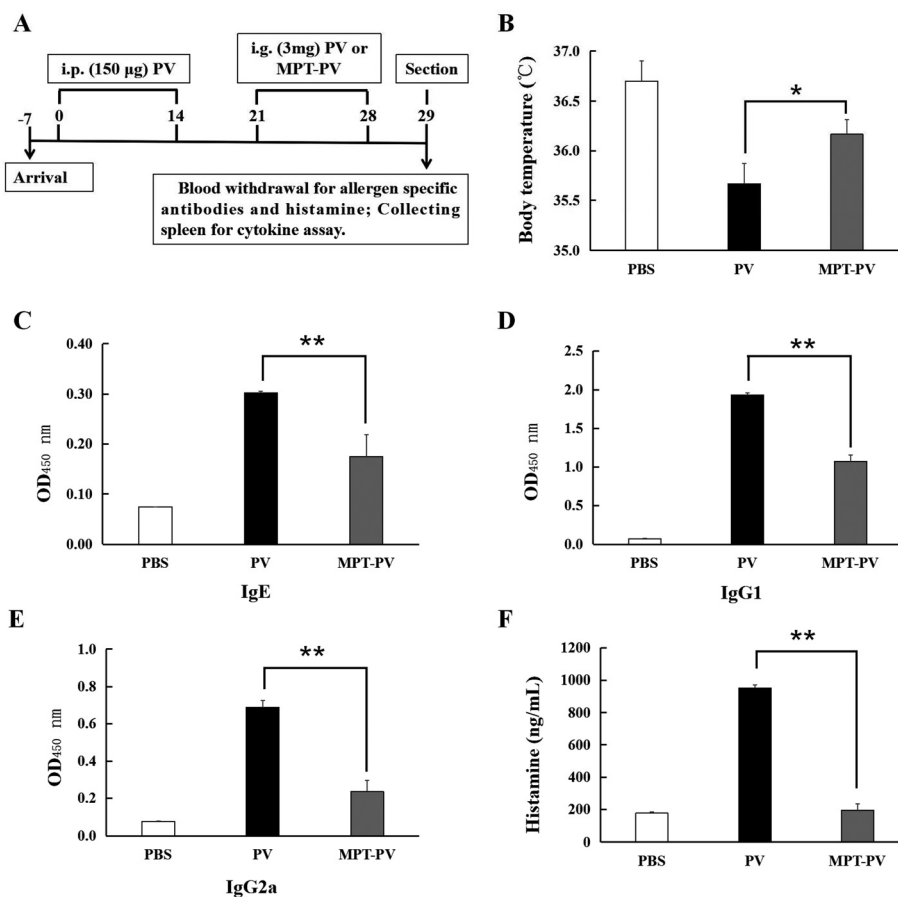


Fig. 4 Sensitizing potential in the mouse sensitization experiment. **A**, Sensitization protocol. Mice were exposed to 150 µL of purified PV (150 µg per mouse, $n = 6$) with alum adjuvant by intraperitoneal (i.p.) injection on days 0 and 14. Mice received 2 mg PV or the PV after the Maillard reaction with pressure treatment (MPT-PV) via intragastric gavage (3 mg per mouse, $n = 6$) on days 21 and 28. Rectal temperatures were measured after the challenge for 30 min on day 28. Blood was obtained on day 29. **B**, The body temperature was measured at 30 min after intragastric gavage on day 28. **C–E**, The levels of IgE, IgG1 and IgG2a were measured on day 29. **F**, The release of histamine was measured in the serum. * $P < 0.05$, ** $P < 0.01$. The data represent the mean $\pm$ SD of triplicate determinations.

assess anaphylaxis in mice. Significant differences in the above index were identified among these mouse groups (Fig. 4B). Second, exposure of the mice to PV led to a similar increase in the levels of PV-specific IgE in their serum. The levels of IgE, IgG1 and IgG2a (Fig. 4C–E) after re-stimulation with PV were higher than those of PBS-exposed mice. The levels of IgE, IgG1 and IgG2a in MR-PV-exposed mice were reduced. What is more, the level of histamine was significantly lower in MR-PV-exposed mice compared to PV-exposed mice (Fig. 4F).

Among the splenocytes (Fig. 5), the percentage of CD19 + B cells increased from 45.06% (PBS group) to 78.22% (PV group). However, the amount of B cells in the MPT-PV group was significantly decreased by 28.13% compared with the PV group.

4. Discussion

Histamine poisoning is the most common cause of scombroid fish poisoning worldwide and results from the ingestion of

histamine-contaminated fish. At the same time, fish is a highly allergenic food responsible for inducing allergic reactions in both children and adults. However, in spite of the high prevalence of histamine fish poisoning and fish allergy, there is a lack of in-depth knowledge of ways of reducing and controlling the associated risks in the food industry.

It is important to note that, once histamine is formed, it is highly resistant to modification, so cooking, smoking and freezing cannot prevent histamine fish poisoning reactions. In this study, we found that the histamine content was stable when it was extracted from fish treated by HT, PT, UV and US, and the level of histamine was not decreased by simple physical processing methods (Fig. 1B). However, the Maillard reaction could reduce histamine levels effectively. When glucose was added to the fish, a Maillard reaction that was affected by the temperature, concentration of glucose and reaction time could reduce the amount of histamine.²⁹ Although the Maillard reaction of Free Amino acids in food processing is the manifestation of nutrient loss, fortunately, fish are rich in pro-

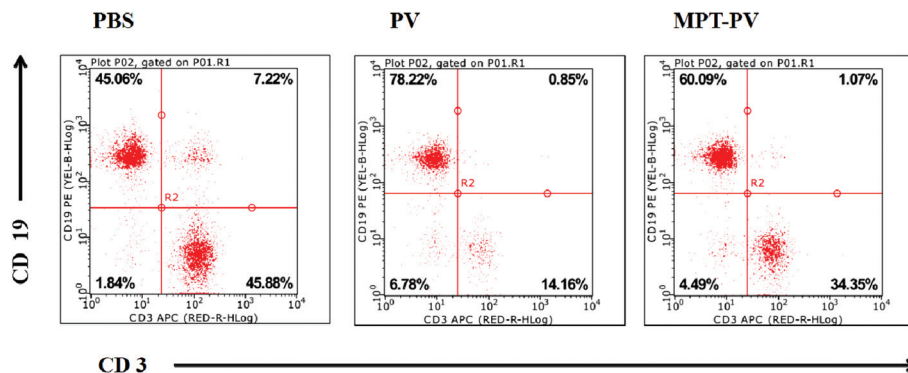


Fig. 5 The influence of MPT products on B/T cells. B/T cells were isolated from the spleens of mice on day 29 and stained for CD3 and CD19 cells. The cells were cultured in the presence of $25 \mu\text{g mL}^{-1}$ anti-CD3-APC and $25 \mu\text{g mL}^{-1}$ anti-CD19 for 30 min.

teins that can provide essential amino acids for the body after digestion in the gastrointestinal tract.

On the other hand, PV is the main allergen in fish.³⁰ Firstly, we explored the effects of the processing methods which shifted the IgG/IgE-binding activity of PV. The IgG binding activity of PV was not reduced after boiling (100°C), due to the presence of Ca^{2+} in the PV structure which can stabilize the tertiary structure of PV. In our study, MPT was the most effective processing method. On the one hand, intensively dry heated has been reported to affect the structures of allergenic proteins.³¹ On the other hand, according to research,^{32,33} the Maillard reaction can reduce the allergenicity of PV effectively. After a Maillard reaction with glucose, PV position was rising and dispersion, along with the degree of the Maillard reaction. During the Maillard reaction the antigenic epitopes of PV may have been inhibited or masked according to Almond.³⁴ The Maillard reaction has many benefits in fish processing, such as improving sensory flavor and color of food. Furthermore, the glycated fish peptide is good for the intestinal flora.³⁵

To verify the influence of the MPT treatment on PV immunoreactivity *in vivo*, a widely used mast mice model was then constructed. Type I hypersensitivity is mediated by IgE binding with FcεRI, which is located on the surface of mast cells. Activated mast cells will release various mediators, including histamine, β -hexosaminidase and other proinflammatory cytokines.¹² The serum IgE antibodies of PV were obtained by injecting PV intraperitoneally into mice.³⁶ The effect of PV on the degranulation of RBL-2H3 cells was studied by the cell-allergic model.³⁷ We found that less β -hexosaminidase was released in the group treated with MPT. These results suggested that PV binds to glucose through the Maillard reaction at canned processing conditions, which influences the structures of histamine and PV. It was noted that MPT was the best method for reducing β -hexosaminidase release, so MPT was considered in subsequent animal experiments. The regulation of IgE production by B cells is very poorly understood. In this study, we found that the total percentage of B cells was still higher in MPT-PV sensitized mice, which explains the higher levels of IgE than in the group administered PBS. More clinical data may be needed to verify

the safety of low-sensitivity products so far, we hope to see such products on the market at an early date for the benefit of allergic consumers. There is a wide market for low sensitivity products, to date, fish with low sensitivity products are relatively rare.

In addition, different animal models were constructed to analyze the difference between histamine poisoning (scombrotoxic food poisoning) and fish allergy. Fish with higher histamine content may cause a more severe allergic reaction, so this part of the experiment was already being planned. Histamine fish poisoning is usually mild, of short duration and self-limiting.³⁸ Given that symptoms result from excess amounts of histamine, the physical manifestations of histamine fish poisoning are similar to those of an allergic reaction. Ideally, fish could be processed by the Maillard reaction so that the bacteria cannot grow and histidine decarboxylase is not activated. It is necessary to analyze these two homologous risks, which can help consumers identify the root causes of adverse food reactions.

5. Conclusions

In this study, on the basis of the method of the Maillard reaction combined with pressure treatment, a canned processing method for *Decapterus maruadsi* was developed and a Chinese patent (no. CN104855489A) was applied for. In general, a method was found to control the histamine content of and reduce the PV hypersensitivity caused by fish, and animal experiments showed concurrent reductions in the intake of extraneous histamine and degranulation release *in vivo*. This method provides a new basis for producing low allergy fish products in the food industry.

Abbreviations

BT	Boiling treatment
FLH	The fish contained low content of histamine
FHH	The fish contained high content of histamine

PT	Pressure treatment
MR	Maillard reaction
MPT	Maillard reaction combined with pressure treatment
PV	Parvalbumin
US	Ultrasonic
UV	Ultraviolet

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

This work was supported by grants from the National Natural Scientific Foundation of China (U1405214, 31871720), the Marine Scientific Research Special Foundation for Public Sector Program (201505026-03).

References

- 1 Anonymous *China fisheries yearbook*, China Agriculture Press, 2017, p. 41.
- 2 C. Zhong, Q. F. Cai, G. M. Liu, L. C. Sun, K. Hara, W. J. Su and M. J. Cao, Purification and characterisation of cathepsin L from the skeletal muscle of blue scad (*Decapterus maruadsi*) and comparison of its role with myofibril-bound serine proteinase in the degradation of myofibrillar proteins, *Food Chem.*, 2012, **133**, 1560–1568.
- 3 A. Artharn, S. Benjakul, T. Prodpran and M. Tanaka, Properties of a protein-based film from round scad (*Decapterus maruadsi*) as affected by muscle types and washing, *Food Chem.*, 2007, **103**, 867–874.
- 4 S. Qiu, Y. Wan, Y. Cheng, Y. Liu, M. P. Yadav and L. J. Yin, Reduction of biogenic amines in sufu by ethanol addition during ripening stage, *Food Chem.*, 2017, **239**, 1244–1252.
- 5 Decomposition and histamine in raw, frozen tuna and mahi-mahi, canned tuna and related species. Compliance Policy Guide. 7108.240. FDA. 1996.
- 6 L. O. Mahony, M. Akdis and C. A. Akdis, Regulation of the immune response and inflammation by histamine and histamine receptors, *J. Allergy Clin. Immunol.*, 2011, **128**, 1153–1164.
- 7 C. A. Akdis and K. Blaser, Histamine in the immune regulation of allergic inflammation, *J. Allergy Clin. Immunol.*, 2003, **112**, 15–22.
- 8 C. Feng, S. Teuber and M. E. Gershwin, Histamine (*Scombroid*) Fish Poisoning: a Comprehensive Review, *Clin. Rev. Allergy Immunol.*, 2016, **50**, 64–69.
- 9 A. Naila, S. Flint, G. Fletcher, P. Bremer and G. Meerdink, Control of Biogenic Amines in Food-Existing and Emerging Approaches, *J. Food Sci.*, 2010, **75**, 139–150.
- 10 Y. Ma, U. Griesmeier, M. Susani, C. Radauer, P. Briza, A. Erler, M. Bublin, S. Alessandri, M. Himly, S. Vcquez-Cortcs, I. S. Rincon-de-Arellano, E. Vassilopoulou and P. Saxoni-Papageorgiou, Comparison of natural and recombinant forms of the major fish allergen parvalbumin from cod and carp, *Mol. Nutr. Food Res.*, 2008, **52**, 196–207.
- 11 M. F. Sharp, J. N. Stephen, L. Kraft, T. Weiss, S. D. Kamath and A. L. Lopata, Immunological cross-reactivity between four distant parvalbumins impact on allergen detection and diagnostics, *Mol. Immunol.*, 2015, **63**, 437–448.
- 12 A. Kuehn, I. Swoboda, K. Arumugam, C. Hilger and F. Hentges, Fish allergens at a glance: Variable allergenicity of parvalbumins, the major fish allergens, *Front. Immunol.*, 2014, **5**, 1–8.
- 13 Y. Hamada, T. A. Sato, S. I. Shizaki, Y. Nagashima and K. Shiomi, Expression and evaluation of IgE-binding capacity of recombinant pacific mackerel parvalbumin, *Allergol. Int.*, 2004, **53**, 271–278.
- 14 E. Ridolo, I. Martignago, G. Senna and G. Ricci, Scombroid syndrome: it seems to be fish allergy but. it isn't, *Curr. Opin. Allergy Clin. Immunol.*, 2016, **16**, 516–521.
- 15 W. Tapingkae, S. Tanasupawat, K. L. Parkin, S. Benjaku and W. Visessanguan, Degradation of histamine by extremely halophilic archaea isolated from high salt-fermented fishery products, *Enzyme Microb. Technol.*, 2010, **46**, 92–99.
- 16 Y. Hu, Z. Huang, J. Li and H. Yang, Concentrations of biogenic amines in fish, squid and octopus and their changes during storage, *Food Chem.*, 2012, **135**, 2604–2611.
- 17 J. Somkuti and L. Smeller, High pressure effects on allergen food proteins, *Biophys. Chem.*, 2013, **183**, 19–29.
- 18 W. Jiang, X. X. He, H. C. Yang, X. W. Xiang, S. W. Hu, S. J. Li and Y. Liu, Histamine reduction by Maillard reaction with glucose, *Food Control*, 2017, 136–144.
- 19 Y. J. Zhao, Q. F. Cai, T. C. Jin, L. J. Zhang, L. J. Zhang, D. X. Fei, G. M. Liu and M. J. Cao, Effect of Maillard reaction on the structural and immunological properties of recombinant silver carp parvalbumin, *LWT – Food Sci. Technol.*, 2017, **75**, 25–33.
- 20 M. Saaïd, B. Saad, N. H. Hashim, M. A. Abdussalamshin and S. Muhammadidir, Determination of biogenic amines in selected Malaysian food, *Food Chem.*, 2009, **113**, 1356–1362.
- 21 J. W. Hu, M. J. Cao, S. C. Guo, L. J. Zhang, W. J. Su and G. M. Liu, Identification and inhibition of histamine forming bacteria in blue scad (*Decapterus maruadsi*) and chub mackerel (*Scomber japonicus*), *J. Food Prot.*, 2015, **78**, 383–389.
- 22 G. M. Liu, N. Wang, Q. F. Cai, T. Li, L. C. Sun, W. J. Su and M. J. Cao, Purification and characterization of parvalbumins from silver carp (*Hypophthalmichthys molitris*), *J. Sci. Food Agric.*, 2010, **90**, 1034–1040.
- 23 M. J. Cao, L. L. Guo, L. J. Zhang, W. J. Su, Y. L. Liang and G. M. Liu, Purification and Allergenicity Identification of Carp Parvalbumin, *Food Sci.*, 2009, **30**, 187–190.
- 24 Y. Shen, M. J. Cao, Q. F. Cai, W. J. Su, H. L. Yu, W. W. Ruan and G. M. Liu, Purification, cloning, expression and immunological analysis of *Scylla serrata* arginine kinase, the crab allergen, *J. Sci. Food Agric.*, 2011, **91**, 1326–1335.

- 25 N. J. Plundrich, R. R. Bansode, E. A. Foegeding, L. L. Williams and M. A. Lila, Protein-bound Vaccinium fruit polyphenols decrease IgE binding to peanut allergens and RBL-2H3 mast cell degranulation in vitro, *Food Funct.*, 2017, **8**, 1611–1621.
- 26 M. Bol-Schoenmakers, R. Bleumink, M. Marcondes-Rezende, E. Mouser, I. Hassing, I. Ludwig, J. J. Smit and R. H. H. Pieters, Diclofenac enhances allergic responses in a mouse peanut allergy model, *Clin. Exp. Allergy*, 2011, **41**, 424–433.
- 27 F. Van-Wijk, S. Nierkens, W. de-Jong, E. J. Wehrens, L. Boon, P. van-Kooten, L. M. Knippels and R. Pieters, The CD28/CTLA-4-B7 signaling pathway is involved in both allergic sensitization and tolerance induction to orally administered peanut proteins, *J. Immunol.*, 2007, **178**, 6894–6900.
- 28 V. Ahuja, M. Quatchadze, D. Stelter, A. Albrecht and R. Stahlmann, Evaluation of biotechnology-derived novel proteins for the risk of food-allergic potential: advances in the development of animal models and future challenges, *Arch. Toxicol.*, 2010, **84**, 909–917.
- 29 Z. Jiang, L. Wang, H. Che and B. Tian, Effect of temperature and pH on angiotensin-I-converting enzyme inhibitory activity and physicochemical properties of bovine casein peptide in aqueous Maillard reaction system, *LWT – Food Sci. Technol.*, 2014, **59**, 35–42.
- 30 L. Tordesillas, M. C. Berin and H. A. Sampson, Immunology of Food Allergy, *Immunity*, 2017, **47**, 32–50.
- 31 F. Liua, M. Teodorowicz, M. A. J. S. van Boekel, H. J. Wichersbc and K. A. Hettinga, The decrease in the IgG-binding capacity of intensively dry heated whey proteins is associated with intense Maillard reaction, structural changes of the proteins and formation of RAGE-ligands, *Food Funct.*, 2016, **7**, 239–249.
- 32 T. Takai, E. Mizuuchi, Y. Kikuchi, T. Nagamune, K. Okumura and H. Ogawa, Glycosylation of Recombinant Proforms of Major House Dust Mite Allergens Der p 1 and Der f 1 Decelerates the Speed of Maturation, *Int. Arch. Allergy Immunol.*, 2006, **139**, 181–187.
- 33 H. J. Jongh, S. L. Taylor and S. J. Koppelman, Controlling the aggregation propensity and thereby digestibility of allergens by maillardation as illustrated for cod fish parvalbumin, *J. Biosci. Bioeng.*, 2011, **111**, 204–211.
- 34 R. J. Almond, B. F. Flanagan, A. Antonopoulos, S. M. Haslam, A. Dell, I. Kimber and R. J. Dearman, Differential immunogenicity and allergenicity of native and recombinant human lactoferrins: role of glycosylation, *Eur. J. Immunol.*, 2013, **43**, 170–181.
- 35 W. Jin, K. Han, S. Dong, Y. Yang, Z. Mao, M. Su and M. Zeng, Modifications in gut microbiota and fermentation metabolites in the hindgut of rats after the consumption of galactooligosaccharide glycated with a fish peptide, *Food Funct.*, 2018, **9**, 2853–2864.
- 36 K. D. Garcia-Orozco, E. Aispuro-Hernandez, G. Yepiz-Plascencia, A. M. Calderon-del-abarca and R. R. Sotelo-mundo, Molecular characterization of arginine kinase, an allergen from the shrimp (*Litopenaeus vannamei*), *Int. Arch. Allergy Immunol.*, 2007, **144**, 535–542.
- 37 J. A. Warner and C. Kroegel, Pulmonary immune cells in health and disease: mast cells and basophils, *Eur. Respir. J.*, 1994, **7**, 1326–1341.
- 38 C. Feng, S. Teuber and M. E. Gershwi, Histamine (Scombroid) Fish Poisoning: a Comprehensive Review, *Clin. Rev. Allergy Immunol.*, 2015, **50**, 64–69.