

MALDI-TOF MS and Magnetic Beads for Rapid Seafood Allergen Tests

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Cite This: <https://doi.org/10.1021/acs.jafc.1c04723>

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ABSTRACT: We developed a strategy using immunomagnetic separation (IMS) coupled with matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) to test seafood allergens. The protocol employed commercial magnetic beads (MBs) functionalized with anti-human IgE antibodies to carry out the IMS of IgEs in blood samples, followed by capture of allergens from seafood protein extracts for allergy analysis. After elution, the captured allergens were identified by MALDI-TOF MS and HPLC-MS/MS. The non-specific adsorption of MBs to biomolecules, the reproducibility and sensitivity of the protocol were investigated. The method shows consistent results with enzyme-linked immunosorbent assay tests. The false positive rate of the present method for the allergy test is 0%. The protocol was applied to detect the allergens in greasy-back shrimp for checking the allergenicity of patients' serum. Cooking fish as soup may effectively decrease the allergenicity. The method can be potentially used to identify unknown allergens of seafood to ensure the safety of allergic patients.

KEYWORDS: magnetic beads (MBs), allergen, sablefish, immunomagnetic separation (IMS), matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS)

INTRODUCTION

Food allergy, an allergic response of mankind to food, is clinically manifested as urticaria, asthma, abdominal pain, diarrhea, and other symptoms and is even serious enough to cause shock.¹ Seafoods are one of the most common triggers of life-threatening anaphylactic reactions.² That is, unlike most other food allergies, seafood allergy persists for life in up to 90% of patients, which is a trend also observed with peanut allergy.³ It is particularly important to develop simple, rapid, and accurate methods to detect allergens in seafood matrices.

A food allergy is considered to be an abnormal response of the body's immune system to certain foods or food protein(s). These abnormal responses of the immune system to certain food proteins are mediated by immunoglobulin E antibodies (IgE-mediated) or by immune cells (non-IgE-mediated). IgE-mediated allergies are defined as type I hypersensitivity reactions, which are also known as immediate reactions. IgE-mediated allergies are caused by the production of IgE antibodies (IgEs) against specific antigens, that is, allergens.⁴ When food allergen-sensitized individuals are exposed to the allergen, an initial immune response triggers IgE-mediated degranulation of immune effector cells, such as mast cells and basophils, resulting in the rapid manifestation of symptoms.⁴

For a non-allergic person, the total concentration of IgEs in blood serum varies according to the life period. The total concentration of IgEs in blood serum increases during childhood until the age of 15–20, stabilizes until approximately age 60, and then slowly decreases. In adults, the normal concentration is around 240 ng/mL (100 IU/mL).⁵ Total IgE concentrations above 100 IU/mL may indicate the sensitivity of the patient to one or more allergens. The concentration of

each kind of allergen-specific IgEs varies greatly for every patient depending on the type of allergen.⁶

Allergy tests can help to determine the specific allergens causing the allergic reaction and/or symptoms. The immunomagnetic enzyme-linked immunosorbent assay (ELISA) method, in which magnetic particles functionalized with polyamidoamine-dendrimers and antibodies, was developed to detect Ara h3/4 peanut allergen in corn flakes and biscuits.⁷ Wang *et al.* proposed an immunomagnetic beads-derived ELISA for parvalbumin (PV) detection, which employed antibody-functionalized graphene oxide (GO) and gold nanoparticles (AuNPs) to amplify the detection signal of PV.⁸ Immunoblots were used to analyze the profiles of patients' IgE reactivity to chicken muscle for the identification and characterization of poultry meat allergens.⁹

Some technologies have been applied for the detection of allergens in seafood matrices. Ion-exchange chromatography (IEC) coupled with capillary zone electrophoresis (CZE) was developed for the detection and quantification of PV, a major allergen in fish.¹⁰ Infrared spectroscopy and Inception-ResNet network modeling were also used to detect PV.¹¹ A polymerase chain reaction (PCR) assay was developed for the detection of seafood allergy. A real-time PCR was developed for the detection of crustacean allergen in foods.¹² Potential allergens of shrimp crustaceans were detected by a real-time PCR

Received: August 4, 2021

Revised: September 22, 2021

Accepted: September 23, 2021



quantitative approach.¹³ A multiplex PCR assay combined with capillary electrophoresis (CE) was developed for the detection of tropomyosin genes of oyster, mussel, abalone, clam, and the 18S rRNA gene of eukaryotes and then for the simultaneous detection of tropomyosin allergens in these seafoods.¹⁴ Pyruvate kinase was identified as a novel allergen in whiteleg shrimp (*Litopenaeus vannamei*) by specific-IgE present in patients with shrimp allergy.¹⁵

Immunomagnetic separation (IMS), based on the use of magnetic beads (MBs), brings important features for high throughput diagnosis, including robust equipment, simple design, high specificity, and low sample volume required.¹⁶ The matrix effect is minimized due to the washing and separation steps.¹⁷ Optimization of the target enrichment for sensitive analysis can be easily realized by adjusting the MB amount and the sample volume applied for analysis.¹⁸ The analysis of samples performed on MBs can be easily achieved without any pre-enrichment, purification, or pretreatment steps, which are normally necessary for standard methods.¹⁷ Magnetic nanoparticles coated with a layer of allergens (peanut protein extract) were utilized to detect allergy indicators in human plasma.¹⁹ Magnetic-assisted aptamer-based fluorescent assay was developed to detect major allergen, tropomyosin, in shellfish.²⁰ However, the study of applying MBs for seafood allergy tests is particularly limited.

Mass spectrometry (MS), as a powerful analytical technique, has been greatly used to identify unknown compounds within a sample and elucidate the structure of different molecules. We previously identified pathogenic bacteria in human blood by using IgG-modified Fe₃O₄ MBs as a sorbent and matrix-assisted laser desorption/ionization time-of-flight MS (MALDI-TOF MS) for profiling.²¹ Micro high-performance liquid chromatography coupled to a dual cell linear ion trap MS (microHPLC-ESI-MS/MS) was used to detect the soy, egg, and milk allergens in a cookie food matrix.²² However, only few MS-based methods have been developed for seafood allergen tests. The major fish allergen, PV, was directly analyzed by selected MS/MS ion monitoring MS.²³ Ayuso *et al.* identified and cloned a new major shrimp allergen, myosin light chain (MLC) protein, by analyzing one-dimensional and two-dimensional electrophoresis of shrimp extracts, followed by IgE immunoblotting and identified by MALDI-TOF MS and Edman sequencing.²⁴

At present, allergen analysis methods based on MS are mainly used for qualitative and quantitative analysis of known allergens;²⁵ no studies are reported to test unknown allergens in seafood matrices to the best knowledge of the authors. The present study developed a strategy for testing unknown allergens in seafoods. This is the first time that allergens in seafood matrices were studied by MALDI-TOF MS and MBs. Specific unknown allergens in seafood matrices were captured by using MBs functionalized with anti-human IgEs and sera of allergic patients, and tested *via* MALDI-TOF MS. The captured allergens were eluted, digested, and identified by MALDI-TOF MS and high-performance liquid chromatography-tandem MS (HPLC-MS/MS) for the elucidation of identities to known allergens. Fifteen serum samples were collected and studied to validate the present method. Our findings for the first time provide evidence of small vasohibin-binding protein (SVBP) as a potential allergen in sablefish. The reproducibility and sensitivity of the protocol for fish allergen tests were investigated. The reliability of the method was testified by using ELISA. The false positive rate (FPR) of the

present method for an allergy test was tested by using normal persons' sera. The possible cross-reacting allergen in crustacean and fish was studied by using the developed protocol. Thermal processing on the allergenicity of sablefish muscle was investigated. With the spectral data sets of crude protein samples of seafoods obtained by IMS and MS, we demonstrated that the method can be promisingly used to test and identify unknown allergens present in seafoods to secure the safety of patients with allergy.

MATERIALS AND METHODS

Chemicals, Seafood, and Serum Samples. Acetonitrile (ACN, 99.90%), acetic acid (AA, 36–38%), ethylene diamine tetraacetic acid (EDTA) (≥99.0%), and 2-propanol (99.80%) were purchased from Shanghai Titan Scientific Co., Ltd. (Shanghai, China). Tris (hydroxymethyl) aminomethane (Tris, 99.5–100.5%) was from Adamas-Beta Co., Ltd. (Shanghai, China). Hydrochloric acid (HCl, 36–38%) was obtained from Collins Reagent Co., Ltd. (Shanghai, China). Trifluoroacetic acid (TFA, ≥99%) was obtained from Fisher Scientific (Loughborough, UK). Phenylmethanesulfonyl fluoride (PMSF) was purchased from LOONGEN Science and Technology Co., Ltd. (Shanghai, China). Phosphate-buffered saline (PBS) solution (10 mM, pH 7.4) was purchased from Shanghai LabTop (LanTuo) Bio-Technology Co., Ltd. (Shanghai, China). Sodium borate buffer (0.1 M, pH 9.5) was obtained from Shanghai Qincheng Biotechnology Co., Ltd. (Shanghai, China). Ammonium sulfate (≥99.0%), sinapinic acid (SA, ≥99.0%), Tween 20, cytochrome *c* from equine heart (≥95%), myoglobin from equine skeletal (≥98%), and trypsin from bovine pancreas (≥10,000 BAEE units/mg protein) were purchased from Sigma-Aldrich (St Louis, USA). Bovine serum albumin (BSA, 96%) was purchased from Shanghai Macklin Biochemical Co., Ltd. (Shanghai, China). Deionized (DI) water was produced using a Milli-Q Simplicity system (Millipore, Bedford, MA, USA) and used for all the solutions. Acetonitrile (ACN, ≥99.8%) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China), formic acid (99%) was purchased from J&K Scientific Ltd. (Beijing, China) for HPLC-MS/MS analysis. Distilled water from Watsons Water Co. Ltd. (Shanghai, China) was used during the HPLC-MS/MS analysis.

Tosyl-activated superparamagnetic polystyrene beads with a diameter of 1.5 μm were obtained from BioMag Scientific Inc. (Wuxi, China). Mouse monoclonal anti-human IgEs were obtained from AbD Serotec (Oxford, U.K.).

Sablefish (*Anoplopoma fimbria*) slices were obtained in October 2020 from the Lingang Ftarest Free Trade Import Food Center (Pudong New Area, Shanghai, China). Greasy-back shrimp (*Metapenaeus ensis*) samples were purchased in November 2020 from a local market (Guzong road, Pudong New Area, Shanghai, China). The photos of the seafood samples used in the present study are shown in Figure S1.

Sera were kindly provided by volunteers. Sera of seven patients with immunoglobulin E (IgE)-mediated food allergy were collected by the Changhai Hospital (Shanghai, China). Eight normal human sera were collected by the Shanghai Tenth People's Hospital (Shanghai, China). The allergy blood tests with the patients' blood specimen were performed by the corresponding hospital. Table S1 lists the information about the sera of allergic patients. The age of the allergic patients ranged from 3 to 65 years. The IgE titers in the sera of patients with IgE-mediated food allergy ranged from 17.8 to 186 IU/mL. The IgE titers for the sera of patients 1 and 4 were 186 and 143 IU/mL, and the corresponding volunteers were aged 3 and 8 years, respectively. The blood samples were collected with the consent of the donors. No research on genetic material was carried out. These samples were in agreement with the World Health Organization's rules for "the clinical use of blood".

Functionalization of MBs. The obtained commercial MBs were functionalized according to the manufacturer's protocol. Briefly, 2.5 μL of MBs (20 mg/mL) were washed three times with sodium borate

buffer (0.1 M, pH 9.5), magnetically separated, and the pellets were collected and re-suspended in 2.5 μ L of sodium borate buffer (0.1 M, pH 9.5). 2.5 μ L of anti-human IgEs (1 mg/mL), 6.3 μ L of sodium borate buffer (0.1 M, pH 9.5), and 5.2 μ L of ammonium sulfate (3 M) were added to the MB suspension. The obtained solution was shaken at 850 rpm overnight at room temperature (RT) on an incubation machine (ThermoMixer C, Eppendorf, Hamburg, Germany) to avoid MB sedimentation.

Then, the mixture was magnetically separated, and the pellets was collected and mixed with 20 μ L of 10 mM PBST (0.05% Tween-20 in PBS, pH 7.4) containing 5% (m/m) of BSA and then incubated at RT for 6 h to coat BSA onto the surface of the MBs for preventing the non-specific binding of reactants, especially proteins in the analyzed bio-samples. The obtained anti-human IgE-functionalized MBs were washed three times with 10 mM PBST (0.05% Tween 20 in PBS, pH 7.4) for further use.

The above-prepared anti-human IgE-functionalized MBs (*ca.* 1 μ L) were transferred into an Eppendorf tube if necessary and then 13 μ L of the serum sample from a fish-allergic patient was added and it was incubated at RT for 5 min. Afterward, the mixture was placed on a magnetic separation rack (BMB-01, 16 holes, for 1.5–2 mL Eppendorf microcentrifuge tubes, BioMag Scientific Inc., Wuxi, China) for collecting the MB pellets. The resultant IgE-coated MB pellets were collected to interact with seafood matrices after rinsing three times with PBST (10 mM, 0.05% Tween 20, pH 7.4) and once with DI water.

Extraction of Proteins from Sablefish and Greasy-Back Shrimp. 6 g of sablefish muscle sample was obtained from four slices of sablefish muscle samples and placed in a beaker with 40 mL of buffer solution (20 mM Tris-HCl containing 5 mM EDTA, pH 7.5) and homogenized for 10 min with an electric homogenizer (F6/10 handheld high-speed homogenizer, Shanghai Jingxin Technology Co., Ltd., Shanghai, China). Then, the suspension was centrifuged (S810 R, Eppendorf, Hamburg, Germany) at 6797g and 4 °C for 30 min. The equivalent content of protein extracted from the sablefish muscle was calculated by taking BSA as a reference with an equation of $A = 0.4758\rho_B - 0.0057$, where A is the measured UV–vis absorbance at 280 nm, ρ_B is the concentration of the protein with unit mg/mL. The UV–vis absorbance of the protein samples from sablefish was measured by using a UV–vis spectrophotometer (UV-1900 UV–vis–NIR Spectrophotometer, Shimadzu, Kyoto, Japan). For comparison purposes, the concentration of the crude fish sarcoplasmic proteins (FSP) of sablefish was tested by a third party (Shanghai Jingkang Biology Engineering Co., Ltd, Shanghai, China) using a BCA kit. The protein samples were filtered with Whatman filter paper prior to the BCA test.

The extraction of protein from muscle sample of greasy-back shrimp (*M. ensis*) follows a previous study.²⁶ In brief, 3 g of muscle sample was chopped into small pieces and put in a beaker with 10 mL of buffer solution [10 mM PBS, with 1 mM of phenylmethane-sulfonyl fluoride (PMSF) as protease inhibitor] and then homogenized for 10 min. The obtained sample was centrifuged at 6797g and 4 °C for 30 min, and the supernatant was collected and interacted with IgE-coated MBs for an allergy test.

IMS for the Fish Allergens. The patient IgE-coated MBs (*ca.* 1 μ L) were incubated with 27 μ L of sablefish sarcoplasmic protein solutions or greasy-back shrimp muscle protein solutions at RT for 20 min to do the allergy test. MBs were then magnetically separated from the solution and rinsed three times with PBST (10 mM, 0.05% Tween 20, pH 7.4) and once with DI water. A control experiment was performed by using a pooled sample comprising eight normal human serum samples from normal individuals (v/v, 1:1). The captured proteins on the surface of MBs were eluted with 2 μ L of 5% (v/v) acetic acid solution, and the supernatant was collected after magnetic separation for digestion.

Digestion of Allergens by Trypsin. The above-eluted allergens were diluted 10-fold with PBS (10 mM, pH 7.4) and digested with trypsin (protein/trypsin = 30:1, w/w) at 37 °C for 14 h. The digestion was terminated by adding TFA to a final concentration of

0.4% (v/v). The obtained digests were analyzed by MALDI-TOF MS and HPLC-MS/MS.

MALDI-TOF MS Analysis of the Samples. A Bruker microflex LRF mass spectrometer (Bremen, Germany) was used for performing all MALDI-TOF MS experiments. A protein aqueous solution containing cytochrome *c* (2 mg/mL) and myoglobin (4 mg/mL) as an external standard was used for the calibration of MALDI-TOF mass spectrometers by utilizing the doubly and singly charged ions of cytochrome C (*m/z* 6181 and 12,361, respectively) and myoglobin (*m/z* 8476 and 16,952, respectively). The matrix for the protein analysis contained 15 mg/mL of sinapinic acid (SA) in a 70:30:0.1 (v/v) acetonitrile/water/TFA mixture. 1 μ L of the eluted samples with allergens were deposited on a MALDI target plate (MSP 96 target ground steel). After air-drying at RT, the sample spots were covered with 1 μ L of SA solution (15 mg/mL). All mass spectra were obtained in the linear and positive ion mode. Mass spectra were acquired in the *m/z* range of 2000–20,000 Da or 2000–40,000 Da. Sum spectra were made from 500 laser shots using the automated sample collection mode. Laser frequency was set as 20 Hz. Detector gain was set as 10.3 $\times$. The laser intensity of SA was 65%. At least three mass spectra were acquired for each sample. All experiments were performed at least in triplicate.

Identification of Allergens Captured by MBs via MALDI-TOF MS. Identification of allergens captured by MBs was achieved by using the search engine (<https://www.uniprot.org>) for searching against the UniProt database and assigning the measured ion peaks. Mass spectrometric data analysis of tryptic digests was performed with flexAnalysis from Bruker and the FindMod tool available on the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) (<http://lexpasy.org/>). The masses and intensities of special peaks were sent to the FindMod tool for database searching. Experimentally measured peptide masses were compared with the masses of the theoretical peptides calculated from a specified Swiss-Prot/TrEMBL entry to finish the peak identification.

Identification of Allergens Captured by MBs via HPLC-MS/MS. The above-prepared tryptic digests of allergens were desalted with a desalting column (MonoSpin C18, GL Science Inc, Tokyo, Japan) on a centrifuge system (Hitachi Centrifuge, Hitachi Koki Co., Ltd.). The obtained solution was dried in a vacuum drying system (LNG-T98 vacuum dryer, Taicang Huamei Biochemical Instrument Factory, Jiangsu, China) at RT for about 40 min. A ChromXP C18 column (120 Å, 0.3 $\times$ 150 mm, 3 μ m, Eksigent AB Sciex, Singapore) was used in this study. The mobile phase was composed of A (98% water with 2% ACN and 0.1% formic acid) and B (98% ACN with 2% water and 0.1% formic acid), with gradient elution of 0–6 min (B, 0%), 6–6.5 min (B, 0–5%), 6.5–95 min (B, 5–28%), 95–100 min (B, 28–55%), 100–105 min (B, 55–80%), 105–112 min (B, 80%), 112–113 min (B, 80–5%), 113–120 min (B, 5%), and 120–124 min (B, 0%). The flow rate was set as 0.012 mL/min (0–6 min), 0.15 mL/min (6–120 min), and 0.02 mL/min (120–124 min), respectively. Column temperature was controlled at 40 °C and the sample injection volume was 27 μ L.

A Q-TOF mass spectrometer (Triple TOF 4600, AB SCIEX, USA) was coupled with the HPLC system (Shimadzu 20A, Shimadzu, Japan) through an electrospray ionization (ESI) source. Ionization was performed in positive mode. ESI was set as 5500 V, and ion source parameters were optimized as follows: gas 1, 25 psi; gas 2, 15 psi; curtain gas, 30 psi; and source temperature, 350 °C. MS/MS data were acquired in the mode of information-dependent acquisition. The maximum number of candidate ions monitored per cycle was 40, and the mass range was 350–1250 (*m/z*). MaxQuant (www.maxquant.org) was used to identify peptides from the HPLC-MS/MS data.

Deallergenization of Fish Muscle by Thermal Processing. 10 g of sablefish muscle sample was added into 200 mL of DI water and boiled on a hot plate (RH digital, IKA, Staufen, Germany) for 5 min, 15 min, 25 min, 30 min, and 1 h. The resultant aqueous extracts were collected and centrifuged at 4 °C 11,000g for 20 min on a centrifuge system (S810 R, Eppendorf, Hamburg, Germany). The resultant supernatant was collected and filtered by using a 0.22 μ m ultrafiltration membrane and collected for MALDI-TOF MS analysis.

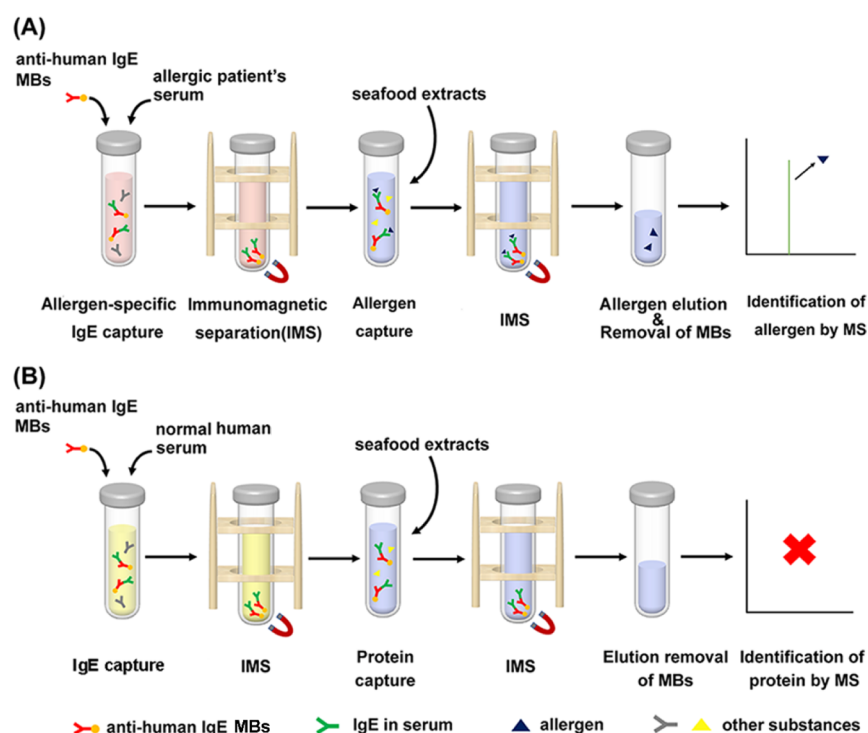


Figure 1. Allergy test procedure based on MBs and MALDI-TOF MS. MBs functionalized with anti-human IgE antibodies were used to carry out IMS of IgE in the blood sample, followed by allergen capture from seafood protein extracts. After elution, the captured proteins were analyzed by MALDI-TOF MS. IMS was operated using a magnetic separation device. The red colored X used in the inlet of (B) shows that allergens cannot be detected in that case.

ELISA Test of the Allergens. A parvalbumin-specific ELISA kit (JLCA4402, 48T, Shanghai Jingkang Biology Engineering Co., Ltd., China) was used to perform the ELISA by following the steps listed in the kit instructions. The FSP of sablefish and eluted solution of the captured allergens in FSP of sablefish by MBs were tested by a third party (Shanghai Jingkang Biology Engineering Co., Ltd, Shanghai, China) using the ELISA kit.

Safety Information. TFA is harmful to the body and has a strong stimulating effect on the eyes, mucous membranes, respiratory tract, and skin. Death may be caused by spasms, symptoms, edema, chemical pneumonia, and pulmonary edema of the larynx and bronchi after inhalation. Acetonitrile (ACN) is flammable and hazardous in case of skin contact (irritant), eye contact (irritant), ingestion, and inhalation. ACN can be slightly hazardous in case of skin contact (permeator). Severe overexposure of ACN can result in death.

RESULTS AND DISCUSSION

Work Flow of Detection of Allergic Proteins by MBs.

Nowadays, allergen analysis methods based on mass spectrometry (MS) are mainly used for qualitative and quantitative analysis of known allergens.²⁵ Herein, a method for testing unknown allergens was developed. During the experiment, IMS was performed to attach the IgE antibodies (IgEs) from the sera of allergic patients. As illustrated in Figure 1, MBs were first functionalized with anti-human IgEs. An allergy test was performed by probing the binding of the obtained anti-human IgEs/IgE immunocomplexes. The FSP of sablefish (*A. fimbria*) were used as a model food matrix containing substances that a patient is allergic/sensitized to. The allergy analysis were performed by MALDI-TOF MS.

After elution of the captured proteins (allergens) from IgEs on the MB surface, the proteins were digested and analyzed by MALDI-TOF MS and HPLC-MS/MS to identify the captured allergens.

Responses of an Allergic Patient's Serum and Normal Human Serum to Seafood Protein Extracts. Fish-induced allergies impact the quality of life of sensitized/allergic individuals. Herein, sablefish, also known as black cod or cod in the Chinese supermarket, was chosen as a model fish to study the allergens' detection by MB-based protocol.

Non-specific adsorption toward biomolecules can perplex the bioanalysis.²⁷ As shown in Figure 2A,B, when the unoccupied binding sites on the MBs were blocked with BSA, the quality of the data obtained by MALDI-TOF MS was greatly improved, illustrating that the blocking step is imperative and improves the signal-to-noise ratio (S/N) of the assay by reducing the background interference. The impact of BSA blockage on the test is further discussed in Supporting Information S3. MBs were blocked with BSA for further analysis in the present study.

The crude FSP of sablefish were extracted to check whether allergen-specific IgEs exist in the human sera. The initial concentration of crude sablefish sarcoplasmic protein solutions is 5.0 ± 0.1 mg/mL ($n = 3$) when extracting 6 g of sablefish muscle sample with 40 mL of buffer solution (20 mM Tris-HCl containing 5 mM EDTA, pH 7.5). During the experiment, sera from allergic individuals were studied. A serum pool from eight normal human serum samples was tested as a control. Figure 2 shows the mass spectra obtained during the sablefish (*A. fimbria*) allergy test of the sera of seafood allergic patients and the normal human serum pooled sample. It can be found that dissimilar to the mass spectrum obtained from the test of normal human serum pooled samples, a clear peak, centered at $m/z = 7766.4 \pm 4.9$ in the mass spectrum of the allergy test of serum of seafood allergic patient can be observed, illustrating that the MB-based protocol can capture allergens from the sablefish protein extracts. Similar experimental phenomena

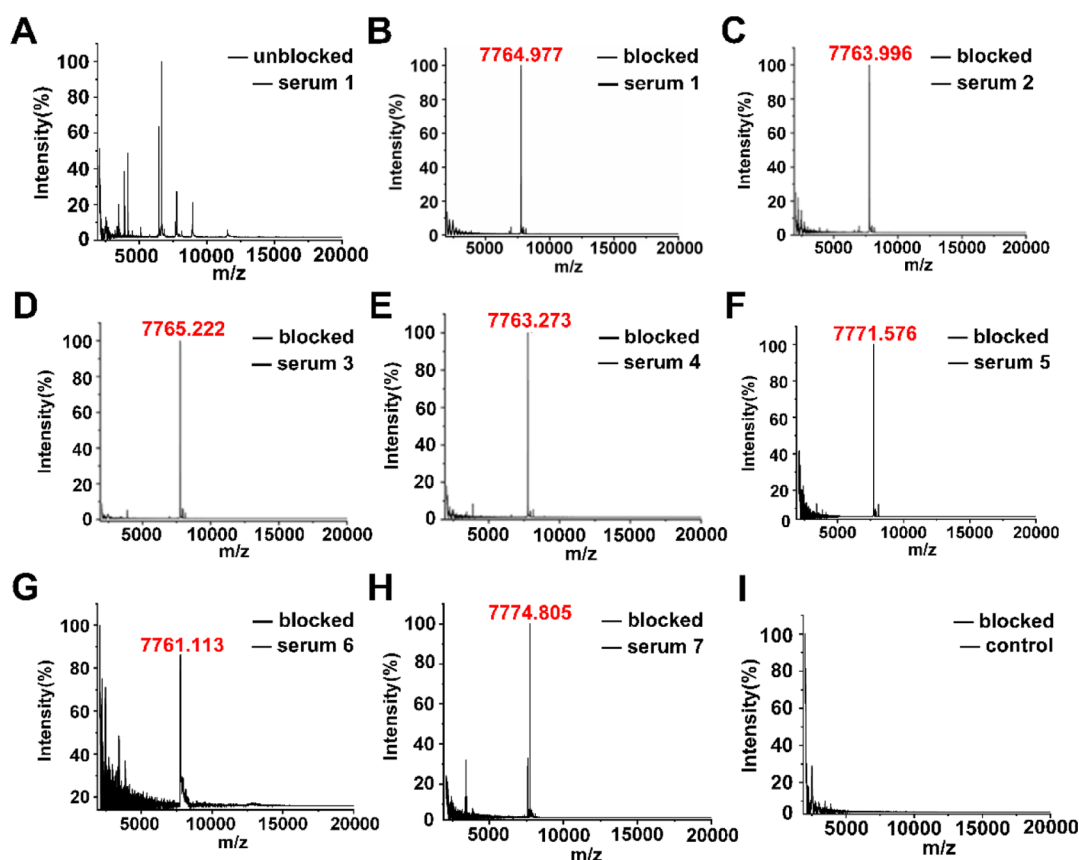


Figure 2. MALDI-TOF mass spectra obtained during the sablefish allergy test of the serum of seafood allergic patients (from A to H) and eight normal human serum pooled sample (I). During the experiment, anti-human IgE-functionalized MBs interacted with human serum and then with sablefish sarcoplasmic proteins. Functionalized MBs were blocked without BSA (A) and with BSA (B–I), respectively. The serum sample was not diluted during the experiment. Human serum samples used here are from patients 1 to 7 listed in Table S1.

were obtained when more sera from sensitized/allergic individuals were tested as shown in Figure 2C–H, indicating that all the analyzed patients are sensitized/allergic to sablefish. The allergy blood test done by the hospital also shows that these individuals are allergic to cod. This result from the present protocol remains consistent with the results of the allergy blood test. Contrarily, in the control experiment, the obtained mass spectra show no obvious ions in the mass range of 2000–20,000, illustrating that no IgE-mediated food allergy issue exists in the analyzed serum mixture which is pooled by normal human sera as shown in Figure 2I.

Reproducibility of the MBs for the Test of Seafood Allergens. Reproducibility can dictate the reliability and generality of results and methods.²⁸ The reproducibility of applying MBs for the food allergen test was investigated. Experimentally, three independent tests were performed to sense the allergens in the sablefish crude protein extracts by using the serum of patient 1. MBs were functionalized with anti-human IgEs and blocked with BSA prior to the allergy test. Figure 3 shows the MALDI-TOF mass spectra obtained from three independent tests of applying MBs for the sablefish allergy test of the serum of a seafood allergic patient. It can be found that in the three runs of measurements, the allergens can be captured, indicated by clear peaks in the obtained mass spectra. The results show that the developed protocol for allergy test is reproducible and capable to be applied for the allergy test.

Sensitivity of the MBs for Allergen Sensing. It has been found that the sensitivity and selectivity of MS analysis are commonly affected by the complexity of matrices.²⁹ The sensitivity of using MBs to detect allergens from crude sablefish sarcoplasmic protein solutions was taken into account. Herein, the crude sablefish sarcoplasmic protein extracts were directly utilized for allergy analysis, which greatly simplified the protocol but increased the complexity of matrices of analytes.

To check the sensitivity of the MBs for allergens in sablefish, during the experiment, crude sablefish sarcoplasmic protein extract was diluted with DI water by different folds, that is, 100-, 500-, 1000-, 5000, 10,000-, 20,000-, 30,000-, and 40,000-fold, and used for the allergy test by MBs.

Figure 4 shows the mass spectra obtained during the fish allergy test of the sera of a seafood allergic patient using a solution of FSP of sablefish, which was diluted by different folds. It can be observed that the suspected allergen (m/z near 7766) can still be observed after a 30,000-fold of dilutions ($S/N = 3$). It can be determined that $0.17 \pm 0.00(3) \mu\text{g/mL}$ ($S/N = 3$) crude sablefish sarcoplasmic protein solutions can still be detectable by the presently developed protocol. That is equivalent to $1.13 \pm 0.02 \mu\text{g/g}$ ($S/N = 3$) of allergen(s) in the sablefish muscle sample under the utilized conditions. The MB-based protocol in the present study may be able to sense a very low amount of allergens from fish muscle matrices.

Identification of Allergens by MALDI-TOF MS and HPLC-MS/MS. The allergens in crude sablefish sarcoplasmic

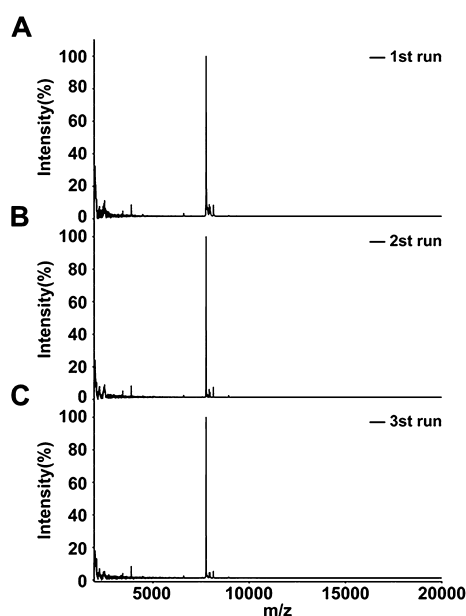


Figure 3. Representative MALDI-TOF mass spectra obtained from three independent experiments during the sablefish allergy test of the serum of a seafood allergic patient. MBs were functionalized and blocked with BSA prior to the test. The MBs were freshly functionalized in each experiment. The proteins were freshly extracted from sablefish muscle samples in each experiment. The tested serum is from patient 1 with information listed in [Supporting Information S2](#).

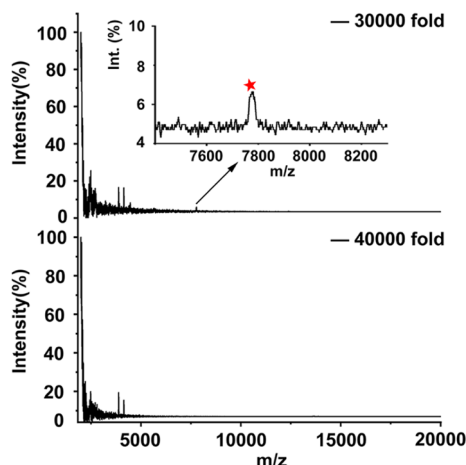


Figure 4. MALDI-TOF mass spectra obtained during the fish allergy test of the serum of a seafood allergic patient using solution of FSP of sablefish, which was diluted by different folds. MBs were functionalized with anti-human IgEs and blocked with BSA prior to the test. The initial protein extract of sablefish is 5.0 ± 0.1 mg/mL ($n = 3$) under the utilized extraction condition and diluted with DI water by serial dilution. The tested serum is from patient 1 with information listed in [Supporting Information S2](#). The peak corresponding to probable allergen is denoted by a red pentagram.

protein extracts captured by MBs were eluted and identified by MALDI-TOF MS analysis. Uniprot database searching shows that the probable allergens, as shown in [Figure 2](#), with an ion peak at $m/z = 7766.4 \pm 4.9$ are SVBP and/or C17orf89 with more information listed in [Supporting Information S4](#), indicating that the patients may be allergic to SVBP and/or C17orf89.

To identify the proteins, the captured allergens in the crude sablefish sarcoplasmic protein extracts were digested with trypsin. The tryptic products of allergens were detected by MALDI-TOF MS and were identified by using the flexAnalysis software from Bruker and the FindMod tool available on the ExPASy (Expert Protein Analysis System) proteomics server of the Swiss Institute of Bioinformatics (SIB) (<http://expasy.org/>).

[Figure 5](#) shows the representative MALDI-TOF mass spectrum of tryptic digests of the captured allergen in FSP of

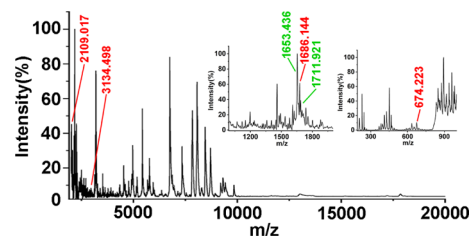


Figure 5. Representative MALDI-TOF mass spectrum of tryptic digests of the captured allergen in FSP of sablefish by functionalized MBs. The tested serum sample is from patient 4. MBs functionalized with anti-human IgEs were used to carry out the IMS of IgE in the blood sample, followed by allergen capture from FSP extracts of sablefish (*A. fimbria*). The captured allergens by MBs were eluted by using $2 \mu\text{L}$ of 5% (v/v) acetic acid solution. The obtained solution of allergens was diluted 10-fold with PBS (10 mM, pH 7.4) and digested with trypsin (protein/trypsin = 30:1, w/w) at 37°C for 14 h. The digestion was terminated by adding TFA to a final concentration of 0.4% (v/v).

sablefish by MBs. The identification results show that the detected ions with peaks at $m/z = 674.223$, 1686.144 , 2109.017 , and 3134.498 can be ascribed to the digests of C17orf89 with mass 673.3991 , 1687.8723 , 2107.9861 , and 3134.5471 , respectively, as listed in [Table S3](#) when considering the resolution of MALDI-TOF MS as 1000 ppm. The detected ion with peaks at $m/z = 1653.436$ and 1711.921 can be ascribed to the digests of SVBP with mass 1651.8735 and 1712.9402 , respectively, as listed in [Table S4](#). Based on the results, the allergen detected from crude sablefish sarcoplasmic proteins may be C17orf89 and/or SVBP.

To elucidate the structure of the allergen, the digests were further analyzed by HPLC-MS/MS. [Table S5](#) lists the predicted and experimentally determined masses of the fragments resulting from tryptic digests of SVBP. The signal observed at m/z 826.3933 Da ([Figure S2](#)) corresponds to a peptide that contains amino acids 26–32 of SVBP with an expected m/z value of 826.39 Da. It can be concluded that the allergen detected from crude sablefish sarcoplasmic proteins by using the serum of seafood allergic patient 4 can be SVBP based on the results of HPLC-MS/MS analysis.

The distribution and functionality of the SVBP in sablefish have not been available in the UniProt database; however, the structure and function of the SVBP in zebrafish are available. It can be found that SVBP enhances the tyrosine carboxypeptidase activity of vasohibin-1 (VASH1) and vasohibin-2 (VASH2), thereby promoting the removal of the C-terminal tyrosine residue of alpha-tubulin. SVBP is also required to enhance the solubility and secretion of VASH1 and VASH2. Besides, SVBP may play a role in axon and excitatory synapse formation (by similarity).

Besides the result obtained from the serum of patient 4, the allergen captured by the serum from patient 1 was analyzed with the results shown in Supporting Information S6. The results show that the allergen may be C17orf89 and/or SVBP. The findings here show that the protein C17orf89 with a molecular weight (M_w) of 7770 and/or SVBP with an M_w of 7767 are potentially recognized by patient IgE and may result in allergic symptoms. Additionally, as shown in the inset of Figure S4, probable allergens with weak signals at $m/z = 11822.031$ and 11838.429 and $S/N = 6$ were also detected. To further identify the allergens captured by using the serum of patient 1, allergens in crude FSP of sablefish captured by IgEs of patient 1 were eluted, digested by trypsin, and analyzed via HPLC-MS/MS. Figure S5 shows the HPLC-MS/MS spectrum of ions of peptides generated by the tryptic digestion of allergens obtained during the sablefish allergy test of the serum of seafood allergic patient 1. It can be found that fragment ions of AFAGMLSDEDIK of b_2 , γ_7 , γ_6 , b_{10} , γ_2 , b_{11} , and γ_1 from parvalbumin beta can be identified by HPLC-MS/MS, illustrating that the allergen captured from crude sablefish sarcoplasmic proteins by using the serum of allergic patient 1 is probably parvalbumin beta. In fact, parvalbumin beta as an allergen in sablefish has been previously reported.³⁰

The reliability of the results obtained by the present method was testified by using ELISA. An ELISA kit of SVBP is not available. The crude sarcoplasmic proteins of sablefish and eluted solution of the captured allergens in crude FSP of sablefish were tested by a third party using a commercial ELISA kit. The results show that the concentration of parvalbumin in crude protein extracts of sablefish is $39.37 \pm 0.22 \mu\text{g/mL}$, equivalent to $0.26 \pm 0.00(1) \text{ mg/g}$ in the sablefish muscle sample. The concentration of parvalbumin in the eluted solution of the captured allergens by using the serum of patient 1 in crude protein extracts of sablefish is $6.27 \pm 0.19 \mu\text{g/mL}$ under the utilized conditions, showing that patient 1 may be allergic to parvalbumin in sablefish. The results provided by the present method are in consistency with the ELISA test.

FPR of the Present Method. False positive rate (FPR) is an important aspect of a test and a measure of accuracy of a test. Herein, during the experiment, 15 serum samples were collected and studied to validate the present method. A pooled serum sample from eight normal persons' sera and four normal persons' sera were individually tested to find out the occurrence of positive diagnosis error. Table S10 lists the results of the allergy test on normal sera by using the present protocol. It can be determined that the FRP of the present method for the allergy test is 0%.

Cross-Reactivity Analysis of Patient 1-Specific IgE with Fish and Crustacean. Patients with specific food allergies are commonly sensitized to related foods.³¹ The cross-reactivity studies are highly necessary to avoid unnecessary dietary restrictions. Herein, greasy-back shrimp (*M. ensis*, ME) was taken as a model of crustaceans to study their cross-reactivity with fish.

Figure 6 shows the mass spectrum obtained during the shrimp allergy test of the sera of seafood allergic patients using a crude protein solution of greasy-back shrimp. It has been reported that the main allergen in shrimp is tropomyosin, whose molecular weight ranges from 30 to 40 kDa.^{32–34} As shown in Figure 6, a signal at $m/z = 30\ 147.340$ was observed on the obtained mass spectra. Probably the detected allergen is tropomyosin. The allergenicity and IgE recognition of

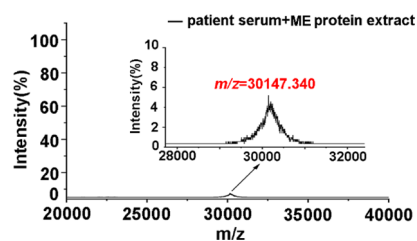


Figure 6. MALDI-TOF mass spectra obtained during the shrimp allergy test of the sera of a seafood allergic patient using a crude protein solution of greasy-back shrimp (*M. ensis*). MBs were functionalized and blocked with BSA prior to further test. The masses in the range of 20,000–40,000 were detected. The serum sample is from patient 1.

tropomyosin has been well documented in crustacean, mollusc, and fish (tilapia).³ However, no tropomyosin was identified in the fish (sablefish) sample analyzed in the present study, showing there may be no cross-reactivity between the analyzed seafood samples. The results here show that the cross-reactivity of allergens between foods can be investigated based on the present protocol. It is promising to apply the present strategy for other food allergen monitoring.

Screening of Thermal Processing on the Allergenicity of Sablefish Muscle. The food processing in different regions may impact the prevalence of seafood allergy. The heating of fish muscle may favor the reduction of allergenicity of fish. The present method was used to investigate the allergenicity of the real food sample.

Considering fish muscles are often consumed after boiling in Asia, herein, the sablefish muscle sample was boiled in water for different periods of time to obtain a soup-like food. The results shown in Figure 7 illustrate that boiling of fish muscle may alter the allergenic properties of fish muscle. The intensity of boiling may vary the allergenic potential. 30 min of boiling can significantly lower the allergenicity of sablefish aqueous extracts. Boiling of sablefish muscle in water may reduce the allergenicity by denaturation of allergenic proteins and/or transfer of low molecular weight (LMW) proteins into cooking water. It can be concluded that the present method can be applied in monitoring the allergenicity of practical foods for human consumption and/or assessing the risk assessment of food allergenicity.

Comparison of Some Modern Methods for Allergy Tests. Table 1 compares the recently developed methods and the present method. It can be found that IMS coupled with MALDI-TOF MS for allergy tests can greatly simplify the procedure by directly analyzing the crude protein extracts of foods and can be applied to sense unknown allergens.

A relatively facile, reliable, and sensitive method is particularly important for the seafood allergy test. The present study demonstrated the feasibility of using IMS coupled with MALDI-TOF MS to detect seafood allergens by using crude protein extracts of sablefish and greasy-back shrimp as model seafood matrices. The blockage of MBs and the sensitivity and reproducibility of the developed protocol were investigated. The results showed that 5% (m/m) BSA in PBST buffer (10 mM, 0.05% Tween 20, pH 7.4) can be used as a satisfactory blocking reagent to inhibit the non-specific adsorption of MBs toward biomolecules. Fifteen serum samples were studied to validate the present method. The signal obtained from the serum of a patient was not observed in the mass spectra during the sablefish allergy test of the normal human serum samples.

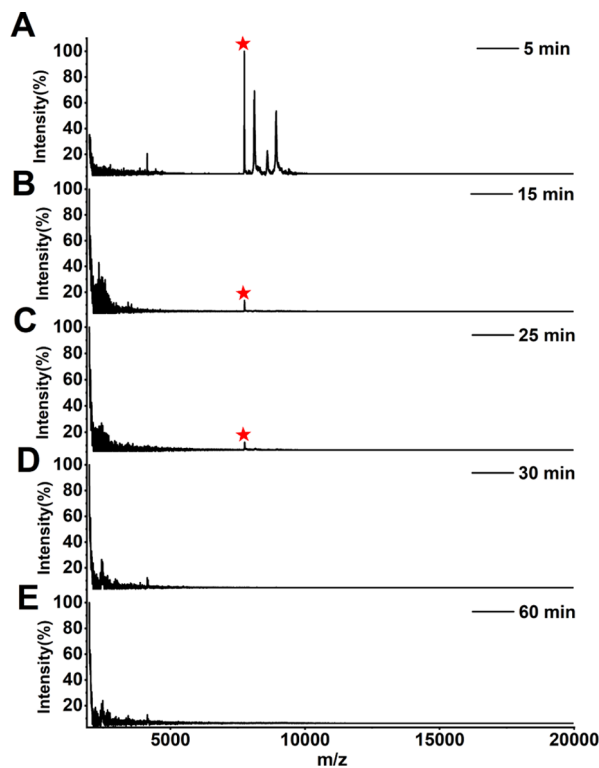


Figure 7. Representative MALDI-TOF mass spectra obtained during the sablefish allergy test by using the serum of a seafood allergic patient. The protein extracts of sablefish were boiled for 5 min, 15 min, 25 min, 30 min, and 1 h. MBs were functionalized with anti-human IgEs and blocked with BSA. The tested serum is from patient 4 with information listed in Table S1.

The allergens, captured from crude sablefish sarcoplasmic proteins by using the sera of seafood allergic patient 4 and patient 1, were analyzed and identified by MALDI-TOF MS and HPLC-MS/MS, and the results show that SVBP may be a potential allergen in sablefish. The fish allergen, parvalbumin beta, was also identified, which was captured from crude sablefish sarcoplasmic proteins by using the serum of allergic patient 1. The present method shows consistent results with the ELISA test. By using eight normal persons' sera, the FPR of the present method for the allergy test was testified as 0%. The MALDI-TOF MS analysis was utilized to investigate the cross-reactivity of patient 1-specific IgE with fish and crustacean, and

the results show that the patient allergic to sablefish may also be allergic/sensitized to greasy-back shrimp. The present method was successfully applied in investigating the impact of food processing on the allergenic potential of practical food samples and/or assessing the risk of food allergenicity. It is promising to apply the presently developed strategy for seafood allergen monitoring and identifying unknown allergens in muscle food matrices.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jafc.1c04723>.

Photographs of the sablefish slice and greasy-black shrimp used in the present study; serum information of allergic patients used in the present work; suppression of non-specific absorption of MBs to other bio-molecules; preliminary results of allergen identification of sablefish; identification of the allergen captured by serum of patient 4; identification of allergen captured by serum of patient 1; and FPR of the allergy test on normal persons' sera (PDF)

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Table 1. Comparison of the Recently Developed Methods and the Present Method where IMS Coupled with MALDI-TOF MS Was Developed for an Allergy Test

method	pros and/or cons	sensitivity (LOD)	references
one-dimensional and two-dimensional electrophoresis, IgE immunoblotting, MALDI-TOF MS, Edman sequencing	purified protein was used; protein extraction and experimental operations are complicated and time-consuming	not available (N/A)	24
immunomagnetic ELISA	long detection and analysis time; complicated and time-consuming protein extraction; a strategy for testing known allergens	5 pg/mL	9
IEC coupled with CZE	complicated and time-consuming protein extraction; a strategy for testing known allergens	0.71 µg/mL	10
PCR assay	genetic methods need complicated pretreatment to extract DNA which is labor-intensive and time-consuming; selection of appropriate primers for DNA amplification is another issue and affects the identification accuracy	1.25 pg	12
infrared spectroscopy and Inception-ResNet network modeling	complicated and time-consuming protein extraction; a strategy for testing known allergens	N/A	11
IMS coupled with MALDI-TOF MS	crude protein extracts were used; simple protein extraction and experimental operation; fast detection; a strategy for testing unknown allergens	0.17 µg/mL	this study

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Funding

This work was supported by the National Natural Science Foundation of China (21804087), and the Science and Technology Commission of Shanghai Municipality (18050502200).

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

AA, acetic acid; Abs, antibodies; ACN, acetonitrile; AuNPs, gold nanoparticles; BSA, bovine serum albumin; CE, capillary electrophoresis; CI, complex I; CZE, capillary zone electrophoresis; DI, deionized; EDTA, ethylene diamine tetraacetic acid; ELISA, immunomagnetic enzyme-linked immunosorbent assay; ESI, electrospray ionization; ExPASy, Expert Protein Analysis System; FAO, Food and Agriculture Organization of the United Nations; FPR, false positive rate; FSP, fish sarcoplasmic proteins; GO, graphene oxide; HCl, hydrochloric acid; HPLC-MS/MS, high-performance liquid chromatography-tandem mass spectrometry; IEC, ion-exchange chromatography; IgE, immunoglobulin E; IgEs, IgE antibodies; IMS, immunomagnetic separation; LMW, low molecular weight; MALDI-TOF MS, matrix assisted laser desorption/ionization time-of-flight mass spectrometry; MBs, magnetic beads; microHPLC-ESI-MS/MS, micro high-performance liquid chromatography coupled to a dual cell linear ion trap mass spectrometry; MLC, myosin light chain; MS, mass spectrometry; M_w , molecular weight; N/A, not available; PBS, phosphate-buffered saline; PBSF, phenylmethanesulfonyl fluoride; PCR, polymerase chain reaction; PMSF, phenylmethanesulfonyl fluoride; PV, parvalbumin; Q-TOF mass spectrometer, quadrupole time-of-flight mass spectrometer; RT, room temperature; SA, sinapinic acid; SIB, Swiss Institute of Bioinformatics; S/N, signal-to-noise ratio; SVBP, small vasohibin-binding protein; TCP, tubulin tyrosine carboxypeptidase; TFA, trifluoroacetic acid; Tris, Tris (hydroxymethyl) aminomethane; UV-vis, ultraviolet-visible; VASH1 and VASH2, vasohibins 1 and 2

REFERENCES

- (1) Valenta, R. Early prevention instead of mending late damage in allergy? *EBioMedicine* **2019**, *45*, 17–18.
- (2) Matricardi, P. M.; Kleine-Tebbe, J.; Hoffmann, H. J.; Valenta, R.; Hilger, C.; Hofmaier, S.; Aalberse, R. C.; Agache, I.; Asero, R.; Ballmer-Weber, B.; Barber, D.; Beyer, K.; Biedermann, T.; Bilò, M. B.; Blank, S.; Bohle, B.; Bosshard, P. P.; Breiteneder, H.; Brough, H. A.; Caraballo, L.; Caubet, J. C.; Cramer, R.; Davies, J. M.; Douladiris, N.; Ebisawa, M.; Elgenmann, P. A.; Fernandez-Rivas, M.; Ferreira, F.; Gadermaier, G.; Glatz, M.; Hamilton, R. G.; Hawranek, T.; Hellings, P.; Hoffmann-Sommergruber, K.; Jakob, T.; Jappe, U.; Jutel, M.; Kamath, S. D.; Knol, E. F.; Korosec, P.; Kuehn, A.; Lack, G.; Lopata, A. L.; Mäkelä, M.; Morisset, M.; Niederberger, V.; Nowak-Węgrzyn, A. H.; Papadopoulos, N. G.; Pastorello, E. A.; Pauli, G.; Platts-Mills, T.; Posa, D.; Poulsen, L. K.; Raulf, M.; Sastre, J.; Scala, E.; Schmid, J. M.; Schmid-Grendelmeier, P.; van Hage, M.; van Ree, R.; Vieths, S.; Weber, R.; Wickman, M.; Muraro, A.; Ollert, M. EAACI Molecular Allergy User's Guide. *Pediatr. Allergy Immunol.* **2016**, *27*, 1–250.

- (3) Ruethers, T.; Taki, A. C.; Johnston, E. B.; Nugraha, R.; Le, T. T. K.; Kalic, T.; McLean, T. R.; Kamath, S. D.; Lopata, A. L. Seafood allergy: A comprehensive review of fish and shellfish allergens. *Mol. Immunol.* **2018**, *100*, 28–57.
- (4) Yu, W.; Freeland, D. M. H.; Nadeau, K. C. Food allergy: immune mechanisms, diagnosis and immunotherapy. *Nat. Rev. Immunol.* **2016**, *16*, 751–765.
- (5) Papamichael, K.; Kreuzer, M.; Guilbault, G. Viability of allergy (IgE) detection using an alternative aptamer receptor and electrochemical means. *Sens. Actuators, B* **2007**, *121*, 178–186.
- (6) Frossard, M.; Gasilova, L.; Dayer, E.; Girault, H. H. Personalized and rapid test for food-related allergy. *J. Allergy Clin. Immunol.* **2018**, *141*, 2297–2300.
- (7) Speroni, F.; Elviri, L.; Careri, M.; Mangia, A. Magnetic particles functionalized with PAMAM-dendrimers and antibodies: a new system for an ELISA method able to detect Ara h3/4 peanut allergen in foods. *Anal. Bioanal. Chem.* **2010**, *397*, 3035–3042.
- (8) Wang, Y.; Zhou, J.; Li, H.; Fu, L.; Fu, L. Graphene oxide and gold nanoparticles-based dual amplification method for immunomagnetic beads-derived ELISA of parvalbumin. *Food Contr.* **2020**, *110*, 106989.
- (9) Klug, C.; Hemmer, W.; Román-Carrasco, P.; Focke-Tejkl, M.; Quirce, S.; Boyano-Martínez, T.; Gaubitz, E.; Wank, H.; Swoboda, I. Gal d 7-a major allergen in primary chicken meat allergy. *J. Allergy Clin. Immunol.* **2020**, *146*, 169–179.e5.
- (10) Fu, L.; Zhou, J.; Wang, C.; Zhang, Y.; Ma, A.; Wang, Y. Determination of a major allergen in fish samples by simple and effective label-free capillary electrophoretic analysis after background suppression in ion-exchange chromatography. *Food Chem.* **2018**, *261*, 124–130.
- (11) Zhang, X.; Li, Y.; Tao, Y.; Wang, Y.; Xu, C.; Lu, Y. A novel method based on infrared spectroscopic inception-resnet networks for the detection of the major fish allergen parvalbumin. *Food Chem.* **2021**, *337*, 127986.
- (12) Herrero, B.; Vieites, J. M.; Espiñeira, M. Fast real-time PCR for the detection of crustacean allergen in foods. *J. Agric. Food Chem.* **2012**, *60*, 1893–1897.
- (13) Fernandes, T. J. R.; Costa, J.; Oliveira, M. B. P. P.; Mafra, I. A new real-time PCR quantitative approach for the detection of shrimp crustaceans as potential allergens. *J. Food Compos. Anal.* **2018**, *72*, 7–14.
- (14) Suh, S.-M.; Kim, M.-J.; Kim, H.-I.; Kim, H.-J.; Kim, H.-Y. A multiplex PCR assay combined with capillary electrophoresis for the simultaneous detection of tropomyosin allergens from oyster, mussel, abalone, and clam mollusk species. *Food Chem.* **2020**, *317*, 126451.
- (15) Lee, C.-H.; Wu, C.-C.; Tyan, Y.-C.; Yu, W.-T.; Huang, E. S.; Yu, H.-S. Identification of pyruvate kinase as a novel allergen in whiteleg shrimp (*Litopenaeus vannamei*) by specific-IgE present in patients with shrimp allergy. *Food Chem.* **2018**, *258*, 359–365.
- (16) Xie, L.; Jiang, R.; Zhu, F.; Liu, H.; Ouyang, G. Application of functionalized magnetic nanoparticles in sample preparation. *Anal. Bioanal. Chem.* **2014**, *406*, 377–399.
- (17) Zacco, E.; Pividori, M. I.; Alegret, S.; Galve, R.; Marco, M.-P. Electrochemical magnetoimmunosensing strategy for the detection of pesticides residues. *Anal. Chem.* **2006**, *78*, 1780–1788.
- (18) Zhong, X.; Qiao, L.; Gasilova, N.; Liu, B.; Girault, H. H. Mass Barcode Signal Amplification for Multiplex Allergy Diagnosis by MALDI-MS. *Anal. Chem.* **2016**, *88*, 6184–6189.
- (19) Ashraf, S.; Qadri, S.; al-Ramadi, B.; Haik, Y. Nanoparticles rapidly assess specific IgE in plasma. *Nanotechnology* **2012**, *23*, 305101.
- (20) Zhang, Y.; Wu, Q.; Sun, M.; Zhang, J.; Mo, S.; Wang, J.; Wei, X.; Bai, J. Magnetic-assisted aptamer-based fluorescent assay for allergen detection in food matrix. *Sens. Actuators, B* **2018**, *263*, 43–49.
- (21) Yi, J.; Qin, Q.; Wang, Y.; Zhang, R.; Bi, H.; Yu, S.; Liu, B.; Qiao, L. Identification of pathogenic bacteria in human blood using IgG-modified Fe(3)O(4) magnetic beads as a sorbent and MALDI-TOF MS for profiling. *Microchim. Acta* **2018**, *185*, 542.

- (22) Monaci, L.; Pilolli, R.; De Angelis, E.; Godula, M.; Visconti, A. Multi-allergen detection in food by micro high-performance liquid chromatography coupled to a dual cell linear ion trap mass spectrometry. *J. Chromatogr. A* **2014**, *1358*, 136–144.
- (23) Carrera, M.; Cañas, B.; Gallardo, J. M. Rapid direct detection of the major fish allergen, parvalbumin, by selected MS/MS ion monitoring mass spectrometry. *J. Proteomics* **2012**, *75*, 3211–3220.
- (24) Ayuso, R.; Grishina, G.; Bardina, L.; Carrillo, T.; Blanco, C.; Ibáñez, M. D.; Sampson, H. A.; Beyer, K. Myosin light chain is a novel shrimp allergen, Lit v 3. *J. Allergy Clin. Immunol.* **2008**, *122*, 795–802.
- (25) Pedreschi, R.; Nørgaard, J.; Maquet, A. Current challenges in detecting food allergens by shotgun and targeted proteomic approaches: a case study on traces of peanut allergens in baked cookies. *Nutrients* **2012**, *4*, 132–150.
- (26) Ayuso, R.; Grishina, G.; Ibáñez, M. D.; Blanco, C.; Carrillo, T.; Bencharitiwong, R.; Sánchez, S.; Nowak-Węgrzyn, A.; Sampson, H. A. Sarcoplasmic calcium-binding protein is an EF-hand-type protein identified as a new shrimp allergen. *J. Allergy Clin. Immunol.* **2009**, *124*, 114–120.
- (27) Bi, H.; Meng, S.; Li, Y.; Guo, K.; Chen, Y.; Kong, J.; Yang, P.; Zhong, W.; Liu, B. Deposition of PEG onto PMMA microchannel surface to minimize nonspecific adsorption. *Lab-on-a-Chip* **2006**, *6*, 769–775.
- (28) Powers, S. M.; Hampton, S. E. Open science, reproducibility, and transparency in ecology. *Ecol. Appl.* **2019**, *29*, No. e01822.
- (29) Careri, M.; Elviri, L.; Lagos, J. B.; Mangia, A.; Speroni, F.; Terenghi, M. Selective and rapid immunomagnetic bead-based sample treatment for the liquid chromatography-electrospray ion-trap mass spectrometry detection of Ara h3/4 peanut protein in foods. *J. Chromatogr. A* **2008**, *1206*, 89–94.
- (30) Fernandes, T. J. R.; Costa, J.; Oliveira, M. B. P. P.; Mafra, I. An overview on fish and shellfish allergens and current methods of detection. *Food Agric. Immunol.* **2015**, *26*, 848–869.
- (31) Kazatsky, A. M.; Wood, R. A. Classification of Food Allergens and Cross-Reactivity. *Curr. Allergy Asthma Rep.* **2016**, *16*, 22.
- (32) Leung, P. S.; Chu, K. H. Molecular and immunological characterization of shellfish allergens. *Front. Biosci.* **1998**, *3*, d306–312.
- (33) Witteman, A. M.; Akkerdaas, J. H.; van Leeuwen, J.; van der Zee, J. S.; Aalberse, R. C. Identification of a cross-reactive allergen (presumably tropomyosin) in shrimp, mite and insects. *Int. Arch. Allergy Immunol.* **1994**, *105*, 56–61.
- (34) Leung, P. S.; Chen, Y. C.; Mykles, D. L.; Chow, W. K.; Li, C. P.; Chu, K. H. Molecular identification of the lobster muscle protein tropomyosin as a seafood allergen. *Mol. Mar. Biol. Biotechnol.* **1998**, *7*, 12–20.