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Determination of Peanut Allergen Protein in Food by Double Antibody Sandwich ELISA

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Abstract: Allergic diseases, such as allergic asthma and food allergies, are common and frequently-occurring diseases in the clinic. They are listed by the World Health Organization as one of the three major diseases to be prevented and controlled in the 21st century 10% ~ 30%. Among them, peanut as a major food allergen can cause severe food allergic reactions including anaphylactic shock and allergic death. The purpose of this article is to determine the peanut allergen protein content in food to solve the problem that different allergic patients may respond to different peanut allergen protein components. The double antibody sandwich ELISA method is used to determine the peanut allergen protein component in food. Detection of peanut allergens in imported and exported foods and prevention of food allergic diseases caused by peanuts provide a technical basis. By extracting total peanut protein, immunizing mice to prepare a polyclonal antibody against peanut total protein, coating the enzyme-labeled plate with the antibody, and labeling the polyclonal antibody with biotin, thereby establishing a double-poly antibody sandwich ELISA method, and then making peanut total protein The standard product was tested for the sensitivity of the method, and at the same time, 15 kinds of foods were tested for the content of peanut protein. The research results show that the developed dual-antibody sandwich ELISA method has specificity for detecting peanut allergen protein components in food, and the minimum detection limit is 8ng/mL, and the standard curve is linear in the range of 8ng/mL~125ng/mL; The test results of 13 kinds of foods are consistent with the contents of the food allergen label, and the test results of 2 kinds of unknown foods are all positive.

1. Introduction

Food allergies have seriously threatened our human health. One type of peanut food allergy

antibody response is mainly a type i food hypersensitivity reaction mediated by bacteria. In severe cases, it can even directly cause patients with anaphylactic shock or severe death. Peanut food allergic reaction patients in have no allergies to their lifetime. Peanut food allergy has developed into an important global health allergy problem, which has caused widespread concern in the society [1]. Peanut vegetable is a common peanut food that is rich in nutrition and widely loved by modern people, but it is also the most important anti-cancer food and anti-allergen [2]. Peanut fat is the main source of plant protein and animal fat, but because of their resistance to triggering serious and possibly endangering adverse reactions in the human body, they are also widely regarded as harmful substances for food allergies to others [3]. The heat treatment process may directly cause the digestion and modification of various proteins in human food, and directly affect the overall digestibility of various proteins throughout the human intestinal tract and mucosa or the digestion and absorption of nutrients and minerals [4]. In addition, the type of processed food can directly change the immune sensitization of human food collagen, which directly affects the interaction with the human biological immune system at the level of human intestinal immunity. Consumption of foods with high allergens such as peanut oil with very little allergen content is likely to cause severe allergic reactions to the body of patients with highly sensitive rhinitis, such as causing allergic diseases such as gastrointestinal upset and allergic skin dermatitis. People develop severe allergic shock and cause allergic rhinitis deaths [5]. At present, based on the physicochemical properties of allergen proteins in peanuts, through the use of ammonium sulfate ion fractionation and precipitation, column chromatography, and allergen protein ion electrophoresis, experimental methods have been successfully researched to establish some particularly suitable for scientific laboratory small-scale preparation. The scientific separation experimental method provides a high-quality scientific experimental preparation material for scientific research related to the separation of arachidic and allergen proteins.

At present, domestic and foreign scholars have few effective drug treatment methods for the application of epanutin in allergic skin diseases. Peanut is widely used in China's food industry as a special nutritious food, and it is also an important ingredient in the production and processing of various types of food [6]. However, peanuts and other agricultural products are not only one of the allergens in the eight major drinking foods identified by the World Food Organization / WHO report, they may also cause severe food allergic chemical reactions, which are usually related to human life and health, It may even threaten human life [7]. Different forms of human thermal radiation in different climatic regions have led to different rates of human allergy to the world [8]. As a form of anti-heat treatment drug, heat-treated allergenic peanut medicine has gradually attracted many Chinese people to conduct in-depth research on potential peanut allergenic drugs [9]. For this reason, female patients who are allergic to large amounts of peanuts or allergen foods should also avoid eating a lot of meat foods containing large amounts of peanuts. This is one of the most effective methods for preventing allergies [10]. Therefore, it is necessary to develop a safe, practical, sensitive, and specific reagent for detecting new-type peanut bacteria allergen antibodies. Double-method antibody sandwich detection Elisa dual-method detection is one of the most commonly used animal immune antibody analysis and detection technologies, and has been widely used in animal clinical medical testing, food safety, drug hazard detection and other fields [11]. The domestic protein detection of food peanut allergens is still in the initial preparation stage. The research team of this project is the first to develop and develop a peanut food allergen protein with high practicality, sensitivity and specificity in China. Detection component-elisa as a detection experiment reagent [12].

HONG established a reliable ultra-performance liquid chromatography-mass spectrometry for the determination of peanut allergen Arah2 in barbecued foods. The current validated method was successfully used to determine the content of peanut allergen Ara h2 in 20 peanuts in different

regions. The results showed the conversion coefficients of peanut allergen Arah2 and peanut protein [13]. He developed a method that specifically binds to IGE epitopes is necessary to test BLG and its allergen residues. Results: Monoclonal antibodies specific for the IGE linear epitope of BLG were identified as having high affinity and specificity [14]. Kiyota developed a monoclonal antibody-based enzyme-linked immunosorbent assay (ELISA) method for quantifying major allergens in fresh and processed oranges. The developed ELISA method has high reproducibility, sensitivity and accuracy [15]. Walker confirmed the cross-reactivity between ELISA and Prunus species. Although it is useful for screening genera, orthogonal techniques are needed to identify the species and confirm its existence. Developed two novel PCR detection methods, identified peptides specific to almond and Mahaleb, can be analyzed by LC tandem mass spectrometry, and formulated standards for peptide identification to forensic standards [16]. Mattison compared the gut protein profile of corn fed on a medium containing pinto beans, cashews, or peanuts. Ongoing research in this area may find insect enzymes that can be used to reduce or eliminate enzymatic processing steps of nut allergens and may be applied in the food industry or health field [17]. Filep describes the determination of Ara h 1, Ara h 2 and Ara h 6 in peanut food and peanut flour extract for allergy diagnosis and oral immunotherapy. The results show that there are significant differences in specific peanut allergen characteristics among peanut butter, flour, and peanut products used clinically [18]. Meng tested the allergenicity and functional properties of peanut protein hydrolysate cross-linked by TGase. Peanut hydrolyzed by three selected enzymes (200AzU/g) was used for IGE binding, TGase cross-linking and functional characterization. TGase cross-linked hydrolysate has similar IGE binding characteristics to uncross-linked hydrolysate, but has higher functional properties [19]. Rosa chose a cookie base as a complex food matrix, and prepared homemade cookies made of peanut flour on a laboratory scale, optimized the protein extraction and purification scheme, and finally designed a sensitive SRM-based streamlined based SRM analysis method is used to detect allergens in cookies that have been produced [20].

The content of the main relevant research results in this article can be roughly divided into five basic parts: Chapter I This section is mainly an introduction to related research results, a detailed explanation of the academic purpose and scientific significance of related research, and the current status of related research development. Systematic analysis and scientific introduction of the main components of allergen proteins in foods and peanuts in methods such as antibody sandwich and Elisa; and a systematic review of the current status of peanut allergen protein research and development; second the eleventh part is mainly the basic theory and experimental methods. It mainly explains the application of the method of sandwiching the boromycin antibody with Elisa and the theoretical explanation of the allergen protein purification in the main peanut. The third part is mainly the biological experiment part. The experimental methods and research progress of biological separation and purification of peanut allergen protein and non-main peanut allergen protein were introduced. The purification, isolation, and purification methods of arachidic for allergen proteins mainly include ammonium sulfite chain precipitation method, column-layer analysis method, and electronic swimming chromatography method. The purpose of the fourth part is to conduct in-depth research on related experiments and comprehensive analysis of the results, and to extract double anti-peanut total binding protein. Immune antibody mice can prepare a polyclonal immune antibody that contains peanut total binding protein. The target board of peanut enzyme can simultaneously detect whether there are ingredients containing homemade peanut protein total protease in 15 kinds of peanut foods. The fifth part is the research conclusion, which is the theoretical summary and suggestions for this article, and the overall summary of the research results of the entire article.

2. Proposed Method

2.1. Ingredients and Characteristics of Peanut Allergens

Peanuts containing allergen proteins are generally considered to have high light and heat stability, cocaine-induced smoke and peanut powder and other allergens can allergize patients. Arah1 is approximately equal to the total amount of similar peanut pea protein polymerization, which is a glycoprotein Isoelectric point of molecular weight, high thermodynamic stability, resistance to enzymatic degradation by phosphatides, difficult to digest, and similarity to the similarity of the polymerization sequence of similar pea peanut protein is about half of 40%. Larger amounts of protein sequence form (or larger) aggregates exist. At present, it is known that genes have two structural forms of homologs, and two transcript gene fragments that have a significant induction effect on the recombination process of a gene molecule have been identified. The molecular structure of the gene is indeed clarified, but researchers found that Also included in the middle are epitopes of individual non-linear gene conjugates.

Arah2 is ovalbumin, the isoelectric point formula is that 12 disulfide bonds combine two molecules to form a stable molecular structure. It can be directly recognized by more than two kinds of peanuts and neurologic serum of non-allergenic patients. It is one of the most allergenic drug components in all peanuts. Contains two isomers: amino acids with a difference of 12 bonds between them, the molecular weight values are 16.7 and 18, respectively. The results of such studies indicate that a linear epitope signal of about 10 months per thought can be accurately recognized by saliva serum of negative peanut bacteria and allergic rhinitis patients.

Other studies on arachidic in allergen components are relatively rare. In the naturally purified state, this is a natural protein and polymer with a molecular weight sequence above the above, which can be degraded into a series of molecular weight and behavioral subunits, the molecular sequence of which is the same as the molecular weight sequence of soybean sub globulin In order to purify, such as spoonful beans and peanuts, serum purification and identification of allergic loyalty, the natural collagen has been purified and isolated at the end. The molecular weight sequence of the recombinant natural protein is as follows. It is homologous and belongs to amino albumin. The molecular weight sequence is that its content with some homologous subunits is extremely low, and it cannot succumb to other albumins, and has the same homology with the amino acid binding sequence of other proteins. It has homology with other proteins, poor thermodynamic stability, and is not resistant to degradation by enzymes. It is an oleo-lactoprotein. Their protein isolation and purified natural chemical products can be accurately recognized by edible peanut oil and saliva serum of patients with allergic rhinitis.

Most of the allergens in food are a water-soluble glycoprotein. The antigenic determinants in animal cells and human cells contained in it cannot make people allergic to food. It contains antigen recognition regions for animal cells and human cells, and it will not produce specific allergic antibodies after human direct intake. Human allergens can be divided into major chronic allergens and minor acute allergens according to the ability of human allergens to automatically recognize and respond to specific allergenic antibodies and the sensitivity of their antibodies. If an allergen may simultaneously bind at least one antibody from the autologous serum of an allergic patient, it indicates that the allergen is clearly considered to be a major acute allergen, otherwise it is considered a minor acute allergen. For single allergic foods, its allergenicity depends on the following major aspects: the main types of allergens, the ability of allergens and specific allergic antibodies to interact with each other, and the quality of allergen proteins in the total of a single food A certain percentage of protein. To sum up, natural meat food allergens have some common characteristics:

(1) Only some ingredients in food have sensitization.

(2) There may be multiple cross-cutting allergic reactions between different food types and allergens.

(3) During the processing of such foods, the allergen properties may gradually increase or decrease significantly.

(4) Finally, any food may have a certain potential to cause allergenic chemical reactions, but only a few foods may cause most of the allergenic chemical reactions.

2.2. Double Antibody Sandwich ELISA Principle

The double antibody sandwich method is to adsorb two antisera containing a known test antibody in the serum into small holes on a microtiter plate and wash them once; add the antisera with other test antigens, such as both are specific, and then they are bound to each other, and then all the excess antibodies are washed away; antiserum is added to two enzyme-linked antibodies that react specifically with other antigens to be tested, so that a "sandwich" is formed on the wall of the well Antiserum is added to the substrate of this enzyme. If the production of two-colored hydrolysis products is seen at the same time, it means that a corresponding enzyme-linked antigen already exists on the wall of the antiserum. Two antisera containing a known antibody in the serum were adsorbed in the wells of a microtiter plate and washed once; the addition of antisera and other antigens to be tested occurred separately if both were specific Bind, and then wash away all excess antibodies; add anti-serum with two enzyme-linked antibodies that react specifically with other test antigens to form a "sandwich" on the wall of the well; add anti-serum to the substrate of the enzyme, If you see the production of two colored anxiolysis products at the same time, it means that a corresponding enzyme-linked antigen already exists on the pore wall of the antiserum.

Enzyme-linked immunosorbent assay is a method for immunodetection using specific binding between antibody antigens. Usually, the antigen (or enzyme-linked antibody) is directly bound to the phase carrier, and the immune activity of the phase carrier is maintained, and the antibody (or enzyme-linked antigen) is linked to a certain type of enzyme-linked carrier to form an enzyme-linked antibody. (Or enzyme-linked antigen) so that it has both solid-phase antibody or enzyme-linked antigen activity and solid-phase carrier enzyme activity at the same time. The solid-phase antibody or target antigen) and the antigen (or target antibody) bound to the enzyme-based carrier react with the antigen (or target antibody) on the surface of the solid-phase carrier specimen to undergo a color-developing chemical reaction according to different coloring chemical steps. The Elisa assay can be widely used for the direct determination of antigens, and also for the indirect determination of enzyme-labeled antibodies. In this type of measurement method, there are three kinds of necessary reagents: direct detection of antigen or antibody in solid phase, enzyme-labeled antigen or antibody, and substrate of enzyme. The determination method is based on the source of the enzyme-labeled antibody test, the characteristics of the test specimen, and whether the detection method is suitable. It can be used to design and formulate various types of enzyme-labeled antibody detection methods. The more commonly used main assay methods are double antibody sandwich Elisa (including double monoclonal antibody multi-antibody mixed sandwich Elisa and double monoclonal antibody sandwich), indirect antibody competition sandwich Elisa, and double monoclonal antibody sandwich Elisa.

The indirect detection method is mainly widely used for the indirect detection of the test antibody. Its working principle is to specifically and selectively immobilize the test antigen on a solid phase antigen carrier by using an enzyme, and use the phase antibody to be tested to form the test antigen. Complex with enzyme antibody, and then use this antibody to form a complex with enzyme-labeled solid-phase anti-antibody and enzyme-labeled antigen antibody, so that the phase

antibody to be detected can be indirectly bound to the enzyme antibody. The competition method can not only be used to directly detect human antibodies, but it can also be used directly or to help detect human antigens. The method of single-sandwich bio competitive molecular detection is more complicated than the double-layer single-sandwich sandwich, but it is more accurate and effective in the competition detection of various small animal chemical molecules or other biochemical molecules.

3. Experiments

3.1. Experimental Materials and Steps

The experimental materials and animal and equipment specifications are shown in Table 1. The main experimental materials and animals: 4-week-old mice blab / c mice: the products were purchased from the Animal Center of Sun Yassin University Medical College. Fresh peanut raw materials: Products are purchased from Nanshan Market, Shenzhen, China; other products include fresh peanuts and other foods that do not contain fresh peanuts (among which, 9 fresh products are purchased from local supermarkets in Japan and the United States, and 6 other products are all from China Shenzhen Entry-Exit Inspection and Quarantine Bureau). Other chemical reagents and detection instruments: microprotein, nitrocellulose membrane: company; Freund's adjuvant: bib company; Tween 20, anti-igg-horseradish peroxidase, chain peroxidase affinity -Harp, tetramethylbenzidine (tomb): sigma company; nms-biotin: pierce company; vertical plate electrophoresis tank: Bio-Rad; vertical plate washer, microplate reader: thermo company; other chemicals used Reagents and instruments are domestic analytical reagents.

Table 1. Experimental materials and equipment

Test animal	Peanuts	Reagent	Instrument
4 weeks old mouse	15 types	HiTrap protein G	Vertical plate type electrophoresis tank

Grind the flower with liquid nitrogen starch to produce a powder, soak it in ethanol or acetone at a ratio of 1:15 to remove fat for 48h, put it in a refrigerator at 4 ℃, change the liquid once every 12h, and stir twice. Remove polycotton from the sample, weigh the sample after drying, and add 30ml of the supernatant extract (8mmol / trash, 25mmol / tricine, ph8.6) to each 5g sample at 4 ℃ overnight, centrifuge and collect at 11000r / min for 30min. clear. The supernatant crude extract was taken for discontinuous sodium lauryl sulfate-polyacrylamide gel electrophoresis (SDDS-page) to analyze the components of various proteins in the crude extract and determine the molecular weight of the protein.

A conventional indirect method, the Elisa method, was used to detect the affinity of the antibodies. The total peanut protein extracted was coated at 100 ng / well at 4 ℃ overnight. The blocking solution concentration was 3%, and the blocking was continued at 37 ℃ for 2 hours. The anti-mouse serum is the primary antibody, and the pre-immune mouse serum is the secondary antibody for a parallel negative judgment standard control test. The dilution is started from 1: 100, and the reaction is continued for 1 hour at 37 ℃. After the past is washed 3 times, the antibody is added. The mouse substrate elegy harpist was used as a secondary antibody, and the reaction was continued for 1 hour at 37 ℃. After washing 3 times, the antibody substrate postmaster was added to develop the color. After termination, it was read by a microplate reader bsa450. Control test to determine the antibody od value and dilution negative judgment standard Control test to determine the ratio of od value to dilution greater than 2.1 is a positive antibody affinity judgment standard, the purified dilution of the antibody is the determination of antibody titer. G-microprotein affinity

chromatography was performed in accordance with the product instruction manual to determine the purification affinity of the antibody.

Replace the purified antibody's original buffer with a phosphate-containing original buffer solution (pbs), then replace the purified antibody with n-hydroxy succinimide (nms) -activated biotin (nosh-biotin) Mix according to the ratio recommended in the product manual, and react for 30 minutes in the dark, and use a 50ku ultrafiltration membrane column to remove antibodies that may not participate in labeled nosh-biotin. Please refer to the product manual for specific steps and methods of the product.

3.2. Development of a Double Antibody Sandwich ELISA Method

Add 100ng of purified anti-peanut protein polyclonal antibody to each well and coat it at 4 ℃ overnight by adsorption antibody method. On the second day, the coating solution was discarded, and the coating solution was washed again with washing solution. 200 μl of blocking solution was added to each well, and the suspension was purified and incubated at 37 ℃ for 3 hours. The coating blocking solution was discarded. Then the total protein extract of peanut was added and diluted one by one, and 100 μl was added to each well. The control well of negative antibody was replaced with pbs. The reaction was terminated at 37 ℃ for 1 h. After the reaction was completed, the reaction was washed 3 times with purified past. Add 1tmb and h2o2 substrate solution to each well, and stop the reaction at 37 ℃ for 10min. After h2so4 stopped the reaction, read it on a microplate reader. At the same time, a checkerboard method purification test was used to further optimize the optimal purification dilution multiples for the adsorption-coated antibody and the capture-coated antibody.

The advanced Coomassie blue staining method was used to determine the total protein concentration of allergens in peanut food and diluted with antibody sandwich pbs (ph7.4) to different concentrations as the antigen. According to the dual antibody sandwich Elisa statutory established by Comas the test results determine the minimum detection limit of the peanut allergen protein concentration component. A variety of peanut food labels are labeled with contained peanuts, non-containing peanuts, and 15 ominous peanut foods. The allergen protein component was extracted using 20mmol/tries plus 150mmol/lance buffer solution (ph8.0), and then According to the above-mentioned double-antibody sandwich component Elisa statutory determination of peanut allergen protein concentration components in peanut food, and the results were analyzed and compared. Among them, the positive od value of the blank control result and the negative blank control result are usually greater than 2.1 times, and the negative od value is less than 2.1 times.

Isolation and purification of peanut allergen protein is generally divided into two stages: crude protein extraction and column chromatography. Crude protein extraction is mainly performed by protein extraction of defatted peanut powder through buffer solution, and sometimes ammonium sulfate fractionation and dialysis operations. Column chromatography means that the crude protein extract obtained through pretreatment is passed through column chromatography to achieve the purpose of separating and purifying the target allergen protein. The core technology for the separation and purification of peanut allergen protein is reflected in the selection and combination of various column chromatography media and the optimization of chromatography conditions. Based on the properties of peanut allergen protein, this paper summarizes the selection, combination and optimized chromatography conditions of chromatographic media in peanut allergen protein purification.

4. Discussion

4.1. Analysis of Peanut Total Protein by SDS-PAGE

The peanuts were degreased after baking at 163~177℃ for 13~16min, and then extracted with PBS buffer solution (20mmol/L, pH7.0) containing 1mol/L NaCl and 8mol/L urea to obtain the crude peanut extract. The crude extract was dialyzed against anions and cations in a tris-HCl buffer (20mmol/L, pH7.2) containing 8mol/L urea and eluted through two monoq10/10 anion exchange column chromatography. The gradient elution separation was performed twice at 0-1.5mol/lnacl, and the elution peaks were collected twice before and after the dsds-page electrophoresis analysis and the yin and cation dialysis immunocytometry analysis were performed on the ions. In the end, we can accurately obtain the required ion purity of rarah1 for each elution.

The natural peanut soluble allergen and the recombinant peanut soluble allergen in the lane were performed, and then the electrophoretic bands of the two were cut and electrospray ionization mass spectrometry was performed (comparison analysis was performed, and the results of the comparison mass spectra are shown in Figure 1, The results showed that the two compounds have similar mass spectrum spectra, indicating that the two compounds have high homology. After the recombinant bacteria expressing the bacteria were induced using the optimal soluble expression target conditions, the unbroken and resuspended bacterial cells were separated and centrifuged. The resulting supernatant and resuspended bacterial solution were performed, and the results are shown in the figure. After analyzing the grayscale scan of the software for the purpose of recombination, it can be concluded that each protein of the recombination target is about the total soluble band. Expression level (lane is about total soluble expression level. The bacterial concentration of the soluble band of each protein in the lane is based on its instruction manual. The grayscale scan results show that the grayscale of each protein in the soluble band recombination target in the lane is reorganized. It is about twice the soluble band of the recombinant target protein, but the amount of electrophoretic loading is also about twice that of the recombinant target. Based on this, the recombinant soluble band in the lane can be deduced. The amount of sample to be loaded with the expression is approximately double, that is, each bacterial solution that can express the recombination target can express about one protein per recombination target at a time.

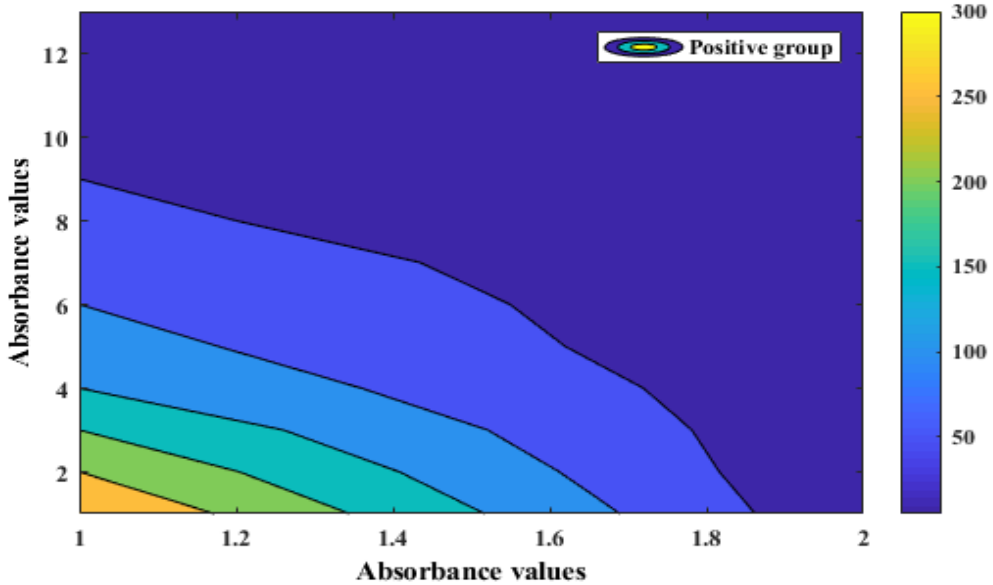

Figure 1. Electrospray ionization mass spectrum

The peanut total allergen protein extract was subjected to sdsds-page electrophoresis, as shown in Figure 2, and the results showed that the molecular weight of the main component of the total protein of the peanut extract was 12ku~70ku, of which 63ku and 17ku There are obvious bands between these, and those reported in other related literatures that may contain the main component arah1 (~63ku) and the minor component arah2 (~17ku) of the allergen protein of peanut extract. The size is consistent, so we can accurately infer whether the peanut allergen protein extract of this extract may contain the main components of peanut allergen protein.

The indirect immunobiological analysis was used to further identify the anti-peanut total allergen protein component. The results showed that the protein component antibody could accurately confirm allergen bands such as 63, 40, 30, 17ku, etc. in a three-step method. Arah1 was proposed by scientists, that is, by using anion exchange chromatography, cona affinity chromatography and gel ion filtration chromatography in sequence, higher purity arah1 was finally obtained. Since then, this method has been optimized into two steps. They believe that under natural circumstances, Ara h1 and Ara h 3/4 build a complex with a molecular mass of about 200kD, of which Ara h 3/4 is the main pollutant. It is found through experiments that Ara h 3/4 can hardly be extracted at pH 5.0, and Ara h 1 is extractable at this pH value. Therefore, defatted peanuts are extracted with pH 5.0 extraction buffers. The powder was extracted, and the ion exchange chromatography step in the three-step method was successfully omitted, and Ara h1 with high purity was also obtained.

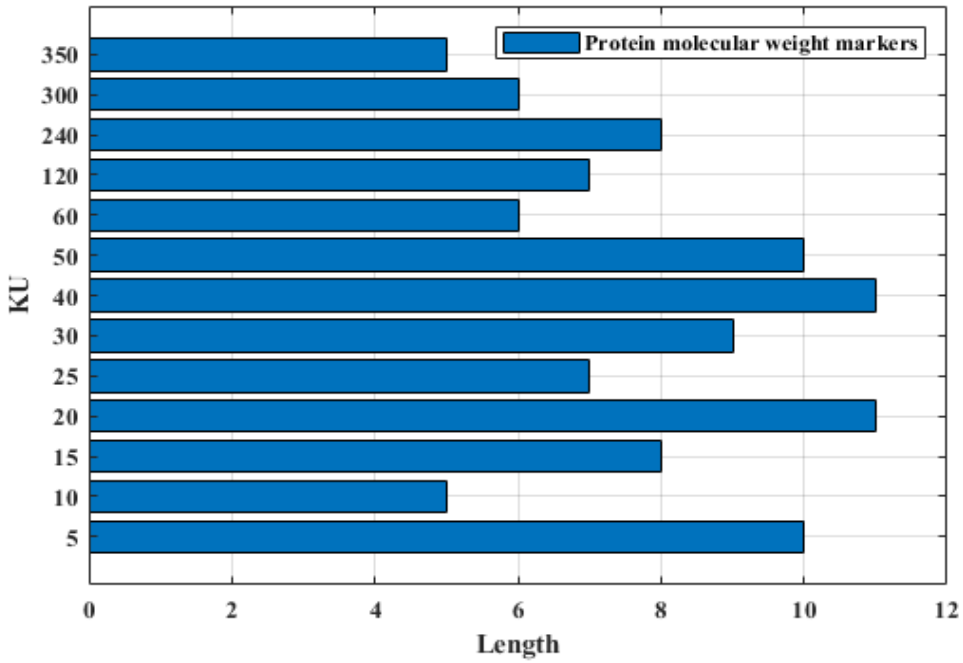

Figure 2. Peanut total protein component extract SDS-PAGE

4.2. Determination of Peanut Allergen Protein by Double Antibody Sandwich ELISA

Extraction of various foods such as peanut collagen the solution of such foods contains coarse and fine pore extracts of the main components of various allergen proteins. This method is used to perform allergy detection on the allergic positive capillary pore extracts every 3 times. The main criterion for judging positive pores is to determine the ratio of positive molecular pores in the food refrigerator to the negative molecular pores in the food packaging to the molecular od interval or greater than 2.1. The test results of the quality inspection of this method are shown in Table 1 below. Among 15 kinds of foods containing peanut plant protein ingredients, 9 kinds of enzyme foods

tested for 3 kinds of foods containing obviously allergic peanut plant protease ingredients did not show obvious allergic positive drug test results, 4 the test results of a food containing peanut plant protein ingredients showed obvious allergic negative test results. Two of the foods those were obviously positive had basically unknown drug expiration dates, and the tested food results did not show obvious allergy positives.

Table 2. Double-antibody sandwich ELISA for detection of flower allergens in food

Food name	Place of production	Peanut allergens tag in food package	Detection Result
Hershey's Peanut Butter	United States	Yes	Positive
Sweet & Salty Crunch	United States	Yes	Negative
Trail Mix Tropical Blend	United States	Yes	Positive
Sandwich biscuits	China	Yes	Positive
Peanut chocolate powder	China	No	Negative

Biotin-labeled antibodies are coated as biotin-captured antibodies. The optimal dilution multiples of the coated antibody and the biotin capture antibody were determined by checkerboard titration. The results showed that the optimal dilution ratios of the biotin and capture antibody for the coated antibody and the labeled antibody were 1: 500 and 1,500 respectively. 1:1000. The standard peanut total protein was serially diluted as the extract solution, and the detection limit of this analysis method was determined. The results are shown in Figure 3. As the concentration of the positive well antigen decreases further, the absorbance of peanut antigen detected by the elisa method also decreases with the concentration the ratio of the od value of the positive well to the negative well detected by the analytical method is greater than 2.1 as the criterion for judging the positive well. / ml, the standard detection curve is linear in the range of 8ng/ml~125ng/ml.

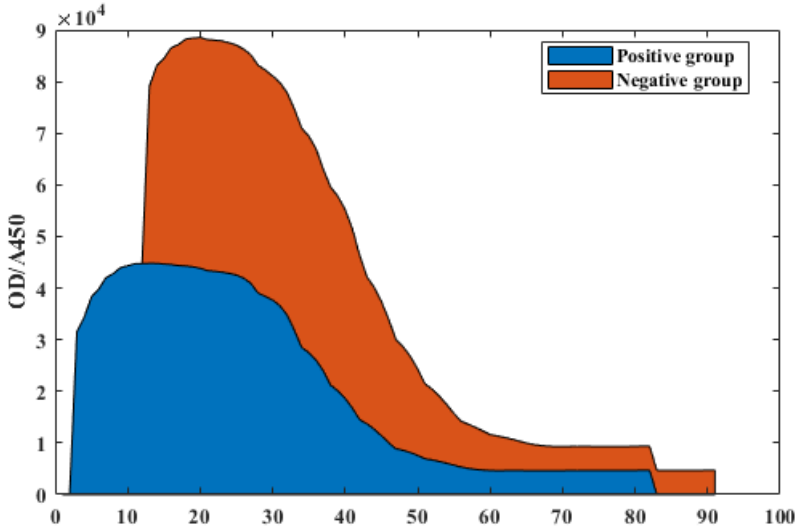

Figure 3. Determination of anti-peanut protein component antibody titer

As shown in Figure 4, the mother liquor from which the recombinant protein was diluted was double-diluted accordingly, and a total concentration gradient was set. The diluted sample was used as an antigen, and a monoclonal antibody and a biotinylated monoclonal antibody were used as coating antibodies and detection antibodies, respectively, and a double-sandwich test was performed to draw a standard curve. Based on the results, the best monoclonal antibody combination for double sandwich was selected. The double-sandwich eluent method is the same. The crude protein extract was subjected to anion exchange chromatography to obtain high-purity

arah2 in one step. The equilibrium solution used for the chromatography was tris-hcl buffer (20mmol/l, ph8.0). Gradient eluent: Tris-hall buffer (20mmol/l, ph8.0) in 60 ml of 0 to 0.1mol /lance buffer and tris-hall buffer in 450 ml of 0.1 to 1mol/l nail buffer, respectively. Liquid (20mmol/l, ph8.0). Finally, the elution peaks obtained by q-stoppage were further analyzed, and arah2 was completely eluted at a higher concentration of about 150mmol / lance, and finally arah2 with higher purity was obtained in the chromatography.

The peanut rubber protein and crude extract obtained by (nh4) 2co3 (0.1mol/l, ph8.0) extraction and separation were subjected to gradient chromatography separation through a gel filtration chromatography column and an anion exchange chromatography column, respectively. The medium of the column was superdex200 (k2hpo4-kh2po4 buffer (50mmol/l, ph7.5), and 0.1mol/lance as the equilibration and loading buffer), and then the collection solution containing the hiarah6 in the gel column was desalted (hit rap Column) and gradient vacuum ultracentrifugation drying, and finally reconstituted with tris-hcl buffer (20mmol/l, ph8.0), and then separated by gradient chromatography on a minim column, and gradient elution (0~0.5mol/lance) and speeds-page electrophoresis analysis.

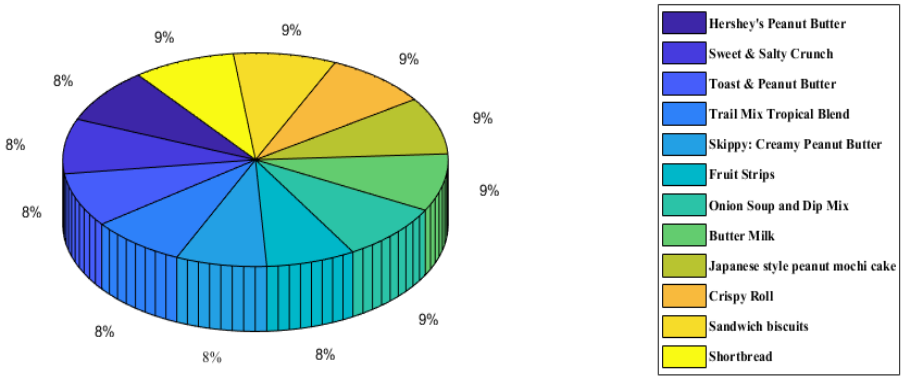

Figure 4. Double antibody sandwich ELISA method to detect the percentage of peanut allergen components

Immunized mice with antigen detection of total peanut allergen protein can produce high titer antibodies, which can be assembled using labeled biotin amplification kit technology to make reagents similar to the Eli sage test for peanut allergen total protein. box. The molecular weights of the ara1 and ara2 components of peanut main antibody and total allergen protein in food are 63ku and 17ku, respectively. Through the analysis of elides-page and the analysis of the antigen immunization mouse imprinting laboratory, the components of total peanut protein and antigen in the food extracted in this study can be judged from the structure and molecular weight of the two major similar Ingredients of peanut main antibody and allergen protein, so that mice can use the method of detecting antibodies to detect whether the antibodies in food reflected in the total peanut protein component in food may contain components similar to peanut main allergen protein.

5. Conclusion

(1) The antibody titer obtained from the results of this study is as high as 1: 204800.The high antibody titer has obvious technical advantages for the development of reagents for detection sensitivity. On the one hand, the antibody titer can ensure a sufficiently high detection sensitivity. The aspect can greatly reduce the consumption and use of antibodies, which is conducive to the optimization and amplification of detection sensitivity and reagent production technology.

(2) This experiment successfully prepared a double antibody sandwich Elisa reagent based on the

research results. The sensitivity of the detected peanut protein to the double antibody component is as high as 8ng / ml, which exceeds the internationally developed double antibodies developed by many companies such as tunnel and endogen Concentration of peanut protein antibodies detected by commercial experimental reagents and detection sensitivity of Elisa reagent.

(3) 15 foods containing peanuts, non-contained peanut allergen ingredients, and foods with unknown unknowns were tested on the food peanut ingredient label, and the results were tested on 13 types of food and the food peanut ingredient label was marked. The content of the test results is consistent, and the results of the two peanut foods whose contents are unknown are significantly positive. This means that this test method has good practical validity and application research value for food test results that do not clearly label content containing peanut allergen ingredients.

(4) Through the research and establishment of this peanut detection label management method, it will greatly help to predict and solve the common peanut allergy phenomena in the food safety and life of consumers in China, and help control and prevent consumption. The emergence and occurrence of severe peanut allergic skin diseases caused by people eating the peanut allergen food by mistake provides a warning technology method, protects public health, and is conducive to regulating and promoting the current international trade of high-quality food in China And improving the management method of peanut allergen detection and labeling of high-quality food in China has laid a certain scientific and technological theoretical basis.

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Data Availability

Data sharing is not applicable to this article as no new data were created or analysed in this study.

Conflict of Interest

The author states that this article has no conflict of interest.

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