AURAPUB

Molecular Nutrition and Health Research

2024 Volume1,Issue1

Application of Modification Treatment to the Detection of Hypoallergenic Peanut Products

Huan Liu[1,a,*], Shuqi Mu[2,b]

[1]School of Food Engineering, Ludong University, Yantai, Shandong, China
[2]Yantai Dacheng Food Co.,Ltd., Yantai, Shandong, China
[a]sd_lh1990@126.com, [b]ytdxs307@163.com,
[*]Corresponding author

Keywords: Modification; Hypoallergenicity; Peanut Products; Allergens; Detection Technology; Desensitization Treatment

Abstract: How to effectively detect and produce low allergenic peanut products has become a key issue that urgently needs to be addressed in the field of food safety. Peanuts, as one of the common food allergens, can cause serious allergic reactions and even endanger life if people who are allergic to peanuts accidentally consume them. Therefore, it is particularly important to develop a technology that can reduce the allergenicity of peanut products. To solve this problem, researchers have adopted advanced modification techniques to modify peanut protein through chemical or physical methods, in order to reduce its allergenicity. The experimental results showed that in specific allergen detection experiments, when the concentrations of unprocessed peanut protein and modified peanut protein were both above 40 μg/mL, the cell viability of modified peanut protein was significantly enhanced, indicating that its allergenicity was relatively low. This discovery confirms the effectiveness of modification technology in reducing peanut allergenicity.

1. Introduction

Peanuts are one of the most widely grown crops in the world. They are not only rich in nutritional value, but also an important raw material for many food processing. However, peanut allergy is a common food allergy reaction that seriously threatens the health of allergic people [1] and may even be life-threatening. As people pay more and more attention to food safety and health, the development and research of low-allergenic peanut products is particularly urgent. Therefore, how to accurately detect and effectively reduce the allergenicity of peanut products has become an important issue that needs to be solved in the current food safety field. Previous studies have mainly focused on the identification of peanut allergens, the exploration of allergenic mechanisms, and the

cultivation of low-allergenic peanut varieties. However, most of these studies focus on a single level and lack systematic solutions. Therefore, finding an efficient, accurate, and low-cost method for detecting low-allergenic peanut products and exploring economically feasible modification and processing technologies became important motivations for this study.

This study aimed to develop a detection method for low-allergenic peanut products based on modified processing technology and explore its feasibility and effectiveness in practical applications. First, this paper selects several common peanut allergen proteins as research objects and modifies them by chemical or physical methods to destroy their allergenic structures or reduce their immunoreactivity. Then, this paper uses biochemical techniques to test the allergenicity of peanut proteins before and after modification and compares the changes in their allergenicity.

2. Related Works

Detecting food allergenicity is an important part of food processing and food health. With the improvement of people's living standards and the enhancement of health awareness, the attention to food safety, especially food allergenicity, is increasing day by day. Luparelli et al. specifically pointed out the allergy risk caused by cross-contamination in food manufacturing and explored the biochemical characteristics and detection methods of these allergens [2]. Hnasko et al. focused on the peanut allergen Ara h1 protein and created a sensitive enzyme-linked immunosorbent assay and lateral flow immunoassay by developing monoclonal antibodies against this protein [3]. Tramuta et al. validated the effectiveness of real-time polymerase chain reaction in detecting peanut traces in five types of food matrices[4]. Schäfer et al. developed an allergen detection method based on synthetic aptamers[5]. Adedeji et al. reviewed the current status and challenges of food allergen detection, pointing out that traditional methods are effective but limited in allergen detection, especially the lack of quantification ability[6].

Although many studies have made progress in the field of food allergen detection, there are still limitations. The above methods are susceptible to interference in complex food matrices, and their stability and reproducibility need to be further verified. Therefore, the method of this paper modifies the peanut protein after preparation and then tests the allergen molecules in the peanut protein to overcome the shortcomings of the existing methods, better ensure food safety, and meet the needs of allergic consumers.

3. Methods

3.1 Structural Characteristics of Peanut Protein

Protein is a complex molecule composed of various amino acids linked in series via peptide bonds. Its structural hierarchy can be divided into one to four levels: the primary structure describes the linear arrangement order of amino acids on the peptide chain, that is, they are connected end to end in a specific order; the secondary structure focuses on the spatial morphology of local fragments within the peptide chain. These local morphologies constitute the basic units of the spatial structure of the complete peptide chain (i.e., the tertiary structure), providing a basic framework for the spatial folding of proteins [7]. The secondary structure characteristically includes two forms: regular α-helix and β-fold; the tertiary structure further extends to the relative positions of all amino acid residues in the entire peptide chain in three-dimensional space, that is, the precise layout of all atoms in the entire peptide chain molecule in three-dimensional space. The main force that maintains the stability of the tertiary structure of proteins comes from secondary bonds, including hydrophobic interactions, ionic bonds, hydrogen bonds and van der Waals forces; as for the quaternary structure, it describes a large complex with a specific spatial arrangement formed by

multiple identical or different subunits that already have three-dimensional structures connected to each other through secondary bonds. The characteristics of peanut protein are shown in Table 1.

Table 1. Characteristics of peanut protein

Types of proteins	Quantity contained(%)	Isoelectric point(pI)	Approximate molecular weight(kDa)
Alajibin protein	10	5.2	30
Peanut associated globulin	30	4.8	55
Peanut agglutinin	5	6.5	40
Peanut globulin	50	5.5	110
Other unknown proteins	5	-	-

3.2 Preparation of Peanut Protein

First, the peanuts were thoroughly washed, then soaked and the red skin removed. Then, the peanuts were pulped at a volume ratio of 1:9 (w/v) and processed by colloid mill to obtain peanut pulp (RPP). For sterilization, the RPP was autoclaved at 121°C for 20 minutes to obtain autoclaved peanut paste (APP).

After APP was cooled to room temperature, it was inoculated with Bacillus natto under strict sterile conditions at an inoculation amount of 9.8×10^5 cfu/mL. Subsequently, fermentation was carried out at 37°C and 150 rpm, and samples were taken after 12 hours, 24 hours, 36 hours, 48 hours, and 60 hours of fermentation.

All samples were pre-frozen, then freeze-dried at -80°C and 9 Pa, and finally made into powder and stored at -20°C. A certain amount of the prepared sample was added with an acetone solution containing 0.07% β-mercaptoethanol at a volume ratio of 17:1 (v/w). After stirring for 2 hours at 4°C and 200 rpm, centrifuge for 20 minutes at 4°C and 6000 rpm, remove the supernatant, and obtain the precipitate, which is the defatted protein. After repeating this defatting process three times, the precipitate is air-dried and stored at -20°C.

The defatted peanut protein was taken and added into Tris-HCl buffer (concentration of 50 mmol/L, pH value of 8.0) at a mass ratio of 1:10 (w/v). After stirring at 4°C for 4 hours, the mixture was centrifuged at 4°C and 8000 rpm for 40 minutes, and the supernatant was collected and stored at -20°C for determination of soluble protein content. The soluble protein content was determined using a BCA kit on the above extracted protein solution.

3.3 Peanut Modification

Peanut modification treatment, as a vital technical means, plays an important role in significantly improving the overall quality of peanuts and derivative products. This processing process mainly involves in-depth modification of the core component of peanuts - peanut protein. The purpose of this is to improve the processing characteristics of peanut protein in multiple dimensions, so as to improve its solubility, emulsification and gelling properties, so that peanuts can perform better in the subsequent processing, whether it is made into food or other derivatives, it can show better quality. Modification treatment is divided into physical modification, chemical modification, enzymatic modification and composite modification. The study uses electrophoresis and electrotransfer in physical modification.

(1) Electrophoresis: Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) [8] was used, with the separation gel concentration of 13.5% and the stacking gel concentration of 5%.

①Sample treatment: Mixing the peanut protein solution with 2×SDS sample buffer thoroughly. Then, heating the mixture to boiling, centrifuge briefly, and let it stand for subsequent use.

②Adding samples: Using a micropipette to accurately add the treated sample and standard molecular weight marker to the comb hole of the electrophoresis instrument.

③ Electrophoresis process: Performing electrophoresis at a constant voltage of 120 V until the bromophenol blue front reaches the lower boundary of the gel. At this point, stopping the electrophoresis, carefully remove the gel, and perform subsequent fixation and staining.

(2) Electrotransfer:

①Preparation: First, marking the nitrocellulose membrane to ensure that its size is exactly the same as the SDS-PAGE gel. Next, preparing 6 filter papers of the same size, divide them into two groups, 3 in each group, and soak them in the anode and cathode electrode buffer respectively.

②Gel balance: After electrophoresis, removing the SDS-PAGE gel from the electrophoresis instrument and soak it in electrotransfer buffer for 20 to 30 minutes to reach equilibrium. Then, laying the "gel sandwich" on the anode in the following order: 3 layers of anode filter paper → nitrocellulose membrane → gel → 3 layers of cathode filter paper. During the laying process, making sure that the layers fit tightly and no bubbles are generated. Finally, gently pressing the "gel sandwich" and cover it with the lid, lower and lock the cathode.

③Electrotransfer operation: Setting the current to 96mA/gel and perform the electrotransfer operation for 2 hours. After the electrotransfer, staining the NC membrane with Ponceau solution for 5 minutes, followed by a 5-minute decolorization treatment. By observing the transfer effect and protein location on the NC membrane, the quality of the electrotransfer can be evaluated and preparations can be made for subsequent immunoblotting experiments.

3.4 In Vitro Digestion Simulation

The in vitro simulated digestion experiment is divided into two parts: simulated gastric digestion and simulated intestinal digestion, both of which are carried out in accordance with the method of the United States Pharmacopoeia. In the simulated gastric digestion experiment, a specific simulated gastric digestion fluid is used to perform in vitro digestion on the sample. The total volume of the reaction system is a preset value. Before the reaction, the protein and the simulated gastric fluid are preheated at a specific temperature to ensure that the volume ratio of the protein to the simulated gastric fluid in the reaction system reaches a predetermined ratio. Subsequently, a simulated gastric digestion experiment was carried out in a water bath at a specified temperature, and the digestion time was set at multiple time points. After the reaction was completed, an appropriate amount of solution was immediately added to neutralize the system to alkalinity, and after boiling, it was frozen for storage.

In the simulated intestinal digestion experiment, the samples were also digested in vitro using a specific simulated intestinal digestive fluid. The total volume of the reaction system, the preheating temperature of the protein and the simulated intestinal fluid, the volume ratio, and the temperature of the water bath were all the same as those in the simulated gastric digestion experiment. The digestion time was also set to multiple time points. After the reaction is completed, a certain proportion of loading buffer is added, and the samples are treated in a boiling water bath and finally frozen for storage. Through these two parts of the experiment, the digestion characteristics of the samples under in vitro simulated digestion conditions can be fully evaluated.

4. Results and Discussion

4.1 Allergen Molecule Detection

In the in-depth study of the interaction of allergen molecules in polymers, the study paid special attention to the formation of intermolecular disulfide bonds, which is one of the key factors affecting protein structure and function. Table 2 lists in detail the number of intermolecular disulfide bonds detected in polymers.

Table 2. Number of disulfide bonds detected between allergen molecules

Allergens	Arah1	Arah2	Arah3	Arah6
Arah1	1	-	-	-
Arah2	14	5	-	-
Arah3	3	12	0	-
Arah6	8	12	10	4
Arah7	2	10	1	7
Arah9	0	1	0	2
Arah13	1	2	0	1

According to the data in Table 2, in the polymer, the number of disulfide bonds formed between different allergen molecules is different. Arah2 is the allergen with the most intermolecular disulfide bonds, and it forms the most cross-linked peptide pairs (12 pairs) with Arah6 and Arah3. Arah1 mainly forms disulfide bonds with Arah2 (14 pairs), while the number of disulfide bonds formed with Arah3 and Arah6 is small or no. In addition to its significant interaction with Arah2, Arah3 also forms 10 disulfide bonds with Arah6. More disulfide bonds were formed between Arah6 and Arah2, Arah3 and Arah7.

The study further measured the IgE (immunoglobulin E) binding ability of the polymers using the enzyme-linked immunosorbent assay (ELISA) method [9-10]. In the ELISA experiment, the specific antibody is coated on a solid phase carrier and combined with the antigen in the sample to be tested. Then, the enzyme-labeled secondary antibody is added to form an antibody-antigen-enzyme-labeled secondary antibody complex. By adding a substrate and causing a color reaction, it can be determined whether an immune response exists. The experimental results are shown in Figure 1.

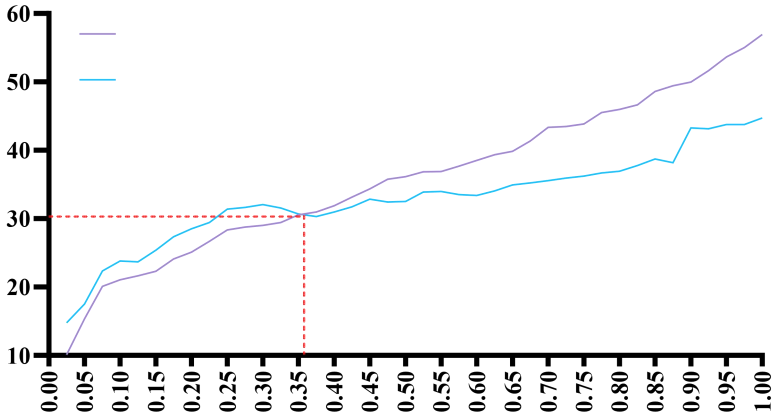

Figure 1. IgE binding capacity of polymers from modified peanuts

In Figure 1, Raw represents the original processed peanut polymer, and Alteration represents the modified peanut polymer. The IgE binding capacity of the polymer in peanuts increases slowly with the increase of protein concentration. Although at low concentrations, the IgE binding rate of the polymer to unprocessed peanut protein is higher than that of modified peanuts, when it reaches 30.62%, the required concentration of modified peanut polymer is significantly higher than that of unprocessed peanut protein, which proves that the IgE binding capacity of modified peanut polymer is lower than that of unprocessed peanut.

4.2 Cell Viability Detection

The study further detected the viability and proliferation ability of Caco-2 cells under the action of Arah2-Raw and Arah2-Alteration proteins. After incubating Caco-2 cells with different concentrations of Arah2-Raw and Arah2-Alteration proteins for 24 h, CCK-8 solution was used to detect the viability of Caco-2 cells. The results are shown in Figure 2.

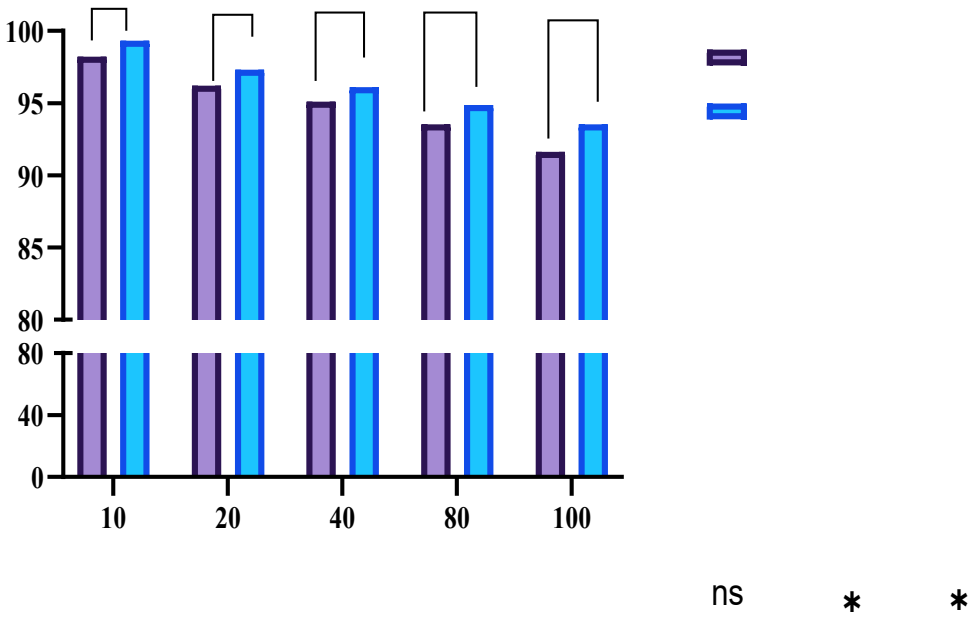

Figure 2. Effect of peanut Arah2 on Caco-2 cell viability

According to the data in Figure 2, the viability of Caco-2 cells shows a downward trend with the increase of the concentration of allergen Arah h2. When the concentrations of Arah2-Raw and Arah2-Alteration are lower than 40 μg/mL, the viability of Caco-2 cells is above 90%. At the same concentration, the effect of Arah2-Raw on Caco-2 cell viability is slightly greater than that of Arah2-Alteration, indicating that Arah2-Raw may have a slightly stronger toxic effect on Caco-2 cells.

The study then further used the EdU method (5-ethynyl-2'-deoxyuridine incorporation method) to detect changes in the proliferation ability of Caco-2 cells after treatment with different concentrations of Arah2-Raw and Arah2-Alteration proteins. The results were observed using a fluorescence microscope and are shown in Figure 3.

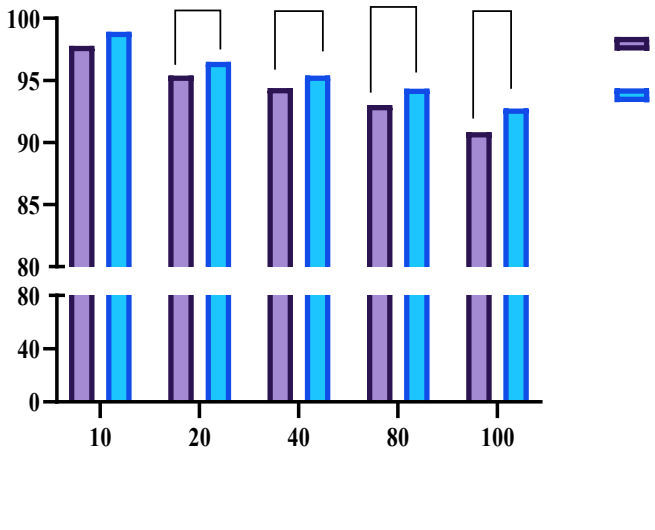

Figure 3. Fluorescence microscopy observation of Caco-2 cell proliferation after peanut Arah2 treatment

According to the data in Figure 3, the proliferation ability of Caco-2 cells shows a downward trend with the increase of the concentration of allergen Arah h 2. At the same concentration, the cell proliferation rate of Arah2-Raw is slightly lower than that of Arah2-Alteration, indicating that Arah2-Raw has a relatively stronger inhibitory effect on the proliferation of Caco-2 cells.

5. Conclusion

In this study, we aimed at the detection and development of low-allergenic peanut products and successfully reduced the allergenicity of peanut protein through modification technology. Compared with traditional detection methods, the method proposed in this study is simple to operate, low-cost, and has a high detection sensitivity, which can accurately assess the allergenicity level of peanut products. However, this study also has certain limitations, such as the modification treatment may have a certain impact on the taste and nutritional value of peanut products, and the differences in the effects of different modification methods. Therefore, this paper suggests further exploring milder modification processing technologies in future research to minimize the impact on taste and nutritional value while ensuring product safety. At the same time, comparative analysis of the effects of different modification methods and study of the mechanism of the effect of modification on the structure and function of peanut protein will help to further optimize and improve the development and detection methods of low-allergenic peanut products.

References

[1] Linacero R, Cuadrado C. New Research in Food Allergen Detection[J]. Foods, 2022, 11(10): 1520-1521.
[2] Luparelli A, Losito I, De Angelis E, et al. Tree nuts and peanuts as a source of beneficial compounds and a threat for allergic consumers: overview on methods for their detection in complex food products[J]. Foods, 2022, 11(5): 728-731.
[3] Hnasko R M, Lin A V, McGarvey J A, et al. Sensitive and selective detection of peanut allergen Ara h 1 by ELISA and lateral flow immunoassay[J]. Food Chemistry, 2022, 396: 133657.
[4] Tramuta C, Decastelli L, Barcucci E, et al. Detection of peanut traces in food by an official food safety laboratory[J]. Foods, 2022, 11(5): 643-647.

[5] Schäfer L, Miskey C, Hein S, et al. DNA Meets Protein — Development, Characterization, and Application of Aptamers against Peanut Allergen[J]. Journal of Agricultural and Food Chemistry, 2024, 72(32): 18225-18233.
[6] Adedeji A A, Priyesh P V, Odugbemi A A. The Magnitude and Impact of Food Allergens and the Potential of AI-Based Non-Destructive Testing Methods in Their Detection and Quantification[J]. Foods, 2024, 13(7): 994-1001.
[7] Luparelli A, Losito I, De Angelis E, et al. Multi-target detection of nuts and peanuts as hidden allergens in bakery products through bottom-up proteomics and high-resolution mass spectrometry[J]. Foods, 2023, 12(4): 726-730.
[8] Pi X, Peng Z, Liu J, et al. Sesame allergy: mechanisms, prevalence, allergens, residue detection, effects of processing and cross-reactivity[J]. Critical Reviews in Food Science and Nutrition, 2024, 64(10): 2847-2862.
[9] Miaw C S W, Martins M L C, Sena M M, et al. Screening method for the detection of other allergenic nuts in cashew nuts using chemometrics and a portable near-infrared spectrophotometer[J]. Food Analytical Methods, 2022, 15(4): 1074-1084.
[10] Hemmings O, Niazi U, Kwok M, et al. Combining allergen components improves the accuracy of peanut allergy diagnosis[J]. The Journal of Allergy and Clinical Immunology: In Practice, 2022, 10(1): 189-199.