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Technology for Detection of Food Allergens based on Immunology

Chengsong An, Yajing Zhao, Qiuyue Wang, Huiwen Wang
Tianjin Agricultural University, Tianjin, 300384, China

Abstract: Food safety is a very important issue in today's society. Food allergens are the leading cause of people's allergies, which have affected nearly a quarter of the world's population. In order to reduce the allergy, it is necessary to avoid food allergy, and it is very important to detect the food allergen, but the traditional detection technology is difficult and does not meet the requirements of high speed and high accuracy. Therefore, a method for detecting allergens by an immunosensor based on immunological techniques was established.

Keywords: Immunology; Allergen detection; Food safety;Immunosensor

1. Introduction

With the continuous improvement of the material level, the quality of food has become an issue that people are increasingly concerned about. The quality of food is closely related to people's health and life safety. The development of science and technology and the promotion of genetically modified products have increased the volume of production, but also increased the hidden danger of food safety. In addition to illegal additions, overdosed pesticides, heavy metal residues, and bacterial contamination, allergens contained in foods are a major problem in food safety, and it seriously endangers people's health and life safety. Allergy is a chronic disease that can be seen everywhere in daily life. The symptoms are complicated and there are many types of allergies that are difficult to cure. In the past few decades, food allergy has become a world-class problem. In recent years, the government and related departments have also begun to pay more attention to allergen problems [1]. In order to prevent people from suffering allergies or educe the harm to the health of consumers, food allergen detection is very important. With the rapid development of immunology technology and continuous research on immune problems, it provides the immune detection technology of high sensitivity, strong specificity and good stability for the people, which is of great significance for the detection of food allergens.

2. Study of Common Food Allergens

Through investigation and research, there are now more than 180 allergic foods that have been identified. Common allergens are found in the following categories:

Crustacean aquatic products. Seafood is very popular with people. Especially in recent years, there have been more and more people eating seafood, and then allergies have emerged. Shrimp allergy is the most common occurrence in crustacean allergy. Researchers have detected that tropomyosin is the major allergen in shrimp meat. Generally speaking, the number of allergen molecules in food is between 10000~75000, usually referring to protein or glycoprotein. The number of tropomyosin molecules is between 35000~38000, and has reached the allergy standard [2].

Egg products. Eggs are a common food in life, which can be cooked readily or made into dishes. Some biscuits and cakes also contain the ingredients of eggs. The crowd that is allergic to eggs is mainly concentrated in children. The allergy of eggs mainly depends on its positive rate. In children's foods, the allergic rate can reach 36%, which is equivalent to 3 times of adults'. The basic allergens in eggs are ovomucoid, ovalbumin, ovotransferrin, lysozyme, and Ot yolk protein. The number of relative molecules in the above allergens is above 28,000, and some even reach 77,000. According to analysis, the protein is more susceptible to allergies than egg yolk because its main allergen is ovomucoid [3].

Peanut products. In past cases in our country, it has been found that peanut and other oil crops are one of the allergens that cause asthma in commonly used foods. Allergies to peanuts can cause bad allergies, and even cause shock and life-threatening. According to the survey, 30% of people with allergies are allergic to peanuts. And unlike milk and other allergens, allergies to peanuts is usually concomitant, and only a few children develop tolerance as they grow.

Table 1. Common Food Allergens

Food	Allergens
Egg	Ovalbumin Lysozyme Ovomucoid Ovotrasferrin Ovomucin
Milk	Casein

	Lactoglobulin Lactalbumin BSA
Shrimp	Tropmyosin
Soybean	Glycinin Soy lectin
Peanut	Peanut Lectin

3. Realization of Food Allergen Immunosensor Technology

The immunosensor is a new type of instrument for detecting food allergens that has emerged in recent years. It has high-quality detection capabilities and can accurately detect small changes in crystal quality. Because of its high sensitivity, simple operation, strong real-time, etc., it is widely used in food safety inspection, medical inspection and other fields. Its composition is shown in Figure 1.

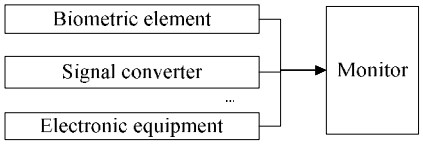

Figure 1. Structure of Immunosensor

3.1. Preparation of experimental materials

Sample preparation. Because there are many kinds of allergy, in order to test the accuracy and contrast, peanut and shrimp antibodies, peanut and shrimp protein were used as test samples respectively.
Solution preparation: phosphate buffer, Piranha solution, nanogold sol, glycine-HC1 buffer, citric acid - sodium citrate buffer Instrument Preparation: Immunosensor

3.2. Methods of experiment

This test will be affected by the temperature, and it takes a certain amount of time for the antibody and the electrodes in the sensor to collect. Therefore, we have to study the conditions produced at 5°C, 15°C, 25°C, and 35°C, respectively. In addition, the time of antibody coating also has an effect on the accuracy of the electrode. As time continues to increase, the frequency is constantly changing. In order to fix the amount of antibody, it is necessary to detect the frequency at different times until the exact time needed to maximize the frequency is determined.
The antibody was detected by placing the electrodes in a solution of ethanol at a concentration of 5 mM, 15 mM, 25 mM, and 35 mM, and the detected frequency was changed with the change of the concentration. As the concentration becomes smaller, the frequency will also become smaller. When the concentration becomes larger, the frequency will also become larger. When the concentration reaches 25mm, the frequency will no longer have a corresponding change. Thus, the higher the concentration, the effect is not necessarily good. The high concentration is not conducive to the continuation of follow-up experiments. If the concentration is too low, it will affect the amount of antibodies, and reduce the sensitivity of the sensor. Therefore, selecting a 25mm ethanol solution in the test can ensure the sensitivity of the sensor and facilitate further testing.
After doing the above work, the shrimp and peanut antibodies were diluted to 1:1000, 1:2000, 1:4000, 1:6000, 1:8000, 1;10000,1:15000. The coating was carried out under different temperature conditions. After 3 hours, they reacted with the corresponding antigens. The results are shown in Table 2.

Table 2. Temperature Differences in Antibody Coating

Temperature	5℃	15℃	25℃	35℃
Dilution times	315.6	262.3	204	166.8
Immune signal	9.5	18.4	10.6	14.9

As can be seen from Table 2, if the dilution factor is too small, the immune signal will be low; if the dilution factor is too large, the antigenic ability will be weak, so the immune signal will not be enhanced bu decreased. Therefore, the dilution ratio of shrimp was 1:6000, and the dilution ratio of peanut antibody was 1:4000, and the selected test temperature was 5°C [4].

3.3. Results of the experiments

Figure 2 shows a graph of the response time of a shrimp allergen protein solution as detected by a sensor. Its rule is that as the immune response progresses, the number of antigenic substances attached to the electrode gradually increases and the time gradually increases. At the 500th s, the curve tends to be gentle,which indicates that the response signal has been stable, as shown in Figure 2. This proves the speed and effectiveness of this testing method.

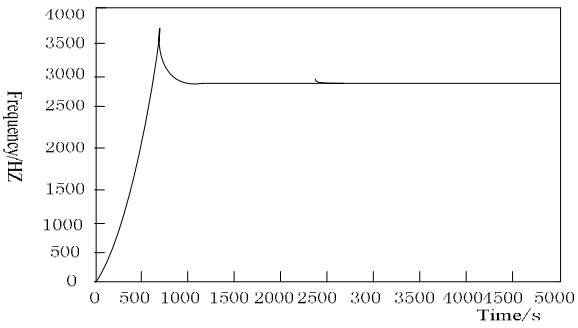

Figure 2. Reaction Time of the Sensor

After the antibody was evenly coated on the electrode surface, a frequency value of F1 was detected. The antibody is then placed in an allergen solution and reacted to form a complex. After 500s, the frequency F2 was meas-

ured again, and the frequency change value calculated by the F2-F1 was calculated.

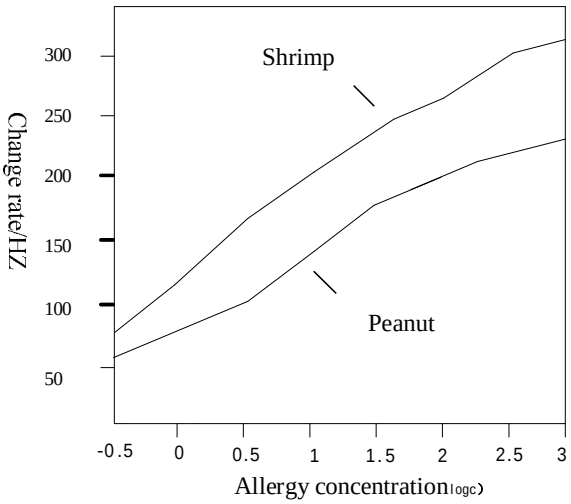

Figure 3. Immune Signals at the Allergy Concentration

Figure 3 is the relationship between the frequency and concentration of the allergenic protein solution of shrimp and peanut at different concentrations, in which the equation of the allergy frequency and concentration of shrimp is y=85.021x+155.35 and R=0.896, and the equation of the allergy frequency and concentration of peanut is y=60.044x+95.12 and R=0.885. As the concentration of allergens increases, the frequency also increases, which is consistent with the sauerbrey equation[5]. It is proved that the detection of allergen based on immunology through sensors is successful.

4 . Conclusions

Although the commonly used immunization method has strong specificity, the operation is tedious, wastes manpower and material resources and time, and some monitoring methods are also accompanied by environmental pollution. Therefore, food allergens cannot be monitored in real time, and the operability is relatively low. The food allergen detection technology based on immunology technology has the advantages of high sensitivity, rapid detection speed, simple operation and accurate quantification, and it has very important practical value in the future detection of food allergens.

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