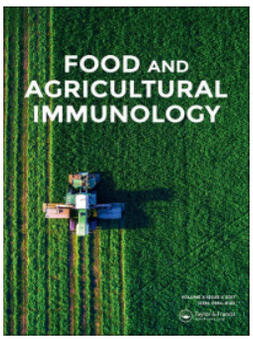

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The influence of pre-treatment methods and matrix effect on sesame (*Sesamum indicum*) sandwich ELISA detection

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ABSTRACT

Food allergy is a serious health risk and severely affects the quality of life in individuals all over the globe. In recent years, there has been an evident increase in the number of food allergy cases due to the consumption of sesame. Hence, to develop a robust and reliable detection method for sesame allergen has become a high priority. In this research, a sandwich ELISA method was established to detect sesame allergens using rat and rabbit antiserums. The limit of detection for sandwich ELISA was estimated at 1 ng/mL, revealing high sensitivity. At the same time, it was found that the most suitable pre-treatment extract for this method was 0.1 M, pH 9.6 Na_2CO_3-$NaHCO_3$ containing 0.5% SDS. This sandwich ELISA method can sensitively and efficiently detect foods containing sesame ingredients, which can protect the sensitive individuals from the exposure to sesame allergenic foods.

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Sesame; allergen; sandwich enzyme-linked immunosorbent assay (ELISA); protein extraction; pre-treatment; detection

1. Introduction

Sesame (*Sesamum indicum*) is a herbaceous annual plant, belonging to the Pedaliaceae family, which is widely planted in tropical and temperate regions. Sesame is one of the main oil crops in China ; due to its high nutrition and delicacy more and more people like to consume sesame products, especially toast or prepare cakes with sesame seeds on them. Sesame allergy is the ninth most common food one (Chadha et al., 2019) and the population allergic to sesame has significantly increased worldwide in recent years (Irani et al., 2011). Sesame allergy is an IgE-mediated food one that tends to appear early in life and is common in the 2 years old, which is conceived as an indicator for early allergy diagnosis (Peters et al., 2013). About 2.5% of infants are allergic to sesame in Australia; about 0.1% of children less than 18 years old are allergic to sesame in

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America; and about 0.93% of young children are allergic to sesame in Israel (Garkaby et al., 2021; Osborne et al., 2011; Soller et al., 2015). But unlike cow's milk and egg allergy, it persists in 80% of the cases (Cohen et al., 2007). On the other hand, around 84% of sesame allergy casualties were also allergic to nut/peanut; sesame should be identified as an ingredient and separated from nuts and peanuts when it may be an allergen contaminant (Derby et al., 2005). Therefore, it is necessary to study sesame allergy in a broad spectrum.

Sesame is one of the main oil crops in China, containing 39.6% fat and 18% protein. So far, seven sesame allergens have been identified: two 2S albumins Ses i 1, molecular weight 9 kDa (Pastorello et al., 2001); Ses i 2, molecular weight 7 kDa (Beyer et al., 2002), one 7S vicillin-like globulin (Ses i3, 45 kDa), two oleosins (Ses i 4, 17 kDa; Ses i 5, 15 kDa) (Leduc et al., 2006), and two 11S globulins (Ses i 6, 52 kDa; Ses i 7, 57 kDa) (Beyer et al., 2007), in which Ses i 1 and Ses i 2 are the most important allergens. According to the previous study on sesame allergy the most sensitive patient reacted to the first dose at 1.02 mg sesame protein (Dano et al., 2015).

Currently, there is no cure for food allergies; the best way is to completely avoid allergenic foods. However, accidental cross-contact between food allergens and other products in production is a serious concern; many foods use or are with contaminate traces of sesame, so it is very difficult to avoid it completely (Wolff et al., 2004), which demands extensive attention (Dreskin et al., 2021). People at potential allergy risk need a clear diagnosis and up-to-date guidance to provide awareness in avoiding such crucial allergens more easily. Thus, it has become a challenging task to find a way to detect sesame in various foodstuffs. Currently, there are many effective approaches for the detection of potential allergens or allergenic proteins in food, for instance, protein detection by Enzyme-linked immunosorbent assay (ELISA) and Liquid chromatography-mass spectrometry (LC-MS), or DNA detection by nucleic acid amplification, with various advantages and disadvantages. Ma developed and utilized stable isotope-labelled internal standard (SIIS) peptides (Ma et al., 2020). López-Calleja developed a method for determining the presence of low amounts of sesame in minced processed foods (López-Calleja et al., 2017). Fatima developed an indirect competitive ELISA to detect traces of sesame in food (Husain et al., 2010), Ecker and Maruyama developed a sandwich ELISA to determine the traces of sesame in food (Maruyama et al., 2016; Redl et al., 2010). Moreover, the sandwich ELISA method is also used to detect other eight types of allergens, especially species with higher homology to sesame like walnuts (Niemann et al., 2009), hazelnuts (Costa et al., 2015) and buckwheat (Panda et al., 2010). But, the sandwich ELISA detection based on single-component allergen has been developed in recent years (Chen et al., 2014; Zhao et al., 2019).

The detection of allergen content is related to the solubility of the protein during the extraction process; only the extracted protein can be detected. Therefore, the quality of the pre-treatment method directly determines the sensitivity of the detection method. The thermal processing of food or the influence of matrix effect may cause the extraction of allergenic protein highly difficult with the buffer, thereby affecting the effectiveness of the detection method. Numerous studies have shown that different pre-processing methods affect ELISA results. Adding buffer additives may increase the extraction rate of food allergenic proteins. The use of buffer additives has been explored in the pre-treatment of shrimp, peanuts and other foods (Albillos et al., 2011; Lasekan & Nayak, 2016; Schmitt et al., 2010), but in the current sesame ELISA detection, there is no research has been

attempted on optimizing the pre-treatment method. Therefore, it is necessary to provide a pre-treatment method compatible with the developed ELISA to improve the efficiency of sesame allergen extraction and the detection sensitivity of the method. In this study, we immunized rat and rabbit with sesame allergen full-component protein to obtain the antiserum and constructed a sandwich ELISA method, revealing higher sensitivity and higher reliability than the previously established methods. The LOD of sesame in the system and real food was analysed, and the influence of food matrix and sesame composition on the detection of sesame was explored. It was found that baking food with sesame, such as biscuits, would reduce the detection efficiency of sesame. At the same time, different extraction buffers and additives were used to extract different heat-processed sesame seeds and tested with the established sandwich ELISA method; According to the level of the ELISA results, a pre-treatment method was determined to make the extraction rate of sesame protein reach a higher value, which provides a reliable method for the establishment of the sesame sandwich ELISA test kit and the optimization of the pre-treatment method.

2. Materials and methods

2.1. Materials and reagents

White peeled sesame was purchased at a local supermarket in China. Grounded pasted sesame was defatted for twelve hours by cold acetone (−20°C) in the ratio of 1:10 (w/v); this was repeated for three times, and then the residual acetone of precipitation was removed in fume hood. Sesame acetone powder was extracted by extraction solution (0.1M Na_2CO_3-$NaHCO_3$, pH 9.6) in the ratio of 1:10 (w/v), stirring at 4°C overnight, then centrifuging the mixture at 12,000 rpm for 20 min. The protein concentration of the supernatant liquid was determined by the BCA test kit, sesame defatted acetone powder and sesame protein were stored at −20°C.

Polyclonal rat anti-sesame antiserums (IgG) and polyclonal rabbit anti-sesame antiserums (IgG) were produced by two different companies in China. The horseradish peroxidase labelled secondary antibody was obtained from Solarbio, Beijing.

The buffer used for protein extraction solution was 0.1M Na_2CO_3-$NaHCO_3$, pH 9.6. Buffers used for SDS PAGE and immunoblotting were staining solution (Coomassie Brilliant Blue) and destaining solution, respectively. Buffers used for sandwich ELISA were: coating solution (0.01 M CBS, pH9.6); phosphate-buffered saline (0.01 M PBS, pH7.4); washing solution (PBST): 0.01 M PBS+ 1% Tween-20; blocking solution: PBST+ 1% BSA; tetramethylbenzidine (TMB) one-component solution was obtained from Solarbio, Beijing and stop solution (2 M H_2SO_4). Buffers used for the processed sesame extraction were as follows: 0.01 M PBS, pH8.6; 0.05 M NH_4HCO_3, pH 9.0; 0.1 M Na_2CO_3-$NaHCO_3$, pH 9.6; 0.1 M Na_2CO_3-$NaHCO_3$+0.5% SDS; 0.1 M Na_2CO_3-$NaHCO_3$+ 0.5% SDS +50 mM DTT. All the experiments were carried out using ultra-pure water, pH was adjusted by 6 mol/L HCl or 6 mol/L NaOH.

2.2. Analysis of the sesame proteins by SDS PAGE

SDS PAGE analysis described by Laemmli (Laemmli, 1970) was carried out to analyse the protein samples. The sesame protein extraction was diluted to 1 mg/mL, and then mixed

with a loading buffer, heated for 7 min to denature the protein; the samples were added with 10 μL of 12% gel. The electrophoresis was performed under 140 V until the end process. Then the gel was stained with a staining solution for 5 min and destained using the destaining solution until the bands were clear.

2.3. Establishment of the sandwich ELISA method

2.3.1. Establishment of standard curve and parameters

Two different mating combinations were carried out in the sandwich ELISA: using rat IgG as the coating antibody, rabbit IgG as the detection antibody and goat anti-rabbit as the secondary antibody or using rabbit IgG as the coating antibody, rat IgG as the detection antibody and rabbit anti-rat as the secondary antibody. The standard curves of the two combinations were used to choose the better mating way.

Microwell plates were coated with 100 μL well^{-1} rat antibody in coating buffer and incubated in 4°C fridge overnight, after washing for 5 min and repeating the washing step three times; 150 μL well^{-1} blocking solution was added into the microwell plates, further incubated for 2 h at 37°C in the incubator and washed three times again. Antigen diluted with PBST 100 μL well^{-1} was added into the microwell plates and incubated for 1 h at 37°C in the incubator, then washed three times. Detection antibody diluted with PBST 100 μL was added into the plate in individual wells, incubated for 1 h at 37°C and washed three times. HRP-labelled secondary antibody diluted with PBST 100 μL was added into the plate, incubated for 1 h at 37°C, then washed three times. TMB one-component solution 100 μL was added into the plate and incubated at 37°C for 10 min, then the stop solution 50 μL was added immediately. The optical density (OD) was measured at 450 and 630 nm. Calibration curve was drawn by the four-parameter method, the protein concentration is on the abscissa and the OD value is on the ordinate.

The calculation of mean coefficients of variation of intra-assay and inter-assay is as follows: seven concentrations and blank control of the standard curve were repeated 10 times. The average and standard deviation (SD) of the 10 parallel values was calculated, and then the coefficient of variation (CV) was calculated by average value by dividing SD and multiplying by 100%. The experiment was carried out for five consecutive days and the standard curves were drawn. The average and SD of five measurements were obtained, and then the CV was calculated by average value by dividing SD and multiplying by 100%.

2.3.2. Cross-reactivity

Twenty-one kinds of foods were tested for cross-reactivity: peanut, walnut hazelnut, pistachio, almond, wheat, soybean, red bean, mung bean, black bean, kidney bean, mango, tomato, peach, yellow croaker, turbot, sea bass, conch, shrimp, egg and milk. After extracting proteins from these foods, the protein concentration was measured by the BCA test kit and adjusted to 0.5 mg/mL. The cross-activity was determined by calculating the equivalent concentration according to the standard curve.

The cross-reactivity rate is the true concentration of the protein (calculated with the sandwich ELISA standard curve) divided by the theoretical concentration (calculated with the BCA test kit).

2.3.3. Spiking of samples

Biscuits, oatmeal and beef sauce were used for spiking. These food matrices were grounded in a grinding machine, different concentrations of sesame powder were added in these chopped food matrices. 5 mL of sesame protein standard solution (2, 1, 0.02 mg/mL) was added to 1 g of the homogenized blank food sample to obtain spike levels of 10, 5, 0.1 mg/g. Later, the foodstuffs were extracted for 1 h; different extracts were diluted at different times with PBST (1:1, 1:5; 1:10), and analysed in sandwich ELISA standard curves. The recovery was calculated from the ratio of the amount of sesame protein determined from the sandwich ELISA to the amount of sesame protein actually added to the sample (Redl et al., 2010).

2.4. Research on the influence of matrix effect

2.4.1. Preparation and extraction of negative and positive food matrix

To explore the influence of matrix effect on detection, sesame seeds were added to the food matrix before and after processing separately. The biscuits were used as a food model, one was to bake after adding crushed sesame powder of different additive concentrations, another was to add roasted or raw sesame powder to blank matrix. Sesame-free cookies were used as control.

The blank biscuits were made of 12 g of butter; 4 g of white sugar was melted in a water bath, then 20 g of low-gluten flour was added. After mixing them thoroughly, they were frozen at −20°C for 30 min and cut into 5 mm slices, then baked at 180°C for 20 min. The production of spiked biscuits is as follows: grounded sesame seeds were gradually diluted with water to different proportions (1:200, 1:2000 and 1:20,000), 4 mL of the spiked solution of different concentrations was mixed into the above blank biscuits, the steps including freezing, slicing and baking are the same as above. Three positive biscuits with different concentrations of 5, 50, and 500 mg sesame/kg were obtained. Then raw or roasted sesame seeds were added to grounded baked biscuits to get 5, 50, 500 mg sesame/g spiked food samples.

The blank biscuits and three different spiked biscuits were extracted with PBST at a ratio of 1:10 for 2 h, the supernatant was for use.

2.4.2. Sandwich ELISA analysis

Protein solutions were diluted in multiple gradients (1:1, 1:10, 1:100) with PBST to perform in sandwich ELISA detection by the above established method. The corresponding concentration of the obtained absorbance value, according to the standard curve, was calculated and then multiplied by the dilution factor to convert it to the original concentration.

2.5. Determination of pre-processing extract

2.5.1. Thermal processing of white sesame seed

According to the way sesame seeds are consumed, two thermal processes of white sesame were chosen: baking (110°C for 15 min); stir-frying (10 min by 400w firepower). Unprocessed sesame was used as a control.

2.5.2. Preparation of sesame soluble protein extracts with additives and without additives

Based on different types of extraction and additives, sesame was grounded using a grinder after each processing method. The selection of the extract was carried out in two steps: the first was the choice of buffer, three different processed sesame seeds (raw sesame, toasted sesame and stir-fried sesame) with different buffers (0.01 M PBS, pH8.6; 0.05 M NH_4HCO_3, pH 9.0; 0.1 M Na_2CO_3-$NaHCO_3$, pH 9.6) to select the most suitable buffer, after selecting the most suitable buffer (Husain et al., 2010; Ma et al., 2020).

The next was the choice of additives, adding a surfactant or/and denaturants to the buffer chosen above, one was to add only 0.5% SDS, another was to add both 0.5% SDS and 50 mM DTT to the buffer, the two steps were combined to select the most optimal extraction method. After 4 h of extraction at room temperature, the mixing solution was centrifuged to obtain a supernatant, getting different protein solutions.

2.5.3. Protein concentration and sandwich ELISA analysis

The concentration of these protein solutions extracted by different buffers and different additives was determined by the Coomassie brilliant blue kit. Furthermore, different protein solutions were diluted in multiple gradients (1:1, 1:10, 1:100, 1:1000) to perform sandwich ELISA detection by the established method, as described above.

3. Results and discussion

3.1. Characterization of sesame proteins

Sesame proteins and two kinds of anti-sesame antiserums were used in sandwich ELISA: rat anti-sesame antiserum (IgG) and rabbit anti-sesame antiserum (IgG). SDS PAGE was carried out to analyse the molecular weights of the sesame proteins. After staining and destaining, the binds of sesame proteins on approximately 7, 9, 15, 17, 20, 23, 32, 34, 45, 52, 57 kDa (Figure 1, lane 1) were clearly shown. SDS PAGE under reducing condition indicates the binds on approximately 20 and 23 kDa may be the basic subunits of binds 52 and 57 kDa, and the binds on approximately 32 and 34 kDa are acid subunits of them, disulphide bonds of the two binds were broken in reducing condition, which is similar to Elide's results (Pastorello et al., 2001).

3.2. Development of the sandwich ELISA

3.2.1. Determination of matching method, working range and parameter of sandwich ELISA

The calibration curves in Figure 2 indicate that rat IgG as a coating antibody is better than others, the first choice could achieve a higher OD value and has a range with a large ordinate span, which is suitable for establishing a standard curve. Therefore, this method was preferred for the optimization of subsequent experiments.

To fit an appropriate standard curve, the antigen range was narrowed down to 10∼1000 ng/mL and drawn by the four-parameter fitting method, which can achieve a higher degree of fit. In addition, the dilution times of coating antibody, detection

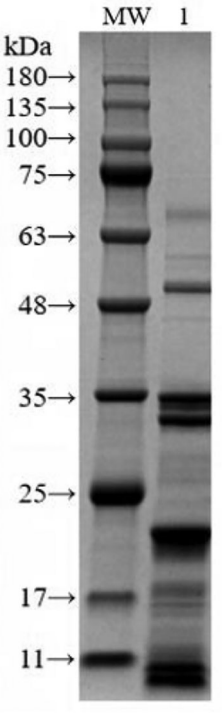

Figure 1. SDS PAGE in 12% acrylamide gel. MW, molecular weight marker; Lane 1, white sesame extract.

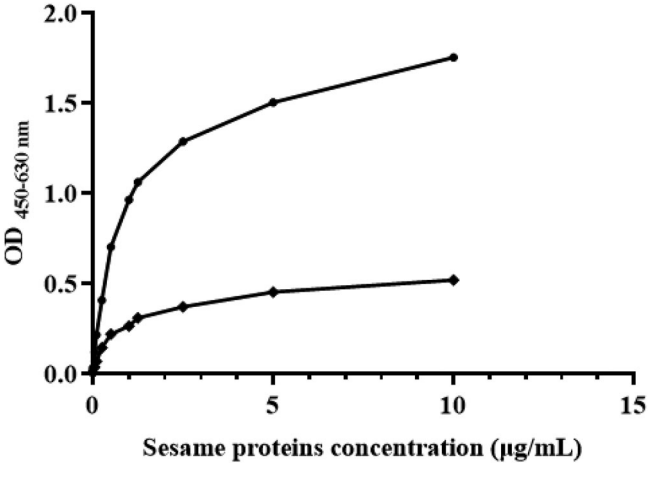

Figure 2. The sandwich ELISA results of antiserum pairing. Rat antiserum (1:10,000 diluted) is used as the capture antibody, rabbit antiserum (1:80,000 diluted) is used as the detection antibody (circle), the HRP-labelled goat anti-rabbit IgG (1:40,000 diluted) as the secondary antibody; Rabbit antiserum is used as the capture antibody (1:50,000 diluted), rat antiserum (1:10,000 diluted) is used as the detection antibody, the HRP-labelled rabbit anti-rat IgG (1:10,000 diluted) as the secondary antibody was diluted 40,000 times (diamond). The range of sesame proteins was 0.001∼10 μg/mL.

antibody and secondary antibody were optimized, a set of data with the highest P/N value and higher absorbance was selected, which can maximize the utilization efficiency of antibody. Although the P/N values are quite different, the coating antibody dilution and detection antibody dilution to OD value were found to have a little influence, but the different dilution of HRP enzyme-labelled secondary antibody revealed a great influence. The standard curve is given as Figure 3, ($R^2 > 0.99$).

3.2.2. Limit of detection (LOD) and limit of quantification (LOQ)

The estimated values of LOD and LOQ were calculated by 20 blanks average adding three times or 10 times standard deviation (SD), then several concentrations of sesame protein based on estimated LOD value were set for determining feasibility; this concentration of protein was determined 10 times in parallel. We chose the concentration with results of 80% exceeding the maximum blank absorbance as the final result LOD of the sandwich ELISA. According to the above evaluation, the LOD of this method was found to be 1 ng/mL, and the LOQ of this method is 10 ng/mL. The LOD of this analytical method for the detection of allergenic foods is lower than 100 ppm (μg allergenic protein/g food) according to Poms et al. (2004). Compared with 4 ng/mL of the sandwich ELISA method LOD (Redl et al., 2010), with our method, the obtained LOD is lower or higher in sensitivity.

3.2.3. Mean coefficients of variation of intra-assay and inter-assay

The calculations of mean coefficients of variation of intra-assay and inter-assay are as follows (Chen et al., 2021): seven concentrations and blank control of the standard

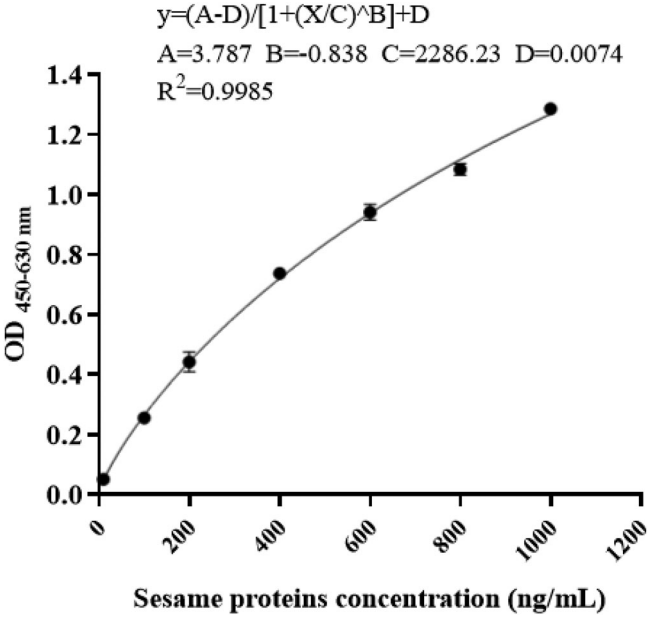

Figure 3. Standard curve of sesame sandwich ELISA drew by the four-parameter method. Sesame proteins' concentrations are between 10 and 1000 ng/mL. The rat IgG antiserum (capture antibody) diluted 1:10,000 in PBST, the rabbit IgG antiserum (detection antibody) diluted 1:40,000 in PBST, and dilution of HRP enzyme-labelled second antibody diluted 1:20,000 in PBST.

curve were repeated 10 times. The average and standard deviation (SD) of the 10 parallel values were calculated, and then the coefficient of variation (CV) was calculated by average value by dividing SD and multiplying by 100%.

The experiment was carried out for five consecutive days and the standard curves were drawn. The average and SD of five measurements were obtained, and then the CV was calculated by average value dividing SD and multiplying by 100%. The results indicate that the CV of precision is all less than 5.8% and the value of accuracy is less than 13.4%, which revealed high stability and repeatability. (Table 1)

3.2.4. Cross-reactivity of the sandwich ELISA

Cross-reactivity of the sandwich ELISA was determined by analysing food ingredients with proteins homologous to sesame proteins, which may cross-react with sesame protein. Twenty one types of foods were tested for cross-reactivity. After extracting these foods, the protein concentration of foods was measured by the BCA test kit and adjusted to 0.5 mg/mL, which was in the mid-range of standard protein, and the equivalent concentration was measured by sandwich ELISA. Cross-reactivity was measured in equivalent concentration. Cross results of 21 species and sesame are shown as Table 2. All 16 kinds of food ingredients showed extremely low response signals, which indicates that they will not or slightly cross-react with sesame, this result is the same as that of Redl (Redl et al., 2010).

3.2.5. Recovery of sandwich ELISA

To determine the accuracy of the sandwich ELISA, different foodstuffs without sesame were used for measuring matrix effects. The different concentrations of mixtures containing 0.01%, 0.5% or 1% sesame powder were extracted in 1:10 with PBST and diluted to different times from 10 to 10000. At the same time, the sesame extraction diluted to similar times with PBST was compared. By analysing the equivalent protein concentrations in the standard curve of sandwich ELISA, the recovery was calculated by the sesame protein concentration of mixture and multiplying by 100%. The results showed that the recovery rate of biscuits, oatmeal and beef sauce is between 72.42% and 128.52%, the matrix influence of oatmeal is more serious than biscuits and beef sauce (Table 3).

3.3. Evaluation of matrix effects

The biscuit food model (including the positive samples with different spiked amounts before or after baking) was crushed with a grinder and extracted by PBST, and diluted

Table 1. Mean coefficients of variation of intra-assay and inter-assay of different sesame proteins.

	Intra-assay		Inter-assay[a]	
Sesame proteins (ng/mL)	Mean	CV (%)	Mean	CV (%)
10	0.061 ± 0.003	4.21	0.059 ± 0.007	12.22
100	0.323 ± 0.015	4.57	0.290 ± 0.043	14.78
200	0.565 ± 0.021	3.65	0.502 ± 0.042	8.36
400	0.821 ± 0.033	4.00	0.803 ± 0.078	9.69
600	1.032 ± 0.032	3.12	1.043 ± 0.087	8.36
800	1.213 ± 0.049	4.12	1.203 ± 0.065	5.39
1000	1.282 ± 0.052	4.07	1.382 ± 0.058	4.15

Results are expressed as means ± SD ($n = 3$).
[a]Inter-assay was performed every 2 days for 10 sequential days.

Table 2. The cross-reactivity of food ingredients analysed by sesame sandwich ELISA.

Food ingredients	Sesame proteins (ng/mL)	Cross-reactivity rate
Peanut	0.0024	0.48%
Walnut	0.0028	0.56%
Hazelnut	0.0029	0.58%
Pistachio	0.0028	0.56%
Almond	0.0030	0.60%
Wheat	0.0022	0.44%
Soybean	0.0020	0.40%
Red bean	0.0019	0.38%
Mung bean	0.0025	0.50%
Black bean	0.0022	0.44%
Kidney bean	0.0026	0.52%
Mango	0.0018	0.36%
Tomato	0.0022	0.44%
Peach	0.0025	0.50%
Yellow croaker	0.0017	0.34%
Trubot	0.0018	0.36%
Sea bass	0.0020	0.40%
Conch	0.0020	0.40%
Shrimp	0.0019	0.38%
Egg	0.0020	0.40%
Milk	0.0021	0.42%

Result is equivalent to the concentration of sesame proteins (ng/mL).

in different multiples for ELISA detection. The results are shown in Table 4, the corresponding concentration of the sample can be obtained according to the standard curve, and the original concentration of the sample can be calculated.

Comparing the test results of adding sesame allergens before and after processing can get the influence of the food matrix on the test, whether the concentration is high or low. The detection concentration of roasted sesame seeds added to the blank biscuits is higher than that of the processed biscuits after adding the sesame seeds; it shows that the processed matrix has a negative impact on the detection of allergenic proteins, which will reduce the rate of protein detection.

Comparing the detection results of raw and roasted sesame seeds in blank samples, we can get the effect of thermal processing on the detection in the food matrix. Roasting will reduce the detection amount of sesame in the food matrix, which is lower than the detection concentration of raw sesame in the food matrix, but the effect seems to have nothing to do with the additional amount; there is no correlation between the detection results obtained with different addition amounts (5, 50, 500 mg/kg) and the additional amount.

Comparing the samples of different dilution multiples, it is found that the detection effect of the sample diluted 100 times is the most effective, which can achieve the maximum detection efficiency. When the sample was not diluted, the food model with three added concentrations could not detect higher absorbance values. When the sample is diluted 10 times, the detection concentration of the sandwich ELISA for

Table 3. Average recovery rate of sesame proteins in three different food matrices.

	Biscuits		Oatmeal		Beef sauce	
Spiked level (mg/g)	Mean recovery (%)	CV (%)	Mean recovery (%)	CV (%)	Mean recovery (%)	CV (%)
10	112.21	6.77	83.16	4.06	95.27	4.95
5	119.59	5.04	79.98	4.81	91.67	3.67
0.1	128.52	9.12	72.42	3.03	87.55	5.18

Table 4. ELISA results obtained with different spiked amounts and different spiked methods.

		Different concentrations of raw sesame powder added before roasting	Different concentrations of toasted sesame powder added after roasting	Different concentrations of raw sesame powder added after roasting
1 Fold diluted	Blank	0.008		
	5 ppm	0.002	0.010	0.006
	50 ppm	0.010	0.016	0.010
	500 ppm	0.046	0.060	0.036
10 Fold diluted	Blank	0.660		
	5 ppm	0.242	0.760	0.399
	50 ppm	0.354	1.177	0.610
	500 ppm	0.924	4.953	2.122
100 Fold diluted	Blank	1.340		
	5 ppm	1.540	1.560	2.240
	50 ppm	3.620	3.410	3.960
	500 ppm	11.580	9.390	13.810
1000 Fold diluted	Blank	–		
	5 ppm			
	50 ppm			
	500 ppm			

In the second column, "ppm" represents mg sesame/kg.
The data unit is mg sesame/kg; "–" represents the results are not within the range of the standard curve, the positive value cannot be detected.

biscuits can only reach the detection limit of 50 mg sesame/kg. When the sample is diluted 100 times, the method can detect a sample of 5 mg sesame/kg, sesame is detected to the greatest extent, and the influence of the food matrix is reduced accordingly. If the dilution multiple is too small, the negative impact on the matrix will be greater, hindering the detection of sesame protein. If the dilution factor is too large, the protein concentration will exceed the detection linear range of the method, and the value cannot be read.

In sum, based on the ELISA method established above to explore the food matrix effect, it is found that the matrix effect (for biscuits) has different effects on samples with different concentrations. There is currently no robust and reliable method in the market to eliminate matrix effect; however, good results can be obtained by a suitable dilution.

Since there is still a conversion of sesame seeds to sesame protein, the absorbance value of the extract obtained by directly adding sesame seeds is much smaller than adding sesame protein. Sandwich ELISA is the same as other measuring methods to determine the concentration of protein. Only the protein is extracted and the soluble protein can be detected. As a result, it is particularly important to increase the content of the soluble protein.

3.4. The influence of different pre-treatment methods on sandwich ELISA detection

3.4.1. Influence of buffer, additive and thermal processing on soluble protein concentration

By comparing the protein concentration of raw sesame and thermal processed sesame in different buffers, it was conceived that different buffers have different extraction efficiencies for different sesame processing methods. For buffers 1 and 2, the extraction efficiency of roasted sesame is higher than that of raw sesame; however, for buffer 3, the extraction efficiency of roasted sesame was lower than that of raw sesame. However, for different

processing methods of sesame, the extraction efficiency of buffer 3 is much higher than that of buffers 1 and 2, the extraction of buffer 2 has the lowest concentration. When three buffers were used for sesame extraction, the soluble protein concentration of stir-fried sesame was lower than that of raw sesame and roasted sesame, and the protein concentration is shown in Figure 4. Buffer 3 with the highest extraction efficiency was selected for subsequent experiments.

SDS and DTT are the two additives that can improve the dissolution rate of protein. The literature reported the use of these two additives to improve protein dissolution rate and their application in ELISA (Lasekan & Nayak, 2016; Watanabe et al., 2005). After adding the surfactant SDS and the denaturant DTT to the buffer 3, the sesame seeds of different processing methods were extracted. It was found that the surfactant and the denaturant had a greater influence on protein extraction. The protein concentration of three different processing methods of sesame seeds extracted with SDS buffer is about

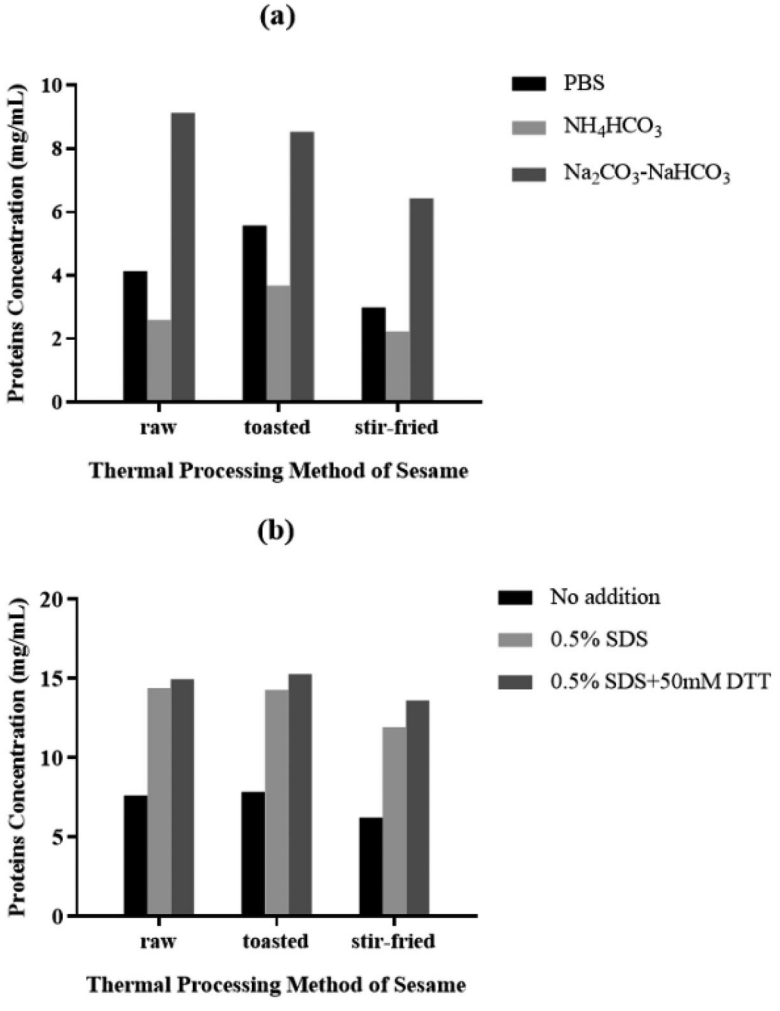

Figure 4. Protein concentration analysis of raw and processed sesame extracts by a different buffer (a) and a different addition (b). Additives in (b) are used on the basis of Na_2CO_3-$NaHCO_3$.

twice as high as that of the buffer without additives and in the buffer with SDS and DTT is compared with the buffer with SDS only. The protein concentration was not significantly increased, even for raw sesame, the protein concentration of the extract with SDS and DTT was lower than that of the extract with SDS only. A similar result was reported that SDS obviously increased the recovery of soluble proteins from raw food without other reducing agent (Watanabe et al., 2005).

3.4.2. Influence of buffer, additive and thermal processing on sandwich ELISA

The established sandwich ELISA method was used to detect the different processing methods of sesame extracts extracted with different buffers and additives. The results in Figure 5(a) were similar to the protein concentration results; the different sesame extracts obtained in buffer 3 still resulted with the highest concentration in the three buffers, which were significantly higher than the extracts obtained from

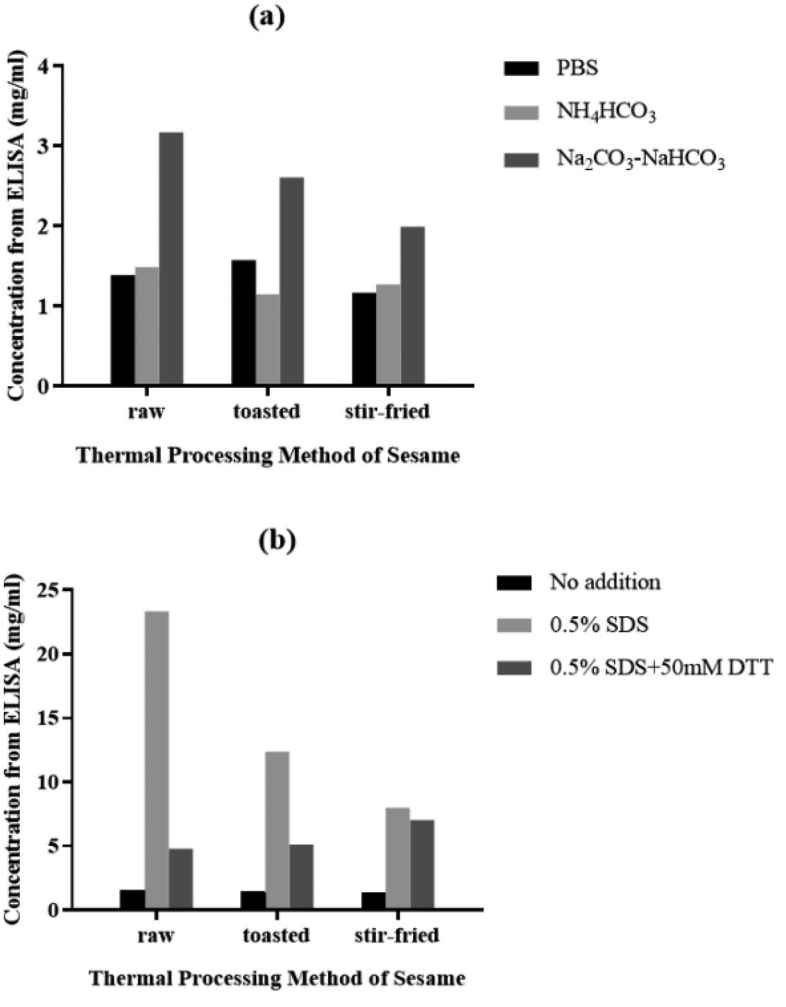

Figure 5. Sandwich ELISA analysis of raw and processed sesame extracts by a different buffer (a) and a different addition (b). Additives in (b) are used on the basis of Na_2CO_3-$NaHCO_3$.

the other two buffers. As for additives (Figure 5(b)), although the protein concentration results show that the two extracts with SDS only and SDS and DTT have similar protein concentrations, in the sandwich ELISA, the sesame extract obtained by the buffer adding SDS only has a higher concentration than adding SDS and DTT; the former is more than twice as much as the latter. An egg protein ELISA kit that used an extraction buffer containing surfactant (SDS) and reducing agents (2-ME) has shown higher protein recovery from foods containing surfactants (Watanabe et al., 2005). In addition, the detection rate of raw sesame in the same extract was much higher than that of roasted sesame and stir-fried sesame and the detection rate of fried sesame was the lowest in the sandwich ELISA.

By comparing the soluble protein concentrations measured by the Coomassie brilliant blue method and the sandwich ELISA method, it was conceived that the two have great differences in the detection results of the additives. In the Coomassie brilliant blue method, the protein concentration obtained from the extraction of SDS and DTT added was higher than the one with only SDS, but in the sandwich ELISA test, the result was the opposite. This may be due to the denaturant DTT interfered with the sandwich ELISA detection and inhibited the antigen-antibody binding epitope, which led to the weakening of the binding site signal, indicating that the buffer solution, containing only the surfactant SDS, can achieve the best detection effect in the sesame sandwich ELISA detection method.

4. Conclusions

The polyclonal antibody sandwich ELISA detection method, based on sesame multi-component proteins, was established and a pre-treatment method suitable for the ELISA method was fabricated. This method can detect sesame protein limit to a Nano gram (ng) level, which is more sensitive than the indirect method and the previous sandwich method. However, the ELISA method showed negative results for the extraction of sesame in food matrix, such as biscuits. In our experiments, this method showed a high sensitivity, precision and stability. We conclude that this sandwich ELISA has a great potential and is a very reliable method that can be used to detect trace amounts of sesame proteins in various commercial foodstuffs.

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