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Short Communication

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Rapid and sensitive real-time PCR-DNA based method to detect *Arachis hypogea* allergens in various food samples

Abstract

Peanut allergy is life threatening health concern that caused by allergenic proteins termed as *Arachis hypogea* that present in peanut. The most fatal allergic reaction to peanuts is anaphylaxis that is harmful to the individuals who are allergic to peanut allergens. Sometimes, traces of peanut allergens contamination are also occurred during food processing. Hence, it is very much important to detect peanut allergen in food products and our proposed study was carried out to detect peanut allergens (*Arachis hypogea* allergen) in various processed and unprocessed foods using real-time Polymerase Chain reaction (real-time PCR) technique as a rapid and sensitive method. First, DNA was extracted from the food samples using commercially available DNA extraction kit and further quantity & quality of the extracted DNA were tested. The extracted DNA was further observed for amplification to test the presence of peanut allergens in chosen food samples to detect *Arachis hypogea* allergen (peanut allergen) positive food samples. The sensitivity and specificity of proposed method was assayed for its limit of detection which was found to be 0.01% and obtained efficiency was 101.2%. Spiking of peanut powder in maize flour was performed at different concentrations.

Keywords: peanut allergen, Delhi-India, DNA isolation, real time-PCR, *Arachis hypogea*, qPCR

Volume 11 Issue 2 - 2023

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Received: May 08, 2023 | **Published:** May 17, 2023

Abbreviations: PCR, polymerase chain reaction; qPCR, quantitative polymerase chain reaction; LOD, limit of detection; ELISA, enzyme-linked immunosorbent assay; LAMP, loop mediated isothermal amplification; RPA, recombinase polymerase amplification

Introduction

Peanut (*Arachis hypogea*) is a leguminous crop and known as the groundnut. It is consumed worldwide for its edible seeds and oil having best nutritional qualities. Peanut allergy is a serious public health issue to those populations who are allergic to peanut allergenic proteins. The major other food allergens are also reports in various preparations of milk, egg, fish, crustacean shellfish (e.g. crab, lobster, and shrimp), tree nuts (e.g. almonds, walnuts, and pecans), wheat, and soybean including peanut which account for total 90% allergenic food reactions.[1–3] Among them, peanut allergy is found to considered more fatal because of its prevalence, persistence and potential for a severe allergic reaction that include anaphylaxis symptoms like itchiness, sneezing, vomiting, diarrhoea, hives, drop in blood pressure, asthma attack and even cardiac arrest. Anaphylaxis is a life-threatening due to peanut allergen caused allergic reaction that results type-I hypersensitivity reaction of the immune system in affected individuals. At present, eighteen peanut allergens have been confirmed ranging from Ara h 1 to Ara h 18. Identified major peanut allergens are Ara h 1 and Ara h 3 that belongs to the cupin superfamily of proteins, and Ara h 2 and Ara h 6 which belongs to prolamin superfamily.[4–7] Hence, various analytical methods have been proposed to detect peanut allergens in food samples like Enzyme-linked Immunosorbent assay (ELISA), Mass spectrometry and protein based method which are reports efficient, precise and sensitive method although heat treatment made during food processing may have a significant impact on the conformation and quality of the proteins that usually interfere in detection of peanut allergens along with other food allergens.[8–12] So, therefore, DNA-based methods have been considered as most rapid, highly sensitive and allergen specific approaches for identifying allergens in food products as compared to protein-based methods or other reported methods. Hence, we proposed DNA based method to detect peanut allergens in different foods by using real-time PCR as a rapid and sensitive method.

Materials and methods

Sample collection

We were procured the different 17 food samples like cookies, protein cookie, chocolate, fruit cakes, cake icing, potato chips, schezwan sauce, aloo bhujia, badam pak and kaju katli randomly from the local market of Sarita Vihar, New Delhi, India and Bhajanpura, North-east Delhi, India.

DNA extraction

The DNA was extracted from the various chosen 17 food samples using the commercially available DNA extraction kit (DSS-DNEUS-011, DSS Imagetech Pvt Ltd., India). 200 mg of food sample was weighed and prepare food sample test solution in 1.5 ml tube by adding 500 µL of lysis buffer and vortexed for 2-3 min, and 15 µL proteinase-K was added in all vortexed test samples and incubated at 65°C for 30 min. After incubation, test food samples were centrifuged at 10,000 rpm for 10 min. After centrifugation, the supernatant was collected in another tube and 300 µL of DNA binding buffer was added followed by mixing. Then, the mixtures were passed through spin column and column was washed with washing buffer 1 and washing buffer 2 respectively. Finally, DNA was eluted in 50 µL of elution buffer for much chosen samples. The quality and quantity of the isolated DNA were checked by Nanodrop (Thermo Scientific) at 260/280 nm. The obtained DNA was stored at -20°C until further their use.

Real-time PCR setup

Peanut allergens were identified in various test food samples by using commercially available kit (DSS-PN-011, DSS Imagetech Pvt Ltd., India). The kit is based on quantitative real-time PCR (qPCR) SYBR protocol. The quantification was carried out by using 16 well Open qPCR real time systems (ChaiBio, USA). For this, DNA from all the chosen test food samples was diluted to make 50 ng/µL and qPCR reaction were performed in 20 µL reactions consisting of 10 µL of SYBR green Dye, 5 µL of DNA and 0.6 µL of primer mix (forward and reverse). The final volume was made up by nuclease free water and all reactions were run in triplicate, and three biological replicates. A positive test control and negative test control reaction provided in kit were set in each batch of amplification in each set of experiment. The thermal condition was used following initial denaturation at 95ºC for 1 min, 40 cycles (denaturation at 95 ºC for 15s, annealing at 60ºC for 1 min). Melting curve analysis was performed at 60ºC up to 95ºC with a 0.5ºC/s transition rate. The specificity and integrity of PCR products was ensured through the melt curve analysis.

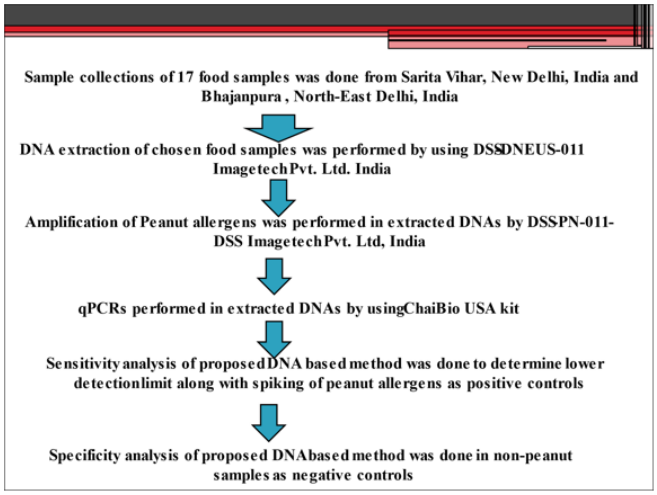
Sensitivity analysis

The sensitivity test or limit of detection (LOD) of the assay was also performed using real-time PCR for the detection of peanut allergens in various chosen food samples. For this the DNA (50 ng/µL) was diluted in different dilutions: 100%, 10%, 1%, 0.1%, 0.01%. The DNA was serially diluted. The total volume was 50 µL. The qPCR reaction was performed as per above dilutions using the same reaction setup and thermal profile. Spiking of peanut powder was performed in maize flour. We generated mixtures with final weight of 200 mg that ranged from 1, 5, 10, 20, 30, 40, 50 mg of peanut powder in maize flour.

Specificity analysis

The specificity of proposed qPCR-DNA based assay was analyzed by amplification of the DNA isolated from a variety of non-peanut raw foods includes cashew, hazelnut, almond, sesame, pistachio, mustard, soya, milk, maize and wheat.

Experimental design of proposed DNA based method



Results

DNA extraction results

Extracted DNA in all chosen 17 food samples for detecting peanut allergens were tested for their quantity and quality by using Nanodrop (Thermo Scientific) at 260/280 nm to determine good yield and highly purified DNA.

Amplification of identified peanut allergen

We have amplified the positive control provided in the peanut allergen detection kit (DSS-PN-011, DSS Imagetech Pvt Ltd., India). DNA extracted from raw peanut was also amplified to validate the kit and the amplification peak was shown in Figure 1 and the respective melt curve shown in Figure 2. Out of the 17 food samples only 6 food samples (Cookie I, Protein cookie, Potato chips I, Aloo bhujia, Schezwan sauce and Badam pak) were tested for presence of peanut allergen (Table 1, Figure 3).

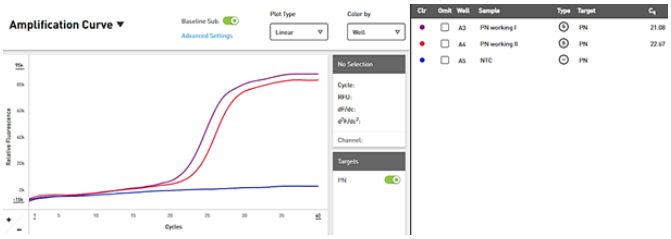


Figure 1 Amplification peaks for identified peanut allergens.

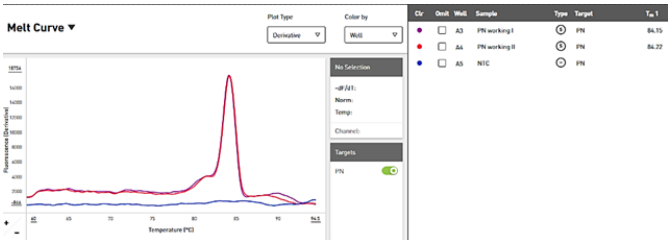


Figure 2 Melt curve for identified peanut allergens.

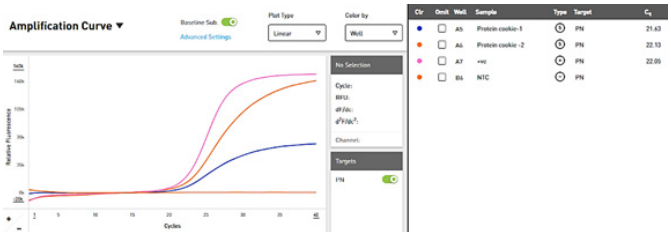


Figure 3 Amplification peaks of the identified peanut allergens in protein cookie food sample.

Table 1 Observations of chosen food samples that analyzed for the presence of peanut allergens

S.No	Food samples	qPCR results
1	Cookie I	Positive
2	Cookie II	Negative
3	Protein cookie	Positive
4	Cake icing I	Negative
5	Cake icing II	Negative
6	Chocolate I	Negative
7	Chocolate II	Negative
8	Chocolate III	Negative
9	Potato chips I	Positive
10	Potato chips II	Negative
11	Potato chips III	Negative
12	Aloo bhujia	Positive
13	Schezwan sauce	Positive
14	Fruit cake I	Negative
15	Fruit cake II	Negative
16	Badam pak	Positive
17	Kaju katli	Negative

Citation: Kondal V, Singh KP, Rani K. Rapid and sensitive real-time PCR-DNA based method to detect *Arachis hypogea* allergens in various food samples. *J Bacteriol Mycol Open Access*. 2023;11(2):82–85. DOI: 10.15406/jbmoa.2023.11.00349

Sensitivity analysis

Sensitivity analysis was performed to determine the limit of detection by using 50ng/µL DNA that was serially diluted up to 0.01%. Observed amplification peaks were shown in Figure 4, melt curve was shown in Figure 5 and the standard curves were shown in Figure 6. The details of the qPCR cycle threshold values (Ct values) and melting temperature were shown in Table 2. Furthermore, we have also checked the sensitivity of the assay by spiking the maize flour with peanut powder and it was obtained in the spiked sample from 10 mg as positive controls.

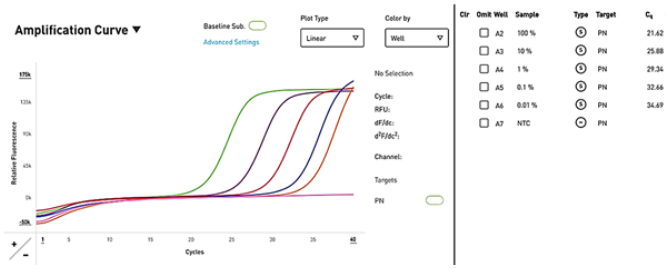


Figure 4 Amplification peaks of the lower detection limit of real-time PCR-DNA method.

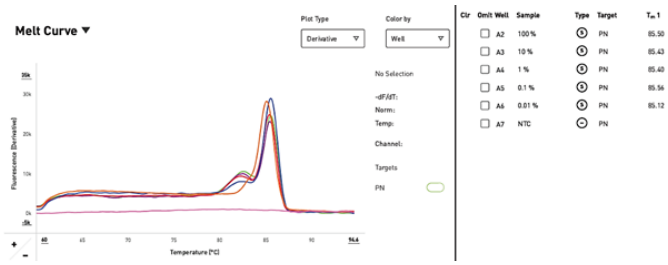


Figure 5 Melt curve of lower detection limit of real-time PCR-DNA method.

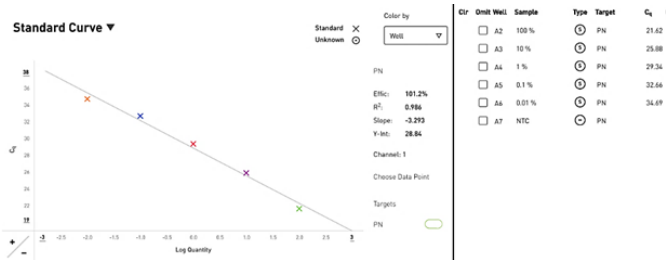


Figure 6 Standard curve of the lower detection limit of real-time PCR-DNA method.

Table 2 Observed qPCR cycle threshold values (Ct values) and melting temperature (Tm) of lower detection limit of real-time PCR-DNA method

Dilutions of the DNA	qPCR cycle threshold values (Ct values)	Tm value
100%	21.62	85.5
10%	25.88	85.43
1%	29.34	85.4
0.10%	32.66	85.56
0.01%	34.69	85.12

Specificity analysis

Identified amplification peaks in isolated DNA from the non-peanut food samples was found to 100% specific for peanut allergen that performed by kit as negative controls (Table 3).

Table 3 qPCR results of non-peanut food samples tested for specificity of qPCR-DNA method

S.No	Food samples	qPCR test results
1	Cashew	Negative
2	Hazelnut	Negative
3	Almond	Negative
4	Sesame	Negative
5	Pistachio	Negative
6	Mustard	Negative
7	Soya	Negative
8	Milk	Negative
9	Maize	Negative
10	Wheat	Negative

Discussion

Extracted DNA in all chosen 17 food samples for detecting peanut allergens were tested for their quantity and quality which was showed good yield and highly purified DNA. Amplification of extracted DNA from raw peanut as positive controls was done and got the positive results for observed amplification peak (Figure 1) and the respective melt curve (Figure 2). Out of the 17 food samples only 6 food samples (Cookie I, Protein cookie, Potato chips I, Aloo bhujia, Schezwan sauce and Badam pak) were found to be positive for peanut allergen (Table 1, Figure 3) whose results were comparable with previous findings.[12,13]

Sensitivity analysis was performed to detect the limit of detection by using 50ng/µL DNA and observed lower detection limit of the assay was 0.01% along with observation of amplification peaks (Figure 4), melt curve (Figure 5) & standard curve (Figure 6). Results of observed qPCR cycle threshold values (Ct values) and melting temperature values (Tm values) were found to similar with previous observations.[13,14] Identified amplification peaks in isolated DNAs of non-peanut food samples was found to 100% specific for peanut allergens confirmed as negative controls (Table 3).[15,16]

Conclusion

Observed amplifications of extracted DNAs were done in all 17 chosen food samples to detect *Arachis hypogea* allergens (peanut allergens) positive food samples. Among them, 6 food samples were found peanut allergen positives (Cookie I, Protein cookie, Potato chips I, Aloo bhujia, Schezwan sauce and Badam pak). The sensitivity and specificity of proposed method was found to be 0.01% and obtained efficiency was 101.2%. Peanut (*Arachis hypogaea*) contains allergenic proteins and reported fatal to the sensitised population. Contamination of peanut allergens in food preparation can occur accidently while processing food or peanut traces mixed in foods to enhance the taste of food preparations. But it must be indicated well on food packet or container ingredient labelling or mentioned by food companies' authorities to prevent accidental consumption by allergic populations. Hence, prevention of peanut caused allergic reaction is to be done by avoiding accidental exposure to peanut allergens by using rapid, convenient, and accurate assay DNA based methods to detect peanut allergens in processed food as compared to protein based other prevailed methods. Nucleic acid amplification methods could further play indispensable role in the future due to its potent lower detection limit and antigen specificity to detect very low concentration of peanut allergens in processed foods. The digital PCR DNA (nucleic acids) based method would get more attention for its rapid quantification ability and cost effective simple device including isothermal amplification such as DNA hybridization based

method, LAMP (Loop mediated isothermal amplification) and RPA (recombinase polymerase amplification) would offer significant breakthrough for on-site detection of food allergy in processed food preparations or samples before selling or consuming commercial food products.

Acknowledgments

I would like also to express my cordially appreciation to Amity University Uttar Pradesh, Noida, India.

Conflicts of interest

The author declares there are no conflicts of interest.

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