

Analytical Methods

Development of loop-mediated isothermal amplification (LAMP) assays for the rapid detection of allergic peanut in processed food

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ABSTRACT

Peanut is a widely and common used in many cuisines around the world. However, peanut is also one of the most important food allergen for causing anaphylactic reaction. To prevent allergic reaction, the best way is to avoid the food allergen or food containing allergic ingredient such as peanut before food consuming. Thus, to efficient and precisely detect the allergic ingredient, peanut or related product, is essential and required for maintain consumer's health or their interest. In this study, a loop-mediated isothermal amplification (LAMP) assay was developed for the detection of allergic peanut using specifically designed primer sets. Two sets of the specific LAMP primers respectively targeted the internal transcribed sequence 1 (ITS1) of nuclear ribosomal DNA sequence regions and the *ara h1* gene sequence of *Arachia hypogaeae* (peanut) were used to address the application of LAMP for detecting peanut in processed food or diet. The results demonstrated that the identification of peanut using the newly designed primers for ITS 1 sequence is more sensitive rather than primers for sequence of *Ara h1* gene when performing LAMP assay. Besides, the sensitivity of LAMP for detecting peanut is also higher than the traditional PCR method. These LAMP primers sets showed high specificity for the identification of the peanut and had no cross-reaction to other species of nut including walnut, hazelnut, almonds, cashew and macadamia nut. Moreover, when minimal 0.1% peanuts were mixed with other nuts ingredients at different ratios, no any cross-reactivity was evident during performing LAMP. Finally, genomic DNAs extracted from boiled and steamed peanut were used as templates; the detection of peanut by LAMP was not affected and reproducible. As to this established LAMP herein, not only can peanut ingredients be detected but commercial foods containing peanut can also be identified. This assay will be useful and potential for the rapid detection of peanut in practical food markets.

1. Introduction

Peanut (*Arachia hypogaeae*) belongs to the botanical family of *Leguminosae*, it also to be called groundnut, earthnut and monkey nut which is a very widely and common used as food ingredient or in many cuisines around the world (Bewley and Black, 1994). At present, in the world, United States, China and India produce most of portion of peanut production. Especially on Americans, over than one million tons approximately has consumed annually for manufacturing peanut butter and its related products (Peanut Inst, 2005). In addition to high content of plant oil and protein supplied for food production, peanut also contains high nutritional content with biological activities such as flavonoids, tocopherols, resveratrol as well as phytosterols for use as supplementary to prevent disease and maintain health (Tuberoso, Kowalczyk, Sarritzu, & Cabras, 2007; Venkatachalam and Sathe, 2006).

Therefore, Peanut is considered as one of important economic crop for application on human benefit. Regardless of its dietary or nutritional value, aflatoxin contamination and allergic characteristic of peanut has long been recognized as major two safety issues in food industry (Wen, Borejsza-Wysocki, DeCory, & Durst, 2007).

Nowadays, total thirteen proteins, Ara h1 to h13, showing allergic characteristics to human in peanut were reported in positive list system by International Union of Immunological Societies (IUIS) Allergen Nomenclature Sub-committee (<http://www.allergen.org/>) of World Health Organization (WHO). Especially on Ara h1 and Ara h2, approximately 65% and 71% of peanut-induced anaphylactic reaction were caused by Ara h1 and Ara h2, respectively (Montserrant et al., 2015). As to allergic characteristic of peanut, the best way to prevent peanut-induced anaphylactic reaction is not consumed the peanut or peanut-related product for the patient. Thus, food labels are relied on

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for the most consumers to manage specific ingredients in their diet. Besides, the related ingredient information on peanut presented in their meal also should be mentioned or described by the manufacturer for reducing the uncertainty of consumer and to guarantee consumers' interests (Vemula, Gavaravarapu, Mendu, Mathur, & Avula, 2014).

To ensure the allergic peanut component in the present of diet, some well-developed detection methods have been reported in practical condition. These techniques for peanut detection were performed basing on either protein or DNA target (Poms, Klein, & Anklam, 2004; Wen et al., 2007). Using immunological assay, the total peanut protein or specific peanut allergen was examined for the evaluation of the presence of peanut in the diet. As to DNA-based assay, a specific DNA region of peanut usually was chosen for amplification using polymerase chain reaction (PCR)-based technique. Albeit protein-based assays, enzyme-linked immunosorbent assay (ELISA), is most frequently used methods with high precision and easy operation for standardizing detection procedure (Poms et al., 2004). However, the higher stability of DNA target than protein under harsh extraction condition it still has a good potential such as higher efficiency, sensitivity as well as specificity for methodological development.

In recent years, a well-established nucleic acid amplification method termed loop-mediated isothermal amplification (LAMP) first reported by Notomi et al. in 2000 was alternatively applied for the diagnosis of biomaterial (Notomi et al., 2000). Four to six primers are used to specifically recognise six to eight distinct DNA sequences on the target gene. *Bst* DNA polymerase with the activity of DNA strand displacement is used, and the LAMP reaction is performed under isothermal conditions. Due to DNA denaturation at a high temperature is not required during the LAMP reaction, thus, a thermal cycler is not strict needed. Presently LAMP is comprehensively applied for detection or diagnostic purposes on bio-sample. For instance, many pathogens including viruses, bacteria, fungi, and parasites are rapid verified using the LAMP assay (Adao and Rivera, 2016; Chen, Lee, Lai, & Lai, 2015; Chen, Ma, Qiang, & Ma, 2016; Huang et al., 2010; Woźniakowski and Samorek-Salamonowicz, 2014; Woźniakowski and Tarasiuk, 2015). Other biomaterials such as food involving animal-, plant-based ingredients as well as genetically modified organisms (GMOs) can be applied as detection targets using the LAMP assay (Abdulmawjood et al., 2014; Lai et al., 2015; Lee, la Mura, Allnutt, & Powell, 2009; Lee, Su, Lien, & Sheu, 2017). Therefore, the LAMP method is considered a potential assay for the development of a rapid, sensitive and specific detection on bio-samples.

Notwithstanding, several works have reported for peanut identification using PCR-based assays. Comparing PCR to LAMP, however, only one previous study reported that peanut identification has performed by LAMP assay. Moreover, very few information were provided from previous work (Li, Wang, Ye, He, & Lei, 2012). Some essential parameters such as primer specificity, integrity of the template DNA as well as sensitivity, they did not systematically addressed. In this study, isothermal DNA amplification was developed for the rapid, sensitive and specific identification of peanut. Sequence of *Ara* h1 and internal transcribed spacer (ITS) of ribosomal DNA were respectively used to design LAMP primer for the investigation of primer's specificity and cross-reactivity. Apart from this, the integrity of DNA in peanut or in commercially processed peanut products was also to be investigated to evaluate the feasibility of the developed LAMP method for the detection of peanut. To the best of our knowledge, this is the first report to verify peanut systematically using LAMP assay.

2. Materials and methods

2.1. Nut samples

The nut samples of peanut (*Arachia hypogea*), walnut (*Juglans regia*), hazelnut (*Corylus avellana*), almond (*Prunus dulcis*), cashew (*Anacardium occidentale*) and macadamia nut (*Macadamia integrifolia*)

Table 1
Sequences of LAMP primers used for the detection of the peanut.

DNA regions	Primers	Sequences
ITS1	PEAF3	5' CGCGGAAAGCGCCAA 3'
	PEAB3	5' GCGTTCAAAGACTCGATGGT 3'
	PEAFIP	3' TGGTCACTCGTCGCCCGA-GGAAGCCAAACGTTTCTGCT 5'
	PEABIP	3' TCGGCAACGGATATCTCGGCT-ACGGGATTCTGCAATTCACA 5'
<i>Ara</i> h1	<i>Ara</i> 1F3	5' ATCTTTTCTAACAACTTTGGGAA 3'
	<i>Ara</i> 1B3	5' TACAGCCACGAGTTCAAG 3'
	<i>Ara</i> 1FIP	3' ACAGGTGAGCATCATGTCCAG-GTTATTTGAGGTGAAGCCAG 5'
	<i>Ara</i> 1BIP	3' AGAGATCAAAGAAGGAGCTTTGATG-CCAGTTCCTTTGTGACGA 5'

were collected from local supermarket (Pingtung, Taiwan). The botanical origin of these plant samples were identified by Professor Wen-Te Chang of the China Medical University (Taichung, Taiwan), and these above samples were deposited at the department of food science of National Pingtung University of Science and Technology.

2.2. DNA extraction

Total genomic DNA from nut samples of peanut, walnut, hazelnut, almond, cashew and macadamia nut were extracted by the genomic extraction kit (AxyPrep Multisource genomic DNA miniprep kit, Axygen Bioscience, CA, USA) according to the previous work (Lee et al., 2017). The concentration of extracted plant genomic DNA was determined by spectrophotometer (NanoVue™, GE Healthcare, Piscataway, NJ, USA). Samples were stored at -20°C until required.

2.3. Design of LAMP primers

The LAMP primers (F3, B3, FIP and BIP) was designed for detecting peanut based on the ITS1-5.8S-ITS2 nuclear ribosomal DNA sequence and *Ara* h1 respectively obtained from GenBank (<http://www.ncbi.nlm.nih.gov>) using commercial software of Primer Explorer V3 (<http://primerexplorer.jp>; Eiken Chemical Co., Ltd., Tokyo, Japan). The accession number of ITS1-5.8S-ITS2 nuclear ribosomal DNA sequence of peanut and *Ara* h1 gene of peanut are presented as AY615267 and AF432231, respectively.

2.4. LAMP reaction

The LAMP reaction was performed in a reaction mixture, as described previously (Lee et al., 2017). Briefly, the reaction mixture contained $1 \times$ *Bst* DNA polymerization buffer, 1 U of *Bst* DNA polymerase (New England Biolabs, Frankfurt, Germany), $0.5 \mu\text{M}$ outer primers (F3 and B3 primers each), $4 \mu\text{M}$ inner primers (FIP and BIP primers each), and $200 \mu\text{M}$ dNTPs each. Finally, the different amount of sample's genomic DNA was used to each LAMP reaction. The mixtures were reacted at $60\text{--}65^{\circ}\text{C}$ for 60 min in a heating block (DNA engine, Biorad, CA, USA).

2.5. Detection of LAMP product

DNA electrophoresis was used to detect the formation of LAMP product in terms of the observation of fluorescence illumination, as described in a previous study (Chen et al., 2015). 2% agarose gel was used to electrophoresis and stained by ethidium bromide for observation of the presence of visible DNA bands.

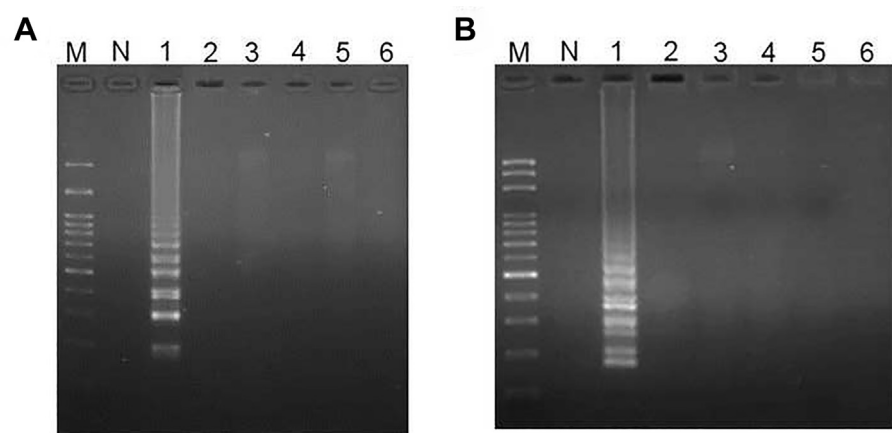


Fig. 1. Primer specificity of the LAMP assay for the identification of peanut. The specificity of ITS-based (A) and *Ara h1*-based (B) LAMP primers used for the detection of peanut. Purified peanut's genomic DNAs were used to perform LAMP. Lanes M and N represent 100 bp of DNA ladder and the negative control, respectively. Lanes 1–6 represent different DNAs: 1, peanut; 2, walnut; 3, hazelnut; 4, almond; 5, cashew; 6, macadamia nut.

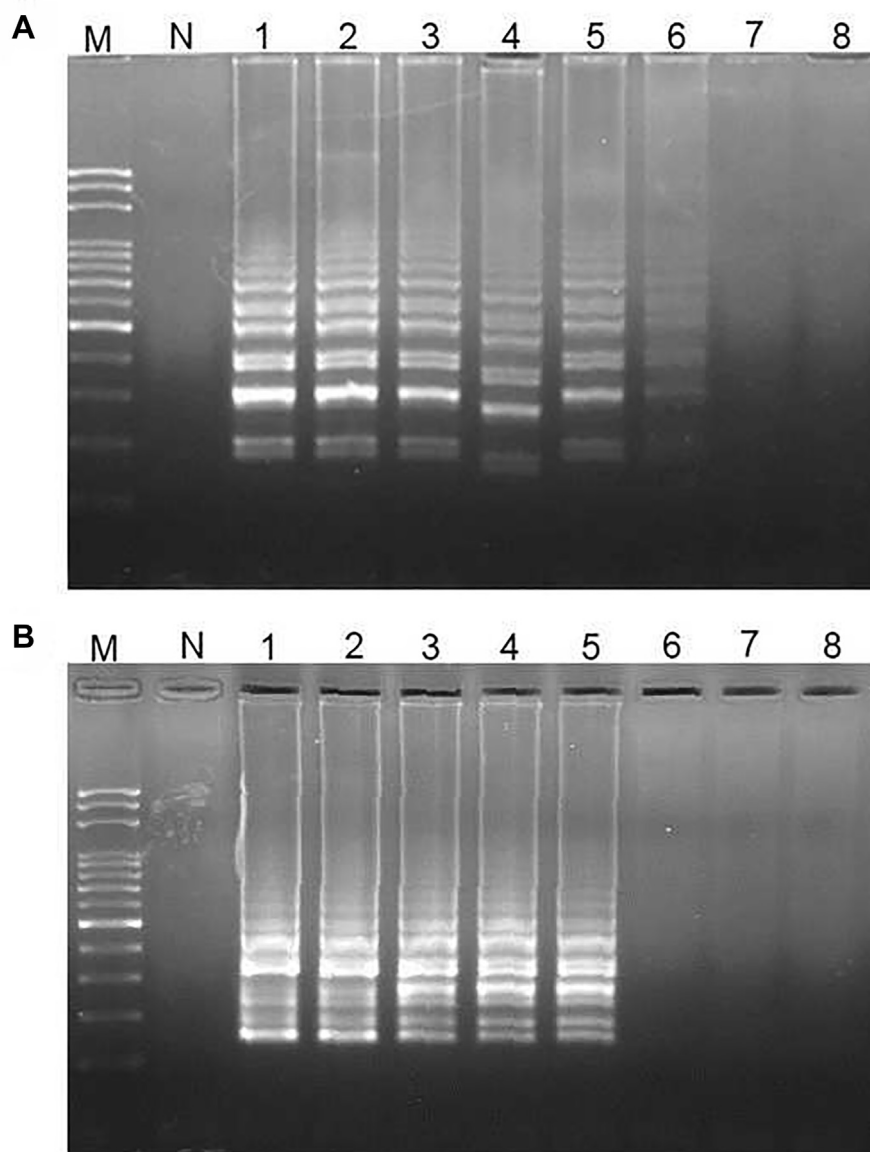


Fig. 2. Reactivity of the LAMP assay for the identification of peanut. The reactivity of ITS-based (A) and *Ara h1*-based (B) LAMP primers used for the detection of peanut mixed with different percentages of the five other DNA of nuts. Lane 1: positive control, Lanes 2–8: 50%, 10%, 5%, 1%, 0.1%, 0.01% and 0% of peanut DNA mixed with DNAs from the five other nuts. Lanes M and N represent 100 bp of DNA ladder and the negative control, respectively.

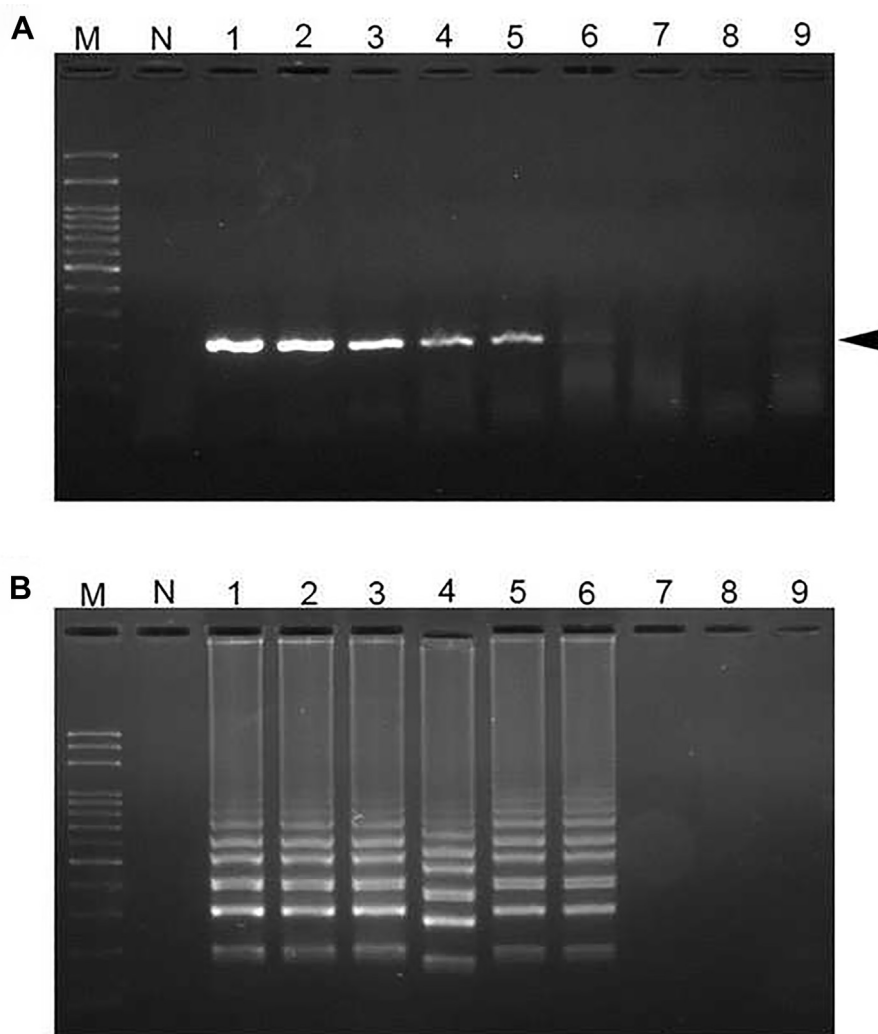


Fig. 3. The sensitivity of specific ITS-based primers used in PCR (A) and LAMP (B) for the detection of peanut. Lanes 1–9 represent the addition of different amounts of peanut DNA: 1, 100 ng; 2, 10 ng; 3, 1 ng; 4, 100 pg; 5, 10 pg; 6, 1 pg; 7, 100 fg; 8, 10 fg; and 9, 1 fg. Lanes M and N represent 100 bp of DNA ladder and the negative control, respectively. Black arrow represents the amplified specific PCR product in the agarose gel.

2.6. PCR

The extracted genomic DNA of peanut was used as template in the polymerase chain reaction (PCR) mixture. PCR was carried out according to previously protocol (Lai et al., 2015) using outer primers (F3 and B3) of ITS-based- and *Ara h1*-based LAMP primers, respectively, as shown as Table 1. The PCR product was subjected to electrophoresis using 2% agarose gel and detected by observation of the presence of visible DNA bands after ethidium bromide staining.

2.7. Preparation of boiled and steamed peanut

Fresh peanut were gained from a local market and crush into pieces and then weighted 20 g for each sample to further treats by boiling and steaming, respectively. For boiling peanut, the peanuts were boiled in hot water with 95 °C for 20, 40, 60, 80, 100 and 120 min, respectively. After cooling in the ice bath, then the cooled peanuts were used to extract genomic DNA according to previous described procedure for performing LAMP and PCR. As to steaming of peanut, peanuts were steamed using autoclave. The crushed peanut were autoclaved at 121 °C for 20, 40 and 60 min (under pressure of 15 psi), respectively. After autoclaving, the steamed peanuts were subjected to ice bath for cooling then to be used for genomic DNA extraction and performing LAMP and

PCR.

3. Results

3.1. Development of LAMP assay for the detection of peanut

To detect peanut DNA by LAMP, two sets of LAMP primers, including F3, B3, FIP and BIP, were respective designed to specifically identify the genomic DNA of peanut, based on the *Ara h1* gene and ITS of nuclear ribosomal DNA sequences available in GenBank (<http://www.ncbi.nlm.nih.gov>) (Table 1). No matter *Ara h1*- or ITS-based primer's sequence, when LAMP primers were used for peanut identification, LAMP products were produced if their target DNAs were present in the samples; and revealed a typical pattern of ladder-like DNA fragments on the agarose gel (Fig. 1A, lane 1; Fig. 1B, lane 1). In contrast, with respect to these two LAMP primer sets for the identification of peanut, our results also demonstrated that no LAMP product was detected on an electrophoresed gel in the non-target genomic DNA samples such as other nuts (lanes 2–6 of Fig. 1A; lanes 2–6 of Fig. 1B). Moreover, when any one set of LAMP primers was used, the LAMP reaction was not influenced by the genomic DNAs of peanut mixing other nut's DNA including, walnut, hazelnut, almond, cashew and macadamia nut with different percentages (Fig. 2). When ITS-based

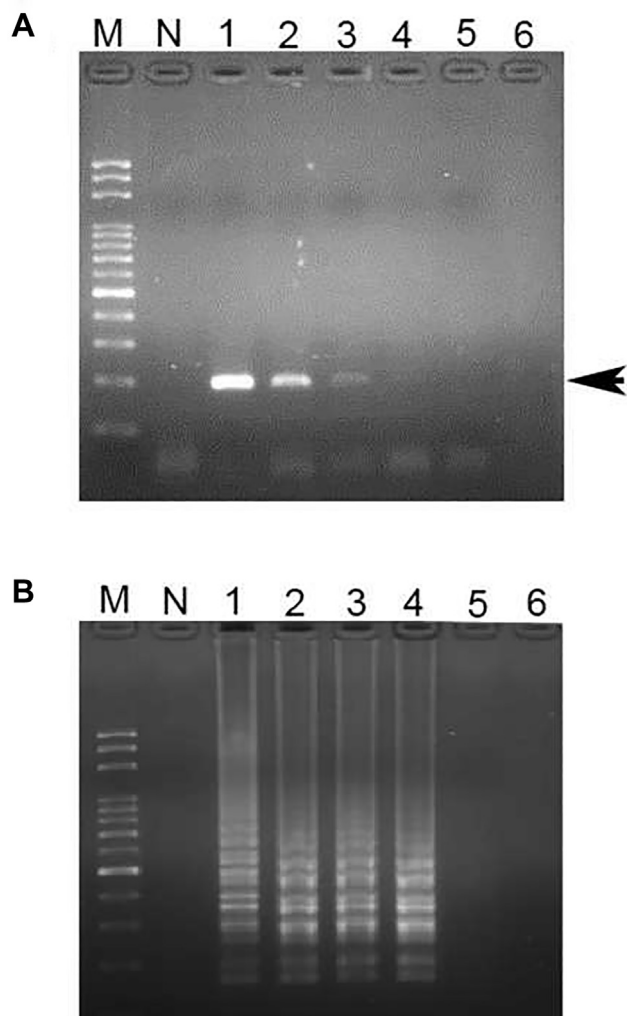


Fig. 4. The sensitivity of specific *Ara* h1-based primers used in PCR (A) and LAMP (B) for the detection of peanut. Lanes 1–6 represent the addition of different amounts of peanut DNA: 1, 100 ng; 2, 10 ng; 3, 1 ng; 4, 100 pg; 5, 10 pg; and 6, 1 pg. Lanes M and N represent 100 bp of DNA ladder and the negative control, respectively. Black arrow represents the amplified specific PCR product in the agarose gel.

LAMP primers were used, at least 0.1% of peanut genomic DNA was required in the total DNA of six kinds of nuts for specifically detection (Fig. 2A). Similarly, *Ara* h1-based LAMP primers maintained their high specificities when at least 1% of peanut DNAs contained in the total DNA of six kinds of nuts, as shown in Fig. 2B. Taken together, these results demonstrate that these two sets of LAMP primers used for the identification of peanut developed herein are specific for the identification of peanut, and have been validated for their use in amplifying peanut target DNAs.

3.2. Sensitivity of LAMP for the detection of peanut

To determine the sensitivity of the LAMP, ITS-based and *Ara* h1-based LAMP primers were respectively used to amplify the target DNAs of peanut. When ITS-based LAMP primers were used, at least 1 pg of peanut genomic DNA was required for performing detection (Fig. 3B). Compared to the peanut PCR primers which specific targeted on ITS sequence, the amount of 10 pg peanut genomic DNA was needed for PCR (Fig. 3A). With respect to the *Ara* h1-based LAMP primers used for the identification of peanut, the detection limits was 100 pg of peanut genomic DNA (Fig. 4B). In contrast, 1 ng of detection limit was determined when *Ara* h1-based PCR primers were used after the

performing PCR (Fig. 4B). All two LAMP reactions were more sensitive than PCR. Taken together, these results indicate that the sensitivities of LAMP methods developed in this work are higher than PCR assays for the identification of peanut DNAs.

3.3. LAMP for the authentication of heat-processed peanut

To evaluate the effect of heating on peanut identification by LAMP assay, peanut was subjected to heat treatment including boiling bath and steam autoclave procedure. when ITS-based LAMP primers were used, Fig. 5B shows that the genomic DNA extracted from peanut following a boiling bath for 20, 40, 60, 80, 100 and 120 min did not influenced the LAMP reaction (Fig. 5B, lanes 1 to lane 6). With regards to the peanut DNA under steam-autoclaved treatment for 20, 40 and 60 min, LAMP products were still revealed following the LAMP reaction (Fig. 5D, lanes 2–4). The PCR results for the detection of peanut DNA were similar to those of LAMP, regardless of whether the template DNA used in the reaction was from boiled or autoclaved peanut (Fig. 5A, lanes 1–6; Fig. 5C, lanes 1–3). Similarly, with respect to *Ara* h1-based LAMP primers, extracted peanut genomic DNAs obtained from the above boiling bath treatments did not amend the performance of the LAMP or PCR (panel B and D of Supplementary Fig. S2). These results indicate that LAMP is well-matched in strength to PCR for the identification of peanut DNAs as shown as panel A and C of Supplementary Fig. S2, respectively. It is worth noting that the specific PCR products or LAMP pattern were not detected in the peanut samples subjected to a steam autoclave procedure for 60 min (lane 3 of panel C and D of Supplementary Fig. S2). In contrast, PCR product and LAMP pattern were only detected in the sample DNA from steam-autoclaved peanut for 20 and 40 min, respectively (lane 1–2 of panel C and D of Supplementary Fig. S2).

3.4. Application of LAMP for the detection of commercial foods containing peanut

To assess whether the LAMP assay developed herein is applicable as a practical tool for the detection of food containing peanut, thirteen commercial foods containing peanut were purchased from a market. The thirteen commercial foods (salted peanut, spicy peanut, wasabi peanut, crispy peanut, peanut candy, meringues peanut, sugar-free peanut butter, peanut butter, spicy peanut bean, peanut pancake, peanut crisp with black sesame, peanut chocolate and wafer with peanut cream) were treated to extract total DNA for use in the LAMP reaction, and two sets of respective peanut LAMP primers were used to authenticate the peanut ingredients. Fig. 6B shows that all commercial peanut foods were detected and the typical gel patterns were showed following LAMP by using ITS-based primers. For the results of PCR, these results were also consistent with those from LAMP detection (Fig. 6A). Similarly, with respect to *Ara* h1-based LAMP and PCR primers, specific amplicon of peanut genomic DNA was detected in the all above commercial peanut foods using LAMP and PCR, respectively (panel A & B of Supplementary Fig. S3). The obtained LAMP results were confirmed by PCR. Taken together, these results suggest that LAMP can be used to rapid and sensitive authenticate peanut containing foods and that LAMP results agree with the labelled formulates of all thirteen commercial peanut foods provided by the manufacturers. Therefore, based on the specificity, sensitivity and validation of the LAMP primers, the practical applications of LAMP can be concluded for use to further authenticate peanut ingredient to provide more precise information of food allergen.

4. Discussion

During the past decade, published literature has stated that LAMP provides a more convenient way to identify organisms including bacteria, fungi, parasites, viruses, animals, and plants (Adao and Rivera,

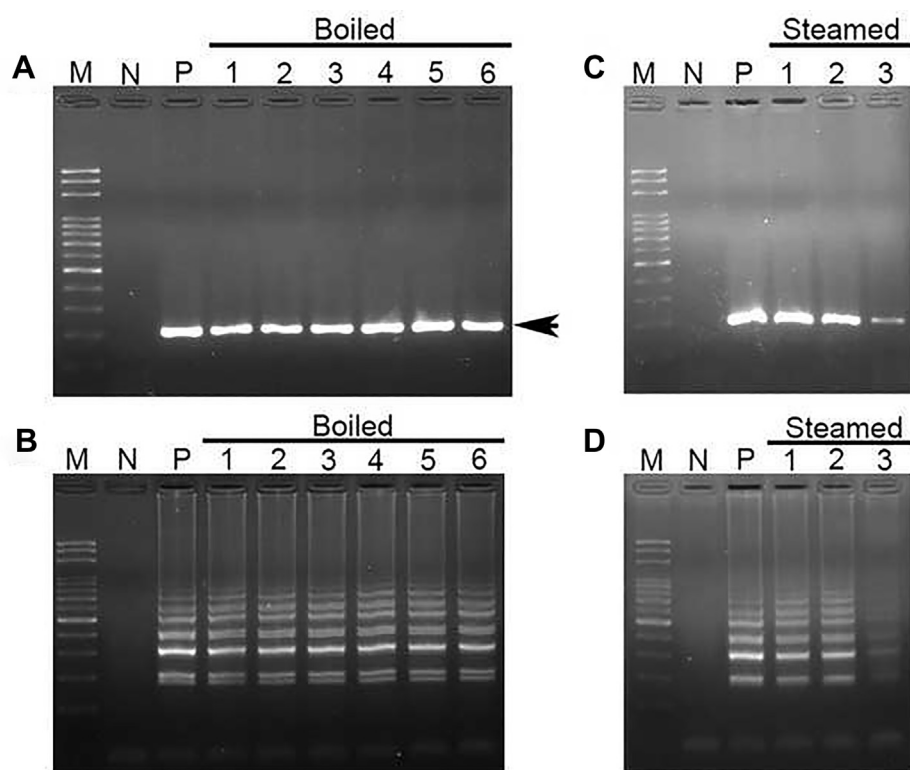


Fig. 5. Analysis of PCR (A, C) and LAMP (B, D) products derived from peanut DNA after boiling and steam autoclaved procedure. When ITS-based PCR and LAMP primers were respectively used, the reactivity of DNA amplification was analysed. Lane M: 100 bp DNA ladder, Lane N: negative control, Lane P: positive control (raw peanut). As to boiling treatment, lanes 1–6 respectively represented that peanut DNA was boiled for 20, 40, 60, 80, 100 and 120 min. For steam autoclaved procedure, lane 1–3 represented the peanut DNA was steamed for 20, 40 and 60 min, respectively. Black arrow represents the amplified specific PCR product in the agarose gel.

2016; Chen et al., 2015, 2016; Huang et al., 2010; Lai et al., 2015; Woźniakowski and Samorek-Salamonowicz, 2014; Woźniakowski and Tarasiuk, 2015). Using DNA polymerase with strand displacement activity, nucleic acid amplification is performed under isothermal condition without a thermal cycler. Thus, LAMP is easier to operate for on-site identification using simple heating with water bath. In this work, the specific aim was to develop a sensitive, specific and rapid method to detect peanut DNA for preventing food allergen. Very few works have systematically described that peanut can be identified using a molecular DNA technique such as LAMP (Li et al., 2012). Thus, further studies of LAMP is needed to satisfy all different conditions if met with some difficulties including the purity of the DNA sample, sensitivity, specificity, or practical application of commercial peanut foods. Even through the weaknesses of PCR for the detection of peanut is continually to be overcome, it is still required to develop an alternative way to more effectively and precisely for use.

For designing specific LAMP primers, in this context, allergen gene, *Ara h1*, of peanut and the sequences of the internal transcribed spacer (ITS) of ribosomal DNA were used. As to *Ara h1* gene, this is most popular target for chosen to perform PCR for the identification of peanut allergen (Wen et al., 2007). The second target for use to peanut identification is ITS of nuclear ribosomal DNA sequences. ITS recently has been recognized as DNA barcode for species identification in the previous studies (Lai et al., 2015; Lee et al., 2017). In our work, *Ara h1* and ITS were therefore to be used for the designation of LAMP primers for the evaluation of relevance when performing LAMP. Here, we successfully developed two sets of LAMP primers such as *Ara h1*-based and ITS-based primers that can be used for the sensitive, specific and rapid identification of peanut. The specificity of LAMP is well-matched in strength to conventional PCR for the identification of peanut DNAs. Moreover, the sensitivity of DNA amplification of LAMP is higher than traditional PCR. The ITS-based primers were more sensitive rather than the *Ara h1*-based primers when performing LAMP assay. It is worth noting that the ITS-based primers exhibited good reproducibility rather than *Ara h1*-based primer when LAMP was performed under 60 °C–65 °C of reaction temperatures. *Ara h1*-based primers were used

for displaying its reproducibility of LAMP on the reaction temperature between 60 and 62 °C (data not shown). This might be the temperature for performing LAMP is very critical for promoting the formation of the loop-mediated secondary DNA structure to initiate the reaction, when *Ara h1*-based primers were used. In Taiwan, there are other two kinds of botanical varieties of peanut, Tainan No. 11 and Black king-kong peanut, were popular consumed dependent on customer's preferences. Using both two sets of LAMP primers in this study, specific amplicon of peanut all can be detected by LAMP (Supplementary Fig. S1). In addition, even further samples from different regions might with genetic variability the LAMP reaction developed herein also to be performed well for the detection of peanut ingredient (Supplementary Fig. S4). As to the sequence specificity, the LAMP primers used in this work they were designed by basing the species-specific and intraspecies-conserved DNA regions. Thus, respect with above further samples (six samples obtained from different countries) testing, the LAMP primers still can be performed for the amplification of peanut amplicon.

In this context, peanut after the boiling and steamed autoclaving treatment were also detected by LAMP when the ITS-based primers were used. Because of the higher amount of template DNA is not required, only a small amount of intact amplicons of the peanut DNA existed, this did not affect the reactivity of the LAMP assay. However, in our cases by using *Ara h1*-based primers, a steam autoclave procedure for 60 min has proved that harsh heat treatment might be result in severe deconstruction of DNA to affect the reactivity of LAMP. Based on this reason, some researchers have reported that the introduction of loop primer sets into the LAMP reaction and the efficiency of LAMP can be improved significantly during DNA amplification (Chen et al., 2015).

Generally speaking, processed commercial foods are seldom to use single ingredient in their formula. Thus, to assess LAMP applicable for peanut DNA identification, more complicate formulation of food ingredient in the processed food should be a challenge to be encountered. Peanut ingredient in commercial foods can be identified by the LAMP method developed herein. Using these validated LAMP primers, even other DNAs obtained from other ingredient were mixed, while the specificity of the LAMP reaction was not altered. These results not only

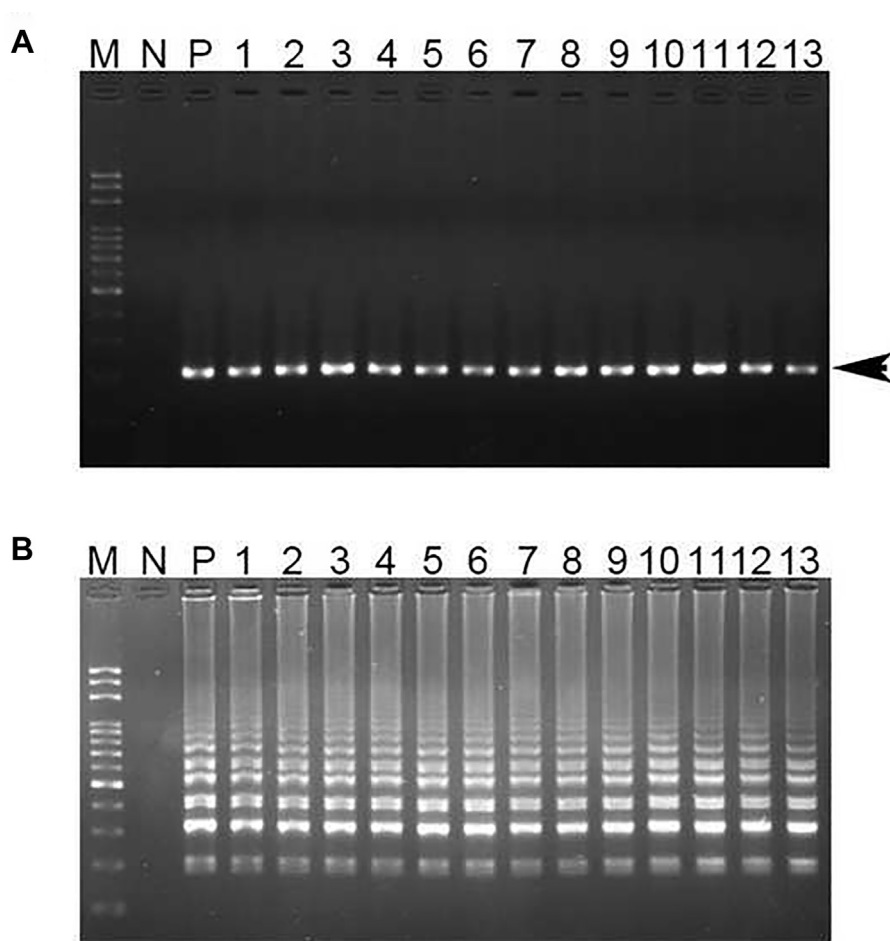


Fig. 6. Electrophoretic analysis of PCR (A) and LAMP (B) products from commercial peanut products. PCR and LAMP assay was performed by using ITS-based primers. Lane M represents 100 bp of DNA ladder, lane N and lane P represents negative and positive control, respectively. Lanes 1–13 represent the sample DNA used for salted peanut, spicy peanut, wasabi peanut, crispy peanut, peanut candy, meringues peanut, sugar-free peanut butter, peanut butter, spicy peanut bean, peanut pancake, peanut crisp with black sesame, peanut chocolate and wafer with peanut cream, respectively.

displayed that the food formulas were in compliance with the food labels provided by the manufacturer, but also showed the use of LAMP for the identification of peanut DNA can be applied. This provides a potential tool for internal auditing to avoid contamination by a manufacturer, even can be used for routine inspections of a food allergen labelling by government authorities (Kao et al., 2004; Lee et al., 2017).

5. Conclusion

In summary, an isothermal condition-based DNA amplification was established in this study for the specific, sensitive and rapid identification of peanut. This method can be applied in processed food for the verification of allergic peanut to prevent allergic reaction.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <https://doi.org/10.1016/j.foodchem.2018.02.124>.

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