



Development and Evaluation of a Loop-Mediated Isothermal Amplification (LAMP) Assay for Rapid Detection of Pistachio (*Pistacia vera*) in Food Samples

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Abstract

Pistachio consumption has increased significantly, mostly due to their potential health effects and their good organoleptic properties. However, pistachio is also an important food allergen and therefore, it is necessary to develop an appropriate method to detect this nut in foodstuffs. In this work, a loop-mediated isothermal amplification (LAMP) assay targeting Pis V 1 allergen coding gene was developed. The developed LAMP assay performed high specificity for pistachio and had no cross-reactions with cashew, mango, walnut, almonds, macadamia nut, hazelnut, peanut, and soybean. The assay can detect at least 10 pg of purified pistachio genomic DNA and 10 mg kg⁻¹ of pistachio nuts in binary mixtures. The autoclave treatment influenced the amplifiability of pistachio; however, this impact was not found after boiling treatment. Practical applicability of the developed LAMP assay has been analyzed by testing different commercial foods and this method may be useful for rapid detection of pistachio in foodstuffs.

Keywords Pistachio · Loop-mediated isothermal amplification · Allergen detection · Commercial foods

Introduction

Belonging to the *Pistacia* genus of the Anacardiaceae family, *Pistacia vera* species is of high economic value due to its fruits, pistachio nuts. Although native to typical countries of Middle and Western Asia, *Pistacia vera* has been cropped widely in Americas, Europe, and Africa (Costa et al. 2019). In 2017–2018, the main producer of pistachio nuts was the USA with 272,292 metric tons (MT), followed by Iran (225,000 MT), Turkey (80,000 MT), Syria (50,000 MT),

and the European Union (15,000 MT) (USDA 2018). Pistachio nuts may have potential health effects on blood lipids, antioxidant, anti-inflammation, blood pressure, glyce-mic control, and weight control, as a result of its high content of healthy components (Dreher 2012). Besides the above nu-tritional benefits, the good organoleptic properties and split shell make pistachio nuts popular worldwide. In 2018, the highest consumption of pistachio nuts was found in the USA (127,434 MT), followed by China (111,500 MT), Turkey (102,800 MT), and the European Union (101,800 MT) (USDA 2018).

Besides being consumed as snack foods, pistachio nuts can also be added as ingredients in various types of food dishes, such as ice cream and confectionery. However, similar to food allergy detected in the consumption of peanut (Boudreau-Romano and Qamar 2018; Wen et al. 2010) and various kinds of tree nuts (Geiselhart et al. 2018; Weinberger and Sicherer 2018), pistachio nuts can also trigger anaphylaxis (Kazuha et al. 2011; Porcel et al. 2006) or even fatality (Bock et al. 2001) as a result of its contained allergens (Costa et al. 2019). Nowadays, five proteins, Pis V 1 to V 5, presenting allergic characteristics in pistachio nuts were officially defined as food allergens by the World Health Organization (WHO, <http://www.allergen.org/>) (Costa et al. 2019; Geiselhart et al.

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2018). Among these allergens, Pis V 4 is recognized as an iron/manganese superoxide dismutase and other four allergens belong to seed storage proteins: Pis V 1 (2S albumin), Pis V 3 (7S globulins or vicilins), Pis V 2, and V 5 (11S globulins or legumins), (Costa et al. 2019; Geiselhart et al. 2018). In respect to its allergy-causing characteristic, strict avoidance of pistachio nuts or pistachio-related product is suggested for the susceptible individuals. Therefore, the presence of pistachio nuts in food must be declared in food labels, which is essential for the sensitive consumers to manage their diet reasonably. However, pistachio nuts may still be contained in food without certain labeling due to the unintentional contamination in food processing. Thus, it is necessary to develop reliable detection methods of the pistachio nuts in food products.

The routine ways to detect food allergens contain protein- and DNA-based strategies, which mainly include the enzyme-linked immunosorbent assay (ELISA) and various types of polymerase chain reactions (PCR), respectively (Prado et al. 2016). ELISA kits detecting pistachio nuts in food products have been available on the market (Liu et al. 2015). However, requiring integral structure or IgE-binding ability of proteins, which can be destroyed during food processing, may be a challenge for protein-based methods (Sanchiz et al. 2018; Vanga and Raghavan 2017). Compared with proteins, the higher stability of DNA makes DNA-based allergen detection methods more promising for processed food products (Holzhauser 2018). Conventional PCR methodology, targeting the COR gene (GenBank accession number: Y07600.2), was designed and applied for detection of pistachio nuts contained in mortadella and 100 mg kg⁻¹ of pistachio nuts can be detected (Barbieri and Frigeri 2006). The ligation-dependent probe amplification (LPA) was used to detect pistachio nuts and nine other food allergens simultaneously and 5 mg kg⁻¹ of pistachio nuts can be detected (Alexandra et al. 2009). At the same time, different real-time PCR systems were designed targeting the specific gene regions of pistachio nuts, which can detect 4 mg kg⁻¹ (Brežná et al. 2008), 0.1 mg kg⁻¹ (López-Calleja et al. 2014), and 10 mg kg⁻¹ (Sanchiz et al. 2016) of pistachio nuts.

Loop-mediated isothermal amplification (LAMP) was first reported in 2000 (Notomi et al. 2000). Targeting six or eight special regions of the DNA template, four or six primers can be obtained. With the help of the strand displacement ability of Bst DNA polymerase, the LAMP assay is carried out at a certain temperature. The amplification of target sequence depends on a repeat of two sequential amplification processes which occur at loop areas composed of the designed primers: elongation of target sequences from a stem loop structure located at the 3'-terminus and then the binding and elongation of new primers to the loop area. As a result of the high activity of the used Bst DNA polymerase, vast amounts of DNA bearing different molecular weight (from low to high) can be obtained in a short time. In less than an hour, approximately 10⁹ copies

of the target region are produced and this DNA amount is higher than that of the conventional PCR (Min et al. 2015). Moreover, LAMP test is performed under a certain temperature and therefore, an expensive thermal cycler may not be necessary. LAMP can be used as an alternative to PCR for disease diagnosis (Seki et al. 2018) or for detection of biomaterials, such as various pathogens including viruses (Song et al. 2018), bacteria (Wang et al. 2019), fungi (Luo et al. 2012) and parasites (Varlet-Marie et al. 2018), food containing animal- (Ran et al. 2016) and plant-based (Lee 2017) components, plant (Focke et al. 2013) and mushroom (Vaagt et al. 2013) species identification, or genetically modified organisms (GMO) (Guan et al. 2010). The LAMP method has also been used as a tool to inspect allergens contained in food products. Using LAMP assay, 4 pg of carrot genomic DNA can be detected and no cross-reactions with other 31 species was evident (Min et al. 2015). Two sets of primers were selected and used for detection of peanut and at least 1 pg of purified peanut DNA can be detected by the internal transcribed spacer (ITS)-based primers (Sheu et al. 2018).

In this work, we aimed to apply the LAMP assay for detection of pistachio allergen coding sequences. Based on the cloned Pis V 1 coding gene, one set of LAMP primers with high specificity and sensitivity was obtained. The influence of food processing, including high temperature and high pressure, on the LAMP assay was analyzed.

Materials and Methods

Samples

Obtained from local markets, the nut samples of pistachios, cashews, mangos, walnuts, almonds, macadamia nuts, hazelnuts, peanuts, and soybeans as well as several commercial brands of processed food were stored at the proper environment. Pistachio nuts produced in the USA, Iran, Turkey, and China were applied for analysis.

In order to analyze the limit of detection (LOD) of the developed LAMP assay, a series of mixtures containing pistachio and wheat were prepared as follows: the first 500-g mixture was obtained by mixing 50-g pistachios flour with 450-g wheat flour and then serial 10-fold dilution was applied. Finally, the mixtures containing 100,000, 10,000, 1000, 100, 10, 1, and 0.1 mg kg⁻¹ of pistachios in wheat flour were obtained and applied to DNA extraction. The purified genomic DNA was applied to LAMP assay and PCR assay as described below to obtain their LOD, respectively.

DNA Extraction

Based on the instruction manual, Dzip (Plant) Genomic DNA Isolation Reagent (Sangon Biotech, China) was applied for

the extraction of genomic DNA of the above nut samples. The quality of the finally obtained genomic DNA, including purity and concentration, was analyzed by a NanoDrop ND-2000 spectrophotometer (NanoDrop Technologies Inc., USA). DNA samples were deposited at -20°C .

Cloning and Sequencing of Pis V 1 Gene

Based on the mRNA sequence of Pis V 1 coding gene (GenBank accession number: DQ631675.1), primers (5'-CGTACTTCTCCTATCTGCC-3') and (5'-CTAGCCACACACTAACAACG-3') were designed and then synthesized by Sangon Biotech. Using the purified genomic DNA of pistachio nuts as DNA template, PCR was performed with an Eppendorf Mastercycler pro (Eppendorf, Germany) in 50 μl , containing 30 ng of DNA, 0.2 mM of each dNTP, 1 U KOD dash DNA polymerase (TOYOBO, Japan), 0.3 μM of each primer, 1 $\times$ PCR buffer, and 1 mM of MgSO_4 . Based on the instruction manual of KOD DNA polymerase, the PCR was carried out with an annealing temperature of 55°C . The PCR products were applied to DNA electrophoresis in 2% agarose gel containing Super Gelred (US Everbright Inc., China) and then sequenced by Sangon Biotech (China).

LAMP Primers Design

Based on the obtained Pis V 1 coding gene, several LAMP primer sets, including F3, B3, FIP, and BIP, were obtained and selected with the help of Primer Explorer V5 software (<http://primerexplorer.jp/e/>). All these primers were synthesized by Sangon Biotech (China). Among these primer sets, one set (Table 1) was finally chosen to establish LAMP assay depending on the experimental verification.

LAMP Assay

LAMP reaction was made in 25 μl , containing 1 $\times$ LAMP PCR Master Mix with 0.16 U DNA polymerase (Sangon Biotech, China), 0.2 μM F3 and B3, 0.8 μM FIP and BIP. The purified genomic DNA was added at different amount for each reaction. Finally, the reaction solution was maintained at 65°C for 1 h with the help of a Mini Heating Dry bath (Major Science, China) to complete the reaction. All LAMP assays were performed at least three times.

LAMP Products Detection

Five microliters of the reaction solution was applied to DNA electrophoresis in 2% agarose gel stained by Super Gelred (US Everbright Inc., China). Additionally, the amplified products can be visually observed by mixing 1 μl of SYBR Green I dye (1/100-diluted, Sangon Biotech, China) with the reaction solution and observing its color change.

PCR Assay

Containing the purified genomic DNA of pistachio nuts, PCR reaction was performed in 50 μl , containing 1 $\times$ San Taq PCR Mix (Sangon Biotech, China), 0.4 μM primer F3, and 0.4 μM primer B3. Based on the instruction manual of 1 $\times$ San Taq PCR Mix, PCR reaction was carried out with an annealing temperature of 55°C . As described above, the PCR products were analyzed by DNA electrophoresis. All PCR assays were performed at least three times.

Preparation of Boiled and Autoclaved Pistachio Nuts

Obtained from local markets, fresh pistachio nuts were crush into pieces. Thirty-gram samples were applied to boiling and steam autoclaving treatments, respectively. For boiling treatment, samples were boiled for 25, 50, 75, 100, 125, and 150 min, respectively. For autoclaving, samples were treated for 15, 30, 45, and 60 min at 121°C , 103.4 kPa, respectively. Then, the cooled samples were applied to DNA extraction and the purified genomic DNA was applied to LAMP and PCR test as described above, respectively.

Results

Sequence of Pis V 1 Coding Gene and LAMP Primers Design

Based on the mRNA sequence of Pis V 1, primers were designed and applied to amplify its coding gene. A specific amplicon with approximately 600 bp was detected (data not shown) and this was consistent with the expected amplicon size of using the mRNA sequence as template directly. Finally, repeated bi-directional sequencing was applied and obtained a

Table 1 DNA sequences of primers used for LAMP assay

Primers	Sequences (5'-3')	Position
F3 (outer forward primer)	AGAGCAAATGGTGAAGCG	nt289-306
B3 (outer reverse primer)	CTGCAACACATATCACGC	nt494-477
FIP (inner forward primer, F1c-F2)	CACATGCGAGGCAATTCAGTGGCAGTTCAGAGGTGAGA	nt384-365, nt320-338
BIP (inner reverse primer, B1c-B2)	CATTTCACCCAGTCAGGGGTGTCAG-TCTCCATATGCTGGCGATGATG	nt412-388, nt448-469

610 bp DNA sequence. Using the obtained sequence as template, various sets of LAMP primers were obtained and the finally selected primer set is shown in Table 1. The position of the selected LAMP primers in Pis V 1 coding gene is indicated.

Specificity

As the primary concern, specificity was analyzed using the purified genomic DNA from pistachio nuts, cashews, mangos, walnuts, almonds, macadamia nuts, hazelnuts, peanuts, and soybeans as template, respectively. When LAMP primers (Table 1) were applied to detect the sample containing pistachio nuts' genomic DNA, the characteristic LAMP product with a ladder-like DNA pattern on gels (Fig. 1B, lane 1) and the change of solution color from orange (the negative control, Fig. 1C, tube N) to green (Fig. 1C, tube 1) by adding SYBR Green I were detected, respectively. In contrast, there was no detectable LAMP product when primers were used in LAMP reactions containing the genomic DNA purified from other nuts mentioned above (Fig. 1B, lanes 2–9, and Fig. 1C, tubes 2–9). Therefore, it demonstrates that this selected set of LAMP primers is highly specific for pistachio nuts and can be applied for further study. At the same time, PCR assay was applied using out primers F3 and B3, and a specific amplicon of approximately 205 bp was detected in the sample containing pistachio nuts' genomic DNA (Fig. 1A, lane 1). There was no detectable amplicon in PCR reactions containing the genomic DNA purified from other nuts (Fig. 1A, lanes 2–9).

Sensitivity

To analyze the detection limit, different dilutions of the genomic DNA of pistachio nuts were firstly applied for LAMP

reaction. Based on the ladder-like DNA pattern detected on the gel (Fig. 2B) and the change in color of the mixture by adding dye (Fig. 2C), this developed LAMP assay can detect at least 10 pg of pistachio nuts genomic DNA (Fig. 2B, lane 5 and Fig. 2C, tube 5) (Fig. 2A). In respect to conventional PCR, there was an evident amplicon in tube containing 100 pg pistachio nut genomic DNA (Fig. 2A, lane 4). However, the amplicon was not detected in tube containing 10 pg pistachio nut genomic DNA (Fig. 2A, lane 5). Taken together, it can be concluded that the developed LAMP test has a high sensitivity for detecting pistachio nut genomic DNA and its sensitivity is higher than the conventional PCR assay.

Using DNA purified from the series of mixtures containing pistachios and wheat flour as template, LAMP and PCR tests were carried out. According to the ladder-like DNA pattern detected on the gel (Fig. 3B) and the color change by adding SYBR Green I (Fig. 3C), the LOD of the LAMP reaction was 10 mg kg⁻¹ of pistachios contained in wheat flour (Fig. 3B, lane 5 and Fig. 3C, tube 5). However, the LOD of conventional PCR was 100 mg kg⁻¹ of pistachios contained in wheat flour (Fig. 3A, lane 4).

LAMP Assay for Detection of Heat-Processed Pistachio Nuts

After being treated with boiling and steam autoclaving, pistachio was applied to genomic DNA purification and the obtained DNA was used for the analysis of the influence of heat treatment on LAMP assay. For boiling bath procedure, the purified genomic DNA from samples with a treatment for 25, 50, 75, 100, 125, and 150 min had no influence on the LAMP reaction (Fig. 4B, lanes 1–6 and Fig. 4C, tubes 1–6). Figure 4 A shows that the boiling treatment also did not influence the PCR assay. With regard to the purified genomic DNA from pistachio nuts under steam

Fig. 1 Specificity analysis of the LAMP and PCR assay for the detection of pistachio nuts. (A) DNA electrophoresis of PCR products, (B) DNA electrophoresis, and (C) visual detection of LAMP products. Lanes M, 100 bp DNA marker; Lanes or tube N, negative control; Lanes or tubes 1–9 represent genomic DNA from different nuts: 1, pistachio nut; 2, cashew; 3, mango; 4, walnut; 5, almond; 6, macadamia nut; 7, hazelnut; 8, peanut; 9, soybean. The arrow indicates the specific amplicon obtained by PCR

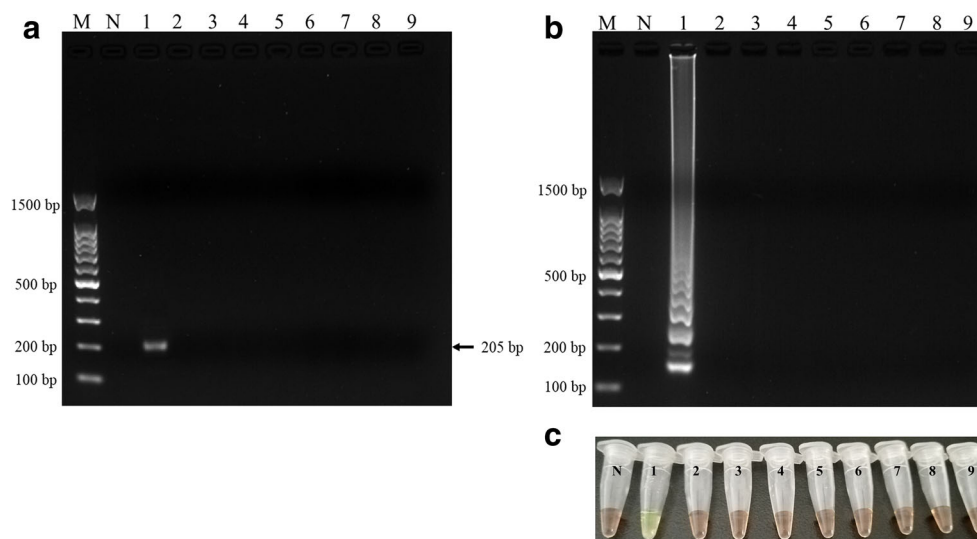
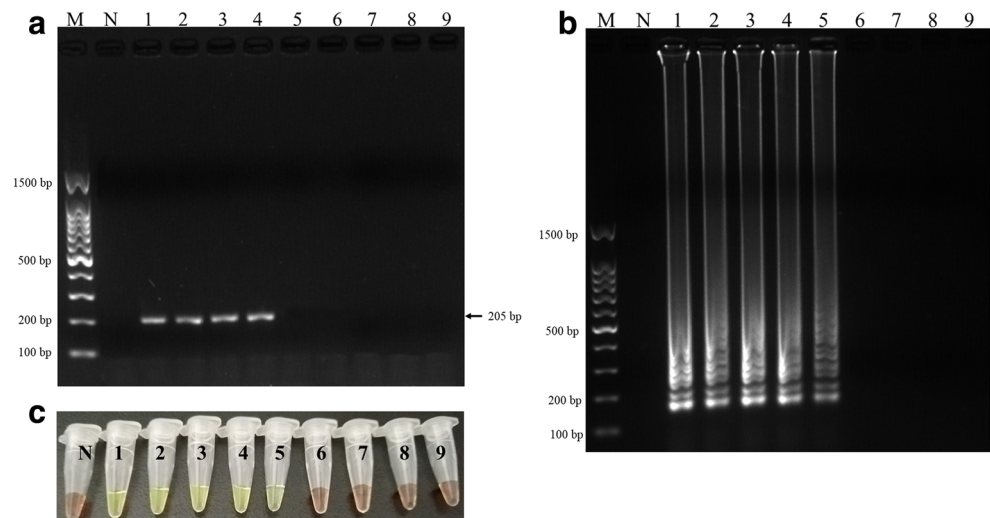


Fig. 2 Sensitivity analysis of PCR (A) and LAMP assay (B and C) for the detection of the purified pistachio nuts genomic DNA. Lanes M: 100 bp DNA marker; Lanes or tube N: negative control; Lanes or tubes 1–9 represent various amounts of pistachio nuts genomic DNA: 1, 100 ng; 2, 10 ng; 3, 1 ng; 4, 100 pg; 5, 10 pg; 6, 1 pg; 7, 100 fg; 8, 10 fg; 9, 1 fg. The arrow indicates the specific amplicons obtained by PCR



autoclaving for 15, 30, 45, and 60 min, positive reactions can only be detected in samples from steam-autoclaved pistachio nuts for 15, 30, and 45 min by DNA electrophoresis (Fig. 5B, lanes 1–3) or by adding SYBR Green I (Fig. 5C, tubes 1–3). However, the specific LAMP products were not detected in samples from steam-autoclaved pistachio nuts for 60 min. The similar influence of the steam autoclaving treatment on PCR assay was found as shown in Fig. 5A.

Commercial Foods Tested with the LAMP Assay

To analyze the adaptability of this developed LAMP test to inspect pistachio nuts contained in commercial foods with an evident label declaration, ten commercial foods containing pistachio nuts were obtained from local markets. The purchased commercial foods (chocolate, cereal bar, biscuit, vegetal burger, meat product, ice cream, bread, bonbon, pesto sauce, jam) were applied to total DNA extraction and the

purified DNA was used for LAMP tests to authenticate the pistachio nut ingredients. According to the results (Fig. 6), pistachio nut genomic DNA contained in all these ten commercial foods were detected by DNA electrophoresis (Fig. 6B, lanes 1–10) or by adding SYBR Green I (Fig. 6C, tubes 1–10). The test results of PCR assay (Fig. 6A) were in accordance with those detected in LAMP assay and therefore, the results obtained from LAMP tests were confirmed by PCR assay.

At the same time, 60 commercial food products with a vague description “may conceal traces of nuts” and 92 commercial foods with no labeling of pistachios or nuts anywhere were obtained from local markets and applied to DNA extraction, followed by LAMP and PCR assay, respectively. Based on the results of the LAMP assay, Pis V 1 gene was detected in 8 commercial foods with a vague description “may conceal traces of nuts” and in 11 commercial foods with no mention of pistachios or nuts (Table 2). Among these commercial foods with positive results detected by LAMP assay, 1 of 8 commercial foods with a vague

Fig. 3 Detection of different amounts of pistachio nuts contained in wheat flour by the LAMP and PCR assay. (A) DNA electrophoresis of PCR products, (B) DNA electrophoresis, and (C) visual detection of LAMP products. Lanes M, 100 bp DNA marker; Lanes or tube N, negative control; Lanes or tube P, positive control; Lanes or tubes 1–7 represent different pistachio concentrations: 1, 100,000 mg kg⁻¹; 2, 10,000 mg kg⁻¹; 3, 1000 mg kg⁻¹; 4, 100 mg kg⁻¹; 5, 10 mg kg⁻¹; 6, 1 mg kg⁻¹; 7, 0.1 mg kg⁻¹. The arrow indicates the specific amplicons obtained by PCR

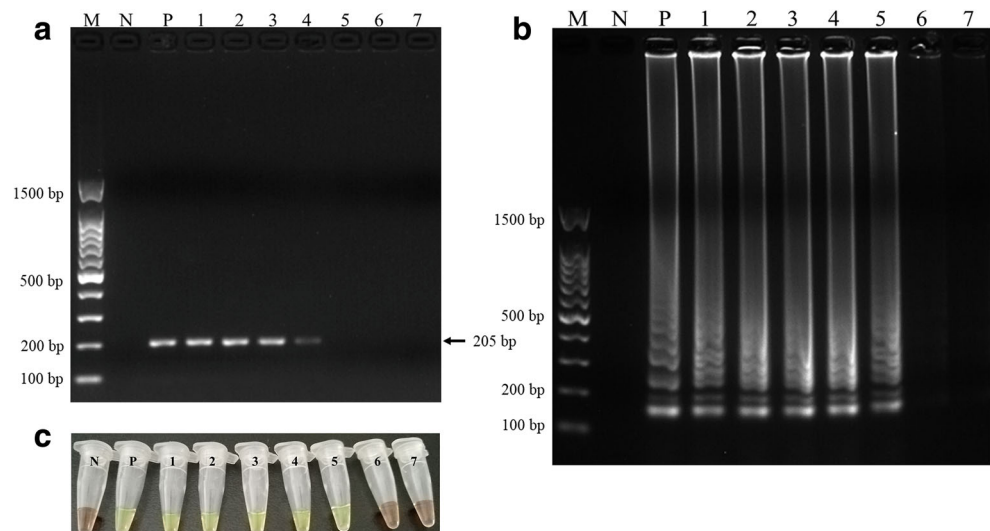
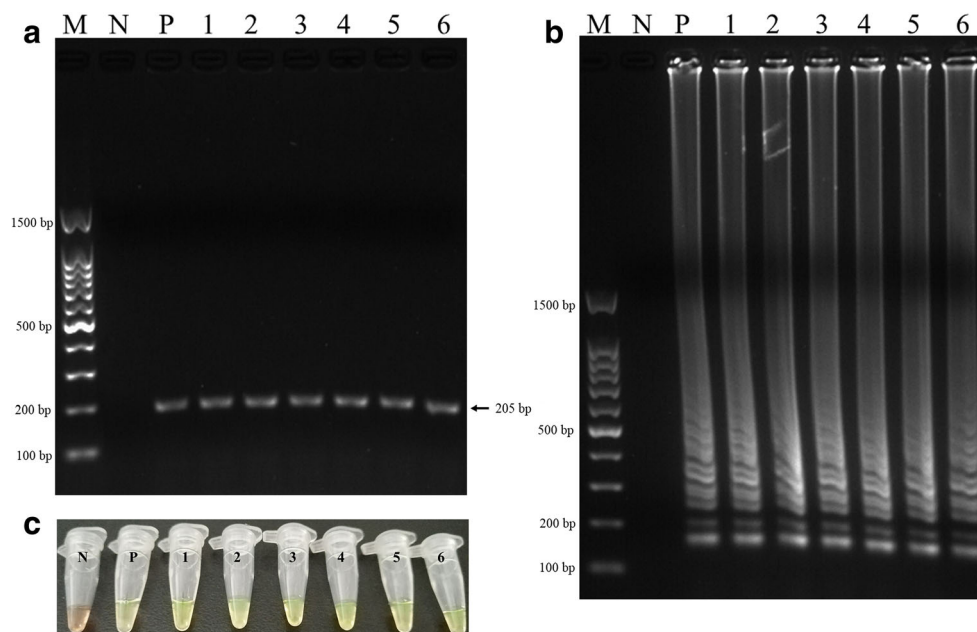


Fig. 4 Analysis of the influence of boiling treatment on the PCR (A) and LAMP (B and C) assay for detection of pistachio nuts. Lanes M: 100 bp DNA marker; Lanes or tube N, negative control; Lanes or tube P, positive control (raw pistachio nuts); Lanes or tubes 1–6 represent the pistachio nuts were boiled for 25, 50, 75, 100, 125, and 150 min, respectively. The arrow indicates the specific amplicons obtained by PCR



description “may conceal traces of nuts” and 2 of 11 commercial foods with no mention of pistachios or nuts yielded no positive amplification by PCR assay (Table 2).

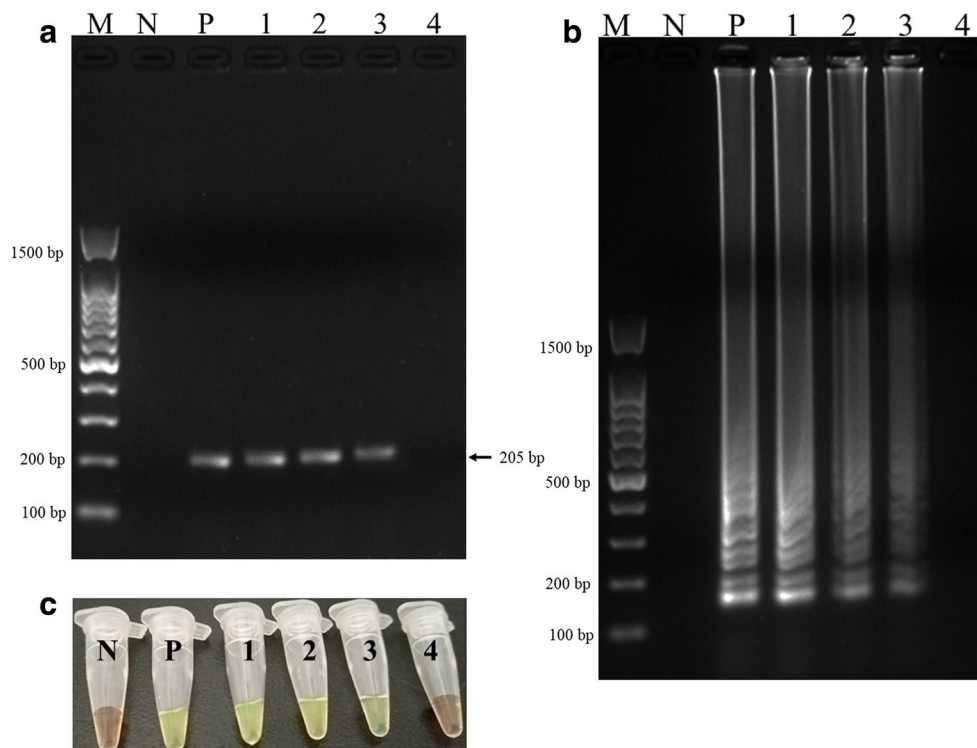
Discussion

As one of nucleic acid’s isothermal amplification methods with promising application for point-of-care (Yan et al.

2014), LAMP is widely designed and applied to identify various kinds of bio-samples in recent years. With respect to the prevalence of pistachio nuts allergy around the world (Costa et al. 2019), we want to develop a LAMP assay to detect pistachio nuts in food samples rapidly, thereby preventing food allergy effectively.

The finally selected set of primers in this work makes a specific identification of pistachio nut DNA without false detection of cashew and other nuts listed above. Compared with

Fig. 5 Analysis of the influence of steam autoclaving on the PCR (A) and LAMP (B and C) assay for detection of pistachio nuts. Lanes M: 100 bp DNA marker; Lanes or tube N, negative control; Lanes or tube P, positive control (raw pistachio nuts); Lanes or tubes 1–4 represent the pistachio nuts were autoclaved for 15, 30, 45, and 60 min, respectively. The arrow indicates the specific amplicons obtained by PCR



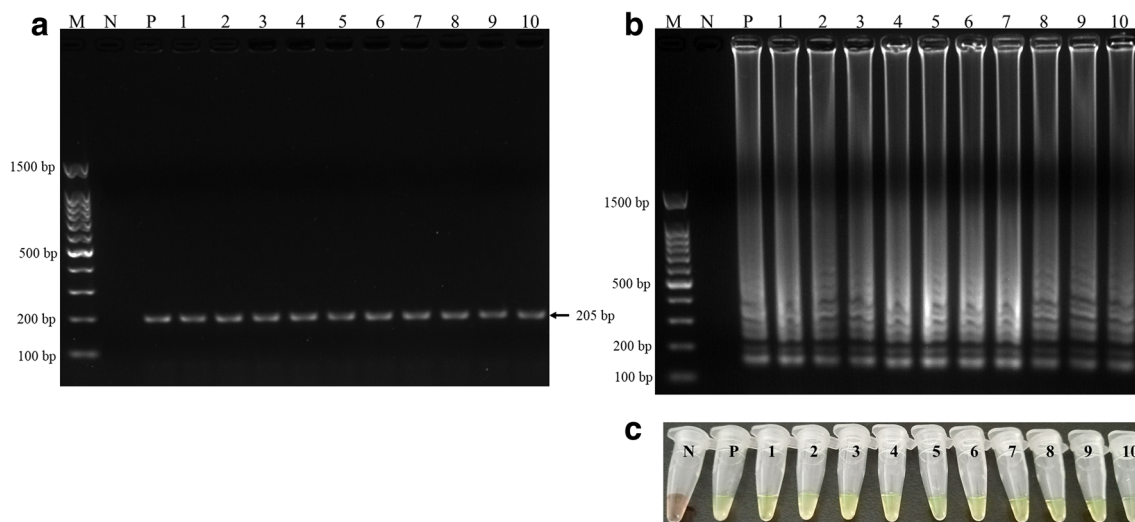


Fig. 6 Application of PCR (A) and LAMP (B and C) assay for detection of pistachio nuts contained in commercial foods. Lanes M: 100 bp DNA marker; Lanes or tube N, negative control; Lanes or tube P, positive control (purified pistachio nuts genomic DNA); Lanes or tubes 1–10

represent the DNA extracted from chocolate, cereal bar, biscuit, vegetal burger, meat product, ice cream, bread, bonbon, pesto sauce and jam, respectively. The arrow indicates the specific amplicons obtained by PCR

the conventional PCR assay (with a sensitivity of 100 pg pistachio nut genomic DNA) performed in this work, the developed LAMP assay shows a higher sensitivity since it can detect 10 pg pistachio nut genomic DNA. The sensitivity of this developed LAMP assay is comparable with the qPCR with SYBR green also targeting Pis V 1 coding sequence (with a sensitivity of 12.5 pg pistachio nut genomic DNA) (Sanchiz et al. 2016). Moreover, when used for detection of

pistachio in wheat flour, the developed LAMP assay has a similar LOD (10 mg kg^{-1}) with locked nucleic acids probe-based qPCR (Sanchiz et al. 2016) and the LOD of the developed LAMP assay is higher than that of the conventional PCR (100 mg kg^{-1}) performed in this work. However, probe-based qPCR targeting the ITS1 region (GenBank accession number: AY677201), 4 mg kg^{-1} (Brežná et al. 2008) and 0.1 mg kg^{-1} (López-Calleja et al. 2014) of pistachio nuts can be detected and this may benefit from the high copy number of the ITS1 region. The influence of the copy number of the targeted gene on the sensitivity of LAMP assay was also reported. Using ITS-based LAMP primers, 1 pg of peanut DNA can be detected. However, only 100 pg of peanut DNA can be detected by targeting *Ara h1* sequence, which is the allergen coding gene with a low copy number (Sheu et al. 2018).

Nowadays, various techniques have been used in food processing, such as thermal, chemical, mechanical, and irradiation treatments. The impact of these processing methods to the allergenicity of various tree nuts has been summarized and reviewed by Vanga and Raghavan (2017). For pistachio nuts, various thermal treatments have been reported to reduce its IgE-reactivity (Noorbakhsh et al. 2010; Sanchiz et al. 2018). And this reduced IgE-reactivity may be due to the alterations of those allergenic proteins happened during thermal treatments. These protein alterations may be a challenge to the commercially used protein-based methods for detecting pistachio contained in foodstuff, such as ELISA tests. As a result of the high stability of DNA, PCR methods show promising in food allergen detection (Böhme et al. 2019; Prado et al. 2016). In this context, the impact of boiling and autoclave treatments to this developed LAMP assay was analyzed. Boiling treatment, even with 150 min, had no influence on the LAMP detection of the Pis V 1 gene as well as the PCR assay. And

Table 2 Results of commercial foods detected by LAMP and PCR assay

	Number of samples	LAMP result	PCR result
Foods with labeling of traces of nuts (60)			
Chocolate	12	+(3)/(−9)	+(2)/(−10)
Cereal bar	5	+(1)/(−4)	+(1)/(−4)
Biscuit	13	+(2)/(−11)	+(2)/(−11)
Bread	17	+(2)/(−15)	+(2)/(−15)
Bonbon	8	−(8)	−(8)
Meat products	5	−(5)	−(5)
Foods with no mention of pistachios or nuts (92)			
Chocolate	16	+(3)/(−13)	+(3)/(−13)
Cereal bar	9	−(9)	−(9)
Biscuit	11	+(1)/(−10)	−(11)
Bread	14	+(2)/(−12)	+(2)/(−12)
Bonbon	13	+(3)/(−10)	+(2)/(−11)
Meat products	6	+(1)/(−5)	+(1)/(−5)
Ice cream	8	+(1)/(−7)	+(1)/(−7)
Yogurt	5	−(5)	−(5)
Precooked meal	3	−(3)	−(3)
Rice cake	7	−(7)	−(7)

+, positive amplification result; −, negative amplification result

this is consistent with the previous report which shows that boiling treatment made no influence on the detection of the same targeted gene using qPCR (Sanchiz et al. 2016). The same influence of boiling treatment on the *Ara h1*-based LAMP detection, targeting the allergen coding gene of peanut with a low copy number, was reported (Sheu et al. 2018). Therefore, it may be concluded that few DNA is degraded during the boiling treatment and there is enough amount of template DNA for LAMP assay, no matter the initial copy number of the targeted gene. When applied to autoclaving treatment (121 °C, 103.4 kPa) for 60 min, no targeted gene can be amplified by the developed LAMP assay. At the same temperature with a higher pressure (121 °C, 120 kPa) for only 15 min, the sensitivity of qPCR for inspecting *Pis V 1* gene decreased drastically (Sanchiz et al. 2016). Thus, the thermal processing with pressure may destroy the DNA integrity severely and the resulted DNA fragments have no enough size for LAMP assay. When autoclaved at 121 °C, 103.4 kPa for 60 min, ITS-based primers performed a better assay of peanut than *Ara h1*-based primers (Sheu et al. 2018). In respect to this phenomenon, the initial copy number of the targeted gene may be a key factor to consider for developing DNA-based detection methods.

Generally, the complicate formulation of commercial foods may be a challenge to detect pistachio by the developed LAMP assay. Based on the validated LAMP primers, pistachios contained in different commercial foods with a declaration of containing pistachio nuts can be detected by the developed LAMP assay, indicating that the specificity or reactivity of the LAMP test was not influenced by other DNA contained in samples. At the same time, commercial foods with a vague description of “may conceal traces of nuts” or with no labeling of pistachios or nuts anywhere were applied to LAMP assay and pistachios were detected by the developed LAMP assay in 8/60 or 11/92 the above commercial foods, respectively. However, conventional PCR failed to recognize pistachios in some commercial foods with a positive LAMP result and this may be due to that this developed LAMP assay has a higher LOD (10 mg kg⁻¹) than conventional PCR (100 mg kg⁻¹). The presence of pistachios in commercial foods without a certain indication in their label was also recognized by qPCR (López-Calleja et al. 2014; Sanchiz et al. 2016). The appearance of pistachios in these commercial foods may be derived from the contamination happened during food processing. However, pistachio nuts contained in these commercial foods without a certain indication in their label may be a potential threat to the sensitive individuals. Therefore, the LAMP assay proposed herein offers a rapid and effective way to detect traces of pistachio nuts in commercial foods and could be used for internal auditing to prevent contamination during food processing or for routine inspections in product quality control.

Conclusion

In respect to its allergy-causing characteristic, a rapid LAMP assay for pistachio with high specificity and sensitivity was developed in this work. Targeting *Pis V 1* coding gene, the developed LAMP assay can detect at least 10 mg kg⁻¹ of pistachio nuts and 10 pg of purified pistachio nut genomic DNA. The developed strategy is applicable for heat-processed pistachio nuts and commercial food products. Consequently, this LAMP assay may be used for on-site detection of pistachio nuts possible contained in foodstuffs, thereby protecting sensitized individuals against hidden allergens.

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Compliance with Ethical Standards

Conflict of Interest Ruifeng Mao declares that they have no conflict of interest. Kaiwen Xie declares that they have no conflict of interest. Menghuan Zhao declares that they have no conflict of interest. Mengqiu Li declares that they have no conflict of interest. Lu Lu declares that they have no conflict of interest. Yu Liu declares that they have no conflict of interest. Qian Wu declares that they have no conflict of interest. Yingying Chen declares that they have no conflict of interest. Tong Zhang declares that they have no conflict of interest. Enjie Diao declares that they have no conflict of interest.

Ethical Approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent Not applicable.

References

- Alexandra E, Anja D, Christine H, Ulrich B, Karl-Heinz E (2009) Simultaneous detection of DNA from 10 food allergens by ligation-dependent probe amplification. *Food Addit Contam Part A* 26(4):409–418
- Barbieri G, Frigeri G (2006) Identification of hidden allergens: detection of pistachio traces in mortadella. *Food Addit Contam Part A* 23(12):1260–1264
- Bock SA, MunOz-Furlong A, Sampson HA (2001) Fatalities due to anaphylactic reactions to foods. *J Allergy Clin Immunol* 107(1):191–193
- Böhme K, Calo-Mata P, Barros-Velázquez J, Ortea I (2019) Review of recent DNA-based methods for main food-authentication topics. *J Agric Food Chem* 67(14):3854–3864
- Boudreau-Romano S, Qamar N (2018) Peanut allergy: changes in dogma and past, present, and future directions. *Pediatr Ann* 47(7):e300–e304
- Brežná B, Dudášová H, Kuchta T (2008) A novel real-time polymerase chain reaction method for the qualitative detection of pistachio in food. *Eur Food Res Technol* 228:197–203

- Costa J, Silva I, Vicente AA, Oliveira MBPP, Mafra I (2019) Pistachio nut allergy: an updated overview. *Crit Rev Food Sci Nutr* 59(4):546–562
- Dreher ML (2012) Pistachio nuts: composition and potential health benefits. *Nutr Rev* 70(4):234–240
- Focke F, Haase I, Fischer M (2013) Loop-mediated isothermal amplification (LAMP): methods for plant species identification in food. *J Agric Food Chem* 61(12):2943–2949
- Geiselhart S, Hoffmann-Sommergruber K, Bublin M (2018) Tree nut allergens. *Mol Immunol* 100:71–81
- Guan X, Guo J, Shen P, Yang L, Zhang D (2010) Visual and rapid detection of two genetically modified soybean events using loop-mediated isothermal amplification method. *Food Anal Method* 3(4):313–320
- Holzhauser T (2018) Protein or no protein? Opportunities for DNA-based detection of allergenic foods. *J Agric Food Chem* 66(38):9889–9894
- Kazuha A, Daisuke W, Yasuhiko T, Yoshinari M (2011) Oral allergy syndrome with severe anaphylaxis induced by pistachio. *Int J Dermatol* 50(5):632–633
- Lee MS, Su TY, Lien YY, Sheu SC (2017) The development of loop-mediated isothermal amplification (LAMP) assays for the rapid authentication of five forbidden vegetables in strict vegetarian diets. *Sci Rep-UK* 7:44238
- Liu C, Chhabra GS, Sathe SK (2015) Pistachio (*Pistacia vera* L.) detection and quantification using a murine monoclonal antibody-based direct sandwich enzyme-linked immunosorbent assay. *J Agric Food Chem* 63(41):9139–9149
- López-Calleja IM, Cruz SDL, González I, García T, Martín R (2014) Survey of undeclared allergenic pistachio (*Pistacia vera*) in commercial foods by hydrolysis probe real-time PCR. *Food Control* 39(1):49–55
- Luo J, Vogel RF, Niessen L (2012) Development and application of a loop-mediated isothermal amplification assay for rapid identification of aflatoxigenic molds and their detection in food samples. *Int J Food Microbiol* 159(3):214–224
- Min S, Gao H, Xiao X, Chen J, Liu C, Feng L (2015) A novel loop-mediated isothermal amplification method for detection of the carrot materials in foods. *Eur Food Res Technol* 241(2):295–302
- Noorbakhsh R, Mortazavi SA, Sankian M (2010) Influence of processing on the allergenic properties of pistachio nut assessed in vitro. *J Agric Food Chem* 58(18):10231–10235
- Notomi T, Okayama H, Masubuchi H, Yonekawa T, Watanabe K, Amino N (2000) Loop mediated isothermal amplification of DNA. *Nucleic Acids Res* 28:e63
- Porcel S, Sanchez AB, Rodriguez E, Fletes C, Alvarado M, Jimenez S, Hernandez J (2006) Food-dependent exercise-induced anaphylaxis to pistachio. *J Investig Allergol Clin Immunol* 16(1):71–73
- Prado M, Ortea I, Vial S, Rivas J, Calo-Mata P, Barros-Velázquez J (2016) Advanced DNA- and protein-based methods for the detection and investigation of food allergens. *Crit Rev Food Sci Nutr* 56(15):2511–2542
- Ran G, Ren L, Han X, Liu X, Li Z, Pang D, Ouyang H, Tang X (2016) Development of a rapid method for the visible detection of pork DNA in halal products by loop-mediated isothermal amplification. *Food Anal Method* 9(3):565–570
- Sanchiz A, Ballesteros I, Martin A, Rueda J, Pedrosa MM, Dieguez MDC, Rovira M, Cuadrado C, Linacero R (2016) Detection of pistachio allergen coding sequences in food products: a comparison of two real time PCR approaches. *Food Control* 75:262–270
- Sanchiz A, Cuadrado C, Dieguez MC, Ballesteros I, Rodríguez J, Crespo JF, Cuevas NL, Rueda J, Linacero R, Cabanillas B (2018) Thermal processing effects on the IgE-reactivity of cashew and pistachio. *Food Chem* 245:595–602
- Seki M, Kilgore PE, Kim EJ, Ohnishi M, Hayakawa S, Dong WK (2018) Loop-mediated isothermal amplification methods for diagnosis of bacterial meningitis. *Front Pediatr* 6:57
- Sheu SC, Tsou PC, Lien YY, Lee MS (2018) Development of loop-mediated isothermal amplification (LAMP) assays for the rapid detection of allergic peanut in processed food. *Food Chem* 257:67–74
- Song H, Bae Y, Park S, Kwon H, Lee H, Joh S (2018) Loop-mediated isothermal amplification assay for detection of four immunosuppressive viruses in chicken. *J Virol Methods* 256:6–11
- USDA (2018) Tree nuts: world markets and trade. Publisher <https://apps.fas.usda.gov/psdonline/circulars/TreeNuts.pdf>. Accessed October 2018
- Vaagt F, Haase I, Fischer M (2013) Loop-mediated isothermal amplification (LAMP)-based method for rapid mushroom species identification. *J Agric Food Chem* 61(8):1833–1840
- Vanga SK, Raghavan V (2017) Processing effects on tree nut allergens: a review. *Crit Rev Food Sci Nutr* 57(17):3794–3806
- Varlet-Marie E, Sterkers Y, Perrotte M, Bastien P (2018) A new LAMP-based assay for the molecular diagnosis of toxoplasmosis: comparison with a proficient PCR assay. *Int J Parasitol* 48(6):457–462
- Wang H, Ma Z, Qin J, Shen Z, Liu Q, Chen X, Wang H, An Z, Liu W, Li M (2019) A versatile loop-mediated isothermal amplification microchip platform for *Streptococcus pneumoniae* and *Mycoplasma pneumoniae* testing at the point of care. *Biosens Bioelectron* 126:373–380
- Weinberger T, Sicherer S (2018) Current perspectives on tree nut allergy: a review. *J Asthma Allergy* 11:41–51
- Wen HW, Borejsza-Wysocki W, Decory TR, Durst RA (2010) Peanut allergy, peanut allergens, and methods for the detection of peanut contamination in food products. *Compr Rev Food Sci Food Saf* 6(2):47–58
- Yan L, Zhou J, Zheng Y, Gamson AS, Roembke BT, Nakayama S, Sintim HO (2014) Isothermal amplified detection of DNA and RNA. *Mol Biosyst* 10(5):970–1003

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