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Extracting genomic DNA of foodstuff by polyamidoamine (PAMAM)–magnetite nanoparticles

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ABSTRACT

Although genetically modified (GM) food is becoming increasingly available, consumers are showing a growing awareness about the need to identify GM and non-GM foodstuff: the reliable identification of GM/non-GM food is therefore an important tool in the social, health and safety debates. The present research responds to this need (i) through developing a novel "single-pot" preparation of PAMAM magnetite nanoparticles (PMNPs) and by fully defining their specific characteristics; (ii) by demonstrating the capability of the PMNPs to isolate genomic DNA from different sample foods; and (iii) by experimentally demonstrating the identification of the isolated DNA by gel-electrophoresis, thus being capable of screening GM and non-GM food.

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1. Introduction

In recent years, a great variety of genetically modified (GM) food has been produced and introduced into worldwide agro-industrial markets [1]. The paper will firstly provide a brief background on the GM crops, well-developed by recombinant DNA technology, which allows the production of plants that are resistant to herbicides and pests, that increase shelf-life in the case of supermarket products and improve their nutritional value [2]. Many GM plant cultivates have spread worldwide. The increasing consumer awareness regarding food safety in general and GM crops and their use as food sources in particular, calls for a more stringent quality control of foodstuffs [3,4]. In order to guarantee the food safety, several methods have been established to detect transgenes, including nucleic acid-based and protein-based detection methods [5–7]. Among these techniques, the polymerase chain reaction (PCR) has been widely used [8–10]. The successful extraction plays a very important role in detecting GM organisms with good quality, adequate amount, integrity and high purity as paramount assessment criteria.

Magnetite nanoparticles have been widely studied for their applications in biology and medicine, i.e. to immobilize enzymes [11–13] and proteins [14,15]; to purify RNA and DNA [16,17]; to magnetically separate and purify cells [18,19]; and to magnetically control the transport of anticancer drugs [20,21]. Generally, these magnetite particles are of a core and shell structure: the biological species cells, nucleic acids, or proteins are coating the magnetite core through an organic or polymeric shell. The shells are either biocompatible (such as gum Arabic and polyethylene glycol) [22,23], or possess active groups which can conjugate with biomolecules such as proteins [24,25] and nucleic acids [26].

In order to practically apply this new methodological framework, the problems of surface modification require careful consideration. Puniredd et al. [27] adopted an approach which involved the formation of Pt nanoparticles within an ultra-thin film matrix formed by covalent layer-by-layer (LbL) assembly of pyromellitic dianhydride (PMDA) and a second generation of polyamidoamine (PAMAM) dendrimer in supercritical carbon dioxide ($SCCO_2$) to synthesis dendrimer-encapsulated Pt nanoparticles. Kavas et al. [28] synthesized a series of Co nanoparticles by employing PAMAM dendrimers with different generations as templates and sodium borohydride as a reducing agent. Matsunaga et al. [29,30] developed a DNA extraction method using PAMAM modified bacteria magnetic particles which extracted DNA from fluid suspensions and blood.

In this work, we developed a novel "single-pot" preparation of PAMAM magnetite nanoparticles (PMNPs) with uniform size, large surface area and high magnetism and adopted a DNA extraction method using PAMAM-modified magnetic particles. Compared with conventional methods, it takes advantages of quick

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processing times, reduced chemical needs, and easy magnetic separation. Furthermore, PMNPs were applied for DNA extraction from GM/non-GM food, tentatively explained by a mechanism whereby DNA can reach the surface of the dendrimer-modified magnetic nanoparticles through electrostatic interactions. The further analysis will demonstrate that such dendrimer-coated particles have a high potential for extracting a large variety of nucleic acids.

2. Experimental

2.1. Materials

Five soy products were purchased at a local retailer, including soybeans, soybean milk, soybean milk powder, soybean flour, soybean sprouts. GM soybeans, to be used as GM positive controls were kindly provided by the Chinese Academy of Inspection and Quarantine. RNAse A was obtained from Sigma. The 1 kb DNA ladder and DL2000 were bought from New England Biolabs and Beijing QXTD-Biotechnology Go. Ltd., respectively. PCR primers, with sequences listed in Table 1, were synthesized by Sunbiotech (Beijing, China). PAMAM–NH_2 G4 dendrimer was fabricated according to Tomalia's method [31]. All other chemicals used were of analytical purity.

2.2. Procedures for synthesis of PAMAM–magnetite composites

A novel method to produce PMNPs was developed during the research, comprising a straightforward "single-pot" process.

Firstly, 3.7 mmol ferric chloride ($FeCl_3{\cdot}6H_2O$, ~1.00 g) and 2.0 mmol ferrous chloride tetrahydrate ($FeCl_2{\cdot}4H_2O$, ~0.4 g) were added to 20 ml ethylene glycol (EG) under ultrasonication at room temperature to form a clear solution. Secondly, 24.2 mmol anhydrous sodium acetate (NaAc, ~2.00 g) was poured into the previous mixture, whilst magnetic stirring formed a uniform suspension. Thirdly, 0.5 g PAMAM was carefully added into the above mixture, with ultrasonication used to form a uniform suspension. The suspension was topped up with EG and sealed into a Teflon-lined autoclave [35], heated at 180 °C for 24 h. The black or gray particles were separated under the effect of an external magnet, rinsed in three cycles of centrifugation, washing and re-dispersion in water. They were finally oven-dried at 60 °C for 8 h.

2.3. Isolation of genomic DNA

The procedure of Davies [36] was applied: the different soy-samples (30 mg each) were placed in a 1.5 ml microcentrifuge tube and re-suspended by vortexing in 0.1 M Tris–HCl buffer (pH 8.0) containing 0.05 M EDTA and 0.5 M NaCl (400 μl). 16 μl of 10% (w/v) sodium dodecylsulfate and RNAse A (10 mg/ml, 89 μl) were added, and the sample was vortexed and incubated at 65 °C for 10 min. The tube was thereafter placed on ice for 5 min, 5 M potassium acetate (13.5 μl) was added and the sample was vortexed once more. The tube was once more placed on ice for a further 20 min and the resulting suspension was centrifuged at 12,000 rpm for 20 min at 4 °C. The supernatant was removed and transferred to a fresh 1.5 ml microcentrifuge tube and an equal volume of binding buffer containing 20% (w/v) polyethylene glycol-8000 and 2 M NaCl was added. Finally, 30 mg PMNPs, after washing in PBS (pH 8.0), were added.

The suspension was vortexed, then gently shaken for 15 min and kept still for 10 min. The PMNPs were magnetically decanted and were recovered. They were washed twice using binding buffer and 70% ethanol. The PMNPs were dried by ambient air. Finally the DNA absorbed on the PMNPs was eluted by TE buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0).

2.4. Characterization of PMNPs

Various analytical techniques were combined to determine the relevant characteristics of the PMNPs.

Transmission electron microscopy (TEM-Hitachi H-800): the samples were first suspended in ethanol, and then placed as a droplet onto a microporous carbon-coated copper TEM grid. Specimens were allowed to air-dry and imaged at 200 kV.

Scanning electron microscopy (SEM-Zeiss SUPRA 55): the samples were first suspended in ethanol, and then placed onto a piece of silica wafer previously treated with methanol, acetone and isopropanol before use. Specimen were air-dried and imaged.

Powder X-ray diffraction (XRD) data were taken on a D/max-Ultima (Rigaku) using Cu Kα radiation (40 kV, 30 mA, λ = 1.5418 Å). The samples, as un-oriented powders, were scanned in steps of 0.02° (2θ) in the range 3–70° using a count time of 4 s per step.

The *Zeta potential* (surface charge) of the samples was measured Zetasizer (Malvern Instruments) after dispersing the solid samples in de-ionized water, while sonicating for over 10 min to form a transparent dispersion.

FTIR spectra (Varian 3100 FT-IR) were determined to study the surface composition of the nanoparticles in the 400–4000 cm^{-1} spectral range and a resolution of 2 cm^{-1}. The hydrophilic PMNPs were mixed with KBr powder, then milled and compacted into thin disk-shaped pellets.

The thermal behavior of the PMNPs was determined by *thermogravimetry*, using a Thermo plus TG8120 (Rigaku), with Ar as protecting gas, and at a temperature ramp of 10 °C/min from room temperature to 800 °C. They were allowed to regain room temperature by natural cooling.

2.5. Polymerase chain reaction (PCR) conditions and gel electrophoresis

The PCR was performed using a DNA Engine (PTC-200 Peltier Thermal Cycler, MJ Research, Waltham, MA). Amplifications were carried out with a final volume of 20 μl containing 2.5 mM dNTPs, EasyTaq 10× buffer, 10 μmol/l of each primer, the DNA templates of the samples and 5 units/μl EasyTaq DNA Polymerase. PCR products were analyzed together with a molecular weight ladder (1 kb DNA ladder, DL 2000) by electrophoresis on a 1–2% agarose gel containing DNAGREEN which was diluted 20 times with de-ionized water.

3. Results and discussion

3.1. Analysis of PMNPs

The crystalline structure and phase purity were determined by power X-ray diffraction. As shown in Fig. 1, the sharp, strong peaks confirmed that the products are well crystallized and presented the typical cubic iron oxide Fe_3O_4 (JCPDS, # 65–3107), which is indexed to the (2 2 0), (3 1 1), (4 0 0), (4 2 2), (5 1 1) and (4 4 0) from left to the right. Consistently, the peak shape and broadening in XRD patterns indicates that polymer affects the crystallinity of magnetic nanoparticles [37]. However, those PMNPs showed good magnetic properties and could be drawn to the wall easily by an outer magnet (shown by Fig. 3C).

The size and morphology of PMNPs were determined by TEM and SEM. Fig. 2A shows the typical TEM images of the as-prepared magnetite nanoparticles. The average diameter is between 150 nm and 200 nm, and the surface of the particles is well modified by hydrophilic dendrimers: even a strong magnetic force is not capable of provoking particle contact, and aggregation may not take place in a period of several hours, as shown by the digital image

Table 1
Primers used for PCR analysis.

Primer	Sequence (5′–3′)	Gene	Amplified fragment (bp)	Ref.
9 F 13 R	ATGGTTTCTCTTGGCTATATGG GCATACGTTTCATGCTTGTTTGAG	Chloroplast ndhB	1000	[32]
Lec1 Lec2	GATGCCTCCACCAGCCTCTTGG GTCGAGTCCCGTGGCAGCAGAG	Soybean lectin	141	
RRS-F RRS-R	CCTTTAGGATTTCAGCATCAGTGG GACTTGTCGCCGGGAATG	CP4-EPSPS	121	[33]
35S-1 35S-2	GCTCCTACAAAT GCCATCATTG GATAGTGGGATTGTGCGTCAT	CaMV35S promoter	195	[34]
NOS-1 NOS-2	GAATCCTGTTGCCGGTCTTG TTATCCTAGTTTGCGCGCTA	NOS terminator	180	[34]

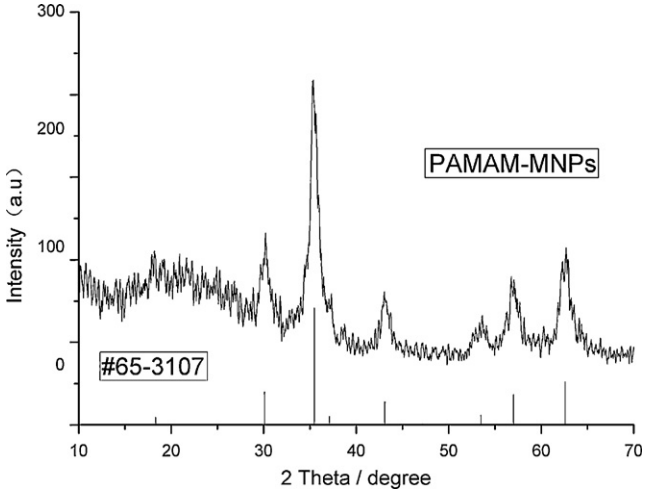

Fig. 1. X-ray diffraction patterns, comparison with standard iron oxide.

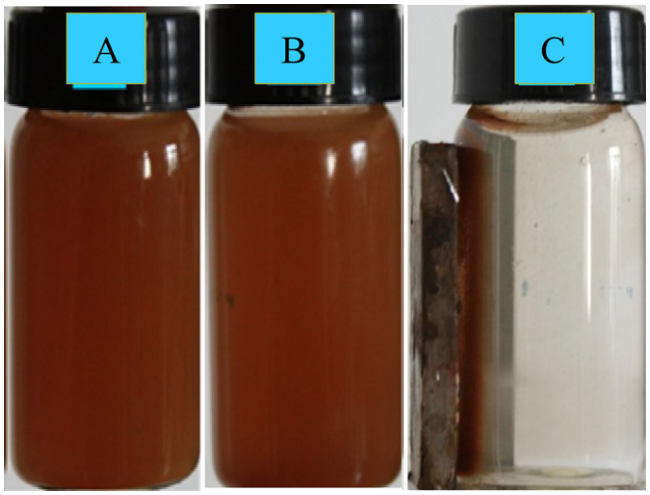

Fig. 3. Visual magnetic properties of PMNPs: (A) just dispersed in water; (B) 6 h after being sonicated; (C) easily drawn to the wall by outer magnet.

of Fig. 3B. Zeta potential measurement, as described further in the text, gave strong support that the magnetic nanoparticles are monodisperse and positively charged (zeta potential is as high as 30.2 mV). SEM images of Fig. 2B coincide well with TEM images, which show that the surface morphology of particles corresponds to rough particles instead of smooth spheres, which can benefit bimolecular conjugation and bioseparation.

Normally, a zeta potential value in excess of 30 mV indicates that stable colloidal suspension of nano-regime can be easily formed through electrostatic repulsion. The Zeta potential of PMNPs is nearly 30.2 mV. They can truly be dispersed into water by outer oscillation or mild sonication. The IR spectroscopy was performed to reveal evidence of the modifying reagent present on the nanocrystalline surface. Fig. 4 shows a typical IR spectrum for PMNPs. Strong bands around 590 cm^{-1} correspond to the Fe–O stretching modes of the magnetite lattice [38]. The intense band between 3400 and 3500 cm^{-1} indicates the stretching vibration

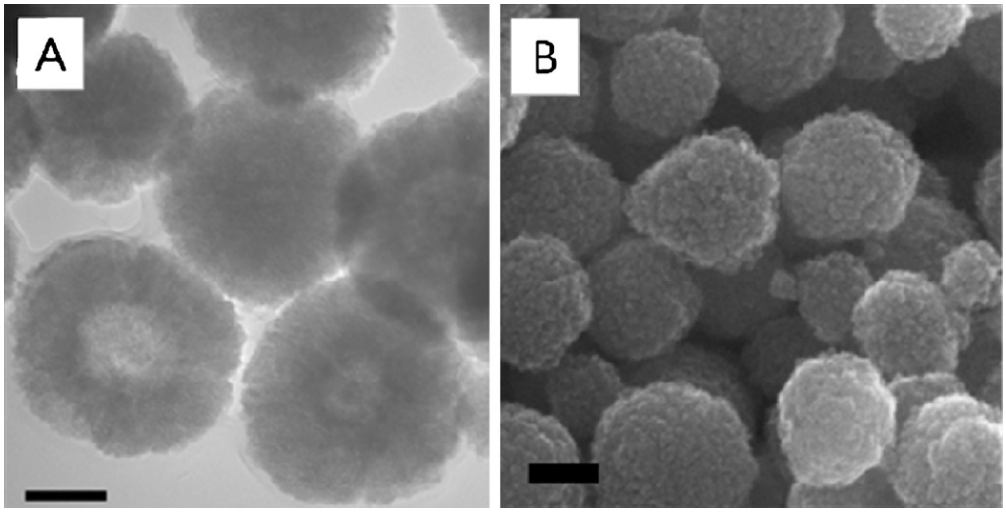

Fig. 2. TEM (A) and SEM (B) images of PMNPs, bar = 100 nm.

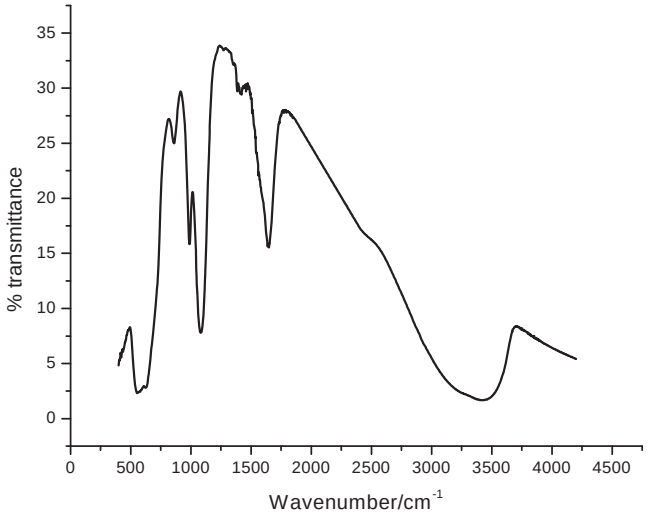

Fig. 4. The FT-IR analysis of the PMNPs.

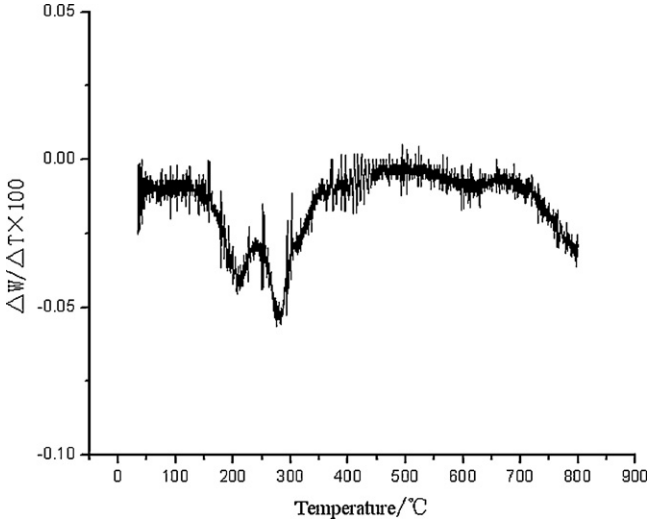

Fig. 6. The differential TG analysis of the PMNPs.

of free N–H. Bands around 1576 cm^{-1} correspond to N–H bending vibration. A series of bands around 1000–1200 cm^{-1} corresponds to the stretching vibration of the C—C bond. These results indicate that PAMAM was successfully bound to Fe_3O_4 magnetite nanoparticles.

The TGA and DTGA profiles (Figs. 5 and 6) of PMNPs further supported that organics had been successfully bound onto the magnetic iron oxide nanoparticles, and mainly consisted of three typical stages: 200–250 °C, 250–300 °C and 600–700 °C. The first slight weight loss is due to the absorbed water molecules, and the second sharp drop under high temperature probably correspondences to the loss of NH_3 converted from organics, The final drop may due to the carbo-thermic reduction to form α- or γ-Fe [39].

From the assessment by the different analytical techniques, it can be concluded that the "single-pot" preparation of PMNPs is successful and provides a stable modified method with interesting properties and behavior. The uniform size, large surface area and high magnetism make PMNPs ideal for application in bioseparation sciences.

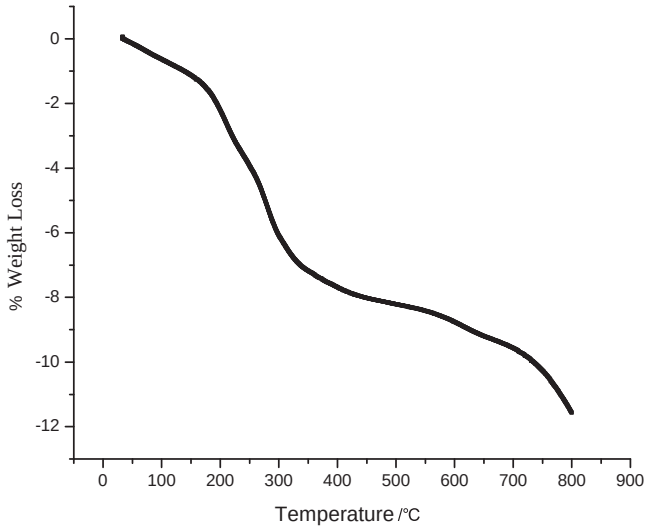

Fig. 5. The TG characterization of the PMNPs.

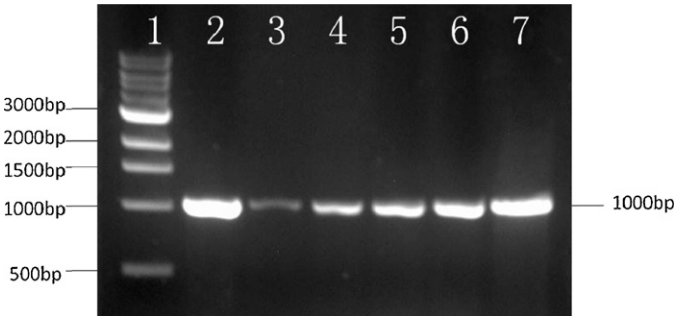

Fig. 7. 1% Agarose gel electrophoresis of amplified fragments corresponding to chloroplast ndhB gene: lane 1, 1 kb marker; lane 2, soybeans; lane 3, soya-bean milk; lane 4, soybean milk powder; lane 5, bean flour; lane 6, soybean sprouts; lane 7, GM soybean (GTS-40-3-2).

3.2. Application of PMNPs

The resulting PMNPs were implanted for DNA extraction from foodstuffs including GM soybeans and other food bought from market. The protocol used for plant genomic DNA isolation was based upon published literature methods with the inclusion of an adsorption step in the presence of sodium chloride and PEG 8000. Under these conditions, adsorbed DNA can be eluted directly into water for immediate applications, without the need for precipitation. The *A*260/*A*280 ratios of isolated genomic DNA of food stuffs were

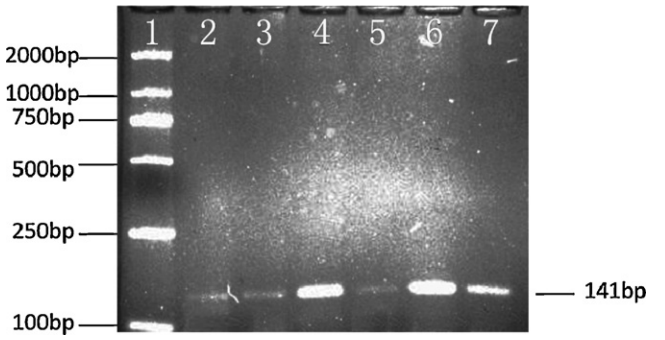

Fig. 8. 2% Agarose gel electrophoresis of PCR products corresponding to lectin gene: lane 1, DL 2000; lane 2, soybeans; lane 3, soya-bean milk; lane 4, soybean milk powder; lane 5, bean flour; lane 6, soybean sprouts; lane 7, GM soybean (GTS-40-3-2).

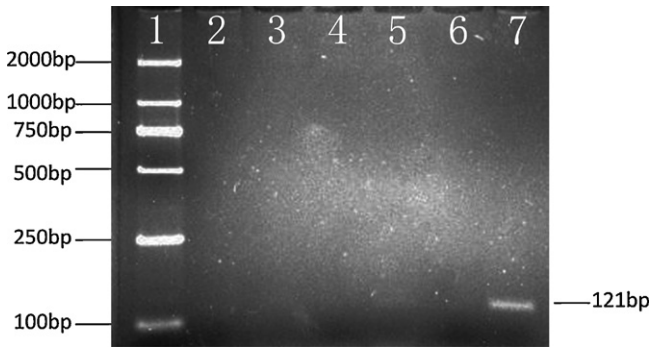

Fig. 9. 2% Agarose gel electrophoresis of PCR products corresponding to CP4-EPSPS gene: lane 1, DL 2000; lane 2, soybeans; lane 3, soya-bean milk; lane 4, soybean milk powder; lane 5, bean flour; lane 6, soybean sprouts; lane 7, GM soybean (GTS-40-3-2).

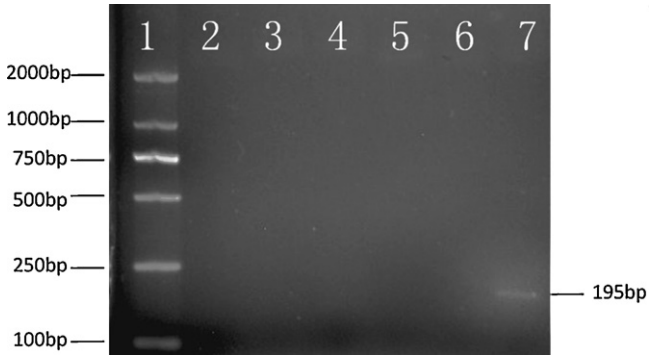

Fig. 10. 2% Agarose gel electrophoresis of PCR products corresponding to CaMV35S promoter gene: lane 1, DL 2000; lane 2, soybeans; lane 3, soya-bean milk; lane 4, soybean milk powder; lane 5, bean flour; lane 6, soybean sprouts; lane 7, GM soybean (GTS-40-3-2).

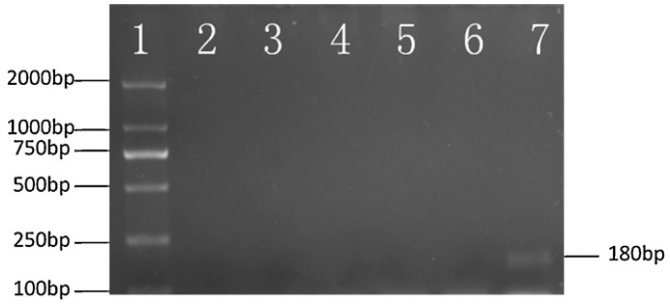

Fig. 11. 2% Agarose gel electrophoresis of PCR products corresponding to NOS terminator gene: lane 1, DL 2000; lane 2, soybeans; lane 3, soya-bean milk; lane 4, soybean milk powder; lane 5, bean flour; lane 6, soybean sprouts; lane 7, GM soybean (GTS-40-3-2).

calculated as a measure of purity. The *A*260/*A*280 ratios for the majority of sample are around 1.6, which displayed reasonable purity of the isolated DNA by UV absorbance. Genomic DNA extracted from foodstuffs could not be detected directly by spectrometry or electrophoresis due to low DNA contents and high degradation of DNA during productive processes. However, the yield of DNA recovered using PMNPs was more than sufficient for further application. Furthermore, PCR of chloroplast sequences was employed as a functional test for the magnetic particles. As shown in Fig. 7, the amplicated products of 1000 bp relevant to chloroplast ndhB gene were observed in all the samples tested.

Lectin is one of the housekeeping genes of soybean. The primer pairs, LEC-1 and LEC-2 (141 bp product), targeting the endogenous lectin gene, were used to confirm the presence of amplifiable soy DNA. The expected fragment was observed in all the foodstuffs that were tested (Fig. 8).

The primer pairs, RRS-F and RRS-R, targeting the CP4-EPSPS gene were used to detect the presence of GMOs. After the PCR, the amplications of 121 bp was observed for the GM soybean, while none was present in the non-GM soybean (Fig. 9). Meanwhile, the primer pairs, CaMV35S promoter and NOS terminator were also viewed for the GM soybean and none in the non-GM soybean (Figs. 10 and 11).

4. Conclusions

The present research developed a straight forward “single-pot” preparation methods of PAMAM modified magnetite nanoparticles. The different analytical techniques used to characterize the PMNPs confirm their stable structure and excellent properties.

The PMNPs where thereafter applied in the adsorption and purification of DNA from various foodstuffs, followed by an appropriate gel-electrophoretic distinction between GM- and non-GM food. The findings provide a facile approach and lay the foundation for the genomic analysis.

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