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Short Communication

DNA extraction from ghee and beef species identification using polymerase chain reaction (PCR) assay

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Abstract

A technique to isolate DNA from ghee was developed for the authentication of beef fat product. The method was based on pre-mixed ghee with phosphate buffer solution (PBS) prior to DNA extraction using Epicentre extraction method. The recovery of beef DNA was then analysed by polymerase chain reaction (PCR) using beef species-specific oligonucleotide primers which targeted the mitochondria DNA (mtDNA) of cytochrome b (cyt b) gene. The amplicon was 274 bp in size. The developed ghee extraction method offers a high yield of DNA providing 100 ng per µl and useful for validating beef fat product.

Introduction

The DNA-based approaches of polymerase chain reaction (PCR) techniques are well known as a specific, reproducible, sensitive, rapid processing time and low costs. However, the successful of this approach is limited by the presence of inhibitors in food matrix. The recovery of good quality DNAis vital in the PCR process. Without DNA or poor quality of DNA, the oligonucleotide primers are not able to amplify the targeted gene in the PCR assay.

In meat specification, either nuclear or mitochondrial DNA (nDNA and mtDNA) genes have been targeted in PCR analysis. Nuclear DNA is larger molecule arranged into chromosomes and contains a greater variation in its type of sequences compared to mtDNA. The mtDNA is commonly used for species identification in food analysis (Meyer *et al*., 1994; Matsunaga *et al*., 1999; Che Man *et al*., 2007; Sahilah *et al*., 2011) especially cytochrome b (cyt b) gene. High copy number of mtDNA is found in the cells, it remain intact during food processing thereby minimizing DNA degradation and does not contain any introns (Unseld *et al*., 1995). The major problem in DNA extraction from ghee is due to high fats content that may inhibit PCR analysis. The inhibitors in food can be in the form of polysaccharides, protein, humic acids and others. Some of the inhibitors are difficult to remove during DNA extraction process and remain until the final DNA preparations (Di Pinto *et al*., 2007). Thus, DNA purity and reliability from DNA extraction technique is very crucial to yield as much DNA as possible from the sample which the technique must beimproved inrecovery of nucleic acid and removing inhibitors prior PCR analysis.

There are many commercial DNA kits available for DNA extraction. Epicentre extraction kit is chosen due to its DNA purity and eliminates the inhibitor presence in food samples. This kit includes rapid desalting process to remove contaminating macromolecules and avoiding toxic organic solvents (Miller *et al*., 1988; Shimizu and Burns, 1995). In the presence study, we report a protocol to extract the beefDNA from ghee using Epicentre kit, identified and evaluate it quality using PCR analysis.

Materials and Methods

Ghee and palm oil samples

Ghee (1) and palm oil samples (3 brands) were purchased from supermarket in the area of Selangor in December 2012. The DNA samples were extracted from each sample in triplicates.

DNA extraction

Ghee fat was melted and 30 ml of ghee oil

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was added with 30 ml of phosphate buffer solution (PBS) (Sigma) (pH 7.0, 0.1 M). The mixturesolution was centrifuged at 13,000 rpm for 20 min and the upper layer was discarded. The upper layer was centrifugedat the same speed for 20 min and pellet was formed. Repeat this step if necessary and pellet were transferred into 1.5 ml centrifuge tube and incubated overnight at 65°C. Similar treatment was also conducted on palm oil samples. Thegenomic DNA wasthen extracted using the Epicentre MasterPure™ DNA purification kit. The volume of proteinase K and RNase A were increased if necessary. Thegenomic DNA was analyzed by electrophoresis in a 1.5% (w/v) agarose 1X TBE (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA) at 120 V for 60 minutes. Gels were stained with ethidium bromide. The amplicons were visualized with UV transilluminator (Alpha Imager TM2200). The 1 kb (Promega) was used as a DNA size marker.

Quantifying of DNA yield

Quantification of DNA extraction was done using UV/Vis spectrophotometer (BioPhotometer plus, Eppendorf).

PCR amplification

The extracted DNA was then subjected to PCR amplification. Amplification of genomic DNA was performed using the SIMB primers which targeted mtDNA of cyt b of beefspecies (Matsunaga *et al*., 1999). The sequences of forward and reverse of oligonucleotide are:

SIMB (F) : 5' GAC CTC CCA GCT CCA TCA AAC ATC TCA TCT TGA TGA AA 3'

SIMB (R) : 5' CTA GAA AAG TGT AAG ACC CGT AAT ATA AG 3'

The assay was performed in a 25 µl containing 1 µl of 100 ng DNA, 12.5 ul of the universal PCR mastermix (Promega), 8.5 µl of sterile distilled water and 1 µl pM each of the forward and reverse primers (Helix Biotech). Amplification was performed in Thermal-cycler (BioRad) with a temperature program consisting of the initial denaturation at 95°C for 2 minutes followed by 35 cycles of denaturation at 95 °C for 30 seconds and annealing at 55ºC for 30 seconds and extension at 72ºC for 40 seconds. The amplicons were analyzed by electrophoresis in a 1.5% (w/v) agarose 1X TBE (0.1 M Tris, 0.1 M boric acid, 0.2 mM EDTA) at 120 V for 60 minutes. Gels were stained with ethidium bromide. The amplified fragments were visualized with UV transilluminator (Alpha Imager TM2200). The 100 bp and 1 Kb DNA ladder (Promega) was used as a DNA size marker.

Result and Discussion

DNA is crucial in any polymerase chain reaction (PCR) analysis. Without DNA, PCR process lacks of results. The DNA quality is important prior PCR analysis. In the present study, using the above method the beef DNA was successfully extracted from ghee oil. As shown in Figure 1, the DNA showed very sharp band on 1.5% (w/v) agarose gel which explained the quality of extracted DNA was good. High volume of ghee oil was used due to its greater chances in extracting as much as possible of DNA from this fat product. The overnight incubation time at 65°C helped increase the recovery of DNA molecules using Epicentre Kit with final product of 100 ng of DNA per µl. Cooking oil of palm olein samples were also extracted using the above methods but no band was shown (Figure 1). The DNA extracted from ghee is possible since it also reported by other workers where they were successful in extracting olive DNA from oil (Fernando de la *et al*., 2004).

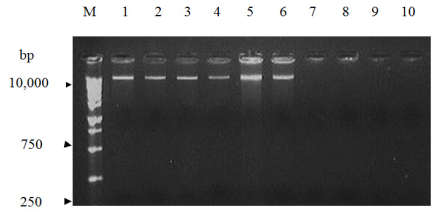

Figure 1. Genomic DNA extracted from ghee oil. Lane M: 1 kb ladder; Lane 1-6: Beef DNA; Lane 7-9: palm oil (Brand A, B and C) and Lane 10: Negative control (palm olein)

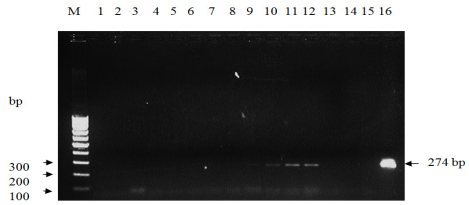

Figure 2. Amplicon of beef DNA isolated from ghee product. Lane M: 100 bp ladder; Lane 1: No DNA was added; Lane 2: negative control; Lane 3-5; palm oil brand A; Lane 6-8: palm olein brand B; Lane 9-12: ghee oil; Lane 13-15: palm olein brand C; Lane 16: positive control (Beef DNA)

The existence of beef DNA in ghee was confirmed by PCR analysis, the PCR mixed was amplified using SIMB oligonucleotide primers. The PCR analysis was also conducted for palm olein extracted sample to ensure there was no trace of beef DNA in the products.All ghee samples were positive with beef DNA, indicated a single band with a molecular weight

of 274 bp. While, none for palm olein samples using the above method. Our finding confirmed that the DNA extracted from ghee was a beef DNA using the oligonucleotide primers as described by Matsunaga *et al.* (1999).

Conclusion

In conclusion, we have demonstrated the pre-mixed of ghee with PBS and extracted beef DNA using Epicentre kit is useful for validating the animal-based fat from beef source.

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