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Identification of the species origin of commercially available processed food products by mitochondrial DNA analysis

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Abstract: Legislation concerning the safety assessment and labelling of foodstuffs has been implemented in many countries. Consequential to a number of cases of food adulteration reported globally, a fast and reliable detection method for the food traceability is required in ensuring effective implementation of food legislation in a country. In this study, PCR-RFLP technique based on cyt b gene has been tested for its suitability for these purposes. This method combines the use of a pair of universal primer that amplifies a 359 bp fragment on the cyt b gene from meat muscle DNA and restriction enzyme analysis. Analysis of experimental beef frankfurter, minced beef, pork frankfurter and pork cocktail samples demonstrated the suitability of the assay for the detection of the beef (Bos taurus) and pork (Sus scrofa), but not applicable for some processed food, particularly detection of mackerel (Rasterelliger brachysoma), sardine (Saedinella Fimbriata) and tuna (Thunnus tonggol) origin in canned food. Commercial frauds through species mislabelling or misdescribed were not detected. The assay is demonstrated applicable for routine analysis of meat traceability of foodstuffs and legislation purposes, if sufficient availability of detectable mtDNA in the foodstuffs is ensured.

Keywords: species detection, mitochondrial DNA, commercially available processed food, meat fraudulent detection

Introduction

In many countries, great value is placed on labelling requirements to facilitate accurate and safe animal identification by the consumers. The risk and threat of food adulteration and mislabelling have become a large concern and challenge for the food control authorities and consumers. Therefore, to enhance food security, fast and reliable detection methods are indispensable for the food industry. In order to enable food control authorities to supervise compliance with labelling requirements, suitable detection methods which could allow unambiguous identification of animal or fish in foodstuff are prudent.

The development of methods for food traceability has been the subject of intense research in many countries. In Europe, food traceability and authentication were seriously considered following EC regulation that requires source of all raw materials in food to be identified (EC, 2002).

A number of Polymerase chain reaction (PCR) based assays have been developed, evaluated and have shown superior for species detection in foodstuff (Jonker et al., 2008) and mislabeling discovery (Machado-Schiaffino et al., 2008). In fact, identification of a mixture of more than one species is possible by PCR (Hubalkova et al., 2008). The genetic identification of different species is made possible by using a lot of molecular markers such as forensically informative nucleotide sequencing (FINS) (Blanco et al., 2008), randomly amplified polymorphic DNA (RAPD) (Calvo et al., 2001), amplified fragment length polymorphism (AFLP) (Fumiere et al., 2003), RFLP (restriction fragment length polymorphism) (Bellagamba et al., 2001), Real-time polymerase chain reaction (qPCR) (Jonker et al., 2008; Prado et al., 2007) and single nucleotide polymorphisms (SNP) (Zhao et al., 2006). These methods differ in genetic information, in standardization of protocols,

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in the interpretation of results and in equipments that are required.

Foodstuff containing even trace amounts of meat must be labeled correctly. Indeed, falsely label food product could be perilous for consumers with chronic illnesses who require specific dietary. For religious concern, consumers require clear and accurate information to make choices about their diet (Woolfe and Primrose, 2004). Recently, we have developed a meat traceability technique based on mtDNA, which is rapid, robust, accurate and cost-effective method for determining the species origin of meat (Chandrika et al., 2009). Given the increasing importance of food traceability for safety, quality and typicalness issues, the restriction fragment length polymorphism-polymerase chain reaction (PCR-RFLP) fingerprinting protocol described in our previous study may represent a suitable tool for tracing the meat or fish origin in processed foodstuff. The methodology afforded by this helpful technique could be well suited for food traceability application due wholly to the specificity and sensitivity, which would allow the unambiguous identification of animal species in food. Consequently, validation studies of existing methods based on commercially obtained processed product are required to ensure the effectiveness of the technique in detecting food adulteration and for the implementation of food legislation.

The applicability of PCR-RFLP method, as outlined above, has been readily embraced by researchers for the study of food research (Fernandez-Tajes and Mendez, 2007; Girish et al., 2007; Lahiff et al., 2001). For several reasons, we are interested in developing a traceability method based on PCR-RFLP and mtDNA for processed food analysis. Firstly, the technique of PCR-RFLP is less costly for a routine food traceability analysis as compared to sequencing technique which is a non-cost effective especially for large-scale food traceability. Also this technique is rapid and not excessively time consuming to be performed. MtDNA gene analysis has been found to be a reliable tool to detect or authenticate meat origin (Fajardo et al., 2007). In particular, the cyt b locus which consists of highly conserved sequences has been chosen for the performance of PCR-RFLP. Cyt b gene sequence has been well characterized among different vertebrate groups, thus allows the recognition of genetic variation between species. (Irwin et al., 1991; Hatefi, 1985). Targeting sequence from mtDNA would certainly facilitate the PCR amplification of DNA from processed food, where the food processing technique could severely damage or degrade genomic DNA, as compared to ntDNA which relatively much larger in base pairs and consist of low copy quantity in cells.

Although the current information on the use of mtDNA for performing PCR-RFLP assay on processed food is available, there has been an increasing interest in recent years for applying this method on commercially available processed food, thus to facilitate the detection of species adulteration and implementation of food legislation. This study focused on the validation of PCR-RFLP assay, based on mtDNA, as a suitable tool to perform safety assessment and traceability of foodstuffs. The present study was also undertaken to authenticate meat and fish origin in several commercially available processed food using PCR-RFLP technique.

Materials and Methods

Sample collection and processing

Processed food samples were purchased from local wet markets around Selangor and Kuala Lumpur, Malaysia. Several widely available processed foods of meat and fish in Malaysia markets, such as beef frankfurter, pork frankfurter and pork cocktail, minced beef, beef burger, chicken meatball, chicken cocktail, pork pepper corn, honey cured ham, pork black pepper, tuna fish sausage, fish cake, fish nugget, fish finger, canned mackerel, canned sardine, tuna in water, tuna in mayonnaise, tuna in vegetable oil and tuna in soya sauce were chosen for analysis. The number of samples collected for each processed food varies from 2-6 samples. The processed food samples were stored at 4°C without removing their container or package. The container or package is uncovered prior to analysis. Then after, 120 mg of ground meat or fish muscle obtained form the respective processed foods was transferred into a 1.5 ml micro centrifuge tube for DNA extraction.

Extraction of genomic DNA

DNA was extracted from the processed food samples using Dneasy Protocol for Animal Tissue provided with the Dneasy Tissue kit (Qiagen, Germany). As a control, genomic extraction was also performed on raw meat and fish samples. One hundred and fifty microliters of elution buffer (Buffer AE) was pipetted directly onto the DNeasy membrane and incubated at room temperature at 1 min. This was then spun at 12,000 g for 1 min to elute. The DNA solution was stored at 4°C. Briefly, ground meat or fish muscle was incubated at 55°C with one hundred and eighty microliters of tissue lysis buffer (Buffer ATL) and 20 µl of 20 mg/ml Proteinase K (Qiagen, Germany) to disintegrate the muscle tissue and divest

the DNA product from contaminating proteins. After overnight precipitation, an extended 70°C for 10 min treatment with two hundred microliters of lysis buffer (Buffer AL) were performed to the sample. Following cellular lysis, the samples were incubated in two hundred microliters of ethanol (96–100%) to allow a formation of homogenous solution. After transferring to DNeasy mini column and spunning at 12,000 g for 1 min, the sample were washed several times using the washing buffers added with ethanol. DNA stock samples were eluted from the DNeasy mini column using one hundred and fifty microliters of elution buffer. DNA concentration was estimated by UV absorption spectrophotometry at a wavelength of 260 nm and stored at 4 °C until further use.

Genomic DNA detection

The integrity of the DNA extracts was determined by electrophoresis by running 10 µl of the extracted DNA on a 1% agarose (Sigma, Germany) at 76 V in 1x Tris-Boric acid-EDTA (TBE) buffer, pH 8.0 [0.089 M Tris Base, 0.089 M Boric Acid and 0.002 M Ethylenediaminetetraacetic Acid (EDTA)]. Three microliters of 1 kb DNA Ladder (Bioron, Germany) was used as size reference. The DNA was dissolved in gel-loading buffer [0.25% (w/v) Bromophenol blue, 0.25% (w/v) Xylene cyanol, 40% (w/v) Sucrose]. The electrophoresis was run, using Bio-Rad Power Pac 300 power supply (Hercules, USA). The gel was stained with etidium bromide (1 µg/ml) for 30 s and destained in sterile water for 30 min before viewing using gel documentation (Syngene, Frederick, USA). The presence of an intense band, with minimal degradation, indicated intact genomic DNA.

PCR amplification

A pair of universal primer, cyt b1 and cyt b2, which was published by Kocher et al. (Kocher et al., 1989) was used to amplify the cyt b gene. Primers sequences were as follows: forward (cyt b1), 5'- CCA TCC AAC ATC TCA GCA TGA TGA AA-3') and reverse (cyt b2), 5'-GCC CCT CAG AAT GAT ATT TGT CCT CA –3'). The primers were purchased from Research Biolabs (Selangor, Malaysia). Reactants for amplification were combined in 0.5 ml polypropylene tubes to give a final volume of 50 µl. Each reaction mixture contained 200 µM nucleotides (dNTP) mix (Finnzymes, Finland), 1x PCR reaction buffer containing 10 mM Tris-HCl, pH 8.8, 50 mM KCl, 0.1% Triton X-100 and 1.5 mM MgCl2 (Finnzymes, Finland), 10 pmol of each primer (Research Biolabs, Selangor, Malaysia), 100-150 ng template DNA and 1.25 unit Taq DNA polymerase (Finnzymes, Finland). PCR was carried out in a Gene-Amp PCR system 2400 thermocycler (PerkinElmer, USA). The cycling conditions included a single initial denaturation at 94ºC for 2 min followed by 40 cycles of 94ºC for 5 s (denaturation), 55ºC for 30 s (annealing), 72ºC for 40 s (primer extension) and a final extension step at 72ºC for 2 min. Negative controls (water) were included in each PCR amplification, in order to verify the PCR efficiency and to detect contamination. Amplified PCR products were detected by running 10 µl of the PCR mixture on a 2% agarose (Sigma, Germany) at 76 V in 1x Tris-Acetate-EDTA (TAE) buffer, pH 8.3 (0.04 M Tris-acetate and 1 M EDTA). The gels were viewed using gel documentation (Syngene, Frederick, USA). Quality control included both positive and negative controls and PCR amplified in parallel with all specimens. The PCR amplifications were done several times for samples which provide negative PCR results to confirm the suitability of performing PCR for sample which has little trace amount of genomic DNA. In order to minimize the potential for contamination, DNA extractions, PCR setup, and agarose gel electrophoresis were performed in three separate rooms.

Enzymatic digestion of amplified DNA

The PCR products of mitochondrial cyt b gene obtained from processed feedproducts were digested with AluI, BsaJI, RsaI, restriction enzymes (New England Biolabs, UK), the suitability of the chosen enzymes to restrict 359 bp cyt b of chicken, beef, pork, tuna, mackerel and sardine was conformed by NEBcutter V2.0. Digestions were performed in a total volume of 20 µl containing 10 µl of amplified DNA, 5 U of restriction enzyme and 2 µl of 1x digestion buffer, whereas digestions with BstNI and MseI enzymes were supplemented with 0.5 µl, 100 µg/ml BSA (bovine serum albumin). The digestion mixture was incubated for 16 h. The incubation temperature was chosen as recommended by the manufacturer for optimal result. The digested samples were analyzed by electrophoresis by using 2% agarose gel in 1x TBE buffer, pH 8.0 for 1 h at 90 V and stained by ethidium bromide. A 100 bp DNA ladder (New England Biolabs, UK) was used as size reference. The gels were visualized using the gel documentation (Syngene, Frederick, USA). Quality control included undigested PCR amplified product was run in parallel with restriction enzyme treated PCR product.

Results

DNA quantification and amplification

Intact, high molecular weight genomic DNA was extracted from some processed food samples.

As shown in Figure 1, some processed food samples no longer contained an intact, high molecular weight band, demonstrating significant levels of DNA degradation. Beef frankfurter, minced beef, pork frankfurter, pork cocktail and other processed food samples had A260/A280 ratios between 1.409-1.841. The control raw samples of beef, pork, mackerel, sardine and tuna DNA have A260/A280 ratios between 1.419-1.88. The PCR performed on the mtDNA from control meat and fish samples with primers cyt b1 and cyt b2 resulted in specific amplification of a 359 bp DNA fragment, as found in Figure 2. Similarly, the PCR performed on the DNA from beef frankfurter, some of minced beef product, pork frankfurter and pork cocktail with these primers produced specific amplification of a 359 bp DNA fragment. Negative PCR amplification results of cyt b gene were found for chicken meatball, chicken cocktail, pork peppercorn, spam, tuna fish sausage, fish cake, fish nugget, fish finger, canned mackerel, canned sardine, canned tuna in water, canned tuna in vegetable oil and canned tuna in soy sauce samples.

PCR-RFLP analysis

Restriction fragment length polymorphism of the cyt b gene of beef frankfurter, minced beef, pork frankfurter, pork cocktail and the others food were shown in Figure 3 and Table 1. The restriction enzyme profiles for chicken product when subjected with Rsa1 endonuclease digestion showed bands of 149 bp and 210 bp. The amplified DNA fragments of pork frankfurter, pork cocktail, pork black pepper and honey cured ham were digested into two fragments which migrated to nearly the same positions respectively on agarose gels were subjected to Alu1 and BsaJ1 digestions. The Alu1 digestion generated 244 bp and 115 bp and the BsaJ1 digestion generated 131 bp and 228 bp for pork frankfurters, pork cocktails, pork black pepper and honey cured ham samples. As expected, referring to the RFLP profile of the raw meat, Rsa1 enzyme did not digest the cyt b gene from pork frankfurter and pork cocktail but did produce bands for beef and chicken processed food. The restriction profiles for chicken cocktail, spam, tuna fish sausage, fish cake, fish nugget, fish finger, canned mackerel, canned sardine, canned tuna in water, canned tuna in vegetable oil and canned tuna in soy sauce samples were not available due to the absent of PCR amplification, could be mainly consequential of contamination or degraded mtDNA. We concluded that processed foods tested, referring to the samples which provided successful PCR amplification, were free from adulteration and not misdescribed as the expected meat were present in the tested food samples.

Discussion

Ensuring food safety has resulted in increased interest in the development of food detection technique. In Britain alone, there was a decline in beef sale as consumers are aware of Bovine spongiform

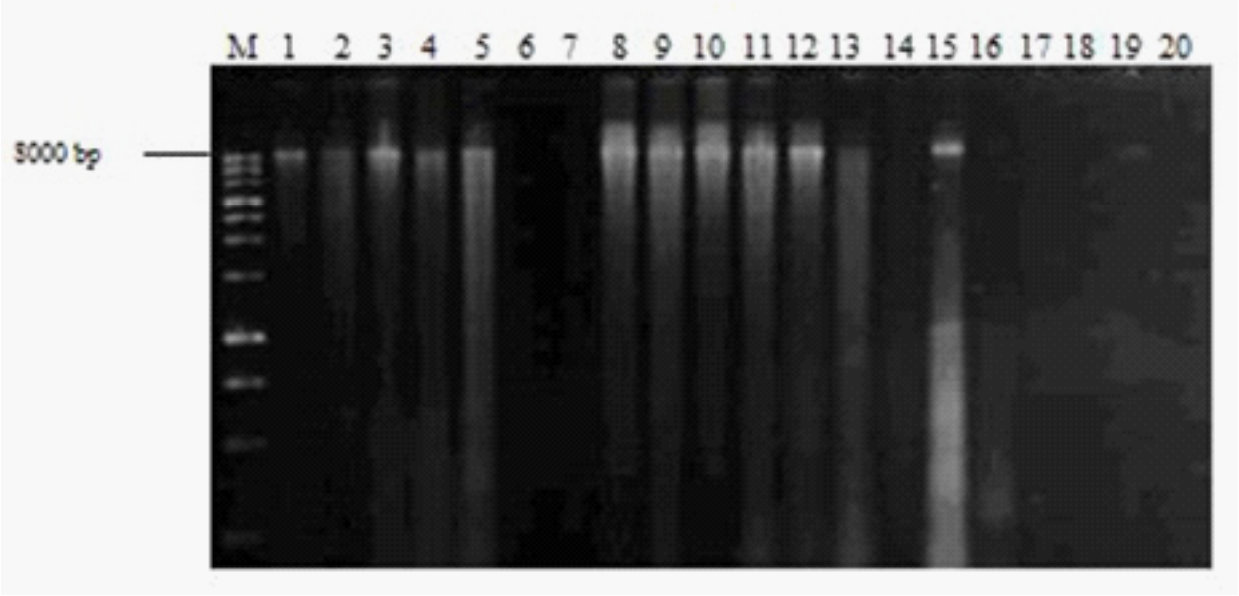

Figure 1. Gel electrophoresis of genomic DNA of processed foods. Lanes: M, 1 kb DNA ladder; 1, Beef Burger; 2, Beef Frankfurter; 3, Minced Beef; 4, Chicken Meatball; 5, Chicken Cocktail; 6, Spam; 7, Pork Peppercorn; 8, Pork Frankfurter; 9, Honey Cured Ham; 10, Pork Cocktail; 11, Pork Black pepper; 12, Tuna Fish Sausage; 13, Fish Cake; 14, Fish Nugget; 15, Fish Finger ; 16, Canned Mackerel; 17, Canned Sardine; 18, Tuna in Water; 19, Tuna in Vegetable Oil; 20, Tuna in Soya Sauce

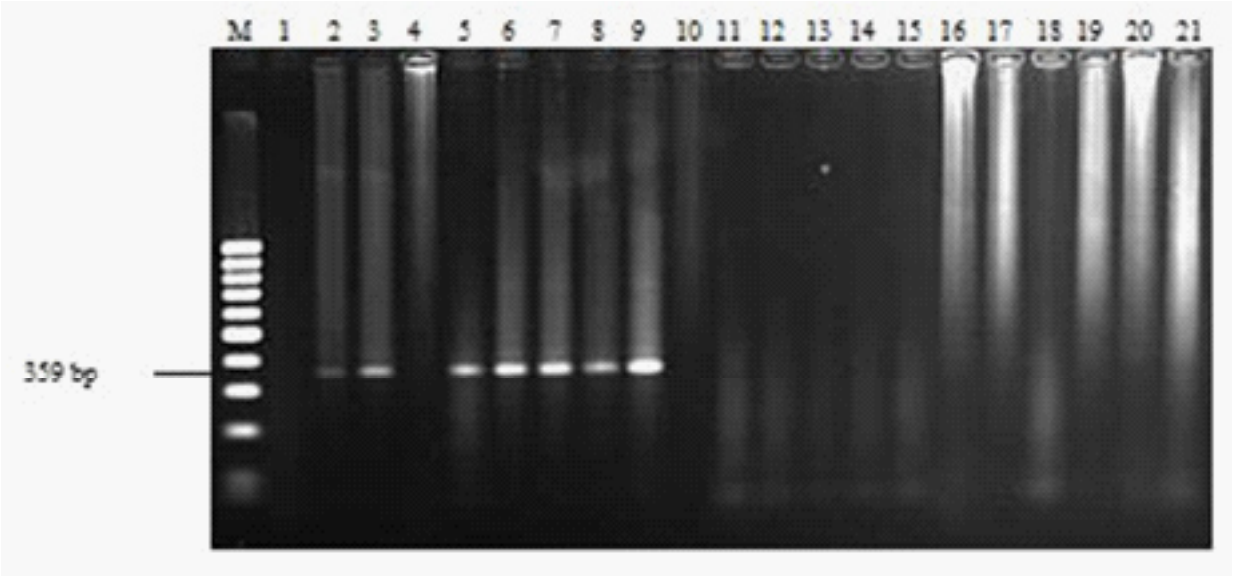

Figure 2. PCR amplification results of cyt b gene of beef frankfurter, minced beef, pork frankfurter, pork cocktail and processed food used as control for samples two. Lanes: M, 1 bp ladder; 1, Beef Burger; 2, Beef Frankfurter; 3, Minced Beef; 4, Chicken Meatball; 5, Chicken Cocktail; 6, Pork Frank; 7, Honey Cured Ham; 8, Pork Cocktail; 9, Pork Black pepper; 10, Pork Peppercorn; 11, Spam; 12, Fish Sausage Tuna; 13, Fish Cake; 14, Fish Nugget; 15, Fish Finger ; 16, Canned Mackerel; 17, Canned Sardine; 18, Tuna in Water; 19, Tuna in Vegetable Oil; 20, Tuna in Soya Sauce; 21, control

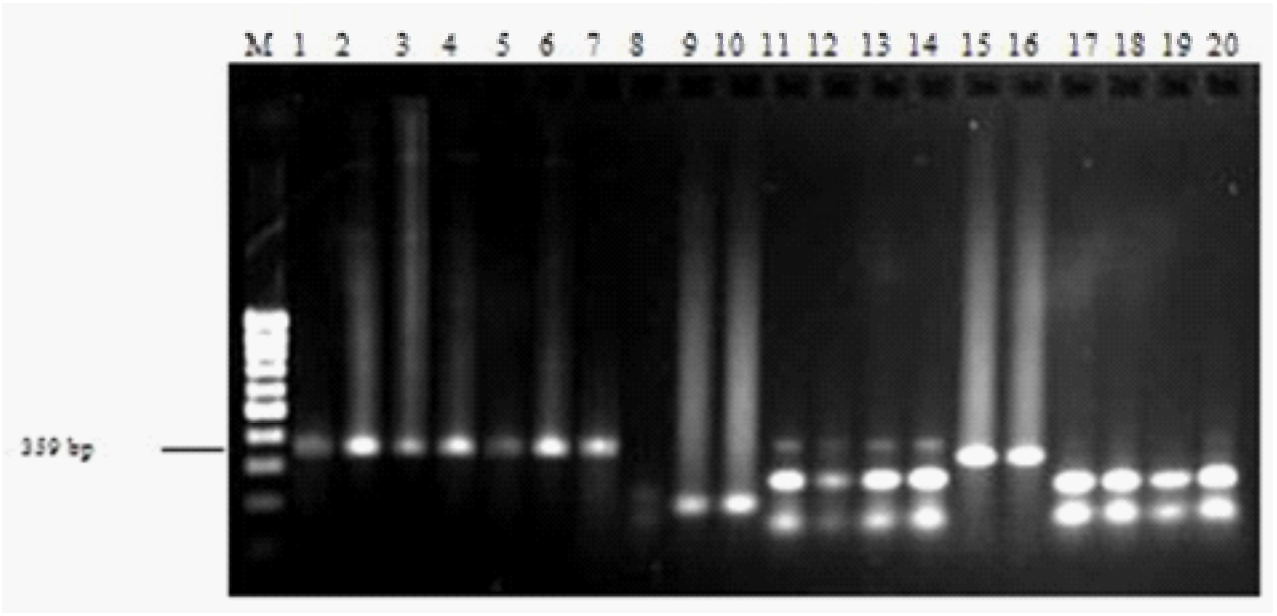

Figure 3. PCR-RFLP of the cyt b gene of processed foods. Lanes: M, 100 bp DNA ladder; 1-7, amplified cyt b from minced and beef frankfurter, c hicken cocktail and pork frankfurter, honey cured, cocktail and black pepper; 8, chicken cocktail digested with Rsa1; 9 and 10, minced and beef frankfurter digested with Alu1; 11-14, pork frankfurter, honey cured, cocktail and black pepper digested with Alu1; 15-16, minced and beef frankfurter digested with BsaJ1; 17-20, pork frankfurter, honey cured, cocktail and black pepper digested with BsaJ1

Table 1. Restriction fragment length polymorphism of the cyt b gene of different processed food

Meat Based Processed Food					
Type	Replicates	cyt b Y	Restriction Enzyme Profile		
			RsaI	AluI	BsaJI
Pork black pepper	1	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	2	+	n/a	244 bp + 115 bp	228 bp + 131 bp
Pork Cocktail	1	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	2	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	3	+	n/a	244 bp + 115 bp	228 bp + 131 bp
Honey cured ham	1	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	2	+		244 bp + 115 bp	228 bp + 131 bp
Pork Frankfurter	1	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	2	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	3	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	4	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	5	+	n/a	244 bp + 115 bp	228 bp + 131 bp
	6	+	n/a	244 bp + 115 bp	228 bp + 131 bp
Pork Pepper Corn	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Beef Frankfurter	1	-	n/a	n/a	n/a
	2	+	n/a	190 bp	320 bp
	3	-	n/a	n/a	n/a
	4	+	320 bp	190 bp	320 bp
Minced Beef	1	+	320 bp	190 bp	320 bp
	2	-	n/a	n/a	n/a
	3	-	n/a	n/a	n/a
	4	-	n/a	n/a	n/a
Beef Burger	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Chicken cocktail	1	+	149 bp + 210 bp	n/a	n/a
	2	-	n/a	n/a	n/a
Chicken Meatball	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Fish Based Processed Food					
Type	Replicates	cyt b*	Restriction Enzyme Profile		
			RsaI	AluI	BsaJI
Tuna Fish sausage	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Fish Nugget	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Crumbed fish	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Fish Finger	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Canned Tuna in Vegetable oil	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Canned Tuna in Soya Source	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Canned Tuna in Mayonnaise	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Fish Nugget	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Canned Mackerel	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a
Canned Sardine	1	-	n/a	n/a	n/a
	2	-	n/a	n/a	n/a

Note:

Y	PCR amplification
+	successful PCR amplification
-	unsuccessful PCR amplification
n/a	not assessed

encephalopathy (BSE) disease, a transmissible, neuro-degenerative fatal brain disease of cattle. It has caused a serious food safety concern among buyers regarding cattle meat and bone meal products, they remain not confident in the safety of beef on sale. In many countries, food traceability systems are becoming mandatory, they are particularly important for tracing livestock and species in animal products. Indeed food fraudulent is widely reported in many countries, direct substitution of one species by another and of mixed species products have been proven by analysis. This has resulted in consumers' lack of confidence towards the species origin in the processed food and also the safety of the food.

Molecular identification techniques are specifically developed to detect species origins in feedstuff or processed food (Khairalla et al., 2007; Jonker et al., 2008). Accurate traceability of feedstuff has been based primarily on PCR-based assays. Other interesting works have been done to detect species origin using different molecular methods in different meat matrices, which was also found to be highly feasible (Chen et al., 2009). Measurements of DNA of species in processed food can, however, be difficult to standardize due to sample processing and contaminants factors. Recently, a PCR-RFLP method for species detection has been developed in our laboratory. The PCR-RFLP assay described is both sensitive and specific in identifying species origin; it principally confirmed detection of species in raw meat. In this study, we explore and report the possibility of detecting species origin from commercial available processed food samples using a PCR-RFLP based on cyt b gene.

A number of investigators have attempted to detect species origin from foodstuffs, in particular processed foodstuff (Prado et al., 2007). In some of their experiments, processed food samples were prepared with added ingredients such as sugar and spices in laboratory (Calvo et al., 2002), followed by performing standardized methodologies to identify species origin in that sample. Performance of PCR assay on these prepared food samples has provided positive detection for some processed food sample. In this study, we have decided to obtain commercially available processed food samples as such to avoid prompt analysis. This measure is important to be considered as the shelf-life and added ingredients to the processed food could highly influence the integrity of the mtDNA. Moreover, availability of DNA quality is influenced by the whole food chain processing. We believe that prompt analysis to be performed in the laboratory could persistently yield in positive mtDNA amplification, given that detectable mtDNA for PCR technique could constantly be available at trace amounts. In this context, it is noteworthy that the availability of detectable mtDNA from processed food is to be the most critical point in PCR-RFLP assays. Therefore, for a technique to be developed based on mtDNA for legislation purposes, it is imperative that such measures to be taken.

Detection of DNA from canned products was assessed by few investigators and was reported to be impractical. Food processing involving excessive heat treatment such as processing canned food product causes a significant degradation to the DNA structure (Quinteiro et al., 1998; Miguel and Begona, 2004). In this study, we noticed that applying molecular technique based on mtDNA on canned processed food could be more challenging that other processed food which basically involved processing under ambient temperature. Although, the DNA extraction technique used here utilizes silica that may improve the yield of small to moderate length DNA fragments, better DNA yield was not obtained from the canned processed food.

Applying silica technique to obtain the highest quantity of mtDNA from meat mixed with plant derivatives certainly would prevent or negatively influence the sensitivity of PCR-RFLP, but in practice it is not complicated to achieve. We concluded that even with added ingredients, which could act as PCR inhibitors, this assay could be successfully be performed to commercial processed food, as positive species detection were available for large number of meat products, some constitute with honey, plant derivatives and other many ingredients.

Despite reports of difficulties, however, some researchers have optimized detection assays to quantify DNA from processed food samples (Woolfe and Primrose, 2004). Woolfe and Primrose have described that PCR assay is effective in measuring DNA from processed food (Woolfe and Primrose, 2004). Although successful for some analytical studies, it is not practical to confirm the availability of detectable DNA from severely damaged DNA by PCR assay. Interpretation of restriction profiles could be complicated for PCR-RFLP assay performed using severely degraded DNA samples. Thus, factors that promote degradation can pose challenges for PCR-RFLP data interpretation. Availability of good, intact DNA from processed food samples is highly dependent on the thermal treatment applied during the processing procedure. Perhaps a non-thermal food preservation technique such as High hydrostatic pressure processing (HHP) or high pressure processing (HPP), used for microbial and enzyme inactivation, that reduces the effects on nutritional and quality

parameters could limit the damages impose to the DNA.

Quantification of trace amount of DNA from degraded DNA has proven to be problematic. A commonly used approach that is to apply the most sensitive PCR method, which could amplify fragment copies of amplicons from degraded DNA, could not constantly provide positive PCR detection. Even with trace amount of DNA availability, detection levels are generally could be low following PCR amplification. But, the availability of low quantity of amplicons does not necessarily reflect that the RFLP could be performed using the low amplicons obtained. This trend is consistent with work conducted in this study, for some of the processed food samples where faint band was detected, particularly from the canned fish products, where the performance of RFLP is not practical. This work has highlighted the difficulties of applying PCR-RFLP base on mtDNA for detecting specific origin in certain processed food products. Although this approach provides reliable analysis of species detection in meat processed food, the requirement for performing PCR-RFLP is largely dependent on the availability of detectable mtDNA from the processed products. Despite these difficulties, due to the application of mtDNA, successful performance of PCR-RFLP could be achieved for certain processed food. The sensitivity of this technique to authentic the meat present in the tested food confirms the suitability of the proposed method for food traceability analysis.

Conclusions

Commercial adulteration of processed meat samples was not detected. The method developed based on mtDNA may be exceptionally handy for food adulteration or fraudulent detections. Based on the result obtained from this study, detection of cyt b gene of species included in a particular processed food may largely dependent on the processing technique applied. In this respect, identifying the suitability of using PCR-RFLP assay according to the processing technique of food will most likely circumvent disappointment in getting negative species detection. The information presented here, suggests that PCR-RFLP are useful tools for detecting food adulteration, depending on the processed food chosen, and will help to protect consumers' rights by enabling the enforcement of labelling regulations in a country.

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