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PERPUSTAKAAN UTAMA

DETECTION THRESHOLD OF THE CYTOCHROME B GENE IN MEAT SAMPLES

ANNIE WONG FUI LING

THIS THESIS IS PRESENTED TO FULFILL THE REQUIREMENT TO OBTAIN A BACHELOR OF BIOTECHNOLOGY DEGREE WITH HONOURS

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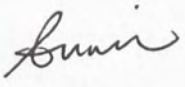

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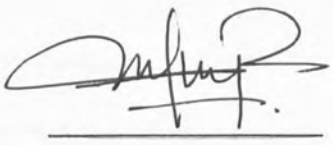

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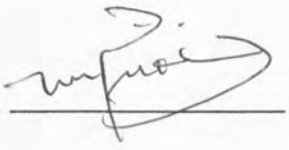

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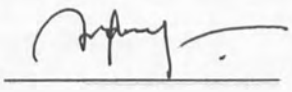

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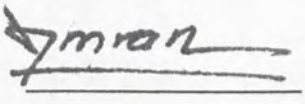

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ABSTRACT

The morphological ambiguities regarding to the traditional methods of animal species classification has lead to the requirement of a more reliable and practical method of species identification. A polymerase chain reaction-based analysis has been developed by amplification of the conserved region of the mitochondrial DNA (mtDNA) cytochrome b (cyt b) gene using universal primers CYTB1 and CYTB2. A good quality of total DNA was extracted from mixtures of raw chicken and turkey and also beef and buffalo and was subsequently subjected to PCR amplification yielding distinct bands of approximately 360 bp in all species. The threshold detection of the mixtures was determined by RFLP analysis by selected restriction enzymes (*Rsa*I, *Bst*nI, *Taq*I and *Alu*I). *Rsa*I has successfully discriminated both chicken and turkey resulting a detection sensitivity limit of 5% turkey. While beef and buffalo was differentiated by two restriction enzymes *Bst*nI and *Taq*I producing fragments of 318bp and 42bp for buffalo and 191bp and 168bp for beef . *Alu*I has failed to produce variable RFLP pattern in this mixture. However, the sensitivity of the beef and buffalo mixture with beef as the minor component cannot be determined as species substitution might have occurred due to the similar banding pattern observed in the gel where beef was suspected to be mislabeled with buffalo. The cyt b PCR-RFLP threshold detection of raw meat samples has proved to be sufficient in both sensitivity and species identification assay.

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ABSTRAK

Ketidakpastian di dalam kaedah tradisional dalam penentuan spesies haiwan telah mencetuskan keperluan terhadap kaedah yang lebih mantap dan praktikal untuk tujuan tersebut. Satu kaedah berdasarkan analisis tindak balas rantaian polimerase (PCR) telah dijalankan ke atas gen sitokrom b daripada mitokondria hasil daripada pencirian asid deoksiribonukleik (DNA) daripada campuran daging ayam dan turki dan juga kombinasi di antara daging lembu dan kerbau. Gen ini telah dimanipulasikan dengan menggunakan satu pasang primer universal untuk menghasilkan produk bersaiz kira-kira 360 pasangan bes. Kaedah tindak balas rantaian polimerase (PCR) di atas telah digabungkan dengan kaedah 'restriction fragment length polymorphism' (RFLP) untuk menentukan kesensitifan bagi keseluruhan kaedah-kaedah ini untuk membezakan di antara spesies haiwan di dalam dua campuran tersebut. Empat jenis enzim pembatasan telah digunakan (*Rsa*I, *Bst*nI, *Taq*I dan *Alu*I) di mana *Rsa*I telah berjaya membezakan spesies ayam dan ayam turki sehingga kepada tahap 5%. Manakala bagi campuran spesies lembu dan kerbau, enzim pembatasan *Bst*nI dan *Taq*I adalah lebih sesuai, dengan menghasilkan fragmen-fragmen bersaiz 318bp dan 42bp bagi kerbau dan 191bp dan 168bp bagi lembu . Walaubagaimanapun, *Alu*I tidak dapat membezakan dua spesies ini kerana data menunjukkan kesamaan di dalam profil RFLP mereka. Walaubagaimanapun, tahap kesensitifan bagi pengenalpastian spesies tidak dapat ditentukan di dalm campuran kedua ini kerana terdapat kemungkinan bahawa daging lembu telah disalahlabelkan dengan daging kerbau berdasarkan kepada profil yang sama di antara kedua-dua spesies. Kaedah PCR-RFLP ini telah terbukti berkesan di dalam penentuan kesensitifan dan pengenalpastian spesies.

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LIST OF ABBREVIATIONS

ATP	Adenosine triphosphate
BERNAMA	Berita Nasional Malaysia
bp	Base pair
cm	centimeter
cyt b	Cytochrome b
ddH_2O	Double distilled water
DNA	Deoxyribonucleic acid
dNTPs	Deoxyribonucleotides
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
g	gram
JAKIM	Jabatan Kemajuan Islam Malaysia
kb	kilobase
M	Molar
MITI	Malaysian International Trade and Industry
mol	molarity
mRNA	messenger ribonucleic acid
mtDNA	Mitochondrial DNA
mL	milliliter
mg	milligram
min	Minute
ng	nanogram
OD	Optical density
PAGE	Polyacrylamide gel
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction-Restriction Fragment Length Polymorphism
pmol	picomol

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RE	Restriction enzyme
RFLP	Restriction Fragment Length Polymorphism
RNA	Ribonucleic acid
rRNA	ribosomal ribonucleic acid
SDS	Sodium dodecyl sulfate
Sec	second
TBE	Tris-borate-EDTA buffer
TE	Tris-EDTA
tRNA	transfer ribonucleic acid
UV	Ultra violet
V	Volt
µL	microliter
µg	microgram
°C	degree celcius

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Label vs. Reality. Consumers are getting more and more alert nowadays regarding to food authenticity and adulteration which has arose from various religious strictures, perceived or real health concerns and cultural likes and dislikes. Numerous major religions have strict nutritional laws concerning meat origin. Hindus forbid the consumption of cattle as it is regarded sacred, while Buddhist practices meatless meal to avoid cruelty to animal. For Islam, Muslims are prohibited from eating pork as it is considered haram. Some people follow a vegetarian diet as their daily eating habit and others are allergic for some food component but what ever the motives are, consumers have the right to get what they are paying for, thus, tagging must provide truthful and no ambiguity information to avoid legal action by the authorities.

Many queries have been rose by consumer on how and why food contamination occurs. Food contamination as in this case is the intentional (by mean illicit species substitution) or unintentional (preparation hygiene during processing) mixing of

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undeclared species of meat other than indicated by the label itself of the meat products. Species substitution for illicit financial gain is perhaps as old as trade itself. It is very regular that declared or implied meat product is being replaced with cheaper one by some selfish trader. While some raw meats can be easily differentiated from others based on color and texture others are often analogous in appearance making substitution fairly easy, and once integrated in a comminuted product; identification based on appearance is utterly lost or not obvious. This phenomenon often leads to mislabeling. For example, turkey meat can be easily substituted by chicken meat and beef to be replaced by buffalo as they share a quite similar physical appearance. Species substitutions also arise where availability is a major concern. Trade barriers are often based on quotas by species every now and then, thus, the raw material is replace by other type of the same kind. Equipments and machineries utilized in any stages of the food processing from slaughtering to distribution and storage, if not thoroughly cleaned after being used on other animal species, will impart in the contamination.

In Malaysia, its Halal food production has its pivotal role among the hearts of 60% of the Muslim population. However, Malaysia is still relying on the imports because the halal food production is insufficient. Regarding to this matter, the government has acknowledge the halal food industry as a new source of economic activity in conjunction of the recent 2004/2005 economy reports in the announcement of 2005 budget. Due to its emphasis on agro-based industries in the field of Biotechnology, a special fund for halal food product's development and promotion is being raised up to finance technology and market expansion and business plan in order to increase the productivity and quality to

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obtain international certification and exports. Various continuous efforts in making Malaysia a reliable halal food centre have been carried out. The efforts include advancement in various skills divisions like inspection, standardization, certification and marketing as being carried out by government agencies like Malaysian International Trade and Industry (MITI) and the Ministry of Agriculture and Agro-based Industry. Jabatan Kemajuan Islam Malaysia (JAKIM) is the sole agency in Malaysia for certification of domestic and international market with a strict standard halal accreditation. The government has previously launched the Standard Halal Malaysia MS-1500:2004 for this purpose.

Concerning to this matter and in order to comply with increasingly strict necessities regarding to tagging accuracy, analyst are required to provide determination of species with maximum certainty but on the other are expected to charge a minimum of the service. Various methods have been done by many researchers. Thus far, quantitative detection of meat species in mixed sample are based on biochemical analysis in one form or another as qualitative and quantitative biochemical traits set all individuals separately from each another. Methods to determine species have kept pace with biochemical technology. Prior to the 1990s, the main methods for species recognition were based on the properties of proteins including immunological, ELISA and amino acid and peptide composition. A major hitch with these protein-based methods is that they do not work well with cooked meats and meats products. On heating, proteins will be apt to become insoluble, which means they are complicated to extract and evaluate on gels. Moreover,

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immunological methods are based on shape identification, and the native shape of proteins is lost when they are heated to cooking temperatures during processing.

To overcome these limitations, a DNA-based method for species identification has been developed which has higher stability compared to protein-based method. Deoxyribonucleic acid (DNA) carries an organism's total genetic information and has to be stable in order for lifelong functioning. It is identical in all cell types of an organism, therefore, it can be easily purified from any cell and once isolated it is much more stable compared to other macromolecules. The information content of DNA is superior to that of proteins, due to the degeneracy of the genetic code. Nevertheless, contemporary DNA methods prefer the use of mtDNA sequences rather than nuclear DNA as it has higher copy number than nuclear DNA. This is true even where the cell is multinucleate as in the case of muscle fiber, where only small amount of DNA can be extracted. Animal mtDNA is maternally inherited and evolves faster than nuclear DNA. Cytochrome b (cytb) is the best known mitochondrial gene and is widely used in phylogenetics work as molecular marker. It is considered variable enough for population analysis and sufficiently conservative for phylogenetic analysis among remotely related organisms. The advent of polymerase chain reaction (PCR) has enabled sensitive analysis towards the gene. Universal primers CYTB1 and CYTB2 direct the amplification of the conserved region in the cytb gene to result in a 360 bp amplicons which will be digested with selected restriction enzyme (RE) to exhibit species-specific restriction fragment length polymorphism (RFLP). PCR-RFLP using universal primers is the best method for species identification of admixtures detection when the sequence of the animal species is not

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available. Since sequencing of amplicons from dual origin is not preferable, it provides a rapid, simple and less expensive method alternative to sequencing of the pcr product.

The purpose of this research is to determine the detection threshold for cytochrome b gene in raw meats. It is significant to develop a sensitive method to detect species contamination so that even a minute admixture can be detected. The use of DNA extracts from meat admixtures for the starting material has an advantage over DNA admixtures as the developed method will be more readily applicable for the detection of admixtures in marketed meat products in real life. The objective of this research is to develop a sensitive method for the threshold detection of admixtures in raw meat.

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CHAPTER 2

LITERATURE REVIEW

2.1 Non-DNA-based species identification analysis.

Existing methods for the issue under study includes the use of proteins, lipids or DNA analysis (Dooley *et al*., 2004; Fernandez *et al*., 2002; Brodmann, 2002). Protein analysis for speciation uses electrophoresis, chromatography or immunoassay. Immunoassay offers a better specificity over electrophoresis in that it has a broader range of species to work on (Dooley *et al.* 2004). However, heat denaturation of proteins during thermal processing and the non-availability of antibodies of many species set a gap with other methods. It has also been argued that adventitious contamination of meat with blood from other species could lead to false result. Some commercially available immunoassay kits provide for qualitative detection of species, however it is non applicable to poultry species. Isoelectric focusing (IEF) is based on the separation of proteins on polyacrylamide gel by pH-gradient and subsequent staining with coomassive blue or silver staining. However, limitation has occurred on heated or marinated meat products (Brodmann, 2002).

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Microscopic analysis based on morphological characteristics which identify animal species through the examination of the tissue constituents on mainly bone fragments was recommended by the European Union (EU) for the control of meat and bone meal (MBM) (Frezza *et al.* 2003). However, this method is only suitable for animal feedstuff because meat products for human consumption do not contain bones.

On the other hand, lipid analysis is really restricted on cases where species of origin of animal fats is being determined. Some fatty acids or pattern of fatty acids has enabled the detection of pork in beef and mutton (Brodmann, 2002).

2.2 Meat

People are becoming increasingly aware of the importance of nutrition in their long term health prospects. As meat and meat products are being consumed on a daily basis, however, there is abundance of misconceptions including the belief that "meat is bad for you", many related to the cancer and cardiovascular risk (MacDonald, 1991). Physicians and the public alike in their haste to evade saturated fat, have lost sight of the reality that lean meat in reasonable serving sizes poses no threat to health and indeed is an extremely important source of many nutrients.

As stated by Damron (2003), meat is defined as the edible flesh of animals that is used for food. Meat and its product are essential food sources because they are nutrient dense, which means that they have many nutrients compared to their calories, and the

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nutrients are digestible and readily obtainable. Animal products supply about 16% of the total calories consumed in the world while the remaining 84% is supplied by plants. Other than a significant amount of energy, animals are a more important source of protein than they are of calories, supplying 34% of the protein consumed in the world (Damron, 2003). Of protein sources, meat provides approximately 44%. Other than protein and fat, meat and meat products also contain high concentrations of micronutrients like folate, selenium and zinc which are reported to be cancer-preventive (Biesalski, 2002). There are no ambiguities that meat is the most valuable supply of dietary iron. Besides, vitamin A and vitamin B12 appear exclusively in meat as essentials nutrients. These facts make meat a significant component of a balanced diet in a range of age and performance groups, including children and teenagers, pregnant women and nursing mothers, senior citizens and athletes. Hence, health professionals must collaborate in educating the public about choosing a well-balanced diet. Indeed, an inconsistent relation between meat and meat products consumption and risk of cancer merits further investigation (Missmer *et al.*, 2002).

2.3 Mitochondria

Mitochondria are eukaryotic organelle responsible for the processes of respiration and electron transport phosphorylation (Madigan *et al.*, 2003). Mitochondria are bounded by a double-membrane system, where an intermembrane space separates the inner and outer mitochondrial membrane. The inner membrane forms numerous folds namely cristae

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which extend into the interior, matrix, of the mitochondria (Fig 2.1). The energy derived from the breakdown of carbohydrates and fatty acids, which is converted to ATP

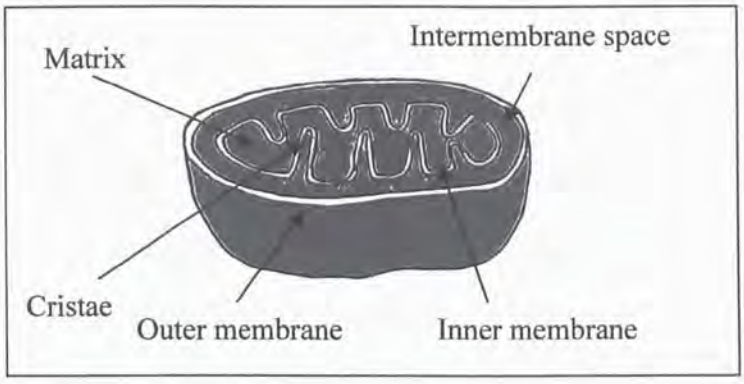

Figure 2.1 Structure of a mitochondrion.

by the process of oxidative phosphorylation is made possible by the mitochondria in its inner membrane (Cooper and Hausman, 2004). The efficiency of ATP generation is greatly enhanced by the increase of its surface area due to the folding of inner membrane into cristae. The proton gradient that drives oxidative phosphorylation is maintained by the inner membrane as it is impermeable to most ions and small molecules between the cytosol and the matrix. Mitochondria show a considerable plasticity for their shape as they show great variability as seen in electron micrographs, nevertheless, the common shape is always nearly spherical rod-shaped. They contain their own DNA located in the matrix, which encodes rRNAs, tRNAs and some mitochondrial proteins, thus making them unique among the cytoplasmic organelles in a cell. Animals mitochondrial DNA

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(mtDNA) is a small circular molecule which is about 15-20 kilobase (Mack *et al.*, 1986) comprised of about 37 genes coding for 13mRNAs, 22 tRNAs, two rRNAs and the rest coding for protein (Kvist, 2000; Desjardins and Morais, 1990). The mitochondrial DNA is arranged very efficiently. It has small intergenic spacers where the reading frames even sometimes overlap and lacks of intron. The control region is the primary non-coding region, and is in charge for the regulation of heavy (H) and light (L) strand transcription and of H-strand replication (Fig. 2.2). mtDNA evolves relatively rapidly compared to the DNA coding for ribosomal RNA (rRNA) for example, thus, mtDNA is very suitable molecular marker for sequence comparison between closely related or even populations of the same species (Campbell and Reece, 2002). The reason for using mitochondrial based DNA analyses originate from the fact that there are many mitochondria per cell, and many mitochondrial DNA molecules within each cell making mitochondrial DNA a naturally amplified source of genetic variation than nuclear DNA. In vertebrates, the mitochondrial genes mutation rate is nearly ten-fold higher than to nuclear genes. Hence, point mutations accumulate rapidly enough to permit the differentiation of even closely associated species (Cheng *et al.*, 2001). The high mutation rate in mtDNA is due to its location at cytoplasm, which is prone to be attacked and there is a poor corrective replication of polymerase and lack of proof reading system (Cheng *et al*, 2003). There are many genes in the mtDNA that were previously studied in species identification. For example mitochondrial 16S rRNA for the identification of clam species (Fernandez *et al.*, 2002). ATPase8 and ATPase6 genes were also successfully employed for the identification and semi quantification of differently

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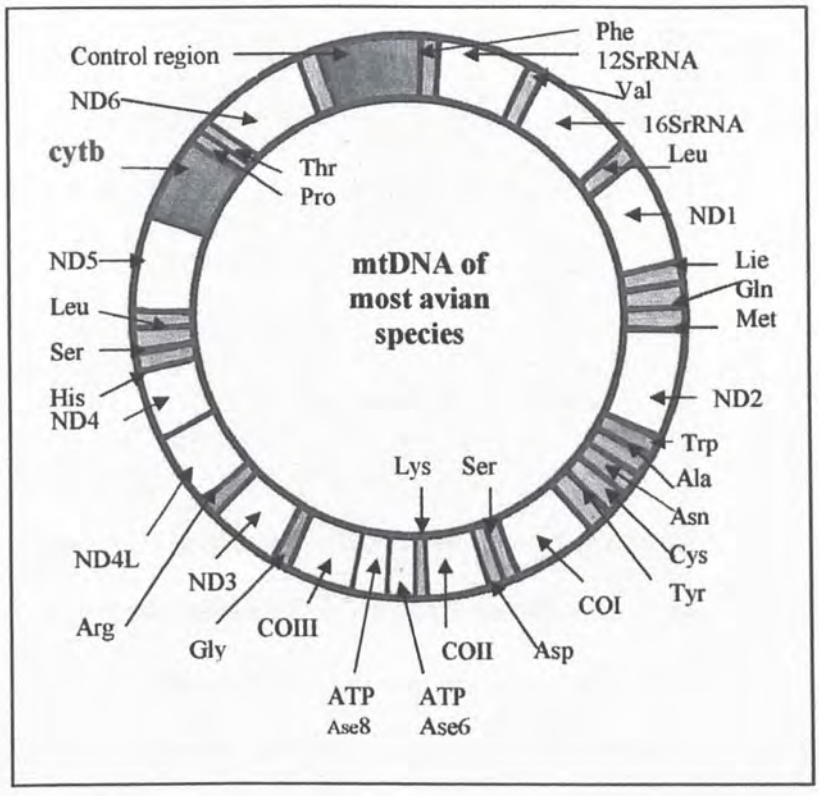

Figure 2.2 Mitochondrial cytochrome b gene locus. Outer circle correspond to the heavy (H) strand while the inner circle the light (L) strand. Cytb-cytochrome b gene; CO I, CO II and CO III-subunit I, II and III of the cytochrome oxidase; NDI-6-subunit I-6 of the NADH reducase; tRNA are represented by their three-letter amino acid abbreviation.

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REFFERENCES

Aberle, E.D., Forrest, J.C., Gerrard, D.E., Mills, E.W., Hedrick, H.B., Judge, M.D. and Merkel, R.A., 2001. *Principle of meat science.* Kendall/Hunt Publishing Company, Iowa.

Aida, A.A., Che Man, Y.B., Wong, C.M.V.L., Raha, A.R. and Son, R., 2004. Analysis of raw meats and fats of pigs using polymerase chain reaction for Halal authentication. *Meat Science* **69**, 47-52.

Bania, J., Ugorski, M., Polanowski, A. and Adamczyk, E., 2001. Application of polymerase chain reaction for detection of goats milk adulteration by milk of cow. *Journal of Dairy Research* **68**, 333-336.

Bellagamba, F., Moretti, V.M., Comincini, S. and Valfre, F., 2001. Identification of species in animal feedstuffs by polymerase chain reaction-restriction fragment length polymorphism analysis of mitochondrial DNA. *Journal of Agricultural Food Chemistry* **49**, 3775-3781.

Biesalski H.K., 2002. Meat And Cancer: Meat As A Component Of A Healthy Diet. *Europe Journal Of Clinical Nutrition* **56** (1), 1-11.

Bloom, M.V., Freyer, G.A. and Micklos, D.A., 1996. *Laboratory DNA Science: An introduction to recombinant DNA techniques and methods of genome analysis.* The Benjamin Cummings Publishing Company, Inc, California.

Brodmann, P., 2002. *Species identification: Development and validation of species identification.* University of Basel.

Brown, T.A., 2001. *Gene Cloning and DNA analysis: An introduction.* Blackwell Science, London, page 33.

Campbell N.A. and Reece J.B., 2002. *Biology.* Benjamin Cummings, San Franscisco.

Champe P.C. and Harvey R.A., 1994. *Lippincott's Illustrated Reviews: Biochemistry.* Lippincott Williams and Wilkins, Philadelphia.

Cheng, Y.H., Wen, C.M., Ding, S.T., Kao, C.C. and Kuo, T.y., 2003. Detecting meat-and-bone meal in ruminant's feeds by species-specific PCR. *Journal of Animal and Feed Sciences* **12**, 851-860.

UMS
UNIVERSITI MALAYSIA SABAH

Frezza, D., Favaro, M., Vaccari, Gabriele., Holst, C., Giambra, V., Anklam, E., Bove, D., Battaglia, P.A., Agrimi, U., Brambilla, G., Marsan, P.A. and Tartaglia, M., 2003. A competitive polymerase chain reaction-based approach for the identification and semiquantification of mitochondrial DNA in differently heat-treated bovine meat and bone meal. *Journal of Food Protection* **66** (1), 103-109.

Gelfand, D.H. and White, T.J., 1990. *PCR protocols: A guide to methods and Applications.* Academic Press, Inc.

Hames B.D. and Hooper N.M., 2001. *Biochemistry.* Viva Books Private Limited, New Delhi.

Horstkotte, B. and Rehbein, H., 2003. Fish species identification by means of restriction fragment length polymorphism and high-performance liquid chromatography. *Food Chemistry and Toxicology* **68** (9), 2658-2665.

Hsieh, H.S., Chai, T., Cheng, C.A., Hsieh, Y.W. and Hwang, D.F., 2004. Application of DNA techniques for identifying the species of different processed products of Swordfish meat. *Food Chemistry and Toxicology* **69** (1) 1-6.

Johnson, K.P. and Sorenson, M.D., 1998. Comparing molecular evolution in two mitochondrial protein coding genes (cytochrome b and ND2) in the dabbling ducks. *Molecular Phylogenetics and Evolution* **10** (1), 82-94.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Paabo, S., Villablanca, F.X. and Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in mammals: amplification and sequencing with conserved primers. *Pro. Natl. Acad. Sci. USA* **86**, 6196-6200.

Kvist, L., 2000. *Phylogeny and phylogeography of European Parids*. Department of Biology, University Of Oulu, Findland.

Lahiff, S., Glennon, M., O'Brien, L., Lyng, J., Smith, T., Maher, M. and Shilton, N., 2001. Species-specific PCR for the identification of ovine, porcine and chicken species in meat and bone meal (MBM). *Molecular and Cellular Probes* **15**, 27-35.

Lenstra, J.A., Buntjer, J.B. and Janssen, F.W., 2001. On the origin of meat-DNA techniques for species identification in meat products. Veterinary Science Tomorrow.

UMS
UNIVERSITI MALAYSIA SABAH

Cheng, C.A., Hsieh, Y.W., Noguchi, T., Arakawa, O. and Hwang, D.F., 2001. Effect of processing on sequence of cytochrome b gene and its restriction site in the meat of Puffer Takifugu Rubripes. *Journal of food and drug analysis* **9** (4), 232-237.

Chua, H.K., 2003. *Determination of the meat samples identities by using polymerase chain reaction.* Bachelor of Science Bioindustry Thesis. University Putra Malaysia.

Comi, G., Iacumin, L., Rantsiou, K., Cantoni, C. and Cocolin, L., 2005. Molecular methods for the identification of species used in production of codfish can detect commercial frauds. *Food Control* **16**, 37-42.

Cooper G. M., and Hausman R.E., 2004. *The Cell: A Molecular Approach.* ASM Press, Washington.

Damron W.S., 2003. *Introduction To Animal Science: Global, Biological, Social and Industry Perspectives.* Prentice Hall, New Jersey.

Darbre P. D., 2001. *Basic Molecular Biology: Essential Techniques.* John Wiley and Sons, New York.

Dawson, M.T., Powell, R. and Gannon, F., 1996. *Gene Technology: Introduction to biotechniques.* BIOS Scientific Publishers Limited, UK.

Desjardins, P. and Morais, R., 1990. Sequence and gene organization of the chicken mitochondrial genome. A novel gene order in higher vertebrates. *Journal of molecular biology* **212** (4), 599-634.

DNA fingerprinting. Birkhauser Verlag Basel, Switzerland.

Dooley, J.J., Paine, K.E., Garrett, S.D. and Brown, H.M., 2004. *Meat Science* **68**, 431-438.

Farias, I.P., Orti, G., Sampaio, I., Schneider, H. and Meyer.A., 2001. The cytochrome b gene as a phylogenetic marker: The limits of resolution for analyzing relationships among Cichlid Fishes. *Journal of Molecular Evolution* **53**, 89-103.

Fernandez, A., Garcia, T., Gonzalez, I., Asensio, L., Rodriguez, M.A., Hernandez, P.E., and Martin, R., 2002. Polymerase Chain Reaction-Restriction Fragment Length Polymorphism Analysis of a 16S rRNA Gene Fragment for Authentication of Four Clam Species. *Journal of Food Protection* **65** (4), 692-695.

UMS
UNIVERSITI MALAYSIA SAB

Linebaugh, K.E.,2000. *Mitochondrial DNA RFLP's give phylogenetic evidence for relatedness among Sculpin populations*. Missouri Western State College.

Lin, Y.S., Poh, Y.P., Lin, S.M., Tzeng, C.S., 2002. Molecular Techniques to Identify Freshwater Eels: RFLP Analyses of PCR-amplified DNA Fragments and Allele-specific PCR from Mitochondrial DNA. *Zoological Studies* **41** (4), 421-430.

Lockley, A.K. and Bardsley, R.G., 2000. DNA-based methods for food authentication. *Trends in Food Science and Technology* **11**, 67-77.

Macdonald H.B., 1991. Meat And Its Place In The Diet. *Canada Journal Of Public Health* **82** (5), 331-334.

Mack, A.L., Gill, F.B., Colburn, R. and Spolsky, C., 1986. Mitochondrial DNA: A source of genetic markers for studies of similar Passerine bird species. *The Auk* **103**, 676-681.

Mackie, I.M., Pryde, S.E., Gonzales-Sotelo, C., Medina, I., Perez-Martin, R., Quinteiro, J., Rey-Mendez, M. and Rehbein, H., 1999. Challenges in the identification of species of canned fish. *Trends in Food Science and Technology* **10**, 9-14.

Madigan M.T., Martinko J.M. and Parker J., 2003. *Brock Biology Of Microorganisms*. Prentice Hall, New Jersey.

Miesfeld, R., 1999. *Applied Molecular Genetics*. Wiley-Liss, New York.

Missmer, S.A., Smith, W.S.A., Spiegelman, D., Yaun, S.S., Adami, H.O., Beeson, W.L., Van den Brandt, P.A., Fraser, G.E., Freudenheim, J.L., Goldbohm, R.A., Graham, S., Kushi, L.H., Miller, A.B., Potter, J.D., Rohan, T.E., Speizer, F.E., Toniolo, P., Willett, W.C., Wolk, A., Zeleniuch, J.A., and Hunter, D.J., 2000. Meat and dairy food consumption and breast cancer: a pooled analysis of cohort studies. *International Journal Of Epidemiology* **31**(1) 78-85.

Myers, M.J., Friedman, S.L., Farrell, D.E., Dove-Pettit, D.A., Bucker, M.F., Kelly, S., Madzo, S., Campbell, W., Wang, R.F., Paine, D. and Cerniglia, C.E., 2001. Validation of a polymerase chain reaction method for the detection of rendered bovine-derived materials in feedstuffs. *Journal of Food Protection* **64** (4), 564-566.

UMS
UNIVERSITI MALAYSIA SAB

Myers, M.J., Yancy, H.F. and Farrell. D.E., 2003. Characterization of a polymerase chain reaction-based approach for the simultaneous detection of multiple animal-derived materials in animal feed. *Journal of Food Protection* **66** (6), 1085-1089.

Paolella, P., 1998. *Introduction To Molecular Biology*. McGraw Hill, New York.

Pereira, S.L., 2000. Mitochondrial genome organization and vertebrate phylogenetics. *Genetics and Molecular Biology* **23** (4), 745-752.

Sanjuan, A. and Comesana, A.S., 2000. Molecular identification of nine commercial Flatfish species by polymerase chain reaction-restriction fragment length polymorphism analysis of a segment of the cytochrome b region. *Journal of Food Protection* **65** (6), 1016-1023.

Saunders, M.A. and Edwards, S.V., 2000. Dynamics and phylogenetic implications of mtDNA control region sequences in New World Jays (Aves:Corvidae). *Journal of Molecular Evolution* **51**, 97-109.

Seidman, L.A. and Moore, C.J., 2000. *Basic Laboratory Methods for Biotechnology*. Prentice Hall, Upper Saddle River, page 488.

Wang, H., Liang, B., Feng, J., Sheng, L. and Zhang, S., 2003. Molecular phylogenetics of Hipposiderids (Chiroptera:Hipposideridae) and Rhinolopids (Chiroptera: Rhinolophidae) in China based on mitochondrial cytochrome b sequences. *Folia Zool* **52** (3), 259-268.

Wang , R.F., Myers, M.J., Campbell, W., Cao, W.W., Paine, D. and Cerniglia, C.E., 2000. A rapid method for PCR detection of bovine materials in bovine feedstuffs. *Molecular and cellular probes* **14**, 1-5.

Walker, J.A., Hughes, D.A., Anders, B.A., Shewale, J., Sinha, S.K. and Batzera, M.A., 2003. Quantitative intra-short interspersed element PCR for species-specific DNA identification. *Analytical Biochemistry* **316**, 259-269.

Walker, J.A., Hughes, D.A., Hedges, D.J., Anders, B.A., Laborde, M.E., Shewale, J., Sinha, S.K. and Batzera, M.A., 2004. Quantitative PCR for DNA identification based on genome-specific interspersed repetitive elements. **Genomics 83**, 518-527.

Weaver, R. F., 2003. *Molecular Biology*. McGraw Hill, New York.

UMS
UNIVERSITI MALAYSIA SAB

Wood, T.C. and Krajewski, C., 1996. Mitochondrial DNA sequence variation among the subspecies of Sarus Crane (Grus Antigone). *The Auk* **113** (3), 655-663.

Yeong, S.L., Yu, P.P., Si, M.L. and Chyng, S.T., 2002. Molecular techniques to identify freshwater eels: RFLP analysis of PCR-amplified DNA fragments and allele-specific PCR from mitochondrial DNA. *Zoological studies* **41** (4), 421-430.

Zehner, R., Zimmermann, S. and Mebs, D., 1998. RFLP and sequence analysis of the cytochrome b gene of selected animals and man: methodology and forensic application. *International Journal of Legal Medicine* **111**, 323-327.

Zischler, H., 1999. *Methods and Tools in Bioscience and Medicine: DNA profiling and DNA fingerprinting.* Birkhauser Verlag Basel, Switzerland.

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