PROCESSED MEAT PROFILING USING DNA BARCODING

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Dedicated to my parents, En Mohd Zawai & Puan Rohana,
who always loving and supporting me
and
deepest gratitude to my supervisor, Dr Faezah Mohd Salleh
for your unwavering support, collegiality and mentorship
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ABSTRACT

More than ever, the globalization together with the changes in consumer food pattern and lifestyle has led to high consumption of processed meat in daily food diet in Malaysia. However, food fraud issues in the form of ingredient substitution, mislabeling, abstraction of valuable contents and adulteration can lead to the consequences like illegal sales of threatened species, causing problem for the diets of certain consumer, such as vegetarians and religious group, and potential health risks to the consumer of this product. Thus, DNA barcoding, a robust and reliable method, were chosen to profile the processed meat up to species level. A total of 10 processed meat products were purchased from local supermarkets chains all around Johor, Malaysia including ground, frozen and canned meats. All the samples were then sequenced across a 300 bp region of the cytochrome b (*cytb*) gene. The resulting sequences were queried against Genbank for species identification. Overall, the results showed that out of 10 samples, 6 (60 %) samples were classified as correctly labeled while; however, another 4 (40 %) samples were found mislabeled, attributed by the false declaration of species. All mislabeled products were claimed to contain only beef on their packaging without any specification on the meat source as required by Malaysia Food Regulation 1985. Buffalo (*Bubalus bubalis*) DNA was found in 3 out of 4 products labeled as beef. Interestingly, 1 out of 4 mislabeled beef products have 99 % genetic similarities with Red Junglefowl (*Gallus gallus*) DNA, thus being classified under mislabeling and substitution case. To sum up, DNA barcoding can be conclude as accurate, sensitive and reliable technique of processed meat authentication that will overcome the ineffectiveness of traditional morphological identification methods and resolve numerous issues regarding food fraud.

ABSTRAK

Kebelakangan ini, arus globalisasi serta perubahan di dalam corak pemakanan dan gaya hidup pengguna telah mendorong kepada pengambilan daging proses yang tinggi di dalam diet pemakanan harian di Malaysia. Walau bagaimanapun, isu penipuan makanan seperti penggantian bahan, kesalahan label, pengurangan kandungan bahan berharga dan pencemaran telah mengakibatkan penjualan spesies terancam secara haram, timbulnya masalah kepada diet pengguna tertentu, serta potensi risiko kesihatan kepada pengguna. Oleh itu, DNA barcoding, sejenis kaedah yang berkesan dan boleh dipercayai, telah dipilih untuk mengenalpasti daging proses sehingga ke tahap spesies. Sebanyak 10 produk daging proses telah dibeli dari rankaian pasar raya tempatan di sekitar Johor, Malaysia termasuklah daging kisar, daging beku dan daging di dalam tin. Kesemua produk ini kemudiannya dijujukan tertakluk kepada kod bar DNA cytochrome b (*CytB*) bersaiz 300 bp. Jujukan yang dihasilkan telah dianalisis menggunakan Genbank untuk pengenalpastian spesies. Secara keseluruhannya, keputusan menunjukkan bahawa daripada 10 sampel, 6 (60 %) sampel diklasifikasikan sebagai dilabel dengan betul; walau bagaimanapun, 4 (40 %) lagi sampel didapati terdapat kesalahan label, disebabkan oleh perisytiharan spesies palsu. Kesemua produk yang telah disalah label menyatakan hanya terdapat daging lembu sebagai bahan pada pembungkusan mereka tanpa sebarang spesifikasi mengenai sumber daging seperti yang dikehendaki oleh Malaysian Food Regulation 1985. DNA kerbau (*Bubalus bubalis*) telah dijumpai di dalam 3 daripada 4 produk yang dilabelkan sebagai daging lembu Menariknya, 1 daripada 4 produk daging lembu yang tidak dilabel secara terperinci mempunyai 99 % persamaan genetik dengan DNA ayam hutan merah (*Gallus gallus*), oleh itu kes ini diklasifikasikan di bawah kes kesalahan label dan juga terdapat penggantian bahan di dalam produk. Sebagai kesimpulannya, DNA barcoding adalah satu teknik pengenalpastian daging proses yang tepat, sensitif dan berkesan dalam mengatasi ketidakcekapan kaedah pengenalpastian tradisional secara morfologi dan juga dapat menyelesaikan pelbagai isu mengenai penipuan makanan.

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LIST OF SYMBOLS AND ABBREVIATION

°C	-	Degree Celcius
bp	-	Basepair
CytB	-	Cytochrome B
g	-	Gram
%	-	Percentage
PCR	-	Polymerase Chain Reaction
COI	-	Cytochrome C Oxidase 1
CBOL	-	Consortium for the Barcode of Life
BOLD	-	DNA Barcode of Life Data System
Kb	-	Kilobase
Min	-	Minute
µL	-	Microliter
V	-	Volt
A	-	Ampere
µM	-	Micromolar
w/v	-	Weight per volume
nm	-	Nanometer
ng	-	Nanogram
BLAST	-	Basic Local Alignment Search Tool

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Globalization led to changes in consumer food pattern and lifestyle. Increasing understanding about food composition and the effects on health has brought great transformation in processed food industry in the past few years (Siro *et al.*, 2008). Meat is an important food commodity as it provides essential nutritions to human such as the presence of dietary fats, essentials amino acids, and B-vitamins. Nowadays, there is high demand from consumers for instant, ready-to-eat and frozen food products because they are convenient. In addition, continuous development of preservation/processing techniques to avoid spoilage of food products has led to the growth of processed meat production. Processed meat including meat products that undergo processes for preservation by smoking, marinating , curing, salting or cooking or those that are in the form of ready-to-eat products (Shan *et al.*, 2017). According to Linseisen *et al.* (2006), examples of processed meat products includes pepperoni, deli meats, burger, nuggets, ham and bacons.

However, arising issues on food traceability and authenticity have been observed globally. Consumer worldwide become aware of the composition of food they consumed and demand for clear and valid information (Sentandreu & Sentandreu, 2014). This is because simple visual identification of meat species is impossible in

processed meat products due to loss of external appearance and sensory characteristics (Flores-Munguia *et al.*, 2000). Hence results in increasing of food fraud cases.

The main areas vulnerable to fraud in the meat industry would be in the following forms: (1) partial or whole substitutions of meat ingredients with an undeclared alternatives (usually cheaper); (2) mislabeling; (3) partial or whole omissions or abstraction of valuable contents and (4) adulteration of the meat products by addition of another substances or undeclared substances to increase product bulk or weight (Hargin, 1996). For example, processing techniques lead to the introduction of secondary species that are not present on the label such cases reported by Kane & Hellberg (2016). Different studies also described mislabeling rates of 20-70% for variety of meat products, including deli meats, ground meat, dried meat and pet food (Cawthorn *et al.*, 2013; Okuma & Hellberg, 2015; Quinto *et al.*, 2016). Other than that, poor meat authenticity evaluations also cause contribute to increased illegal meat substitution and undeclared species in food products (Bottato *et al.*, 2014).

In some cases, food frauds in processed meat affect public health, religious consideration, conservation efforts and economy. The existence of undeclared ingredients in the food products can be dangerous to consumers with allergy to meat. For instance, the study by Masiri *et al.* (2016) reported the risk of pathogen infection increased with the presence of undeclared pork residues in meat products. Moreover, many of developing countries had suffered from the food fraud as the beef consumption has been slow decline and lost market share. Besides, this problem also has create challenges for the beef industry to find new market outlet as the consumer's trust has been affected (Zhao *et al.*, 2014). Additionally, poor authentication of meat products lead to illegal sales of threatened species protected and disrupts the effort of conservation aimed at these animals. One of the consequences of food fraud are it get in the way with religious practices which forbid consumption of particular animal species. For example, according to Muslim dietary laws (Halal) enshrined in the holy Quran, the consumption of pork is

prohibited (Nakyinsige *et al.*, 2012). Even when fraudulent may not impact the public health and food safety, it may cause the loss of consumer's trust into the food supply chain and the authority bodies.

Meat species identification is an outstanding field of food forensics which ensures food safety and quality to the consumers and protects regulatory laws related to meat and meat products. Authentication is a practice in which a food is validate together with its label description (Ashurst & Dennis, 2013). Since ancient times, authenticity has been a major concern of consumers, regulators and producers. Thus, the modern equipment and advances in information technologies and basic sciences have provides variety techniques for meat products authentication. Meat products identification is traditionally based on morphological features such as texture, color, size and odor (Chauhan & Sharma, 2003) as well as microscopic inspection of tissue structure and raw materials arrangement. Both approaches are simple and economical. However, the problem with these techniques is that it is less accurate and requires skills. Due to the facts that processed meats have highly destruction of observable characteristics, it makes this approach rarely useful.

Besides, with the advances of chemical and biochemical technologies, electrophoretic and immunological (Asensio *et al.*, 2001) analyses are used. Various spectrometric instruments such as mass spectrometry (MS), high performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR) are utilized to analyze metabolites. The general objective of the strategies that can be grouped herein would be to identify and quantify the maximum number of low molecular weight compounds contributing to discriminate between samples (Sentandreu & Sentandreu, 2014). However, the chemical fingerprints are complicate in the analysis due to the presence of many secondary metabolites in the processed meat products. Besides, the chemical profile may vary with storage environments and manufacturing process (Lo & Shaw, 2018). Also, the time required for the analysis of each individual sample may be considerably long, thus limiting the performance at the time to get results in control laboratories. One of the

analytical techniques commonly used for meat species authenticity depend mostly on protein-based techniques, include chromatographic, electrophoretic and spectroscopic approaches. However, analysis are limited because most of the soluble proteins denatured during food processing (Fajardo *et al.*, 2010).

Therefore, with the advancement in the molecular technologies, the present study were carried out with the objectives to investigate the capability of DNA Barcoding, a sequencing-based method to identify the authenticity of processed meat products up to species level. DNA barcoding utilize short mitochondrial genetic marker in an organism's DNA to identify the species name and this technique are sensitive, applicable and specific even to products that have undergo numerous processes during manufacturing. Since DNA barcoding is based on genetic variation within the standardized genetic region, we are focusing on the cytochrome B (*CytB*) as this mitochondrial gene is a standardized region in animals. This target is relatively conserved within species and display divergences between species allowing the samples to be identified at the species level at great number of cases.

1.2 Problem statement

Processed meat is highly consumed in daily food diet in Malaysia. However, numerous issues regarding non-authenticity of the processed meat products have arised and been observed globally. Food fraud, in the forms of intentional or unintentional ingredient substitution and mislabeling of food products which may be perform for reasons such as economically motivated lucrative benefits and poor food law enforcement can lead to outcome like illegal sales of threatened (i.e. endangered, critically endangered, vulnerable) species preserved by the Endangered Species Act (ESA). Poor meat authentication is a major concern as this might also cause troubles for the diets of particular consumers, such as vegetarians and religious groups, and potential health risks, resulting in decreasing

of consumer's confidence in the food supply chain. More than ever, meat safety and value related issues have drawn public scrutiny due to increase in public awareness among consumers on getting clear information. Manufacturing of meat products undergo various treatment such as heating and extreme low pH results in degradation of DNA fragment size. Hence, traditional authentications via morphological identification by sensory analysis of texture, odor, shape and colour as well as microscopic assessment of tissue structure are rarely useful due to destruction of the visible characteristics during the process. Therefore, in this study, the advances of molecular technologies via sequence-based techniques such as DNA barcoding can profile the meat source up to species level using generic mitochondrial marker, thus allowing more precise content description for processed food products.

1.3 Objectives of the study

The objectives of this study were:

1. To isolate genomic DNA (gDNA) from processed meat samples.

2. To amplify mitochondrial DNA barcode from selected processed meat samples via PCR.

3. To apply DNA barcoding for authentication of processed meat via bioinformatics analysis.

1.4 Scope of study

In the current study, a total of 10 meat products were purchased from the various supermarkets chains in Johor, Malaysia consisting of both local and

imported brands. Five products are categorized as beef product and another five categorized as chicken products. Genomic DNA extraction was carried out on processed meat products by using Qiagen DNeasy Blood & Tissue Kit. Then, the 5' region of Cytochrome B (*CytB*) gene were subjected to amplification through Polymerase Chain Reaction (PCR) using *CytB* universal primer. Next, the generated sequences were then analysed against GenBank (http://www.ncbi.nlm.nih.gov) in order to profile processed meat product up to species level.

1.5 Significance of study

In the present study, cytochrome B (*Cyt*B), a systematize region of mitochondrial gene in animals were used as a DNA barcode to profile processed meat products up to species level. This accurate, sensitive and reliable technique of meat authentication can overcome the ineffectiveness of the traditional authentication methods and sort out numerous issues regarding food fraud such as substitution or mislabeling. The discovery of this work also confirms the presence of specific species in processed meat by comparing the amplified DNA barcode to the Genbank database. This will assist in the future meat authentication and accurate declaration of meat species in commercial meat products to ensure the fair trade, freedom of choice and agreement with legislation.

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