ZIBELINE INTERNATIONAL PUBLISHING

Science Heritage Journal (GWS)

DOI : http://doi.org/10.26480/gws.01.20198.11.16

ISSN: 2521-0858 (Print)
ISSN: 2521-0866 (Online)
CODEN : SHJCAS

REVIEW ARTCLE

PROTEOMICS AS TOOLS FOR BIOMARKERS DISCOVERY OF ADULTERATION IN SLAUGHTERING PROCEDURES

Azura Amid[1*], Norshahida A. Samah[2]

[1]International Institute for Halal Research and Training (INHART), International Islamic University Malaysia, P.O. Box 10, 50728 Kuala Lumpur, Malaysia.
[2]Faculty of Agro-Based Industry, University Malaysia Kelantan, Jeli Campus, Locked Bag No.100, 17600 Jeli, Kelantan, Malaysia.
****Corresponding Author E-mail: azuraamid@iium.edu.m***

ARTICLE DETAILS

Article History:

Received 26 December 2018
Accepted 26 January 2019
Available online 14 February 2019

ABSTRACT

Slaughtering is an unavoidably sensitive issue among Muslim and Jews. This paper gives an outlook on possible detection methods in resolving the dilemma of religious slaughtering status. It will be rather easier to differentiate meat of different animal origins due to the exclusive genetic blueprint. However, in the case of adulteration in slaughtering procedure, the meat was taken from a similar source, thus, complicating the detection process. Therefore, an alternative approach employing proteomics were developed to identify protein expression patterns after external stimulation with electrical treatment. In the slaughtering process, the pain which is triggered by an external stimulus is expected to influence the protein profiles. Therefore, variations in stunning treatments which result in different patterns of protein profiles will pinpoint the specific biomarker for over stunned animals. This will inevitably help to detect adulterations in slaughtering procedure.

KEYWORDS

Biomarkers, halal, proteomics, slaughter, stunning.

1. INTRODUCTION

In the Third National Agricultural Policy, the government has emphasized on developing Malaysia as International Halal Food Hub. In line with this aim, The Halal Malaysian Standard MS 1500:2004 was developed by the Technical Committee on Halal Food . Shariah law is the fundamental guide in developing the Halal standard. The law of Islam applicable is the Mazhab of Shafie and any other Mazhabs of Maliki, Hambali, and Hanafi approved by the Yang di-Pertuan Agong to be in force in the Federal Territory, or the Ruler of any State to be in force in the states, or any fatwa approved by the Islamic Authority. Halal products are fast gaining worldwide recognition, and the average global halal food trade is estimated at RM 600 billion per year (approximately 190 billion USD). Tremendous potential in the development and production of halal products is available, and greater efforts should be placed to expand the market share.

Slaughtering is an act of taking the life of animals and plays a major role in halal food production. In Malaysia, for instance, the production of halal food is regulated by the Department of Islamic Development Malaysia (JAKIM). The Government has streamlined the implementation of the Halal certification in 2003 and JAKIM is appointed as the lead agency in the conferment of the halal certificates. In addition, this body is also responsible for issuing licenses to the slaughterhouse. The Fatwa Council has decided that the use of water stun in slaughtering poultry is considered as permissible (mubah). The standard set for water bath stunning in Malaysia is the use of current in the range of 0.25 A to 0.5 A with 40 V for the duration of 3 to 5 seconds (Malaysian Standard, 2004). At this mild electrical treatment, the chickens will be rendered unconscious, thus, easing up the slaughtering process in mass production. However, there are reported cases of food adulterations where the voltage was increased up to 100 V . At this high voltage, the chickens are no longer alive during the slaughtering process. In such a situation, the slaughtering act is considered as void, and the chicken is categorized under carcass, thus, unlawful for the Muslim consumption.

Therefore, it is highly beneficial to distinguish between the allowed and the adulterated slaughtering process. The genetic blueprint which is DNA is exclusive for different types of organism thus making the detection of meat originating from different animal sources to be rather easier. However, on the issue of slaughtering, the meat originates from a similar source. Therefore, another approach is taken by employing differences in protein expression due to an external stimulus which is stunning by electrical treatment.

2. FOOD AUTHENTICATION: HALAL MEAT

Halal meat is one of the main criteria in the production of food for Muslim consumption. In general, Halal meat is exposed to adulteration, and such irresponsible act includes contamination with non-Halal meat such as porcine and meat originating from Halal animals but not undergoing required religious slaughtering process. It is much easier to differentiate contaminated meat as different species of animals carry distinct patterns of genetic makeup. In the case of adulteration in slaughtering technique, the identification is vague and lack of scientific basis. Previously, modern molecular genomics and the science of genomics have opened up new and exciting possibilities to dissect complex phenotypic traits. These advances have made it possible to develop comprehensive genetic linkage maps in the animals such as in pig . To date, over 4000 genes and markers have been added to the gene map of the pig. Mapping of genes exclusive from pig indirectly provides a database in identifying meat contaminated with the nonhalal swine meat.

A real-time quantitative PCR detection method has been developed by Tanabe and colleagues to detect trace amounts of pork, chicken, beef, mutton, and horseflesh in food [1]. The primers were designed on the gene

encoding cytochrome b for the specific detection of each species. In Japan, in 2007, there was an incident of the meat processing company disguised pork mince as beef, and this has focused attention on the labeling of mince in processed food. As a result, the Ministry of Health, Labor, and Welfare of Japan has notified their recommendations to declare three meat items which are beef, chicken and pork. Some methods have been developed in detecting the meat ingredients in processed foods to verify the labeling. Such methods include the detection of species-specific proteins by enzyme-linked immunosorbent assay (ELISA) and of species-specific DNA molecules by polymerase chain reaction (PCR). About ELISA, developing a specific antibody against skeletal muscle is difficult and tricky.

Currently, species identification had been achieved through different methods using protein and DNA based methods. Protein-based methods include sodium lauryl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), isoelectric focusing (IEF), ELISA and HPLC. However, the protein profiles are tissue-dependent, and these proteins may be denatured on processing and heating, leading to subsequent loss of analytical specificity . Protein techniques also require blotting, a long period for result production, and preparation of antibodies. Such drawbacks have limited the use of protein techniques in food analysis. However, in cases where the meat origin is similar, such as in the issue of adulteration in the slaughtering process, proteomics approach seems to have the advantage over the DNA based methods.

In the halal and haram study, most of the studies focus on how to detect porcine contamination in food. Table 1 summarizes the food authentication studies that have been conducted using various methods and approaches. Despite the numerous researches conducted in food authentication and halal related issues, none of them has ever focused on authentication of the halal status in slaughtered chicken or any other domesticated animals.

Table 1: Summary on food authentication studies with various methods and approaches

System	Focus	Finding	Limitation	Reference
Pork meat	Identifying genes for meat quality in pork	Halothane gene and Napole gene have been recognized for their effects on quality of pork meat.	This study is only limited to pork.	[2]
Animal meats: cattle, sheep, swine, rabbit, chicken, turkey and sea bream.	Detection of animal contaminants in feedstuffs.	Effective detection of up to 0.5 % of different animal meals (bovine, porcine, ovine, rabbit, chicken, turkey, and fish).	This study is limited to monitoring the quality of animal feedstuffs.	[3]
Animal meats: pork, beef, poultry, and kangaroo.	Detection of meat's origin in heat processed products at concentrations below 1.5 % using counter immuno-electrophoresis.	The sensitivity of counter immuno-electrophoresis for the detection of chicken, swine, beef, and kangaroo proteins against immunization antigens was 1.5 %, 5 %, 5 %, and 5 % respectively.	This method requires the development of species specific antisera against heat-stable soluble proteins.	[4]
Muscle tissue samples: beef, goat, sheep, pig, horse, cat, and dog.	Identification of meat in meat mixtures by the polymerase chain reaction (PCR) using species specific primers.	PCR can detect the origin of meat in mixtures of less than 0.5 %.	The PCR amplification cycles need to be optimized for meat in mixtures of less than 0.5 %.	[5]
Raw meat and autoclaved treated meat.	Identification of most species (ruminant, poultry, and porcine) by multiplex PCR assay on conserved region from mitochondrial 12S rRNA and 16S rRNA genes	The sensitivity, high specificity and repeatability of the multiplex PCR assay is feasible and ideal for rapid analysis on sausages, cold cut and other industrial meat products or in feedstuffs.	This technique is only applicable in identifying different origin of meat.	[6]
Meat samples (pig, chicken, cow)	Detection and discrimination of three meat species (pork, cow, and chicken) by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP)	The amplified 359 base pairs portion of the mitochondrial cyt b gene of pure and mixed samples was cut with three different restriction enzymes (Alu I, Bsa JI, and Rsa I) resulting in species specific RFLP.	It will only identify the meat origin adulteration.	[7]
Pork meat	Detection of porcine contaminants by improved DNA extraction and PCR.	Amplification of species specific 152 bp porcine leptin gene fragment in processed pork samples.	This study is only limited to detection of leptin gene.	[8]
Porcine genes	Identification of single nucleotide polymorphism (SNP) in porcine genes encoding enzymes in hepatic metabolic pathways.	Identification of GNMT, ESTL147, and HDG on SSC 7, 12, and 13 respectively, as potential candidate genes for carcass and meat quality.	The identified genes are only useful for evaluating carcass and meat quality of pork.	[9]

Porcine organs and tissues	Detection of pork in cooked meat products by enzyme-linked immunosorbent assay (ELISA) using a monoclonal antibody.	The detection limit was determined as 0.5 % (w/w) pork in heterologous meat mixtures.	This method requires the development of antibody.	[10]
Pork, Chicken, Beef, Mutton, and Horseflesh	Detection of trace amounts of pork, chicken, beef, mutton, and horseflesh in foods using a real time quantitative PCR.	The limit of quantification of this method was found to be 100 fg/µl of each mitochondrial DNA in 10 ng/µl of the wheat mitochondrial DNA matrix. The calculated R^2 values of the standard curves for the five species ranged between 0.994 and 0.999.	This study only detects different meat origins.	[1]

3. ISLAMIC PERSPECTIVE ON SLAUGHTERING

The ritual slaughter in Islam is termed as Zabah. Zabah means to purify, and in slaughtering, it means to purify the flesh of the animal from flowing blood [11]. In Islam, flowing blood is impure and thus, prohibited for consumption. A scientific study has proven that blood is a good medium for the growth of germs and bacteria. In line with this finding, Islam had long ago emphasis on the essential of draining out the blood from the animals through the slaughtering process. In Islamic slaughtering, the blood vessels together with the esophagus and trachea are cut while the spinal cord is left remaining. If the spinal cord is cut, the nerve fibers to the heart might be damaged leading to cardiac arrest, thus, resulting in stagnation of blood in the blood vessels.

Al Qaradawi also discussed several reasons on the prohibition of eating dead animals [12]. The first reason is that eating the flesh of the dead animal is repugnant to civilized taste and is considered to be contrary to human dignity. Besides that, it also helps mankind to maintain health status as animals which died naturally without slaughtering is quite likely to have an acute or chronic disease that might be harmful when consumed. The same problem of possible harms exists in animals dying because of starvation and aging. The wisdom of the Islamic slaughtering is to take the animal's life in the quickest and least painful way. It is forbidden to rend the throat by using teeth or nails since this will cause pain to the animal.

4. STUNNING PRACTICE IN SLAUGHTERING ANIMALS

The Humane Methods of Slaughter Act of 1958 mandates the use of humane methods for pre-slaughter immobilization of all livestock under USDA inspection. The law also dictates that animals be made insensible to pain (rendered unconscious) before bleeding. Poultry Inspection Regulations of the USDA state that poultry should be slaughtered in accordance with good commercial practices in a manner that will result in thorough bleeding of the carcasses and assure breathing has stopped before scalding. Pre-slaughter immobilization can be classified as chemical, mechanical and electrical. Electrical immobilization is accomplished by passing a sufficient amount of electrical current through the brain of birds for a given amount of time. Today, the most common electrical stunning method employed in commercial operations is the water bath stunning. It is claimed not only comply with humane slaughter practice, but also help to relax the neck muscles and contact wing muscles for proper positioning of the head for automatic killers, prevent excessive struggling and wing flapping during bleed out, facilitate rapid bleeding, and relax or loosen feathers.

At the poultry slaughter plant, each day thousands of birds are crammed inside crates stacked on trucks waiting to be slaughtered. The birds are manually pulled from crates on the flatbed trucks and hung upside down in the live-hang area of the slaughterhouse. These birds are suspended with their huge, heavy breasts upside down by their legs from the shackles . The bird's head and upper bodies are dragged through an electrified water bath, and the paralyzed birds will have their necks cut. After 90 seconds of bleeding out time, the birds are then dropped, dead or alive into tanks of scalding water. In demand for an ever-growing trade, most chickens are slaughtered at 6 to 7 weeks old and weigh 4 to 5 pounds, which is several times heavier than a normal chicken of that age. Therefore, these younger and heavier birds with extremely fragile blood vessels are more susceptible to hemorrhages under an electrical treatment and stunning process.

Birds experience pain and suffering in the same way as humans and other mammals as they have the same nociceptors pain receptors as a human does. Therefore, they are expected to have the same capacity for fear, misery, and terror. They possess a conscious awareness of their surroundings and their experience. As human may experience, the painful sensation carried by sensory neurons will only cease after tissue damage has ended if the stimulus or source of pain remained. A minimum of 120 mA for chicken has been proven to rupture the bird's fragile blood vessels. Similar amperage electroconvulsive treatments given to human have been reported to give thunderbolts effects in their heads. This minimum current result in heart failure or immediate death. However, it was also well documented that stunning at high current is one of the major factors that cause downgrading in meat and carcass quality [13-15].

In an electrical stunning, the birds' heads and upper bodies are dragged through an electrified water bath through which is intended to immobilize them. Immobilization keeps them from desperately flapping and jerking while hanging head down from the conveyer belt. The immobilization will paralyze the muscles of their feather follicles to facilitate feather removal and to induce certain characteristics desired by customers. In the West, the humane method of slaughtering is pictured through the ability to induce cardiac arrest with sufficient current to ensure most birds will be rendered permanently unconscious during the stunning and slaughtering process . Van de Nieuwlaar described the method of stun to kill method as a frustrating cat and mouse game between the inspection authorities and poultry processing industry as even though the method is promoted for humane slaughtering it will also result in major carcass downgrading [16].

After being dragged through the electrified water bath, the paralyzed birds have their necks partially cut by a machine blade or manual neck cutter. The fastest method of inducing brain death in birds is by neck cutting where the two carotid arteries and jugular veins will be severed. Carotid arteries supply the brain with freshly oxygenated blood that maintains consciousness, whereas, the jugular veins carry spent blood away from the brain. Failure to cut both carotids can add two minutes to the time taken for brain failure to occur in birds. The worst case is reported at an incidence of severance of only one jugular vein, which can cause birds to retain consciousness while in severe pain for up to 8 minutes . The instrument used to perform slaughtering must be extremely sharp to facilitate the quick cutting of the blood vessels. The rapid drainage of blood causes anoxia and often prevents birds from regaining consciousness during the subsequent 80 to 90 seconds [17].

In the water bath stunning, birds are shackled upside down on a moving conveyer belt that takes them to the electrified water bath. Inadequate

stunning of birds is a common problem with this method, especially with ducks and geese that tend to raise their heads when entering the water bath. It was also noted that chickens could and do inhale water during electric stunning in a water bath. In recent years, the strength of electric current has been raised to ensure the death of the birds by cardiac arrest. However, the number of carcass defects increased with the increase of the stunning electrical voltages. High stunning voltages (103 to 193 V Alternating Current) are sufficient to satisfy birds' welfare issues but resulted in other side effects such as physical damage, breast blood spots, red wingtips, and broken bones. High stunned carcasses were classed as B grade. Moderate stunning (53 to 63 V AC) seemed to be more effective in bleed out (50 %) and resulted in better carcass quality which was graded as A .

According to Federation of Veterinarians of Europe (FVE) Code of Good Veterinary Practice, veterinarians shall endeavor to ensure the welfare and health of the animals under their care, in whichever section of the veterinary profession they work. According to Council Directive 93/119/EC, animals shall be spared from any avoidable pain or suffering at the time of slaughter or killing. Therefore, ruminants, pigs, rabbits, and poultry shall be stunned before slaughter or killed instantaneously. However, as certain religious groups require that animals should not be stunned before slaughter, the directive allows limited derogations to take account of the particular requirements of certain religious rites. Most Member States did use the derogations to allow the slaughter of animals without prior stunning. Outside the European Union, however, in countries such as Switzerland, slaughter without prior stunning is prohibited. It is also noteworthy that in other parts of the world, in New Zealand in particular, protocols have been developed, which allow specific methods of stunning while meeting the requirements of some of the religious rites. It is clear that animal welfare is no longer merely a European Union (EU) issue, rather it is being accorded a growing level of importance in civil society around the world.

5. PROTEOMICS

Proteomics is the study of the entire protein complement of the genome . The term "proteome" was first coined in the early 1990s, and the "omics" term symbolizes a redefinition of how we think about biology and the workings of living systems . Unlike the genome, which is comparatively static, protein is a highly dynamic entity, as the protein content of a given cell will vary concerning changes in the surrounding environment, the physiological state of the cell (e.g., Position in the cell cycle), stress, drug administration, health, and disease . Therefore, proteomics could be considered as a study on how the genetic information stored in the DNA is expressed in the form of proteins. The research trend seems like focusing on the proteins for a greater and deeper understanding of biological processes such as signal transduction, growth and differentiation, and cellular response to a variety of agents and conditions .

Proteomics deals with more complex mixtures than most protein biochemistry experiments, the science relies more heavily on the separation of components in protein mixtures. 2-D gel electrophoresis and liquid chromatography offer method of physical separation of protein mixtures. Denaturation and enzymatic digestion are used to cleave the intact proteins into peptides. Mass analysis has been the driving engine of proteomics. Essentially, the adaptation of mass spectrometry to proteins has greatly increased the amount of data obtained from these experiments. Each mass spectrometer has three major elements which are the ion source, a mass analyzer, and detector. Ionization in proteomics is generally handled via matrix-assisted laser desorption ionization (MALDI) or electrospray ionization (ESI). Mass analysis is typically managed through time-of-flight (TOF) or quadrupole analyzers. A detector records the ion current resulting from ions exciting the analyzer. Mass spectrometry produces proteomics data, but proteomics information requires that the spectra be interpreted. Many programs interaction makes the interpretation possible. First, mass spectrometry is conducted via instrument control software. The peptides usually identified by the sequence database search algorithms.

Proteomics study aims to compare the spectrum of proteins expressed in cells or tissue under different environmental conditions or from different disease states . In a 2D-gel, the intensity of a protein spot is assumed to be directly proportional to the amount of protein in a given cell at a particular time. Therefore, any changes in the intensities of protein spots in the gel image could be correlated to the amount of protein expressed. In addition to the change in intensity, any changes in protein structure associated with post-translational modifications such as phosphorylation, oxidative modification or glycosylation may result in changes in the pI or molecular weight of the protein. Phosphorylation alters the pI and surface charge, while glycosylation can alter the protein size, pI, charge, and hydrophobicity. As a result of such modifications, target proteins may appear in the gel by a change in the vertical or horizontal position. This technique is aimed to detect any differences in protein intensities or complexity between groups of gels .

6. BIOMARKER DISCOVERY THROUGH PROTEOMICS

The past decade has witnessed a surge in technological developments in various biomedical related disciplines, namely, genomics, transcriptomics, and proteomics. These advances have changed the way in which experiments are designed. The core technologies that have been responsible for this growing trend include high-speed DNA sequencing (genomics), microarray technologies for the study of mRNA (transcriptomics) and mass spectrometry (MS) for studying protein function and expression (proteomics). These three major scientific areas have been developed independently from one another, but all are biologically and computationally connected. For example, global proteomics, where the goal is to characterize as many proteins as possible within a given system, is made possible only through the generation of large genomic databases. Apart from that, a complete understanding of cell function is only achieved by integrating data acquired at the genomic, transcriptomic, proteomic, as well as metabolite levels. When genetic analyses aim at identifying individuals with a predisposition to certain diseases, and therefore, the long-term risk, proteomic analyses provide the opportunity to detect diseases as they occur .

Alterations in protein abundance, structure, and function act as useful indicators of pathological abnormalities before developing clinical symptoms, thus, often serve as useful diagnostic and prognostic biomarkers. A biomarker is a substance found in the blood, urine, cerebrospinal fluid, or tissues and is often detected in higher than normal amounts in patients with a certain disease or condition. A biomarker can include patterns of single nucleotide polymorphisms (SNPs), DNA methylation, or changes in mRNA, protein, or metabolite abundances, providing that these patterns can be shown to correlate with the characteristics of the disease [18]. It has been demonstrated, however, that there is no predictive correlation between mRNA abundances and the quantity of corresponding functional protein present within a cell. Hence, direct measurement of biologically active protein expression is believed to be more accurate to indicate the cellular dysfunction underlying the development of the disease.

After the completion of the Human Genome Project in 2003, there are ongoing efforts to identify genetic polymorphisms that may point to disease predisposition or unique response to therapy such as towards drug side effects . Development of micro hybridization arrays has powered the functional genomics phase in which gene expression profiling is being used to correlate gene expression patterns with disease classification and predict response to therapy. Although the blueprints of human disease may be genetically encoded, the execution of the disease process occurs through altered protein function. While gene microarray studies elucidate gene expression patterns associated with disease, little is known about the complexity of protein-protein interactions, protein localization, or modifications. For many diseases, such as cancer, protein function is altered in the context of key signaling pathways that regulate critical cellular functions including proliferation, apoptosis, differentiation, survival, immunity, metabolism, invasion, and metastasis .

A biomarker should be presented in an easily obtainable sample such as urine or blood to give the greatest impact. The biomarker must be capable of screening a large number of samples in a high-throughput manner.

Validation of a biomarker requires the analysis of thousands of samples to ensure that the potential biomarker is indeed related to a disease state and is not simply a function of the variability within the samples due to differences in diet, genetic background, lifestyle, and so on [19,20].

Future developments in biomarker-based proteomics technologies will be dramatically impacted by the recent realization that a high percentage of the diagnostically useful lower molecular weight, serum protein entities are bound to higher molecular weight carrier proteins such as albumin . These carrier proteins likely serve to amplify and protect lower molecular weight biomarkers from clearance by the renal system [21-25]. Conventional protocols for biomarker discovery begun by discarding the abundant high molecular weight carrier species without realizing the valuable cargo they harbor. In the future, the development of novel nanotechnology platforms that allow the amplification and abundant harvests of diagnostic low molecular weight biomarkers *in vivo* or *ex vivo* is expected and anticipated [26]. Such nanotechnology tools might consist of derivatized gold nanoparticles that actively bind biomarkers, providing enriched signature profiles elucidated via mass spectrometry platforms. Initial studies with magnetic nanoparticle probes coated with bait antibodies and unique barcode DNA fragments can amplify signals of low abundant biomolecules at concentrations as low at 3 attomolar (approximately 18 to 20 copies per 10 µl of fluid) [27-31]. This amplification is comparable to PCR amplification of nucleotide sequences and can theoretically be used to detect hundreds of protein targets at a time in patient samples. The proteomics approach has been widely used in various fields and research purposes [32-39]. The applications vary regarding the system used and the focus of the research.

7. PERSPECTIVE

Food authentication studies have been conducted by using various approaches mainly by exploiting the genetic differences in different meat origins. At the same time, the employment of proteomics in studying expression levels of different proteins in various systems, including plants and animals have been actively investigated [40-46]. However, it appears that, to the best of our knowledge, there has no report on the differences in the expression levels of proteins in animals during slaughtering and stunning. As improper stunning is included as one type of food adulteration, therefore, proteomics could serve as a tool in verifying the authentication level of the slaughtered animal. We have embarked on the use of proteomics to discover potential biomarkers for slaughter and stunned chicken, and we foresee the biomarkers will provide a new revolution for both proteomics and food authentication studies.

ACKNOWLEDGMENT

Authors would like to thank the Ministry of Higher Education Malaysia for providing Fundamental Research Grant Scheme (FRGS 0207-43) to support this project and Ministry of Science and Technology Malaysia for providing National Sciences Foundation Scholarship for Norshahida Abu Samah for her MSc study.

REFERENCES

[1] Tanabe, S., Hase, M., Yano, T., Sato, M., Fujimura, T., Akiyama, H. 2007. A real-time quantitative PCR detection method for pork, chicken, beef, mutton, and horseflesh in foods. Bioscience Biotechnology Biochemistry, 71 (12), 3131-3135.

[26] Nam, J.M., Thaxton, C.S., Mirkin, C.A. 2003. Nanoparticle-based bio-bar codes for the ultrasensitive detection of proteins. Science, 302, 1884-1886.

[2] Houde, A., Godbout, D., Gariepy, C. 2001. Major genes and meat quality. Advances in Pork Production, 12, 139-142.

[3] Elisa, B., Andrea, G., Graziella, B. 2006. Development of a "one step Real Time PCR" method for animal meat detection in feedstuffs. [cited 2006 16th July 2010]; Available from: http://www.istitutospallanzani.it/doc/229.pdf.

[4] Rencova, E., Svoboda, I., Necidova, I. 2000. Identification by ELISA of poultry, Horse, kangaroo, and rat muscle specific proteins in heat processed products. Veterinarni Medicina, 45, 353-356.

[5] Ilhak, O.I., Arslan, A. 2001. Identification of meat species by polymerase chain reaction (PCR) technique. Turkish Journal of Veterinary and Animal Sciences, 31 (3), 159-163.

[6] Ghovvati, S., Nassiri, M.R., Mirhoseini, S.Z., Moussavi, A.H., Javadmanesh, A. 2009. Fraud identification in industrial meat products by multiplex PCR assay. Food Control, 20, 696-699.

[7] Ong, S.B., Zuraini, M.I., Jurin, W.G., Cheah, Y.K., Tunung, R., Chai, L.C., Haryani, Y., Ghazali, F.M., Son, R. 2007. Meat molecular detection: sensitivity of polymerase chain reaction - restriction fragment length polymorphism in species differentiation of meat from animal origin. ASEAN Food Journal, 14 (1), 51-59.

[8] Alaraidh, I.A. 2008. Improved DNA extraction method for porcine contaminants, detection in imported meat to the Saudi market. Saudi Journal of Biological Sciences, 15 (2), 225-229.

[9] Ponsuksili, S., Chomdej, S., Murani, E., Blaser, U., Schreinemachers, H.J., Schellander, K., Wimmers, K. 2005. SNP detection and genetic mapping of porcine genes encoding enzymes in hepatic metabolic pathways and evaluation of linkage with carcass traits. Animal Genetics, 36, 477-483.

[10] Chen, F.C., Hsieh, Y.H. 2000. Detection of pork in heat-processed meat products by monoclonal antibody-based ELISA. Journal of AOAC International, 83, 79-85.

[11] Desai, E. 2009. The Ritual Islamic Slaughter. [cited 2010 16th July]; Available from: http://al-inaam.com/library/rslaughter.htm.

[12] Al-Qaradawi, Y. 2001. The Lawful and The Prohibited in Islam. Islamic Book Trust. Kuala Lumpur.

[13] Veerkamp, C.H. 1987. Stunning and killing broilers. in European Symposium on Poultry Meat Quality. Budapest, Hungary.

[14] Gregory, N.G., Wilkins, L.J. 1989. Effect of stunning current on carcass quality in chickens. Veterinary Record, 124, 530-532.

[15] Ali, A.S.A., Jensen, J.F., Jensen, J.A. 1996. Effect of different electrical stunning voltages on blood loss, carcass quality and breast muscle pH. Archiv fur Geflugelkunde, 3, 126-131.

[16] van de Nieuwelaar, J. 1993. Technical and technological aspects. in EC Workshop on Pre-slaughter Handling and Stunning of Poultry. Brussels, Belgium.

[17] Bilgili, S.F. 1999. Recent advances in electrical stunning. Poultry Science, 78,282-286.

[18] MacNeil, J.S. 2004. Better biomarkers for the diagnostics labyrinth. Genome Technology, 24-33.

[19] Rabilloud, T., Humphery-Smith, I. 2000. Introduction: the virtue of proteomics. Proteome research: two-dimensional gel electrophoresis & identification methods, ed. T.H. Rabilloud. Springer. New York.

[20] Gygi, S.P., Corthals, G.L., Zhang, Y., Rochon, Y., Aeberson, R. 2000. Evaluation of two-dimensional gel electrophoresis-based proteome analysis technology. Proceedings of the National Academy of Sciences of the United States of America, 97 (17), 9390-9395.

[21] Zainomi, M.J. 2004. Malaysian standard MS 1500:2004, Halal food – production, preparation, handling and storage – general guidelines, in Standards and Quality News SIRIM. SIRIM: Malaysia p. 2-3.

[22] Calvo, K.R., Liotta, L.A., Petricoin, E.F. 2005. Clinical proteomics: from biomarker discovery and cell signaling profiles to individualized personal therapy. Bioscience Reports, 25, 107-125.

[23] Liotta, L.A., Ferrari, M., Petricoin, E. 2003. Clinical proteomics: written in blood. Nature, 25 (6961), 905.

[24] Mehta, A.I., Ross, S., Lowenthal, M.S., Fusaro, V., Fishman, D.A., Petricoin, A.F., Liotta, L.A. 2003. Biomarker amplification by serum carrier protein binding. Disease Markers, 19 (1), 1-10.

[25] Geho, D.H., Petricoin, E.F., Liotta, L.A. 2004. Blasting into the microworld of tissue proteomics: a new window on cancer. Clinical Cancer Research, 10 (3), 825-827.

[26] Badano, J.L., Katsanis, N. 2002. Beyond Mendel: an evolving view of human genetic disease transmission. Nature Reviews Genetics, 3 (10), 779-789.

[27] Gregory, N.G., Wilkins, L.J. 1984. A practical guide to neck cutting in poultry. in Meat Research Memorandum No. 54. United Kingdom.

[28] Fazallah, P., Rosniza, M.T. 2002. Pusat Sembelihan Ayam Perlu Diawasi, in Berita Harian. Kuala Lumpur.

[29] Ellegren, H., Chowdhary, B., Johansson, M., Anderson, L. 1994. A primary linkage map of the porcine genome reveals a low rate of recombination. Genetics, 137,1089-1100.

[30] Archibald, A.L., Brown, J.F., Couperwhite, S., Mc Queen, H.A., Nicholson, D., Haley, C.S. 1995. The PiGMaP consortium linkage map of the pig (Sus scrofa). Mammalian Genome, 6, 157-175.

[31] Rohrer, G.A., Alexander, L.J., Hu, H., Smith, T.P.L., Keele, J.W., Beattie, C.W. 1996. A comprehensive map of the porcine genome. Genome Research, 6,371-391.

[32] Hunt, D., Parkes, H.C., Davies, I.D. 1997. Identification of the species of origin of raw and cooked meat products using oligonucleotide probes. Journal of Food Chemistry, 60, 437-442.

[33] Ali, S.A. 2009. Halal: The Most Humane Slaughter. The New Nation. Dhaka.

[34] Bilgili, S.F. 1922. Electrical stunning of broilers – basic concepts and carcass quality implications. The Journal of Applied Poultry Research, 1,135-146.

[35] Dickens, J.A., Shackelford, A.D., Dickens, J.A., Shackelford, A.D. 1988. Feather-releasing forces related to stunning of chickens, and its effect on the quality of the meat. Fleischwirtschaft, 68, 1416-1420.

[36] Davis, K. 2002. The Need for Legislation and Elimination of Electrical Immobilization. [cited 2006 13th September]; Available from: http://www.upc-online.org/slaughter/report.html.

[37] Martini, F.H. 2004. Fundental of Anatomy anf Physiology. 6th Edition ed., Pearson Education. San Francisco.

[38] Grandin, T. 2002. Animal Care and Handling. in American Meat Institute Stunning Conference. Illinois.

[39] Heath, G.B.S. 1984. The slaughter of broiler chickens. World's Poultry Science Journal, 40, 151-159.

[40] Ali, A.S.A., Lawson, M.A., Tauson, A., Jensen, J.F., Chwalibog, A. 2006. Influence of electrical stunning voltages on bleed out and carcass quality in slaughtered broiler chickens. Archiv fur Geflugelkunde, 71 (1), 35-40.

[41] Wasinger, V.C., Cordwell, S.J., Cerpa-Poljak, A., Yan, J.X., Gooley, A.A., Wilkins, M.R., Duncan, M.W., Harris, R., William, K.L., Humphery-Smith, I. 1995. Progress with gene product mapping of the mollicutes: Mycoplasma genitalium. Electrophoresis, 16, 1090-1094.

[42] Liebler, D.C. 2002. Proteomic approaches to characterize protein modifications: new tools to study the effects of environmental exposures. Environmental Health Perspectives, 110, 3-9.

[43] Hanash, S.M. 1998. Two-dimensional gel electrophoresis. 3rd ed. Gel electrophoresis of proteins: a practical approach ed. B.D. Hames. Oxford University Press. Oxford.

[44] Palzkill, T. 2002. Proteomics. Kluwer Academic Publishers. The Netherlands.

[45] Meleth, S., Deshane, J., Kim, H. 2005. The case for well-conducted experiments to validate statistical protocols for 2D gels: different pre-processing = different lists of significant proteins. BMC Biotechnology, 5, 7.

8. [46] Bock, C., Coleman, M., Collins, B., Davis, J., Foulds, G., Gold, L., Greef, C., Heil, J., Heilig, J.S., Hicke, B., Nelson-Hurst, M., Husar, G.M., Miller, D., Ostroff, R., Petach, H., Schneider, D., Vant-Hull, B., Waugh, S., Weiss, A., S.K., Wilcox, Zichi, D. 2004. Photoaptamer arrays applied to multiplexed proteomic analysis. Proteomics, 4, 609-618.