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Agronomy

Application of DNA Based Molecular Assays for Halal Meat Products Authentication-An overview

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Abstract Review Article

Religion is a robust social dynamic in human food consumption and purchasing decision. Muslim consumers often ready to pay higher prices for the inherent natured Halal meat products. However, the meat products where physical features are disrupted by food processing steps is more prone to fraudulent admixing or alteration. Hence to secure human health, keep consumer trust and religious faith species authentication of Halal meat product is important. DNA biomarkers based assay provide an excellent opportunity to authenticate animal species with better specificity and stability under different food processing condition. Therefore, here we have described DNA based molecular assay platforms that could be used for the detection of meat animal species from raw and commercial meat products for halal meat product verification. It will facilitate for the development of improve species detection techniques and "Halal" food authentication to secure consumer health and belief.

Keywords: DNA, Halal, Meat, Authentication.

INTRODUCTON

The Arabic term "Halal" means permissible or lawful things for Muslim consumers. According to Islam, the foods for Muslim consumer are defined by Quran (the divine book), Hadith (Prophet Muhammad SWA teachings including words, actions, and his approval) and Fiqh (judgements using the evidence from the Quran and Hadith, and from consensus of Islamic scholars). Thus, in reference to the food and Islamic dietary standard, meat is most strictly regulated such as swine, carnivorous and meat of the dead animals or those immolated to other than Allah strongly prohibited [1, 2]. Furthermore, meats of the certain animals are careers of certain zoonotic diseases such rabies by canine species [3], swine-influenza virus H1N1 (S-OIV) by swine species [2]. Therefore, animal species authentication of the meat products is an important issue to anchor religious faith and human health.

Malaysian food authority has defined halal food through MS1500:2009. It includes the guideline for Halal food production, processing, handling and storage condition those are permitted according to Islam along with food safety or "tayyiban" issue. The 'Halal' logo on food products defines that the products are prepared following the Shariah law of Islam and with proper hygienic condition and trusted by Muslims (about 1.8 billion currently).

The recent porcine DNA scandal in the chocolate product in Malaysia [4]; horse meat scandal in Europe and rat meat scandal in China has given us brain storming apprehension for the detection of animal species in the foods [5]. Due to the inherent nature, specialized preparation and comparatively higher price of halal foods fraudulent labelling of halal logos is taking place [6]. Thus authentication of meat species is an essential part to regulate the Halal food market and secure consumers' trust. Halal status of the food cannot easily determine by the organoleptic test. To ensure the Halal foods in compliance with religion and health, DNA based technique provide a highly precise and sensitive species detection platform.

HALAL FOOD MARKET

The Muslim population is growing at a faster rate than the non-Muslim in the world. The estimated annual growth rate is 1.5% and 0.7% for muslim and non-muslim respectively. Thus in the subsequent two decades Muslim population of the world is predict to be increase about 35%. Thus by the next 20 years the Muslim population will reach 2.2 billion if follow the current growth trend and will construct 26.4% of the world human population (**Fig-1**). This vast Muslim population is the dormant consumer of the Halal meat products. Beside the Muslim population Halal food is

one of the choices for non-Muslim consumer too due to ethical and safety issue. For instance, there are 2 million Muslim in UK, but the consumer of Halal meat is about 6 million. In the Netherlands, the estimated market value of Halal food products is about USD 3 billion on an annual basic where it includes the non-Muslim Dutch consumers. Although only 20% of the global Muslim population from Arab and Middle East, but the consumer of these countries have higher purchasing power to buy the Halal product [8].

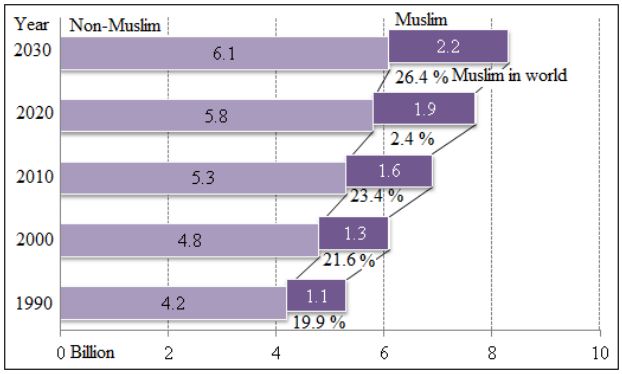

Fig-1: Projection of world Muslim population by the year 2030; (Source: [7]).

SPECIES AUTHENTICATION TECHNIQUES

For determining the animal species in meat or foods current assays are based on analysis of lipid [6], proteins [9], and nucleic acids [10] biomarkers. For determining the animal species in food, lipid-biomarker assays are based on the positional analysis of fatty acid triacylglycerol (TAG) and 2-monoacylglycerol (2-MAG). But the drawbacks of the lipid based methods in species authentication is the alteration of the positional distribution of fatty acids with TAGs and MAGs during the cooking process (Ali et al., 2012). It has been described for the utilization of protein as a biomarkers for animal species authentication using different techniques such as electrophoretic, chromatographic and spectroscopic [11]. However, the denaturation of soluble protein during the thermal treatment of process foods reduces the potentialities of protein based approaches. Furthermore, to determine the antibodies raising against a specific protein for animal species authentication in foods by immunoassays have the possibility of interruption by the cross-reactions from closely related species ([12]. For overcoming these limitations DNA based assays demonstrated it potentiality to determine the animal species both in raw or processed foods.

DNA BASED SPECIES AUTHENTICATION

Species Specificity Detection

To distinguish the animal species, DNA biomarkers have enough discriminating power depending on the variation of the sequence in the genome (**Fig- 2**). The detection of animal species based on sequence analysis of DNA created by most of the neutral molecular evolutionary force that accumulate at different lineages over a time period [13]. To determine the animal speices, both nuclear DNA or mitochondrial DNA (mt-DNA) target are useful [11]. The major characteristic of the DNA molecule that makes it intensely useful for species detection is the more informative unique genetic code [14]. Thus DNA based PCR assays are commonly use for detection of animal species from raw or meat product such as pork [15] and beef [16]. To define the animal specificity of the DNA marker it is important to calculate the mismatch at the primer binding site before running the PCR. The specific primers set can be blast against NCBI data base for detecting cross species amplification. Further rechecking can be done by aligning the specific primer with other common meat species using multiple sequence alignment tools. Finally in wet lab, common meat species which are mostly available in the meat market can be used to cross check the specificity and standardization of the PCR assay. Thus DNA based assays are promising field not only for halal species authentication in the food products but also for detection of food borne pathogens, biodiagnostics, forensic and halal food analysis analysis[17].

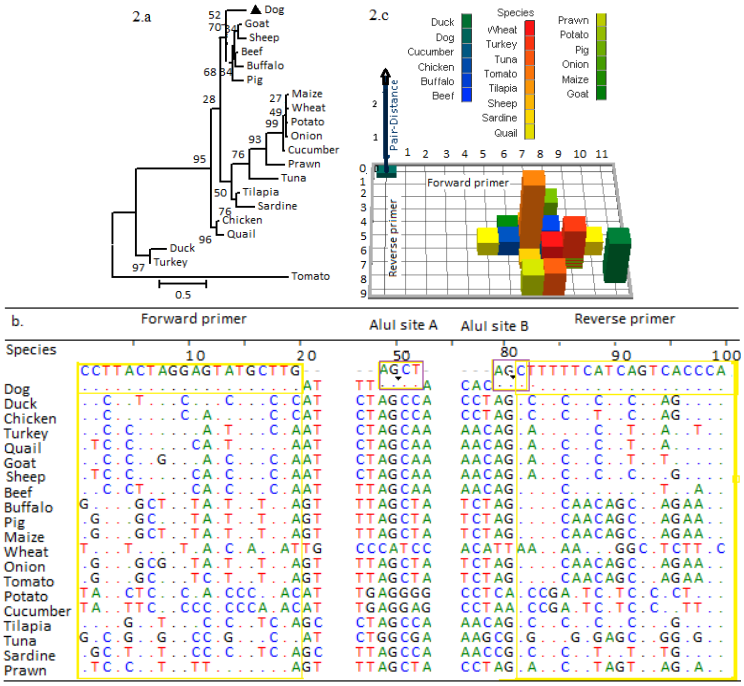

Fig-2: In-silico analysis of species detection primers and discrimination properties using DNA sequences of species specific target with other animal, plan and fish species

(a) Construction of dendogram using Neighborhood-Joining method for the specific species target such as 100 bp regions of cytb/cob-gene sequences of dog and other 19 plant, animal and fish species. (b) Mismatch bases analysis with dog specific primers and the positions of *Alu*I restriction sites. (c) 3D plot using species specific primer mismatch and the pairwise distance. (Adapted from [18]).

Stability of the DNA based assay

DNA based biomarkers amplify more efficiently and able to separate with higher resolution with better recovery in degraded sample analysis. Thus DNA biomarkers of have huge interests for the development of biochip, biosensor and forensic applications due to their superior stability. However, the stability of the species specific marker depends on the successful amplification of the specific fragment which can be determined by certain electrophoresis technique. Thus the separation of canine specific DNA fragment was amplified PCR strategy using agarose gel from raw or food processing conditions (**Fig- 3**). The digestion of PCR products with restriction endonuclease and PCR-RFLP assay was the provide more authentic specie detection platform which was further enhanced by application of capillary electrophoresis. PCR-RFLP analyses, showing 100 bp PCR and *Alu*I restriction digestion products obtained from raw, autoclaved and ready to eat model dog burger (**Fig-3**).

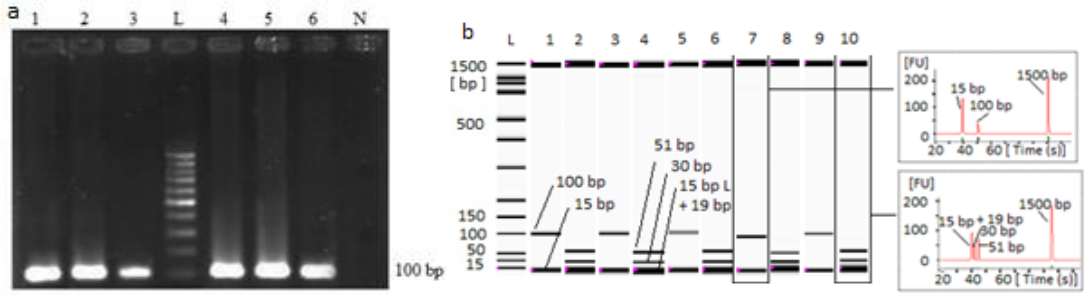

Fig-3: Stability analysis of the DNA based species specific PCR assay under different processing conditions.

Clear 100 bp PCR products was obtained from different thermally treated dog meat samples using PCR and agarose based end point detection. Lane 1: Raw dog meat, Lane 2: 90 min boiled dog meat; Lane 3: Autoclaved dog meat at120°C under 45 psi for 2.5 h.; Lane L: 100 bp ladder; Lane 4 to 6: autoclaved dog meat at120°C for 50 min; 110°C for 12 min, and 133°C for 20 min under 43.51 psi respectively, Lane N: Negative control (Adapted from [10]). (b) PCR-RFLP analyses, showing 100 bp PCR and *Alu*I restriction digestion products obtained using capillary electrophoresis based end point detection from raw, autoclaved and model dog burger sample. Lane L: Ladder; Lane 1, 3, 5, 7, 9 before restriction digestion and Lane 2, 4, 6, 8, 10 after

restriction digestion of PCR product obtained from raw, autoclaved meat and raw, autoclaved and ready to eat model dog burger, respectively. Corresponding electropherograms as demonstrated by respective labels (Adapted from [18]).

Validity of the DNA marker

To protect foods frauds, it is very important to prove the validity of the assay in highly process foods as most of the adulteration occur in mincemeat or processed food products. The substituted lower cost meat may affect consumer health and lifestyles, such as vegetarianism, or religious practices. For example, Muslims have the food taboo for pork meat consumption. However, the detection of specific target by traditional agarose gel image provides only a simple present or absent answer [19]; while sometime it is necessary to know the amount of the adulterant or count the microbial threat in the food. The real-time PCR assay allows the quantitative analysis of the specific target using a fluorescent reporter molecule. However, the presence of various additives and inhibitors in commercial meat and food products might prevent the primer binding at specific sites and reduce the amplification efficiency, diminishing the sensitivity and specificity of the assay [20]. Therefore, it is important to validate the the DNA based assays by preparing model experimental samples as well as commercial food products such as Meatball (**Table -1**) and chicken nuggets (**Table-2**). The performance of the canine meat specific DNA based assay under pure and spiked chicken and beef meatball formulation, a constant detection limit of 0.2% (0.04 ng DNA) was obtained and no commercial samples were found to be positive [10].

Table-1: Validity of DNA based assay for meatball analysis result using DNA based PCR assay. (Adapted from [10])

Meatball sample	Day1	Day2	Day3	≥ 0.2 % Dog DNA Detection	Detection probability
Pure chicken meatball	3	3	3	0/9	100
Pure beef meatball	3	3	3	0/9	100
Pure Dog meatball	3	3	3	9/9	
Dog meat spiked with chicken	9	9	9	27/27	100
Dog meat spiked with beef	9	9	9	27/27	100
Commercial chicken meatball					100
A	3	3	3	0/9	
B	3	3	3	0/9	100
C	3	3	3	0/9	100
D	3	3	3	0/9	100
E	3	3	3	0/9	100
Commercial beef meatball					
A	3	3	3	0/9	100
B	3	3	3	0/9	100
C	3	3	3	0/9	100
D	3	3	3	0/9	100
E	3	3	3	0/9	100

For determining the validity of the RT-PCR assay, a good linearity ($R^2 = 0.999$) was demonstrated by canine specific assay while tested by using chicken nugget sample. The construction of the model for using canine meat contaminated nuggets sample showed an excellent recovery rate of 87±28% to 112±19% (Table 2), where different percentage of the deliberately canine meat spiked were used [21]. Latest nano-platform and biosensors come forward with attractive materials with excellent physio-chemical properties to create building block for DNA molecule reorganization. Thus, the DNA based biomarker assay showed its potentiality for both qualitative and quantitative detection of species in food product and can be used in the food industries as well as in Halal food authentication laboratories.

Table 2. Validation of the real time qPCR assay for determining canine meat/DNA using deliveratelty contaminated and qPCR predicted value of canine meat/DNA in chicken nuggets (Adapted from [21])

Canine meat (contamination)	qPCR predicted value		Canine DNA (contamination)	qPCR predicted value	
Admixed %, w/w	Admixed % w/w	Recovery w/w (%)	Concentration ng/µl	Concentration ng/µl	Recovery ng/µl (%)
100	94.79±8.39	94.56±8	20	19±1.712	95±9
10	11.29±1.95	112±19	2	2.24±0.389	112±19
1	1.07 ± 0.18	107±18	0.2	0.20±0.028	101±14
0.10	0.10± 0.03	87± 28	0.02	0.02±0.005	89±26
0.01	0.01±0.00	108±21	0.002	0.002±.0004	108±21

SUMMARY

Although there are advances in halal authentication and food pathogen detection platform, there still some challenges and opportunities to improve. The PCR based DNA detection techniques have paved the way for the rapid and sensitive detection of animal or microbial species in foods. Thus nano-biotechnology opened the field with biosensor with miniaturization handy devices. The collaborations between the researcher in the fields of food science, engineering, molecular biology and nanotechnology can achieve better portable, hand-held devices and biosensors for the detection of "Halal" foods to secure consumer religious faith and health.

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REFERENCES

1. Khattak JZ, Mir A, Anwar Z, Abbas G, Khattak HZ, Ismatullah H. Concept of halal food and biotechnology. Advance Journal of Food Science and Technology. 2011 Oct 25;3(5):385-9.
2. Peiris JM, Poon LL, Guan Y. Emergence of a novel swine-origin influenza A virus (S-OIV) H1N1 virus in humans. Journal of Clinical Virology. 2009 Jul 1;45(3):169-73.
3. Ajoke E, Solomon A, Ikhide E. The role of dog trading and slaughter for meat in rabies epidemiology with special reference to Nigeria—a review. J Exp Biol Agric Sci. 2014 Apr;2(2):130-6.
4. Chakravorty S. Pork DNA found in two chocolate products of Cadbury Malaysia: Report. Reuters. Retrived from: http://www. reuters. com/article/2014/05/26/us-mondelez-intl-recallidUSBREA4P0CH20140526. 2014.
5. Ali ME, Razzak MA, Hamid SB. Multiplex PCR in species authentication: probability and prospects—a review. Food Analytical Methods. 2014 Nov 1;7(10):1933-49.
6. Rohman A, Erwanto Y, Man YB. Analysis of pork adulteration in beef meatball using Fourier transform infrared (FTIR) spectroscopy. Meat Science. 2011 May 1;88(1):91-5.
7. The Pew Research Center's Forum on Religion & Public Life, The Future of the Global Muslim Population. 2011 January 27 [cited 2015 January 15]; Available from: http://www.pewforum.org/2011/01/27/the-future-of-the-global-muslim-population/.
8. Hughes R, Malik R (2014). *The Global Halal Industry: An Overview*. [cited 2015 January 14]; Available from: http://www.gifr.net/gifr2013/ch_13.PDF.
9. Asensio L, González I, García T, Martín R. Determination of food authenticity by enzyme-linked immunosorbent assay (ELISA). Food control. 2008 Jan 1;19(1):1-8.
10. Rahman MM, Ali ME, Hamid SB, Mustafa S, Hashim U, Hanapi UK. Polymerase chain reaction assay targeting cytochrome b gene for the detection of dog meat adulteration in meatball formulation. Meat science. 2014 Aug 1;97(4):404-9.
11. Montowska M, Pospiech E. Authenticity determination of meat and meat products on the protein and DNA basis. Food Reviews International. 2010 Dec 13;27(1):84-100.
12. Ayaz Y, Ayaz ND, Erol I. Detection of species in meat and meat products using Enzyme-Linked Immunosorbent Assay. Journal of Muscle Foods. 2006 Apr;17(2):214-20.
13. Kimura M. Evolutionary rate at the molecular level. Nature. 1968 Feb 17;217(5129):624-6.
14. Pereira F, Carneiro J, Amorim A. Identification of species with DNA-based technology: current progress and challenges. Recent patents on DNA & gene sequences. 2008 Nov 1;2(3):187-200.
15. Ali ME, Hashim U, Dhahi TS, Mustafa S, Man YB, Latif MA. Analysis of pork adulteration in commercial burgers targeting porcine-specific mitochondrial cytochrome B gene by TaqMan probe real-time polymerase chain reaction. Food analytical methods. 2012 Aug 1;5(4):784-94.
16. Mane BG, Mendiratta SK, Tiwari AK. Beef specific polymerase chain reaction assay for authentication of meat and meat products. Food Control. 2012 Dec 1;28(2):246-9.
17. Iwobi AN, Huber I, Hauner G, Miller A, Busch U. Biochip technology for the detection of animal species in meat products. Food Analytical Methods. 2011 Sep 1;4(3):389-98.
18. Rahman MM, Ali ME, Hamid SB, Bhassu S, Mustafa S, Al Amin M, Razzak MA. Lab-on-a-chip PCR-RFLP assay for the detection of canine DNA in burger formulations. Food Analytical Methods. 2015 Jul 1;8(6):1598-606.
19. Woolfe M, Primrose S. Food forensics: using DNA technology to combat misdescription and fraud. TRENDS in Biotechnology. 2004 May 1;22(5):222-6.
20. Di Pinto A, Forte VT, Conversano MC, Tantillo GM. Duplex polymerase chain reaction for detection of pork meat in horse meat fresh sausages from Italian retail sources. Food control. 2005 Jun 1;16(5):391-4.
21. Rahman MM, Hamid SB, Basirun WJ, Bhassu S, Rashid NR, Mustafa S, Mohd Desa MN, Ali ME. TaqMan probe real-time polymerase chain reaction assay for the quantification of canine DNA in chicken nugget. Food Additives & Contaminants: Part A. 2016 Jan 2;33(1):10-8.