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RESEARCH PAPER

Detection of Porcine Based Materials in Processed Food Using Polymerase Chain Reaction Method

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Abstract

The detection of halal adulteration in processed food, particularly the presence of porcine based material, be it human or animal food is very important to safeguard the interest of Muslim consumers. Many techniques have been developed to determine such adulteration which can ascertain the halal status of food products. In this paper, the presence of porcine based materials in few processed foods namely meat products, candy products, and animal feeds obtained from Kota Samarahan, Sarawak, Malaysia were screened by using Polymerase Chain Reaction (PCR) method. Findings revealed that all processed meat samples showed a positive result of the presence of porcine DNA. A similar result was also obtained for a few samples of animal feeds and candy products. As such, further study is required to verify the results.

Keywords: Halal authentication, processed foods, Polymerase Chain Reaction

Abstrak

Pengesanan pencemaran halal dalam makanan yang diproses, terutamanya kehadiran bahan berasaskan khinzir, sama ada makanan manusia atau haiwan adalah sangat penting untuk menjaga kepentingan pengguna Islam. Banyak teknik yang telah dibangunkan untuk menentukan pencemaran sedemikian yang dapat memastikan status produk makanan halal. Dalam makalah ini, kehadiran bahan berasaskan khinzir dalam beberapa makanan yang diproses iaitu produk daging, produk gula-gula, dan makanan haiwan yang diperoleh dari Kota Samarahan, Sarawak, Malaysia telah diperiksa dengan menggunakan kaedah Reaksi Polimerase Berantai (PCR). Penemuan menunjukkan bahawa semua sampel daging yang diproses menunjukkan hasil positif kehadiran DNA khinzir. Hasil yang sama juga diperolehi untuk beberapa sampel makanan haiwan dan produk gula-gula. Oleh itu, kajian lanjut diperlukan untuk mengesahkan keputusan.

Kata kunci: Pengesahan halal, makanan olahan, reaksi polimerase berantai

INTRODUCTION

Halal is an Arabic word which refers to what is lawful or permissible in Islamic law. By contrast "Haram" means forbidden in all area of life. The term halal is a rule in Islam for Muslim to follow when they decide to consume food and other things. Naturally, halal is related to food, but the concepts of halal has a huge meaning in our livelihood. It covers economic, financial, consumer goods as well as family relationships.

Halalan toyyiban is the specific concept when Muslim related consuming goods which is allowed according to the syariah law. Consumable according to halalan toyyiban concepts must be safe, hygiene and the quality must be assured by the Islamic authority (Ambali & Bakar, 2013).

In between halal and haram, there is the grey area that we classify as syubhah. The concept of syubhah occurs when we face a confusing situation where we could not determine the validity of halal and haram products. The Prophet (peace be upon Him) has given a guideline concerning Syubhah matters as reported by Bukhari, Muslim, Abu Daud, Ibn Majah and Darimi, as follow: Which means:

> *'What is Halal is clear. And what is Haram is also clear. And in between those two is a dubious area in which many people do not know about. So whoever distanced himself from it, he has acquitted himself (from blame). And those who fall into it, he has fallen into a state of Haram'.*

In Islam consuming halal goods is not a matter of principle, but it is also a way of life. Muslim should consume halal products not only for the sake of pleasure but also as part of performing duties and responsibilities and mission in this world.

In the process of reaching a developed country by 2020, Malaysia as an Islamic country aims to be one of the largest world halal hub. Besides becoming an industrial nation that caters for its own market, the halal industry is aiming to supply halal consumables to the global consumers. This initiative is not only meant for food industry players but the members of the authorities and financial supports must give their commitment as what the nations has planned in the Third Industrial Master Plan (IMP3) (Ambali & Bakar, 2013).

In 2013, The Dubai Chamber of Commerce estimated the value of halal food consumer purchases in the global industry is about US$1.1 trillion. The market value of the halal product in the regions like Indonesia and Turkey in 2012 are worth up to $197 million and $100 million, respectively. It shows that the increasing needs of halal product throughout the world. Even the European Union market for halal food increased by 15% and is worth an estimated US$30 billion. This could be due to the awareness of consumers towards halal products through the halal certification by producing countries (Buang & Mahmod, 2012).

Hence, halal authentication on food is deemed very significant and important for the halal food industry. In meeting the needs of society for safe and good quality foods in a sustainable way, science and technology have always played a vital role in the food industries. People are more aware about the food that they consume, the ingredients as well as the process of preparing the end products. The term from farm to table deem the whole chain of halalan toyyiban concepts. Due to that, the food that we eat, had undergone many level of production and quality control processes before distributed to all over the world. People tend to be more particular and raise their curiosity about the processed food products whether it contains any haram substances. The demand for transparency in the food industries has increased which brings the enhancement of the analytical methods of halal food ingredients (Che Man et al., 2007).

In some cases, the origin of some of the ingredients and the compositions, which might come from non-halal sources is not clearly stated on the food label. Thus, certain extent of protection is needed for the consumer especially the Muslim consumer to ensure the food ingredients are stated clearly and precisely (Eliasi, 2002). The support for the validity and accuracy of food labelling are needed to provide the development of analytical techniques, such as the halal analysis of food ingredients. Halal analysis on food has been performed in many types of foods namely cake (Che Man et al., 2005), chocolate (Che Man et al., 2005) biscuits (Che Man et al., 2011), meat (Al-Jowder et al., 1997), ghee and butter (Farag et al., 1983) and

vegetable oils (Marikkar et al., 2005). Several analytical techniques have been employed in halal analysis using Polymerase Chains Reaction technique (PCR), Gas Chromatography-Mass Spectrometry (GCMS), Fourier Transform Infrared Spectroscopy (FTIR), Liquid Chromatography-Mass Spectrometry (LCMS), High Pressure Liquid Chromatography (HPLC), Differential Scanning Calorimetry (DSC) and Random Amplified Polymorphic DNA (RAPD) techniques.

In this study, the halal analysis screening process of few food products were performed using Polymerase Chains Reaction technique (PCR). This PCR techniques which is based on porcine DNA analysis, is highly sensitive, reliable and valid to judge the result.

MATERIALS AND METHODS

Sample Preparation

Three groups of samples were collected from local supermarket in Kota Samarahan area as shown in the following Table 1 below.

Table 1. Category of samples

Category of Sample	Number of Sample
Processed Meat (Burger patty)	6
Sweet and Candy	6
Pet Food	9

Preparation of Samples and Controls

25 mg of each sample was weighed and carefully placed into the labelled 1.5 mL Eppendorf tube separately. 100 µL of ultra-high quality water was used as the negative control while 25 mg of porcine based product was used as positive control.

DNA Extraction

The extraction conducted according to manufacturer's protocol (QIAGEN-QIAamp DNA Mini Kit). The protocol shall be 100 µL of ATL buffer was added into each sample and control. The samples and controls were then gently mixed well before left in a room temperature for 30 min. 20 µL proteinase K was used and briefly mixed the sample in a vortex with the enzyme at 56°C for 3 h. The action of enzyme was optimized by incubating the mixture in water bath and then was put in vortex three times per hour for dispersion of samples. The samples were centrifuged for one min at 2000 rpm. 100 µL buffer AL was then added into the samples and was put in vortex for 15 s. After that, the samples and controls were left in a room temperature for 30 min before being placed into an oven at 70°C for 10 min. 200 µL of 95% ethanol was added into the sample and was put in vortex again for 15 s before briefly centrifuged for one min at 2000 rpm. The samples and controls were then transferred into a QIAamp Mini spin column without wetting the rim as an act of caution. The samples and controls were centrifuged at 8000 rpm for one min after closing the lid. The spin columns were transferred into clean collection tubes. The QIAamp Mini spin columns were open carefully to add 250 µL Buffer AW1 and centrifuged for one min at 8000 rpm. While the filtrates discarding, the QIAamp Mini spin column placing in a clean 2 mL collection tube was repeated and 250 µL Buffer AW2 was added. The mixtures and controls were centrifuged at full speed of 14,000 rpm for three min. The QIAamp Mini spin columns were placed again in another new 2 mL collection tube and centrifuged at full speed of 13,000 to 14,000 rpm for 1 min. The extracted DNA from samples and controls were then dried in oven at 70˚C for 5 min. The collection tubes were replaced by clean 1.5 mL micro-centrifuge tubes and were added with 200 µL Buffer AE. The samples and

controls were incubated for 1 min at room temperature and the centrifugation was conducted for one minute towards the samples and controls at 8000 rpm.

PCR Amplification

The DNA amplification was performed based on the method used by Farouk et al. (2006); In a 25 µL reaction, 1 µL of eluted DNA, 2.5 µL 10x PCR buffer containing 1.5 mM Mg^{2+}, 0.25 µL each of forward and reverse primers (20 mM), 5 µL 5x Q-Buffer, 15.25 µL UHP water, 0.5 µL dNTPs mix (10 mM) and 0.25 µL Taq DNA polymerase (Vivantis) were used. The forward primer followed the sequence of 5'-TGCAGTCTGTCTCCTCCTCCAAA-3' while the reverse primer was 5'-CGATAATTGGATCACATTTCTG-3'. The PCR cycle was programmed according to the following conditions: 95˚C for 15 min incubation for the activation of taq DNA polymerase, then 30 cycles of 95°C for 1 min, followed by 55°C for 1 min, 72°C for 1 min, and lastly 72°C for 10 min using Veriti 96 well Thermal Cycler.

PCR Electrophoresis

The PCR samples were then run in a 1.5% agarose gel in 1x TBE buffer stained with Ethidium Bromide (EtBr) using electrophoresis equipment at a voltage of 150 V. By using Bio-Rad Gel Doc 1000 gel documentation system, the results were then visualized and photographed. The molecular marker used was 100 bp ladder.

RESULTS AND DISCUSSION

The results were visualized on the gel image with the 100bp ladder provide the size reference information about the amplification of the targeted DNA. The amplification size should be approximately 152 bp. Table 2 showed the result from all of the samples and Figure 1 shows the electrophoresis gel image.

Table 2. Results PCR for three categories of samples

Category of Sample	Sample ID	Type of samples	Result (+/-)
Processed Meat (Burger)	B1	Beef	+
	B2	Beef	+
	B3	Beef	+
	B4	Beef	+
	B5	Beef	+
	B6	Lamb	+
Sweet and Candy	S1	Local; without halal logo	-
	S2	Imported; with halal logo	-
	S3	Imported; with halal logo	-
	S4	Local; with halal logo	-
	S5	Local; with halal logo	-
	S6	Imported; without halal logo	-
Pet Food	PF1	Fish food	-
	PF2	Fish food	-
	PF3	Cat Food	-
	PF4	Fish food	-
	PF5	Cat Food	+
	PF6	Bird Feed	-
	PF7	Bird Feed	-
	PF8	Cat Food	-
	PF9	Bird Feed	-

This conventional PCR technique was successfully performed as the expected band of the positive control is emerged while there is no band appeared for the negative control. The

visualization on the gel image (Figure 1) showed all the samples have the bands at around 152 bp ladder, as also indicate by the positive control.

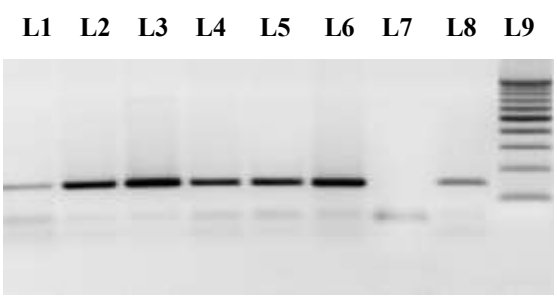

Figure 1a: Electrophoresis image for processed meat

L=Lane; L1; B1, L2: B2, L3; B3, L4; B4, L5; B5, L6; B6, L7; Negative Control, L8; Positive Control and L9; marker 152bp.

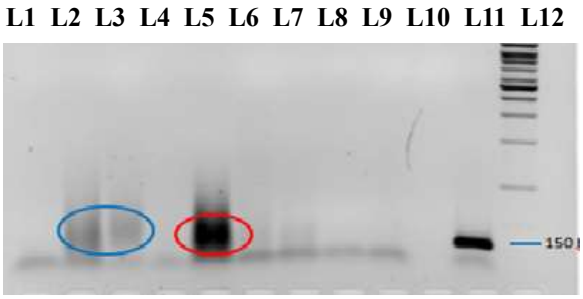

Figure 1b: Electrophoresis image for pet food

L=Lane; L1; PF1, L2: PF, L3; PF3, L4; PF4, L5; PF5, L6; PF6, L7; PF7; L8; PF8, L9; PF 9, L10; Negative Control, L11; Positive Control and L12; marker.

Figure 1. Electrophoresis gel image

CONCLUSION

The findings indicated that the tested food samples are suspected to contain porcine based material as the ingredients of the sample. Analysis of porcine contaminant food products without the Halal logo or 'pork/porcine free' on the ingredients list which obtained from the local market and retail stores could be confirmed by using the PCR-based method because the method is proposed to be a reliable and sensitive protocol for routine analysis. In addition, the suspected band (Figure 1b) in circle required further investigation. In conclusion, further study is utmost needed using the HPLC or LCMS spectroscopic methods to confirm the results for this PCR analysis.

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