# Detection of Pork in Canned Meat using TaqMan Real-time PCR

Nihad A.M. Al-Rashedi , Emad Uldeen Hateem
[1,2] *Biology Department , College of Science , Muthanna University*
*Received 20/4/2016, Accepted: 21/5/2016, Published 30/10/2016*

[*] *Corresponding Author: nihadalrashedi1@gmail.com*

**Abstract:** This study was aimed to detection of pork contamination in commercial canned meat product in Iraq market using real-time PCR method through analysis of mitochondrial cytochrome *b* gene. Method based on porcine-specific primers and TaqMan probe were designed to amplify 130 bp for assessing pork adulteration in canned meat. Testing the twenty samples of local market that show no pork detection to have pork contamination in nineteen meat cans. In conclusion, specific PCR amplification of cytochrome b gene is useful in detection of hidden pork meat mince in processed foods because of its rapid, specificity and sensitivity. This method will ensure that trust of the customers with these products.



***Keywords***: Real-time PCR; cytochrome *b*; canned meat; pork.

## Introduction

The Detection of the source of meat is a critical and important issue in food products especially in muslim populations which are considered the food safety related to health and hygienic a *halal* authentication. In this incurrent study, the cytochrome b region of the mitochondrial DNA (mtDNA) was selected to be tackled to detect each meat sample. The advantage of mitochondrial DNA-real time PCR analysis derives from the high copy number of mtDNA in cells [1].

The methods are already used routinely depended on fatty acid metabolites or species specific proteins for identification of meat species, so that difficult predict to components of samples because exposed to high temperature and others processes prior to prove examination [2], in contrast to DNA tests are durable components to such these processes.

## Materials and Methods

The sample preparation and DNA extraction of meat cans that were randomly collected from shops in different regions of Iraq. Preparation of 50 mg of canned meat was blended using a disposable homogenization pestle and placed in a 1.5 ml microcentrifuge tube. DNA was extracted from canned meat samples using food and feed DNA extraction kit (USbiological, USA) are designed for the rapid isolation of highly pure genomic DNA from feed and food. The meat samples were used for the investigation of the pork containing meat that collected from market included in Table(1). The resulting DNA extraction was DNA suspended in TE buffer and stored at −20°C for further amplification process.

Real-time PCR of target in cytochrome *b* was performed triplicate on 96-well plate using Agilent Mx3000p qPCR system. The final volume of PCR reaction was 20 µl final volume included of 2.5 µl of DNA template, 12.5 µl of kappa probe fast master mix, 1.6 µl of 10 µM forward and reverse primer, 0.2 µl of 10 µM probe and 0.4 µl of low Rox dye.

The set of primers and probe consisted of Cyt b-forward and reverse oligonucleotides [3] as follows:

Probe 5-(FAM)-AAC ATC AGG ATT TTT GCT GCA TTT GC-(TAMRA)-3
Forward primer 5-CAC AGC AAG CCC CTT AGC CC-(TAMRA)-3
Reversed primer 5-CCC ATG AAA GAC GGT ACA AGG TAT ACT G—3

**Table (1): The meat samples were collected from the Iraqi markets**

| No. | Product name | Product type | components of meat | Manufacture company | Origin |
|---|---|---|---|---|---|
| 1 | SPAM Lite | barbecue kebabs | 68% pork 32% Chicken | Hormel Food corporation | USA |
| 2 | Bavaria | Chopped meat | beef and chicken [1] | Technical Food Industries company | UAE |
| 3 | Ghadeer | Luncheon beef meat | beef[1] | Jordanian Advanced for Food Industry | Jordan |
| 4 | AlBassateen | Corned beef Loaf | beef [1] | Technical Food Industries company | UAE |
| 5 | Bodroon | corned beef meat | beef [1] | Technical Food Industries company | UAE |
| 6 | Kawthar Al Diar | Luncheon beef meat | beef[1] | Jordanian Advanced for Food Industry | Jordan |
| 7 | Amani | Chicken luncheon meat | chicken [1] | special packed for Sameer Nouri Mahmoud Al-Janabi | Brazil for Iraq |
| 8 | Bravo | Chicken luncheon meat | chicken 81% | National Poultry company | Joradan |
| 9 | Al-Mara'i | luncheon meat | Chicken[1] and beef | Amana Food Industries | Jordan |
| 10 | AlTaghziah | Chicken luncheon meat | chicken 80% plant 18% | Al-Taghziah company | Lebanon |
| 11 | AlTaghziah | Beef luncheon meat | beef 70% chicken 10% | Al-Taghziah company | Lebanon |
| 12 | Americana | Chicken luncheon meat | chicken 82% plant 18% | Interbrands cairo | Egypt |
| 13 | Baider | Beef Luncheon | Beef[1] | Al-Faris Al Arabi Trading company | Kingdom of Saudi Arabia |
| 14 | Baider | Chicken Luncheon | Chicken[1] | Al-Faris Al Arabi Trading company | Kingdom of Saudi Arabia |
| 15 | Kalleh | Chicken frank | Chicken[1] | Kalleh amol | Iran |
| 16 | Hana | Beef Luncheon meat | Beef[1] | Nile Group for Trading company | Egypt |
| 17 | Hana | Luncheon meat | Chicken[1] | Nile Group for Trading company | Egypt |
| 18 | Hana | Mortadella | beef[1] | Nile Group for Trading company | Egypt |
| 19 | Bavaria | Corned meat | beef and | Technical Food | UAE for |

| | | | | | |
|---|---|---|---|---|---|
| | | hash | plant [1] | Industries company | Iraq |
| 20 | Bavaria | Beef Luncheon meat | beef[1] | Technical Food Industries company | UAE for Iraq |

[1] It did not specify the proportion of meat component in the can.

The amplification was performed according to the thermal curve of PCR: enzyme activation at 95 °*C* for 3 min, followed by 40 cycles of start at 95°C for 3 seconds and 60 °C for 20 seconds.

This research applied and evaluated a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) using cytochromegene to detect pork contamination in meatballs from local markets in Surabaya and Yogyakarta regions, Indonesia. To confirm the effectiveness and specificity of this fragment, thirty nine DNA samples from different meatball shops were isolated and amplified, and then the PCR amplicon was digested byrestriction enzyme to detect the presence of pork in meatballs.restriction enzyme was able to cleave porcine cytochromegene into two fragments (131 bp and 228 bp). Testing the meatballs from the local market showed that nine of twenty meatball shops in Yogyakarta region were detected to have pork contamination, but there was no pork contamination in meatball shops in Surabaya region. In conclusion, specific PCR amplification of cytochrome b gen and cleaved byrestriction enzymes seems to be a powerful technique for the identification of pork presence in meatball because of its simplicity, specificity and sensitivity. Furthermore, pork contamination intended for commercial products of sausage, nugget, steak and meat burger can be checked. The procedure is also much cheaper than other methods based on PCR, immunodiffusion and other techniques that need expensive equipment.

**Result and Discussion**

The consumption of meat in developing countries is increased with time and which required to protection from falsely label food for religious and health reasons. So, there is demand to provide a system to detect contamination of pork because some contamination was mixed with cattle.

The present study aimed to apply real-time PCR method using cytochrome *b* gene that has already confirmed for pork detection in meat cans products in Iraqi local markets (4).

Genomic meat DNA was extracted for template of amplification by real-time PCR with the specific primers and Taq-MAN probe. The extraction genomic DNA from the twenty local meat cans samples showed the bands in electrophoresis system of genomic DNA from meat cans of Iraq local market as shown in figure (1).

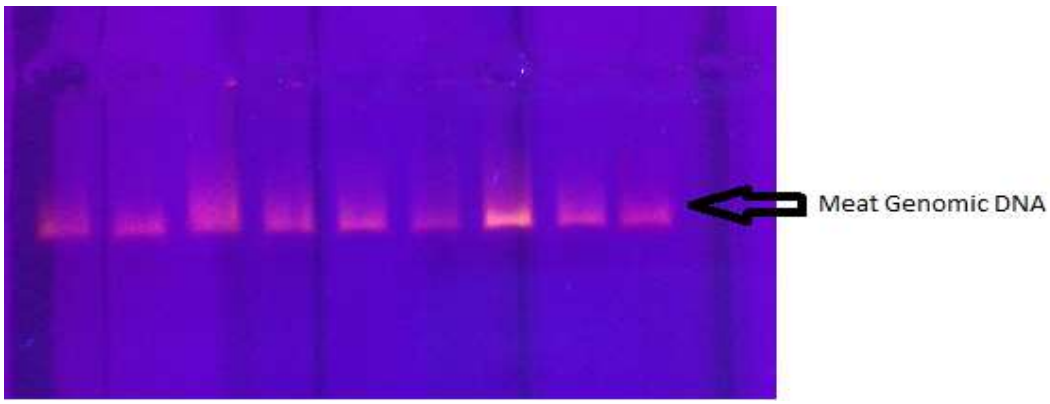


**Fig. (1): Agarose gel electrophoresis of DNA extraction of meat samples**

There are several different methods are currently used for detection of pork when is subject to authentication of meat species, usually depend on detecting properties of proteins or immunological assays. These methods were faced drawbacks in cooking temperature prior to the investigation.

Real-time PCR of species-specific mitochondrial DNA sequences is best choosing for identification of meat species and candidate to be the routine investigation and quality control for pork detection in meat products because of real-time PCR method has potential sensitivity and specificity to targeted the template DNA sequence, and addition to rapid, easy and allows the detection of several species at the same run (1). In this study is ideal for amplified the small target DNA sequence 131 bp on cytochrome *b* gene by real-time PCR from a meat cans to ensure the amplification of DNA target where genome is commonly degraded [4].

Pork meat of Hormel Food trade mak as standard of real-time amplification 131 bp fragment in sample 1 and other samples of different trade marks from sample 2 to 20 showed negative for beef and /or chicken of amplification. The aim of this study was fast, simple and sensitive, reliable method for detection of pork in meat cans from different manufacturing sources of Iraq market by real-time PCR, the primer pair and probe determined for the cytochrome b gene amplifies a fragment of 131 base pairs length as shown in figure (2). The mitochondrial DNA is maternally inherited which present in highly redundant about thousands of copies per cell (Greenwood and Paboo, 1999) as against single copy of genomic DNA. Thus, making it increases the probability of finding any trace of interested DNA fragment and severe manufactured conditions of meat [5]. Some studies that details mitochondrial DNA markers are more efficient than nuclear markers for the identification and authentication of meat species [6].

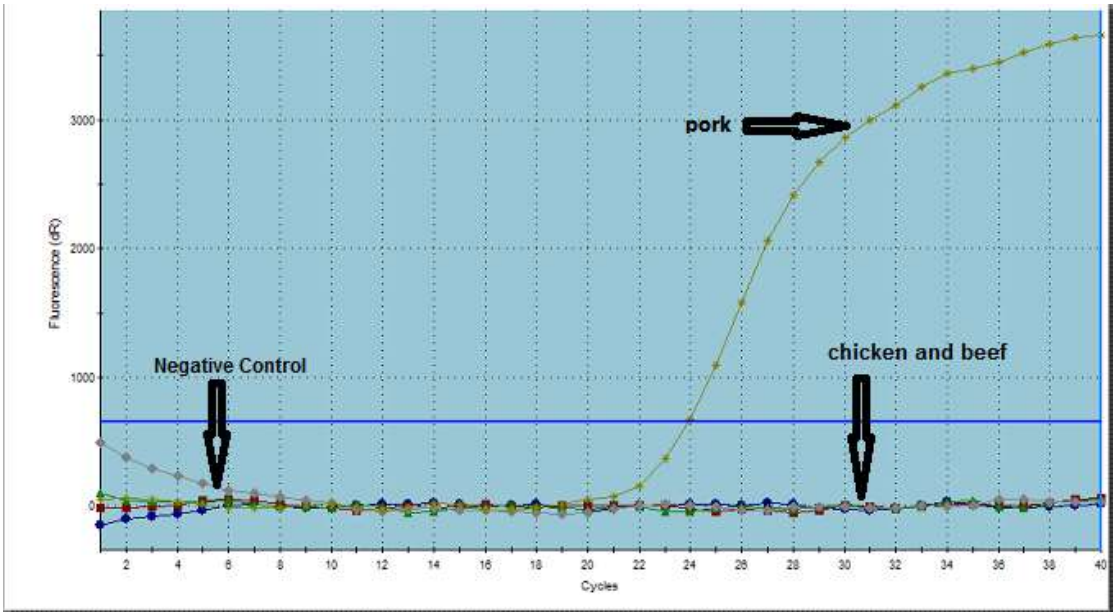


**Fig. (2): Pork as standard of real-time amplification 131 bp fragment in sample 1, other samples 2-20 showed negative [beef and chicken) of amplification and negative control of amplification**

**Conclusion:** In the real-time PCR, the probe and primer pair of cytochrome *b* gene has amplifie a fragment of 131 bp length in it is possible to detect the obvious presence of pork.

**References**

[1] Kesmen, Z.; Gulluce, A.; Sahin, F. and Yetim, H. (2009). Identification of meat species by TaqMan based real-time PCR assay. Meat Sci.; 82: 444-449.

[2] Chen, E-C and Hsieh, Y-HP (2000). Detection of pork in heat processed meat products by monoclonal antibody-based ELISA. J AOAC Intl, 83(1): 79-85.

[3] Tanabe, S.; Hase, M.; Yano, T.; Sato, M.; Fujimura, T. and 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.

[4] Edris, S.; Mutwakil, M.H.Z.; Abuzinadah, O.A.; Mohammed, H.E.; Ramadan, A. *et al*. (2012) Conventional multiplex polymerase chain reaction (PCR) versus real-time PCR for species-specific meat authentication. Life Science Journal; 9(4):5833-5837.

[5] Bellagamba, F.; Moreti, VM; Cominicini, 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 Agriculture and Food Chemistry 49:3775-3781.

[6] Rastogi, G.; Dharne, MS; Walujkar , S.; Kumar; A.; Patole, MS and Shouche YS (2007). Species identification and authentication of tissues of animal origin using mitochondrial and nuclear markers. Meat Sci, 76: 666-674.