Detection of Pork in Canned Meat Products by using DNA-based Methods

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Abstract

Adulteration of meat products became a matter of great concerns of religious, economical, legal and hygienic aspects. Canned meat is one of the most favorable in a lot of countries, which makes it prone to adulteration. The objective of the current study was to identify pork in canned meat for the presence of adulteration in commercial market of Basrah city/Iraq. Thirty canned meat were collected from commercial market. The possibility of a species mixture was tested with polymerase chain reaction (PCR), targeting pork (290bp). Analysis of canned meat revealed negativity results of all samples to pork meat. In conclusion, samples analysed in the current study showed that there was no adulteration by mixing pork meat in canned meat products due to absent production of pork flesh for religion and hygienic aspects. Beef and mutton flesh might be replaced in chicken, horse, and donkey flesh for economic reason.

Key words: *Canned meat, fraud, meat species identification, mislabeling, PCR.*

Introduction

Meat is the muscle tissue of an animal that is consumed as food. It is composed of high amount of water (75%), protein (20%), fat (5%) and small amount of carbohydrates, vitamins, and minerals [1]. Meat products are considered a favorite item in a lot of countries and due to the consumption of meat products continues to elevate, mislabeling and adulteration of meat products have become common [2]. Consumers usually rely on food labeling to make right choices for religious and public health reasons [3]. The success this kind of adulteration in processed meat is invisible changes in the appearance, color, texture, and flavor of the processed meats. Under food labeling regulations, adulteration by mixing or replacement undeclared species in meat products is illegal. Recently, detecting the meat species in meat product is considerable importance issue to ensure the food safety for public health [4].

Methods have been used to detect species of meat are based on either protein or DNA detections. The protein detection techniques are unable to identify between close relatives species. In addition, these techniques are required complicated isolation procedure and time consuming [5]. However, DNA detection technique is considered as reliable, efficient, simple and a quick method to identify meat species [6]. In previous studies, testing processed of meat products by DNA detection technique revealed the use of a label that is incorrect [(7)]. Since becoming aware of these issues, this work aimed to identify pork in canned meat products by detection the meat specie in it under laboratory conditions.

Materials and Method

2.1 Sample collection

A total of 30 sample (3 canned meats from each product) were collected from various commercial markets in Basrah city/ Iraq. These samples including products labeled as beef luncheon meat(Baidar, Kingdom of Saudi Arabia), beef luncheon meat(Hena, United Arab Emirates), beef luncheon meat (Ghadeer, Jordan), corned beef loaf(Burdon, Brazil), beef hot dog (AlTaghziah, Lebanon) ,beef hot dog(Al Qaisar, Kingdom of Saudi Arabia),chicken luncheon meat (Hena, United Arab Emirates),chicken luncheon meat (Baidar, Kingdom of Saudi Arabia),chicken luncheon meat(Alatyab, Turkey),and chicken hot dog(AlTaghziah, Lebanon). Following collection, samples were kept at room temperature until analysed. The collected samples were marked numerically (Table 1).

Table 1: Canned meat products analysed for authentication

Sample ID	Product label	Trademark	Source
1	Beef Luncheon Meat	Baidar	Kingdom of Saudi Arabia
2	Beef Luncheon Meat	Hena	United Arab Emirates
3	Beef Luncheon Meat	Ghadeer	Jordan
4	Corned Beef Loaf	Burdon	Brazil
5	Beef Hot Dog	AlTaghziah	Lebanon
6	Beef Hot Dog	Al Qaisar	Kingdom of Saudi Arabia
7	Chicken Luncheon Meat	Hena	United Arab Emirates
8	Chicken Luncheon Meat	Baidar	Kingdom of Saudi Arabia
9	Chicken Luncheon Meat	Alatyab	Turkey
10	Chicken Hot Dog	AlTaghziah	Lebanon

2.2 Sample Preparation for DNA extraction

The sample (2 g) was aseptically collected using sterile forceps, cut using a sterile scalpel, and mixed thoroughly with distal water (60 ml) in a blender (230 rpm for 120 sec) to homogenize (Figure 1). The sample was then transferred into two microcentrifuge tube (1.5 ml). To prevent DNA degradation, samples stored at -20 °C until analysis [8].

2.3 DNA extraction and detection

DNA extraction was carried out for all canned meat samples in triplicate using the DNA extraction kit GsyncTMDNA (Geneaid Biotech Ltd., Taiwan). The extraction procedure was done according to the manufacturer's instruction. Briefly, the tissue sample (25 mg) was lysed with ATL buffer (200 µL) and Proteinase K (20 µl), vortexed at 30 min, and incubated overnight at 60 °C. Then, GSB buffer (200 µl) and absolute ethanol (200 µl) was added and vortexed (10 sec). The sample was then transferred to GC columns, washed with W1 buffer (400 µl), centrifuged (14,000 xg for 30 sec), followed by second washed with W2 buffer (600 µl), centrifuged (16,000 xg for 30 sec), and discard the flow through. To dry the column matrix, the GC column centrifuged at 16,000 xg for 3 min. After that, preheated elution buffer (100 µl) was added, stand at room temperature for 3 min to allow elution buffer, and finally centrifuged (16,000 xg for 30 sec) to elute purified DNA. DNA quantity was determined by using a Nano-Drop 2000 spectrophotometer (Nano Drop

Technologies, Wilmington, USA)[9].

2.4 PCR primers and amplification

The primers described in previous study were used to amplify a 12S rRNA region

(10)(Table 2).

Table 2: Oligonucleotide Primer sequence and target fragment for PCR assay

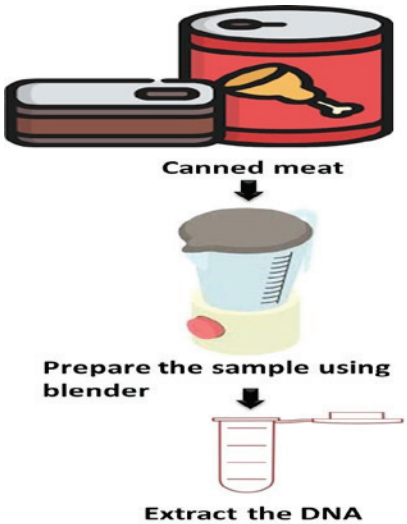
Species	primer sequence	Target fragment
Pork	5'-CTACATAAGAATATCCACCACA-3' 5'-ACATTGTGGGATCTTCTAGGT-3'	290 bp
Chicken	5'-TGAGAACTACGAGCACAAAC-3'	183 bp

2.5 Polymerase Chain Reaction (PCR) assay for gene amplification

DNA from the samples was amplified in a total 25 µL reaction volume containing genomic DNA (1µg) of each specie, primers (1µM), $Mgcl_2$ (2mM), dNTP (0.2mM), PCR buffer (2.5µL of 10X) and the enzyme Taq DNA polymerase (1unit). The Polymerase chain reaction assay conditions were performed by thermal cycler as follows: initial denaturation step (94°C for 4 min) followed by 30 cycles of: denaturation step (94◦C for 30 sec), annealing step (57-64°C for 30 sec), extension step (72°C for 30 sec), and a final elongation (72◦C for 30 sec))[10].

2.6 PCR products detection

The PCR production was detected on agarose gels (1.5%) prepared with agarose in Tris-borate-EDTA buffer (1x) at 100 V for 30 min, stained with a fluorescent stain (Ethidium Bromide) and images by gel- documentation systems (UVIDOC UK). The size of the band was determined by comparison with a standard DNA ladder [10].



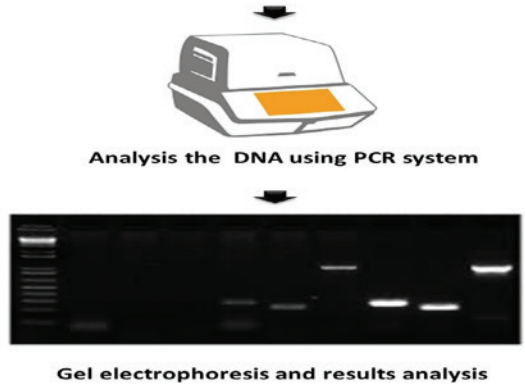


Figure 1: Testing canned meat for detected animal DNA

Results and discussion

Under food labeling regulations, adulteration by mixing undeclared species in meat products is illegal. Recently, detecting the meat species in meat product is important issue for public health. A target fragment of pork (290 bp) was amplified. PCR analysis of canned meat revealed negativity results of all samples for regarding to pork meat (Table 3)(Figure 2). This find is in agreement with previous studies, in which there was no adulteration by mixing pork meat in meat products [10, 11, 12]. It has been found that pork meat replacement in some countries is rarely due to absent production of pork flesh for halal food status. However, the tendency to mix pork meat and fat into the processed meats is more frequent in other countries due to high production of pork flesh and its cheapness. The previous study found that meat products that were declared as buffalo contained pork [7, 13]. In addition, beef and mutton meat might replace in chicken, horse, and donkey meat. In Turkey, meat products (uncooked beef burger, kofta, sausage, and luncheon) that were declared as beef contained poultry and donkey meat [10]. In addition, raw meat that was declared as beef contained mix of horse and deer meat

[11]. In china, meat products that were declared as buffalo contained cattle, pork, and duck meat [7]. In Iran, raw burgers that were declared as beef contained poultry [14]. One of the reasons for the replacement of chicken, horse, and donkey flesh in beef and mutton flesh is economic. The success of this kind of adulteration in processed meats is invisible changes in the visual inspection (appearance, color, texture, and flavor) of the processed meats [4].

Table 3: PCR results for canned meat samples

Sample ID	Product label	Adulteration ingredients PCR results (Pork)
1	Beef Luncheon Meat/ Baidar	-
2	Beef Luncheon Meat/ Hena	-
3	Beef Luncheon Meat/ Ghadeer	-
4	Corned Beef Loaf/ Burdon	-
5	Beef Hot Dog/ AlTaghziah	-
6	Beef Hot Dog/ Al Qaisar	-
7	Chicken Luncheon Meat/Hena	-
8	Chicken Luncheon Meat/ Baidar	-
9	Chicken Luncheon Meat/ Alatyab	-
10	Chicken Hot Dog/ AlTaghziah	-

Note: (+) denotes for presence and (-)stands absence

Figure 2: PCR product with 290 bp using pork specific primer in 1.5% agarose gel electrophoresis. PCR analysis showing the negativity result of all samples for regarding to pork meat (L1- L10). M: molecular marker (100 bp ladder); lane 1: beef luncheon meat(Baidar, Kingdom of Saudi Arabia) ;lane 2:beef luncheon meat(Hena, United Arab Emirates);lane 3: beef luncheon meat (Ghadeer, Jordan); lane 4: corned beef loaf(Burdon, Brazil);lane 5: beef hot dog(AlTaghziah, Lebanon); lane 6: beef hot dog(Al Qaisar, Kingdom of Saudi Arabia); lane 7: chicken luncheon meat(Hena, United Arab Emirates); lane 8: chicken luncheon meat(Baidar, Kingdom of Saudi Arabia); lane 9: chicken luncheon meat(Alatyab, Turkey); lane 10: chicken hot dog(AlTaghziah, Lebanon).

Conclusion, samples analysed in the current study revealed that there was no adulteration by mixing pork meat in canned meat products. Pork meat replacement in some countries is rarely due to absent production of pork flesh for halal food status. However, Beef and mutton flesh (more expensive) might be replaced in chicken, horse, and donkey flesh (cheaper). To allow the consumer to make right choices when purchasing canned meat, canned meat must be frequently analysed using effective methods by governmental institutions.

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