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Molecular Assay for Fraud Identification of Handmade Hamburgers

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Background: Meat products could be sources of enter pathogens. Identification of meat species in different foods could help us in molecular epidemiological studies of pathogens transmitted by meat.
Objectives: In this study, we targeted cytochrome b for identification of beef in handmade hamburgers.
Patients and Methods: A total of 110 raw handmade hamburgers were collected from different areas of Yazd city, Iran, during spring of 2013. Genomic DNA was extracted using the salting out method. The beef cytochrome b gene was amplified using specific primers. Analysis of the amplicons was done with agarose gel electrophoresis usinga100 base pair (bp) DNA ladder.
Results: The results showed that among the 110 handmade hamburger samples, 10 (9.09%) samples did not containany cow meat while 100 samples contained cow meat.
Conclusions: We used an appropriate molecular method for controlling raw and processed products. Therefore, this study would be useful for control of correct labeling and protection of consumer's rights.

Keywords: Meat Products; Cytochromes b; Molecular Diagnostic Techniques

1. Background

Meat could be a source of enter pathogens such as E*nterococci, corynebacterium, Mycobacterium avium subspecies Para tuberculosis, Campylobacter, Clostridia, Coronavirus, Rotavirus, Torovirus, Calicivirus, Astrovirus, Canine parvovirus, Bovine virus, Diarrhea virus, Rinderpest virus*, Coccidia, *Cryptosporidium, Taenia saginata* and *Taenia solium*; the latter could be transmitted by pork. Identification of meat species in different foods is an appropriate action for the recent crisis in the meat sector (1) and could help us in molecular epidemiological studies of pathogens transmitted by meat. Therefore, uncovering of adulterated meat products is very important. Furthermore, identification of meat species is also important for allergic individuals and people with religious beliefs that specify allowable intake of certain species.

Nowadays, determination of meat species is mainly based on DNA methods such PCR based techniques and protein methods such as ELISA (2-5). High cooking temperature in food making process could change the structure of protein and this make narrow down the use of protein methods. However, DNA molecule has high thermal stability and a species specific sequence. Molecular techniques with target of genomic and mitochondrial genes are an appropriate method for species detection (6-10).

2. Objectives

In this study, we targeted cytochrome b for identification of beef in handmade hamburgers.

3. Materials and Methods

3.1. Sampling

A total of 110 raw handmade hamburgers were collected from west, east, north, south and center of Yazd city, Iran, during the spring of 2013. Each sample was cut into 5-mm slices and five slices from different parts of each sample were selected and mixed, and then preserved in 70% ethanol at -20°C for further studies.

3.2. DNA Extraction

Genomic DNA was extracted using the salting out method. Approximately 30 mg of each sample was suspended in 900 μLof buffer (50 mM NaCl, 25 mM EDTA, pH = 8.0, 50 mM Tris-HCL pH = 7.6), with 10 μL proteinase K (20 mg/mL), and SDS with an end concentration of 1%, followed

by rapid mixing and overnight incubation at 56°C. The purification of DNA was performed by adding 250 μL of 6 M NaCl. After centrifugation, the supernatant was transferred to a new sterile 1.5 mL micro tube. Next, DNA precipitation was performed, by adding double volume of chilled absolute ethanol. After washing with 70% ethanol, the DNA pellet was resuspended in 100μLof sterile double distilled water and preserved at -20°C until use for the next steps.

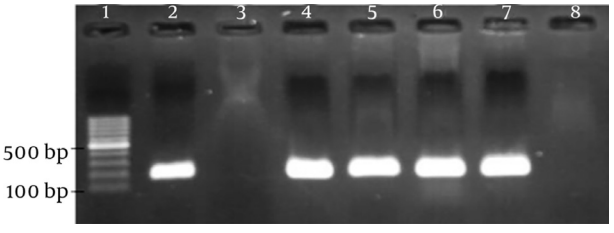
3.3. Detection and Identification

To ensure the presence of beef, the beef *cytocrome b* gene was amplified using specific primers;cytbF 5'-CTGCCTAATCCTACAAATCCTC-3' and *cytbR* 5'-CGTAATATAAGCCTCGTCCTAC-3'. Amplification was done using 1X PCR buffer, 1.5 mM $MgCl_2$, 0.2 mM of each dNTPs, 1 U of Taq DNA polymerase, 10 pmol of each primer and 100 ng genomic DNA as a template in a 25 μL PCR reaction mixture. The PCR assay was performed in VeriPlex (ABI). The cycling conditions included an initial denaturation at 94°C for five minutes, followed by 30 cycles at 94°C for 30 seconds, 58°C for 30seconds and 72°C for 30 seconds and final extension at 72°C for five minutes. This amplification was performed for both the negative and positive controls. Presence of an amplicon with a 197 bp size could verify the existence of beef products. Analysis of the amplification results was done by agarose gel electrophoresis alongside a100-bp DNA ladder.

4. Results

DNA extraction was performed with high quality and quantity. Amplification with designed specific primers showed an amplicon with a length of 197 bp (Figure 1). Results showed that among the 110 handmade hamburger samples, 10 (9.09%) samples did not have any cow meat while 100 samples had cow meat. The analysis of samples was performed using agarose gel electrophoresis alongside apositive and negative control.

Figure 1. Agarose Gel Electrophoresis



Lane1: 100-bp DNA ladder; lane2: positive control; lane3: negative control; lanes4-7: samples with cow meat; lane8: sample without any meat cow.

5. Discussion

The need for gathering data about the content of food products, especially meat products, is constantly increased. Thus, identifying meat species is an important goal for food inspection programs, underlined by the enforcement of community laws. This study describes a conventional PCR assay for detection of beef materials in handmade hamburgers. We used *cytochrome b* gene as a target to set up a PCR techniques for detection and identification of cow meat in hamburgers. The *cytochrome b* locus has been well characterized among different vertebrate groups (11). Such DNA-based methods have some advantages; DNA molecules are strong enough that will be maintenance during the food processing and therefore are good target for identification of different animal species, and higher thermal stability compared to proteins, while they are present in the majority of cells (12).

Some investigations have indicated the existence of mislabeled products such as adulteration of sausages labeled as ostrich that instead contained pork meat or highly processed meat products labeled as turkey, which had been adulterated by chicken (13). Our work showed that 9.09% of the products were not in agreement with their label. The present study like previous studies showed considerable adulteration in hand made hamburgers in Iran. The results of this study are useful for effective control of adulterated consumer products and fraud in foods comprised of meat, and violations of labeling requirements for meat products.

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