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Detection of Chicken Meat Adulteration in Raw Hamburger Using Polymerase Chain Reaction

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Abstract

Background: Detection of food adulteration is an important issue from aspects of food control and food regulation. This study aimed to detect adulteration of chicken meat in raw hamburger using species specific Polymerase Chain Reaction (PCR).
Methods: Raw hamburgers including 42 handmade and 48 industrial samples were collected from 90 restaurants and supermarkets. Following genomic DNA extraction from raw hamburgers which were claimed to be made of beef meat, PCR was performed to detect chicken (*Gallus gallus*) meat as an adulterant.
Results: The oligonucleotide primers amplified mitochondrial DNA sequences under selected conditions and revealed specific 183 and 300 base pair fragment for chicken and cattle, respectively. Results showed that 94.4% of all hamburgers, including 100% of handmade and 89.6% of industrial samples, contained undeclared chicken meat.
Conclusion: This high rate of undeclared chicken meat in hamburger samples may be related to mixing beef with cheaper parts of chicken. The outcomes of this study suggest that this method of detection can be applied by quality control laboratories and inspection services to determine adulteration in different kinds of meat products.

Introduction

"Hamburger" is a popular meat product consumed by many people all over the world which is prepared from ground red meat, particularly beef as raw material. However, some other undeclared types of meat may also be substituted as adulterants. Iranian hamburgers are categorized into two groups of handmade and industrial ones. National Standard of Iran classifies industrial hamburgers into three categories based on their beef content, 30%, 60-74% and 75-95% (ISIRI, 2007). According to Iranian Food and Drug Organization, manufacturers are obliged to inform consumers about the used raw ingredients content, but the kind and the quantity of meat are not clear in the case of handmade hamburgers. These are prepared and sold in fast food restaurants and no considerable action has been applied to control their safety (Hajimohammadi et al., 2014).

The meat used in raw hamburger is exposed to severe morphological changes due to grinding operation. Such condition increases the possibility of fraudulent activities by some producers regarding to economical point of view. Earlier studies have widely reported the fraudulent substitution of cheaper meat for more expensive one. In a study, the analysis of 100 samples of meat and meat products showed that 22% of samples contained undeclared meats in which chicken meat were substituted for beef more than other meat species (Ayaz et al., 2006).

By regard to the above facts and the high demand for more

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transparency in food industry, it is critical to develop precise and reliable methods intended to control the species origin of meat used in hamburgers. Different analytical techniques based on protein analysis have been applied for meat fraud identifications which are time consuming, expensive and not specific enough. In comparison, DNA based methods are fast, inexpensive and more reliable (Girish et al., 2005; Jiaqin et al., 2008; Yin et al., 2009). Polymerase Chain Reaction (PCR) technique has been used for specific identification of chicken (*Gallus gallus*) adulteration in different meat products (Dalmasso et al., 2004; Ghovvati et al., 2009; Mane et al., 2009).

The objective of this study was applying PCR method as a sensitive and specific tool to detect chicken adulteration in raw hamburger samples sold in restaurants and supermarkets.

Materials and methods

Sample preparation

Raw hamburgers including 42 handmade and 48 industrial samples were randomly collected from 90 restaurants and supermarkets of Iran. Also, one sample of fresh raw chicken meat was provided from a certified butchery to be used as positive control. All samples were transported to the laboratory under refrigeration, and were immediately processed or stored frozen at -20 °C for the next steps.

DNA extraction

DNA extraction was carried out from 100 mg of hamburger samples based on the salting out extraction method (Aljanabi and Martinez, 1997; d'Angelo et al., 2007). This method includes the following steps: first, 400 µl of lysis buffer (10 mM Tris-HCl, pH 8.0; 2 mM EDTA, pH 8.0; 0.4 M NaCl) was added to each sample and mixed. Then, 40 µl of 20% SDS (Merck, 8220500100) and 20 µl of proteinase K (10 mg/ml, Merck, 1245680100) were added and mixed. Following incubation at 65 °C for 1 h, 300 µl of 6 M NaCl (Merck, 1064041000) was added to each sample, mixed for 30 s and then centrifuged at 10000 xg for 30 min. Afterwards, the supernatant was transferred to a new tube, an equal volume of isopropanol (Merck, 1096341000) was added and mixed. Samples were incubated at -20 °C for 1 h and were then centrifuged at 10000 xg for 20 min. The DNA pellet was washed with 70% ethanol (Merck, 1009832500), dried and redissolved in 100 µl of sterile deionized water.

Concentration and purity of extracted DNA

Following nucleic acid extraction, the concentration of DNA was measured by UV absorption at 260 nm using BioPhotometer plus apparatus (Eppendorf), and the purity of DNA was evaluated on the basis of absorbance ratio of 260 to 280 nm.

Oligonucleotide primers

Oligonucleotide primers were purchased as purified and desalted specimen from Eurofins. These primers were published by Dalmasso et al. (2004) and Kotowicz et al. (2007) for chicken and cattle, respectively. The primers were diluted to a final concentration of 10 µM with sterile double distilled water and stored at -20 °C for the next step of investigation. The sequences of oligonucleotide primers are given in Table 1.

Amplification

DNA extraction was followed by PCR protocols using DNA template of chicken and cattle in reaction with species specific oligonucleotide primers. Conventional PCR was carried out for the detection of *Gallus gallus* and *Bos taurus* using a thermocycler instrument (Eppendorf) in a final volume of 20 µl with the following reagent concentrations: 2 µl of 10 X PCR buffer (CinnaGen, CG7507C), 0.4 µl of dNTP (10 mM, CinnaGen, DN7603C), 0.6 µl $MgCl_2$ (50 mM, CinnaGen, TP7506C), 0.2 µl of Taq DNA polymerase (5 U/µl, CinnaGen, TA7506C), 1 µl of each forward and reverse primers (Eurofins MWG Operon), 100 ng of DNA as a template and nuclease free water to adjust the volume (CinnaGen, DW8505). In order to obtain reliable results, positive and negative controls were used for each set of primers during the PCR reactions.

Thermal cycler conditions were as follows: pre-incubation at 94 °C for 4 min followed by 35 cycles consisting of dsDNA denaturation at 94 °C for 30 s, primer annealing at 60 °C for 40 s; primer extension at 72 °C for 30 s; and then a final elongation at 72 °C for 5 min.

Sensitivity of PCR amplification

PCR assay was performed for different known mixtures of chicken meat in beef to detect the minimum quantity of chicken DNA and to verify the sensitivity of this method. The percentages of chicken meat are as 0.1, 0.5, 1, 2 and 5% (w/w). Cattle and chicken specific primer pairs were crossed checked with DNA segments of cat, donkey, sheep and pig to verify the specificity of the assay.

Agarose gel electrophoresis of PCR products

PCR products were analyzed using agarose gel electrophoresis. The 1.5% agarose (Merck, 1168010025) gel was made using 0.5 X Tris Borate EDTA (TBE) buffer (Sigma, 93309-1L) and 10 µg/ml of DNA Safe Stain (CinnaGen, PR881603) as gel visualizing agent. The condition was constant voltage at 100 V for 1 h and the PCR products were finally analyzed using UV transilluminator (Vilber lourmat).

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Results

Following DNA isolation from raw hamburger samples by the salt extraction method, spectrophotometric results revealed that the extracted DNA had high quality and purity. DNA concentrations were between 250-1200 µg/ml with the A260/A280 ratio ranging from 1.6 to 1.9.

Species specific primers used under the selected conditions amplified chicken and cattle genes with expected bands of 183 and 300 bp, respectively (Fig. 1 and Fig. 2). According to the mentioned figures, there isn't observed cross reaction of our interested primers with genomic DNA from sheep, pig, donkey and cat that confirm the high specificity of the assay.

The sensitivity of the applied method was tested in 0.1% chicken meat content in beef (Fig. 3). According to negative controls, no environmental contamination was detected.

Analysis of 90 raw handmade and industrial hamburgers verified the presence of bovine DNA in all samples as claimed by restaurants or labeled by manufacturers. All 42 handmade hamburger and 43 of 48 industrial hamburger samples contained chicken DNA (Table 2). Fig. 4 shows the presence of chicken DNA in some of the tested hamburger samples.

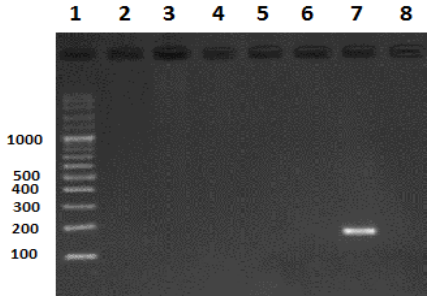

Fig. 1: Specificity of PCR assay of extracted DNA from different meat species with chicken primers. Lane 1: 100 bp DNA ladder; Lanes 2-6: extracted DNA of donkey, cat, pig, sheep and cattle, respectively; Lane 7: chicken DNA with the expected fragment of 183 bp; Lane 8: negative control

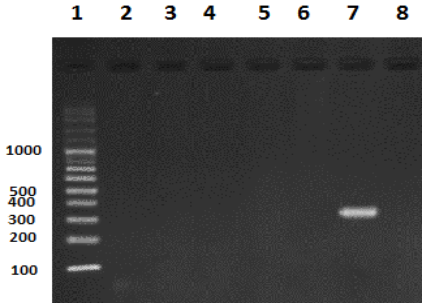

Fig. 2: Specificity of PCR assay of extracted DNA from different meat species with cattle primers. Lane 1: 100 bp DNA ladder; Lanes 2-6: extracted DNA of donkey, cat, pig, sheep and chicken, respectively; Lane 7: cattle DNA with the expected fragment of 300 bp; Lane 8: negative control

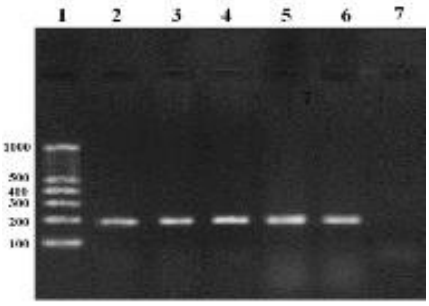

Fig. 3: Agarose gel electrophoresis of PCR product from different mixtures of chicken meat and beef; Lane 1: 100 bp DNA ladder; Lanes 2-6: chicken DNA in 0.1, 0.5, 1, 2, and 5%, respectively; Lane 7: negative control

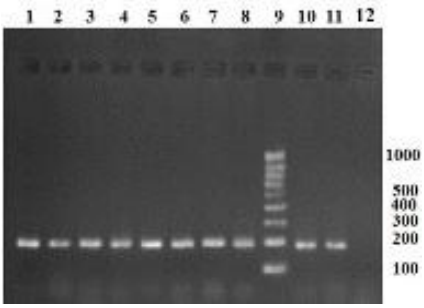

Fig. 4: Agarose gel electrophoresis of PCR product from hamburger samples; Lanes 1-8 and 10: chicken DNA (183 bp) in nine hamburger samples; Lane 9: 100 bp DNA ladder; Lanes 11 and 12: positive & negative controls, respectively

Table 1: Primers used in this study, PCR product length and annealing temperature

Species	Primer sequences 5'-- 3'	Amplicon length	Annealing temperature (°C)	Gene name
Cattle	F-CAATAACTCAACACAGAATTTGC R-CGTGATCTAATGGTAAGGAATA	300 bp	52	D-LOOP
Chicken	F-TGAGAACTACGAGCACAAAC R-GGGCTATTGAGCTCACTGTT	183 bp	60	12S rRNA

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Table 2: Rate of chicken meat adulteration in 90 raw hamburger samples

Hamburger type	Sample size	Samples contained beef No. (%)	Samples contained chicken meat No. (%)
Handmade	42	42 (100)	42 (100)
Industrial	48	48 (100)	43 (89.6)
Total	90	90 (100)	85 (94.4)

Discussion

Food safety and quality is a critical subject and meat species identity is a very important issue from health and regulatory aspects. Food adulteration is a legal term meaning noncompliance of food with health or safety standards. It is important that food control laboratories are able to detect animal species in meat products which may be substituted or mixed with other undeclared species. Since meat adulteration is of great importance from both economic and health point of view, the demands for development of accurate, rapid and inexpensive analysis methods are increasing.

Protein based techniques such as HPLC (Aristoy and Toldrá, 2004; Chou et al., 2007) and ELISA (Aslaminejad et al., 2010; Girish et al., 2005) have been established for food identification, but their detection limits are restrictive. These methods are mainly used for unprocessed foods and are not able to differentiate between closely related animal species. DNA based techniques are gaining popularity in meat species identification. Mitochondrial DNA (mtDNA) primers are considered suitable for this purpose due to its higher DNA stability and copy number (Aslaminejad et al., 2010; Ballin et al., 2009; Jia-qin et al., 2008). PCR techniques based on conserved mtDNA primers have been developed for species identification in foods as rapid and inexpensive methods (Girish et al., 2005).

It has been shown that species specific PCR technique described here is suitable enough for meat and meat products fraud identification. The results of this study showed that 85 raw hamburger samples (94.4%) contained chicken DNA. This method was also able to detect 0.1% of target species which confirms high sensitivity of species specific PCR technique for meat adulterant identification.

A considerable number of studies have been previously conducted on fraud identification of meat and meat products in which chicken meat was detected as undeclared meat. In a similar study, different meat samples were tested using multiplex PCR and the results demonstrated that 40% of the sausages and 30% of the cold cut samples contained chicken meat (Ghovvati et al., 2009). In a more recently study, a variety of DNA based methods including species specific PCR was used for meat identification of 14 animal species in a total of 139 processed meat products. Results revealed that 95 of 139 (68%) samples were contained non-declared species; chicken meat was found as one of the most commonly species in 23% of total samples (Cawthorn et al., 2013).

Quality of meat derived food products is an important issue and meat adulteration by means of mixing beef with chicken is a worldwide problem (Ayaz et al., 2006; Doosti et al., 2011). Primary reason for this type of adulteration is lower price of chicken meat compared with beef. Chicken waste products, called trimmings such as fat connective tissue, blood vessels, nerves, cartilage, sinew, bloody effluvia and even pieces of bone may be mixed with meat and used as adulterants. These waste products have lower nutritional value rather than meat. They may also be contaminated with food borne pathogens. Therefore, the probable presence of these pathogens due to insufficient cooking temperature in final products poses a potential health risk for consumers.

Conclusion

The outcome of this study showed suitability of PCR method for identification of meat fraud and clearly demonstrated the presence of chicken meat in hamburger. Considering high rate of undeclared chicken meat in hamburger samples, it is necessary to apply this technique by quality control laboratories for routine assessment of meat fraud in a rapid and reliable way.

Conflicts of interest

The authors declare no conflicts of interest.

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