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Molecular and histological investigation of adulterated ready-to-eat heated meat products with chicken substances

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Abstract

The likelihood substitution of low priced meat or tissues for that of high priced one has increased as the replacement of chicken meat instead of beef. So, the idea of this study were to find out this fraud by detection of chicken DNA and to discover foreign chicken tissue types histologically, especially in heated ready to eat (RTE) beef products. Fifty samples of ready to eat (RTE) Kofta and hawawshy (25 each) were collected in Assiut city. Polymerase chain reaction technique was applied on 12S rRNA gene for detection of chicken DNA in the collected samples. Out of the total examined kofta and hawawshy samples, 78% (39/50) of the examined samples were found to be adulterated with chicken DNA. Different foreign tissues were found histologically. The results of this research indicated that the quality of examined RTE meat products was very bad and therefore strict control on such products should be applied by appropriate authorities.

Introduction

High economic value of meat is one of the causes of adulteration of meat by substitution of low priced rather than high priced one, as the substitution of beef meat with chicken meat. Another explanation for the adulteration in processed meat products may occur through accidental contamination due to inappropriate handling or processing. As in case, if the meat processor is not appropriately cleansed prior to use in the next meat processing, minced meat can embrace the tiny amount of minced meat previously processed (Abd El-Nasser *et al.,* 2010).

Chicken meat might cause allergy to some people so it is required to detect trace quantity of the ingredient, especially in the heated products as it is very difficult to be recognized visually. Identifying the species of animal can be doubtful when the common species characteristics such as size, shape and appearance (the morphological characters) are removed in processing or heating (Latorre *et al*., 2015).

Fraudulent substitution of meat can involve both species and tissues. The inclusion of unwanted organs, such as the visceral organs, hyaline cartilage, skin, fat and bone of slaughtered animals as alternative meat substitute in heated meat products can be regarded as fraud.

Consumers are concerned with animal tissues, such as nervous tissues being contaminated with infectious agents. Moreover, addition of some plants in meat products might cause allergic response (Latorre *et al.*, 2015). Previously, some researchers described that histological methods have been successfully used to detect unwanted tissues in some meat products (Rezaian and Rokni, 2001; Prayson *et al*., 2008b). The advantages by using histological method are determination of food constituents, and subsequently the food quality can be evaluated (Latorre *et al*., 2015). The quality of meat and meat products is affected, especially by fat and connective tissue contents as well as muscle fiber characteristics (Joo *et al*., 2013).

Protein based techniques have been described to be inappropriate for species detection in heated meat products due to alteration of this substance by heating during food processing which result in changes in the antigenic structure of the molecules (Giovannacci *et al*., 2004). Therefore, DNA based method (PCR) has been developed for this purpose.

DNA based techniques have caused a change in species detection methods to replace the protein based techniques. The use of PCR in DNA analysis has been found to be simple, sensitive, specific and time saving method to detect the species of origin exposed to diverse processing conditions. Moreover, in contrast to proteins, DNA is more stable when intensive processing such as heating, pressures, or chemical processing is applied. PCR technique has a large detection power because it relies on the identification of specific sequence DNA segment of a definite tissue or animal (Rashid *et al*., 2014).

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Matsunaga *et al.* (1999) used the polymerase chain reaction (PCR) to identify, chicken as raw substance for products. A forward primer was planned on a conserved mitochondrial DNA sequence in the cytochrome-b gene. Identification is achievable by electrophoresis of PCR yield. Chicken fragment was designed for detection from cooked meat heated at 100 or 120°C for 30 mins. Haunshi *et al*. (2009) prepared specific primers for pigeon detection based on cyt b and species-specific markers for chicken, duck and pig D-loop mitochondrial genes that could recognize the previous species in fresh and processed meats. Mane *et al.* (2009) confirmed that no adverse effects of cooking and autoclaving were found on amplification of chicken DNA, when applied designed primer pair based on mitochondrial D-loop gene for specific detection of chicken meat, and production of 442 bp DNA fragments from fresh, processed and autoclaved meat and meat products.

The aim of the study was to evaluate the species-specific PCR assay for detection and identification of the chicken species in retail heated RTE meat products. Also, the detection of added chicken tissues or parts morphologically, as a confirmation of adulteration.

Materials and Methods

Collection of samples

Fifty samples of retailed heated Reatdy-to-Eat (RTE) kofta and hawawshy (25 each) were collected. The samples were stored at – 20°C till used for DNA extraction.

DNA extraction

The extraction was carried out according to the kit protocol (Wizard® Genomic DNA Purification Kit, Promega)

Primer

The primer sets used for detection of chicken DNA by applying 12S rRNA gene as explained by Dalmasso *et al.* (2004), giving an amplicon size of 183bp. The primers are as follows:

F-TGAGAACTACGAGCACAAAC

R-GGGCTATTGAGCTCACTGTT

Polymerase chain reaction

PCR amplification was performed in a final quantity of 25 µl. The reaction mixture contained 12.5 µl GoTaq® Green Master Mix (Promega, M7122) , 2 µl of DNA sample, 1µl of each of forward and reverse primers, and 8 µl of Ultra-Pure DNase/RNase-Free distilled water (Gibco, Grand Island, NY, USA).

Amplification was performed in a Thermal Cycler ((Techne Cyclgene, UK) with the following cycling conditions; after an initial heat denaturation step at 94°C for 5 mins, 35 cycles were programmed as follows: denaturation at 94°C for 45 s, annealing at 60°C for 1 mins, extension at 72°C for 30 s, and final extension at 72°C for 7 mins. The PCR products were subjected to electrophoresis in 1% (w/v) agarose gel, stained with ethidium bromide and photographed under UV transilluminator then documented with a gel documentation apparatus.

Histological examination

A total of 16 samples of previously examined heated kofta and hawawshy (8 of each) were selected for histological examination for detection of chicken constituents and other unauthorized tissue.

Each sample was divided into three equal parts and then, 2-3 pieces were taken from each part. The tissues were fixed in 10% neutral-buffered formalin and were implanted in paraffin and routinely processed for light microscopy. The paraffin-embedded masses were incised into 6µm sections and stained using hematoxylin and eosin (HE) for histological analysis. The slides were observed under a light microscope (Latorre *et al.,* 2015).

Results

PCR analysis

By using of chicken mitochondrial 12S rRNA gene, the results of this study (Table 1 and Figure 1) Showed that 92% (23/25) of retail heated kofta and 64% (16/25) of retail heated hawawshy were adulterated with chicken substance. High rate of adulteration (39/ 50 (78%)) with chicken parts was confirmed by PCR assay.

Histological inspection

Each of bone and cartilage was detected in one sample of hawawshy. Nerve fiber detected in one sample of kofta. Adipose tissue and color additives each were detected in one sample of kofta and one sample of hawawshy. Liver and smooth muscle were detected in two samples of kofta and two samples of hawawshy, respectively. Presence of smooth muscle indicates illegal addition of viscera or intestine. Skin of chicken was detected in two samples of kota and one sample of hawawshy. Plant substances were detected in 4 samples of kofta and two samples of hawawshy. More than one type of added tissue could be found in each sample. An unpredicted finding was

Table 1. Incidence of adulteration with chicken DNA in the examined heated kofta and hawawshy by PCR.

Type of samples	No. of Samples	Negative samples	Positive samples
Kofta	25	2/25 (8%)	23/25 (92%)
Hawawshy	25	9/25 (36%)	16/25 (64%)
Total	50	11/50 (22%)	39/50 (78%)

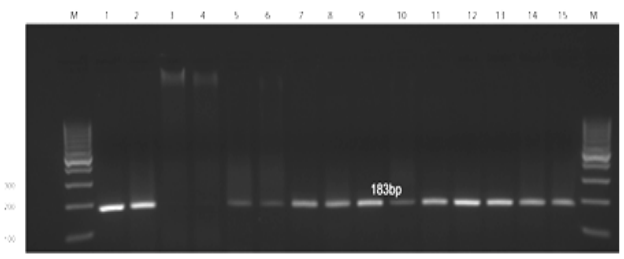

Figure 1. Agarose gel electrophoresis of PCR product from examined kofta and hawawshy samples; Lanes 2 and 5-15 chicken mitochondrial 12srRNA (183 bp); Lane M: 100 bp DNA ladder; Lanes 1 and 3: positive and negative controls, respectively.

the existence of parasite in one sample of kofta (Table 2, Figures 2 and 3).

Discussion

PCR assay for detection of adulteration

The food control organizations should be able to detect animal species in heated meat products which can be easily substituted or mixed with other undeclared species. Meat adulteration has a great significance, as it causes economic and health problems. So, it necessitates development of analytical methods for detection of added meat, especially the relatively cheaper chicken meat for the processed beef meat products. DNA based techniques are the most accepted in meat species identification, especially in processed and heated products, as they are accurate, rapid and inexpensive analytical techniques. Mitochondrial DNA (mtDNA) primers are documented to be suitable for this purpose owing to its DNA stability and high copy number (Ballin *et al*., 2009).

Mitochondrial 12S rRNA gene was used in the development of PCR identification as it's highly conserved in animal species (Dalmasso *et al.*, 2004). The results of this study (Table 1 and fig. 1) Showed that 92% (23/25) of retail heated kofta and 64% (16/25) of retail heated hawawshy were adulterated with chicken material. The high rate of adulteration (39/ 50 (78%)) with chicken parts was confirmed by PCR assay.

Many studies applied for detection of chicken meat in processed or heated meat products using the PCR assay; Cawthorn *et al*. (2013) used species specific PCR for the discovery of 14 animal species in a total of 139 processed meat products. The chicken meat was detected as one of the most added species in 23% of total analyzed, processed meat samples. Hopwood *et al.* (1999) recognized the chicken meat in fresh or cooked meat admixtures containing meat of species as beef, lamb, pork, horse, duck and pheasant by applying the species-specific PCR assay. Mehdizadeh *et al.* (2014) used 12S rRNA gene for detection of chicken DNA in industrial and handmade hamburger. Their study demonstrated that 94.4% of all hamburgers, including 100% of handmade and 89.6% of industrial samples, contained undeclared chicken meat.

Table 2. Tissue type identified in the histologically examined kofta and hawawshy samples.

Added or foreign tissue	Kofta		Hawawshy		Total	
	No.	%	No.	%	No.	%
Chicken Cartilage	-	-	1	12.5	1	6.25
Spongy bone	-	-	1	12.5	1	6.25
Nerve fiber	1	12.5	-	-	1	6.25
color additives	1	12.5	1	12.5	2	12.5
fat cells	1	12.5	1	12.5	2	12.5
Liver	2	25	-	-	2	12.5
Smooth muscle	-	-	2	25	2	12.5
Skin of chicken	2	25	1	12.5	3	18.75
plant cells or material	4	50	2	25	6	37.5
Parasite	1	12.5	-	-	1	6.25

Histological method for detection of adulteration

Meat isn't only muscle tissue as consumers would believe, but all edible organs and tissues of animals as well as birds. Skeletal muscle as adipose tissue, blood vessels, connective tissue, and peripheral nerve might be associated with muscle tissues. The main reason for the adulteration with chicken meat is due to its lower price compared with beef. Chicken trimmings as fat, connective tissue, blood vessels, nerves, cartilage, and bone may be used as adulterants. These waste products have low nutritional value, and may be a source of food borne pathogens for consumers. So, histological analysis was used for detection of added tissue type to detect, and improve the meat product quality (Joo *et al.*, 2013).

As shown in Table 2 and Figures 2 and 3; each of bone and cartilage was detected in one sample of

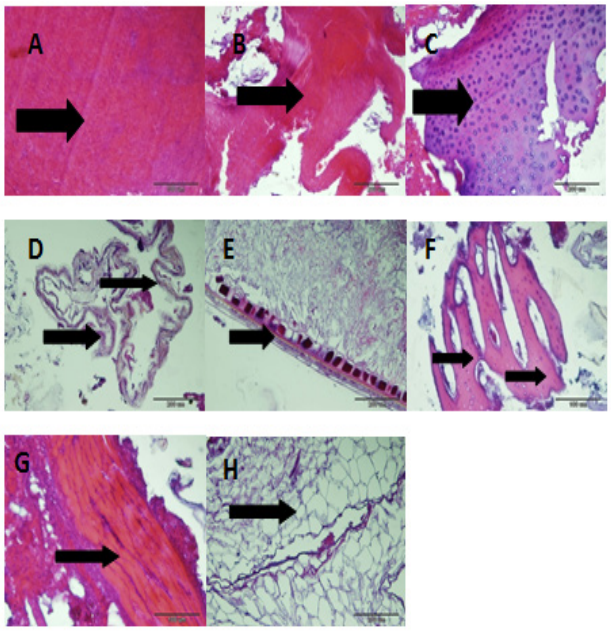

Figure 2. A., and B. Basic staining: Hematoxylin – eosin, histological section show smooth muscle. C. Basic staining: Hematoxylin – eosin, histological section shows hyaline cartilage in hawawshy. D. Basic staining: Hematoxylin – eosin, Photomicrograph of hawawshy containing skin. E. Basic staining: Hematoxylin – eosin, Photomicrograph of hawawshy containing plant cells. F. Basic staining: Hematoxylin – eosin, Photomicrograph of hawawshy containing spongy bone. G. Basic staining: Hematoxylin – eosin, Photomicrograph of hawawshy containing color additive. H. Basic staining: Hematoxylin – eosin, Photomicrograph of hawawshy containing adipose tissue

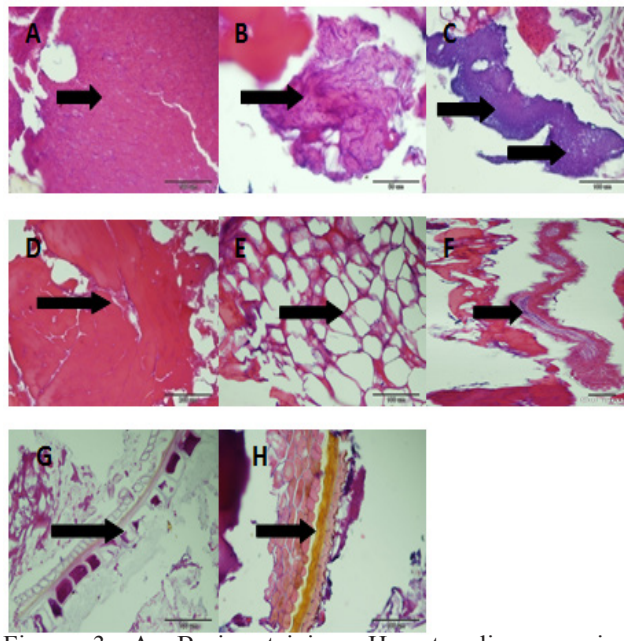

Figure 3. A. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing liver cells. B. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing nerve fibre. C. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing skin. D. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing color additive. E. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing adipose tissue. F. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing parasite. G. and H. Basic staining: Hematoxylin – eosin, Photomicrograph of Kofta containing plant material

hawawshy; they were not authorized especially when referred to other species. Small amounts of bone and cartilage may have been removed during the advanced recovery of meat (separation process) (Prayson *et al*., 2008). Nerve fiber detected in one sample of kofta. Adipose tissue and color additives each were detected in one sample of kofta and one sample of hawawshy. Liver and smooth muscle were detected in two samples of kofta and two samples of hawawshy, respectively. Presence of smooth muscle indicates illegal addition of viscera or intestine. Skin of chicken was detected in two samples of kota and one sample of hawawshy. Plant substances were detected in 4 samples of kofta and two samples of hawawshy, which might be added as extenders to provide bulkiness to the meat products or as spices for flavoring (Prayson *et al*., 2008). More than one type of added tissue could be found in each sample. An unpredicted finding was the existence of parasite in one sample of kofta.

Several studies were performed for the detection of illegal tissues in meat products. According to morphological analysis carried out in USA on eight different brands of hamburgers (Prayson *et al*., 2008b) and hot dogs (Prayson *et al*., 2008a) demonstrated the occurrence of connective tissue, adipose tissue, cartilage, bone, blood vessels, peripheral nerve and plant material. Illegal smooth muscle and soya tissues were observed histologically in meat products by Rokni *et al.* (1999) that is in accordance with our findings. Also, thirty samples from three different types of Kabab Loghme, sausages, hamburger, and minced meat which are marketed in Tehran, Iran were evaluated; chicken skin, hyaline cartilage, adipose, blood vessels, nerves and plant material were found (Sepehri Erayi, 2008). A wide range of unauthorized tissues had been detected by Latorre *et al*. (2015) in 20 examined, processed meat products, including connective tissue, adipose tissue, cartilage, gizzard, soya and ovary. The previous studies revealed that histological examination of commercialized meat products was supportive to identify the type of added tissues in meat products.

Conclusion

The outcomes of this study show that no adverse effects of heating were found on detection and amplification of chicken DNA. The PCR assay was found to be specific and sensitive for rapid

identification of chicken parts in the heated meat products. The use of histological identification method helps in the detection of illegal tissue and further confirmation of adulteration. This study suggests that these methods of detection can be applied by quality control laboratories and inspection services to determine adulteration in heated ready to eat meat products.

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References

Abd El-Nasser, M., Labieb, H. and Doaa, M. A. 2010. Identification of meat species in some meat products in Assiut city. Assiut University Bulletin Environment Research 13 (2):1- 12

Ballin, N. Z., Vogensen, F. K. and Karlsson, A. H. 2009. Species determination–Can we detect and quantify meat adulteration? Meat Science 83:165-174.

Cawthorn, D. M., Steinman, H. A. and Hoffman, L. C. 2013. A high incidence of species substitution and mislabeling detected in meat products sold in South Africa. Food Control 32: 440-449.

Dalmasso, A., Fontanella, E., Piatti, P., Civera, T., Rosati, S. and Bottero, M. 2004. A multiplex PCR assay for the identification of animal species in feed stuffs. Molecular and Cellular Probes. 18: 81

Giovannacci, I., Guizard, C., Carlier, M., Duval, V., Martin, J.L. and Demeulemester, C. 2004. Species identification of meat products by ELISA. International Journal of Food Science and Technology 39: 863–867. *http://dx.doi.org/10.1111/j.1365-2621.2004.00859.x*

Haunshi, S., Basumatary, R., Girish, P. S., Doley, S., Bardoloi, R. K. and Kumar, A. 2009. Identification of chicken, duck, pigeon and pig meat by species-specific markers of mitochondrial origin. Meat Science 83: 454-459. *http://dx.doi.org/10.1016/j.meatsci.2009.06.026*

Hopwood, A. J., Fairbrother, K. S., Lockley, A. K. and Bardsley, R. G. 1999. An actin gene-related polymerase chain reaction (PCR) test for identification of chicken in meat mixtures. Meat Science 53(4): 227−231. *http://dx.doi.org/10.1016/ S0309-1740(99)00060-1*

Joo, S. T., Kim, G. D., Hwang, Y. H. and Ryu, Y. C. 2013. Control of fresh meat quality through manipulation of muscle fiber characteristics. Meat Science 95: 828-836

Latorre, R., Sadeghinezhad, J., Hajimohammadi, B., Izadi, F. and Sheibani, M. T. 2015. Application of Morphological Method for Detection of Unauthorized Tissues in Processed Meat Products. Journal of Food Quality and Hazard Control 2: 71-74.

Mane, B. G., Mendiratta, S. K. and Tiwari, A. K. 2009. Polymerase chain reaction assay for identification of chicken in meat and meat products. Food Chemistry 116: 806-810.

Matsunaga, T., Chikuni, K., Tanabe, R., Muroya, S., Shibata, Yamada, J. and Shinmura, Y. 1999. A quick and simple method for the identification of meat species and meat products by PCR assay. Meat Science 51: 143-148. *http://dx.doi.org/10.1016/S0309-1740(98)001120*

Mehdizadeh, M., Mousavi, S. M., Rabiei, M., Moradian, K., Eskandari, S., Fesarani, M. Abbasi, Rastegar, H. and Alebouyeh, M. 2014. Detection of Chicken Meat Adulteration in Raw Hamburger Using Polymerase Chain Reaction. Journal of Food Quality and Hazards Control 1: 36-40

Prayson, B. E., McMahon, J. T. and Prayson, R. A. 2008a. Applying morphologic techniques to evaluate hotdogs; what is in the hotdogs we eat? Annals of Diagnostic Pathology 12: 98-102.

Prayson, B., McMahon, J. T. and Prayson R. A. 2008b. Fast food hamburgers: what are we really eating? Annals of Diagnostic Pathology 12:406-409.

Rashid, P. M. A., Babashekh, M. O., Marouf, A. S. and Amin, K. M. 2014. Identification of Animal Species in Meat Broth by Simplex and Multiplex PCR. Journal of-Zankoy Sulaimani- Part A (JZS-A) 16(1): 97-102.

Rezaian, M. and Rokni, N. 2001. Histological study of heated meat products of Mazandaran province. Pajouhesh and Sazandegi 15: 61-63.

Rokni, N., Jahed- Khaniki, G. R. and Pousty, I. 1999. Detection of unauthorized tissue in heated sausages by using histological. Pajouhesh and Sazandegi 13: 76-82.

Sepehri-Erayi, S. 2008. Histological methods evaluation for detection of adulteration of raw meat products supplied in Tehran. Tehran, Iran: Faculty of Veterinary Medicine, University of Tehran, DVM thesis.