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Assessment of adulteration in fresh minced beef and cooked kofta by different methods

Hala W. Gaafar1; Abo Bakr M. Edris[1]; Mona N. Abd El-Naeim[2] , Walid S. Arab[1]

[1]*Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Toukh, Qalyubia 13736, Egypt.*

[2] *Department of Histology and Cytology, Faculty of Veterinary Medicine, Benha University*

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ABSTRACT

Concerns about food fraud are important from a public health, legal, economic, and religious perspective. We randomly selected forty samples of fresh minced beef and cooked kofta (twenty of each) from various butcher shops and restaurants in the Gharbia governorate, Egypt. Tests including sulfuric acid heating test, precipitation test, and polymerase chain reaction (PCR) were applied on the samples to see if they had been tampered with by adding illegal meat. Out of the total number of samples, 18 (90%) of the fresh minced beef and 15 (75%) of the cooked kofta were found to be free of adulteration. Two samples of raw minced beef (10%) and four samples of cooked kofta (20%) included horse meat. In cooked kofta, one sample (5%) contained dog meat. All examined samples were free from pork meat. Investigation of meat product adulteration with different species is critical from food safety, and food security points of view.

1. INTRODUCTION

Meat products are widely available in Egypt and are often made from beef. The demand for such ready-to-eat beef products is steadily expanding, owing mostly to socioeconomic development and changing lifestyles. The importance of the quality of meat and meat products in the human diet makes their quality an important issue for consumers, regulatory agencies, processors, and merchants alike (El-Sheikh et al., 2022).

Food adulteration has become a major issue in recent years, stifling progress in food production, consumption, and management (Zhang et al., 2024). It is ubiquitous due to the producer's dishonesty and a drive for quick profit. According to Mokhtar et al. (2018), the high price of meat and meat products encourages adulteration, resulting in various ailments and economic losses for customers. Adulterating superior quality meat with inferior quality meat is a prevalent activity worldwide; hence, the meat species definition is an important issue in maintaining consumer food safety under meat and meat product legislation (El-Shazly et al., 2016). The optimal technique for identifying specific meats is determined by the test's requirements and the state of the meat. Recent sophisticated techniques can detect even traces of undesired meat in the ready-to-eat meat (Chappalwar et al., 2020). Chemical analysis is useful for qualitative assessment of meat product adulteration (Mokhtar et al., 2018). The molecular methodology was demonstrated to be a specific, sensitive, and rapid method for detecting tainted meat (El-Sheikh et al., 2022). PCR applications are more reliable, easier, faster, and generally stable than other methods for detecting meat species adulteration. The presence of target DNA has been successfully discovered, and amplification is unaffected by spice addition or cooking (Hassanin et al., 2018).

Therefore, the current study was done to assess the adulteration in fresh minced beef and cooked kofta in Gharbia governorate, Egypt. The collected samples were subjected to chemical testing such as sulfuric acid heating, immunological tests such as precipitation, and DNA-based molecular tests such as polymerase chain reaction (PCR).

2. MATERIAL AND METHODS

This research was approved by Institutional Animals Care and Use Committee of faculty of veterinary medicine, Benha University (Approved number BUFVTM 01-12-23)

2.1. Collection of samples

Forty samples of fresh minced beef and cooked kofta (20 per each) were randomly selected from various butcher shops and restaurants in Gharbia governorate, Egypt. Samples were individually wrapped in polyethylene bags, placed in an icebox, and sent to the lab for analysis. Hence, many trials were conducted on the collected samples of these meat items, including both simple techniques (the sulfuric acid heating test) and standard techniques (the precipitation test and PCR).

2.2. Methodology

2.2.1. Sulfuric acid heating test (AOAC, 2006)

A few drops of concentrated sulfuric acid were added during the heating of suspected meat samples. The exhibited repulsive odor resembles a horse stable and

Correspondence to: walids444@gmail.com

yellow oily globules appear on the broth during cooking indicating equine meat.

2.2.2. Precipitation test

The analyzed meat products were tested for adulteration with banned meat using the method suggested by Mackie and McCartney (1996).

2.2.2.1. Antisera

The current study utilized patent-specific antisera for various meat types, including beef, equine, dog, and pig. We employed antisera that are unique to patents from Sigma-Aldrich Chemie GmbH in Taufkirchen, Germany, for several types of meat, such as beef (B3759), equine (H8890), dog (D4908), and pig (P3164).

2.2.2.2. Fat extraction

Fifty grams of meat were finely cut and placed in a flask with 100 ml ether chloroform mixture (1:1) with shaking for 24 hours. Discard the ether chloroform mixture and the meat was washed with the addition of normal saline.

2.2.2.3. Dissolving of fat

The meat sample was washed several times (3-6 times) with distilled water, the washing was performed each time by shaking the sample with distilled water for 5 minutes in a tightly closed container (capacity: 120 ml). The distilled water was discarded at each time by squeezing the sample with a double gauze layer.

2.2.2.4. Filtration

The final amount of the meat sample produced from previous stages was weighed and a double volume of normal saline was added to the meat sample and then kept in the refrigerator at 2-4° C for 12 hours. The meat sample was filtered. The filtrate became ready for the subsequent analysis.

Accordingly, the meat extract was tested with patent-specific antisera for different animals including beef, equine, dog, and pig in small precipitation tubes by the addition of one drop of patent-specific antisera to one drop of the extract. The appearance of precipitation on the bottom and the wall of the precipitation tube was considered positive.

2.2.3 Polymerase Chain Reaction (PCR)

Genomic DNA was extracted from each examined sample using the Gene JET Genomic DNA Purification Kit. The PCR master Mix and the 100 bp DNA ladder were purchased from Fermentas (Sambrook et al., 1989).

2.2.3.1. Primer sequences of species-specific genes for PCR identification system

The application of PCR for the identification of cyt b genes to demonstrate diverse meats from different animal species was conducted using the primers in Table 1.

2.2.3.2. DNA Extraction from the examined meat products (Obrovska et al., 2002)

Muscle samples were used to extract mitochondrial DNA. In short, 1 milliliter of lysis buffer-ST was added to 500 milligrams of cryogenically pulverized muscle tissue under liquid nitrogen. To reach a final concentration of 2%, combine 50 mM Tris-HCl (pH 8.0), 10 mM EDTA (pH 8.0), 100 mM NaCl, 150 µg/ml proteinase K, and SDS. After that, the samples were kept in an incubator at 55°C all night long to make sure they were uniform. After the incubation period, the lysate was transferred to a fresh tube and an equivalent volume of tris-saturated phenol was introduced. Following a 10-minute gentle stirring, the liquid was centrifuged at 10,000 rpm for 10 minutes at 15°C. Then, the liquid phase was extracted twice using a 24:1 ratio of chloroform to isoamyl alcohol and once with half the volume of tris-saturated phenol. A fresh tube was used to collect the diluted portion of the previous chloroform/isoamyl alcohol extraction. The next step was to create a precipitate by adding an equal proportion of isopropyl alcohol and a 20th volume of 3M sodium acetate (pH 5.5).

After being rinsed with 70% ethanol, the harvested mitochondrial DNA was dried and then mixed with 500 µl of TE (10 M Tris-HCl, 1 mM EDTA, pH 8.0). We used agarose gel electrophoresis to check the DNA's integrity and purity, and quantitation used UV spectrophotometry.

2.2.3.3. DNA amplification

2.2.3.3.1. Amplification reaction of cyt b genes for cattle, pig, and equine by multiplex PCR (Jain et al., 2007)

Using a thermal cycler (Master cycler, Eppendorf, Hamburg, Germany), the amplification was carried out. For PCR amplification, a 25-µl reaction volume was used, which included 50 mM KCl, 1.5 mM $MgCl_2$, 200 µM dNTP mix, primer mix (4-60 pmol each), 1.25 units of Taq DNA polymerase, and 2 µl (90 ng template DNA). A universal forward primer SIM was used for pig, horse, and cow meat in addition to species-specific oligonucleotide primers. For the multiplex PCR, the primers were mixed in the following proportions: 1:0.6:0.6:2 for SIM, pig, horse, and cow. The amplification process involved a denaturation step at 94°C, followed by 31 cycles of denaturation, annealing, extension, and final extension at 72°C for 10 minutes. Each cycle was performed using a thermal cycler. The PCR amplification products were analyzed by electrophoresis on an agarose gel. The DNA fragments that had been amplified were analyzed by running them through a 4% agarose gel electrophoresis in 5 µl/100 ml of TBE buffer. They were then stained with ethidium bromide and seen using a UV transilluminator. An electrophoresis run at 100 V for 60 minutes was used to separate the DNA fragments. To measure the diameters of the fragments, a 100 bp DNA ladder was used.

2.2.3.3.2. Amplification reaction of cyt b gene for detection of dog meat (Edris et al., 2012)

The following ingredients were utilized in the PCR reaction: 50 µl of 1 ng genomic DNA extracted from the meat that was suspected of being tainted, 25 pmoles of primer, 1x Taq DNA polymerase buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, and 0.2 units of Taq DNA polymerase. The DNA was denaturated at 94°C for 4 minutes before amplification. Then, there were 35 cycles of 94°C for 60 seconds, with annealing temperatures ranging from 48°C to 58°C, each lasting 60 seconds. After subjecting the samples to a polymerization process lasting 60 seconds at 72°C and a final extension lasting 10 minutes at the same

temperature, they were thereafter kept at 4°C. The mtDNA fragments that had been amplified were then separated on a 2% agarose gel. They were stained with ethidium bromide and photographed after being visualized using a UV transilluminator.

2.3. Statistical Analysis

The gathered data were examined utilizing one-way analysis of variance (ANOVA) with Duncan's post hoc test via SPSS® version 16.0, following the protocols suggested by Feldman et al. (2003).

3. RESULTS

3.1. sulfuric acid heating test

The application of sulfuric acid heating test (Table 2) for detection of adulteration by equine meat in fresh minced beef and cooked kofta samples resulted in 18 samples (90 %) and 16 samples (80 %) were free (not adulterated). While, 2 samples (10 %) and 4 samples (20 %) were adulterated by equine meat, respectively.

3.2. precipitation test

The application of the precipitation test (Table 3) for the detection of adulteration in fresh minced beef and cooked kofta samples resulted in 18 samples (90 %) and 15 samples (75 %) being free (not adulterated), respectively. While adulterated samples were 2 samples (10 %) and 5 samples (25 %), respectively.

The application of the precipitation test (Table 4) for the detection of adulteration in fresh minced beef and cooked kofta samples resulted in 18 samples (90 %) and 15 samples (75 %) were not adulterated. While adulterated samples with equine meat were 2 samples (10 %) of fresh minced beef (one sample -5 % mixing between cattle & equine meat and one sample- 5 % adulterated with pure equine meat) and 4 samples (20 %) of cooked beef kofta (3 samples 15 % mixing between cattle & equine meat and one sample 5 % adulterated with pure equine meat). The results also showed that one sample (5 %) of cooked beef kofta was adulterated with dog meat (mixing between cattle & dog meat) while there was no adulteration with pig meat in all examined samples.

3.3. PCR technique

Results in Fig 1&2, and Table 5 showed the application of the PCR technique for the detection of adulteration in fresh minced beef and cooked kofta samples resulting in 18 samples (90 %) and 15 samples (75 %) not adulterated. While adulterated samples with equine meat were 2 samples (10 %) of fresh minced beef (one sample 5 % mixing between cattle & equine meat and one sample 5 % adulterated with pure equine meat) and 4 samples (20 %) of cooked beef kofta (3 samples 15 % mixing between cattle & equine meat and one sample 5 % adulterated with pure equine meat). The results also showed that one sample (5 %) of cooked beef kofta was adulterated with dog meat (mixing between cattle & dog meat) while absence of pig meat in all examined samples.

Table 1 Primers used to demonstrate diverse meats from different animal species

Species	Target gene	Oligonucleotide sequence (5′ → 3′)	Product size (bp)	References
SIM*	*cyt b* (F)	5′CCTCCCAGCTCCATCAAACATCTCATCTTGATGAAA′3		
Cattle	*cyt b* (R)	5′CTAGAAAAGTGTAAGACCCGTAATATAA′3	274	Matsunaga et al.
Equine	*cyt b* (R)	5′ CTCAGATTCACTCGACGAGGGTAGTA ′3	439	(1999)
Pig	*cyt b* (R)	5′ GCTGATAGTAGATTTGTGATGACCGTA ′3	398	
Dog	*cyt b* (F)	5′ GGAGTATGCTTGATTCTACAG ′3	808	Abdulmawjood and
	cyt b (R)	5′ AGAAGTGGAATGAATGCC ′3		Buetter (2003)

Table (2) Incidence of adulteration by equine meat in the examined meat samples (n=20).

Beef Products	Adulterated		Non-adulterated	
	No	%	No	%
Fresh minced beef	2	10	18	90
Cooked beef kofta	4	20	16	80
Total (40)	6	15	34	85

Table (3) Incidence of adulteration in the examined beef products with meat of other animal species by precipitation test (n=20).

Beef Products	Adulterated		Non-adulterated	
	No	%	No	%
Fresh minced beef	2	10	18	90
Cooked beef kofta	5	25	15	75
Total (40)	7	17.5	33	82.5

Table (4) Incidence of adulterated beef products with other animal species by precipitation test (n=20).

Beef Products	Cattle		Equine		Cattle & Equine		Cattle & Dog		Cattle & Pig	
	No	%	No	%	No	%	No	%	No	%
Fresh minced beef	18	90	1	5	1	5	0	0	0	0
Cooked beef kofta	15	75	1	5	3	15	1	5	0	0
Total (40)	33	82.5	2	5	4	10	1	2.5	0	0

Table (5) Adulteration % of beef products with meat of other animal species using PCR technique (n=20).

species	Fresh minced beef		Cooked beef kofta		Total (40)	
	No	%	No	%	No	%
Pure cattle meat	18	90	15	75	33	82.5
Pure equine meat	1	5	1	5	2	5
Cattle & equine meat	1	5	3	15	4	10
Cattle & dog meat	0	0	1	5	1	2.5
Cattle & pig meat	0	0	0	0	0	0
Total (40)	20	100	20	100	40	100

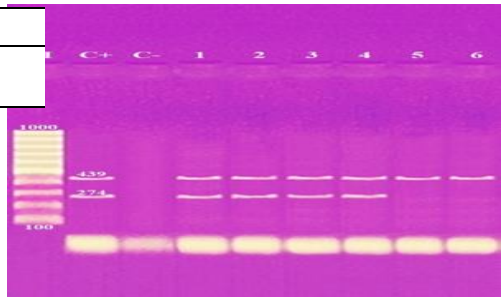

Fig. (1). Agarose gel electrophoresis of multiplex PCR of *cyt b* gene of equine (439 bp) and cattle (274 bp) for detection of adulterated beef products. Lane M: 100 bp ladder. Lane C+: Control positive. Lane C-: Control negative. Lanes from 1 to 4: Cattle meat intermixed with equine meat. Lanes 5 and 6: Pure Equine meat.

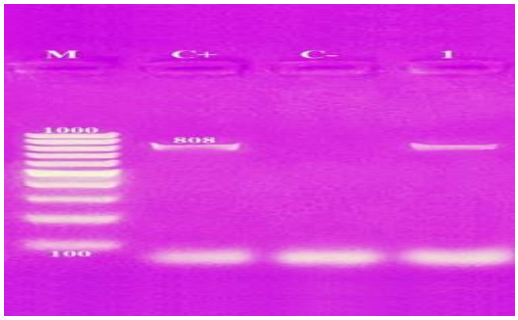

Fig. (2). Agarose gel electrophoresis of PCR of *cyt b* gene (808 bp) detection of dog meat in examined beef products. Lane M: 100 bp ladder. Lane C+: Control positive. Lane C-: Control negative. Lane 1: Cattle meat intermixed with dog meat.

4. DISCUSSION

A worldwide problem that violates food standards is meat adulteration, particularly when it involves species replacement. Because meat adulteration is a common problem in ground and processed meats, it is important to find out what kind of meat is in meat products as part of food safety, especially in processed meat products (El-Sheikh et al., 2022). In the present study, several samples were found contaminated with horse and dog flesh. Likely, El-Shazly et al. (2016) found 90% pure cattle meat with no adulteration with dog or pig meat in examined minced beef and cooked samples, while Hamouda and Abdelrahim (2022) found similar results when they examined fresh minced beef samples. The results from the studies by Abd El-Nasser et al. (2010), El-Shazly et al. (2016), and Hassanin et al. (2018) showed marked adulteration of the Minced meat samples. They found that 35.7% of the meat was pig meat, 7.0% was donkey meat, 10% was mixed cattle and horse meat, and 6.7% was pure horse meat. However, Hamouda and Abdelrahim (2022) reported that 73.3% of the examined samples were pure cattle meat with no adulteration with horse flesh. Because of their high protein, vitamin, and mineral content, major processed meat products, such as minced beef, are a popular ingredient among most people.

Due to their high value and substantial demand, processed meat products are susceptible to fraud. Furthermore, it becomes difficult to visually distinguish authentic minced beef due to the elimination of morphological features (Setiadi et al., 2022). Modern technologies that can quickly, accurately, and reliably find adulteration are necessary for good supervision, which is important for keeping the meat industry growing in the right way. There have been several developments in the past 20 years regarding methods for determining whether meat or meat products are authentic. Some of these methods are spectroscopic, which uses certain metabolites, immunological, which uses proteins, and polymerase chain reactions (PCRs), which use DNAs as their building blocks (Li et al., 2020). Along with that, the results of the study on cooked beef kofta samples agreed with those of Khatun et al. (2021) (no pig meat added), El-Sheikh et al. (2022) (5% pure horse meat and no pig meat added), and Hamouda and Abdelrahim (2022) (no pig meat added). However, Abd El-Nasser et al. (2010) found 45.5% pig and 18.0% donkey meat, Omran et al. (2019) found 20% donkey meat and El-Sheikh et al. (2022) found 85% no adulteration. Hamouda and Abdelrahim (2022) found no adulteration with horse meat and 4% adulteration with dog meat, while El-Sheikh et al. (2022) observed 10% mixing of cattle and equine meat and no adulteration with dog meat, suggesting lower findings. El-Shazly et al. (2016) demonstrated that multiplex PCR can find real meat in mixed meat samples that are raw, cooked, or spoiled, and it can even find as little as 5% (5 pg DNA contaminants) of DNA from other animals

5. CONCLUSIONS

In conclusion, this study shows some of the chemical, immunological, and molecular methods that can identify meat species adulteration. These include adding illegal equine and dog meat to beef products. The current investigation found that compared to fresh minced beef samples; cooked beef kofta samples had a greater rate of meat adulteration especially fresh minced beef due to food additives that were added it and its methods of preparation.

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