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INCIDENCE OF MEAT PRODUCTS ADULTERATION USING DIFFERENT TECHNIQUES

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ABSTRACT

A total of sixty samples of cooked beef products (burger, sausage and beef hawawshi, 20 samples from each) had been collected from some restaurants in the Gharbia district in Egypt. The samples were examined for adulteration with prohibited meat using different techniques, a simple technique (sulphuric acid heating test) and standard techniques (precipitation test and PCR). The results showed the non-adulterated samples were 17 samples (85 %) of burger, 15 samples (75 %) of sausage and 14 samples (70 %) of hawawshi, while the samples which had been adulterated with equine meat were 2 samples (10 %) of burger, 4 samples (20 %) of sausage and 4 samples (20 %) of hawawshi. But only one sample (5 %) of burger and hawawshi had been adulterated with dog meat; moreover, we found pork meat in two samples only, one of the sausages and the other in the hawawshi samples.

Keywords: Adulteration, PCR, burger, equine meat, hawawshi, sausage.

INTRODUCTON

Meat is considered an important food source of protein, vitamins and minerals for humans (Daniel *et al.,* 2011). Because of the increased need for meat and meat products, it is important to take care and ensure that those products are safe and healthy (Mottin *et al.,* 2011).

Meat and its products are foods that have been prepared and sold directly in different places, and must be supplied safely and of good quality because they are considered the major source of daily protein intake for a lot of people (Samapundo *et al.,* 2015). Increased consumers' needs allow greedy meat producers to adulterate meat products, for example, minced meat (El-Sheikh *et al.,* 2022). Due to economic issues and to increase its benefits, most people still adulterate meat and its products, which leads to serious public health risks (Li *et al.,* 2020).

Most Consumers do not face a problem detecting fresh meat when they were been bought from the market because the physical properties of beef are different from equine meat. Meat adulteration in minced meat is a widespread problem in most markets. In addition, mixing different meat species followed by grinding leads to difficulties in detecting this type of meat origin (Hassanin-Faten *et al.,* 2018).

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Using other species than cattle or sheep for meat products, like donkey or pork meat, is a red line to the Egyptian meat production regulation (Donley, 2019).

The public authorities have controlled the main problems of adulteration, which were the mislabeling of meat and its products. A fast and accurate method must be used for inspections (Pu *et al.,* 2023).

The organoleptic and chemical properties of meat and meat products, like physico-chemical methods and immunological techniques, like the precipitation test, have been used for the detection of the meat origin in meat products (Rout *et al.,* 2018).

Chemical examination, along with the PCR technique, may be used to detect meat adulteration (Omran *et al.,* 2019).

Because of its stability in heating and processing, DNA-based molecular techniques are one of the choices for meat species identification; also, due to their sensitivity, accuracy, and low cost (El-Sheikh *et al.,* 2022).

So, this study was conducted to evaluate the adulteration of some ready-to-eat cooked beef samples (burger, sausage and hawawshi) in Gharbia governorate, Egypt by using chemical techniques, including sulphuric acid heating test, immunological techniques such as precipitation test and (PCR).

MATERIAL AND METHODS

1. Collection of samples:

Sixty (60) samples of cooked beef products represented by burger, sausage and hawawshi (twenty samples of each sample) were collected from different restaurants in Gharbia governorate, Egypt. The collected samples were put in a polyethylene bag, then in an icebox and were transferred to the laboratory for the detection of their adulteration with different meats.

2. Methodology:

2.1. Sulphuric acid heating test: (AOAC, 2006).

A few drops of concentrated sulphuric acid were added during the heating of suspected meat samples. The exhibited repulsive odor resembles a stable horse, and yellow oily globules appear on the broth during cooking, indicating equine meat.

2.2. Precipitation test:

The technique recommended by Mackie and McCartney (1996) was carried out for the detection of adulteration of the examined meat products with prohibited meat.

2.2.1. Antisera:

Patent-specific antisera for different animals, including beef, equine, dog and pig meats, were used in the present work. Patent-specific antisera (Sigma-Aldrich Chemie GmbH, 82024 Taufkirchen, Germany) for different animals, including beef (B3759), equine (H8890), dog (D4908) and pig (P3164) meats were used in the present work.

2.2.2. Fat extraction:

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

2.2.3. Dissolving 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 described each time by squeezing the sample through a double gauze layer.

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 a

refrigerator at 2-4° C for 12 hours. The meat sample was filtered. The filtration becomes 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 at the bottom of the wall of the precipitation tube was considered positive.

2.3 Polymerase Chain Reaction (PCR):

2.3.1. Materials used for PCR:

Genomic DNA extraction:

- DNA Using GeneJET Genomic DNA Purification Kit
- DNA amplified products "PCR master Mix" (Fermentis):
- Gel Electrophoresis: Sambrook *et al.* (1989).
- DNA ladder (molecular marker): 100 bp (Fermentas, lot No: 00052518).

3. Statistical Analysis:

One-way analysis of variance (ANOVA) was used for the collected data, Duncan by SPSS® version 16.0, according to the methods recommended by Feldman *et al.* (2003).

RESULTS

Table 1: Incidence of the adulteration of cooked beef samples by equine meat using sulphuric acid heating test (n=20).

Adulteration% / Cooked beef samples	adulterated		non adulterated	
	No	%	No	%
Burger	2	10	18	90
Sausage	4	20	16	80
Hawawshi	4	20	16	80
Total (60)	10	16.67	50	83.33

Table 2: Incidence of the adulteration of cooked beef samples with other animal species meat by precipitation test, (n=20).

Adulteration% / Cooked beef samples	adulterated		non adulterated	
	No	%	No	%
Burger	3	15	17	85
Sausage	5	25	15	75
Hawawshi	6	30	14	70
total (60)	14	23.3	46	76.7

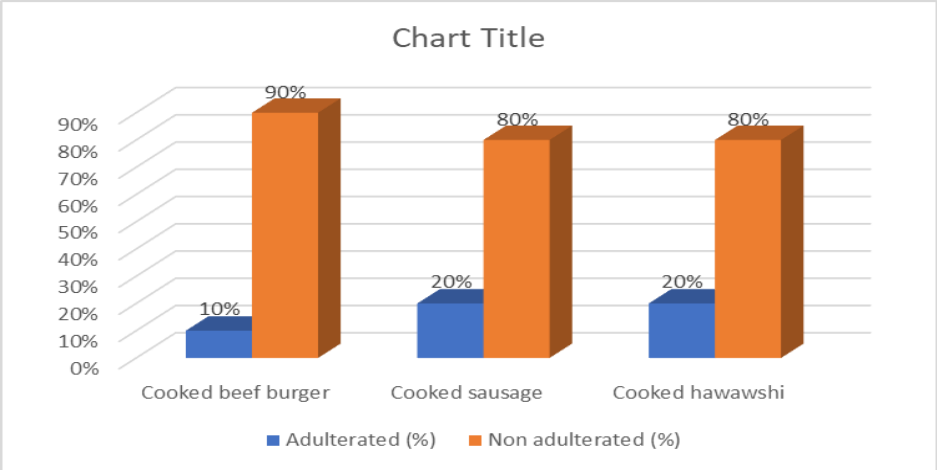


Figure 1: Incidence of the adulteration of cooked beef samples by equine meat using sulphuric acid heating test (n=20).

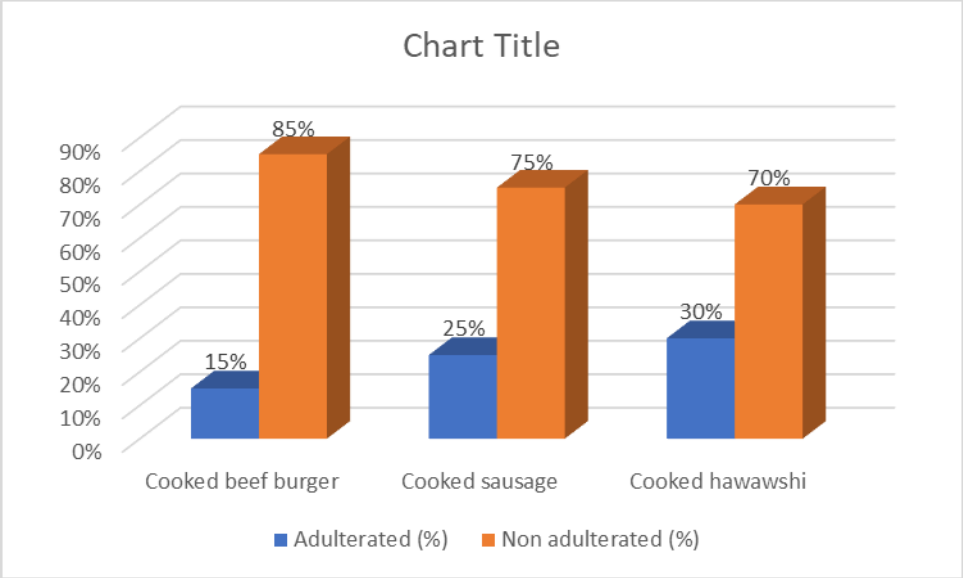


Figure 2: Incidence of the adulteration of cooked beef samples with other animal species meat by precipitation test, (n=20).

Table 3: Detection of the adulteration of cooked beef samples with other animal species using the precipitation test (n=20).

Species / Cooked beef samples	Cattle		Equine		Cattle & equine		Cattle & dog		Cattle & Pork	
	No	%	No	%	No	%	No	%	No	%
Burger	17	85	0	0	2	10	1	5	0	0
Sausage	15	75	0	0	4	20	0	0	1	5
Hawawshi	14	70	2	10	2	10	1	5	1	5
Total (60)	46	76.67	2	3.33	8	13.34	2	3.33	2	3.33

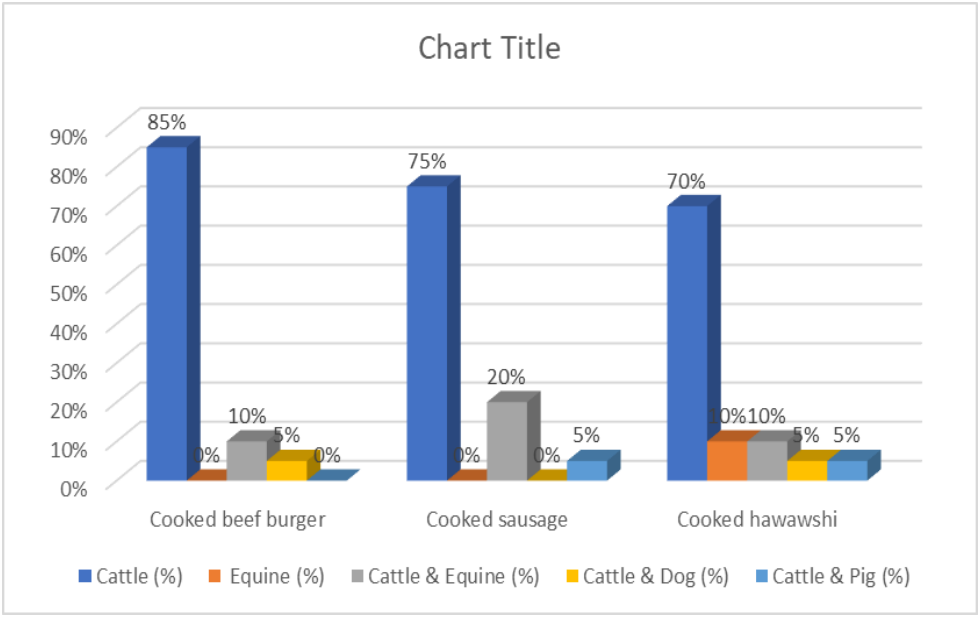
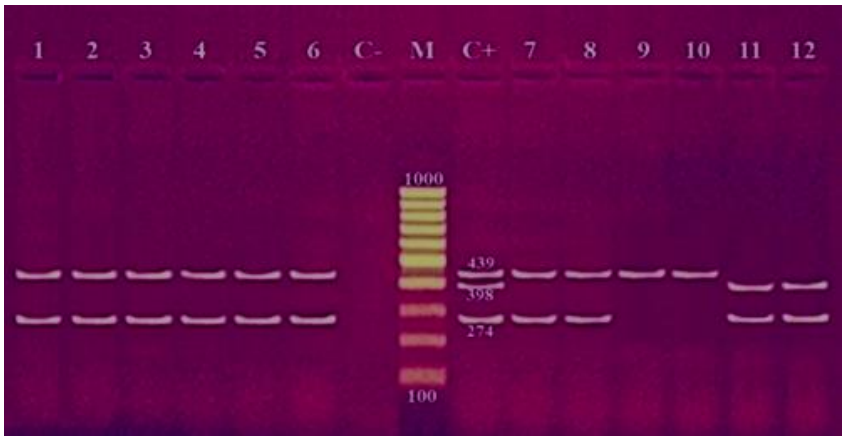
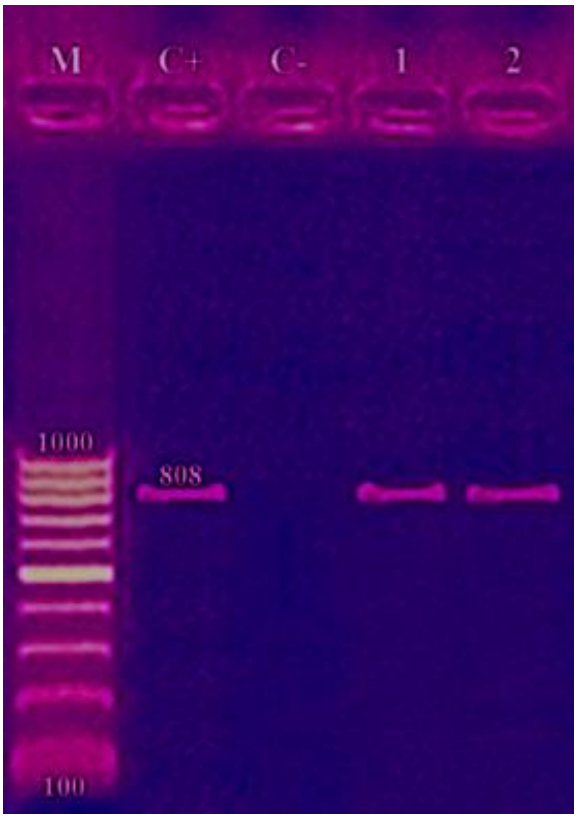


Figure 3: Detection of the adulteration of cooked beef samples with other animal species using the precipitation test (n=20).



Photograph (1): Electrophoresis of multiplex PCR of *the cyt b* gene of equine (439 bp), Pig (398 bp) and cattle (274 bp) to identify the adulteration in beef products.

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for the *cyt b* gene of equine, pig and cattle meat.
Lane C-: Control negative.
Lanes from 1 to 8: Cattle meat intermixed with equine meat.
Lanes 9 & 10: Pure Equine meat.
Lanes 11 & 12: Cattle meat intermixed with pig meat.



Photograph (2): agarose gel electrophoresis of PCR of *the cyt b* gene (808 bp), detection of dog meat in examined beef products**.**

Lane M: 100 bp ladder as molecular size DNA marker.
Lane C+: Control positive for *the cyt b* gene of dog meat.
Lane C-: Control negative.
Lanes 1 & 2: Cattle meat intermixed with dog meat.

Table 4: Detection of the adulteration of cooked beef samples with other meat species using the PCR technique (n=20).

Sampled / Other meat species	Burger		Sausage		Hawawshi		Total (60)	
	No	%	No	%	No	%	No	%
Pure cattle meat	17	85	15	75	14	70	46	76.67
Pure equine meat	0	0	0	0	2	10	2	3.33
Cattle & equine meat	2	10	4	20	2	10	8	13.34
Cattle & dog meat	1	5	0	0	1	5	2	3.33
Cattle & pig meat	0	0	1	5	1	5	2	3.33
Total (60)	20	100	20	100	20	100	60	100

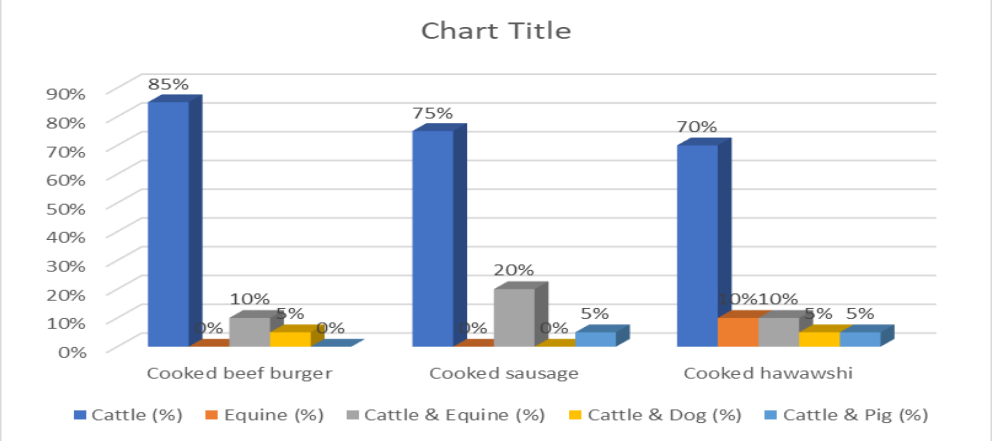


Figure 4: Detection of the adulteration of cooked beef samples with other meat species using the PCR technique (n=20).

DISCUSSION

Meat species speciation is a matter of major concern, including economic and legal as well as health aspects. The number of processes, right from traditional methods to most modern techniques, has been applied for the identification of adulteration (Chappalwar *et al.,* 2020).
The adulteration of meat is considered one of the most common economic problems in meat preparation and industry (Cao *et al.,* 2018). Meat species identification is a major point to control meat products from adulteration (Abbas *et al.,* 2017).

The results in Table (1) and figure (1) showed that the numbers of adulterated cooked beef samples by using sulphuric acid heating test were 2 samples (10%) in burger and 4 samples (20%) in sausage and 4 samples (20%) in hawawshi samples, while the results obtained after using precipitation test (Table 2, 3 and Figure 2, 3) showed 3adulterated samples (15%) in burger and 5 samples (25%) in sausage and 6 samples (30%) in hawawshi.

Using the PCR technique helps us to detect the species of prohibited meat that has been added to samples for adulteration (Table 4 and Figure 4). The results showed 2 adulterated burger samples with equine meat and one sample with dog meat. While in the sausage samples, 4 samples were adulterated by equine meat and only one by pork meat. For hawawshi samples, 6 samples had been adulterated, 2 samples were pure equine meat, 2 samples mixed cattle and equine meat, one sample by dog meat and finally one sample had been adulterated by pork meat.
The results recorded from examination of cooked beef burger samples were similar to Khatun *et al.* (2021) (no adulteration with pig meat) and El-Sheikh *et al.* (2022) (85 %

pure cattle meat; no adulteration with pure equine meat and Pork meat).

Multiplex PCR decreases consumables and personnel time (Villamizar-Rodríguez and Lombó, 2017) and several genes can be detected in one step using it (Ngam-wongsatit *et al.,* 2008). The higher results were detected by Omran *et al.* (2019) (20 % donkey meat); El-Sheikh *et al.* (2022) (15 % adulterated by intermixed cattle & equine meat) and Elmarya (2023) (14.3 % donkey meat).

Moreover, the results obtained from examination of cooked beef sausage samples were nearly similar to El-Shazly *et al.* (2016) (no adulteration with dog meat); Hassanin-Faten *et al.* (2018) (73 % pure cattle meat) and El-Sheikh *et al.* (2022) (75 % pure cattle meat).

Moreover Higher results were been detected by El-Shazly *et al.* (2016) (80 % pure cattle meat and 20 % adulteration with pure equine meat); Hassanin *et al.* (2018) (20 % pure equine meat and 7 % intermixed cattle and pig meat); Omran *et al.* (2019) (10 % donkey meat); El-Sheikh *et al.* (2022) (10 % pure equine meat and 5 % intermixed cattle and dog meat) and Elmarya (2023) (28.6 % adulteration with donkey meat and 14.3 % adulteration with dog meat).

While lower results were previously recorded by El-Shazly *et al.* (2016) (0 % pig meat), Hassanin *et al.* (2018) (7 % intermixed cattle & equine meat), Khatun *et al.* (2021) (no adulteration with pig meat), El-Sheikh *et al.* (2022) (10 % intermixed cattle & equine meat and 0 % adulteration with pig meat) and Hamouda and Abdelrahim (2022) (0 % intermixed cattle & equine meat and 0 % adulteration with pork meat).

In addition to the results of the examined cooked beef hawawshi samples, which were similar to those of El-Sheikh *et al.* (2022) (70 % pure cattle meat; 10 % pure equine meat and 5 % intermixed cattle & dog meat), who also recorded higher results (15 % intermixed cattle and equine meat). While lower results were detected by Hamouda and Abdelrahim (2022) (8.3% adulteration with equine meat and not detected dog & pork meat in the samples).

The results obtained from the current study showed that the highest percent was recorded for the examined cooked beef Hawawshi of meat species adulteration, followed by cooked beef sausage, and then cooked beef burger.

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حالات غش منتجات اللحوم باستخدام تقنيات مختلفة

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تم جمع ستين عينة من منتجات اللحوم البقرى المطهية (البرجر والسجق والحواوشي ،بواقع ٢٠ عينة من كل منها) من بعض المطاعم في محافظة الغربية في جمهورية مصر العربية. تم فحص العينات بحثًا عن الغش باللحوم المحظورة باستخدام تقنيات مختلفة، مثل التقنيات البسيطة (اختبار تسخين حمض الكبريتيك
) وتقنيات قياسية مثل (اختبار الترسيب وتفاعل البوليميراز المتسلسل). أظهرت النتائج أن العينات الغير مغشوشة كانت ١٧ عينة (٨٥٪) من البرجر و١٥ عينة (٧٥٪) من السجق و١٤ عينة (٧٠٪) من الحواوشي، بينما كانت العينات التي تم غشها بلحوم الخيول عينتين (١٠٪) من البرجر و٤ عينات (٢٠٪) من السجق و٤ عينات (٢٠٪) من الحواوشي. و تم غش عينة واحدة فقط (٥٪) من البرجر والحواوشي بلحوم الكلاب؛ وعلاوة على ذلك، وجدنا لحم الخنزير في عينتين فقط، واحدة من السجق والأخرى في عينات الحواوشي. ولذلك توصي الدراسة بتشديد الرقابة على هذه الانواع من اللحوم الجاهزة وعمل تحليل دوري حتى يلتزم المصنعون والتجار بالتعليمات المتبعة بشأن جودة اللحوم وعدم غشها.