Iraqi Journal of Veterinary Sciences

www.vetmedmosul.com

Detection of mutton meat fraud with beef meat as a genetically related species using multiplex PCR and GC/MS/MS

E.A. Abdelrahman[1], M.K. Morsy[2], S.S. Ibrahim[3], E.I. El toukhy[4], E.Y. Abdelhiee[5] and R.A. Elsabagh[6]

[1]Al-Basateen Slaughter House, Cairo Veterinary Authority, Cairo, [2]Department of Food Technology, Faculty of Agriculture, [3]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Benha University, Qaliobiya, [4]Department Biotechnology, Animal Health Research institute, Aldoqqi, [5]Department of Forensic Medicine and Toxicology, Faculty of Veterinary Medicine, Matrouh University, Matrouh, [6]Department of Food Hygiene and Control, Faculty of Veterinary Medicine, Benha University, Qaluobia, Egypt

Article information

Article history:
Received October 14, 2022
Accept March 29, 2023
Available online June 13, 2023

Keywords:
Forensic
Adulteration
Mutton
Beef
Fatty acids

Correspondence:
E.Y. Abdelhiee
ehabyahya76@mau.edu.eg

Abstract

Food safety and forensic applications rely seriously on identifying meat samples of unknown species. Mutton meat is delicious and high-priced meat. Its tallow is a flavoring precursor to being authenticated by using different meat species. Mutton authenticity by beef meat that is genetically related to each other makes it difficult to detect fraud of products, mainly if the substitution is applied with fat, not only meat. So, accurate methods for detection must be applied to evaluate meat fraud based not only on protein but also on fat substitution. This work aimed to evaluate the mutton meat substitution by meat and fat of beef using multiplex PCR and Gas Chromatography-Tandem Mass Spectrometry (GC/MS/MS). Multiplex PCR detects cytochrome b in mutton meat adulterated with different concentrations of beef meat, even with 0.5%. PCR products amplified at 274 bp for mutton and 271 bp for beef. Fatty acid profile of pure mutton meat (tallow) samples and mutton authenticated with beef meat by GC/MS/MS. It was found that it contained 39.48 mg/100 g of total saturated fatty acids (TSFA), 60.48 mg/100 g of total unsaturated fatty acids (TUSFA), and 3.4 mg/100 g of trans-fatty acids (TFA). While after being authenticated with beef, these results changed to 49.58, 49.57, and 6.37 mg/100gm for TSFA, TUSFA, and TFA, respectively. In conclusion, Multiplex PCR and GC/MS/MS evidenced to be accurate and applicable for the recognition of mutton meat authentication.

DOI: 10.33899/ijvs.2023.136444.2583,

Introduction

Many researchers in the fields of Food Hygiene and Veterinary Forensic Medicine in Egypt have been increasingly interested in the adulteration of food, especially meat (1-3). Mutton meat is one of the most delicious and predominant foods to many consumers worldwide as it contains high-quality protein, low cholesterol, and low fat (4). Some consumers prefer mutton products over other meat due to their better sensory attributes (5). Sheep considers common livestock that spread all over the world (6). The national production of 64,758 tons of mutton in 2020 failed to meet the demand, and an inventory of 8.7 million heads was created (7). Mutton has the highest price compared to other meat, changing between 20 and 40 USD/kg (8). Egyptian consumers prefer mutton meat products such as kebab and kofta, which have a higher price than those of beef origin. This mutton product's higher price gives the reason for some illegal traders to substitute other meat species in these products. Mutton products can be substituted by using

only mutton tallow with other meat, such as beef, to produce the flavor of mutton products. Several studies confirmed that the fat of animals is the primary precursor for meat flavor (9,10). Mixing or replacing meat products with other ingredients without labeling considers the authenticity or fraud of food at any step of processing (11). Authenticity or fraud of food considers a severe problem and a threat to health, economic, legal, and religious concerns (12,13). Moreover, it leads to losing trust and confidence between consumers and food safety regulators. Hence, methods for the detection of fraud are required for food inspectors and also, consumers. Many methods have been used for meat fraud detection, such as polymerase chain reaction (PCR), which is a faster and more reliable molecular method (14), real-time PCR (15), and advanced mass spectrometry (MS) methodologies that consider a novel analytical method for detection of food authentication due to its accuracy and sensitivity (16).

Mutton authenticity by beef meat that is genetically related to each other makes it difficult to detect fraud of products, mainly if the substitution is applied with fat, not only meat. So, accurate methods for detection must be applied to evaluate meat fraud based not only on protein but also on fat substitution. This work aimed to evaluate the mutton meat substitution by meat and fat of beef using multiplex PCR and GC/MS/MS.

Materials and methods

Ethical approve

The current work followed the guidelines of The Animal Care Committee, Faculty of Veterinary Medicine, Benha University, Egypt. Ethical number: BUFVTM 20-9-22.

Samples collection and preparation

Fresh meat specimens (mutton and beef) were collected from Al-Basateen Central Abattoir of Cairo, Egypt. Samples were minced and prepared as pure beef meat (100%), pure mutton meat (100), mutton authenticated with beef 0.5%, mutton authenticated with beef 5%, mutton authenticated with beef 30%, and mutton authenticated with beef 50% to be examined by multiplex PCR. While for GC/MS/MS, fatty acid profile evaluation samples were prepared as pure mutton meat (100), pure beef meat (100%), and a mixture of beef and mutton (50%).

Extraction of DNA

QIAamp DNA Mini Kits (Qiagen - Labsave, Germany, Catalogue no. 51304) were used for DNA extraction and purification from the meat specimens. Using silica membranes, different samples can be purified with the QIAamp DNA Mini Kit. It takes only 20 minutes to prepare spin columns without mechanical homogenization. Each sample (25 mg) was incubated overnight at 56 ˚C with 180 µl of ATL buffer and 20 µl QIAGEN protease into the bottom of a 1.5 ml microcentrifuge tube till tissue lysis, then incubated at 72 ˚C for 10 min. The mixture was centrifugated at 4000 rpm for 2 minutes to eliminate drops from the inside of the lid. Transfer the supernatant was diluted with ethanol 96%. The mixture was then put into a QIAamp DNA mini spin column. The DNA attached to the column was washed twice in 2 centrifugation stages using 2 distinct wash buffers. 50 µl buffer AE, pure DNA was eluted from the column to enhance the purity of the eluted DNA. The Spectrophotometer was used to determine the content and purity of DNA (Biometra, Germany) at 260 and 280 nm, respectively (to obtain the ratio of the absorbance) (13). DNA fragments were separated electrophoretically at 100 V for 60 min. A 100 bp plus DNA Ladder (Qiagen, Germany, GmbH) was used to determine the size of the fragments.

Cytochrome-b gene primers

Samples were extracted and examined following Doosti *et al.* (14) depending on the evaluation of the cytochrome-b (Cyt-b) gene in examined samples (Table 1).

Table 1: The sequence of Cytochrome-b gene primers for sheep and beef

Species	Primer Sequence 5'-3.'	Expected size	Reference
Sheep	Forward: ATGCTGTGGCTATTGTC Reverse: CCTAGGCATTTGCTTAATTTTA	274 bp	Doosti *et al.* (14)
Beef	Forward: GCCATATACTCTCCTTGGTGACA Reverse: GTAGGCTTGGGAATAGTACGA	271 bp	

Multiplex PCR

Emerald Amp GT PCR master mix (Takara) (Code No. RR310) kit was used for multiplex PCR amplification on a total volume of 25 µL samples. The mixtures involved in the PCR reaction contained 12.5µL of PCR master mix (RR310A Kit), 1µL forward primer, 1µL reverse primers, 6µL of extracted DNA, and 4.5µL double distilled water. For the multiplex PCR, common forward primers for beef and mutton meat were accepted and utilized in combination with species-specific oligonucleotide primers. The amplification process involved 35 cycles of initial denaturation at 94 C, followed by 10 minutes of final extension at 72 C using a thermal cycler. Molecular size markers were specified on each gel. Agarose gel electrophoresis was used to evaluate the PCR results.

DNA amplification

Finally, the amplified products were separated on ethidium bromide agarose gel and then electrophorized. Gel photographic pictures by the gel documentation system were taken and analyzed using computer software. A 100 bp DNA ladder was used.

GC/ MS/MS for evaluation of mutton meat authentication

Fatty acids profile evaluation including SFA, MUSFA, PUSFA, omega 3, and omega 6 by using GC/MS/MS. Fat was dissolved in a mixture of GC grade solvents (methanol, acetone, *n*-hexane, chloroform, and sodium methoxide) Sigma- Aldrich, USA. Fatty acid was extracted and saponified, then methylated by adding a diazomethane solution to convert to methyl esters. Methyl esters of different samples were dissolved in aliquots and chloroform solution and then analyzed by GC. GC-MS/MS (Agilent 8890-7010B/2019) evaluate fatty acids from C8 to C22 (17).

Statistical analysis

Data are displayed as mean±SD. Statistical analysis was conducted using one-way ANOVA followed by Tukey's post hoc test for multiple group comparisons using SPSS for Windows (Version 21.0; SPSS Inc., Chicago, IL, USA).

Results

Evaluation of mutton meat authentication by using multiplex PCR

The results in the current study presented in figure 1 revealed multiplex PCR product by agarose gel electrophoreses Cyt-b gene for mutton and beef meat, amplified at 274 bp for mutton and 271 bp for beef. Multiplex PCR detects all the authenticated samples at different concentrations for mutton and beef meat (Figure 1).

Evaluation of SFA in mutton, beef, and mutton plus beef meat samples

In the current study and as shown in table 2, the results of the SFA in the samples of mutton plus beef 50% compared to the samples of mutton meat showed a significant increase in the levels of palmitic acid (C16) and stearic acid (C18). Moreover, the levels of pentadecanoic acid (C15), margaric acid (C17), and nonadecanoic acid (C19) were significantly decreased. However, there were no significant changes in the levels of lauric acid (C12), myristic acid (C14), and arachidic acid (C20). Furthermore, when we compared the results of SFA of mutton plus beef with the beef meat, we found there was a significant elevation in the contents of (C15) and (C17) and a significant reduction in the contents of (C12), (C14), (C18), and (C19). There was no significant change in the levels of (C16) and (C20). The total SFA results showed a significant increase in the mutton plus beef 49.58% compared to the mutton meat 39.48% by a percentage of 25.58%.

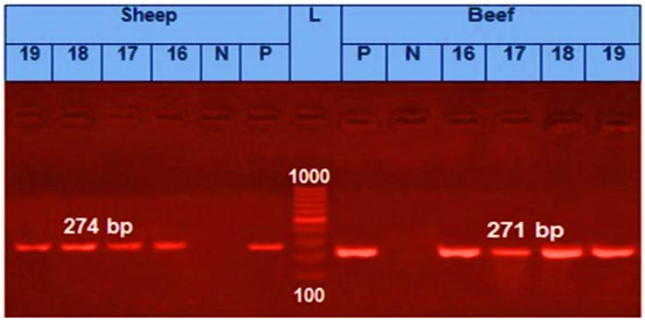

Figure 1: Agarose gel electrophoresis for multiplex PCR product of cyt -b gene of mutton meat authenticated with beef meat. All samples (from lane 16 to lane 19) gave a positive band at 274 bp for mutton and 271 bp for beef. Lane L: Molecular weight marker 100bp DNA ladder. Lane P: Positive control. Lane N: Negative control. Lane 16: Mutton authenticated with Beef 0.5%. Lane 17: Mutton authenticated with Beef 5%. Lane 18: Mutton authenticated with Beef 30%. Lane 19: Mutton authenticated with Beef 50%.

Evaluation of MUSFA in mutton, beef, and mutton plus beef meat samples

The findings of MUSFA in the mutton plus beef meat were significantly reduced for palmitoleic acid (C16:1), 10-heptadecenoic acid (C17:1), and Cis-vaccenic acid (C18:1c6), Trans-vaccenic acid (C18:1n11t), 10-nonadecenoic acid (C19:1), and 11-eicosenoic acid (C20:1) compared to that of mutton meat. While the findings of elaidic acid (C18:1n9t) were significantly elevated in the samples of mutton plus beef meat compared to the samples of mutton meat. No significant changes occurred in the levels of myristoleic acid (C14:1) and oleic acid (C18:1n9) of the mutton plus beef meat compared to the beef meat (Table 3). The results of MUSFA of the samples of mutton plus beef showed a significant elevation of (C14:1) and (C17:1), a significant reduction of (C16:1), and no significant changes in (C18:1n9t), (C18:1c6), (C18:1n9), (C18:1n11t), C19:1), and 11-eicosenoic acid (C20:1) compared to the beef meat samples. The total MUSFA in the mutton-authenticated beef samples was 48.59 mg/100 g (Table 3). According to the previous results, the levels of the total MUSFA of the mutton plus beef samples were reduced by 16.98% compared to the mutton meat.

Evaluation of PUSFA in mutton, beef, and mutton plus beef meat samples

As shown in table 4, the PUSFA results of the mutton plus beef samples exerted a significant elevation in the linolenic acid (C18:3), a significant reduction in the linoelaidic acid (C18:2n6t), and no significant changes in linoleic acid (C18:2n6), 7,10-octadecadienoic acid (C18:1), and 8,11-eicosadienoic acid (C20:3) levels compared to the mutton meat samples. Whereas, the comparison of the results

of mutton authenticated beef meat with the beef meat showed a significant increase in (C18:2n6t), a significant decrease in (C20:3), and no significant change in (C18:2n6), (C18:3), and (C18:1) levels. The total PUSFA contents in the mutton, mutton plus beef, and beef meat samples were 2.09, 1.22, and 0.98 mg/100 g, respectively. From the previous findings, the total PUSFA of mutton plus beef was reduced by 53.11% compared to the mutton meat samples.

Table 2: SFA in mutton, beef, and mutton plus beef meat samples by using GC/ MS/MS

Parameters		Groups			Significant			
		Mutton	Beef	Mutton+Beef	F	P	P post Hoc	LSD
Saturated Fatty Acids	C12	0.10±0.01	0.14±0.01	0.07±.017	22.200	0.002*	A: 0.021* B: 0.066 C: 0.001*	0.02
	C14	2.70±0.36	5.86±0.56	3.20±0.40	42.527	0.000*	A: 0.000* B: 0.420 C: 0.001*	0.62
	C15	2.88±0.20	0.70±0.10	1.51±0.02	204.264	0.000*	A: 0.000* B: 0.000* C: 0.001*	0.18
	C16	14.88±0.51	21.87±1.03	20.95±0.62	75.123	0.000*	A: 0.000* B: 0.000* C: 0.362	1.03
	C17	6.22±0.43	1.57±0.46	3.13±0.54	71.417	0.000*	A: 0.000* B: 0.001* C: 0.018*	0.66
	C18	12.39±0.84	27.33±1.25	20.56±1.09	144.779	0.000*	A: 0.000* B: 0.000* C: 0.001*	0.70
	C19	0.25±0.08	0.10±0.01	0.09±0.02	9.038	0.015*	A: 0.028* B: 0.021* C: 0.970	0.08
	C20	0.06±0.01	0.10±0.01	0.07±0.02	6.500	0.031*	A: 0.031* B: 0.679 C: 0.090	0.02
TSFA		39.48±1.87	57.91±1.89	49.58±0.92	96.586	0.000*	A: 0.000* B: 0.001* C: 0.002*	2.2

F one-way ANOVA, p: significant at level <0.05, P: post Hoc, A: Mutton - Beef, B: Mutton - Mutton+Beef, C: Beef - Mutton+Beef, In the case of Least Significant Differences (LSD), a significant result means at least one group differs from the other groups. (C12): lauric acid, (C14): myristic acid, (C15): pentadecanoic acid, (C16): palmitic acid, (C17): margaric acid, (C18): stearic acid, (C19): nonadecanoic acid, (C20): arachidic acid, TSFA: total saturated fatty acids.

Total USFA content in mutton, beef, and mutton plus beef meat samples

The content of the total USFA in the mutton plus beef samples was significantly reduced compared to the mutton meat and significantly increased compared to the beef meat samples (Table 5).

Trans fatty acids content in mutton, beef, and mutton plus beef meat samples

In our work, the trans fatty acids content of the mutton plus beef samples was significantly increased compared to the mutton meat. It was significantly decreased compared to the beef meat samples (Table 5).

Evaluation of omega 3 and 6 content in mutton, beef, and mutton plus beef meat samples

The results of omega 3 and 6 are illustrated in table 5. There was a significant increase of Omega 3 in the mutton plus beef meat samples compared to the mutton samples. While there was no significant change in the omega 3 of mutton plus beef samples compared to the beef meat samples. Furthermore, the findings of omega 6 content showed no significant change between the specimens of mutton, beef, and mutton plus beef meat.

Table 3: MUSFA in mutton, beef, and mutton plus beef meat samples by using GC/ MS/MS

Parameters		Groups			Significant			
		Mutton	Beef	Mutton+Beef	F	P	P post Hoc	LSD
Mono-unsaturated Fatty Acids	C14:1	0.12±0.02	0.80±0.10	0.22±0.02	111.114	0.000*	A: 0.000* B: 0.155 C: 0.000*	0.08
	C16:1	0.47±0.01	0.23±0.05	0.14±0.02	80.113	0.000*	A: 0.000* B: 0.000* C: 0.026*	0.04
	C17:1	3.85±0.32	0.38±0.08	1.59±0.17	193.881	0.000*	A: 0.000* B: 0.000* C: 0.001*	0.3
	C18:1n9t	0.00±0.00	3.58±0.55	2.48±0.55	47.335	0.000*	A: 0.001* B: 0.000* C: 0.065	1.4
	C18:1c6	15.13±1.14	1.19±1.56	1.95±0.43	139.650	0.000*	A: 0.000* B: 0.000* C: 0.709	1.56
	C18:1n9	35.88±0.96	35.37±1.05	39.34±3.76	2.589	0.155	A: 0.961 B: 0.241 C: 0.172	4.46
	C18:1n11t	2.56±0.25	0.80±0.10	1.06±0.06	106.432	0.000*	A: 0.000* B: 0.000* C: 0.196	0.22
	C19:1	0.29±0.02	0.05±0.01	0.08±0.01	171.000	0.000*	A: 0.000* B: 0.000* C: 0.165	0.02
	C20:1	0.15±0.01	0.07±0.01	0.08±0.01	57.000	0.000*	A: 0.000* B: 0.000* C: 0.483	0.01
TMUSFA		58.39±0.89	40.87±1.57	48.59±1.57	120.9	0.000*	A: 0.000* B: 0.001* C: 0.001*	2.15

F one-way ANOVA, p: significant at level <0.05, P: post Hoc, A: Mutton - Beef, B: Mutton - Mutton+Beef, C: Beef - Mutton+Beef, In the case of Least Significant Differences (LSD), a significant result means at least one group differs from the other groups. (C14:1): myristoleic acid, (C16:1): palmitoleic acid, (C17:1): 10-heptadecenoic acid, (C18:1n9t): elaidic acid, (C18:1c6): Cis-vaccenic acid, (C18:1n9): oleic acid, (C18:1n11t): Trans-vaccenic acid, (C19:1): 10-nonadecenoic acid, (C20:1): 11-eicosenoic acid, TMUSFA: total monounsaturated fatty acids.

Discussion

Meat hygiene and forensic applications must consider the animal species in an unknown meat sample with the rising existence of illegal incidents such as meat fraud. One of the most popular meat products is mutton because of their nutritional properties, sensory attributes, physicochemical composition, and characteristic flavor compared to other meat species (18). As well as its healthier consideration (19). So, many higher educational consumers prefer mutton over other meat (20). The authenticity of mutton meat products to decrease the cost of production widely speeded in Egyptian markets and restaurants.

In this study, the mitochondrial gene cytochrome-b was used to detect mutton meat authenticity even if adulterated with a closely related gene, such as beef meat. Multiplex PCR detected even a small concentration of adulteration, even 0.5%. These results confirmed the viability of multiplex PCR for detecting meat fraud and agree with Dalmasso *et al.* (21), Hossain *et al.* (22), and Wang *et al.* (23). Galal-Khallaf also recommended DNA-based methods, such as multiplex PCR, for the detection of meat authentication due to its accuracy, fast, and sensitivity (24). Species-specific targeting mitochondrial (mt) genes have received significant attention (25,26).

Table 4: PUSFA in mutton, beef, and mutton plus beef meat samples by using GC/ MS/MS

Parameters		Groups			Significant			
		Mutton	Beef	Mutton + Beef	F	P	P post Hoc	LSD
Poly-unsaturated Fatty Acids	C18:2n6	1.09±0.41	1.01±0.01	0.46±0.35	3.591	0.094	A: 0.948 B: 0.107 C: 0.160	0.55
	C18:2n6t	0.80±0.10	0.00±0.00	0.28±0.02	142.615	0.000*	A: 0.000* B: 0.000* C: 0.003*	0.11
	C18:3	0.00±0.00	0.05±0.01	0.03±0.01	28.500	0.001*	A: 0.001* B: 0.010* C: 0.054	0.02
	C18:1	0.10±0.02	0.10±0.01	0.08±0.01	0.67	0.54	A: 1.000 B: 0.001 C: 0.001	0.04
	C20:3	0.00±0.00	0.05±0.01	0.00±0.00	75.000	0.000*	A: 0.000* B: 1.000 C: 0.000*	0.0001
TPUSFA		2.09±0.78	1.22±.02	0.98±0.10	4.863	0.055	A: 0.129 B: 0.052 C: 0.762	0.76

F one-way ANOVA, p: significant at level <0.05, P: post Hoc, A: Mutton - Beef, B: Mutton - Mutton+Beef, C: Beef - Mutton+Beef, In the case of Least Significant Differences (LSD), a significant result means at least one group differs from the other groups. (C18:2n6): linoleic acid, (C18:2n6t): linoelaidic acid, (C18:3): linolenic acid, (C18:1): 7,10-octadecadienoic acid, (C20:3): 8,11-eicosadienoic acid, TPUSFA: total polyunsaturated fatty acids.

Table 5: Omega3, Omega 6, TUSFA, and TFA in mutton, beef, and mutton plus beef meat samples by using GC/ MS/MS

Parameters	Groups			Significant			
	Mutton	Beef	Mutton+Beef	F	P	P post Hoc	LSD
Omega 3	0.00±0.00	0.05±0.01	0.03±0.01	28.500	0.001*	A: 0.001* B: 0.010* C: 0.054	0.02
Omega 6	1.09±0.95	1.01±0.01	0.64±0.06	0.563	0.597	A: 0.983 B: 0.607 C: 0.707	2.86
TUSFA	60.48±0.62	42.09±1.59	49.57±1.15	180.9	0.000*	A: 0.000* B: 0.001* C: 0.002*	2.16
TFA	3.40±0.60	3.69±0.41	6.37±0.82	20.042	0.002*	A: 0.845 B: 0.003* C: 0.005*	5.74

F one-way ANOVA, p: significant at level <0.05, P: post Hoc, A: Mutton - Beef, B: Mutton - Mutton+Beef, C: Beef - Mutton+Beef, In the case of Least Significant Differences (LSD), a significant result means at least one group differs from the other groups. TUSFA: total unsaturated fatty acids, TFA: trans fatty acids.

Moreover, using cytochrome b as a mitochondrial gene is exceptionally conserved in different species of animals (27), because it produces a high copy number of small, circular mitochondrial DNA per cell and resists the processing temperature of food products (28). Many previous studies confirmed the efficiency of PCR sequencing of mitochondrial genes in accurately detecting meat authentication (fresh or processed meat products), including sheep, cattle, buffalo, chicken, and goats (13,29), as it is very powerful in differentiating between different genes (30). Moreover, it is a rapid, sensitive, and accurate method (31).

Concerning the fatty acids profile of mutton meat (tallow) by GC/MS/MS, results revealed that mutton meat containing mutton tallow (MT) is composed of SFA 39.48%.

These results changed after mutton meat was experimentally authenticated by beef 50% to be 49.58% for total SFA, 50.42 for total USFAC, and 6.37 for Trans Fatty Acids. These results are lower than those reported by Liu *et al.* (32), who find that it is about 62% of saturated fatty acids in mutton tallow. The results differ regarding the age, breed, and nutrition of animals. The average percentage of myristic acid (C14) in mutton meat in the current study is in the same range as that reported by Banskalieva *et al.* (33), and USDA (34). Other fatty acids, palmitic acid (C16), stearic acid (C18), palmitoleic acid (C16.1), 7,10-octadecadienoic acid (C18.1), linoleic acid (C18.2), and linolenic acid (C18.3), of mutton meat in our experiment were slightly reduced than those in the previous studies. GC/MS recently proved accurate and sensitive for detecting meat and fat fraud (35,36). Recent studies were performed to examine the food safety of different types of meat (37-39). In Egyptian marketplaces, the addition of an undeclared meat species is previously reported by Zahran *et al.* (3), and Yacoub *et al.* (40) worldwide Jin *et al.* (41), and Li *et al.* (42).

Conclusions

Mutton meat authentication with beef is hardly detected by traditional ways of detection due to the genetic relationship between the two species. Multiplex PCR proved an accurate method for detecting mutton meat adulteration with beef, even at low concentrations. Moreover, GC/MS/MS provides an illustrated fatty acids profile for mutton that is affected if authenticated with other species. Multiplex PCR and GC/MS/MS have been established to be valued techniques for the forensic identification of species and detecting fraud and adulteration of mutton meat.

Conflict of interest

The authors of this article have no conflict of interest.

References

1. Ahmed H, El-Nasser MA, Mohammed D, Mohamed M. Identification of meat species in some raw meat products in Assiut city, Egypt. Proceedings of the XVth International Congress of the International Society for Animal Hygiene. Vienna: Tribun EU; 2011. 973-975 p.
2. Omran GA, Tolba AO, El-Sharkawy EE, Abdel-Aziz DM, Ahmed HY. Species DNA-based identification for detection of processed meat adulteration: Is there a role of human short tandem repeats (STRs)?. Egypt J Forensic Sci. 2019;9(1):1-8. DOI: 10.1186/s41935-019-0121-y
3. Zahran D, Hagag S. Use of molecular biology techniques in the detection of fraud meat in the Egyptian market. Afr J Biotechnol. 2015;14(5):360-364. DOI: 10.5897/ajb2014.14297
4. Sun S, Guo B, Wei Y, Fan M. Multi-element analysis for determining the geographical origin of mutton from different regions of China. Food Chem. 2011;124(3):1151-1156. DOI: 10.1016/j.foodchem.2010.07.027
5. Alanís PJ, Miranda-de la Lama GC, Mariezcurrena-Berasain MA, Barbabosa-Pliego A, Rayas-Amor AA, Estévez-Moreno LX. Sheep meat consumers in Mexico: Understanding their perceptions, habits, preferences and market segments. Meat Sci. 2022;184,108705. DOI: 10.1016/j.meatsci.2021.108705
6. Aziz TA, Lafta IJ. Isolation and antimicrobial resistance of *Staphylococcus spp*., enteric bacteria, and *Pseudomonas spp*. associated with respiratory tract infections of sheep. Iraqi J Vet Sci. 2021;35:53-58. DOI: 10.33899/ijvs.2021.131098.1917
7. SIAP. Servicio de Informacion Agroalimentaria y Pesquera. Ovino. Poblacion ganadera 2011-2020. Cabezas; 2020. [available at]
8. Thomé-Ortiz H. Heritage cuisine and identity: Free time and its relation to the social reproduction of local food. J Herit Tour. 2018;13(2):104-114. DOI: 10.1080/1743873x.2017.1343336
9. Xu Y, Chen Q, Lei S, Wu P, Fan G, Xu X, Pan S. Effects of lard on the formation of volatiles from the Maillard reaction of cysteine with xylose. J Sci Food Agric. 2011;91(12):2241-2246. DOI: 10.1002/jsfa.4445
10. Ma X, Zhan P, Tian H, Wei Z, Wang P. Effects of different enzymatic hydrolyses of mutton tallow on the aroma characteristics of the Maillard reaction of xylose-cysteine based on GC-MS, E-Nose, and statistical analysis. Eur J Lipid Sci Technol. 2020;122(3):1900212. DOI: 10.1002/ejlt.201900212
11. Prusakova OV, Glukhova XA, Afanas'eva GV, Trizna YA, Nazarova LF, Beletsky IP. A simple and sensitive two-tube multiplex PCR assay for simultaneous detection of ten meat species. Meat Sci. 2018;137:34-40. DOI: 10.1016/j.meatsci.2017.10.017
12. Flauzino JR, Alves LM, Rodovalho VR, Madurro JM, Brito Madurro AG. Application of biosensors for detection of meat species: A short review. Food Control. 2022;142:109214. DOI: 10.1016/j.foodcont.2022.109214
13. Hassan KI, Mohamed AA, Mohammed NA. Detection of the origin of animal species in kebab meat using mitochondrial DNA based-polymerase chain reaction (mtDNA-PCR). Iraqi J Vet Sci. 2019;33(1):39-43. DOI: 10.33899/ijvs.2019.125516.1038
14. Doosti A, Ghasemi P, Rahimi E. Molecular assay to fraud identification of meat products. J Food Sci Technol. 2014;51(1):148-152. DOI: 10.1007/s13197-011-0456-3
15. Li W, Luo Y, Liu C, Dou L, Zhao L, Su L, Jin Y. Effects of feeding patterns on volatile flavor components and fatty acid composition of Sunit sheep meat. Food Sci. 2019;40(24):207-213. DOI: 10.7506/spkx1002-6630-20190107-095
16. Valletta M, Ragucci S, Landi N, Di Maro A, Pedone PV, Russo R, Chambery A. Mass spectrometry-based protein and peptide profiling for food frauds, traceability, and authenticity assessment. Food Chem. 2021;365:130456. DOI: 10.1016/j.foodchem.2021.130456
17. Ackman RG. The gas chromatograph in practical analyses of common and uncommon fatty acids for the 21st century. Anal Chim Acta. 2002;465(1-2):175-192. DOI: 10.1016/s0003-2670(02)00098-3
18. Teixeira A, Silva S, Rodrigues S. Advances in sheep and goat meat products research. Adv Food Nutr Res. 2019;87:305-370. DOI: 10.1016/bs.afnr.2018.09.002
19. Coleman G, Jongman E, Greenfield L, Hemsworth P. Farmer and public attitudes toward lamb finishing systems. J Appl Anim Welf Sci. 2016;19(2):198-209. DOI: 10.1080/10888705.2015.1127766
20. Alanís PJ, Miranda-de la Lama GC, Mariezcurrena-Berasain MA, Barbabosa-Pliego A, Rayas-Amor AA, Estévez-Moreno LX. Sheep meat consumers in Mexico: Understanding their perceptions, habits, preferences and market segments. Meat Sci. 2022;184:108705. DOI: 10.1016/j.meatsci.2021.108705
21. Dalmasso A, Fontanella E, Piatti P, Civera T, Rosati S, Bottero MT. A multiplex PCR assay for the identification of animal species in feedstuffs. Mol Cell Probes. 2004;18(2):81-87. DOI: 10.1016/j.mcp.2003.09.006
22. Hossain MM, Ali ME, Abd Hamid SB, Mustafa S, Desa MN, Zaidul IS. Targeting double genes in multiplex PCR for discriminating bovine, buffalo, and porcine materials in food chain. Food Control. 2017;1(73):175-84. DOI: 10.1016/j.foodcont.2016.08.008
23. Wang L, Hang X, Geng R. Molecular detection of adulteration in commercial buffalo meat products by multiplex PCR assay. Food Sci Technol. 2019;39:344-348. DOI: 10.1590/fst.28717

24. Galal-Khallaf A. Multiplex PCR and 12S rRNA gene sequencing for detection of meat adulteration: A case study in the Egyptian markets. Gene. 2021;764:145062. DOI: 10.1016/j.gene.2020.145062
25. Alikord M, Keramat J, Kadivar M, Momtaz H, Eshtiaghi MN. Multiplex-PCR as a rapid and sensitive method for identification of meat species in halal-meat products. Recent Pat Food Nutr Agric. 2017;8:175-182. DOI: 10.2174/2212798409666170113151213
26. Qin P, Qu W, Xu J, Qiao D, Yao L, Xue F, Chen W. A sensitive multiplex PCR protocol for simultaneous detection of chicken, duck, and pork in beef samples. J Food Sci Technol. 2019;56(3):1266-1274. DOI: 10.1007/s13197-019-03591-2
27. Van der Kuyl AC, Kuiken CL, Dekker JT, Goudsmit J. Phylogeny of African monkeys based upon mitochondrial 12S rRNA sequences. J Mol Evol. 1995;40(2):173-180. DOI: 10.1007/BF00167111
28. Partis L, Croan D, Guo Z, Clark R, Coldham T, Murby J. Evaluation of a DNA fingerprinting method for determining the species origin of meats. Meat Sci. 2000;54(4):369-376. DOI: 10.1016/s0309-1740(99)00112-6
29. Mahajan MV, Gadekar YP, Dighe VD, Kokane RD, Bannalikar AS. Molecular detection of meat animal species targeting MT 12S rRNA gene. Meat Sci. 2011;88(1):23-27. DOI: 10.1016/j.meatsci.2010.11.026
30. Sheet OH. Molecular detection of mecA gene in methicillin-resistant *Staphylococcus aureus* isolated from dairy mastitis in Nineveh governorate, Iraq. Iraqi J Vet Sci. 2022;36(4):939-943. DOI: 10.33899/ijvs.2022.132643.2115
31. Abdelmonem GG, Amer AM, Hussein EA, Aboezz ZR, Habashi AR, Sharawi SS. Assessment of multiplex PCR for detection of FMDV, BVDV, BTV, and possible coinfection with *Pasteurella multocida* in cattle. Iraqi J Vet Sci. 2022;36(4):1053-1059. DOI: 10.33899/ijvs.2022.132983.2158
32. Liu J, Zhang W, Liu D, Zhang W, Ma L, Wang S. Physicochemical properties of a new structural lipid from the enzymatical incorporation of flaxseed oil into mutton tallow. Heliyon. 2022;8(6):e09615. DOI: 10.1016/j.heliyon.2022.e09615
33. Banskalieva VV, Sahlu T, Goetsch AL. Fatty acid composition of goat muscles and fat depots: A review. Small Rumin Res. 2000;37(3):255-268. DOI: 10.1016/s0921-4488(00)00128-0
34. USDA. Food Data Central. US Department of Agriculture Agricultural Research Service. [available at]
35. Montowska M, Fornal E. Absolute quantification of targeted meat and allergenic protein additive peptide markers in meat products. Food Chem. 2019;274:857-864. DOI: 10.1016/j.foodchem.2018.08.131
36. Pavlidis DE, Mallouchos A, Ercolini D, Panagou EZ, Nychas GE. A volatilomics approach for off-line discrimination of minced beef and pork meat and their admixture using HS-SPME GC/MS in tandem with multivariate data analysis. Meat Sci. 2019;151:43-53. DOI: 10.1016/j.meatsci.2019.01.003.
37. Al-Naemi HS, Al-Sanjary RA, Faraj RA. Detection of lead, chromium, and cobalt in meats of cattle and buffalo from retails of Mosul city. Iraqi J Vet Sci. 2020;34(2):447-451. DOI: 10.33899/ijvs.2019.126069.1224
38. Shaapan R, Toaleb NI, Abdel-Rahman EH. Detection of *Toxoplasma gondii*-specific immunoglobulin (IgG) antibodies in meat juice of beef. Iraqi J Vet Sci. 2021;35(2):319-324. DOI: 10.33899/ijvs.2020.126829.1390
39. Al-Musawi AM, Awad AH, Alkhaled MJ. Molecular analysis of *Cryptosporidium* species in domestic goat in central Iraq. Iraqi J Vet Sci. 2022;36(4):1041-1045. DOI: 10.33899/ijvs.2022.132974.2155
40. Yacoub HA, Sadek MA. Identification of fraud (with pig stuffs) in chicken-processed meat through information of mitochondrial cytochrome b. Mitochondrial DNA A DNA Mapp Seq Anal. 2017;28(6):855-859. DOI: 10.1080/24701394.2016.1197220
41. Jin P, Ding H, Li P, Fan L, Fu C. Analysis of meat products adulterated in Suzhou area in 2013. Chinses J Food Hyg. 2014;26:168-172. [available at]
42. Li N, Wang J H, Shen Q, Li FQ, Xu J, Jiang T. Survey of duck, chicken and pork component in lamb or beef slices sold in Beijing. Chinses J Food Hyg. 2014;26(03):227-232. [available at]

الكشف عن الغش في لحوم الضأن مع لحوم البقر كأنواع مرتبطة وراثيا باستخدام تفاعل البلمرة المتسلسل المتعدد وتحليل الكروماتوجرافى الغازي/التحليل بالمطياف الكلى

السيد عبدالرحمن عبدالرحمن[١]، محمد خيرى مرسى[٢]، سمر صابر إبراهيم[٣]، عصام إسماعيل الطوخى[٤]، إيهاب يحيى عبدالحى[٥] و رشا عبدالحميد الصباغ[٦]

[١]مجزر البساتين، مديرية الطب البيطري القاهرة، [٢]قسم تكنولوجيا الأغذية، كلية الزراعة، [٣]قسم الطب الشرعي والسموم، كلية الطب البيطري، جامعة بنها، القليوبية، [٤]قسم البيوتكنولوجي، معهد بحوث صحة الحيوان، الدقي، [٥]قسم الطب الشرعي والسموم، كلية الطب البيطري، جامعة مطروح، مطروح، [٦]قسم الرقابة الصحية على الأغذية، كلية الطب البيطري، جامعة بنها، القليوبية، مصر

الخلاصة

يعتبر تحديد الأنواع لعينات اللحوم غير المعروفة ذات أهمية حيوية لتطبيقات الطب الشرعي وسلامة الأغذية. لحم الضأن هو لحم لذيذ وغالي الثمن. شحمه له نكهة مميزة تجعله في مقدمة الغش باستخدام أنواع مختلفة من اللحوم. غش لحم الضأن عن طريق لحوم البقر المرتبطة ببعضها البعض وراثيًا تجعل من الصعب اكتشاف الغش في المنتجات خاصةً إذا تم تطبيق الاستبدال بالدهون وليس اللحوم فقط. لذلك، يجب تطبيق طرق دقيقة للكشف عن الاحتيال على اللحوم ليس فقط على أساس البروتين ولكن أيضًا على استبدال الدهون. تهدف هذه الدراسة إلى تقييم استبدال لحم الضأن باللحوم ودهن اللحم البقري باستخدام تفاعل البلمرة المتسلسل المتعدد وتحليل الكروماتوجرافى الغازي/التحليل بالمطياف الكلى. يكشف تفاعل البلمرة المتسلسل المتعدد عن السيتوكروم ب في لحم الضأن المغشوش بتركيزات مختلفة من لحم البقر حتى مع ٠,٥٪. ظهرت منتجات تفاعل البلمرة المتسلسل المتعدد عند ٢٧٤ نقطة أساس للضأن و ٢٧١ نقطة أساس للحوم البقر. ملف تعريف الأحماض الدهنية لعينات لحم الضأن النقي ولحم الضأن المخلوط مع لحم البقر بواسطة تحليل الكروماتوجرافى الغازي/التحليل بالمطياف الكلى وجد أنه يحتوي على ٣٩,٤٨ مجم/١٠٠جم من إجمالي الأحماض الدهنية المشبعة، ٦٠,٤٨ مجم/١٠٠جم من إجمالي الأحماض الدهنية غير المشبعة و٣,٤ مجم/١٠٠جم من الأحماض الدهنية المتحولة. بينما بعد الخلط مع لحم البقر، تغيرت هذه النتائج إلى ٤٩,٥٨ و ٤٩,٥٧ و ٦,٣٧ مجم/١٠٠جرام لكل من إجمالي الأحماض الدهنية المشبعة، إجمالي الأحماض الدهنية غير المشبعة والأحماض الدهنية المتحولة على التوالي. في الختام، أثبتت طرق تفاعل البلمرة المتسلسل المتعدد وتحليل الكروماتوجرافي الغازي/التحليل بالمطياف الكلى أنها دقيقة وقابلة للتطبيق للكشف عن الغش في لحم الضأن.