البحث الرابع

Zagazig Veterinary Journal

Volume 35
Number 2

E-mail: zvj@mail.zu.edu.eg

Zag. Vet. J. (ISSN. 1110-1458) Vol. 35, No.2 (2007) PP.10-18

Identification Of Meat Species In Some Ready To Eat Meat Products Sold In Egyptian Markets

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ABSTRACT

Identification of species adulteration in ready-to-eat meat products is important for consumer protection, food labeling law enforcement and in forensic analysis. In this study, 225 samples from kabab, grilled kofta and meat loaves (75 of each) were collected from Kalubia,Gharbia and Menuofia governorates for detection of forbiddened meat added to such popular food. All samples were analyzed by precipitation test, glycogen, sulphuric acid heating test. Erlich test and ELISA assay.

From our study we noted that all the examined kabab samples were free from canine meat but contain 4%,28% and 12% equine meat in Kalubia, Gharbia and Menuifia governorated. respectively, but in case of grilled kofta, canine meat was detected in 4% of examined samples in Gharbia governorate only, while equine meat was present in 8%,32% and 16% of the analyzed samples in Kalubia, Gharbia and Menufia governorates respectively, by ELISA assay. Moreover, 4% of the examined meat loaves were adulterated with canine meat in Gharbia governorate, while 8.32 and 28% of the examined meat loaves samples was adulterated with equine meat. Comparing the different methods used in this study we can conclude that the Erlich test is indicative only in positive cases, while precipitaion and glycogen tests are highly sensitive in negative cases of meat adulteration, when comparing with the more accurate ELISA assay. Also, using the ELISA, techniques the determination of meat species content in cooked mixed meat product is posible and preferable.

INTRODUCTION

It is important for consumers, retails and food regulatory bodies that meat products are of a consistently high quality acuthentic, and have not been subjected to adultration by any lower-grade material either by accident or for economic gain *(1)* because adultration of high quality meat and meat products with their inferior/cheaper counterprts is a problem in meat industry *(2)*. The detection of species adultration in meat products is important for consumer protection and food-labeling law enforcement *(3)*.

It has been reported that some opportunist people may market the meat of animal species that the society normally does not consume to meet the demand and increase their profit. This unethical practice occurs primarily by mixing the meat of unacceptable species into that of livestock meat through grinding and/ or processing, or less commonly by direct marketing of the flesh *(4)*. It is largely agreed that this practice is adulteration in regard to religious, ethical, economic and health aspects *(5)*.

Preventing adulteration of meat foods with less desirable or objectionable meat species is important for economic, religious and health reasons. In addition, determination of the species of origin of the meat components in meat products is an important task in food hygiene, food control, food codex and veterinary forensic medicine. Adulteration of meat species is important for people whose religious practices limit the types of meat they eat, and for people who have allergies to certain types of meat proteins *(6)*.

Single and multispecies adulterations have been reported in commercial meat products. The addlteration rate of cooked meat products (22.9%) was higher than that of raw meats (15.9%) *(7)*.

Most of the methods used for identification of species of meat have been reported to have limitations in use because of problems in specificity (i.e., sensory analysis, glycogen level, histological differentiation, properties of tissue fat, immunological methods, complexity (i.e., electrophoresis, DNA hybridization), high cost (i.e., NDA hybridization) and some requirements for baseline data about the differences in protein composition (i.e.; isoelectrofocusing) *(5).* Also, meat species can be identified by serological *(8)*, histological *(9)*, immunochemical *(10)* or molecular biological *(11)* methods , but enzyme-linked immunosorbent assay (ELISA) which is highly sensitive , specific and practical, is the most effective and widely used method for detecting meat species in meat and meat products *(12)* .

The objective of the present study was to identify meats of mutton , beef, equine and canine meat in three different products (Kabab,grilled kofta and meat loaves) in three governorates (Kalubia, Gharbia and Menufia) by precepitation, glycogen test and then confirmation for equine meat by sulphuric acid and Erlich test and for equine and canine by monoclonal antibodies Sandwich ELISA test kits.

MATERIAL AND METHODS

A total of 225 random samples of ready-to-eat kabab,grilled kofta and meat loaves (75 of each) were equally collected from Kalyobia ,Gharbia , and Menufia governorates for detection of forbidden meat added to such popular food items. All collected samples were directly transferred to the laboratory for detection of the kind of meat by the following tests:

Precipitation test *(13)*

All the examined meat products were free from fat and salt. Thus, fat dissolvement was obtained using ether chloroform mixture. On the other hand, salt extraction was carried out by washing the sample several times with distilled water till complete removal of the salt. Accurately, 100ml of physiological normal saline was added to 50 grams of the sample (free from fat and salt) and left for 12 hours in a refrigerator. The mixture was then filtered to obtain clear meat extract. Moreover, the meat extract was tested with sheep, bovine, horse, dog and cat antisera in small precipitating tubes by the addition of one drop of antiserum to one drop of extract. Appearance of precipitation on the bottom or the wall of precipitating tube was considered positive reaction.

Glycogen test *(14):*

Twenty grams of the sample were minced and digested by 80 ml alcoholic solution of potassium hydroxide (10%). The mixture was filtered and neutralized. The glucose content was estimated by using spectrophotometer (Spectronic 21). Further, the glycogen percentage was calculated by the following formula:

Glycogen % = glucose % x 0.927

All samples contained higher glycogen contents (more than 0.25 %) were subjected to confirmatory tests for detection of equine meat as follows:

Sulphuric acid heating test *(15)*

Few drops of concentrated sulphuric acid were added during heating of suspected meat sample. The equine meat exhibited repulsive odour resemble horse stable as well as yellow oily globules were appeared on the broth during cooking.

Erlich test *(15)*

It is applied for demonstration of equine meat. The suspected flesh was soaked in formaline for 48 hours. Smelling of an intensive characteristic odour resemble that of roasted geese indicated equine meat.

Application of Enyme-linked Immunosorbant Assay (ELISA)

Identification of animal species in meat by ELISA was carried out according to the recent adapted method *(6).* At least, 25 g of meat sample were prepared for analysis by blending in 100 ml distilled water. The resulting mixture was then heated at 100 °C for 15 minutes followed by liquid filtration through Whatman filter paper (No.4)

Therefore, the cooked meat species identification kits (ELISA kits) for equine and canine meat were used by Sonicor microcomputer emiautomatic ELISA reader in the control laboratory of Fac.of Vet.Med. Benha Univ.. The kits were purchased from Welcome diagnostic A division, Dartford. England, DA 15 AH.

Statistical analysis of the obtained results was carried out using *ANOVA* test *(16)*

RESULTS

Results of this study are presented in the following Tables.

Table 1. Detection of different meats used in kabab manufacture in three different governorates in Egypt by precipitation test (n = 25).

Meat type	Kalubia Governorate		Gharbia Governorate		Menofia Governorate	
	No.	%	No.	%	No.	%
Mutton	19	76	14	56	20	80
Beef	5	20	4	16	2	8
Equine	1	4	7	28	3	12
Canine	-	-	-	-	-	-
Total	25	100	25	100	25	100

Table 2. The glycogen content % of kabab samples (n= 25) collected from the three the Egyptian governorates

Governorate	Min.	Max.	Mean+ S.E.	Adultrated samples	
				No.*	%
Kalubia **	0.13	0.97	0.21 ± 0.03	2	8
Gharbia	0.18	1.79	0.55 ± 0.04	9	36
Mehofia	0.14	1.15	0.34 ± 0.03	4	16

* No. of samples had higher glycogen content as compared with normal standard limits (up top 0.25)
** ANOVA test indicate significant differences (P<0.01)

Table 3. Confirmatory tests for detection of equine meat in adultrated kabab samples (n=25).

Test Governorate	Adultrated samples by p.p.t. test		Adultrated samples by glycogen test		+ve sulphuric acid test for equine meat		+ve Erlich test for equine meat		ELISA test	
	No.	%	No.	%	No.	%	No.	%	No.	%
Kalubia	1	4	2	8	1	4	1	4	--	--
Gharbia	7	28	9	36	7	28	5	20	6	24
Menofia	3	12	4	16	3	12	3	12	2	8

Table 4. Detection of different meats used in grilled kofta manufacture in three different governorates in Egypt by precipitation test (n = 25).

Meat species	Kalubia Governorate		Gharbia Governorate		Menofia Governorate	
	No.	%	No.	%	No.	%
Mutton	1	4	3	12	2	8
Beef	22	88	13	52	17	68
Equine	2	8	8	32	6	24
by ELISA	2	8	8	32	4	16
Canine	-	-	1	4	-	-
Total	25	100	25	100	25	%

Table 5. The glycogen content % of grilled kofta samples (n= 25) collected from the three the Egyptian governorates

Governorate	Min.	Max.	Mean+ S.E.	Adultrated samples	
				No.*	%
Kalubia **	0.14	1.09	0.33 ± 0.04	3	12
Gharbia	0.17	2.13	0.64 ± 0.07	10	40
Mehofia	0.16	1.30	0.49 ± 0.04	6	24

• No. of samples had higher glycogen content as compared with normal standard limits (up top 0.25)
** ANOVA test indicate significant differences (P<0.05)

Table 6. Confirmatory tests for detection of equine meat in adultrated grilled kofta samples (n=25).

Test Governorate	Adultrated samples by p.p.t. test		Adultrated samples by glycogen test		+ve sulphuric acid test for equine meat		ve Erlich test fo equine meat		ELISA test	
	No.	%	No.	%	No.	%	No.	%	No.	%
Kalubia	2	8	3	12	2	8	2	8	2	8
Gharbia	8	32	10	40	8	32	7	28	8	32
Menofia	6	24	6	24	5	20	3	12	4	16

Table 7. Detection of different meats used in meat loaf manufacture in three different governorates in Egypt by precipitation test (n = 25).

Meat species	Kalubia Governorate		Gharbia Governorate		Menofia Governorate	
	No.	%	No.	%	No.	%
Mutton	--	--	1	4	---	---
Beef	23	92	12	48	17	68
Equine	2	8	10	40	7	28
Canine	--	--	2	8	1	4
By ELISA	--	--	1	4	--	--
Total	25	100	25	100	25	100

Table 8. The glycogen content % of meat loaf samples (n= 25) collected from the three the Egyptian governorates

Governorate	Min.	Max.	Mean+ S.E.	Adultrated samples	
				No.*	%
Kalubia **	0.17	1.15	0.39 ± 0.05	4	16
Gharbia	0.17	1.52	0.56 ± 0.04	11	44
Mehofia	0.20	2.27	0.80 ± 0.06	14	56

* No. of samples had higher glycogen content as compared with normal standard limits (up top 0.25)
** ANOVA test indicate significant differences (P<0.01)

Table 9. Confirmatory tests for detection of equine meat in adulterated meat loaf samples (n=25).

Test Governorate	Adultrated samples by p.p.t. test		Adultrated samples by glycogen test		+ve sulphuric acid test for equine meat		ve Erlich test fo equine meat		ELISA test	
	No.	%	No.	%	No.	%	No.	%	No.	%
Kalubia	2	8	4	16	3	12	1	4	2	8
Gharbia	10	40	14	56	10	40	7	28	8	32
Menofia	7	28	11	44	9	36	5	20	7	28

DISCUSSION

Adulteration of meat species is important for people whose religious practices limit the types of meat they eat, and for people who have allergies to certain types of meat proteins. Therefore, determination of the species of the meat components in meat products is an essential task in food hygiene and veterinary forensic medicine.

Depending on the precipitation test, it is obvious from the results recorded in Table 1 that the types of meat used for kabab manufacture were mutton, beef and equine with percentages, of 76,20 and 4% for Kalubia Governorate, 56,16 and 28% for Gharbia Governorate and 80,8 and 12% for Menofia Governmorate respectively. While, all examined kabab samples were free from dog meat in the three Governorate.

Meat substitution and/or adulteration was previously recorded many investigators *(7, 17,18)* who reported that 13.33% of meat sold at butcher shops in Gharbia Governorate were falsified by equine meat using precipitation test.

The problem of substitution or adulteration of costly meat with a cheaper one, whether by accident or intention, it not a new one. Also, determining the species which meat originated from is an integral part of food regulatory control with respect to economic fraudulence *(19)*. Incorrect species labeling can result in economic fraud or can mislead persons wishing to avoid certain meats or poultry for health or culture reasons *(20)*. Moreover, the nature of some meat products offers many possibilities for adulteration. Cheaper cuts or offal may be substituted for more expensive cuts and water or vegetable matter may be added *(21)*.

Generally, single and multi-species adulteration were reported in commercial meat products and the adultration rate of cooked meat products was higher than that of raw meats *(6)*. Unfortuntly, the sensitivity of precipitation test is affected by cooking of meat where the cooked meat extracts failed to produce a precipitation reaction *(17)*. Thus. there is an urgent need for applying other techniques to confirm the results of precipitation test, particularly, for cooked meat products.

Data in Table 2 indicated that the glycogen percentages in examined kabab samples were varied from, 0.13 to 0.97 with an average of 0.2 ±0.03 in Kalubia, 0.18 to 1.79 with an average of 0.55 ± 0.04 in Gharbia and 0.14 to 1.15 with an average of 0.34 ± 0.03 in Menufia. Differences associated with the examined kabab samples in the three governorates as a result of their glycogen contents were significant ($P<0.05$).

Actually, it has been recommended *(22)* that the glycogen should not exceed 0.25% in beef and mutton, but it may exceed 1.50% in equine meat. Conseqently, the adultration rates of kabab sold in Kalubia ,Gharbia, and Menofia Governorates according to its glycogen %were 8,36, and 16 %, respectively Table 2. In other words. equine meat was detected in 1,9, and 4 kabab samples in such three Governorates. respectively.

The present results nearly similar with those recorded by several investigators *(21, 23)*.

Substitution of mutton by meat of other animal species (e.g. equine) is essentially done for economic purposes, but such substitution may induce allergic reactions in sensitized individuals and may constitute a public health hazard *(24)*. Therefore, detection of this substitution is important only from the religious aspect of consumer especially in most Islamic countries who forbid the consumption of pork or horse meat . This type of adulteration may be occurred accidentally whereas the butchers did not routinely clean their grinders in their shops. Similarly, ***Hsieh et al. (25)*** recorded that the market mangers

readily admitted that they did not routinely clean grinders when changing from ground pork to another meat.

Comparison between the accuracy and sensitivity of different tests for detection of equine meat in adultrated kabab samples was shown in Table 3. The results of single qualitative tests such as sulphuric acid and Erlich were nearly similar . However, the most accurate ELISA test indicated that 24% and 8% of examined kabab samples were substituted by equine meat in Gharbia and Menoufia governorates, respectively. Lower results were obtained by Erlich test which may give false negative results. In contrast, higher results of adultrated kabab samples were recorded by application of both precipitation and glycogen tests which may give false positive results.

It is of great concern to mention that ELISA test proved that all kabab samples in Kalubia governorate were free from equine meat, while precipitation (4%), glycogen (8%), sulphuric acid (4%) and Erlich (4%) tests revealed positive reactions. This attempt can confirm the differences between the sensitivity of these techniques for detection of meat adulteration Table 3.

These findings come in accordance with those previously reported several researchers ***(5, 6, 26, 27).***

In general, the methods used for identification of meat species have been reported to have limitations in use because of problems in specificity as glycogen and precipitation tests ***(28).*** While, ELISA is highly sensitive, specific and more effective for detecting species in fresh meat and cooked meat products ***(12).***

Results achieved in Table 4 declared that the percentages of identified meat by precipitation test used for kofta manufacture in Kalubia, Gharbia, and Menufoa Governorates were 12,8 and 4% of mutton, 88,52, and 68% and of beef and 8,32, and 24% of equine meat, respectively. Only dog meat was detected in one sample (4%) of kofta sold in Gharbia governorate.

Dog meat is cheaper than buffalo and camel or even has negligible price, indicating that the possibility of intentional substitution is for economic reason. The reason for substituting more expensive meat like beef and lamb for cheaper meat such as poultry include the use of the unmarketable trimmings from expensive meat and improper cleaning of the grinding. The widespread species adulteration in retail markets may be attributed to inadequate meat inspection and the lack of a suitable and affordable analytical method ***(3, 4, 9, 18, 29).***

As a result of demand for meat and meat products has been higher, some opportunist people may market the meat of animal species that the society normally does not consume to meet their demand and increase their profit. This unethical practice occurs primarily by mixing the meat of unacceptable species into that of food animals through grinding and / or processing ***(5).***

Moreover, the acceptability of examined samples of grilled kofta according to their glycogen contents of is demonstrated in Table 5. Respectively, 12,40, and 24 % of grilled kofta sold in Kalubia , Gharbia and Menofia Governorates were unaccepted due to their higher glycogen content (more than 0.25%) The mean values of glycogen % in grilled Kofta samples were 0.33 ± 0.04. 0.64±0.07, and 0.49 ±0.04 in Kalubia,Gharbia, and Menoufia Governorates, respectively. These differences were significant ($P<0.05$).

Subsequently, the high glycogen contents in examined samples of grilled kofta may be originated from addition of equine meat and/ or offal tissues especially liver which increases the glycogen level without authentication by equine meat ***(21).***

On the other hand, application of ELISA test revealed that 8, 32, and 16 % of

examined grilled kofta samples in Kalbia, Gharbia, and Menoufia governorates were adultrated by equine meat respectively (table, 6). Different results were obtained by application of precipitation (8,32, and 24 %), glycogen (12,40, and 24 %), sulphuric acid (8,32, and 20 %) and Erlich test (8,28, and 12 %) for examined kofta in such three Governorates, respectively.

However, the result of ELISA clearly demonstrated the usefulness of antisera species in the identification of the species origin of cooked meat providing the method's superiority and sensitivity *(6).*

Table 7 proved that the percentage of meat loaf samples manufacture from beef and equine meat were 92 and 8% in Kalubia, 48 and 40% in Gharbia, and 68 and 28 % in Menoufia Governorates, respectively. Also 8 and 4% of meat loaves were substituted by dog meat in both Gharbia and Menoufia Governorates, respectively , depending on precipitation test.

On contrast, the glycogen % in meat loaves was ranged from 0.17 to 1.15 with an average of 0.39 ± 0.05 in Kalubia ,0.20 to 2.27 and an average of 0.80 ±0.06 in Gharbia,and 0.17 to 1.52 and an average of 0.56 ± 0.04 in Menoufia governorate (table 8).

In general, the highest rate of meat loaves adulteration may be attributed to the nature of the products manufactured from various meat cuts collected from different sources and the light regulatory control on most ready-to-eat meat products sold in Egyptian markets.

Results given in Table 9 indicated that the percentages of adulterated meat loaf samples with equine meat by ELISA were 8,32, and 28 % in Kalubia, Gharbia, and Menoufia Governorates, respectively. Lower results were recorded by Erlich test (4,28, and 20), however, higher findings were obtained by precipitation, glycogen and sulphuric acid tests.

In this respect, it has been found that the precipitation reaction was visible only above a minimum level of 10 % in the meat mixture containing adulterants while ELISA could detect the adulterants down a minimum level of 1% *(17)*.

As conclusion, the highest rate of meat adulteration and/or substitution was detected in Gharbia governorate. Also the current results allow to recommend that Erlich test is indicative only in positive cases while precipitation and glycogen tests are highly sensitive in negative cases of meat adulteration depending on the comparison with the most accurate ELISA technique

ELISA provides a rapid and reliable means to detect the contamination of meat with equine and canine meat to ensure product quality and safety. To safeguard consumer rights, the legislation of each country should therefore impose a labeling of food products declaring the species used in their manufacture and food laboratories need to have a available techniques to ascertain the species used in the manufacture of meat products.

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الإستعراف على أنواع اللحوم فى بعض منتجات اللحوم الجاهزة للأكل والمباعة فى الأسواق المصرية

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إن الإستعراف على الغش فى منتجات اللحوم الجاهزة للأكل مهم لحماية المستهلك والقوانين المنظمة لذلك وكذلك فى التحاليل الطبية الشرعية. وفى هذه الدراسة تم تجميع ٢٢٥ عينة من الكباب ، الكفتة المشوية والحواوشى (٧٥ عينة من كل نوع) من محافظات القليوبية ، الغربية ، المنوفية لاكتشاف نوعية اللحوم المضافة لهذه الأغذية وتم تحليل هذه العينات بواسطة اختبار الترسيب ، الجليكوجين ، حمض الكبريتيك ، ايرلش والإليزا.

ومن هذه الدراسة لاحظنا أن كل عينات الكباب تحت الفحص كانت خالية من لحم الكلاب ولكنها تحتوى على ٤% ، ٢٨% ، ١٢% لحم خيول فى محافظات القليوبية ، الغربية ، المنوفية على الترتيب . ولكن فىحالة الكفتة المشوية تواجد لحم الكلاب فى ٤% فقط من العينات فى محافظة الغربية بينما وجد لحم الخيل فى ٨%، ٣٢% ، ١٦% فى محافظات القليوبية والغربية والمنوفية على التوالى وذلك بواسطة اختبار الإليزا. وبالإضافة الى ذلك فإن ٤% من عينات الحواوشى التى فحصت فى محافظة الغربية وجدت مختلطة بلحوم الكلاب بينما ٨% ، ٣٢% ، ٢٨% من هذه العينات تم غشها بلحوم الفصيلة الخيلية فى نفس هذه المحافظات.

ومن الأختبارات التى تم أجراؤها يمكن أن نستخلص أن اختبار أيرلش مفيد فى حالة الإيجابية فقط بينما أختبار الترسيب والجليكوجين مفيد فى حالة السلبية فى غش اللحوم وذلك بالمقارنة بطريقة الإليزا وهى **الأكثر دقة. ويفضل استخدام الإليزا فى تحديد أنواع اللحوم فى منتجات اللحوم المطهية الجاهزة للأكل.**