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BIOLOGICAL AND CHEMICAL IDENTIFICATION OF ADULTERATION OF BEEF AND ITS PRODUCTS IN GHARBIA GOVERNORATE

By

Nabila. :, M.A and Hassan**, M.A*

** Department of Forensic Medicine & Toxicalogy, Fac. Vet. Med., Moshtohor, Zagazig University, Benha Branch*

*** Department of Food Control, Fac. Vet. Med., Moshtohor, Zagazig University, Benha Branch*

ABSTRACT

Ninety random samples of minced beef (45), beef burger (45), kofta (45), and sausage (45),were examined for their adulteration by substitution with other species and/or some food additives. Minced beef samples were collected from butcher shops, restaurants and processing plants located in Gharbia governorate (15 of each) for species identification by application of precipitation and glycogen tests. However, determination of nitrites, connective tissues, starch, dried skim milk and soy bean flour in examined samples of beef burger, kofta and sausage were carried out for comparison with their standard limits. The results of precipitation test indicated that 13.33% and 20% of minced beef at butcher shops and restaurants were substituted by equine meat, respectively. Moreover, the mean values of glycogen content in examined minced beef samples of butcher shops, restaurants and processing plants were 0.41 ± 0.06, 0.52 ± 0.08 and 0.28 ± 0.03 with unacceptable rates 33.33%, 53.33% and 13.33%, respectively. In regard to nitrites, 26.67%, 40% and 60% of examined samples of beef burger, kofta and sausage exceeded the safe permissible limits, respectively. The mean values of hydroxy proline contents were 0.112 ± 0.012, 0.123 ± 0.014 and 0.137 ± 0.020 which represented 1.50%, 1.27% and 1.81% connective tissues in beef burger, kofta and sausage, respectively. Accurately, 5,4 and 2 samples of beef burger, kofta and sausage exceeded the standard limit of starch, respectively. On the other side, dried skim milk and soy bean flour were detected in 53.33% & 73.33% of beef burger, 33.33% & 46.67% of kofta and 26.67% & 20% of sausage, respectively. Importance of adulteration of beef and its products with other species and certain food additives as well as safety of such products for human consumption were discussed.

INTRODUCTION

Meat adulteration is an age old vice where it has hygienic, ethical and economical problems in addition to religious reasons ***(Hsieh et al., 1995).*** Detection of such deception

is not always an easy task particularly when the meat is cut into small pieces where the anatomical features are disappeared *(Aulakh et al., 1995).*

An average of 25% to 30% of the edible meat sold in various parts of India is adulterated *(Jacob, 1995).* Furthermore, ***Hsieh et al. (1995)*** recorded that the rates of substituted species in both raw and cooked beef samples collected from Florida retail markets in USA were 15.9% and 22.9%, respectively.

Identification of meat may be achieved by physical, chemical and biological trails. In this respect, the biological technique (precipitation test) for meat identification is more sensitive, specific and rapid method where it depends on antigen antibody reaction for detection of any type of meat in a complex mixture *(Allen, 1990).*

In developing countries, particularly China, the incidence of tumors among people is higher due to consumption of meat products containing excessive amounts of nitrites which have carcinogenic effect through formation of nitrosamines in the digestive tract *(Song and Hu, 1988).*

Determination of hydroxy proline as a measure of collagen, starch and dried skim milk in meat products are of great value from the economic stand point of view *(Pearson, 1984).* Also, soy bean flour may be used as extender in the production of ground beef patties to increase the protein content of the product *(Potter, 2001).* Consequently, the meat products may be adulterated by one or more of these trails.

Therefore, the current study was carried out to detect the adulteration of beef and its products and to compare the levels of certain food additives in such products with the standard limits stipulated by Egyptian Organization for standardization and Quality Control *"E.O.S.Q.C" (1991).*

MATERIAL AND METHODS

A total of 90 random samples of minced meat (45), beef burger (15), kofta (15) and sausage (15) were collected from different localities in Gharbia governorate for detection of their adulteration. Minced beef samples were collected from butcher shops, restaurants and processing plants (15 of each) and subjected to precipitation and glycogen tests for species identification. While, beef burger, kofta and sausage were examined for their contents of nitrites, connective tissues, starch, dried skim milk and soy bean flour.

Precipitation test *(Mackie and Mc Carteny, 1996):*

The examined minced meat should be free from fat and salt. Fat dissolvement was obtained by addition of 100 ml ether chloroform mixture to 50 g minced meat 24 hours. While, salt extraction was carried out by washing the sample with distilled water several times. Accurately, 100 ml of physiological saline was added to 50 grams of minced meat (free

from fat and salt) and left for 12 hours and then filtered to obtain clear meat extract. Furthermore, the meat extract was tested with horse and dog antisera already prepared in laboratories of Ministry of Health. In small precipitating tube, one drop of antiserum was added to one drop of extract. Appearance of precipitation on the bottom or wall of the tube was considered positive reaction.

Glycogen test *(Pearson, 1984):*

Twenty grams of minced meat were digested by 80 ml alcoholic solution of potassium hydroxide (10%). The mixture was filtered and neutralized by diluted hydrochloric acid. The glucose content was estimated by using spectrophotometer (spectronic 21, Fischer model, Germany). The glycogen % was calculated by the following formula:

Glycogen % = glucose % x 0.927.

Quantitative analysis of nitrite, starch and soy bean flour in meat products was performed according to official methods of analysis ***(A.O. A .C.,1990).*** While, the standard method recommended by **International Organization for Standardization *"ISO" (1994)*** was applied for determination of hydroxy proline % by which the connective tissues added to meat products were calculated. Average connective tissue % was estimated according to hydroxy proline % and fat % in examined samples.

Detection of dried skim milk in examined samples of meat products was adopted according to the technique recommended by ***Egyptian Standard Legalization (1993).***

RESULTS

Table (1): Incidence of falsified samples of minced beef marketed at butcher shops, restaurants and processing plants (n = 15).

Source of minced meat	+ve ppt test for horse meat		Glycogen %				
	No.	%	Min	Max	Mean ± S.E	Falsified samples	
						No.*	%
Butcher shops	2	13.33	0.15	1.39	0.41 ± 0.06	5	33.33
Restaurants	3	20.00	0.24	1.47	0.52 ± 0.08	8	53.33
Processing plants	-	-	0.13	0.59	0.28 ± 0.03	2	13.33

Table (2): Acceptability of examined samples of meat products based on their contents of nitrites (n = 15)

Meat Product	Permissible limit (ppm)	Samples exceeded permissible limit		N	Max	Mean ± S.E
		No.	%			
Beef burger*	Not more 125	4	26.67	83	164	118.67± 6.31
Kofta**	Not more 125	6	40.00	96	179	129.93 ± 7.72
Sausage***	Not more 125	9	60.00	101	213	159.80 ± 10.48

* Egyptian Standard No. (1681/1991).
** Egyptian Standard No. (1673/1991).
*** Egyptian Standard No. (1672/1991).

Table (3): Contents of connective tissue (tendons) in examined samples of meat products (n= 15).

Meat product	Hydroxy proline %			Average connective tissue %
	Min	Max	Mean ± S.E	
Beef burger	0.091	0.143	0.112 ± 0.012	1.50
Kofta	0.097	0.156	0.123 ± 0.014	1.27
Sausage	0.108	0.164	0.137 ± 0.020	1.81

Table (4): Acceptability of examined samples of meat products based on their contents of starch (n = 15).

Meat product	Permissible limit*	Falsified samples		N	Max	Mean ± S.E
		No.	%			
Beef burger	Not more 5%	5	33.33	4.2	7.1	5.35 ± 0.29
Kofta	Not more 5%	4	26.67	3.9	6.4	5.08 ± 0.19
Sausage	Not more 5%	2	13.33	3.5	6.0	4.61 ± 0.18

* E.O.S.Q.C. (1991)

Table (5): Contents of food additives which not advertised in labels of examined samples (n = 15).

Meat product	Dried skim milk		Soy bean flour	
	Positive samples	%	Positive samples	%
Beef burger	8	53.33	11	73.33
Kofta	5	33.33	7	46.67
Sausage	4	26.67	3	20

DISCUSSION

Falsification of minced beef by other prohibited meats was determined by precipitation and glycogen tests as shown in table (1). Respectively, 13.33 % and 20% of minced

beef samples marketed at butcher shops and restaurants in Gharbia governorate were substituted by horse meat as indicated by precipitation test. However, all examined samples of minced beef of processing plants were not falsified by addition of horse meat.

Accordingly, species substitution appeared to be more serious problem in minced beef sold in butcher shops and restaurants as compared with that of processing plants.

Beef adulteration by other species was previously recorded by ***Aulakh et al. (1995); Hsieh et al. (1995) and Calvo et al. (2002).***

Apparently, substitution of beef by other animal species (equine) is obviously done for economic purposes. Such substitution is unethical and objectionable for health and religious reasons.

Ingestion of undeclared meat protein may induce allergic reactions in sensitized individuals ***(Hayden, 1991)*** and infection of human being with organisms of public health concern ***(Potter, 2001).***

On the other hand, high glycogen contents were obtained in 5 (33.33%),8(53.33%) and2 (13.33%) minced beef samples marketed at butcher shops, restaurants and processing plants, respectively. However, the glycogen % in examined samples of minced beef varied from 0.15 up to 1.39 with an average of 0.41 ± 0.06 for butcher shops, 0.24 up to 1.47 with an average of 0.52 ± 0.08 for restaurants and 0.13 up to 0.59 with an average of 0.28 ± 0.03 for processing plants (table 1).

Accurately, the maximum glycogen content in beef is 0.25% above which the beef is falsified ***(Warries, 2000).*** Thus, the high glycogen contents in minced beef samples may be originated from addition of horse meat and/or offal tissues especially liver which rise the glycogen content without authentication by horse meat ***(Potter, 2001).***

The present results agree with those reported by ***Al-Jowder et al. (2001) and Al-Jowder et al. (2002)*** who discriminated between pure beef and beef containing 20% potential adulterants such as heart, tripe kidney and liver.

Table (2) revealed that the mean values of nitrites in examined samples of beef burger, kofta and sausage were 118.67 ± 6.31, 129.93 ± 7.72 and 159.80 ± 10.48 ppm, respectively. Nitrite content in meat products should not exceed 125 ppm according to ***Egyptian Standards (1991)*** stipulated for beef burger (1881/1991), kofta (1973/1991) and sausage (1972/1991). Accordingly, 26.67%, 40% and 60% of examined samples of beef burger, kofta and sausage exceeded the permissible limit.

These findings come in accordance with those obtained by ***Cardova et al. (1990) and Zhukova et al. (1999).***

Nitrite is added to meat products for its colouring, flavouring and antimicrobial properties. In details, nitrite

is converted into nitric oxide which reacts with metmyoglobin to form nitrosyl myoglobin responsible for attractive pink colour of meat products ***(Wirth, 1998)***. Also, nitrite is very effective as antibotulinum factor where it combines with heme of meat which is very essential nutrient required for the growth and multiplication of *Clostridium botulinum* ***(Warries, 2000)***.

In contrast, Nitrite can react with amines in the gastric acid to form the highly carcinogenic nitrosamines ***(Archer, 2002)***. Moreover, the consumption of meat products by women one time/week was associated with childhood brain tumors and acute lymphocytic leukemia among children as a result of high nitrite contents in such meat products ***(Sarasua and Savitz, 1994, Knekt et al., 1999 & Huncharek and Kupelnick, 2004)***.

Consequently, the risk of botulism should be considered in relation to the carcinogenic effect of nitrite as suitable inhibitory substance to replace nitrite in meat products. Thus, the addition of nitrite should be limited to a few special meat products in which *Clostridium botulinum* really represents a public health hazard.

Results achieved in table (3) declared that the mean values of hydroxy proline contents were 0.112 ± 0.012, 0.123 ± 0.014 and 0.137 ± 0.020% in examined beef burger, kofta and sausage samples, respectively. Thus, the hydroxy proline % represented 1.50%, 1.27% and 1.81 % connective tissues in beef burger, kofta and sausage, respectively.

Nearly similar results were reported by ***Vanden et al. (1990) & Hassan and Daoud (1997)*** who found the mean connective tissue contents in examined samples of beef burger and sausage were 1.12 % and 1.61 %, respectively.

Addition of meat rich in connective tissues during the manufacture of certain meat products is considered adulteration where their addition can affect other nutrients particularly the real protein contents constituting unfair and dishonest competition.

Starch contents in examined samples of meat products are declared in table (4). In general, the starch levels ranged from 4.2 to 7.1 with a mean value of 5.35 ± 0.29% for beef burger, 3.9 to 6.4 with a mean value of 5.08 ± 0.19 % for kofta and 3.5 to 6.0 with a mean value of 4.61 ± 0.18% for sausage.

Egyptian Standards (1991) stipulated that the addition of starch to meat products should not be more than 5%. Thus, 33.33%, 26.67% and 13.33% of beef burger, kofta and sausage samples exceeded this limit and disagreed with the standard specifications.

It is of interest to mention that starch is partially gelatinized during the heat treatment of meat products so that it absorbs the liberated water and acts as a binder. Further, the flavour of starch tends to be less fl-

oury and more nutty as a result of processing temperature ***(Cross and Overby, 1988).*** Also, the starch is added to meat products for economic reasons where the starch increases the degree of product swelling after storage period ***(Loffe et al., 2002).***

In the present study, the accurate starch contents are not written on the label of the product. This may results in some problems in peoples interested with regime to obtain the correct calories in their diet. In addition, increased starch level affects the digestibility of amino acids and other nitrogenous components ***(Mitsin and Sauer, 1992).***

Table (5) indicated that the dried skim milk and soy bean flour were detected in 53.33% & 73.33% of beef burger, 33.33% & 46.67% of kofta and 26.67% & 20% of sausage samples, respectively. Generally, such food additives were not mentioned in the labels of examined meat products.

Technologically, milk powders are used as emulsifiers and water binders in applications like sausages and other emulsified meat products ***(Warries, 2000).***

Concerning soy bean flour, it is used as meat replacement in developing countries to compensate the shortage of animal proteins ***(Nasser, 1985).*** Moreover, ***Rackis et al. (1991)*** stated that the soy bean flour should not be used in meat products due to its off-flavour and bad taste. Soy bean has bad effects on iron absorption, serum ferritin and gastric acid secretion as well as gastrointestinal hormone release ***(Mcarthur et al., 1988).***

Finally, the constituents of meat and its products are greatly varied from one producer to another and they may contain different kinds of tissues and sometimes a mixture of meat of various origin. Economically, meat products may be adulterated by high levels of food additives such as starch and soybean as well as exaggerated amount of nitrite which constitute a public health hazard. Thus, great efforts should be done by the local authorities and public health agencies as well as food hygienists to protect the consumers against all these cases of meat adulteration and to limit this serious phenomenon in Gharbia governorate .

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الملخص العربى

الاستعراف على غش اللحوم ومنتجاتها بيولوجيا وكيميائياً فى محافظة الغربية

نبيلة محمود عبد العليم* محمد أحمد محمد حسن **

فرع بنها - جامعة الزقازيق - كلية الطب البيطرى بمشتهر -*قسم الطب الشرعى والسموم جامعةالزقازيق- فرع بنها -** قسم مراقبة الأغذية- كلية الطب البيطرى بمشتهر

انتشرت فى الأونة الأخيرة ظاهرة غش اللحوم ومنتجاتها عن طريق استخدام لحوم حيوانات أخرى وذلك لتحقيق أعلى عائد مادى ممكن. هذا وقد يتم التدليس عن طريق بيع لحوم حيوانات محرمة طبقا للشريعة والقانون مما يشكل خطورة صحية على المستهلك علاوة على التحريم الشرعى لها. لذلك قامت فكرة هذه الدراسة للوقوف على حجم هذه الظاهرة من جهة، وكذا دراسة حالات غش بعض منتجات اللحوم بالإفراط فى استخدام مضافات اللحوم مثل فول الصويا والنشا وكذا النيتريت بقيم أعلى من المسموح به طبقاً للمواصفات المصرية.

أجريت هذه الدراسة على عدد تسعين (٩٠) عينة من اللحوم ومنتجاتها بواقع خمسة وأربعين (٤٥) عينة من اللحم البقرى المفروم وخمسة عشرة (١٥) عينة من كل من البيف برجر، الكفتة والسجق. وقد تم جمع عينات اللحم البقرى المفروم من مختلف محلات الجزارة، المطاعم ومصانع اللحوم (١٥ عينة لكل موقع) لتحديد مدى غشها ببعض لحوم الحيوانات الأخرى عن طريق إجراء اختبار الترسيب وتحديد نسبة الجليكوجين فى تلك العينات. كما تم قياس نسب النيتريت، النشا، بودرة اللبن وفول الصويا فى عينات البيف برجر، الكفتة والسجق لمقارنتها بالحدود المسموح بها.

وقد دلت نتائج الدراسة على أن ١٣,٣٣% و ٢٠% من اللحم المفروم الخاص بمحلات الجزارة والمطاعم يمت للعائلة الخيلية وليس لحم بقرى كما هو معلن عنه. كما كانت متوسطات نسب الجليكوجين فى عينات اللحم المفروم لمحلات الجزارة، المطاعم ومصانع اللحوم هى ٠,٤١ ± ٠,٠٦، ٠,٥٢ ± ٠,٠٨، و ٠,٢٨ ± ٠,٠٣، على التوالى، مما يؤكد غشها عن طريق إضافة لحوم الخيل أو بعض الأحشاء مثل الكبد. وبمعنى آخر، فإن ٣٣,٣٣%، ٥٣,٣٣% و ١٣,٣٣% من عينات اللحم المفروم الخاص بمحلات الجزارة، المطاعم ومصانع اللحوم غير مطابقة وقد تم غشه استناداً إلى اختبار الجليكوجين، على الترتيب.

وبالنسبة لنتائج فحص منتجات اللحوم، فإن ٢٦,٦٧%، ٤٠% و ٦٠% من عينات البيف برجر، الكفتة والسجق قد تجاوزت الحدود المسموح بها للنيتريت، على التوالى، مما يشكل خطورة صحية على المستهلك. كما تبين أن عينات البيف برجر، الكفتة والسجق قد تم غشها بإضافة بعض الأنسجة الضامة مثل الأوتار والأربطة بنسب ١,٥٠%، ١,٢٧% و ١,٨١%، على الترتيب.

وبالمثل فإن عدد خمس عينات (٥) من البيف برجر، أربع عينات (٤) من الكفتة وعينتان (٢) من السجق غير مطابقة بالنسبة لمحتواها من النشا حيث تجاوزت نسبة النشا بها الحدود المسموح بها. وعلى الجانب الآخر، فإن بودرة اللبن والفول الصويا قد تم اكتشافها فى ٥٣,٣٣% و ٧٣,٣٣% من عينات البيف برجر، ٣٣،٣٣ % و ٤٦،٦٧ % فى عينات الكفتة، ٢٦,٦٧ % و ٢٠% فى عينات السجق، على التوالى، بالرغم من عدم ذكر وجودها على أغلفة تلك المنتجات.

هذا وقد تمت مناقشة الأهمية الصحية لعمليات غش اللحوم ومنتجاتها سواء بإضافة لحوم حيوانات أخرى أو بعض المواد الحافظة، علاوة على تحديد حجم المشكلة للجهات المختصة لتفادى تلك الظاهرة الخطيرة.