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DETECTION OF NATIVE AND MODIFIED SOYBEAN IN SOME MEAT PRODUCTS IN ASSIUT CITY, EGYPT

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ABSTRACT:

High meat prices prompted the meat industries in Egypt to produce various meat brands extended with soybean proteins. Genetically modified foods are often in the news. Much of the world has experienced strong and increasing resistance to the introduction of any genetically modified foods to the market place. Agar gel immunodiffusion (AGID) and polymerase chain reaction (PCR) were used to detect soybeans in some meat products (minced meat, raw kofta, sausage and beef burger). PCR was applied due to stability of deoxyribonucleic acid (DNA) at high temperature and highly conserved structure of DNA within all tissues of an individual. Soybean was detected with AGID at 12%, 30% and 20% in raw kofta, sausage and beef burger, respectively, but not detected in minced meat. By using PCR native and modified soybeans were detected in 100% and 69%, respectively in beef burger and at lower rates in other products.

INTRODUCTION:

The use of soybean proteins as meat extenders has spread significantly due to the interesting nutritional and functional properties that are present in soybean proteins. Together with these properties, health and economical reasons are the major causes for the addition of soybean proteins to meat products. Nevertheless, despite the good properties associated to soybean proteins, there are many countries in which the addition of these proteins is forbidden or in which the addition of soybean proteins is allowed up to a certain extent. Thus, the need of analytical methods enabling the detection of added soybean proteins in meat products is obvious. Microscopic, electrophoretic, immunologic, and chromatographic methods are the most widely used for this purpose (Belloque *et al.,* 2002).

Genetically modified foods are often in the news. While genetic modifications have made improvements in many crops and helped to increase yields. Much of the world, in contrast, has experienced strong and increasing resistance to the introduction of any genetically

modified foods to the market place (Brandner, 2002).

Most of the developed analytical methods for GMO detection are DNA-based, since protein-based assays are not suitable for processed food. Polymerase chain reaction (PCR) and real time PCR-based methods have been generally accepted for regulatory compliance. (Rodriguez-Lazaro *et al.*, 2007).

Therefore, the objectives of this study were designed for estimation of the adulteration of meat products with soybean in Assiut retail markets. Also, detection of the accuracy of meat products labeling in the samples and comparison between the results of identification of species by AGID and PCR technique was also one of the objectives.

MATERIALS AND METHODS:

Samples:

Two hundred random beef meat products samples of minced meat, raw kofta, sausages and beef burger (50 of each) were collected from Assiut city retail markets, Egypt and subjected to analysis for detection of meat adulteration with soybean.

Preparation of soybean antigens:

Antigens from raw and heat treated soybean were prepared and kept frozen at -20 till be used for analysis. Female New Zealand white breed rabbits at 10–12 weeks age were used for antiserum preparation. They were subjected to examination for health signs, free from any abnormalities, vaccination with bacterial and viral vaccines before the experiment. Rabbits were divided into 2 groups according to the number of antigens used. Three rabbits were used for each group and three rabbits were kept as control. Rabbits groups were immunized for production of the target antiserum.

Raw soybean protein extraction:

Raw soybean protein can be extracted by grinding of soybean seeds, then extraction of fat with acetone, after that it is preferred to perform extraction of protein with alkaline water (pH 9) at volume 1:5, filtration and centrifugation of the sample. For immunization of rabbits, the supernatant must be filtered through bacteriological filters (Zheng *et al.*, 2007).

Heated soybean protein extraction:

Heated soybean protein extracted by heating of soybean seeds in alkaline water at 100 °C for 30 minutes in water bath, cooling, filtration and centrifugation. For immunizing rabbits antigen must be filtered through bacteriological filters (Carp *et al.*, 1999).

Preparation of meat product samples extract:

Two hundred meat products samples of minced meat, raw kofta, sausages and beef burger (50 samples of each) were tested for the presence of raw and heated soybean. 25 gm of product samples were mixed with 50 ml physiological saline and stomached. The samples were held for 1 hr at room temperature for extraction of water soluble proteins.

Samples were centrifuged. Supernatant was filtered through whatman filter paper (Hsieh *et al.*, 1995).

Immunization:

For immunization of rabbits extract filtered by bacteriological filter 0.22 µ.Well mixing of 1 ml of soybean extract with 1 ml of freund adjuvant till formation of milky substance. Injection of the antigen into four sites subcutaneously at the back of the female New Zealand rabbits (10-12 months). Rabbits received 3 doses with intervals 3-4 weeks (until decline of antibodies) (Macedo-silva *et al.*, 2000).

Testing of a blood sample by taking the sample from marginal ear vein by sterile needle at the base of the ear as possible after disinfection with alcohol 70%. The sample should not be more than 3 ml, if more amount needed it can be taken from the central ear vein up to 15% of the total blood volume based on the animal body weight.

Bleeding of rabbits occurred by 10-14 days after the last injection. Leave the blood to clot at room temperature for 1hr. The blood left at the refrigerator overnight. Centrifugation for collection of serum which contain the antibodies. If will not be used immediately stored at -20°c. To prevent contamination add sodium azide 0.02 % (Hayden, 1979).

Methods for amplification and detection of soybeans:

1-Agar Gel Immunodiffusion Test (AGID):

- Modified immune precipitation method based on the method of Ouchterlony (Siklenka *et al*, 2004). The bottom of a 6 cm Petridish was covered with 5 ml of 1% agarose solution cooked in 0.02 M Phosphate buffer solution (PBS), pH 7.4. After agarose coagulation and equilibration at 4ºC for 30 min, a cutter used to drill 7 holes (1 central and 6 peripheral) in agarose.

-Rabbit immune-serum was added to the central well and the examined meat product extract samples to the peripheral ones. Antigens for testing different heat processed products (50 µl) were added to the six peripheral wells.

-The precipitation reaction was considered positive if a pronounced positive reaction zone occurred in the central line between the central and the peripheral wells (between antiserum and antigen, respectively).

2-Polymerase Chain Reaction method (PCR):

Fifty samples (14 samples of minced meat, 11 of raw kofta, 12 of sausages and 13 of beef burger) were chosen from the suspected and negative adulterated samples examined by AGID to be reexamined by PCR.

A-Extraction of DNA:

DNA extracted by using QIAamp DNA Mini Kit (Catalog no. 51304, Qiagen Pvt. Ltd).

B-Polymerase chain reaction:

Lectine gene was used for amplication of sequence for detection of native soybean and CP4EPSPS gene was used for amplification of

sequence for detection of modified soybean (synthesized by Bio Basic Inc.).

Species	Product size (bp)	Sequence	Name
Native Soybean	118	GCCCTCTACTCCACCCCCATCC	LE103
		GCCCATCTGCAAGCCTTTTTGTG	LE104
Modified Soybean	172	TGATGTGATATCTCCACTGACG	EPSPS-B1
		TGTATCCCTTGAGCCATGTTGT	EPSPS-B2

References for native and modified soybean was Lin *et al.,* (2006)
LE: Lectine gene
CP4EPSPS: 5-enolpyruvylshikimate-3-phosphate synthase from *A. tum-efaciens* strain CP4.

Steps of PCR:

Initial denaturation at 94°C for 4 minutes, then denaturation at 94°C for 1 minute, Annealing at 55°C for1 minute, extension at 72°C for 30 seconds and final extension at 72°C for 10 minutes. The basic three steps; denaturation, annealing and extension repeated 35 cycles.

RESULTS:

Table 1: Incidence of adulteration of the examined beef meat products samples with soybeans using Agar Gel Immunodiffusion test (AGID) (n=50 for each)

Type of soybean	Minced meat		Raw kofta		Sausage		Beef burger		Total Samples	
	No	%	No	%	No	%	No	%	No	%
Heated soy	-	-	4	8	14	28	9	18	27	13.5
Raw soy	-	-	2	4	1	2	1	2	4	02.0
Total Samples	-	-	6	12	15	30	10	20	31	15.5

Table 2: Incidence of native and modified soybean in the examined beef meat products samples using PCR

Type of soybean	Native Soybean		Modified Soybean			Total Samples selected for PCR
	No	N.S. %	No	G.M.S. %	G.M.S. % from N.S.	
Minced meat	7	50	4	28.6	57	14
Raw kofta	8	72.7	5	45.5	62.5	11
Sausage	9	75	6	50	66.7	12
Beef burger	13	100	9	69	69	13
Total Samples	37	74	24	48	65	50

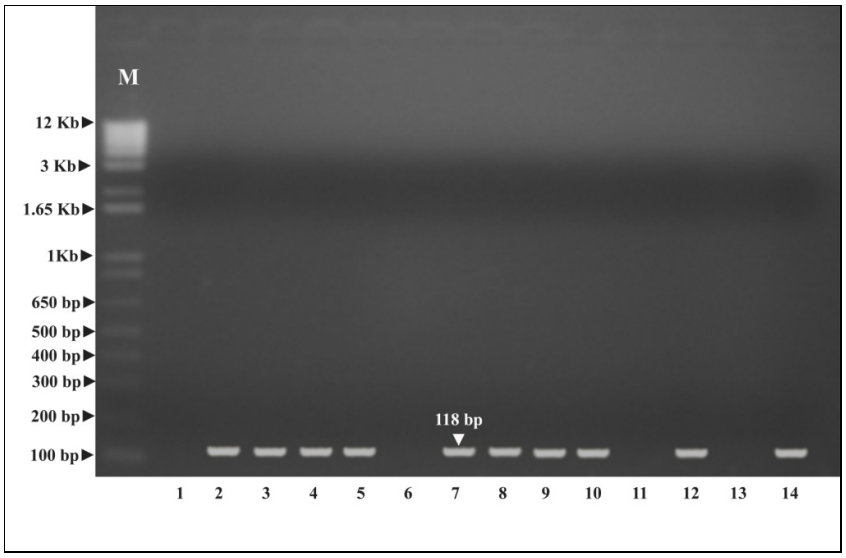

Figure 1: Agarose gel electrophoresis of PCR amplicon (118bp) showing presence of native soybean in beef meat products samples from 1 to 14 at lanes 2, 3, 4, 5, 7, 8, 9, 10, 12and 14. Lane M, 1 kb plus DNA ladder

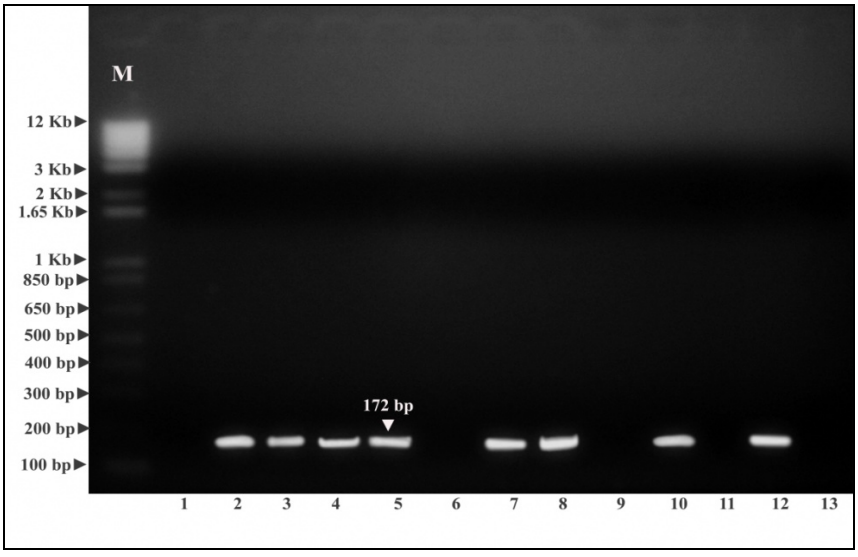

Figure 2: Agarose gel electrophoresis of PCR amplicon (172bp) showing presence of genetically modified soybean adulteration in beef meat products samples from 1 to 13 at lanes 2, 3, 4, 5, 7, 8, 10 and 12. Lane M, 1kb plus DNA ladder.

DISCUSSION:

AGID was applied on 50 samples of each of the following beef meat products; minced meat, raw kofta, sausage and beef burger for detecting the adulteration with soybeans. The results of AGID test presented in Table 1 revealed that the adulteration rates for raw kofta with heated and raw soybean were detected at rates 8% and 4%, respectively. The adulteration rates in sausage were 28% and 2% for heated and raw soybean, respectively. Heated and raw soybeans were detected at rates 18% and 2%, respectively in beef burger.

One of the goals of this study was to apply PCR to detect efficiency of detection of adulteration with soybean. DNA is a rather stable molecule, allowing analysis of processed and heat-treated food products (Unseld *et al*., 1995); it contains higher information than proteins because of the degeneracy of the genetic code, and, due to the ubiquity of DNA, all kinds of tissues can be analyzed. Out of fifty samples from each product suspected and negative adulterated meat products samples examined by AGID technique, an alternative method based on conventional PCR analysis to confirm the results of the adulteration which recorded by AGID technique. The adulteration rates for minced meat, raw kofta, sausages and beef burger with native soybean were 50%, 72.7%, 75% and 100%, respectively. While the adulteration of the same meat products with modified soybean were 28.6%, 45.5%, 50% and 69%, respectively as presented in table 2. The results proved that all samples give positive with genetically modified soybean were previously detected positive with the LE gene primer which present in all soybean products which also described by Lin *et al*. (2006).

In this study the presence of soybean DNA was determined with two pairs of oligonucleotides from the soybean lectin *Le1* gene. The primer pair is specific for the single copy lectin gene LE in soybeans and yields a PCR product of 118 bp size as shown in figure 1. The LE primer, which is routinely used for confirmation of the endogenous gene, is recommended for product-specificity detection. Using the PCR method to identify GM products, the primer was designed based on the regulatory sequence or structural gene in the inserted gene fragment. The primer EPSPS which is specific to the structure gene of herbicide-tolerant was recommended to be used for detection of GM-soybeans products. The primer EPSPS yields a PCR product of 172 bp as shown in figure (2). This primer also used by Lin *et al*., (2006) in screening and specific traits for detection of GM-soybeans products.

From the study it was cleared that native or modified soybean can be easily detected by PCR inspite of the great processing of soybean before and after its addition. This may be due to using of short primer fragments which can be detected over the whole food chain, but long fragments may not detected in highly processed samples. This also noted by Bogani *et al.* (2009). It was noticed that the sensitivity and accuracy of PCR in detection of soybean in meat products greatly overcome potency of AGID test.

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الكشف عن فول الصويا الأصلية والمهجنة في بعض منتجات اللحوم في مدينة أسيوط

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يعد غش اللحوم واحداً من أهم مشاكل الغش والتدليس التجاري التي يتعرض لها المجتمع المصري في الآونة الأخيرة، وذلك لعمل منتجات رخيصة الثمن، والذي أصبح مشكلة هامة في مصر خاصة مع وجود الدخل المنخفض وارتفاع أسعار اللحوم بشكل ملحوظ. يعانى سوق اللحوم من ضعف الرقابة وانتشار الغش والتلاعب حيث يمكن أن يحدث غش اللحوم بإضافة بروتين غير حيواني مثل فول الصويا وعدم الإعلان عن ذلك في البطاقة المطبوعة على المنتج مما يؤدى إلى تكلفة اقتصادية أعلى على المستهلك مما يجب، بالإضافة إلى أن هناك من المستهلكين من يعانى من الحساسية لبعض هذه البروتينات التي من الممكن أن تكون غير مدونة على البطاقة الخاصة بالمنتج. وقد ذكر في بعض المراجع العلمية أن المواد الغذائية المهجنة من الممكن أن تسبب حساسية للمستهلك وكذلك قد تؤدى إلى مقاومة الميكروبات للمضادات الحيوية.

تم في هذه الدراسة جمع عدد 200 عينة من منتجات اللحم البقرى من مدينة أسيوط خلال عام 2008م، وقد احتوت هذه العينات على 50 عينة من كل من اللحم البقرى المفروم والكفتة النيئة والسجق البقرى والبيف بيرجر.

وقد تم إجراء كل من اختبار الترسيب خلال الطبقة الجلاتينية واختبار تفاعل البلمرة المتسلسل للتعرف على غش اللحوم بإضافة فول الصويا وكان معدل الغش بفول الصويا المعامل حراريا والنيئ في الكفتة النيئة 8٪، 4٪، وفى السجق 28٪، 2٪، وفى البيف برجر18٪، 2٪ على الترتيب ولكنه لم يتم العثور عليهما في اللحم المفروم، وذلك باستخدام اختبار الترسيب خلال الطبقة الجلاتينية.

وقد تم اختيار 50 عينة من العينات التي كانت نتائجها سلبية أو مشكوك فيها بالاختبار السابق لكي يعاد تحليلها باختبار تفاعل البلمرة المتسلسل وكانت نسبة الغش بفول الصويا الأصلية 50٪، 72.7٪، 75٪، 100٪ في اللحم المفروم، الكفتة النيئة، السجق والبيف برجر على الترتيب. بينما كان معدل الغش بفول الصويا المهجنة 28.6٪، 45.5٪، 50٪، 69٪ في المنتجات السابقة على الترتيب. وقد لوحظ أنه كان 57٪، 62.5٪، 66.7٪، 69٪ من فول الصويا المستخدم كان من النوع المهجن في اللحم المفروم، الكفتة النيئة، السجق والبيف برجر على الترتيب. أي أنه كان 65٪ من فول الصويا المستخدمة في المنتجات كانت مهجنة. من هذه النتائج يتضح ضرورة تحرك جمعيات حماية المستهلك لضبط حالات الغش والاستعانة بخبراء الطب البيطري لعمل التكييف القانوني لهذه الحالات والتطبيق الحازم لقانون حماية المستهلك.