



Investigation of genetically modified additives in meat and meat products in Mosul city

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Article information

Article history:

Received 11 March 2025

Accepted 24 May 2025

Published 21 June 2025

Keywords:

Meat
Transgenic
Soybean
PCR

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Abstract

Consumer demands for meat and meat products have increased over the last years; Therefore, meat yields were improved using biotechnology. Gene modification techniques have been applied for these purposes using transgenic plants. The goal of this study is to investigate the presence of genetically modified additives in meat and meat products sold in local markets in Mosul city; DNA extraction of meat samples was screened for the presence of common regulatory genes represented by Cauliflower mosaic virus *P-35S* promoter and the *Agrobacterium nopaline synthase T-nos* terminator as well as the specific target Roundup Ready soybean gene (*RR*) using polymerase chain reaction assay to amplify sequences of these elements. The results displayed the existence of Roundup-ready soybean gene in processed meat products sold in Mosul city market 73%, especially in beef and poultry luncheon compared to sausage and mortadella with high prevalence in imported poultry meat 41.67% in comparison to other types of meat. The results also revealed that 58.33% of poultry luncheon and 50% of beef mortadella were positive for the presence of the 35S promoter gene, while only 3 (%4.69) of processed meat samples showed positive results for the presence of the *nos* terminator gene. Phylogenetic analysis revealed genetic diversity compared to global genes of transgenic plants recorded in Genbank. According to the results, the application of a monitoring system to detect genetically modified additives in meat products is recommended to protect consumer health.

DOI: [10.33899/ijvs.2025.158126.4178](https://doi.org/10.33899/ijvs.2025.158126.4178), ©Authors, 2025, College of Veterinary Medicine, University of Mosul.

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Introduction

Meat is highly consumed throughout the world; in developing countries, meat is accounted as a source of protein in addition to its organoleptic features (1); the production is necessary to meet the requirements, and genetic modification has recently been used to yield a high meat production and to reduce the impact of the high cost of meat by adding vegetable protein sources (2-4). The most vegetable protein used in the meat industry is soybean protein, which has high water-holding capacity and emulsifier properties improving the final meat product texture. Additionally, soybean protein in meat production indicates reduced cholesterol levels showing health benefits

(5,6). Although many GMOs are introduced in meat production, it's still under unacceptable level by consumers due to some ethical and health causes (7,8); some countries restricted the use of soybean protein to a certain limit, the US regulation permits a level of 2% for sausage, 0.04% of soybean in mortadella and hot-dog in Brazil (9,10). Screening of trace amounts of soybean in some meat products is necessary to assess the adulteration in some meat products that are widely consumed in developing countries (2,11,12). Polymerase chain reaction (PCR), as a sensitive method was used to detect the soybean in meat (13,14). For routine screening of GMOs in meat, the Cauliflower mosaic Virus 35S gene (CaMV 35S) is taken into account as a promoter gene along with Nopaline Synthase (*T-nos*) as the

terminator gene. The screening of GMO additives in food has been handled by many studies (15-18).

The target of the current investigation was designed to screen (for the first time) the presence of GMO materials in some meat products commercially available in the Mosul city market depending on the presence of *RR* genes; sequencing of DNA from *RR* genes is described in the current paper.

Materials and methods

Ethical approval

The study protocol was approved by the Institutional Animal Care and Use Committee at the College of Veterinary Medicine, University of Mosul, and included an authorized ID of UM.VET.2024.045 at 9.7.2024.

Samples

One hundred twelve samples of meat from various sources were collected randomly from Mosul city markets in the period between August to December 2024. The collected meat samples were distributed to 64 samples of processed meat and 24 samples each of imported meat and local meat, including both beef and poultry meat. The processed meat included luncheon meat samples, sausage meat samples, and mortadella. All samples were transported to the lab using

clean containers and preserved at chilling temperatures till analysis was done.

DNA extraction

DNA was extracted from meat samples according to a DNA extraction kit (Add Bio, Korea). 20 mg of meat samples were held in the bottom of a 1.5 ml microcentrifuge tube and mixed with 200 µl lysis buffer, 20 µl proteinase K (20 µg/ml), and incubated overnight at 56°C for tissue lysis. After incubation of the mixture for 10 minutes at 72°C, 200 µl of binding solution was added, mixed with vortex, and incubated for 10 minutes. Centrifugation was done at 8000 rpm for one minute, and the supernatant was transferred into the new 1.5 ml tube. Then 200 µl ethanol 100% was added to the mixture, put into a mini spin column for 15 seconds, and centrifuged at 13000 rpm for 1 min. Following centrifugation, the DNA was washed and centrifuged twice using 500 µl of both wash buffer 1 and 2 at 13000 rpm for 1 min to evaporate the ethanol residues. The DNA was eluted with 100 µl of elution buffer and then incubated for 1 min at room temperature; the obtained DNA was stored at -20°C. DNA extracted from meat samples was screened for 35S Promoter and nos Terminator region using primers provided by (MacroGen/Korea) to identify GM DNA in meat and provide a specific gene represented by Roundup Ready soybean gene for conventional PCR assay (Table 1).

Table 1: Primers used to screen GMO additives in meat

Primers	Sequence (5– 3)	GM gene	DNA size (bp)	Temperature (°C)	References
35S -F	CCACGTCTTCAAAGCAAGTGG	P-35S	123	60	19
35S -R	CCTCTCCAAATGAAATGAACTTCC				
<i>nos</i> -F	GCAATGACGTTATTTATGAGATGGG	T-nos	118	60	19
<i>nos</i> -R	GACACCGCGCGCGATAATTTATCC				
RR-F	CAT-TCC-CGG-CGA-CAA-GTC-	RR	172	60	20
RR -R	TTG-ATG-ACG-TCC-TCG-CCT-TC				

PCR Reactions

PCR assay has been used to identify 35S, *nos*, and *RR* genes. The reactions were accomplished using 1 µl from each pair of primers (F and R), 12.5 µl of master mix (Add Bio, Korea), and 2 µl of DNA template was added followed by adding 8.5 µl of nuclear-free water to achieve 25 µl total volume of final product. PCRs reactions were done in Thermocycler (BioRad, USA) using proper thermal cycling conditions, including predenaturation at 95°C for 5 minutes followed by subjected DNA to heat at 95°C for 30 s (35cycles), the primer annealed at 60 °C for 30 S, 45 s extended at 72 °C, and 10 min ultimate extension at 72 °C. Target DNA bands were visualized by applying gel electrophoresis using 1.5% agarose gel to separate amplified DNA and imagined fragments. DNA ladder 100 bp as a marker was loaded into wells along with target DNA. DNA amplicons were illustrated using a UV transilluminator with gel captured using the Gel Documentation System (BioRad, USA),

DNA Sequencing

The amplicons of Roundup ready soybean gene (*RR*) were excised from the agarose gels and purified; the sequences were assessed according to Sanger dideoxy sequencing at NCBI server with BLAST (Basic Local Alignment Search Tool) software. The structure of phylogenetic analysis was done using the Maximum likelihood method depending on the Tamura-Nei model in MEGA12 software.

Results

The screening results of genetically modified additives in meat and meat products sold in Mosul city markets revealed the presence of 22.32% of 35S promoter gene and 2.68% of the *nos* terminator gene, while the specific Round ready soybean gene revealed 29.46%, Higher percentage for *P-35 S* gene, *T-nos* gene with *RR* soybean gene were present in processed meat reached to 28.13%, 4.69% and 37.5%

respectively compared to other meat samples (Table 2). The distribution percentage of specific *RR* soybean gene in processed meat was 73% compared to 15% and 12% in both imported and local meat, respectively (Figure 1). The screening of GM additives in beef meat samples showed that mortadella has 50% 35S promoter followed by luncheon 41.67% associated with a high percentage of *RR* soybean gene in beef luncheon 75% then mortadella 50% and beef sausage 33.33% (Figure 2). Poultry meat samples recognized the existence of GM additives genes in luncheon at 58.33% and imported poultry meat at 33.33%, followed by local meat at 25% for the *P-35S* promoter gene. In comparison, the *RR* soybean gene were recognized with the same percentage, 50% in both of poultry luncheon and mortadella, followed

by 41.67 and 33.33% in imported and local poultry meat subsequently. Still, the poultry sausage detected less *RR* soybean gene 16.67% (Figures 3-6). The sequencing of the *RR* gene revealed were nucleotide identity percentage of 92.96% recorded in the Genebank database from transgenic plants from different countries, including Belgium, USA, China, and Switzerland (Table 3). A phylogenetic tree of *RR* gene in six positive samples from our study was constructed and bootstrap analysis with 1000 re-samplings. The phylogenetic tree illustrates the evolutionary relationship among our positive samples with a high degree of genetic similarity, especially the *RR-55* and *RR-6*, by a bootstrap value of 93% (Figure 7).

Table 2: Screening of GM additives in meat at Mosul city

Samples	No.	<i>P-35S</i>		<i>T-nos</i>		<i>RR</i>	
		Positive No.	%	Positive No.	%	Positive No.	%
Processed meat	64	18	28.13	3	4.69	24	37.5
Imported meat	24	4	16.67	-	-	5	20.83
Local meat	24	3	12.5	-	-	4	16.67
Total	112	25	22.32	3	2.68	33	29.46

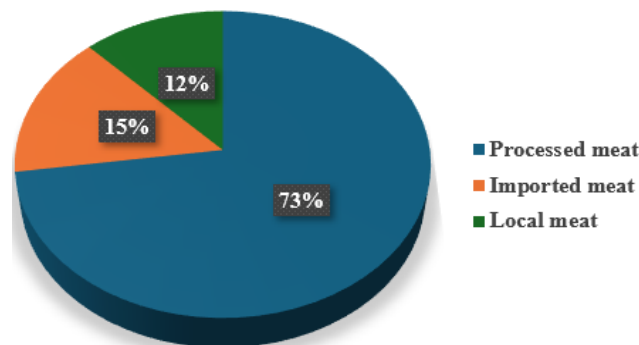


Figure 1: Distribution of Roundup-ready soybean gene in meat and meat products in Mosul city.

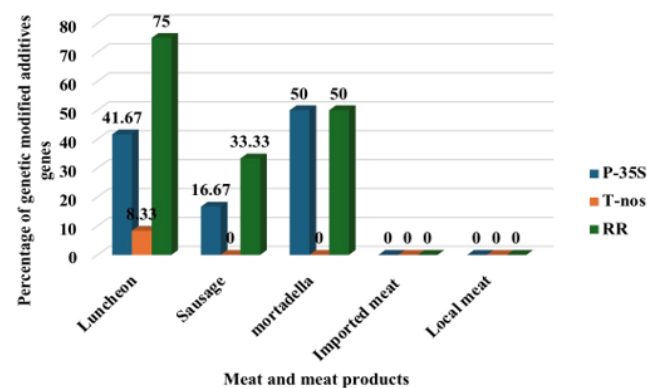


Figure 2: Prevalence of genetically modified additives genes in beef meat and meat products.

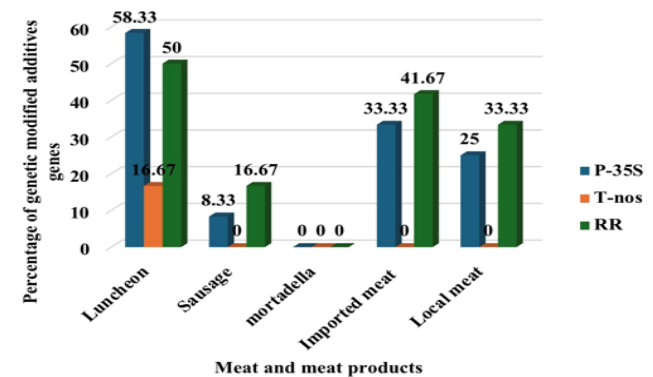


Figure 3: Prevalence of genetically modified additives genes in poultry meat and meat products.

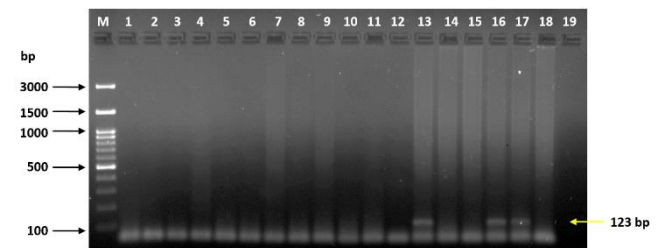


Figure 4: Gel-electrophoresis image displaying the amplified product of the *P-35S* gene for GM additives in meat. Lane 13,16,17 positive with a product size of 123 bp, Lanes 1-12,14-15,18 represent the negative sample, and lane 19 represents the negative control. The Lane M is the DNA marker 100 bp.

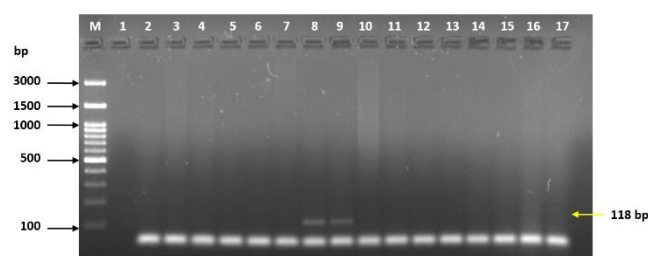


Figure 5: Gel-electrophoresis image displaying the amplified product of the *T-nos* gene for GM additives in meat. Lanes 8,9 positive with a product size of 118 bp, Lane 1-7,10-17 represents a negative sample, and Lane 1 represents the negative control. The Lane M is the DNA marker 100 bp.

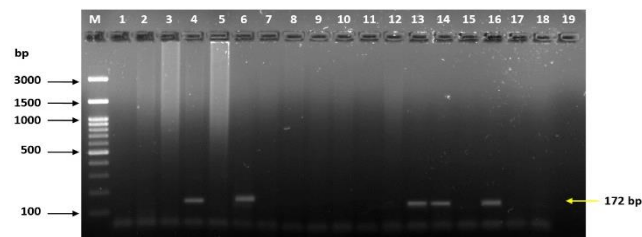


Figure 6: Gel-electrophoresis image displaying the amplified product of *RR* soybean gene for GM additives in meat, Lane 4,6,13,14,16 positive samples with a product size 172 bp, Lane 1-3,5, 7-12,15,17-18 represents negative samples, lane 19 represents the negative control. The Lane M is the DNA marker 100 bp.

Table 3: Percentage distribution of Roundup-ready soybean gene depending on the blast in GenBank of NCBI

Scientific Name	Query	Identity	Country	Accession Number
Gateway expression vector pAGRIKOLA-CATMA1a11610	13	92.96	Belgium	LT724735.1
Expression vector pOsAct2-1-Tnos	13	92.96	USA	EU259514.1
Gateway expression vector pAGRIKOLA-CATMA4a19057	13	92.96	Belgium	LT725408.1
Cloning vector pMono_T-vector	13	92.96	USA	JN681269.1
Transformation vector pC23HC	13	92.96	China	EU327493.1
Cloning vector pCASP1::CASP1:mTurquoise	13	92.96	Switzerland	HQ699545.1
Cloning vector pSOL9LHGRC	13	92.96	Germany	JX185747.1

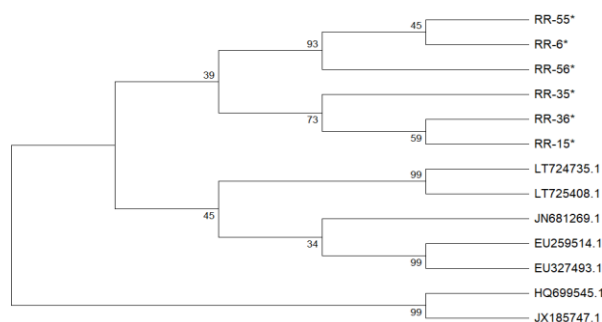


Figure 7: Phylogenetic analysis of *RR* gene (6 samples) from our study (*) by MEGA12 software.

Discussion

Meat safety is an important concept related to consumers' health; nowadays, to cover the demands for meat consumption around the world, various strategies have been improved to increase production (21,22); therefore, genetically modified (GM) plant species have significantly elevated over the past years, and the GM remnants for both human and animal food proportionally rose. The study investigated for the first time the residence of the *RR* soybean gene in meat sold in Mosul city reached 29.46% which in agreement with the percentage of *RR* soybean gene in processed food in Malaysian markets (23) and 24% of

processed meat in Serbian positive for both *RR* soybean gene and *P-35S* promoter (24) but it is less than the existence of *RR* soybean gene 43.75% in meat provided in Riyadh town confirmed the presence of specific GM additives represented by *RR* gene in poultry luncheon 66.67% more than in sausage 47.06% (25), consistent with another study in Syrian the permanence of GM additives in mortadella revealed high percentage to both of *P-35S* gene and *RR* soybean gene (26). The presence of target-specific Round ready soybean gene in processed meat consumed in Mosul city reaches 37.5%, close to the 38% of Hungarian food positive for soybean gene (27-29).

The differences may relate to the heat treatment of processed meats, which leads to decreased DNA quality and becomes, to a lesser extent, undetectable by the action of polymerase inhibitors (30,31). Earlier studies referred to that genetically modified soybean protein may added to processed meat as a trace ingredient during manufacturing to improve sensory traits and tenderness of products through its water-binding ability and its low cost compared to other protein sources (32-34) or it may enter the meat supply through animal diets which are derived from GM crops especially soybean and corn to attained healthier meat products; thus the meats from such animals could have a remnants of GM ingredients (35,36). The origin of soybean used as an additive in processed meat is imported from countries with a history of encouraging plant genetic engineering (24,28). Also, conventional PCR can be used as

a sensitive technique to detect the common regulatory genes of genetic modification, including the *P-35S* promoter and *T-nos* terminator, as well as the specifically modified soybean gene additives (37). The phylogenetic analysis suggests genetic differentiation compared to other global sequences referring to region evolutionary patterns and the strong bootstrap indicating high reliability of relationships; further research should be incorporated with a large number size to refine transgenic modification more accurately. Although the using of biotechnology to produce healthier meat (38,39), the availability of genetically modified products gave attention to monitoring these transgenic additives in meat products and applying legislation recommended the labeling of meat products derived from these materials to save consumers public health and to detect meat adulteration earlier (40,13,2).

Conclusion

Various types of meat sold in our local markets with the high demand of consumers give us a new contact to monitoring about residues of GM additives in these meats. Genetically modified additives in meat are a term of significant debate, including ethical and regulatory considerations. The screening of GM additives in meat at the local market in Mosul city displayed the existence of GM additives in meat products without labeling refers to the addition of genetically modified ingredients; the GM additives may be added indirectly through ingredients to processed meats as fillers derived from genetic modified soybean or corn. Positive findings indicate a commercial adulteration and indicate that PCR assay is a useful molecular tool to identify GM additives. The finding suggests that it is necessary to monitor the GM additives in meat with the urgent need to establish a genetically modified legislation program, and checking the GM additives in meat products will be necessary to save consumers health.

Acknowledgment

The authors are grateful to the University of Mosul, College of Veterinary Medicine, for supporting the study.

Conflict of interest

The authors of the manuscript confirmed no conflict of interest during data analysis.

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الكشف عن الإضافات المعدلة وراثيا في اللحوم ومنتجاتها في مدينة الموصل

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الخلاصة

ازدادت في السنوات الأخيرة متطلبات المستهلكين إلى اللحوم ومنتجاتها الأمر الذي دعا إلى تطوير استخدام بعض تقنيات الهندسة الوراثية في إنتاج اللحوم. ويطبق تقنيات التعديل الوراثي لهذا الغرض باستخدام نباتات معدلة وراثيا. هدفت الدراسة الحالية إلى التحري عن وجود الإضافات المعدلة وراثيا في اللحوم ومنتجات اللحوم التي تباع في الأسواق المحلية لمدينة الموصل، حيث تم استخلاص دنا عينات اللحوم للكشف عن الجينات التي تنظم التعديل الوراثي والمتمثلة بالجين المحفز ($P-35S$) والجين النهائي للتعديل الوراثي ($T-nos$) بالإضافة إلى الكشف عن وجود جين الصويا المعدلة وراثيا (RR) باعتماد تقنية تفاعل البلمرة المتسلسل. أظهرت النتائج وجود جين الصويا المعدلة وراثيا في منتجات اللحوم المصنعة التي تباع في أسواق مدينة الموصل ونسبة ٧٣٪ وخاصة في عينات لانشون الأبقار والدواجن مقارنة بعينات الصوصج والمارتديلا مع ارتفاع نسبة تواجد هذا الجين في لحوم الدواجن المستوردة ونسبة ٤١,٦٧٪ مقارنة بباقي أنواع اللحوم في الدراسة، كما وبيئت النتائج ان ٥٨,٣٣٪ من عينات لحوم لانشون الدجاج و ٥٠٪ من عينات مارتديلا الأبقار موجبة لتواجد الجين المحفز للتعديل الوراثي $P-35S$. وظهرت نتائج تحليل الشجرة الوراثية وجود تباين وراثي في جينات الصويا المعدلة وراثيا عند مقارنتها بجينات النباتات المعدلة وراثيا المسجلة في بنك الجينات العالمي من دول أخرى. وتبعاً لهذه النتائج توصي الدراسة باعتماد نظام مراقبة دورية على اللحوم للتحري عن الإضافات المعدلة وراثيا في منتجات اللحوم لحماية صحة المستهلكين.