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# Food adulteration with genetically modified soybeans and maize, meat of animal species and ractopamine residues in different food products

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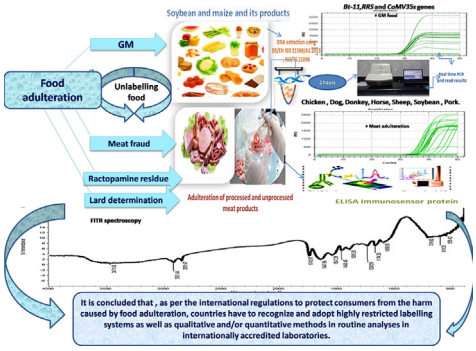
## GRAPHICAL ABSTRACT

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## ABSTRACT

*Background:* Governments around the world have developed a variety of strategies to address the long-standing food crisis. Food contaminated by genetically modified organisms (GMOs) and meat residues from hormonally treated animals, has recently received increased attention, posing serious health risks to consumers. The aims of this study are to detect recombinant DNA in genetically modified maize, soybeans, and fruits. Furthermore, meat adulteration by mixing meat from different animal species and ractopamine residues (RAC) in imported and local food products were detected using qualitative and quantitative methods.

*Results:* Sixty local and imported food samples were collected from different supermarkets, local markets, street vendors, and slum areas in Egypt. The results revealed that the recombinant DNA targeted sequences were detected in 25 samples, with the common regulatory genes (*CaMV35s*) found in 16 of them. The Bt-11 and RRS genes were both detected in maize and soybean samples. However, 35 were used for a screening of meat adulteration with meat from different animal species using qualitative real-time PCR and RAC residue detection using ELISA. The results revealed that 11 samples of pork were positively adulterated, and six samples of meat were positively adulterated (dog, donkey, pork, horse, sheep, chicken, and soybean). Finally, lard was detected in three positively adulterated porcine meats.

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*Conclusions:* It is concluded that, as per the international regulations, in order to protect consumers from the harm caused by food adulteration, countries must recognize and implement highly restricted labelling systems, as well as qualitative and/or quantitative methods in routine analyses in internationally accredited laboratories.

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## 1. Introduction

Food control and safety must be evaluated on a regular basis through risk assessments as it is a universal concern that affects human health. When the food is free of any contaminants, meets all the nutritional requirements, and has reliable labeling, then it is considered safe. Some issues related to food safety include food adulteration, toxicity, illegal food additives, pesticides, and hormone residues that encourage almost all countries to tighten food quality regulations [1]. Consequently, every government pays attention to the instructions stated by the World Health Organization (WHO) in order to prevent possible health problems that might be caused by a lack of food safety regulations [2]. Food adulteration entails the use of different animal species (dog, donkey, pork, horse, sheep, chicken and cat) mixed with bovine meat, as well as the use of some feed additives promoting growth such as RAC residue to increase quantity and reduce production costs [3]. The examination of adulteration should be performed frequently due to the religious affairs, fraud and malicious marketing practices, health risks such as specific food allergies and mutations besides the economic and legal concerns [4].

GMOs are considered one of the main food adulteration technologies and illegal additives that involves inserting foreign genes from animals, bacteria, viruses, or other plant species into crops [5]. Modern agricultural technologies are used to maximize production (quantity and quality) through better control of breeding against pests and insects. Apart from the public debates concerning GM technology, several GM crops have been permitted worldwide since the 1990s under certain regulations, and many transgenes, such as soybeans and maize, are accepted globally for cultivation and consumption [6]. Some researchers have stated that the consumption of GM food has a high potential risk of inducing allergies, toxicity, and contributing to the development of cancer via increasing DNA mutations [7]. Despite all of these human health risks, few countries, including Brazil, Argentina, USA, Canada and China, authorize the use of GMO with specified procedures in the regulatory status of the applications in their own biosafety legislation [8,9,10]. There are several cantons that have presented laws prohibiting the use of GMOs in agriculture [11]. More than 101 communes and rules have certified that they are free of GMOs. According to United Nations' Food and Agriculture Organization, Egypt has an obligatory negative labeling regulation on food products, which requires them to be labeled GMO-free in order to be imported.[12].

Meat products have several nutritional values and are suggested for daily use although the nutrients and minerals in meat vary according to meat ingredients, composition, and processing/-manufacturing conditions [13]. As a result, a high quality of meat should be available with all the nutritional values and without any contaminants or unknown animal species [14]. Due to the high consumption and over price of meat, producers tend to use unauthorized species in the production of processed and unprocessed meat products. Meat adulteration, which involves mixing bovine meat with meat from other animals like donkey, dog, pork, chicken, sheep and horse, is becoming a common practice in many countries [15,16].

RAC residue is a synthetic feed additive with pharmacological and structural characteristics that are very similar to catecholamine. It acts an energy repartitioning agent by diverting nutrients through increasing protein synthesis ratio and/or through decreasing protein degradation, which promotes muscle growth by inducing muscle hypertrophy, decreasing fat deposition, improving feed conversion, and therefore increasing average daily weight gain to improve carcass yield and meat quality, thereby increasing financial profit [17,18,19,20]. In numerous countries, RAC is permitted to be used in animal production. The Codex Alimentarius Commission (Codex) has recognized RAC maximum residue limits of 90, 40, 10, and 10 μg/kg for kidney, liver, fat, and meat, respectively [21].

Various countries have rejected its use and recognized strict traceability programs due to the toxicological and pharmacological side effects of RAC residues in meat products [20]. It can cause poisoning effects and therefore the consumption of meat products containing RAC residues may induce tachycardia, headache, spasm, high vital sign, muscle tremor, restlessness, apprehension, and anxiety, according to European Food Safety Authority [22]. Cooking methods can reduce the RAC residues by up to 47.52%, according to Hassan et al. [19]. Therefore, detecting meat from unknown sources or from growth-promoted animals is critical in order to apply food safety and protect consumers from illegal adulteration regarding health, economic, and religious issues [23,24]. This detection allows for an upgrade of risk assessments related to meat manufacturing and meat products if bovine meat is mixed with meat from other animal species, which can have harmful effects on human and animal health [22].

Nowadays, the most commonly used analysis techniques for qualifying the detection of GMOs and meat animal species are qualitative Real time-PCR analysis using SYBR GREEN and TaqMan probe [25], while the determination of RAC residues using ELISA technique [20]. To the best of our knowledge, the presence of the GM food, detection of commercial fraud with meat from different animal species, and RAC residue have not been studied on a variety of processed food gathered from various markets. The aim of this study was to investigate the presence of GM soybeans and maize in imported and local food products in order to detect meat adulteration and RAC residues.

## 2. Material and methods

### 2.1. Certified reference material

FAPAS Certified reference material (CRM) (GM accredited by the United Kingdom Accreditation Service (UKAS) as complying with the requirements of ISO/IEC 17025) was used for standard curve generation in real-time PCR analysis. CRMs from GM lines were used as a positive control for the evaluation of soybean and maize samples. The indicated CRMs must cover the CaMV35S promoter

and NOS terminator lines to be able to screen for GMOs while enhancing specific genes for detection of the endogenous targets (soybean, maize, and fruits). In this investigation, the same protocol was used in this investigation to determine the transgenic content in food samples with soybean or maize for the specific events *Roundup (RRS*) and *Bt11* genes, respectively. For each sample, appropriate CRMs and sterile ultra-pure water were used as controls to reduce the risk of false negative/positive contamination during the DNA extraction method and qualitative PCR analysis.

### 2.2. Sample collection

Sixty local and imported food samples were collected from different supermarkets, local markets, street vendors and slum areas in Egypt. However, 25 samples were prepared for the detection of GM sequences, 35 were prepared for screening of meat adulteration with animal species, RAC residues, and lard detection. In 2020, 25 commercially processed soybeans and maize samples from various brands (13 soybean, 9 maize and 3 fruits) were randomly purchased from the Egyptian markets. The 13 soybean samples include cake mix ($n$ = 1), biscuits ($n$ = 7), powder drink ($n$ = 2), spices ($n$ = 1), chips ($n$ = 1), and soybean protein ($n$ = 1), while the 9 maize samples include cake mix ($n$ = 1), powder drink ($n$ = 1), corn flakes ($n$ = 1), canned corn ($n$ = 1), popcorn ($n$ = 1), chips ($n$ = 1), powder drink ($n$ = 2), and baking powder ($n$ = 1). The origins of the collected samples, which came from different countries with varying GMO legislations are presented in Table 1.

The 35 meat samples were divided into two categories: processed and unprocessed meat products. Out of them, 16 meat samples were processed meat products that include hot dog ($n$ = 3), canned beef ($n$ = 2), pastrami ($n$ = 2), salami ($n$ = 1), sausages ($n$ = 2), luncheon ($n$ = 3) and, burger ($n$ = 3), while the other 17 samples were unprocessed meat products that include frozen meat ($n$ = 6), kofta ($n$ = 3), raw steak ($n$ = 2), shawarma ($n$ = 1), liver ($n$ = 1), minced meat ($n$ = 4), and veal ($n$ = 2), as shown in Table 2. As a positive control, one fresh sample each of donkey (Equus asinus), dog (Canis familiaris), chicken (*Gallus gallus*), pig (Sus scrofa), cat (Felis catus), and sheep (*Ovis aries*) was used. The samples were homogenized and stored frozen at −20°C until the DNA extraction process began. Following the detection of DNA by real-time PCR, the food samples were considered for analysis in order to detect the presence of GMOs, animal species, hormone residues, and lard. After the collection of samples, they were delivered directly to the laboratory and each package was labeled with an external code. For the prevention of enzymatic degradation, all the samples were homogenized and stored at −20°C until the DNA extraction process. This work was performed in accredited ISO21571 GMO laboratories at Research Park, Faculty of Agriculture, Cairo University and the Ministry of Higher education.

### 2.3. DNA extraction from different food samples and meat

The DNA extraction method for the food and meat products was performed according to the joint Research Center of the European commission and ISO21571/2013(26) with some modification. The maize, soybean, fruits and meat samples were ground with liquid nitrogen, and approximately 50 mg were weighted for each sample before being moved into a sterilized 1.5 ml microcentrifuge tube comprising 500 µl of cetyltrimethylammonium bromide (CTAB) extraction buffer and vortexed for 15 s. The mixture was placed in a dry block thermostat TDB-120 (BIOSAN_16003) for 30 min at 65°C and then 7 µl of proteinase K was added and incubated overnight at room temperature. The mixture was treated with 10 µl of RNase A (100 mg/ml) and centrifuged at 12,000 × g for 45 min before adding 700 µl of phenol–chloroform isoamyl alcohol and was again centrifuged at 12,000 × g for 20 min. Then, 350 µl of the extracted DNA was transferred into a sterile microtube containing 600 µl of CTAB precipitate and incubated for 75 min at room temperature. After removing the supernatant and allowing the precipitate to dry, 350 µl of NaCl followed by 350 µl of phenol–chloroform isoamyl alcohol were added. After that, 200 µl of ethanol absolute alcohol was added to the mixture and left overnight. The mixtures were then centrifuged at 12,000 × g for 20 min, the supernatant was discarded, and 700 µl of 70% ethanol was added. The samples were centrifuged again at 12,000 × g for 15 min and the supernatant was discarded. The DNA pellet was left to dry in ethanol before being dissolved in 100 µl Tris–EDTA (TE) buffer. The DNA was cooled and stored at −20°C for further use [27]. DNA extraction from reference materials were performed with CTAB method in ISO21571. The DNA purity and concentration was measured by (Nanodrop™ 2000, Thermo Scientific™) and dissolved in a final concentration of 20 ng/µl.

### 2.4. Determination of the concentration and purity of the extracted DNA

The measurements were taken using Nanodrop 2000 c spectrophotometer. The concentration of the extracted DNA was determined by comparing it to a blank solution at 260 nm. The ratio 260/280 was used to estimate the purity of the extracted DNA. The ratio of 260/280 nm for all extracted DNA ranged between 1.7 and 2. Atypical working concentration of 100 ng/µl was prepared for each sample for further analysis.

### 2.5. PCR primers

To qualify GMOs, specific primer sequences for soybean (*lectin* gene), maize (*starch synthase IIb* gene, *SSIIb*), and the construct-specific GM sequences, *RRS* and *Bt11,* were performed using the real-time PCR, while common regulatory sequences (35S promoter,

**Table 1**
The 25 unlabeled maize, soybean and fruit samples analyzed.

| Product's name | Number of sample | Types of species | Domestic/Imported | GM label |
|---|---|---|---|---|
| Corn flakes | (2) | Maize | Imported | Absent |
| Popcorn | (1) | Maize | Imported | Absent |
| Canned corn | (1) | Maize | Imported | Absent |
| Chips | (2) | Maize soybean | Imported | Absent |
| Biscuits | (7) | Soybean | Imported | Absent |
| Soybean protein | (1) | Soybean | Imported | Absent |
| Cake mix | (2) | Maize soybean | Imported | Absent |
| Spices | (2) | Maize soybean | Imported | Absent |
| Baking powder | (1) | Maize | Domestic | Absent |
| Powder drink | (3) | 2 Maize, 1soybean | 2 imported, 1 domestic | Absent |
| Fruits | (3) | Fruits | Imported | Absent |

**Table 2**
The 35 animal meat species analyzed.

| Types of products | Product's name | Sample NO. | Local/Imported | Label |
|---|---|---|---|---|
| Processed Meat | Hot Dog | (3) | Imported | Absent |
| | Canned beef | (2) | Imported | Absent |
| | Salami | (1) | Imported | Absent |
| | Burger | (3) | Imported | Absent |
| | Luncheon | (3) | 2 Imported<br>1 local | Absent |
| | Pastrami | (2) | Imported | Absent |
| | Sausages | (2) | Imported | Absent |
| Unprocessed Meat | Frozen meat | (6) | 3 Imported<br>3 local | Absent |
| | Kofta | (3) | Local | Absent |
| | Shawarma | (1) | Local | Absent |
| | Liver | (1) | Imported | Absent |
| | Raw steak | (2) | Local | Absent |
| | Veal | (2) | Local | Absent |
| | Minced meat | (4) | 2 Imported<br>2 Local | Absent |

*NOS* terminatorgenes) according to DS/EN ISO 21569/A1 2013 and ISO/TS 21098 [28,29] were used. Besides, specific primers for different animal meat species were used, that is, *12 S RNA-tRNA val* for pork, 12S RNA for Poultry, Cytochrome b for dog, horse, and sheep, NADH-ubiquinone oxidoreductase chain 2 (ND2) for donkey, and ubiquinone oxidoreductase chain4 (ND4) for cat, as clearly listed in Table 3.

### 2.6. Qualitative Real-Time PCR assay

Real-time PCR amplification was performed using BRO8301 (TaKaRa, Shiga, Japan) for a total volume of 20 μl samples. The PCR mixtures were 20 μl in total volume, with10μl SYBER GreenReal-Time PCR master mix (KAPA Kit), 0.5 μl of forward and reverse primer, 2 μl of extracted DNA (10 ng) from each sample, and 7 μl of distilled water. Thermal cycler conditions were performed using the following conditions: preincubation at 95°C for 5 min, 45 cycles comprising of dsDNA denaturation at 95°C for 30 s, primer annealing for 1 min at 65°C, and fluorescence signal collection at the end of each cycle. Real-time PCR of all genes were performed as triplicate, where the temperature was increased by 0.5°C and ranged from 65°C to 94°C. Each PCR amplification was performed in triplicate, and a negative control of deionized water, which comprised a no-template control (NTC) with all sets of responses was used, as well as positive controls, were used during the PCR reactions. For each set of primers, data were collected and processed using the real-time detection system software version. The PCR products were evaluated using agarose gel electrophoresis, with the gel dissolved in 1.5% agarose with 1× Tris Buffer EDTA (TBE) running buffer. The run was performed at 80 V for 180 min, after which the gel was stained with 0.1% ethidium bromide (EtBr). A 100 bp DNA ladder was used as molecular size standards. The DNA bands were visualized under ultraviolet light, and the gels were analyzed with a gel imager (Bio-Rad – Gel Doc™ EQ).

**Table 3**
The primer sequences used to identify transgenic DNA and species- specific sequences in food product.

| Species | Primer | Product length (bp) | Target genes |
|---|---|---|---|
| Common regulatory gene | F:GCATGACGTTATTTATGAGATGGG<br>R:GACACCGCGCGCGATAATTTATCC F:GCTCCTACAAATGCCATCA | 118 | *T-NOS* |
| | R:GATAGTGGGATTGTGCGTCA | 195 | *CaMV35s* |
| Genetic-modified Maize<br>Housekeeping gene for maize | F:TGTGTGGCCATTTATCATCGA<br>R:CGCTCAGTGGAACGAAAACTC | 68 | *Bt-11* |
| | F:CTCCCAATCCTTTGACATCTGC<br>R:TCGATTTCTCTCTTGGTGACAGG | 151 | *SSIIb gene* |
| Genetic-modified Soybean<br>Housekeeping gene for soybean | F:TGATGTGATATCTCCACTGACG<br>R:TGTATCCCTTGAGCCATGTTGT | 172 | *Ready Roundup Soybean* |
| | F:GACGCTATTGTGACCTCCTC<br>R:TGTCAGGGGCATAGAAGGTG | 87 | *Lectin gene* |
| Pork | F:CTACATAAGAATATCCACCACA<br>R:ACATTGTGGGATCTTCTAGGT | 290 | *12 S RNA-tRNA val*gene |
| Dog | F:AAACCCTTCTTCCCTCCCCT<br>R:TGCATTCGGTTACTGCTGACA | 143 | Cytochrome b |
| Horse | F:CTATCCGACACACCCAGAAGTAAAG<br>R:GATGCTGGGAAATATGATGATCAGA | 153 | Cytochrome b |
| Donkey | F:CATCCTACTAACTATAGCCGTGCTA<br>R:CAGTGTTGGGTTGTACACTAAGATG | 145 | ND2 |
| Sheep | F: TTAAAGACTGAGAGCATGATA<br>R: R:ATGAAAGAGGCAAATAGATTTTCG | 225 | Cytochrome b |
| Poultry | F: TGAGAACTACGAGCACAAAC<br>R: GGGCTATTGAGCTCACTGTT | 183 | 12S RNA |
| Cat | F:CATGCCTATCGAAACCTAACATAA<br>R: AAAGAAGCTGCAGGAGAGTGAGT | 274 | ND4 |

### 2.7. Ractopamine determination and ELISA analysis

A competitive colorimetric assay was used to determine RAC residue using My BioSource supplies Enzyme Linked Immunosorbent Assay (ELISA) kits (ELx8081U No. 20397). The Immunosorbent Assay (ELISA) kits are used for the detection of a wide range of antigens, proteins, and peptides in a variety of species reactivity. Sinogenclon Co., Ltd (China) coated the plate well with RAC antigen. Two grams (±0.05 g) of homogeneous tissue samples were oscillated in 8 ml of acetonitrile solution for 2 min before being centrifuged at room temperature at 4000 r/min for 10 min to remove fat. 5 ml of the supernatant was dried at 50–60°C. A volume of 50 μl was used for the assay, according to the procedure described by the manufacturer.

### 2.8. FTIR spectroscopy analysis

Fourier-transform infrared spectrophotometer (FT-IR) (Thermo Scientific Nicolet 380) was used to determine the presence of lard in meat samples. The functional group generated was observed using spectrophotometer in the mid infrared region (500–4000 cmG1). This instrument is outfitted with a deuterated triglycine sulphate (DTGS) detector and a KBR beam splitter with an 8 cm G1 resolution and 32 scanning. After every image, a new reference air background spectrum was reserved. The KBr plates must be exhaustively washed after this procedure to avoid contamination of future samples. The windows were wiped down with a tissue and then washed several times with diethyl ether and ethanol. The polishing kit was used in the lab to polish the window surface and dried with a soft tissue before being filled into the next sample.

## 3. Results

### 3.1. DNA concentration

The screening of GMO in food products and undeclared animal species were carried out using DS/EN ISO 21569/A1:2013, ISO/TS 21098 [28,29], and qualitative real-time PCR methods. Regarding CRM testing, the sensitivity of the qualitative analyzing method for GMO detection of soybean and maize, as well as undeclared animal species in meat product, was extracted with the appropriate amount of DNA and adequate quality for more accurate GMO and animal meat species testing in qualitative real-time PCR reaction. The absorbance ratios of extracted DNA at 260 nm ranged from 1.7 to 2.0 ng/μl, and the concentration of DNA was ranging from 30 to100 ng/μl for soybean, maize, and fruit product, while it ranged from 20 to 100 ng/μl for processed and unprocessed meat products. Furthermore, the results also proved that the extraction procedure was accurate, reliable, and integrated, and that the extracted DNA from raw or processed food samples was of high quality.

### 3.2. Detection of recombinant DNA target sequences from genetically modified soybean, maize, and fruits in food products using Qualitative Real-time PCR

The GM sequences from genetically modified soybean, maize, and fruits were screened in food products. To detect *CaMV35s, T-NOS, Bt-11* and *RRS* genes using qualitative PCR, a total of 25 non-labeled samples were collected, including 13, 9 maize and 3 fruits. Our results suggested that the intrinsic SSIIB and specific lectin should be available for further investigation of GM sequences for soybean and maize, respectively. The results of the present study revealed that 16 out of 35 (12 soybean, 2 maize, and 2 fruits) samples were positive for screening targets (*CaMV 35S*), as presented in (Table 4 and Table 5). The positive signals or |PCR amplification products were detected at position 192 of *CaMV 35S* sequence and displayed in samples [5,9,10,11,13,14,15,16,17,19,20,21,22,23,24,25], as presented in Fig. 1. According to the results of qualitative real-time PCR, 16 samples yielded positive results with *CaMV35s* sequence, indicating the presence of GM sequences in their genome, as shown in Fig. 2, Table 4, and Table 5. It is worth mentioning that the other common regulatory gene (*T-NOS*) was also detected at 118 bp in only sample 13, as presented in Fig. 3. Further evaluation revealed that all 12 soybean and 9 maize samples out of the 16 GM positive samples

**Table 4**
Foods analyzed for adulteration with genetically modified maize, soybean, and fruits.

| Code | Product's name | Types of species | Domestic/Imported | *Common regulator genes CaMV35s* | *TNOS* | *GM Specific BT-11* (Maize) | *events RRS* (Soybean) |
|---|---|---|---|---|---|---|---|
| 1 | Corn flakes | Maize | I | - | - | - | - |
| 2 | Cake mix | Maize | I | - | - | - | - |
| 3 | Cake mix | Soybean | I | - | - | - | - |
| 4 | Corn flakes | Maize | I | - | - | - | - |
| 5 | Biscuits | Soybean | I | Detected | - | - | - |
| 6 | Canned corn | Maize | I | - | - | - | - |
| 7 | Popcorn | Maize | I | - | - | - | - |
| 8 | Fruit | Fruit | I | - | - | - | - |
| 9 | Fruit | Fruit | I | Detected | - | - | - |
| 10 | Powder drink | Soybean | I | Detected | - | - | - |
| 11 | Spices | Soybean | I | Detected | - | - | - |
| 12 | Chips | Maize | I | - | - | - | - |
| 13 | Biscuits | Soybean | I | Detected | Detected | - | - |
| 14 | Chips | Soybean | I | Detected | - | - | - |
| 15 | Biscuits | Soybean | I | Detected | - | - | - |
| 16 | Powder drink | Maize | D | Detected | - | Detected | - |
| 17 | Spices | Maize | I | Detected | - | - | - |
| 18 | Baking powder | Maize | D | - | - | - | - |
| 19 | Powder drink | Soybean | I | Detected | - | - | - |
| 20 | Soybean powder | Soybean | I | Detected | - | - | Detected |
| 21 | Biscuits | Soybean | I | Detected | - | - | - |
| 22 | Biscuits | Soybean | I | Detected | - | - | - |
| 23 | Biscuits | Soybean | I | Detected | - | - | - |
| 24 | Biscuits | Soybean | I | Detected | - | - | - |
| 25 | Fruit | Fruit | I | Detected | - | - | - |

I: Imported, D: domestic – : Not detected for GM sequence (Negative results); Detected: for GM sequence (positive results).


**Fig. 1.** PCR amplification of GMO-specific regions using CaMV35S primers. Lanes 1–25 extracted from food products containing maize, soybean, and fruits samples. M: Molecular weight marker (100 bp ladder) + ve: positive sample (Certified reference material) and – : negative sample (sterile ultra-pure water), NTC non-template control.

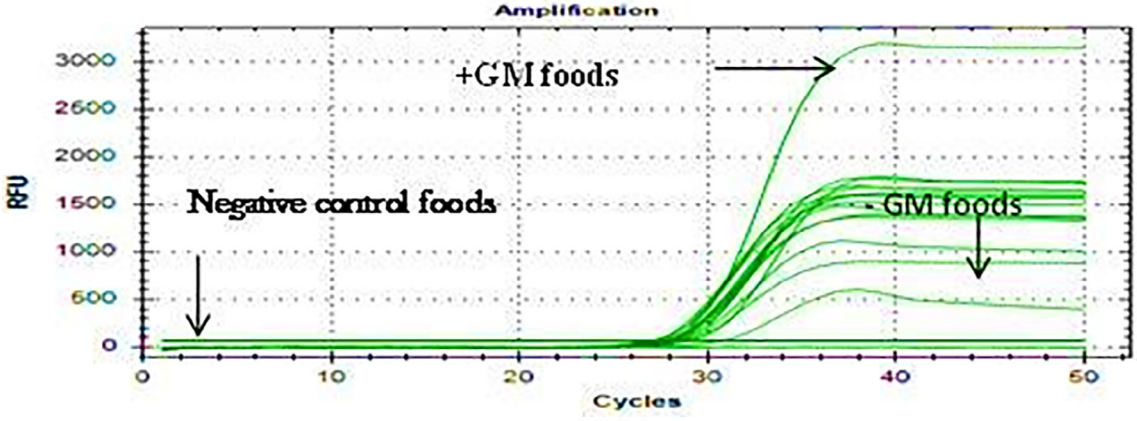


**Fig. 2.** GM food as standard of real-time amplification at 195 bp bands in 25 samples (+GM) and negative control of amplification (–GM).

**Table 5**
The Detection of endogenous genes and transgenic DNA sequences of soybean and maize product samples in 2020 using qualitative Real-time PCR method.

| Food products | NO. of samples | *SSIIb* | Lectin | Common regulatory *CaMV35sNOS* | | *GM Specific events* Bt-11 RRS | | GMO% percentage |
|---|---|---|---|---|---|---|---|---|
| Soybean product | 13 | - | 13 | 12 | 1 | - | 1 | 92% |
| Maize product | 9 | 9 | - | 2 | - | 1 | - | 22% |
| Fruits | 3 | - | - | 2 | - | - | - | 66% |
| Total | 25 | 9 | 13 | 16 | 1 | 1 | 1 | - |

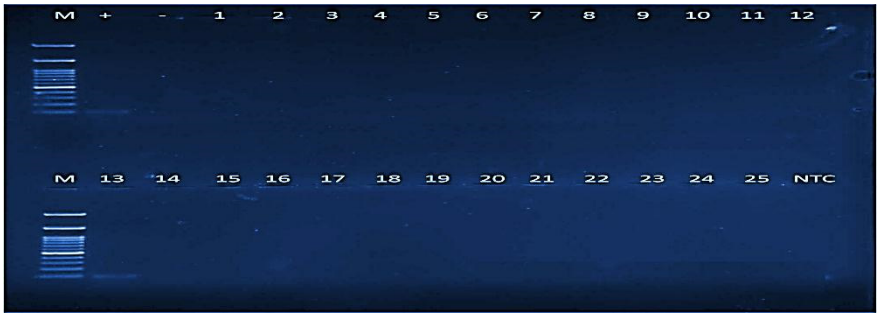


**Fig. 3.** PCR amplification of GMO-specific regions using primer pairs: TNOS for/TNOS rev. Lanes 1–25 extracted from food products containing maize, soybean, and fruits samples. M: Molecular weight marker (100 bp ladder) +ve positive sample, i.e., certified reference material, –ve negative control. NTC non-template control.

were detected using the *RRS* gene (soybean) and *Bt-11* gene (maize), respectively. Our results revealed that the *RRS* sequence was present in the genome of soybean powder sample (NO. 20) (Fig. 4A), while *Bt-11* sequence was present in the genome of powder drink sample (NO. 16) (Fig. 4B). The results confirmed the presence of GM sequences in soybean and maize genomes, as presented in Fig. 4. The qualitative real-time PCR results of the 12 food samples containing soybean, which included chips, biscuits, soybean powder protein, spices and powder drink were positive for screening targets (*CaMV35s, NOS, RRS*). *CaMV35s, NOS* and *Bt 11* were also identified in two maize samples, that is, powder drink and spices. However, as presented in Table 4, two fruit samples tested positive for*CaMV35s*. The results of the present study (Table 5) indicated that 92%, 22%, and 66% of soybean, maize and fruit samples, respectively, tested positive for screening targets GM sequences. Egypt has imported different types of transgenic crops, such as soybean and maize, but the cultivation of these plants is still prohibited.

### 3.3. Determination of commercial adulteration with different animal meat species using the real-time PCR

The animal species declared on the product label was detected in all thirty five products revealed that the PCR amplifiable DNA was successfully extracted from all processed and unprocessed meat products according to DS/EN ISO 21569/A1:2013. The mitochondrial DNA of 35 meat samples representing different animal species were successfully amplified using specific primers. In this study, we propose a qualitative real-time PCR analysis for accurate quantification of pork, soybean, chicken, dog, cat, donkey, sheep, and horse using specific primer sequences targeting the *lectin* gene of soybean, *12SRNA- tRNA val* of pork, *cytochrome b* of dog horse and sheep, *ND2* of donkey, and *ND4* of cat. Through real-time PCR, the primers generated specific fragments, that is, 290, 143, 153, 145, 225, 183, and 274 bps for pork, dog, horse, donkey, sheep, chicken and cat, respectively. The quantitative real-time PCR results are recommenced and compared with the labeled data regarding the addition of pork, soybean, chicken, dog, cat, donkey, sheep, and horse to the 35 samples of processed and unprocessed meat products presented in Table 6. The results revealed that clear and positive data findings for the porcine virulent gene (*12SRNA-tRNA val*) were scored in 11 meat samples [12,14,19,21,22,23,16,25,17,26,28], as presented Table 6 and Fig. 5. Moreover, the virulent genes in different animal meat species were amplified in five samples, that is, for the dog [29], horse [31], donkey [28], sheep [30], and chicken [33], while cat virulent genes were not detected in any of the meat samples, as shown in Fig. 5. The results revealed that pork is the most common undeclared species in burgers, luncheons, hog dogs, veal, liver, burgers, and frozen meat. Furthermore, the results showed that the imported minced meat was contaminated and adulated with dog, horse, and sheep (Table 6).

### 3.4. Determination of RAC residues using ELISA technique

The results of RAC revealed that all the tested samples contained RAC, but none of them exceeded the maximum limit specified by Codex [21]. Therefore, the RAC limit ratio was found in 19 out of 35 collected processed and unprocessed meat samples. The results showed that the RAC residues in liver tissues were the highest among samples. Furthermore, the highest detected RAC concentrations were associated with samples contaminated with pork. The data in Table 7 show that the unprocessed samples exhibited a higher RAC value than heat processed samples. In this connection, Pastrami had a RAC concentration of6.3 μg/kg when prepared by dehydration at room temperature, compared to luncheon, which had RAC concentration of 3.44–4.63 μg/kg when prepared by boiling or steaming. These results prove that heat treatment can decrease the RAC concentration in processed meat samples.

### 3.5. Determination of lard in meat samples using FTIR spectroscopy

Fourier-transform infrared spectroscopy "FTIR" peaks highlight the presence of specific hydroxide groups, thereby identifying fatty acids. However, the FTIR spectrophotometry analysis was used to determine the presence of a fatty acid called nervonic acid that indicates the contamination of meat with lard. The results revealed that only 3 out of 35 meat samples showed positive results, which were shawarma, liver, and Hawawshi [14,22,24], respectively. According to the results, the peaks of the carboxylic group are flat, and the wave numbers for these three samples are 3441, 3431, 3471 cm, respectively. Furthermore, the ketone peaks are sharp and the wave numbers are 1742 $cm^{-1}$ for all of these samples (Fig. 6). Moreover, the concentration value of nervonic acid in samples 14, 22, and 24 were 89.95, 73.38, and 74.88, respectively, indicating the presence of lard contamination. The remaining processed and unprocessed meat samples that showed negative results, indicating a lack of broadband at the hydroxide group and the absence of nervonic acid. The results proved the presence of lard contamination in three processed and unprocessed meat products.

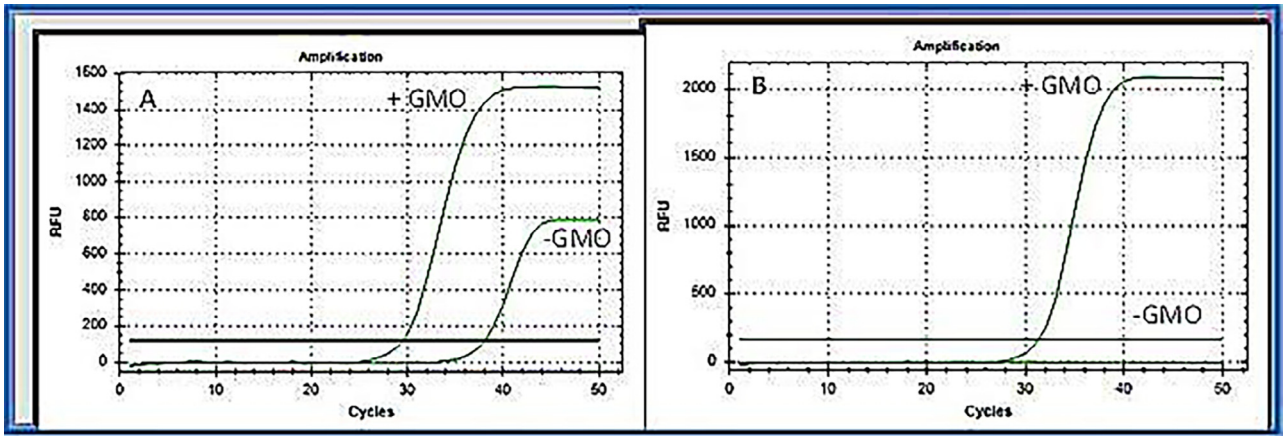


**Fig. 4.** GM food as standard of real-time amplification for both genes Ready Roundup Soybean (A) at 172 bp in sample 20 and Bt-11 (B) at 68 bp bands in sample 16, Negative control of amplification.

**Table 6**
Meat products analyzed for adulteration with other species.

| Code | Product's name | Processed/ Unprocessed | Undeclared animal meat species detected | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Domestic/ Imported | (Pork) *12 S RNA-tRNAval* | (Poultry) *12S RNA* | (Dog) *Cytochrome b* | (Sheep) *Cytochrome b* | (Horse) *Cytochrome b* | (Donkey) *ND2* | (Cat) *ND4* | Soybean Lectin |
| 1 | Hot Dog | P | I | - | - | - | - | - | - | - | - |
| 2 | Canned | P | I | - | - | - | - | - | - | - | - |
| 3 | Pastrami | P | I | - | - | - | - | - | - | - | - |
| 4 | Salami | P | I | - | - | - | - | - | - | - | - |
| 5 | Frozen meat | U | I | - | - | - | - | - | - | - | - |
| 6 | Hot Dog | P | I | - | - | - | - | - | - | - | - |
| 7 | Canned | P | I | - | - | - | - | - | - | - | - |
| 8 | Sausages | P | I | - | - | - | - | - | - | - | - |
| 9 | Kofta | U | L | - | - | - | - | - | - | - | - |
| 10 | Kofta | U | L | - | - | - | - | - | - | - | - |
| 11 | Raw steak | U | L | - | - | - | - | - | - | - | - |
| 12 | Burger | P | I | Detected | - | - | - | - | - | - | - |
| 13 | Luncheon | P | L | - | - | - | - | - | - | - | - |
| 14 | Shawarma | U | L | Detected | - | - | - | - | - | - | - |
| 15 | Frozen meat | U | L | - | - | - | - | - | - | - | - |
| 16 | Frozen meat | U | L | Detected | - | - | - | - | - | - | - |
| 17 | Luncheon | P | I | Detected | - | - | - | - | - | - | - |
| 18 | Sausages | P | I | - | - | - | - | - | - | - | - |
| 19 | Hot Dog | P | I | Detected | - | - | - | - | - | - | - |
| 20 | Frozen meat | U | I | - | - | - | - | - | - | - | - |
| 21 | Veal | U | L | Detected | - | - | - | - | - | - | - |
| 22 | Liver | U | I | Detected | - | - | - | - | - | - | - |
| 23 | Burger | P | I | Detected | - | - | - | - | - | - | - |
| 24 | Raw steak | U | L | - | - | - | - | - | - | - | - |
| 25 | Frozen meat | U | I | Detected | - | - | - | - | - | - | - |
| 26 | Luncheon | P | I | Detected | - | - | - | - | - | - | - |
| 27 | Pastrami | P | I | - | - | - | - | - | - | - | - |
| 28 | Frozen meat | U | I | Detected | - | - | - | - | Detected | - | - |
| 29 | Minced meat | U | I | - | - | Detected | - | - | - | - | - |
| 30 | Minced meat | U | I | - | - | - | Detected | - | - | - | - |
| 31 | Minced meat | U | I | - | - | - | - | Detected | - | - | - |
| 32 | Minced meat | U | L | - | - | - | - | - | - | - | - |
| 33 | Veal | U | I | - | Detected | - | - | - | - | - | - |
| 34 | Burger | P | I | - | - | - | - | - | - | - | Detected |
| 35 | Kofta | U | L | - | - | - | - | - | - | - | - |

P: processed; U: unprocessed; I: imported; L: Local.

## 4. Discussion

Food safety is a major concern around the world, owing to the increased attention to the concept of food adulteration, which affects people of all genders and ages. The Imported and food markets have a high influence on public health as different strategies have been developed to increase food grains production using GM material, a mixture of meat animal species, and meat from hormonally exposed animals. Therefore, this comprehensive screening study was designed to demonstrate and detect economically encouraged food adulteration with GMOs, animal meat species, and RAC in 60 local and imported products. It is generally considered difficult to accurately determine the food adulteration with GMOs and meat animal species using the same qualitative specific methods. As a result, this study was performed according to DS/EN ISO 21569/A1:2013, ISO/TS 21098 and qualitative real-time PCR methods due to their reliability, cost effectiveness, and high sensitivity in the detection of any contaminates in meat and food products.

Food samples containing soybean and maize have been selected for GM sequence detection since soybean and maize are ranked as the two most widely cultivated GM crops in the world. Results of GM sequence detection revealed that using the common regulatory genes (CaMV35s, NOS terminator) and specific genes (*Bt-11* and *RRS*) for soybean and maize, respectively, the recombinant DNA target sequences were detected in 16 out of 25 non-labeled samples using qualitative real-time PCR. The results revealed that the recombinant DNA target sequences were detected in some imported products, such as chips, biscuits, soybean protein, spices, powder drink, and fruits, but not in domestic food production. Results of the present study indicated that the majority of GM positive samples contained soybean (95%) while only two of the positive samples contained maize (22%). Our results agree with several studies (Sieradzki et al. [30] in Poland, Ujhelyi et al. [31] in Hungarian, Greiner and Konietzny [32] in Brazil and Arun et al. [33] in Turkey). In many countries, these studies found a high percentage of GM sequences in food and feed products, including soybean products. Our findings for the non-labeled food samples were consis-

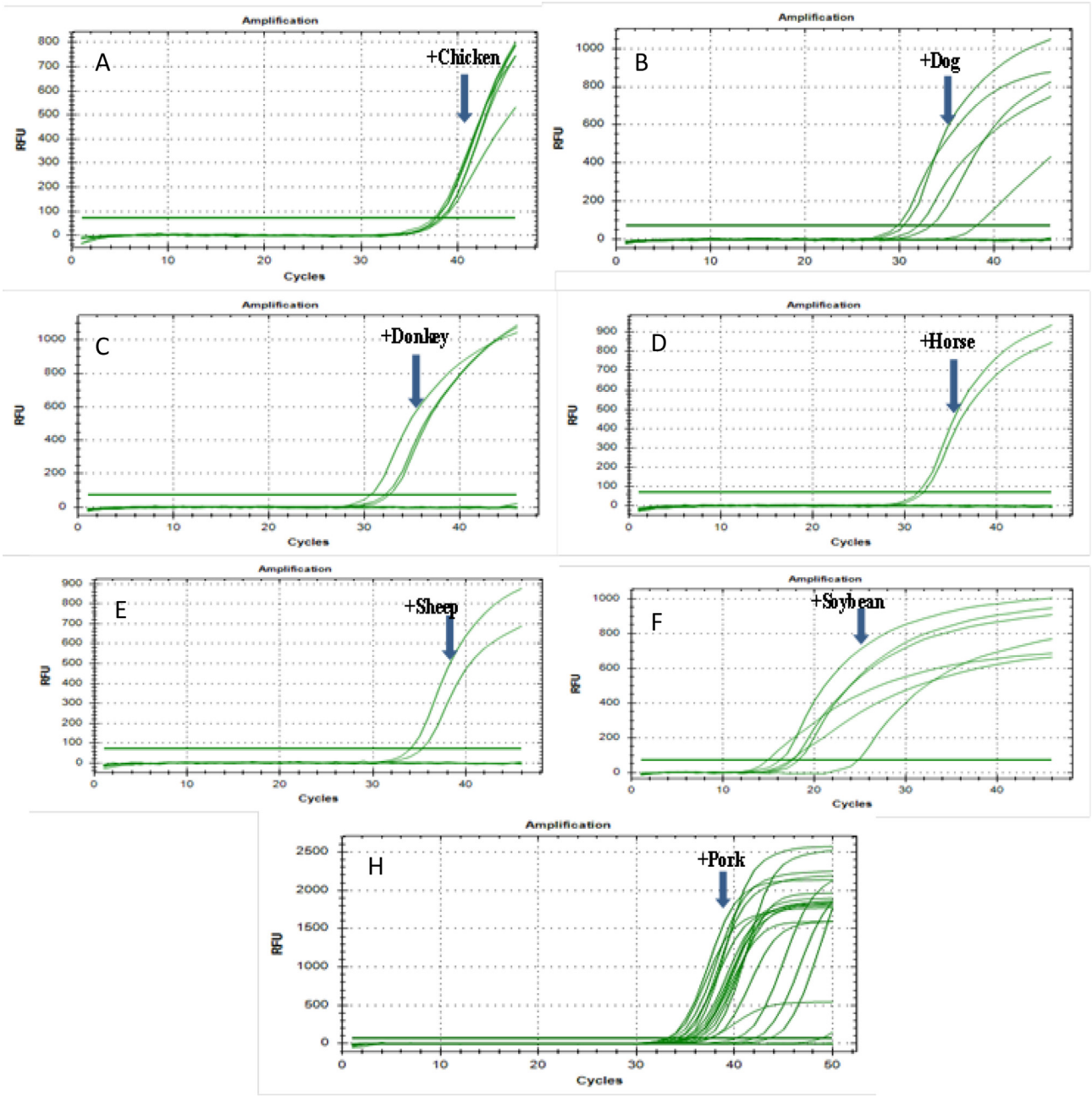


**Fig. 5.** Real-time PCR amplification of 34 animal species: (A) chicken, (B) dog, (C) donkey, (D) horse, (E) sheep, (F) soybean, and (H) pork.

tent with the results of Rabiei et al. [34], who used qualitative real time-PCR toscreen25 food samples from Iranian markets using *CaMV35s* and *Bt11* primers, but only 5 were positive for GM maize. Similarly, Kaur et al. [35] found CaMV 35 S promoter or NOS terminator and Bt-11 sequences inthe genomes of 13 out of 20 screened non-labeled maize samples from the Malaysia market. CaMV 35 S promoter and NOS terminator are the two most important screening common regulatory genes for qualitative PCR analyzes in most of the commercialized transgenic crops [36]. Furthermore, Holden et al. [37] revealed that the CaMV 35 S exists in 95% of GM foods in Europe. Safaei et al. [38] used *CaMV35S*promoter and *NOS* terminator for the identification of GM rice sequences by PCR in non-labeled rice samples from the Iran market Furthermore, Oraby et al [39] used the*CaMV35s promoter* and *NOS* terminator genes for GM sequence detection in food products using the PCR technique in Egypt.

Our results in Egypt, which used specific events such as the*Bt-11* and *RRS* genes for soybean and maize, respectively, suggest the need for further evaluation and confirmation of the GM sequence in food products. The results indicated that the *Bt-11* and *RR soy* genes were present in both the GM positive soybean and maize samples, proving the presence of GM sequences in their genomes. Our results agree with Zdjelar et al. [40], who indicated that eight non-labeled soybean samples from the EU countries, Argentina, the USA, Thailand, and Brazil eventually yielded positive results for *RRS* sequence. RRS specific gene sequence is the only transgene plant variety permitted for consumption in the EU market, but it is not permitted to be cultivated. The gene RRS has been encoded to be glyphosate-resistant during the cultivation. Glyphosate is a nonselective chemical substance that is commonly used in RR herbicides although its accumulation in soil and plants may have unintended consequences for the environment and human

**Table 7**
Meat products analyzed for adulteration with RAC residue.

| | Product's name | Domestic/Imported | Adulteration | RAC (μg/kg) |
|---|---|---|---|---|
| Processed meat | Burger | I | Pork | 2.74 |
| | Canned beef | I | ND | 0.89 |
| | Salami | I | ND | 3.44 |
| | Luncheon | I | Pork | 4.63 |
| | Hot Dog | I | Pork | 1.14 |
| | Sausage | I | ND | 2.44 |
| | Pastrami | I | ND | 6.30 |
| Unprocessed meat | Veal | L | Pork | 1.28 |
| | Liver | I | Pork | 2.02 |
| | Raw steak | L | - | 2.34 |
| | Frozen meat | I | Pork | ND |
| | Minced meat | I | Dog | 0.30 |
| | Minced meat | I | Sheep | ND |
| | Minced meat | I | Horse | ND |
| | Veal | I | Poultry | ND |
| | Frozen meat | I | Donkey | 1.48 |
| | Kofta | L | ND | 3.33 |
| | Shawerma | L | Pork | 2.75 |
| | Frozen meat | L | Pork | ND |

ND: Not detected; L: local; I: Imported.

health [41]. For instance, Mesnage et al. [42] revealed that glyphosate has adverse effects such as neurotoxicity, carcinogenicity, hepatic, and kidney toxicity when used within regulatory limits. The present study indicated the maize events *Bt-11* were detected in a single product like tortilla spice. However, the Maize event *Bt-11* is designed to provide resistance to an insect that has been approved for use in food and feed products by the EU. Hence, food safety concerns necessitate the detection of residue concentrations and GM materials in food products, especially in glyphosate-resistant crops.

The results of the study in 2005 and that of the present study indicated the increasing rate of GM products' availability in Egypt. Therefore, the necessity of a monitoring system to provide a good reliable control of GM materials in food products, and subsequently, on their labeling is obvious. In spite of the Egyptian legislation requiring the labeling of food materials derived from GMOs, none of the collected samples in 2020 were appropriately labeled. Egypt has imported different types of transgenic crops, including soybean and maize, but the cultivation of these plants is prohibited till date. Additionally, in order to control these products and protect the consumers' concerns about their biosafety, adopting regulation and reliable monitoring program is recommended. However, the several risks that estimated from the use of GM food product, as stated in several studies around the world, has led to a mandatory labeling system indicating that food contains GM products to save consumer's right and protect public health [43,44,45]. According to the European Union (EU) legislation and several other countries' rules and restrictions, products containing GMO must be labeled with "GMO-free" to be legalized and accepted for entering the Egyptian markets [7]. Moreover, the consumption of food and fruit products in the developing countries has increased, necessitating heightened awareness of unlabeled food for the protection of public health. A restricted system should be developed to allow for the detection of the GMO products found in fruits, food, and feed.

The detection of animal species in processed and unprocessed meat products is causing widespread concern due to medical concerns and customer rights. Consequently, many analytical techniques such as RFLP and RAPD were used for the identification of meat species using DNA-based or PCR-based techniques [46]. Recently, real-time PCR has been recommended as the most accurate technique for screening of animal meat species in individual or in mixed samples to protect consumers from adulterated food and save public health. In the present study, real-time quantitative PCR method was used to detect animal meat species of pork, chicken, dog, cat, donkey, sheep, and horse in meat products according to DS/EN ISO 21569/A1:2013. Then, for the detection of each species, specific primers were designed for the gene encoding *12S RNA,* 12SRNA-*tRNA val*, *cytochrome* b, *ND2* and *ND4*.

The results indicated that the specific sequence of each species was detected in 17 out of 35 meat samples, including 11 that were adulterated with pork, and only one sample was found to be positive for each species (dog, donkey, horse, sheep, soybean, and poultry). The majority of the positive meat samples were unprocessed and imported, while only three samples were domestic from slum area. In parallel, it also agrees with the study of Rashid et al. [47] because they used similar primers for the detection of meat animal species. Another agreement, on the other hand, used the real time-PCR for the detection of the adulteration in animal meat species [48]. In Bangladesh, Farag et al. [46] reported the presence of dog, donkey, chicken, pork, sheep, and horse in 15 meat samples using DNA-based techniques, particularly the PCR-based techniques such as RFLP and RAPD.

The results revealed that the real-time PCR systems were established for the specific detection of each species, whether it was a GM maize/soybean or mixture of meat animal species. Meanwhile, it clearly proved to be an easy and accurate method for applying to various food and meat products, and it is globally used due to its high quality and reliability of results.

In the scanning study, the Enzyme-Linked Immunosorbent Assays (ELISA) technique was used to quantify RAC residues in meat products. This method can detect RAC accurately without the need for complicated purification due to the specificity of the antibody used [49]. The results revealed that the RAC limit ratio was shown in 19 out of 35 collected processed and unprocessed meat samples. In this respect, Chai et al. [50] have established this technique as a screening method for RAC residues in imported and exported meat. Besides, Dong et al. [51] proved that the concentration of the RAC in tissues is ascends as follows: stomach > kidney > large intestine > small intestine > liver > heart > muscle. This finding may be related to the high temperature exposure during preparation, as suggested by Hassan et al. [19]. In addition that may explain the decrease in RAC residues in heat-processed meat. Our results revealed that

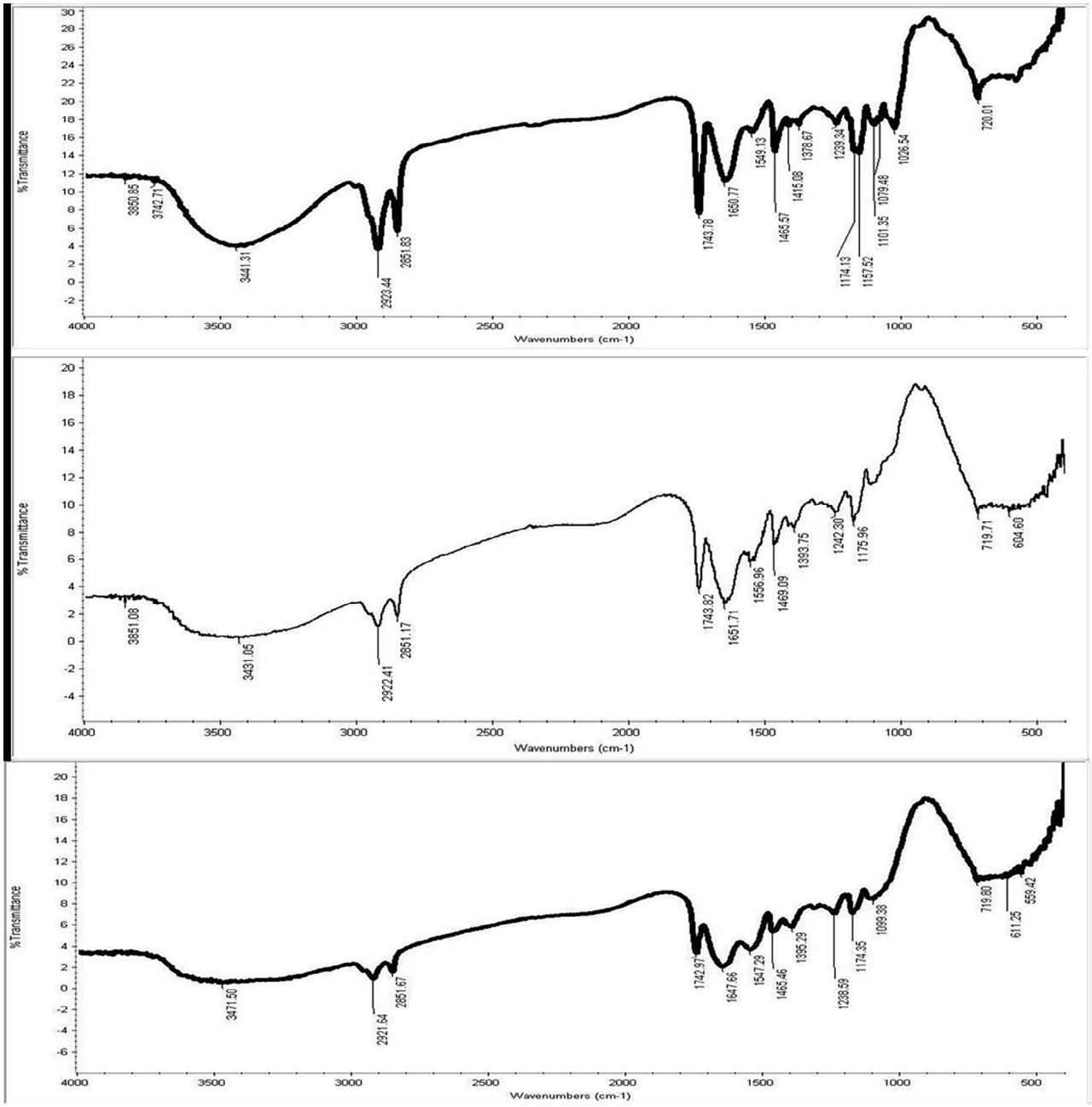


**Fig. 6.** Showing the FT-IR curve of the positive lard samples.

untreated samples (kofta, shawarma) had a higher RAC value than heat-treated samples (pastrami, luncheon). However, the concentrations remain below the maximum safe limit specified by Codex, 2012 [21].

FTIR analysis was used to detect the presence of Lard in processed and unprocessed meat. Our results revealed that only three samples were identified as containing lard in processed and unprocessed meat products, while the remaining meat samples were not identified as containing lard. Our results agree with Ramli et al. [52], who reported that the FTIR analysis can provide a low-cost and rapid method with minimal usage of chemicals to identify the presence of lard in meat samples. However, the discriminant FTIR analysis performed was able to categorize the samples into their specific groups, permitting the detection of lard.

The detection of GMi soybeans, maize and fruits, undeclared animal species, RAC residue and lard presence revealed the need for comprehensive studies as well as studies of the physiological effects after long-term consumption by humans. Likewise, there are many previous studies that prove the existence of several risks to human health where the governments have the responsibility of developing and implementing regulations to protect consumers worldwide from the harm caused by food adulteration. Previous studies on genetically modified plants has raised severe safety concerns about their use as food or feed [53].

## 5. Conclusions

The current study was conducted to detect the economically adulterated food products containing GMOs, undeclared animal meat species, lard and RAC residue in several local and imported products to ensure the consumer protection and his/her right to

choose. According to our results, it could be concluded that DS/EN ISO 21569/A1:2013, ISO/TS 21098, qualitative real-time PCR, FTIR spectroscopy, and ELISA methods have high sensitivity, accuracy and cost effectiveness for detecting and monitoring of adulteration in food and meat products. The results clearly presented the existence of transgenic sequences (GM) in soybean and maize food products. Besides, the presence of lard, high RAC concentrations and undeclared animal meat species in processed/unprocessed meat products has been documented. The obtained data clearly showed that all the detected positive samples were unlabeled, providing consumers with reliable information. The present study emphasizes the urgent need for a strict legislative and regulation system in the sector of local/imported food products to emphasize the labeling compliance, and hence, protecting the human public health.

## Conflict of interest

The authors declare no competing financial interest.

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