Journal Pre-proofs

Research Article

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PII: S0717-3458(21)00054-3
DOI: https://doi.org/10.1016/j.ejbt.2021.11.005
Reference: EJBT 531

To appear in: *Electronic Journal of Biotechnology*

Received Date: 1 May 2021
Accepted Date: 15 November 2021

Please cite this article as: A.A. Mostafa, A.E-H. Abu-Hussein, M.T. ElRouby, F. Abou-Hashim, H.S. Omar, Food adulteration with genetically modified soybean and maize, meat of animal species and ractopamine residues, in different food products, *Electronic Journal of Biotechnology* (2021), doi: https://doi.org/10.1016/j.ejbt.2021.11.005

EJBT-D-21-00112
Original Research Article

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

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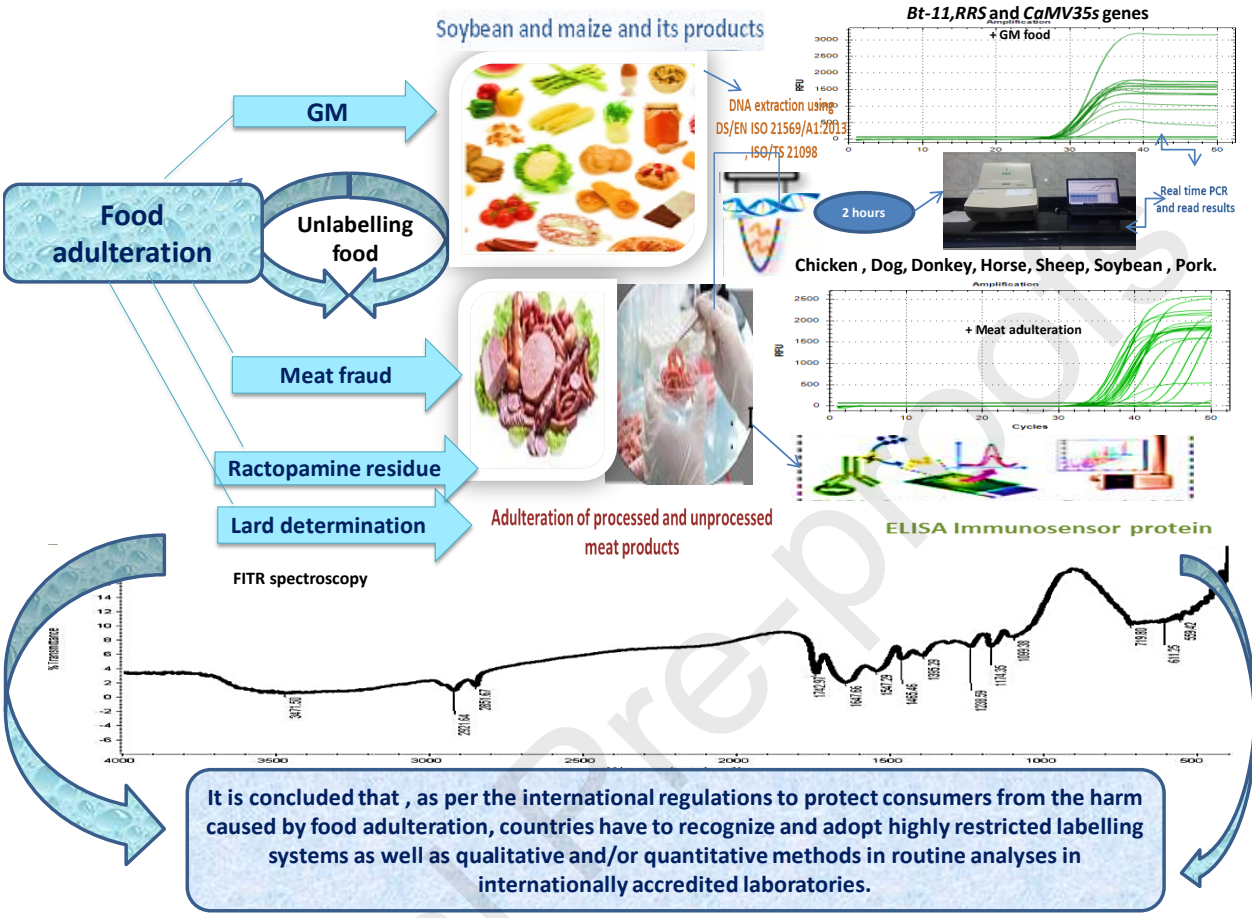
Abstract

Introduction: Worldwide, governments have developed many strategies to overcome the long-standing food problem. Recently, increasing attention has been paid to food contaminated by GMOs and residues of meat from hormonally treated animals, which leads to high health risks for consumers. The aims of this study were to detect recombinant DNA from genetically modified maize, soybeans, and fruits. Besides, the adulteration of meat by mixing meat from different animal species and ractopamine residues (RAC) using qualitative and quantitative methods in imported and local food products was detected.
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*) in 16 samples. The *Bt-11* and *RRS* genes were both detected in maize and soybean samples, respectively. However, thirty-five were used for a screening of meat adulteration with meat from different animal species using qualitative real-time PCR and detection of RAC residues using ELISA. The results revealed that 11 samples were positively pork adulterated and 6 meat samples were positively adulterated (dog, donkey, pork, horse, sheep, chicken, and soybean). Finally, lard was detected in three positively adulterated porcine meats.
Conclusion: It is concluded that, as per the international regulations to protect consumers from the harm caused by food adulteration, countries have to recognize and adopt highly restricted labelling systems as well as qualitative and/or quantitative methods in routine analyses in internationally accredited laboratories.

Graphical abstract



Keywords: CaMV35s; ELISA; Food adulteration; Food Safety; FTIR; Gmos; Maize; Meat; Ractopamine; Real time PCR; Recombinant DNA; Soybean.

1. Introduction

Food control and safety needs to be evaluated regularly by risk assessments as it is a universal concern that affects human health. When the food is free from any contaminants, contains all the nutritional requirements and reliable labeling, then it is safe. Some issues are related to food safety as food adulteration, toxicity, illegal food additives, pesticides and hormone residues that encourage almost all countries to increase regulation regarding the food quality (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 due to the lack of food safety regulations (2). Food adulteration involves the use of different animal species (dog, donkey, pork, horse, sheep, chicken and cat) to be mixed with bovine meat and the use of some feed additives promoting growth such as RAC residue in order to increase the quantity and reduce the

production cost (3). The examination of adulteration should be performed frequently due to the religious affairs, fraud and malicious marketing practices, health risks produced as specific food allergies, mutations besides the economic and legal concerns (4).

GMOs are considered as one of the main food adulterations technologies and illegal additives that are generated by inserting foreign genes from animals, bacteria, viruses or other plant species into crops (5). Modern agricultural technologies are used to maximize the production (quantity and quality) through better controlling breeds 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 while many transgenes are accepted globally for cultivation and consumption as soybean and maize (6). Some researchers had stated the consumption of GM food as a high potential risk factor in triggering allergies, toxicity and contributing to the development of cancer by enhancing DNA mutations (7). Although all of these human health risks, few countries accept the use of GMO as Brazil, Argentina, USA, Canada and China but with specific procedures in the regulatory status of the applications in their own biosafety legislation (8,9,10). There are several cantons that have presented laws in contradiction of GMOs in agriculture (11). More than 101 communes and rules have confirmed themselves free of genetically modified organisms. According to Agriculture Organization of the United Nations and Food, Egypt has an obligatory negative labeling regulation on food products as it must be labeled with GMO free to permit import of the product (12).

Meat products have several nutritional values and suggested for daily use whereas the nutrients and minerals in meat varies 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 is becoming a common practice in many countries by mixing bovine meat with different animal species' meat like donkey, dog, pork, chicken, sheep and horse (15,16).

RAC residue is a synthetic feed additive and its pharmacological and structural characteristics are extremely nearby to catecholamine. It performances an energy repartitioning agent by diverting nutrients through increasing protein synthesis ratio and/or through decreasing protein degradation that promotes muscle growth inducing muscle hypertrophy, decreasing fat deposition, improving feed conversion and therefore increasing average daily weight gain to improve carcass yield and meat quality which will subsequently increase the 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 90, 40, 10 and 10 µg/kg as RAC maximum residue restrictions 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). The cooking methods may reduce the RAC residues up to 47.52% according to Hassan et al. (19). Therefore, detection of meat from unknown sources or from growth-promoted animals is highly required to apply food safety, protect consumers from illegal adulteration regarding health, economic and religious issues (23,24). This detection allows upgrade of risk assessments related to meat manufacturing and meat products - if bovine meat mixed with different meat from other animal species- that cause harmful effects concerning human and animal health (22).

Nowadays, the common analysis techniques for qualifying 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 our knowledge, the presence of the GM food, detection of commercial fraud with meat from different animal species and RAC residue have not previously been studied on several processed foodstuffs gathered from various markets. The aim of this study was to investigate the presence of GM soybean and maize to detect meat adulteration and RAC residues in imported and local food products.

2. Material and Methods

2.1 Certified reference material

Certified reference material (CRM) from FAPAS (GM accredited by United Kingdom Accreditation Service (UKAS) as complying with requirements of ISO/IEC 17025) was used for standard curve generation in real time PCR analysis. CRMs of 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 GMO presence while enhancing specific genes for detection of the endogenous targets (soybean, maize and fruits). In this investigation, the same protocol was done for the determination of the transgenic content in food samples with soybean or maize for the specific events *Roundup (RRS*) and *Bt11* genes, respectively**.** Both appropriate CRMs and sterile ultra-pure water were used as control for each sample for the reduction of false negative/positive risk concerning the contamination during DNA extraction method and qualitative PCR analysis.

2.2 Sample collection

Total of sixty local and imported food samples were collected from different supermarkets, local markets, street vendors and slum areas in Egypt. However, twenty five samples for detection of GM sequences, thirty five for screening of meat adulteration with animal species, RAC residues and lard detection were prepared. A total of 25 commercially processed soybean and maize samples from various brands (13 soybean, 9 maize, 3 fruits) were purchased randomly from the Egyptian markets in 2020. The 13 soybean samples include (cake mix (n = 1), biscuits (n = 7), powder drink (n = 2), spices (n = 1), chips (n = 1), 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 collected samples

were from different countries with some variations in GMO legislations as presented in **Table 1**.

The thirty five meat samples were categorized as two types of meat products included 16 processed meat products i.e. (hot dog (n = 3), canned beef (n = 2), pastrami (n = 2), salami (n = 1), sausages (n = 2), luncheon (n = 3) and burger (n = 3)), whereas 17 unprocessed samples include (frozen meat (n = 6), kofta (n = 3), raw steak (n = 2) , shawerma (n = 1), liver (n = 1), minced meat (n = 4) and veal (n = 2) as shown in **Table 2**. Also, one fresh sample as donkey (*Equus asinus*), dog (*Canis familiaris*), chicken (*Gallus gallus*), pig (*Sus scrofa*), cat (Felis catus) and sheep (*Ovis aries*) were used as a positive control. Samples were homogenized and stored frozen at -20°C until the DNA extraction process. The food samples were considered for analysis in order to detect the presence of GMOs, animal species, hormone residues and lard following the detection of DNA by real-time PCR. After collection of the samples, they were transported 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 process of DNA extraction. This work was performed on accredited ISO21571 GMO laboratories in Research Park, Faculty of Agriculture, Cairo University and 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 samples containing maize, soybean, fruits and meat samples were grinded by the aid of liquid nitrogen, approximately 50 mg were weighted for each sample and moved into a sterilized 1.5 ml microcentrifuge tube comprising 500 µl of cetyltrimethylammonium bromide (CTAB) extraction buffer and mixed by vortexing for 15 s. The mixture was placed in a dry block thermostat TDB-120 (BIOSAN_16003) for 30 minutes at 65ºC and a total of 7 µl of proteinase K was added and incubated overnight at room temperature. The mixture was added with 10 µl of RNase A (100 mg/ml) and centrifuged at 12,000×g for 45 min then added 700 µl of phenol-chloroform isoamyl alcohol and centrifuged at 12,000×g for 20 min. Transferred 350 µl of the extracted DNA into a sterile microtube containing 600 µl of CTAB precipitate and incubated for 75 min at room temperature. The supernatant was removed and the precipitate was left to dry followed by adding 350 µl of NaCl then 350 µl of phenol-chloroform isoamyl alcohol. The mixture was then added with 200 µl of ethanol absolute alcohol and left overnight. The mixtures were then centrifuged at 12,000×g for 20 min, discarded the supernatant and 700 µl of 70% ethanol was added. The samples were centrifuged again at 12000×g for 15 min and supernatant was discarded, DNA pellet was left to dry ethanol and dissolving the extracted DNA sample 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 performed using Nanodrop 2000 c spectrophotometer. The concentration of the extracted DNA was determined by measuring 260 nm against a blank solution. The ratio 260/280 was used to estimate the purity of the extracted DNA. The ratio of 260/280 nm for all extracted DNA were between 1.7 and 2. For each sample and a typical working concentration of 100 ng/µl was prepared for further analysis.

2.5 PCR primers

The specific primer sequences for soybean (*lectin* gene), maize (*starch synthase IIb* gene, *SSIIb*), and the construct specific GM sequences as *RRS* and *Bt11* were performed to qualify GMOs using the real-time PCR while common regulatory sequence *(35S promoter, NOS* terminator genes) according to DS/EN ISO 21569/A1 2013 and ISO/TS 21098 (28,29). Besides, specific primers for different animal meat species were used i.e. *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 and 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 was 20 µl as final volume containing 10 µl SYBER Green Real-Time PCR master mix (KAPA Kit), 0.5 µl of forward and reverse primer, 2 µl of extracted DNA (10 ng) of 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 with the collection of fluorescence signal 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 from 65°C to 94°C. Each PCR amplification was applied in triplicate and the negative control of deionized water that comprised no-template control (NTC) with all sets of responses was used and also 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 and the gel was disolved with 1.5% agarose with 1x Tris Buffer EDTA (TBE) running buffer. The run was performed at 80 V for 180 minutes then the gel was stained using 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 using a gel imager (Bio-Rad – Gel DocTM EQ).

2.7. Ractopamine determination and ELISA analysis

The method of RAC residue using My BioSource supplies Enzyme Linked Immunosorbent Assay (ELISA) kits (ELx8081U No. 20397) is based on a competitive colorimetric assay. The Immunosorbent Assay (ELISA) kits for the detection of many antigens, proteins and peptides in a wide-ranging selection of species reactivity. The antigen of RAC has been coated in the plate well by Sinogenclon Co., Ltd (China). Two grams (± 0.05 g) of homogeneous tissue samples were oscillate to 8 ml of acetonitrile solution for 2 min and centrifuged at room temperature at 4000 r/min for 10 min to remove fat. 5 ml from the

supernatant were dried at 50-60°C. A quantity 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 for determination of lard presence in meat samples. The functional group generated was observed using spectrophotometer in the mid infrared region (500-4000 cmG1). This instrument is prepared with deuterated triglycine sulphate (DTGS) detector and KBR as beam splitter with a resolution of 8 cm G1 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. Wiped the windows with a tissue and then washed for 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 filling into the next sample.

3. Results

3.1 DNA concentration

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 and also undeclared animal species in meat product in qualitative real-time PCR reaction were extracted with the appropriate amount of DNA and adequate quality for more accurate GMO and animal meat species testing. The absorbance ratios of extracted DNA at 260 nm ranged from 1.7-2.0 ng/µl, and the concentration of DNA was ranging from 30-100 ng/µl for soybean, maize and fruit product while the concentration of DNA was ranging from 20-100 ng/µl for processed and unprocessed meat. Also, results proofed that the extraction procedure was accurate, reliable and integrated while the extracted DNA was having a high quality from raw or processed food samples.

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

The screening of the GM sequences from the genetically modified soybean, maize and fruits in food products were carried out. A total of twenty-five non-labeled samples were collected including thirteen soybean, nine maize and three fruits to detect *CaMV35s, T-NOS, Bt-11* and *RRS* genes using qualitative PCR. Our results suggested that the intrinsic SSIIB and specific lectin are to be available for further investigation of GM sequences for soybean and maize, respectively. The results of the present study revealed that the 16 out of 35 were 12 soybean, 2 maize, and 2 fruit 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 192 of *CaMV 35S* sequence and displayed in samples (5, 9, 10, 11, 13, 14, 15, 16, 17, 19, 20, 21, 22, 23, 24 and 25) as presented in **Fig. 1**. According to the results of qualitative real-time PCR, 16 samples showed positive

results with *CaMV35s* sequence which proved the presence of GM sequences in their genome as shown in **Fig 2**, **Table 4** and **Table 5**. It 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 of the 12 soybean and 9 maize samples out of the 16 GM positive samples were detected using specific using the *RRS* gene *(*soybean) and *Bt-11* gene (maize). Our results revealed that the soybean powder sample (NO. 20) contained the *RRS* sequence in their genome (**Fig.4 A**). While, the powder drink sample (NO. 16) included *Bt-11* sequence in their genome (**Fig. 4 B**). The results proved the presence of GM sequences in soybean and maize genome as presented in **Fig. 4**. In the light of qualitative real-time PCR results of the twelve food samples containing soybean including chips, biscuits, soybean powder protein, spices, powder drink were positive for screening targets (*CaMV35s, NOS, RRS*). Also, *CaMV35s, NOS* and *Bt 11* were identified in two maize samples i.e. powder drink and spices. While, two fruit samples were positive with *CaMV35s* as presented in **Table 4**. The results of the present study (**Table 5**) indicated that 92%, 22%, and 66% of soybean, maize and fruit samples, respectively were positive for screening targets GM sequences. To date, Egypt has imported different types of transgenic crops such as soybean and maize, but the cultivation of these plants is prohibited yet.

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 thirty-five meat samples representing different animal species were successfully amplified using specific primers. In this study, we propose the qualitative real-time PCR analysis for accurate pork, soybean, chicken, dog, cat, donkey, sheep and horse quantification 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 .The primers generated specific fragments i.e 290, 143, 153, 145, 225, 183, and 274 bps for pork, dog, horse, donkey, sheep, chicken and cat , respectively by real-time PCR. Results of the quantitative real-time PCR are recommenced and compared with the labelled data regarding the addition of pork, soybean, chicken, dog, cat, donkey, sheep and horse of the 35 samples of processed and unprocessed meat product as presented in Table 6. The results revealed that clear and positive data findings for pork virulent gene (*12SRNA- tRNA val)* were scored in eleven meat samples (12, 14, 19, 21, 22, 23, 16, 25, 17, 26 and 28) as presented **Table 6** and **Fig. 5**. Moreover, the virulent genes in different animal meat species were amplified in five samples i.e. for the dog (29), horse (31), donkey (28), sheep (30), and chicken (33), while the detection of cat virulent genes was negative for all meat samples as shown in **Fig. 5** .The results revealed that pork is the main undeclared species in burger, luncheon, hog dog, veal, liver, burger and frozen meat. Moreover, the results showed the imported minced meat contaminated and adulated with dog horse and sheep (**Table 6**).

3.4 Determination of RAC residues using ELISA technique

The results of RAC showed that all the tested samples contained RAC, but no sample exceeded the maximum limit stated by Codex (21). Therefore, the RAC limit ratio was showed in 19 out of 35 collected processed and unprocessed meat samples. The results revealed that the RAC residues in liver tissues were the highest among samples. Moreover, the highest detected RAC concentrations were associated with samples contaminated with pork. The data existing in Table 7 show that the unprocessed samples exhibited high RAC value than those of heat processed. In this connection Pastrami showed RAC concentration of 6.3 µg/ Kgas prepared by dehydration under room temperature conditions compared with luncheon, (RAC ranged between 3.44-4.63 µg/Kg) prepared by boiling or steaming. These results prove that heat treatment can decrease the RCA concentration in processed meat sample.

3.5 Determination of lard in meat samples using FTIR spectroscopy

Fourier-transform infrared spectroscopy "FTIR" peaks are highlighting the presence of specific hydroxide groups and hence identifying fatty acids. However, the FTIR spectrophotometry analysis was used to determine the presence of a fatty acid called nervonic acid indicating the contamination of meat with lard. The results revealed that only 3 out of 35 meat samples showed positive results that were shawarma, liver and hawawshi (14, 22 and 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. Moreover, the peaks of ketone are sharp and the wave numbers are 1742 cm -1 for all of these samples (**Fig. 6**). In addition, the samples 14, 22, and 24 containing the concentration value of nervonic acid 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 that indicating there was no broadband at the hydroxide group and absence of nervonic acid. The results proved the presence of lard contamination in three processed and unprocessed.

4. Discussion

Food safety is a main concern worldwide due to the increased attentions toward the concept of food adulteration that affects human of every gender and age. The Imported and food markets have a high influence on public human health as different strategies were developed to increase food grains production by GM material, mixture of meat animal species and meat from hormonally exposed animals. Therefore, this comprehensive screening study was designed to demonstrate and detect the economically encouraged food adulteration with GMOs, animal meat species and RAC in sixty local and imported products. It is generally considered difficult to accurately determine the food adulteration with GMOs and meat animal species in the same qualitative specific methods. Accordingly, 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 effective and high sensitivity in the detection of any contaminates in meat and food product.

Food samples containing soybean and maize have been selected to be analyzed for GM sequence detection. Since soybean and maize are ranked as the two main GM crops

cultivated worldwide. Results of GM sequence detection revealed that the recombinant DNA target sequences were detected in 16 out of 25 non-labeled samples using the common regulatory genes (CaMV35s, NOS terminator) and specific genes (*Bt-11* and *RRS*) for soybean and maize, respectively 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 detected in domestic food productions. Results of the present study indicated 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. These studies showed a high percentage of GM sequences in food and feed products in many countries included soybean products. Our findings of the collected non-labeled food samples were consistent with results of Rabiei et al. (34) who screened twenty-five food samples from Iranian markets using *CaMV35s* and *Bt11* primers by qualitative real time-PCR but only 5 were positive GM maize. Similarly, the screening of food samples by Kaur et al. (35) indicated that 13 out of 20 non-labeled maize samples from the Malaysia market included CaMV 35 S promoter or NOS terminator and Bt-11 sequences in their genome. CaMV 35 S promoter and NOS terminator are the two main important screening common regulatory genes for qualitative PCR analyzes in most of the commercialized transgenic crops (36). In addition, Holden et al. (37) revealed that the CaMV 35 S exists in 95% of GM foods in Europe. Moreover, Safaei et al. (38) studied that the non-labeled rice samples from the Iran market using *CaMV35S* promoter and *NOS* terminator for the identification of GM rice sequences by PCR. In addition, in Egypt Oraby et al (39) used the *CaMV35s* and *NOS* terminator genes for GM sequence detection in food products using the PCR technique.

Our results in Egypt using specific events i.e. 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 two the GM positive soybean and maize samples contained the *Bt-11* and *RR soy* genes, which proved the presence of GM sequences in their genome. Our results agree with Zdjelar et al. (40) indicated that eight non-labeled soybean samples eventually from EU countries, Argentina, USA, Thailand and Brazil, were positive results for *RRS* sequence. RRS specific gene sequence is the only transgene plant variety permitted for consumption in the EU market, but it's not permitted to be cultivated. The gene RRS has been encoded to be glyphosate-resistant during the cultivation. Glyphosate is a nonselective chemical substance commonly used in RR herbicides although the accumulation of this Glyphosate herbicide in soil and plants may due to unintended influence on the environment and human health (41). For instance, Mesnage et al. (42) revealed that glyphosate has adverse effects under regulatory limits such as neurotoxicity, carcinogenicity, hepatic and kidney toxicity. 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 resistance for an insect that has been acceptable by the EU in food and feed products. Accordingly the food safety concern it is necessary to detect the residue concentration and GM materials in food products especially in glyphosate-resistant crops.

The results of the study in 2005 and our 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 regarding the labeling of food materials derived from GM products, none of the collected samples in 2020 were appropriately labeled. To date, Egypt has imported different types of transgenic crops such as soybean and maize, but the cultivation of these plants is prohibited. Additionally, in order to control these products and protect the consumers' concern 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). 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, consumption of food and fruit products in the developing countries increased which requires a high degree of awareness against unlabeled food for the protection of the public health. A restricted system should be accomplished 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 possing a high concern as medical aspects and customer's right in worldwide. Consequently, many analytical techniques were used for the identification of meat species using DNA-based techniques or techniques based on PCR such as RFLP and RAPD (46). Recently, real-time PCR have recommended as the most accurate technique for screening of animal meat species either 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, specific primers were designed as the gene encoding *12S RNA,* 12SRNA- *tRNA val, cytochrome* b, *ND2* and *ND4* for the detection of each species.

The results indicated that the specific sequence of each species were detected in 17 out of 35 meat samples including 11adulterated with pork and only one sample as 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 agrees with the study of Rashid et al. (47) as they used similar primers for the detection of meat animal species. In this regards whereas, another agreement used the real time-PCR for the detection of the adulteration in animal meat species (48). While Farag et al. (46) reported that the presence of dog, donkey, chicken, pork, sheep and horse in fifteen meat sample using DNA-based techniques especially the techniques based on PCR such as RFLP and RAPD in Bangladesh.

The results revealed that the real-time PCR systems were established for the qualification of specific detection of each species either for GM maize/soybean or mixture of meat animal species. Meanwhile, clearly proved to be as an easy and accurately applied to various food and meat products, and used internationally for 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 is able to detect RAC accurately without complicated purification, and 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) has established this technique as a screening method for residues of RAC in imported and exported meat. Besides, Dong et al. (51) proved that the concentration of the RAC in tissues is ascending arranged as follows: stomach>kidney>large intestine>small intestine >liver>heart>muscle. This finding may be related to exposure to high temperature during preparation, as suggested by Hassan et al. (19). In addition that may explain the reduction of RAC residues in heat processed meat. Our results revealed that the untreated samples (kofta, shawarma) had a high RAC value than the samples treated by heat (pastrami, luncheon). However, the concentrations still under the maximum safe limit stated by Codex, 2012 (21).

Lard presence detection in processed and unprocessed meat using FTIR analysis was evaluated. Our results revealed that only three samples were identified with the lard presence in processed and unprocessed meat product, while the other meat samples were not identified as lard-containing samples. Our results agree with Ramli et al. (52) as they reported that the FTIR analysis is able to provide a low cost and rapid method with lower 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 and therefore permitting the detection of lard presence. .

The results of soybean, maize and fruits GM detection, undeclared animal species detected, RAC and lard presence detection 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 responsibility for making regulations to protect consumers against harm arising from food adulteration worldwide. Previous studies on genetically modified plants cause high risks and safety concerns regarding their consumption as food or feed (53).

5. Conclusion

The current study was conducted to detect the economically adulterated food products with GMOs, undeclared animal meat species, lard and RAC residue in several local and imported products to ensure the consumer protection and his 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 were with high sensitivity, accuracy and cost effective 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 concentration and undeclared animal meat species in processed / unprocessed meat products are documented. The obtained data clearly showed all the detected positive samples were unlabeled which in turn provide reliable information to consumers. The

present study highlights 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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Table1. The twenty five unlabelling maize, soybean and fruit samples were 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)	2Maize, 1soybean	2 imported, 1 domestic	Absent
Fruits	(3)	Fruits	Imported	Absent

Table 2. The thirty five animal meat species were 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 1 local	Absent
	Pastrami	(2)	Imported	Absent
	Sausages	(2)	Imported	Absent
Unprocessed Meat	Frozen meat	(6)	3 Imported 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 2 Local	Absent

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

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

Code	Product's name	Types of species	Domestic /Imported	*Common regulator genes*		*GM Specific BT-11*(Maize)	*events RRS*(Soybean)
				CaMV35s	*TNOS*		
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)

Table 5 .Detection of endogenous genes and transgenic DNA sequences of soy bean and maize product samples during 2020 by qualitative Real time PCR method.

Food products	**NO. of samples**	***SSIIb***	**Lectin**	**Common regulatory *CaMV35s* *NOS***		***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	-

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

				Undeclared animal meat species detected							
Code	Product's name	Processed /Unprocessed	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.

Table7: 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

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 (100bp ladder) +ve: positive sample (Certified reference material) and - : negative sample (sterile ultra-pure water) ,NTC non template control .

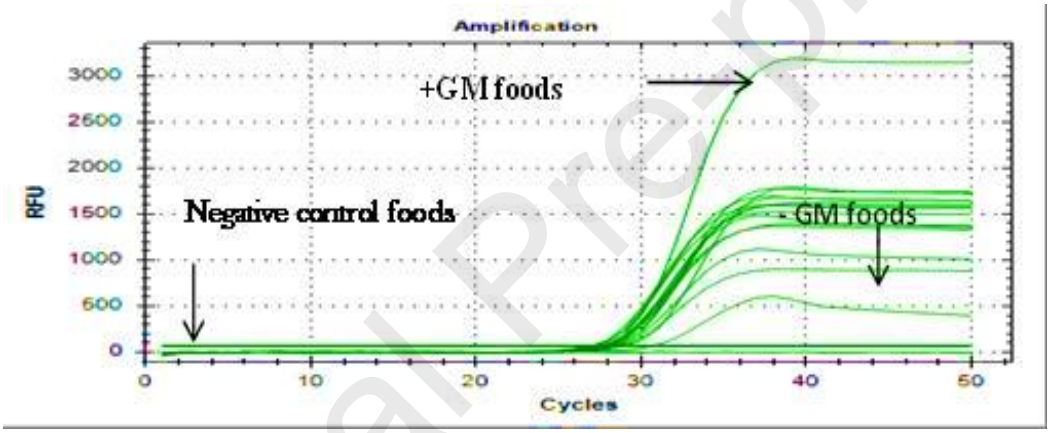

Fig. 2. GM food as standard of real-time amplification at 195 bp bands in 25 samples (+GM) and negative control of amplification (-GM).

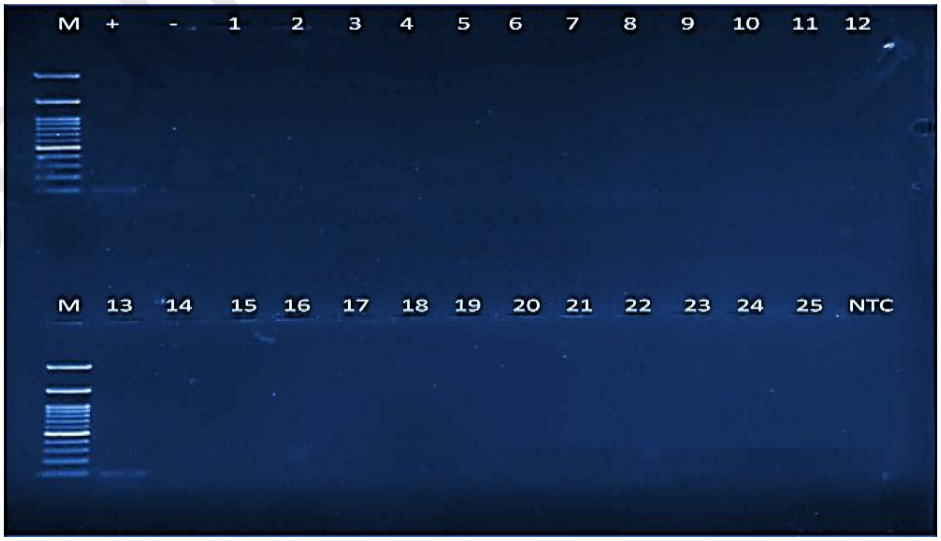

Fig. 3. PCR amplification of GMO-specific regions using primer pairs: TNOS for/TNOS rev. Lanes 1-25 extracted from food products containing maize, soybean and fruits samples. M: Molecular weight marker (100bp ladder) +ve positive sample i.e. Certified reference material, -ve negative control. NTC non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

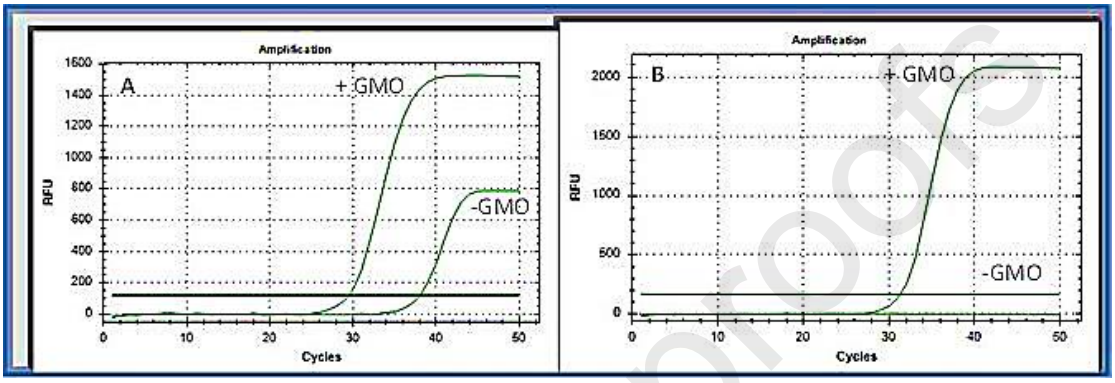

Fig. 4. GM food as standard of real-time amplification for both genes Ready Roundup Soybean (A) at 172bp in sample 20 and Bt-11 (B) at 68bp bands in sample 16, Negative control of amplification.

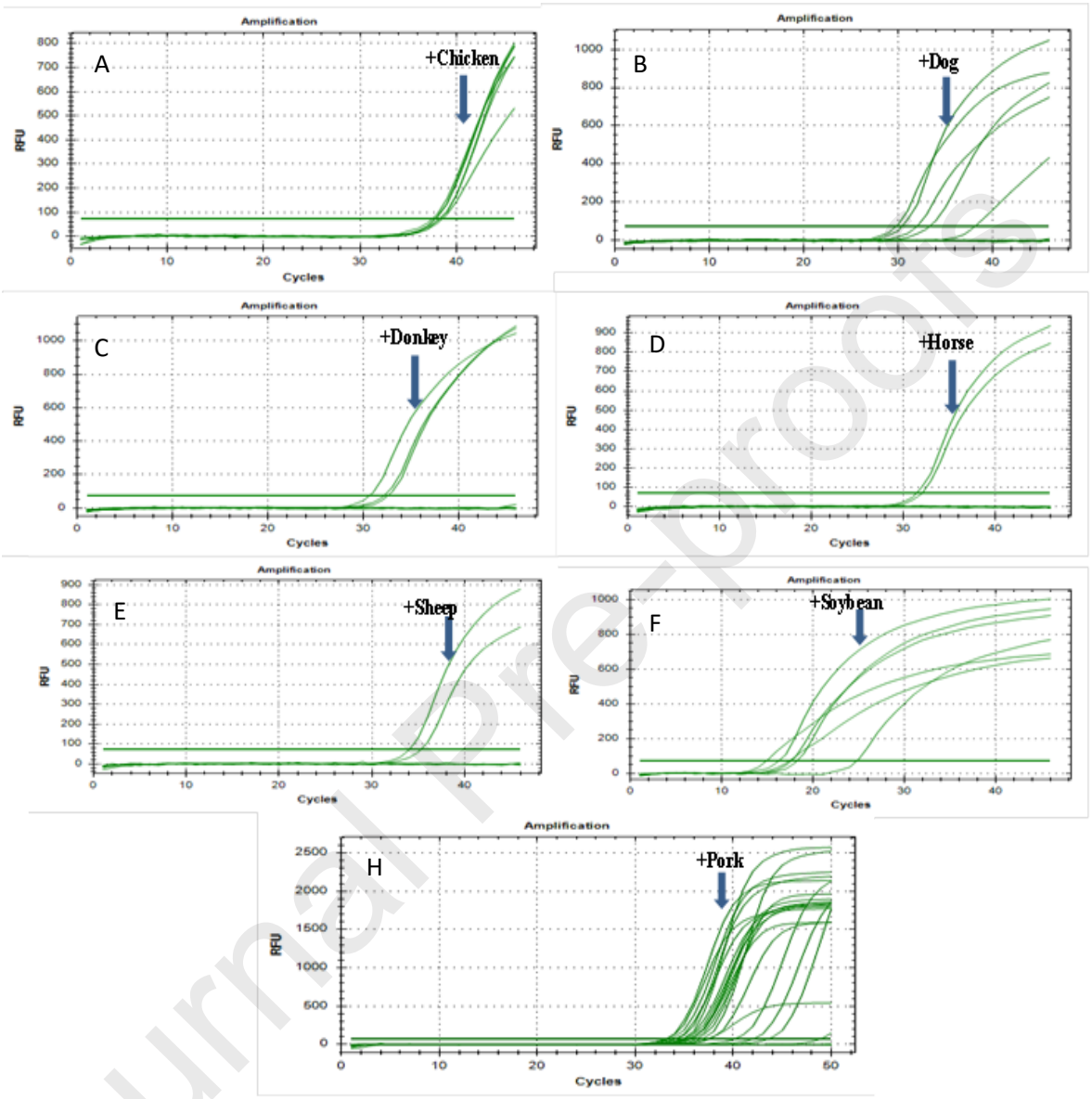

Fig. 5. Real-time PCR amplification of thirty four animal species, a) chicken , b) dog, c) donkey, d) horse, e) sheep, f) soybean and H) pork.

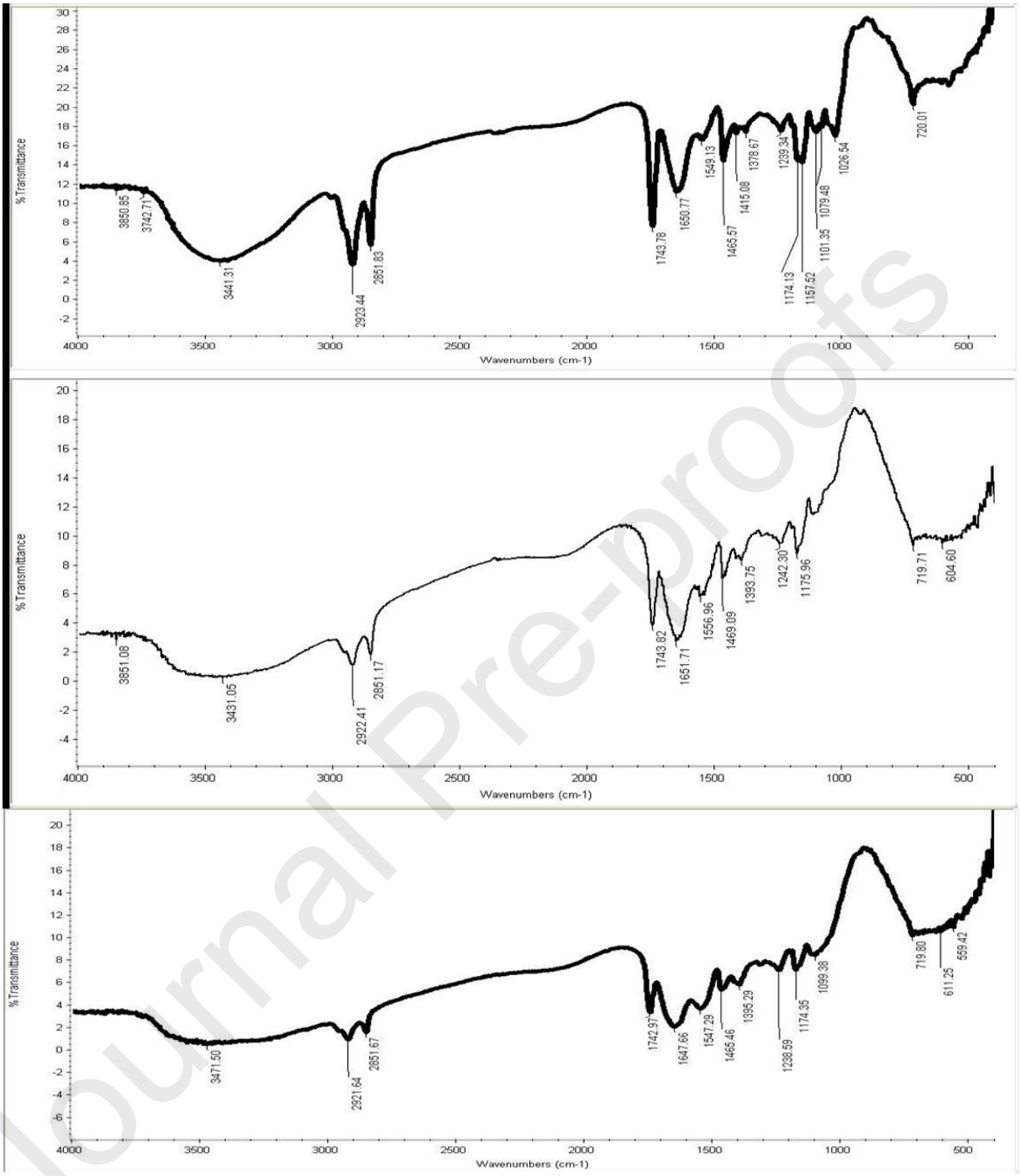

Fig.6. Showing the FT-IR curve of the positive lard samples.