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# Applications of PCR Technique to Identify the Raw Materials in Different Foods and Feed Products

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**Abstract** The aim of this work was to evaluate the possibility of using PCR technique to identify raw materials in different food and feed products. As materials for analysis food products based on soybean, feeds and cold meat products were used. The DNA extracted from all types of materials was amplifiable, even if the concentration and quality were affected by the high degree of processing. As it was expected, the lectin, soy bean specific gene was present in all of the soy bean food products and feeds and also in three of the cold meat products. The GM (genetically modified) sequence was identified only in three types of feeds, being absent from each food product. Besides, for the cold meat products the animal species origin was determined. It was pointed out that the labeling meet the legislation regarding the GM products, but it is not accurate for the animal originated food. It was demonstrated that PCR can be a valuable tool, with a high specificity which can be used to evaluate the food authenticity.

**Key words**

PCR, raw materials, GMO, food authenticity

The polymerase chain reaction (PCR) technique is an *in vitro* enzymatic amplification method of a specific DNA sequence. Discovered in 1983 by Kary B. Mullis, it is based on the discovery of T*aq* polymerase, a thermo-stable enzyme that can withstand temperatures higher than 90°C, in the presence of which the two chains forming the double helix structure separate allowing each chain to be copied.

The PCR technique consists of a series of heating and cooling cycles: an initial DNA denaturation at 94-98°C, followed by a repetition of a DNA denaturation step at 94-98°C, primer annealing step at 50-65°C, and an extension step at 72-80°C, followed by a final extension at 70-74°C [9].

The aim of this paper was to use the PCR technique in order to detect and identify the raw materials in feed and food products based on soybean and meat.

It is known that the soybean is a plant from the Fabaceae family, also known by its binomial name Glycine max. It is an annual plant possessing hairy stocks with few ramies and hairy trifoliate leaves. It has small flowers that are white or violet in colour, existing in groups comprising of three to nine pieces, on the axil of the leaf, on a short raceme. It produces cylindrical pods, usually containing one to five globular seeds, rich in high quality protein. [1]

It is a herbaceous pant originating from East Asia, where it has been cultivated for a very long time and it is considered to be a very important plant species, along with rice, wheat, barley and millet. In Asia, the soybean is nicknamed *boneless meat* or *Chinese beef*. Soybean entered Europe around 1700 CE, the United States of America in 1765, and Romania in 1876 through Transylvania.

It is appreciated for its nutritive values, which are comparable to food of animal origins, but also because of the health benefits it provides and it is considered one of the contributing factors to Asians' longevity. Correctly processed soybeans are the only food of vegetal origin that can supply the body with all the essential amino-acids.

In the USA following the First World War, during the recession, soybean due to its attribute of fixing nitrogen played an essential part in soil regeneration. [3]

Today soybean is a major part of human and animal nourishment alike. It can be found in cold meats, it is used as a protein source by people who choose to observe religious customs such as fasting and people who choose not to eat food of animal origins, and it can be found in feeds.

In a constant search for betterment, agriculture and biotechnologies have evolved throughout the years. One biotechnological process that is relevant to this paper is genetic modification. For example, plants can be genetically modified to make them resistant to various pests and diseases, or to increase their productivity levels [11].

Genetic modification is subjected to a comprehensive set of rules and regulations, that differ from country to country, and that depending on the region they are implemented in, may be stricter or

more relaxed. The European Union for example, takes a harsh stance on the cultivation and distribution of genetically modified crops and any usage of such crops has to be declared [12, 13]. In order to verify whether the food and feed people and animals consume really is as it is declared by the manufacturers, it can be tested using the PCR technique. In the same time the food composition was verified to emphasize if the raw materials listed on labels are true.

In order to verify whether the extracted DNA is of high enough quality to be amplifiable or not, primers specifically designed for the analyzed species were used. For the purposes of this paper, where we wanted to detect whether soybean DNA was successfully extracted, the presence of lectin was tested for. Lectin is a gene that is specific to soybean, and is present in one single copy in its genome.

In order to detect the presence of genetically modified soybean, the DNA sequence introduced in Roundup Ready (RR) soybean was taken into account (Fig. 1). The detection method used in our work was developed for the T-nos sequence - primers HA-nos118 [5, 7, 8, 10, 15].

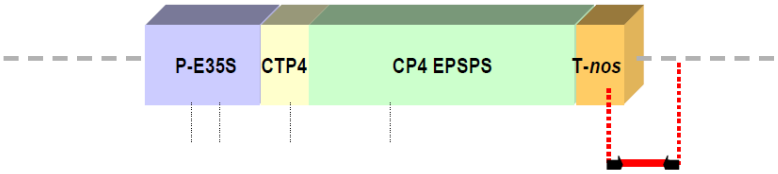


Fig. 1 The elements of the GM (genetically modified) construct introduced in RR soybean
P-E 35S- enhanced CaMV 35S promoter; CTP4- chloroplast transit peptide from Petunia Hybria EPSPS gene
CP4 EPSPS – EPSPS from agrobacterium sp. Strain CP4; T-nos – nos terminator

In order to emphasise different animal species in meat products the primers developed for specific mithocondrial genes namely 12S rRNA-tRNA for swine and 12S rRNA for poultry were used [2, 4, 14].

## Materials and Methods

### Biological materials

Different soybean food, feeds and meat products were analyzed: 1- Soy noodles, 2- soy cubes (a), 3- soy grains (a), 4- soy slices, 5- soy cubes (b), 6- soy grains (b), 7- ground feed, 8- soy feed, 9- feed (a), 10- feed (b), 11- feed (c), 12- salami (a), 13- salami (b), 14- sausage (a), 15- sausage (b), 16- chicken sausage, 17- pork sausage (a), 18- pork sausage (b).

### Working methods

### DNA extraction and analysis

To extract the DNA from the samples we worked with, we followed the CTAB extraction protocol [6]. 100 mg of previously ground sample were mixed with 300 µl sterile distilled water in tubes. Then 500 µl CTAB extraction buffer was added (CTAB - 20g/l; NaCl-1.4 M; Tris-HCl – 0.1 M; $Na_2EDTA$-20 mM), followed by homogenizing the mixture through vortexing.

Following that, 20µl K-proteinase was added and the mixture was incubated for a period of 30 minutes at a temperature of 65°C and then centrifuged for a period of 10 minutes at 16000xg. The resulting supernatant was then transferred into a new tube and 500 µl of chloroform was added.

The resulting mixture was centrifuged for 5 minutes at 16000xg, the resulting supernatant was transferred into a new tube and 1 ml CTAB precipitation solution was added (CTAB -5g/l; NaCl-0,04M). The mixture was homogenized and then left at room temperature. After 60 minutes, the mixture was centrifuged for 5 minutes at 16000xg and the resulting supernatant was discarded, keeping only the sediment on the bottom of the tube. 350 µl NaCl 1.2 M was added and the tube was agitated until the sediment dissolves. 350µl chloroform was added and the mixture was centrifuged for 10 minutes at 16000xg and the resulting supernatant was transferred into a new tube. 200 µl Isopropanol was added, the tube contents was gently mixed together and then kept at room temperature for 20 minutes. The mixture was centrifuged for 10 minutes at 16000xg, the supernatant was discarded and 500µl of 70% ethanol was added. The mixture was shaken until the sediment on the bottom of the tube was completely dissolved and then centrifuged for 10 minutes at 16000xg and the resulting supernatant was discarded. 100µl of sterile distillated water was added and the tube was shaken until the sediment on its bottom dissolves completely.

Following its purification, the quantity and quality of the previously extracted DNA was assessed using the spectrophotometre to measure its concentration and 260/280 absorbance ratio.

### PCR analysis

The amplification mixture was obtained from adding the following solutions: 12.5 µl GoTaq Green Master Mix (Promega, USA), 2 µl Primer 1 20 µM , 2µl Primer 2 20 µM, and 6.5 µl sterile distillated water

in as many tubes as samples available, and then in each tube 2 µl sample DNA was added.

The tubes containing the PCR mixture and the DNA samples were placed in the thermo-cycler for the allotted time necessary for them to undergo DNA amplification.

In order to amplify the lectin gene the following primers were used: GMO3: 5'-GCCCTCTACTCCACC CCCATCC-3' and GMO4: 5'- GCCCATCTGCAAGCCTTTTTGTG-3'.

The program used to amplify the lectin gene consisted of several heating and cooling cycles: (i) initial DNA denaturation at $95^0$C for a period of 10 minutes, followed by (ii) 35 cycles of denaturation at $95^0$C for 30 seconds, primer annealing at $60^0$C for 30 seconds, and extension at $72^0$C for 60 seconds and (iii) final extension at $72^0$C for 3 minutes.

For the purpose of amplifying the artificially inserted gene the following primers specific for the terminal sequence t-nos sequence were used: HA-nos118-f: 5'-GCATGACGTTATTTATGAGATGGG-3' and 5'-GACACCGCGCGCGATA ATTTATCC-3'.

The program used for this amplification consisted of the following steps: (i) an initial denaturation at $95^0$C for 3 minutes, (ii) 50 cycles of: denaturation at $95^0$C for 25 seconds, primer annealing at $62^0$C for 30 seconds, and extension at $72^0$C for 45 seconds and (iii) the final extension at $72^0$C for 7 minutes [5].

In order to amplify genetic material originating from swine meat the following primers were utilized: 12S rRNA-tRNA 5'-CTACATAAGAATATCCACCACA-3' and 5'-ACATTGTGGGATCTTCTAGGT-3' (290bp).

For amplifying genetic material of poultry origin the following primers were used: 12S rRNA 5'-TGAGAACTACGAGCACAAAC-3' and 5'-GGGCTATTGAGCTCACTGTT-3' (224bp).

The program used for this amplification was: (i) after an initial heat denaturation at 94 °C for 10 min, (ii) 35 cycles conswasting of: DNA denaturation at 94°C for 30 seconds, primer annealing at 60°C for 1 minute, and extension at 72°C for 1 minute, and (iii) the final extension at 72°C for 5 minutes. [2]

The amplification products were separated by agarose gel electrophoresis prepared as follows: 100 ml TAE solution and 1.5 g agarose were heating to boiling, 2 µl Ethidium bromide was added and the mixture was poured into a mould. Once the gel has solidified, it was placed in the electrophoresis tank and covered withTAE solution.

Then, after removing the DNA samples from the thermo-cycler, 25 µl of amplification products were introduced in each well formed in the gel. In the last well 6 µl molecular weight marker was placed.

The gel was then subjected to and electrical current of 100 V in the electrophoresis tank, for a period of 40 to 50 minutes, allowing the DNA fragments to migrate.

Following the electrophoresis process, the gel was analyzed under a UV light to assess how much the DNA fragments have migrated within the gel.

## Results and Discussions

In our work 18 samples were analyzed, out of which samples 1 through 6 were soybean based products, samples 7 through 11 were feeds and samples 12 through 18 were cold meats. The soybean based products and the feeds were grounded and measured 100 mg per sample. With the cold meats, before grinding and measuring 100 mg per sample they were air dried.

The quality and quantity of the extracted DNA was heavily influenced by the degree of processing the raw materials were subjected to (Table 1 and Fig. 2). As such, the lowest quality and quantity of DNA was found in cold meats, which undergo intense processing.

It is known that the ratio between the absorbance at 260nm and 280nm respectively is an indicator of the DNA quality, considering that if the value is closer to 2 the samples are pure, with low content of proteins.

Table 1

**The concentrations and quality of the extracted DNA samples**

| Sample No. | Sample | DNA concentration (ng/l) | 260/280 | 260/230 |
|---|---|---|---|---|
| 1 | Soy noodles | 206.5 | 2.13 | 2.34 |
| 2 | Soy cubes | 154 | 2.06 | 2.46 |
| 3 | Soy grains | 112.6 | 2.05 | 2.39 |
| 4 | Soy slices | 109.7 | 2.07 | 1.89 |
| 5 | Soy cubes (different manufacturer) | 64.53 | 2.04 | 2.27 |
| 6 | Soy grains (different manufacturer) | 118.3 | 2.05 | 2.23 |
| 7 | Ground feed | 35.27 | 1.56 | 0.93 |
| 8 | Soy feed | 36.64 | 1.37 | 0.58 |
| 9 | Feed 1 | 23.47 | 1.98 | 1.64 |
| 10 | Feed 2 (GMO) | 64.96 | 1.85 | 0.98 |
| 11 | Feed 3 | 29.95 | 1.65 | 1.03 |
| 12 | Salami 1 | 33.43 | 1.65 | 0.73 |
| 13 | Salami 2 | 19.35 | 1.85 | 1.43 |
| 14 | Sausage 1 | 15.96 | 1.81 | 1.72 |
| 15 | Sausage 2 | 17.51 | 1.89 | 1.69 |
| 16 | Chicken sausage (crenvursti) | 34.13 | 2.04 | 1.78 |
| 17 | Pork sausage 1 (crenvursti) | 12.24 | 1.58 | 1.27 |
| 18 | Pork sausage 1 (polonez) | 17.58 | 1.8 | 1.44 |

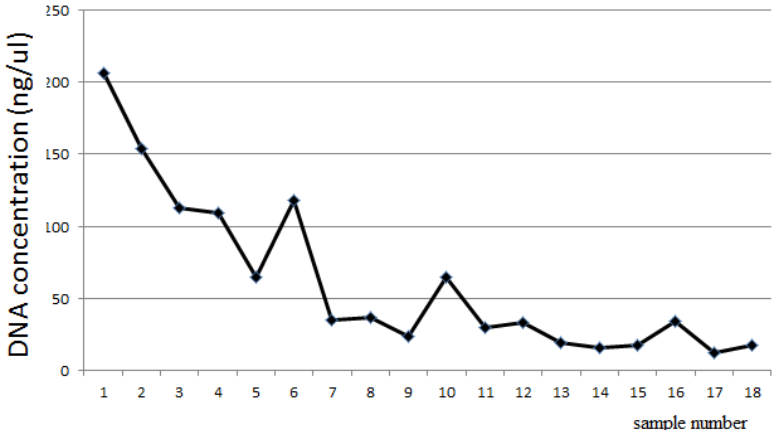


Fig. 2 The concentrations of the extracted DNA

First the samples were analysed with the primers specific for lectin genes (Fig 3).

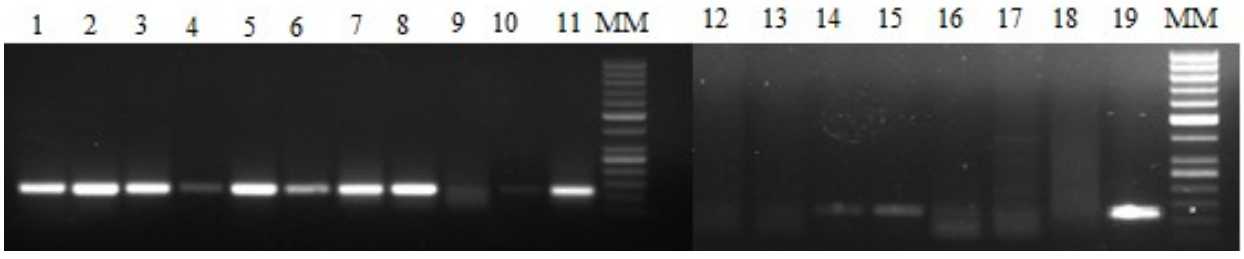


Fig. 3 The agarose gel electrophoresis of the amplification products with the lectin specific primers

1- Soy noodles, 2- soy cubes (a), 3- soy grains a, 4- soy slices, 5- soy cubes (b), 6- soy grains (b), 7- ground feed, 8- soy feed, 9- feed (a), 10- feed (b), 11- feed (c), 12- salami (a), 13- salami (b), 14- sausage (a), 15- sausage (b), 16- chicken sausage, 17- pork sausage (a), 18- pork sausage (b), 19- positive control, MM- molecular weight marker (100bp DNA ladder)

It can be observed that the lectin gene was present in all the soybean based product samples and feed samples (less in samples 4, 9 and 10) and in some of the cold meets samples, namely in samples 14 and 15. The amplified fragments were similar in size with the positive control (sample 19), which was extracted from certified soybean materials. In the same time it was pointed out that the extracted DNA was amplifiable.

Further on all the samples the DNA were amplified with the t-nos specific primers, in order to determine if they contain GM soybean (Fig. 4).

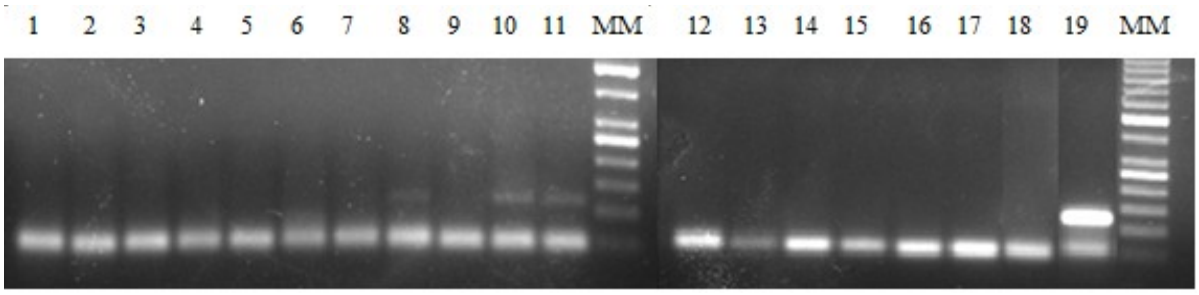


Fig. 4 The agarose gel electrophoresis of the amplification products with the t-nos specific primers

1- Soy noodles, 2- soy cubes (a), 3- soy grains a, 4- soy slices, 5- soy cubes (b), 6- soy grains (b), 7- ground feed, 8- soy feed, 9- feed (a), 10- feed (b), 11- feed (c), 12- salami (a), 13- salami (b), 14- sausage (a), 15- sausage (b), 16- chicken sausage, 17- pork sausage (a), 18- pork sausage (b), 19- positive control, MM- molecular weight marker (100bp DNA ladder)

Analyzing the gel it turned out that none of the cold meats samples contain genetically modified raw materials and that genetically modified soybean was used in samples 8, 10 and 11, which are all feeds. Genetically modified soybean was absent from all soybean based products.

The DNA samples extracted from cold meat products were analyzed with primers specific for swine and poultry because they are the most frequently raw materials used for this kind of food (Fig. 5).

Therefore it was possible to identify whether the cold meets samples contained DNA of swine and/or poultry origin. Most cold meets contained swine, less so in the case of sample 16, which was labeled as chicken sausage. The poultry material was identified in almost all of the samples, with exception of one salami sample (12).

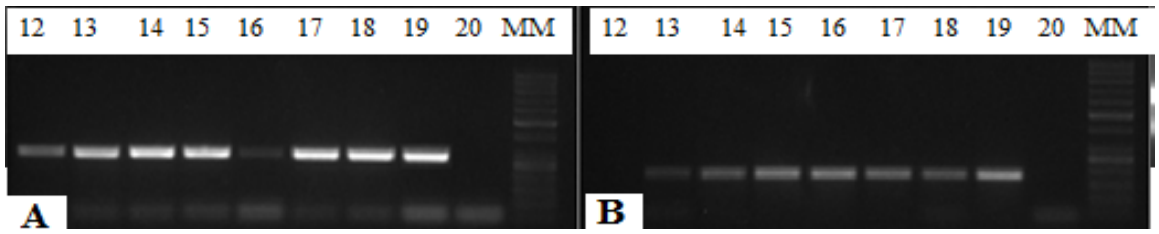


Fig. 5 The agarose gel electrophoresis of the amplification products with the swine (A) and poultry (B) specific primers for the meat origin products

12- salami (a), 13- salami (b), 14- sausage (a), 15- sausage (b), 16- chicken sausage, 17- pork sausage (a), 18- pork sausage (b), 19- positive control, 20- non template control, MM- molecular weight marker (100bp DNA ladder)

## Conclusions

Considering the obtained results the DNA was correctly extracted regardless of sample type.

By assessing the extracted DNA through spectrophotometre measurements and electrophoresis, it was ascertained that the type of sample the DNA was extracted from highly influences its quantity and quality.

Even when working with small quantities of low quality extracted DNA, amplification was possible, demonstrating the accuracy of the PCR technique and the species-specific primers' performance.

The transgene's presence was highlighted through PCR analysis, using HA-nos 118 primers for feeds.

None of the food products analyzed contained genetically modified ingredients. Furthermore, where

meat products were involved, identifying the raw materials, whether it was swine and/or poultry, was possible.

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