# Detection of Residues of Genetically Modified Soybeans in Breaded Fried Turkey Cutlets

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**ABSTRACT: The presence of genetically modified organisms (GMOs) in food products is usually ascertained by the polymerase chain reaction (PCR) or nested PCR if sensitivity has to be increased. Since most, if not all, GMO products are of plant origin, the target sequences are the 35S promoter or NOS terminator. The extreme sensitivity of nested PCR can be misleading if the results are not interpreted correctly, since contamination of non-GMO products with residual amounts of GMO may be positive. We report that breaded turkey breast cutlets labeled as containing GMO soybean products were actually prepared from wheat flour that had been contaminated with transgenic soybean.**

**Key Words: PCR, nested PCR, GMO, soybeans, 35S promoter**



## Introduction

RECENT ADVANCES IN CREATING PLANTS WITH USEFUL TRAITS (Oeller and others 1991; Vardi and others 1993; Falco and others 1995; Worrall and others 1998; Rao and others 1998), and their approval by government authorities has made it possible for food manufacturers to market genetically modified food products. Transgenic soy was one of the first plants to be genetically modified and soy products are widely used today in food processing (Shirai and others 1998). Transgenic soy constitutes a large proportion of the total soybean crop in the USA, which is the world's major producer. Pressure from consumer groups and public demand have caused several governments, particularly in Western Europe, to impose labeling for the presence of genetically modified products when used as a components of foods.

Labeling policies require the availability of systems that identify the presence of genetically modified products. The polymerase chain reaction (PCR) is the current method of choice. PCR uses specific primers for several sequences that are used in creating transgenic plants: the cauliflower mosaic virus (CaMV) 35S promoter, neopaline synthetase (NOS), octopine synthetase (OCS) and CaMV terminators (Beck and others 1982; Depicker and others 1982; MacCormick and others 1998). Detection of very small amounts of genetically modified organisms (GMOs) products, or products in which the DNA is destroyed during processing, the more sensitive method of nested PCR is used (Studer and others 1997). However, in using PCR techniques or the more sensitive nested PCR to detect specific DNA, the risk is that contamination of raw material with genetically modified products prior or during processing can be misinterpreted. This paper reports an incident in which breaded turkey cutlets produced in Israel were labeled as prepared with genetically modified soybean products Actually, it was found that wheat flour used in the process was contaminated with soybean product held in common storage facilities such as silos and grain ships.

## Results and Discussion

TO EXAMINE THE PRESENCE OF TRANSGENIC MATERIAL IN breaded turkey breast cutlets, DNA was extracted from whole breaded turkey cutlets and the breaded coating and analyzed for the presence of 35S promoter sequences. Both DNA samples tested positive for these sequences (Fig. 1, lanes 1, 2). To our surprise, whole wheat flour that was purchased in a local grocery store and intended to serve as a negative control, was also positive for the presence of the 35S promoter sequences (Fig. 1, lane 3). Both water-extracted samples were negative (Fig. 1 lanes 4, 5) and DNA extracted from transgenic soybeans was positive for the 35S promoter sequences (Fig. 1, lane 7).

To identify the origin of the transgenic plants, the same samples were checked for the presence of soybean or corn products. We amplified the samples with primers targeting to soybean *le1* sequences and the maize zein gene. All three 35S positive samples were also positive for soybean *le1* (Fig. 2, lanes 2 and 4) and negative for maize (data not shown). DNA samples containing the *le1* sequences (Fig. 2, lanes 1, 2 and 4) produced bands similar in size to that of the control soybean DNA (Fig. 2, lane 5), whereas the reactions with DNA extracted from water failed to show any products (Fig. 2, lane 3).

These results suggest that the breaded turkey cutlets contained soybean residues sufficient to support the heminested PCR. To identify the soy source, we tested for its presence in each of the breaded turkey cutlet coating components. DNA was isolated from the breading and wheat flour used to attach the breading to the meat and examined for soybean *le1* sequences. We could show that *le1* sequences were in the flour (Fig. 3, lanes 3 and 7) from two sources, but not in the breading (Fig. 3 lane 5). All negative controls, maize and Israeli wheat grain DNA (Fig. 3, lanes 1, 2, respectively) and extracted water (Fig. 3, lanes 4 and 6)

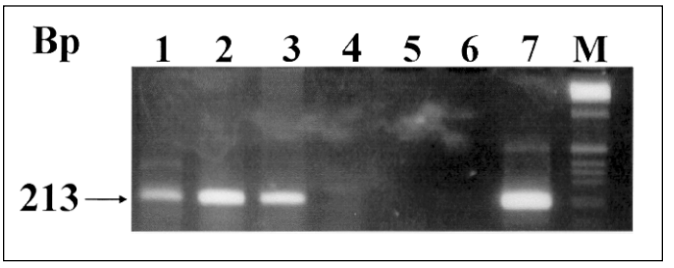


**Fig. 1—Detection of 35S promoter sequences in Of-Tov breaded turkey cutlet by heminested PCR. The PCR incorporated primers #1 and #2 and the heminested step with primers #1 and #4. Lane 1, DNA from total breaded turkey cutlet. Lane 2, DNA from breaded turkey cutlet coating. Lane 3, DNA from wheat flour purchased in a grocery store. Lanes 4 and 5, extracted water. Lane 7, DNA from soybean flour. M, 1 kb ladder.**

did not support the reaction.

To corroborate our findings that the grocery wheat flour was contaminated with soybean, we examined a variety of Israeli wheat grains, commercial flour, flour specially prepared from Israeli wheat grain and also an imported flour prepared after meticulous separation of wheat grains from soybean residues. The only sample that was positive in this set of reactions was the flour made from the imported wheat grains (Fig. 4, lane 7). The weak signal seen indicates that soybean residues were not completely removed, resulting in poor amplification. All other flour samples prepared from Israeli wheat grains or imported grains (Fig. 4, lane 3, 5) were negative, the negative controls (Israeli wheat grain, maize and two water-extracted DNA) and the positive soybean flour control showed the expected result (Fig. 4, lanes l, 2, 4, 6, 8 and 9, respectively).

The results clearly show that the wheat grain used commercially in Israel were contaminated with soybean residues that were easily detected by nested PCR. Since most of the wheat grains used in Israel are imported from the USA, it seems that the wheat had been contaminated with soybeans during storage, either in the USA or in Israel, or during shipment to Israel.

Public awareness and legislation in several countries require public notification where food products contain a genetically modified food product. The method for detecting such products is to use PCR to detect 35S promoter sequences and termination sequences of NOS, OCT, and CaMV. In some cases DNA can be destroyed; thus it is desirable to increase PCR sensitivity by the use of nested or heminested PCR. However, their extreme sensitivity can give misleading results.

In this work, we attempted to trace the source of the GMFP in turkey cutlets even though GMO products were not deliberately used in the processing of the breaded turkey cutlet or in other products produced by this particular food processing plant. We found that the coating of bread crumbles and wheat flour was contaminated with transgenic soybean residue (Fig. 1 and 2). When we tested each component separately, we were able to identify that soybean residue was present in the flour component, but not in the bread crumbles. To validate this finding, we demonstrated that flour from various mills and flour purchased at regular food markets were also contaminated with soybean (Fig. 1, 2 and 4). Since the bread crumbles are also prepared from wheat flour, it seems that the elevated temperature (above 120 °C) in preparing the crumbles destroyed DNA present in the flour to a level that it can not serve as a template for PCR. The fact that we could not find soybean products in flour prepared from Israeli or American wheat prepared in our laboratory raises the possibility that most of the commercial flour in Israel is contaminated with soybean. Inquiries as to the way that wheat grain is stored and transported, demonstrated that the same silos store wheat and soybean grains in turn, and the same grain cargo vessels carry soybean and wheat grains. Wheat flour prepared from soybean-contaminated grains will invariably contain soybean DNA and, if it is transgenic soybean, it will be detectable using nested PCR with soybean primers.

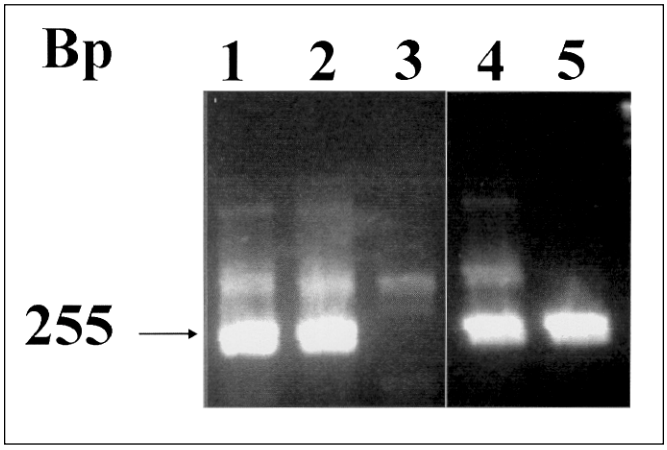


**Fig. 2—Testing the presence of soybean residues in Of-Tov breaded turkey cutlet by heminested PCR with primers targeting to soy *le1* sequences. For the first reaction, *le1* GMO1 and GMO2 primers were used, for the second reaction, GMO1 and GMO4 primers were used. Lane 1, DNA from total breaded turkey cutlets. Lane 2, DNA from breaded turkey cutlet coating. Lane 3, extracted water. Lane 4, DNA from wheat flour bought in a grocery store. Lane 5, DNA extracted from soybean.**

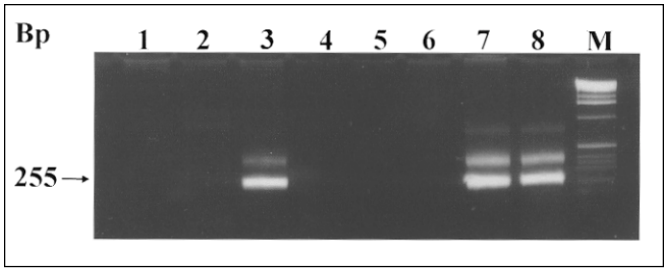


**Fig. 3—Examination of soybean residues in cutlets coating components by heminested PCR with primers targeting to soy *le* 1 sequences as done in Fig. 2. Lane 1, maize DNA. Lane 2, DNA from Israeli wheat grain. Lane 3, DNA prepared from flour used in breaded turkey cutlet coating. Lane 4, DNA extracted from water. Lane 5, DNA from bread crumbles. Lane 6, DNA extracted from water. Lane 7, DNA from a second source of wheat flour for breaded turkey cutlet coating. Lane 8, DNA from soybean flour.**

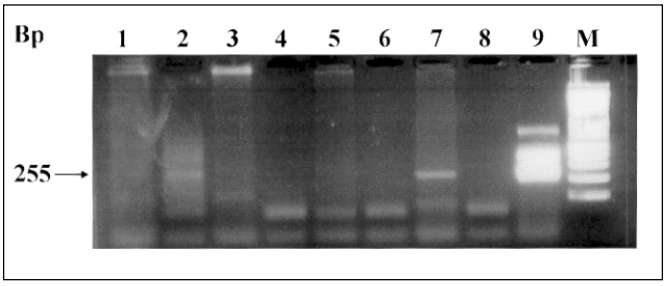


**Fig. 4—Detecting soybean residues in flour from various sources by heminested PCR with primers targeting to soy *le1* 1 sequences as done in Fig. 2. Lane 1, DNA from Israeli wheat grains. Lane 2, maize DNA. Lane 3, DNA from Israeli wheat flour. Lane 4, extracted water. Lane 5, DNA from hard-wheat imported flour prepared after manual removal of soybeans. Lane 6, extracted water. Lane 7, DNA from soft-wheat imported flour prepared after manual removal of soybeans. Lane 8, reaction without DNA. Lane 9, DNA from soybean flour. M, 1 kb ladder.**

## Materials and Methods

### DNA extraction

Two volumes of water were added to soybean and wheat grains and incubated at room temperature for 14 to 18 h and then homogenized in a blender. For extraction, 300 mg of each paste was added into 860 μL extraction buffer (Studer and others 1997), 100 μL of 5 M guanidine hydrochloride and 40 μL 2.5 mg/mL proteinase K (Sigma Chemical Co., St. Louis, Mo., U.S.A.) and incubated at 60 °C for 3 h and then centrifuged at

12,000 × *g* for 15 min at room temperature. The clear solution was used to isolate the DNA using Wizard mini prep (Promega, Madison, Wis., U.S.A.). Five hundred μL of the clear solution was added to 1 mL of miniprep resin and applied to the kit's mini column using a vacuum manifold. The columns were washed with 2 mL 80% isopropanol and DNA was eluted with 50 μL water preheated to 75 °C and centrifugation at 12,000 × *g* for 30 sec after 1 min incubation. DNA was kept at –20 °C.

### Heminested PCR

To detect soybean we used 4 primers corresponding to the soybean lectin gene *le*I. The outer primers were*:* GM01 5'-TGCCGAAGCAACCAAACA TGATCCT-3' at position 1099-1124. The reverse primers GM02: 5' TGATGGATCTGATAGAATTGACGTT at position 1512-1536. The inner primers were GM03: 5' GCCCTCTACTCCACCCCCATCC 3' at position 1215-1136 and the reverse primers GM04: 5' GCCCATCTGCAAGCCTTTTTGTG at position 1332-1354 (Studer and others 1997). The first reaction consisted of reaction buffer (75 mM Tris-HCl, pH 8.5, 15 mM $(NH_4)_2SO_4$, 2 mM $MgCl_2$), 100 μM each dNTP, 100 ng of each outer primer (#1 and #2), 0.375 U Taq polymerase (MBI, Fermentas, Vilnius, Lithuania) and 2 μL of extracted DNA in a total volume of 25 μL. The reaction conditions were 95°C for 3 min, then Taq polymerase was added at 80 °C for "hot start," then 45 s for each step of 55, 72, and 94 °C for 35 cycles; the conditions for the last step was 55 °C for 45 s and 72 °C for 3 min. Heminested PCR was performed using 2 μL of the 1st PCR with the same reaction mixture as described except that the primers were #1 and #4, or #2 and #3, and #3 and #4. The reaction conditions were one cycle of 95 °C for 1 min, 57 °C for 45 s 72 °C for 50 s, then 10 cycles of 94, 57, and 72 °C for 45 s each and extention at 72 °C for 3 min.

The outer primers for detecting the CaMV 35S promoter sequences were: 35S1, 5'-GACAGTAGAAAAGGAAGGTG-3' at position 35-54, and the reverse primers 35S2 were 5'-TATATAGAGGAAGGGTCTTG-3' at position 254-273. The inner primers were 35S3 5'-GCTCCTACAAATGCCATCA-3' at position 54-73, and the reverse primers were 35S4 5'-GATAGTGGGATTGTGCGTCA-3' at position 229-248. Reaction conditions were the same as for soybean *le1* described above, except that the annealing temperatures were 50.9 °C for the first and the second reactions. PCR products were visualized after electrophoresis in ethedium bromide-stained 2% agarose gels.

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