

## **DETECTION OF GENETICALLY MODIFIED SOYBEANS DNA IN A CHEESE LIKE PRODUCT AND SOME HEAT-TREATED PRODUCTS AS FOOD MODEL**

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### **ABSTRACT**

This work was conducted to detect of recombinant DNA of genetically modified (GM) in soybean like cheese and heat-treated GM soybean milk. Genomic DNA was extracted from soybean like cheese and heat-treated GM soybeans milk by using the cetyltrimethylammonium bromide (CTAB) method and vivants DNA extraction kit. Primer pair P35s-f2 /petu-r1 amplify the junction region between CaMV35S promoter and part of CP4EPSPS and GMOScreen kit that amplify CaMV35S promoter and or NOS terminator were selected to detect the recombinant of DNA in Roundup Ready soybean. They gave PCR products of band sizes, 172 and 123 bp. PCR products were detected when DNA solution extracted from soybean like cheese and heat-treated GM soybean milk by the vivants DNA extraction kit. PCR products of the expected 172, and 123 bp were detected by agarose gel electrophoresis at the end of storage period 60 days. However, PCR products were not detected in DNA extracted from soybean like cheese and heat-treated GM soybean milk by the CTAB method. These results indicate that judicious selection of DNA extraction methods and target sequences are important to detect DNA from soybean like cheese and recombinant DNA can be detected in soybean like cheese

**Keywords:** GM soybeans, DNA extraction, processed food modal, PCR.

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### **INTRODUCTION**

Soy products play an important role in prevention of chronic diseases such as menopausal disorder, cancer, atherosclerosis and osteoporosis (Anderson *et al.*, 1999). Soymilk is rich in high quality proteins and suitable for the growth of bifidobacteria (Shimakawa *et al.*, 2003) because this product contains sucrose, raffinose and stachyose, used by most of the strains that belong to this genus during fermentation (Scalabrini *et al.*, 1998). Due to great advances in agricultural biotechnology, scientists are able to use artificial genetic manipulations to successfully transfer genes for herbicide tolerance, and insect resistance into traditional crops. Many genetically modified (GM) plant cultivars have been registered worldwide. On the other hand, consumers' increasing awareness regarding food safety has created the need for more stringent food quality control. GM crops and their use as food sources have aroused public safety concern. Polymerase chain reaction (PCR) has become a common method of screening, detecting or quantifying GM content (Berdal *et al.*, 2001; Lau *et al.*, 2004; Peano, 2004; Watanabe *et al.*, 2004, and Elsanhoty *et al.*, 2005 and 2006). The are increasing in consumption of foods derived from soybean such as soy milk, soy yoghurt, tofu and some meat products. Foods made from soybeans are among the most traditional and familiar foods in some countries around the world.

However, most of the soybeans consumed in the world are imported from United States, where GM soybeans are grown. Thus, detection or quantification of GM soybeans in processed foods is an important issue for food quality control. Temperature and pH are important DNA degrading factors (Bauer *et al.*, 2003; and Alexander *et al.*, 2004). pH is the major effective parameter affecting DNA in food independent of the matrix and its degrading effect depends on exposure time and temperature. Although some reports have examined the detection of GM soybeans in processed foods (Chen *et al.*, 2005; Liu *et al.*, 2005; and Wang 2005), little information about the detection of GM soybeans in soybean cheese or cheese analogue. It is often thought that extraction or detection of genomic DNA from refined or fermented foods would be impossible because of removal or degradation of genomic DNA by purification or fermentation processes. Many reported previously that a fragmented endogenous gene of soybean (*lectin 1* gene) in natto could be extracted by the cetyltrimethylammonium bromide (CTAB) or alkaline lysis methods and detected by PCR using a primer pair producing 100-bp amplicons (Kakihara *et al.*, 2007). In addition, the fragmented lectin 1 gene in soy sauce could be extracted by the alkaline lysis method, but not the CTAB method (Kakihara, 2006). These results suggest that judicious selection of DNA extraction methods and target sequences makes it possible to detect soybean genomic DNA in fermented foods. The objective of this study was to examine the detection of genetically modified DNA of soybean in heat-treated GM soybean milk and in a cheese-like product.

## **MATERIALS AND METHODS**

### **Materials**

The genetically modified was developed by Monsanto, USA. The soybean is designed to resist glyphosate Roundup Ready herbicides and produce increased yields for farmers. The target transgenic soybean is Roundup Ready, a soybean which is resistant to the weed killer glyphosate. The resistance is the result of the incorporation of a 5-enol-pyruvyl-shikimate-3-phosphatesynthase (EPSPS) gene. The genetically modified Roundup Ready was obtained from (Monsanto Co. USA), and non genetically modified soybean was obtained from Egyptian Agricultural company for seed production (EGASEED), Dokki, Cairo, Egypt. Certified reference materials genetically and non genetically Round up Ready soybean (CRM) powders, containing 5% and 0% were obtained from Fluka Chemie GmbH (Buchs, Switzerland). Both genetically modified and nongenetically modified Roundup Read soy bean were authorized depending on DNA based method as described by Mayer *et al.* (1996).

### **Dehulled soybean and preparation of soybean milk**

Soybean seeds were dehulled in Food Technology Research Institute Agriculture Research Center, Giza-Egypt in soybean pilot plant. Dehulled soybeans were soaked in deionized water (water/bean ratio=6:1) overnight. After rinsing, the soaked beans were ground and soymilk was obtained after sieving through 200 meshes and heated at 95 °C for 15 min and cooked at that temperature for 7 min. The final product was cooled and stored at 5°C until used

### **Other materials**

Raw cow's milk was obtained from Department of Dairy Technology, Food Technology Research Institute and stored at 5°C until used. Rennet and calcium chloride were obtained from local market. Cow's milk was heated at 95°C for and 15 min and cooled and stored at 5°C until used.

### **Bacterial strains**

*Bifidobacterium lactis Bb-12*, *Lactobacillus acidophilus LA-5* (Freeze-Dried Red-Set) were obtained from Chr. Hansen laboratories, Copenhagen, Denmark. Culture was prepared by the activating in reconstituted and sterile (121°C/ 2 min) before using.

### **Procedure for cheese like samples**

Each treatment was carried out as the follows. Soy and cow's milks were mixed to obtain 4L of blend of desired proportion (100/0, 25/75, 50 / 50 and 75/ 24 soy/cow's milk, v /v). The blend was heated to 42°C, the blend was fermented by 3% bacteria was made according to the method by Hanet *al.* (2001). Soymilk was fully coagulated after 2 h of fermentation; the rennet was added and then incubated at 42 °C for 4 min to accelerate coagulation. When the soft curd was transferred to moulds for draining and pressing, the cured was cut into cubes of the size 3.0×3.0×1.5 cm. The cubes were placed in jars containing NaCl2 brine and salted for 3 days at 4°C. After the brine was drained, the cubes were placed in sealed jars for storage at 5°C. The cheese samples were collected and stored at -20 °C at the following storage period 0, 15, 30, and 60 days.

### **DNA isolation and quantification**

All DNA analysis was done in kingdom of Saudi Arabia, department of food science and human nutrition, biotechnology laboratory. Genomic DNA was extracted from soybean seeds, certified references materials and soy bean like cheese during storage period by using CTAB method according to official German methods for soybean (Ananyonus 2002) and vivants DNA extraction kit according to producer's instructions, all investigated samples were extracted twice in independent procedures. Furthermore, a blank sample consisting of 200 µl autoclaved bi-distilled water was used to control reagents used in the work.

### **Samples**

Heat-treated GM soybean milk as a model processed food that prepared above was packed in autoclaveable conical flask 250 ml and autoclaved at 121°C for 20 min (this time does not include the time for raising and lowering the temperature.

### **Quantification of genomic DNA**

The concentration and purity of the extracted DNA were measured by the NanoDrop™ Wilningto, DE, USA, and spectrophotometer according to producer's instructions. Spectrophotometer optical densities of 260 nm and 280 nm were used to investigate the DNA quantity. DNA purity was measured using the appropriate ratio of OD260: OD280 (1.65-1.85). Concentrations (ng/µl) and A260/A280 readings were recorded for each sample. The extracted DNA concentration was measured and adjusted by dilution to conc. 20-25 ng/µl prior to PCR, using bi-distilled sterile water.

Table (1): Oligonucleotide primer pairs sequence and their target element.

Primer	Sequence	Fragment length	Target element	References
GM03 / GM04	5'- gCC CTC TAC TCC ACC CCC ATC C 3' 5- gCC CAT CTg CAA gCC TTT TTg Tg 3'	118 bp	Soy lectin gene.	Meyer et al. (1996)
P35s-f2 / Petu-r1	5' - TgA TgT gAT ATC TCC ACT gAC g -3' 5' -TgT ATC CCT TgA gCC ATg TTg T-3'	172 bp	Transition site from the CaMV35S promoter sequence to the petunia hybrida chloroplast-transit-signal sequence in RRS.	Wurz et al. (1997)

### Oligonucleotide primers

Primers used in this study together with their target specific part of the investigated DNA are listed in Table (1). All primers were synthesized by Biosynthesis, Inc USA and obtained in a lyophilized state. All primers were solved before use to obtain a final concentration of 20 pmol/ $\mu$ l of each.

### DNA amplification and PCR condition

PCR was carried out on thermocycler (Biometra, T1) using a master mix was prepared. Each PCR reaction mix had 25  $\mu$ l total volume and contained 2.5  $\mu$ l Reddy Mix buffer (10x concentrate, Thermo Scientific), 2  $\mu$ l MgCl<sub>2</sub> solution (25 mM), 1  $\mu$ l dNTPs solution (0.2 mM each of dATP, dCTP, dGTP and dTTP), 0.5  $\mu$ M of each primer, 0.625 Unit Thermoprime Taq polymerase (Thermo Scientific), 2  $\mu$ l of template extracted DNA and was completed to 25  $\mu$ l with water. Table (2) explains the time/temperature profiles used in PCR for each primer pair including the conditions. All amplicons were stored at 4 °C until gel electrophoresis.

Table (2): Time / temperature profiles for qualitative PCR with DNA extracted from maize and soybean samples using the primer pairs described in Table (1).

Primer pair	Initial denaturation	Denaturation	Annealing	Extension	Cycles	Final elongation
GM03 /GM04	10 min. at 95 °C	30 sec. at 95 °C	30 sec. at 60 °C	1 min. at 72 °C	35	3 min. at 72 °C
P35s-f2 /petu-r1	10 min. at 95 °C	30 sec. at 95 °C	30 sec. at 62°C	25 sec. at 72 °C	35 - 40	10 min. at 72 °C

### Gel electrophoresis

Agarose gel preparation as well as electrophoresis were carried out using Tris-base/borate (TBE) buffer solution (pH 8.0), containing 45 mmol/L Tris-base / boric acid and 1 mmol/L EDTA adjusted with hydrochloric acid. To determine the size of the DNA fragments, DNA of known size (50, 100 bp DNA marker, Roche Germany) together with the different amplicons were separated on 2% w/v agarose gel (LE, Roche) TBE buffer stained with 0.01% ethidium bromide solution (0.5 mg/L). 10  $\mu$ l of all amplicons and DNA marker were stained before gel electrophoresis by 2  $\mu$ l xylene cyanol dye solution (1 mg xylene cyanol, 400 mg sucrose and completed to 1 ml with water), and then subjected to electrophoresis for 45 min. The amplicons were made visible by ethidium bromide staining and documented using UV transillumination and Dophine-View WealTech.

## RESULTS AND DISCUSSION

### DNA extraction from soy bean cheese and heat-treated GM soybean milk.

DNA extracted from heat-treated GM soybean milk or soybean like cheese by the CTAB or vivants kit methods at different storage period was applied to agarose gel electrophoresis. It revealed that DNA in heat-treated GM soybean milk and soybean like cheese was highly degraded (Fig. 1).

Interestingly, the sizes of DNA extracted by both extraction methods were entirely different. The CTAB method unlikely to extract of DNA of all treatments whereas, the vivants kit more likely to extract of DNA from all samples at the different storage period (Fig. 2.) The purity of the extracted DNA was evaluated using the absorbance ratio of 260 and 280nm (A260/A280) using NanoDrop™ technology. The DNA concentration in the extracted solutions was determined by measuring the absorbance at 260nm. The Vivants DNA extraction kit was given high purity and concentration of the extracted DNA from different investigated samples (data not shown). In general, DNA isolated from processed food must be purified to remove other, interfering substances such as protein, fat and polysaccharides (A260/A280D1.6-2.0), and must be diluted prior to PCR (5-50ng are used per reaction Meyer, 1999). Therefore, it was thought that the DNA solutions obtained from heat-treated GM soybean milk and soybean like cheese by the vivants method were adequate purity and yield for applying PCR analysis. On the other hand, the DNA extracted from heat-treated GM soybean milk and soy bean like cheese by the CTAB method was of insufficient purity. The DNA solutions extracted from the heat-treated GM Soybean milk by the CTAB method and vivants DNA kits were applied to PCR.

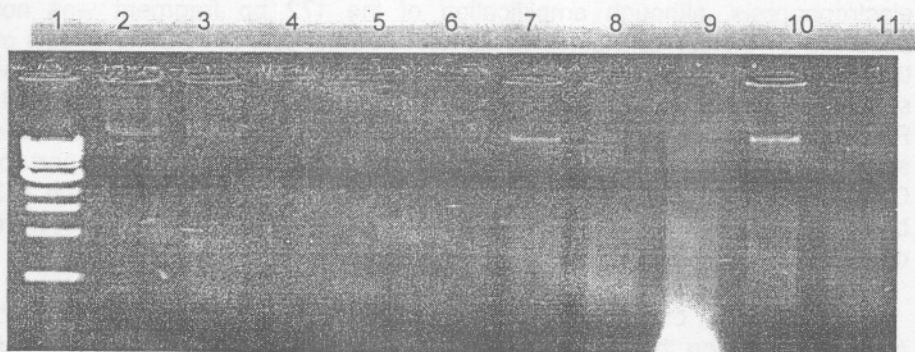


Fig.1. Example of agarose gel electrophoresis of DNA extracted from heat-treated GM soybeans milk and soybean like cheese. DNA extracted from heat-treated GM soybean cheese during storage period 0, 10, 15, 30, and 60 days by the CTAB method respectively, lane 1: DNA ladder 100 base pair, lanes 2, 3, 4, 5, 6 DNA extracted from heat-treated GM soybeans milk, lanes: 7, 8, 9, and 10 DNA extracted from soybean like cheese with different percent of cow milk 25, 50, 75 and 100 % at 0 times, Lane 11 DNA extraction control.

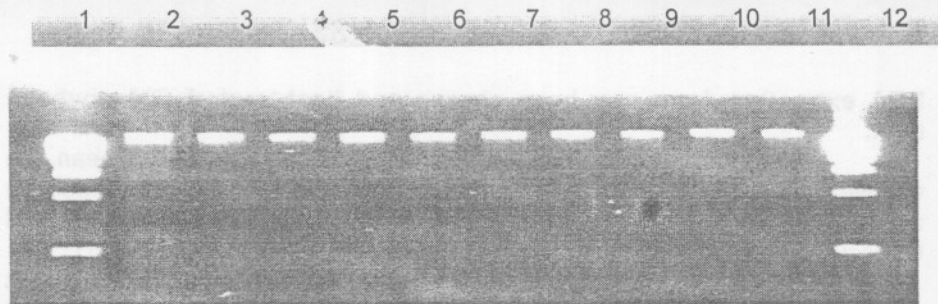


Fig.2. Example agarose gel electrophoresis of DNA extracted from heat-treated GM soybeans milk and soybean cheese DNA extracted from heat-treated GM soybean like cheese during storage period 0, 10, 15, 30 and 60 days by Vivants DNA extraction kits respectively, lane 1 and 12: DNA ladder 100 base pair, lanes 2, 3, DNA extracted from heat-treated GM soybeans milk at the 30 and 60 days end of storage period, lanes: 4, 5, 6 and 7 DNA extracted from soybean like cheese after 30, lanes 8, 9, 10 and 11 DNA extracted from soybean like cheese after 60 days of storage period.

Although DNA extracts obtained by the CTAB method could not be amplified by the primer pairs GM03/GM04 and GMOScreen kits (GenScan), the PCR products of the 118 bp fragments from the DNA solution extracted by the vivants method were detected (Fig. 3 and figure 4). On the other hand, when the DNA solutions extracted from soybean like cheese by CTAB methods were applied to PCR, amplification was not observed.

For DNA extracted from heat-treated GM soybean milk and soybean like cheese by the CTAB method and vivants DNA extraction kit, the PCR products of the expected fragments were detected in agarose gel electrophoresis, although amplification of the 172 bp fragment was not observed by the CTAB method (Data not shown). Also, the PCR products of the expected extracted from soybean like cheese and heat-treated GM soybean milk were applied to PCR, PCR products by using the primer pair P35s-f2 /petu-r1 and products using GMO Screen kit derived from the amplifying the junction region between CaMV35S promoter and part of CP4EPSPS and products using GMO Screen kit amplifying (172 bp and 123 bp from CaMV35S only in DNA extracted by vivants DNA extraction kit were detected (Fig. 5 and 6).

Detection of genetically modified DNA using of primer pair P35s-f2 /petu-r1 amplifying the junction region between CaMV35S promoter and part of CP4EPSPS and using GM Screen kit for NOS terminator and or CaMV35S promoter



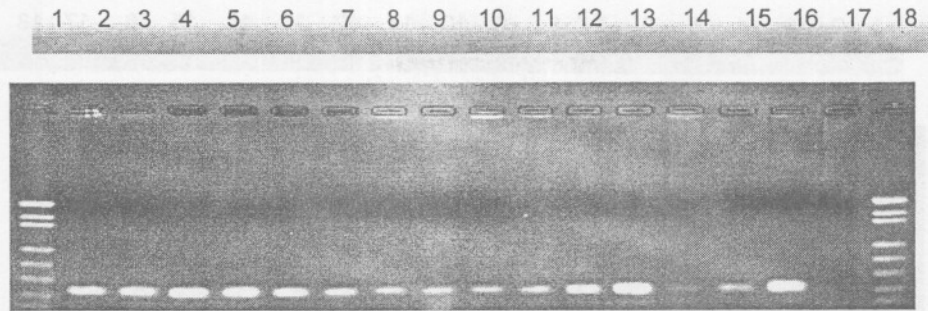


Figure 3. Example of agarose gel electrophoresis of DNA extracted from heat-treated GM soybeans milk and soybean like cheese, DNA extracted from heat-treated GM soybean cheese during storage period by Vivants DNA extraction kits respectively and examined by PCR-analysis using primer GM03 and GM04. Lane 1 and 18: DNA ladder 50 bp, lanes 2, 3, 4, and 5 PCR products of DNA extracted from heat-treated GM soybeans milk, at storage period 0, 10, 15, 30 and 60 days lanes: 7, 8, 9, 10 and 11, DNA extracted from soybean like cheese with different percent of cow milk 25, 50, 75 and 100 % at 0 times, lanes 12, 13, 14, 15 and 16 PCR products of DNA extracted from soybean like cheese with different percent of cow milk 25, 50, 75 and 100 % after 60 days of storage period, lane 17: PCR products negative control with out DNA template

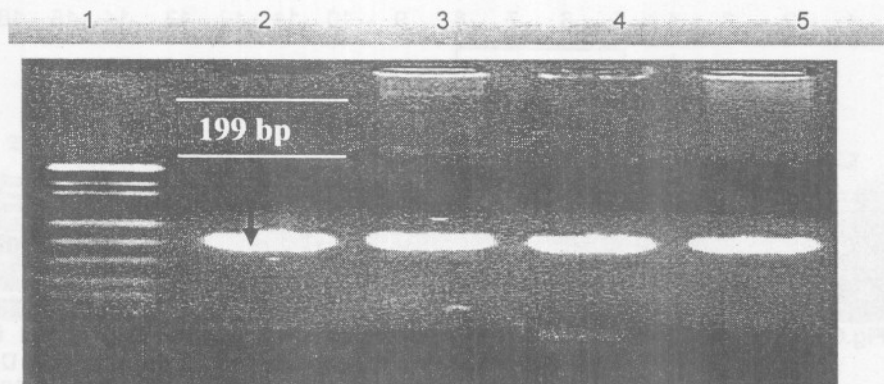


Fig. 4. Agarose gel electrophoresis of examples of DNA extracted from soybean like cheese at the end of storage period 60 days by Vivants DNA extraction kits respectively and examined by PCR-analysis using GMOScreen kit, lane1: DNA ladder with GMOScreen, lane 2 PCR product of DNA extracted from heat-treated GM soybeans milk, lanes: 3, 4 and 5, PCR products of DNA extracted from soybean like cheese with different blended percent of cow's milk.

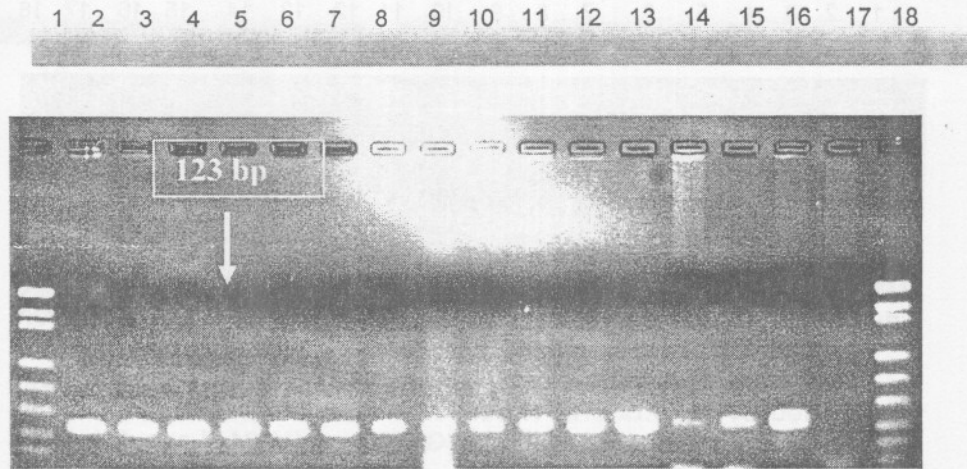


Fig.5. Agarose gel electrophoresis of DNA extracted from heat-treated GM soybeans milk and soybean cheese during storage period 60 days. DNA extracted by Vivants DNA extraction kit and amplified by using of GMOScreen kit. Lanes 1 and 18: DNA ladder with kit, lanes 2, 3, 4, 5: PCR products from DNA extracted from heat-treated GM soybeans milk, lanes: 6, 7, 8, 9, and 10: PCR products from DNA extracted from soybean like cheese after 15 day storage, lane 11, 13, 14, 15 DNA extracted from soybean cheese after 60 days storage, lane 16 PCR products from DNA of 5% RR positive control, lane 17 PCR products from DNA of negative control 0% genetically modified Roundup Ready soybean.

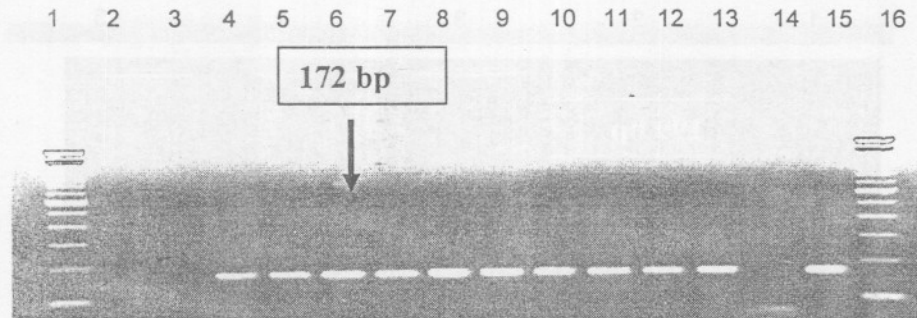


Fig.6. Agarose gel electrophoresis of DNA extracted from heat-treated GM soybeans milk and soybean cheese during storage period 60 days. DNA extracted by Vivants DNA extraction kit and amplified by using of P35s-f2/Petu-r1. Lanes 1 and 16: DNA ladder 100pb, lanes 2 PCR control , lane 3: PCR products from DNA of negative control 0% genetically modified Roundup Ready soybean, lanes 4, 5: PCR products from DNA extracted from heat-treated GM soybeans milk in 0 and 60 days of storage period, lanes: 6, 7, 8, and 9, PCR products from DNA extracted from soybean like cheese in 0 time, lanes: 10, 11, 12, 13 PCR products of DNA extracted from soybean like cheese after 60 days storage, lane 14: PCR of extraction control, lane 15: PCR product of DNA from 5% RR positive control.



## Discussion

The results obtained from this study indicated that recombinant DNA could be detected in soybean like cheese and soybean milk heat-treated extracted by the vivants DNA extraction kit and could not be detected by CTAB methods and by PCR using primer pairs P35s-f2 /petu-r1 amplifying the junction region between CaMV35S and to the petunia hybrida chloroplast-transit-signal sequence in RRS. In addition, *lectin* 1 and chloroplast DNA kit from GMOScreen kit were detectable from both soybean like cheese and soybean milk heat treated at the different storage period even if the primer pairs the expected band size of 118 bp from lectin gene and chloroplast DNA were used. The results obviously indicate that judicious selection of DNA extraction methods and target sequences is important to detect recombinant DNA from processed soy bean products such soybean like cheese and soybean milk heat treated. Using primer pairs P35s-f2 /petu-r1 that amplified the transition site from the CaMV35S promoter sequence to the petunia hybrida chloroplast-transit-signal sequence in RRS. PCR products could be detected in soybean cheese extracts. Judging from the detection of expected PCR products in GM soybeans or heat-treated GM soybeans, and there are the failure implies the presence of PCR inhibitors in such soybean like cheese and soybean milk heat treatment and extracts or lower stability of DNA during the manufacturing process of soybean like cheese and soybean milk heat treatment. The obtained results were disagreements with the results obtained by Meyer (1999) and (Ogasawara *et al.*, 2003) who reported that no genomic DNA is detectable in highly heat-treated food products, hydrolyzed plant proteins, starch derivatives, or defined chemical substances such as defined soy oils. Kakahara *et al.* (2007) reported that by using of CTAB method for DNA extraction from natto could be amplified the DNA of lectin gene from soy treated products. Our results imply that the DNA sequences between junction region between CaMV35S and to the petunia hybrida chloroplast-transit-signal sequence and by using of GMOScreen kit for detection both CaMV35S promoter and NOS terminator would be easier to fragment than the sequences between NOS and part of CP4EPSPS. Bauer, (2003) stated that temperature and pH are important parameters for evaluation of DNA degradation in food. Ogasawara *et al.* (2004) examined genomic DNA fragmentation of GM corn during food processing and concluded that the SSIIb gene (an endogenous gene of maize) would be easier to fragment than an inserted gene with extruder processing. Several factors, such as temperature, pH, pressure, and fermentation, had a causal influence on DNA fragmentation in food processing. On the other hand, DNA regions containing high GC contents are generally considered to be stable when exposed to high-temperatures. Yoshimura *et al.* (2005) reported that the regions of GM maize MON810 and p35S were more highly degraded than those of SSIIb in the early stages of heat-treatment because the GC contents of the PCR products for MON810 specific detection and p35S universal detection were lower than GC contents of the products of SSIIb. Thus, GC content in the target sequence of recombinant DNA may be an indicator of stability or fragmentation of the target gene in processed foods. This study indicated that recombinant DNA can be detected in soybean like cheese and heat treatment soybean milk. It is important to develop the Egyptian regulation concerning genetically modified food, feed and crops special their

label determine the contents quantitatively because in Egypt food products require crops in their materials. In general, the contents of GM crops can be estimated from the ratio of copy numbers of recombinant DNA and endogenous DNA using quantitative PCR in instances when target DNAs are not fragmented or degraded. As shown in Fig. 5 and 6, PCR products of 172 and 123 bp in vivants kit extracts from soybean like cheese and heat treated soybean milk were observed, although that of 172 and 123 bp in CTAB extracts were not observed. This result strongly suggests that a part of the sequences in junction region between CaMV35S and to the petunia hybrida chloroplast-transit-signal sequence in RRS and GMOScreen kit for detection of NOS terminator and CaMV35S promoter to detect the genetically modified Roundup Ready soybean in soybean like cheese and heat treated soybean milk during storage period 60 days. To determine the contents quantitatively, further investigations are needed.

#### **Conclusion**

Soybeans and their products were analyzed by PCR to detect the presence of GM soybeans. Most soy-derived products are generated through a heating process, which damages the DNA template used for the PCR reaction. However, PCR which generates small DNA fragments can still detect the presence of DNA. Fermentation and storage period are also an important part of the process in making soybean products. The DNA of soybean after a long period of fermentation, such as 60 days, was seriously damaged. As a result, the vivants DNA extraction kit could be used to extract high purity DNA from soybeans and their products and the primer P35s-f2 /petu-r1 amplifying the junction region between CaMV35S and to the petunia hybrida chloroplast-transit-signal sequence in RRS and GMOScreen kit which identify both CaMV35S and Nos in transgenic crops could be detected by PCR. Finally, a quantitative assay of Roundup Ready soybean was performed by real-time PCR. Using this method, it was possible to make an accurate assessment of the percentage of GM soybeans mixed in with non-GM soybeans.

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الكشف عن الـ د ن أ الصويا المعدلة وراثيا في مشابيه الجبن (جبن الصويا) وبعض  
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رأفت محمد السنهوتي  
معهد الهندسة الوراثية والتكنولوجيا الحيوية، قسم البيوتكنولوجيا الصناعية -جامعة المنوفية.

تم إجراء هذا العمل للكشف عن الـ د ن أ المعاد تركيبة الخاص بالصويا المهندس وراثيا في مشابيهات الجبن المحتويه علي لبن الصويا وفي لبن الصويا المعامل حراريا علي درجة حرارة عالية (الآتوكلاف). تم استخلاص الـ د ن أ من كل من المنتجين السابقين باستخدام طريقة السيتاب وباستخدام الكيتس المسمي باسم فيفيتنس. وتم اختيار كلا من البريمر P35s-f2 /petu-r1 والكيتس المسمي باسم GMO Screen للتعرف علي الـ د ن أ المعدل وراثيا الموجود في الصويا حيث ان البريمر يقوم باجراء عملية تعريف وتكبير لجزء من Ca MV 35S promoter and part of CP4EPSPS ويقوم الـ GMO Screen kit بالتعرف علي كلا من NOS terminator and or Ca MV 35S promoter حيث يعطي البريمر ناتج من الـ PCR مقداره ١٧٢ قاعدة نيروجينية ويعطي الكيتس ناتج من الـ PCR مقداره ١٢٣ قاعدة نيروجينية . وقد وجد ان الـ د ن أ المستخلص من هذان الموديلين بواسطة الكيتس المسمي باسم فيفيتنس أعطي ناتج من الـ PCR مقداره ١٧٢، ١٢٣ قاعدة نيروجينية من كلا من الكيتس والبريمر وذلك الي نهاية فترة التخزين ٦٠ يوم، علي العكس فان الـ د ن أ المستخلص بواسطة طريقة السيتاب لم يمكن التعرف من خلال تكبيره بواسطة PCR بواسطة نفس البريمر والكيتس. النتائج السابقة توضح ضروره اختيار البريمر والتتابع من الـ د ن أ أو طريقة استخلاص الـ د ن أ الذي يعطي نتائج جيدة يمكن بها التعرف علي الـ د ن أ الصويا المعدل وراثيا في منتجات الصويا التي تجري عليها خطوات تصنيعية تؤثر علي الـ د ن أ.