

Detection of genetically modified DNA in fresh and processed foods sold in Kuwait

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Developments in genetic engineering technology have led to an increase in number of food products that contain genetically engineered crops in the global market. However, due to lack of scientific studies, the presence of genetically modified organisms (GMOs) in the Kuwaiti food market is currently ambiguous. Foods both for human and animal consumption are being imported from countries that are known to produce GM food. Therefore, an attempt has been made to screen foods sold in the Kuwaiti market to detect GMOs in the food. For this purpose, samples collected from various markets in Kuwait have been screened by SYBR green-based real time polymerase chain reaction (RT-PCR) method. Further confirmation and GMO quantification was performed by TaqMan-based RT-PCR. Results indicated that a significant number of food commodities sold in Kuwait were tested positive for the presence of GMO. Interestingly, certain processed foods were tested positive for more than one transgenic events showing complex nature of GMOs in food samples. Results of this study clearly indicate the need for well-defined legislations and regulations on the marketing of approved GM food and its labeling to protect consumer's rights.

Introduction

There has been exponential growth in the field of application of plant genetic engineering for trait improvements during the past 15 years. This has resulted in adoption of this technology to genetically modify agricultural crops for potential improvement in crop quality and productivity. Plants produced by transgenic technology or genetic engineering are called genetically modified organism (GMO). In recent years significant number of crop plant species have been genetically modified to attain traits such as biological defense against diseases and insects, thus reducing the need for chemical pesticides and introducing genetic traits that enable crops to better withstand drought, heat and saline conditions.¹⁻⁷ In addition, genetic modification has been used to improve various characteristics such as color, flavor, texture, nutrient value and shelf life of foods.^{8,9} The most important and successful genetically modified traits are herbicide tolerance, insect resistance and virus resistance.¹⁰⁻¹⁸ In recent years several GM products with similar traits have been successfully commercialized.¹⁹ However, use of GMOs in the food commodities still remains to be a controversial issue. Although there is hardly any evidence about the potential risk aspects of GMOs, the safety assessment of food and feed containing transgenic material follows a stringent approach. Generally the transgenic materials are compared with their non-GM counterparts in order to test intended and unintended effects and their potential impact on the nutritional quality, environment, humans and animals. In view of this, currently extensive animal

studies are being performed before the release of GMOs into the market.²⁰

There has been enormous growth in the total area of cultivation of GM crops. In 2010, the accumulated hectareage planted with GM crops during the 15 y from 1996 to 2010 exceeded one billion hectares.²¹⁻²³ According to estimates, there was a massive 87-fold increase in hectareage between 1996 and 2010, making biotech crops the fastest adopted crop technology in the history of modern agriculture.²³

Given the status of the global food chain, and given the substantial proportion of Kuwait's food supply that is imported, it is very likely that GMOs are present in a significant portion of foods sold in Kuwait. Currently, a number of countries are actively pursuing detection and monitoring of GM products in food commodities. Some examples of those studies performed in various countries include reports from Malaysia,^{24,25} Brazil,²⁶ China,²⁷ Saudi Arabia,²⁸ etc.

Yet, to date, the actual status of GMO in foods sold in the Kuwaiti market is unknown, because no testing has been done to empirically assess foods in the marketplace. A number of countries in the world have put in place laws or regulations requiring transparency regarding the presence of GMOs or derivatives of GMOs in food products.²⁹⁻³² Thus, there is a real need to put in place the capacity to verify the claims that importers make to both regulatory authorities and the food industry. Moreover, it is important to protect the consumer's right to know the nature of food to make their own choice to accept or reject food that contains genetically modified DNA.

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Table 1. Results of screening of food samples for the presence of DNA sequences containing CaMV 35S Promoter, TNOS Terminator, 5-Enolpyruvyl Shikimate-3-Phosphate Synthase (EPSPS) Gene, Cry1AB and PAT

Sample	*CaMV 35S	*TNOS	[†] EPSPS	[‡] Cry1AB	[§] PAT
Wheat thins (USA)	-	-	+	-	-
Basmati rice (Pakistan)	-	-	-	-	-
Corn Flakes (Germany)	-	-	-	+	-
Shaker Rice (Pakistan)	-	-	-	-	-
Rice Crisp (Egypt)	-	-	-	-	-
Alfa Alfa (USA)	-	-	-	-	-
Sunflower seeds (USA)	-	-	-	-	-
Wheat bran (USA)	+	-	+	-	-
Harees (Australia)	-	-	-	-	-
Potato Sticks (USA)	-	-	-	-	-
Tostitos (USA)	+	+	+	+	+
Raw corn (Brazil)	+	+	-	+	-
Lay's potato chips (USA)	-	-	-	-	-
Raw soy (India)	+	+	-	-	-
White corn meal flour (USA)	+	+	+	-	+
Ketchup (USA)	-	-	-	-	-
Barley (Australia)	-	-	-	-	-
Barley (Oman)	-	-	-	-	-
Barley (UAE)	-	-	-	-	-

The reference materials used as positive control are: *Herbicide resistant transgenic soy GTS-40-3-2; [†]Herbicide resistant transgenic soy GTS-40-3-2 and canola GT73 ; [‡]Transgenic maize expressing *phosphinothricin N-acetyltransferase* and *Cry1Ab delta-endotoxin* BT11 or transgenic maize expressing truncated version of the *Cry1Ab* insecticidal protein MON810; [§]Transgenic maize expressing a gene for herbicide resistance *phosphinothricin N-acetyltransferase* BT11.

The objective of this research was to detect GMOs in food samples imported to Kuwait. The need to monitor and verify the presence and amount of GMO-derived ingredients in food products demands analytical methods capable of detecting and quantifying the foreign DNA that has been introduced. In this paper we provide the results of screening of fresh and processed food samples collected from Kuwait markets for the detection of GMOs. We also present the results of quantitative analysis of various transgenic elements present in selected food samples.

Results

The screening results showed that a number of samples found to be positive for the presence of transgenic-DNA sequences such as the CaMV 35S promoter, NOS terminator and other trait related genes.

The results of SYBR green based qPCR analysis showed that out of 89 food samples examined (raw and processed), 19 samples contain genetically modified DNA elements and that represents approximately 21% of the total analyzed samples. Analysis of wheat bran (animal feed), Tostitos, raw corn, raw soy and white corn meal flour showed the presence of CaMV 35S promoter sequence (promoter derived from the cauliflower mosaic virus). In addition to that, Tostitos, raw soy and white corn had NOS terminator sequence (*Agrobacterium Nopaline synthase* terminator). Several other samples had gene for either herbicide resistance or gene for insecticide protein (Table 1). In order to validate the GMO detection method optimized in the laboratory, selected samples have been sent to a certified laboratory (Eurofins GeneScan GmbH, Engesserstraße, Freiburg, Germany) to revalidate the results of analysis and to investigate more food samples for the presence of GMO. The results of this analysis confirmed our screening results and demonstrated that potato, rice, tomato, red apple, raw wheat, raw soy, white flour, rice flour, ketchup and corn flakes contain genetically modified DNA such as CaMV 35S promoter/NOS terminator (data not shown). With these results it is very clear that significant portion of food sold in Kuwait contains one or more genetically modified ingredients that are not labeled.

Based on the outcome of the screening results, foods containing transgenic DNA samples were subjected to further analysis to identify the type of genetic modification and the percentage proportion of genetically modified DNA in those samples. Taqman real-time quantitative PCR confirmed the presence of multiple transgenic events in Tostitos and raw corn samples. The Ct values obtained for each event was compared with the Ct value of the control corn specific DNA. The results indicated the presence of three events: DAS59122 (39.77%), MON810 (0.12%) and TC1507 (100%) in Tostitos (Table 2). Analysis of another sample of raw corn showed that this contains 15.17% GM corn MON810 (Table 2).

Discussion

There is a lack of information about the prevalence of GMOs in the consumer markets of gulf countries in general and Kuwait in

particular. Therefore no legislation has yet been enacted in this region to regulate the marketing of fresh or processed food that contains genetically modified organisms. This requires adoption of reliable and sensitive methods for the detection of GMOs sold in the Kuwaiti market without proper labeling. An attempt has been made to screen potential GMO foods that are being sold in the Kuwaiti market without any label about GMO status. In this context, the study is not an extensive survey about the extent of GMOs in the market. We strongly recommend that there is a need for a large-scale survey on prevalence of unlabelled food that contains GMOs.

One of the most challenging tasks in detection of GMOs in food is isolation of good quality DNA from processed foods. In heavily processed food samples, the intact DNA is completely absent, present in very low concentrations or heavily damaged, making it difficult to detect and quantify through even the most sensitive molecular methods. Although there are well-standardized methods for DNA isolation for the analysis of samples to detect presence of GMO elements, a great deal of effort is required to get good quality of DNA, especially from the processed foods. DNA extracted from processed food are often poor quality, contaminated with PCR inhibitors at high degree.³³ Therefore, for those kinds of processed samples, method of DNA isolation needs further optimization. Although several DNA extraction methods have been developed, it is not always possible to extract good DNA with the required high quality and purity, and that could lead to errors in RT-PCR quantification. In this study, various DNA isolation methods had been employed to achieve high quality DNA for molecular analysis. Among the tested methods, CTAB-based DNA isolation was found to be highly suitable for various types of rice, including rice infusions, rice flakes, corn flakes and Japanese sushi rice; the wizard DNA purification kit was found to be good for vegetables, corn seeds, harees, barley, corn meal, corn flakes, etc. (data not shown). Isolation of good quality DNA from red apple, green apple, grapes, sweet corn cob, sweet kernel corn, soy bread, potato chips, corn snacks, etc. was found to be very difficult with both the methods. This clearly would indicate the complexity involved in DNA isolation from various types of samples. Therefore, it can be concluded that careful evaluation of DNA isolation methods for each type of sample is the most important requirement for obtaining reproducible and credible results in GMO detection and quantification.

There are a number of methods described in the literature for the detection of GMO by PCR.³³⁻⁵³ PCR-based GMO detection is used for amplifying a fragment of DNA that has one or more GMO element. Currently, quantitative PCR (Q-PCR) is the method of choice to detect and quantitatively measure amounts of transgenic DNA in a food or feed sample. This method uses fluorescent dyes, such as SYBR Green, or fluorophore-containing DNA probes, such as TaqMan, and allows determination of number of copies of the transgene.⁵⁴ In this study, SYBR green based qPCR method was adopted for the initial screening of the samples. It is important to note that the screening process does not reveal the characteristics and type of transgenic element. Also, this would not reveal the degree of modification and the percentage of genetically modified DNA in any food sample. Therefore,

Table 2. Quantification of various GMO Events in individual food samples as determined by Taqman PCR

Material	GMO events	Target Ct	Control Ct	Δ Ct	% of GMO-DNA
Tostitos, USA	DAS59122	24.9	22.5	2.5	39.77
	MON810	35.5	32.9	2.6	0.12
	TC1507	24.1	24.2	-0.1	100
Raw corn, Brazil	MON810	27.1	24.4	2.4	15.17

in selected GMO-positive food samples, detailed investigations have been performed to understand the specific events present in the tested foods. Taqman-based qPCR was applied to confirm the preliminary screening results. During this analysis, selected samples have been tested for the presence/absence of individual genetic elements that are commonly used in genetic engineering. Based on these results the genetic modifications have been traced back to the event level. During this analysis it was evident that some samples had multiple GM events.

Due to the enormous expansion of GM industry, more and more food commodities containing GM ingredients have been observed all over the world. To protect the consumer's freedom of choice, over 40 countries have formulated and issued GMO labeling regulations. In the European Union (EU) and other regions, the use of this technology, the consequent release of GMOs in the environment and the marketing of GMO-derived food products are strictly regulated. GM foods must be labeled at the threshold of 0.9% in EU, 3% in Korea and 5% in Japan.⁵⁵ In China, no matter what the percentage of GMO is, 17 types of GM products must be labeled.⁵⁵ In Kuwait, most of the processed foods are imported from other countries, and there are no regulations set in place for GMO levels in food commodities. Certainly, the tremendous increase in area of acreage of GMO cultivation indicates its success due to the benefits to the farmers and the industries, but the possible negative factors associated with GMOs cannot be ruled out without concrete scientific evidence about its safety to the environment and health in a case-by-case basis. The pros and cons of GMOs have been described comprehensively in published papers.⁵⁶⁻⁶⁰ This is the first scientific report about the occurrence and nature of GMOs in fresh and processed foods sold in Kuwait. A detailed study with a representative large scale sampling strategy would certainly provide more information about the proportion of fresh or processed food that contains GMO in the Kuwaiti market. Lack of regulations in Kuwait for labeling foods for the presence of GMO does not provide an opportunity for the people to make a personal choice about the consumption of GMOs in their food. Therefore, this investigation would be useful in demanding a need for an in-depth survey and legislations that strictly implement food labeling to protect the consumer's right to accept or reject GMOs in the food.

Material and Methods

Eighty-nine non-labeled samples imported from countries that produce genetically modified foods were collected from ten local

Table 3. Primers used in qPCR for the detection of genetically modified DNA in food samples

Primer Name	Specific target DNA
Primers used for Taxon specific RT-PCR	
RBCL	Ribulose 1,5-biphosphatase gene for specific detection of any plant DNA.
LECTIN	Lectin gene for specific detection of soy DNA
ADH	Alcohol dehydrogenase gene for specific detection of maize DNA.
Cru A	Acetyl-CoA carboxylase gene for specific detection of oilseed rape DNA.
Primers used for transgene specific RT-PCR	
p35s	Specific for the 35S promoter of the <i>Cauliflower Mosaic Virus</i>
tNOS	Specific of the terminator of the nopaline synthase gene from <i>Agrobacterium tumefaciens</i>
CP4 (EPSPS)	Specific for the 5-enolpyruvyl shikimate-3-phosphate synthase (EPSPS) transgene from <i>Agrobacterium</i> (Strain CP4)
SAD1	Specific for the stearyl-acyl carrier protein desaturase gene from cotton
PFMV	Specific for the 34S promoter of the <i>Figwort Mosaic Virus</i>
PLD	Specific for the phospholipase D gene from rice
CryIAb	Specific for the CryIAb transgene coding for a <i>Bacillus thuringiensis</i> insecticidal delta endotoxin gene.
Cry3Bb	Specific for the Cry3Bb1 transgene coding for a <i>Bacillus thuringiensis</i> insecticidal delta endotoxin gene.
Pat/Pat	Specific for the phosphinothricin-acetyl-transferase transgene from <i>Streptomyces viridochromogenes</i>
Pat/Bar	Specific for the transgene phosphinothricin-acetyl-transferase transgene from <i>Streptomyces hygroscopicus</i>
CRT	Specific for the reverse transcriptase gene of the <i>Cauliflower Mosaic Virus</i>

Table 4. Primers used in Taqman PCR for quantification of individual transgenic events in selected food samples

Primer	Transgenic Event	Reference Material
CryIAb	MON810	MON810 (1%)
Pat/Bar	TC1507	Bt176 (1%)
CryIAb and Pat/Pat	DAS59122	Bt11 (1%)
ADH, tNOS, and EPSPS	NK603	GTS-40-3-2 (1%) and GT73

supermarkets and food shops in Kuwait and analyzed to detect the presence of genetically modified DNA. Samples were collected periodically from various supermarkets in Kuwait from 2009–2010. They were categorized as raw samples (Table S1) and processed foods (Table S2). Screening of food samples for the presence of foreign DNA from GMOs in food samples was

performed by isolation of DNA, followed by analysis to detect the presence of transgenic DNA using SYBR Green based real time PCR. Total DNA extracted from the samples was used in combination with specific primer pairs^{61,62} targeting distinct marker sequences. List of names of oligonucleotide primers and the target genes used for GMO screening have been listed in Table 3. TaqMan probes were used for event-specific detection of GMOs and quantification of genetically modified DNA of individually identified events in food samples. They have been listed in Table 4. Certified reference materials used in the study are GTS-40-3-2 (1%), Bt11 (1%), GT73, LL62 (1%), MON810 (1%), MON1445 (1%), Bt11 (1%) and Bt176 (1%). The certified reference materials and oligonucleotide primers were provided by the Scientific Institute of Public Health, Brussels, Belgium.

For fresh food, cereals and grains, one kilogram of each food sample was collected from various supermarkets and ground individually. After grinding, an aliquot of 1 g of each sample was pooled to form representative sample for DNA extraction and GMOs detection. For processed foods, sample from the same brand was collected from four different supermarkets. Then, 200 g of each sample was ground individually, and an aliquot of 1 g of each sample was pooled together to form a representative sample for further analysis.

DNA isolation protocols were optimized on a case-to-case basis. Various DNA isolation protocols were adopted to obtain sufficient quantity of high quality DNA. Commercial kits used for DNA isolation include the Nucleospin food isolation kit (Macherey-Nagel, GmbH and Co.K. Germany) and the Wizard Magnatic DNA purification system for food (Promega Corporation). CTAB DNA extraction procedure was also used in certain instances.^{61,63} The quality and quantity of the isolated DNA samples was determined by UV spectrophotometry and agarose gel electrophoresis. The DNA bands were visualized by UV transilluminator and photographed using gel documentation unit.

Food samples were screened for the presence of GMO using SYBR Green based real-time PCR (qPCR). Total DNA extracted from the food samples were subjected to qPCR analysis using various primer pairs targeting distinct marker sequences that are commonly used in plant genetic engineering. qPCR assays were performed on an ABI 7300 PCR System (Applied Biosystems). The master reagents (Applied Biosystems) were allowed to thaw and then mixed by vortexing and centrifuged before use. The qPCR was performed in 25 µl total reaction volume. The reaction mixture had 1× SYBR Green PCR Master mix, forward and reverse primers, 50 ng genomic DNA. Positive control reactions had genomic DNA of reference materials and negative controls (non-template control or NTC) received equal amounts of water. PCR reaction was performed in duplicate. The reaction plate was sealed with flat optical adhesive cover (Applied Biosystems), briefly centrifuged and placed in the thermal block of the qPCR instrument. The program consisted of a single cycle of DNA polymerase activation for 10 min at 95°C followed by 40 amplification cycles of 15 sec at 95°C (denaturing step), 1 min at 60°C (annealing step) and 1 min at 60°C (extension step). Corresponding cycle threshold (Ct) values

were determined for each reaction. Ct value of 40 and above was considered as negative. The screening was performed with duplicate samples.

Event-specific GMO detection was performed by qPCR method based on *Taqman* chemistry.⁶⁴ Assays were performed on an ABI 7300 PCR System (AB Applied Biosystems) using reagents from Applied Biosystems. The assay was performed in 50 µl total reaction volume containing 12.5 µl of 1× *Taqman* Master Mix, 0.375 µl of 20 µM of each primer pair and 0.4 µl of 10 µM corresponding fluorescent probe in eppendorf tube. The mixture was mixed well and centrifuged briefly. A total of 40 µl master mix was dispensed in appropriate wells of a 96-well reaction plate, followed by the addition of 10 µl of template (10 ng/ µl) genomic DNA of samples to be tested. DNA from certified reference material served as the positive control and water was used in negative control reactions. The plate was closed with flat optical sealed cover, briefly centrifuged and placed in the thermal block of the qPCR instrument. The following thermal program was applied; a single cycle of DNA polymerase activation for 10 min at 95°C followed by 45 amplification cycles of 15 sec at 95°C (denaturing step), 1 min at 60°C (annealing step) and 1 min at 60°C (extension step). Data analysis was performed in automatic mode using ABI Sequence Detection Software version 1.4 (Applied Biosystems) Ct value was determined for each sample.

Detailed characterization of selected food materials were performed by quantification of GM event/events by quantification of the copy number of a DNA sequence unique to a particular GM event in relation to the copy number of a taxon-specific gene. Standard curve was prepared from transgenic and endogenous DNA according to Marc et al. (1999). The assay was performed in 50 µl total reaction volume containing 12.5 µl of 1× *Taqman* Master Mix, 0.375 µl of 20 µM of each primer pair, and 0.4 µl of 10 µM corresponding fluorescent probe (Table 4) in eppendorf tube for each GM event. The mixture was mixed well and centrifuged briefly. The reaction mixture (40 µl) was dispensed in a 96-well reaction plate. Genomic DNA template at 10 µl of various concentrations (15, 5, 2 ng/µl of the tested

samples) were added to individual wells in a 96-well reaction plate. Positive control and negative controls have been used as explained in previous sections. Certified reference materials (Table 4) with specific GMO events have been used to prepare standard curves that are used for event specific quantification of GMO in food. The plate was closed with flat optical sealed cover, briefly centrifuged and placed in the thermal block of the qPCR instrument. The following thermal program was applied. A single cycle of DNA polymerase activation for 10 min at 95°C followed by 45 amplification cycles of 15 sec at 95°C (denaturing step), 1 min at 60°C (annealing step) and 1 min at 60°C (extension step). Primers used in this study were selected from published reports.^{61,62} Data analysis was performed in automatic mode, according to the ABI Sequence Detection Software version 1.4 (Applied Biosystems). The percentage of individual GMO events in a food sample was determined by calculating the corresponding Ct value of individual events in each sample. The copy numbers of the transgenic DNA and taxon-specific DNA from each sample was calculated using standard curves prepared for certified materials.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Supplemental Materials

Supplemental materials may be found here:
<http://www.landesbioscience.com/journals/gmcrops/article/21364/>

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