Short communication

Prevalence of genetically modified foods (GM foods) in the United Arab Emirates

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A B S T R A C T

The real and/or perceived risks of genetically modified organisms (GMO) prompted food safety regulators to label the GM products. Although there are no legislations on GM labeling and cultivation of GM crops in the UAE, the present study aims to monitor the status of GM foods in the UAE market using Light cycler real time PCR technology and GMO screening kit. The yield and purity of DNA extracted by CTAB method was higher when compared to Qiaqen plant kit with an exception of soya products for which Qiagen kit yielded better results.

Out of 128 samples tested, 16 were positive for plant, 35S promoter and Tnos fragment. In conclusion, GMO screening assay applied in this study confirms the presence of genetically modified food in the UAE market. The rapidly growing GM market with multiple events and the threat from unapproved events signifies the value of surveillance program for monitoring the status of GM foods.

1. Introduction

Genetic modification refers to any change in the heritable traits of an organism achieved by means of intentional manipulation (Health Canada, 2003). The real and/or perceived risks of genetically modified organisms (GMO) prompted food safety regulators to label the GM products allowing the consumers to identify the product of interest and its contents. Several countries have imposed stringent monitoring programs to identify direct or indirect, immediate and or delayed adverse effects of GMO after their release on the market (de Jong, 2010). It is also important to counteract the application of unapproved transgenic events and to cope with the remaining scientific uncertainties inherent to risk analysis of approved GM crops (Wilhelm, Sanvido, Castanera, Schmidt, & Schiemann, 2009). Furthermore, the labeling information should reflect the actual content of the product so that consumer can choose the food item that interests them. Thus, monitoring GM foods has become a routine parameter for verification of food labeling and other legislation purposes.

At the international level, there is no consistent and harmonized set of rules to facilitate international trade of approved GM crops and related products due to substantial differences in the laws and regulations (Premanandh, 2010). For instance, in the United States, Canada, Japan, Philippines, Thailand and Taiwan, food with a content of up to 5% of approved GM material does not require labeling while Brazil, Australia, New Zealand, and Saudi Arabia, labeling is mandatory for GM content of more than 1% (Gruebe & Rao, 2007). Many developing countries have adapted de facto wait and watch policy on labeling legislations. In the European Union (EU), initial labeling legislation was framed by the European Union (EU) in 1997 and further refinements were made later (Jankiewicz, Broll, & Zagon, 1999). According to the European Regulation, adventitious presence of up to 0.9% does not require labeling on approved GM crops and the producers have to supply an event specific detection method for each new GMO variety (European Commission Regulation, 1829/2003). In the case of non-approved GMO, zero tolerance is applied.

Numerous detection techniques have been applied in the recent years to detect GM content from various matrices (Lipp, Anklam, & Stave, 2000; Rogan et al., 1999; Stave et al., 2000; Truckess, 2001). Most notably, nucleic acid based real time PCR assays are applied globally for qualitative and quantitative detection of GM foods (Dinon, de Melo, & Arisi, 2008; Hardegger, Brodmann, & Hermann, 1999; Holst-Jensen, Ronning, Lovseth, & Berdal, 2003; Vaitilingom, Pijnenburg, Gendre, & Brignon, 1999). The analytical techniques whether protein based or nucleic acid based, complement or overpower each other in certain cases (Zhang & Guo, 2011). Nevertheless, both techniques are currently in use for the detection of genetically modified organisms in food.

Although the Gulf cooperation council (GCC-5) countries do not produce transgenic crops, they are mainly dependent on imports such as corn, soybean and products thereof from GM producing...
countries including the United States. The Gulf standards organization (GSO) formed a subcommittee in 2008 to develop standards and regulations related to biotechnology (GAIN Report, 2010). To date, there is no published information on the status of GM foods imported in to the UAE. Thus, the present study aims to monitor the status of GM foods in the UAE market using Light cycler real time PCR technology and commercially available kit for the detection of 35S promoter and/or Tnos sequences. Since most of the commercially grown crops contain 35S and or Tnos elements (Holden, Levine, Scholdberg, Haynes, & Jenkins, 2010), GM screening method has been applied to the samples collected from UAE market followed by event related test for specific GMO detection.

2. Materials and methods

2.1. Samples

A total of 128 samples were applied to the present study (Table 1). The samples were imported from USA, UK, EU, Brazil, South Africa, India, Thailand, Philippines, Egypt and China. Depending on the nature of the samples, appropriate storage conditions were provided to store the samples until further use.

Table 1
Food samples applied in the present study and results of GMO analysis.

<table>
<thead>
<tr>
<th>Sample matrix</th>
<th>Number of samples</th>
<th>Plant gene</th>
<th>35S</th>
<th>Tnos</th>
<th>lectin</th>
<th>zein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize and maize products</td>
<td>26</td>
<td>26</td>
<td>04</td>
<td>06</td>
<td>ND</td>
<td>06</td>
</tr>
<tr>
<td>Soya and soya products</td>
<td>16</td>
<td>16</td>
<td>06</td>
<td>06</td>
<td>06</td>
<td>ND</td>
</tr>
<tr>
<td>Wheat and wheat products</td>
<td>26</td>
<td>26</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Vegetables and noodles</td>
<td>27</td>
<td>25</td>
<td>02</td>
<td>02</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Rice</td>
<td>24</td>
<td>24</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Pizza</td>
<td>09</td>
<td>09</td>
<td>06</td>
<td>06</td>
<td>04</td>
<td>06</td>
</tr>
</tbody>
</table>

ND; not detected.

2.2. DNA extraction

The DNA extraction was performed using two different techniques depending on the nature of the samples. The anion exchange resin based DNA extraction (DNeasy plant mini kit, Qiagen, Germany) was applied to soya samples as per the manufacturer’s instructions with overnight incubation of samples for proteinase K digestion.

All other samples were DNA extracted using hexadecyltrimethyl ammonium bromide (CTAB) method (Doyle & Doyle, 1987) with some modifications.

Approximately 100–120 g of material from each sample were finely ground using a high-speed knife mill (Grindomix GM 200, Retsch, Germany). Prior to extraction, β-mercaptoethanol (for 5.0 ml of buffer, add 25 μl of β-mercaptoethanol) was added to the CTAB buffer (1 M Tris HCl pH 8.0; 5 M NaCl; 0.5 M EDTA; 2 g of CTAB). From the homogenized sample mix, 100 mg was transferred to an eppendorf tube to which 500 μl of CTAB buffer was added. The tubes were mixed well to make sure the sample is in solution. The samples were incubated at 55 °C for at least 2 h in water bath. After the incubation, 500 μl of chloroform was added and mixed gently by shaking the tubes. The samples were centrifuged for 7 min at 16,000 g. The top layer containing the aqueous phase was transferred to a new tube and labeled. The volume of the aqueous phase was estimated and accordingly 0.08 volumes of 7.5 M cold ammonium acetate and 0.54 volumes of cold isopropanol were added. The tubes were mixed by inverting 20–30 times and incubated on ice for 30–40 min. After incubation, the samples were centrifuged for 3 min at 16,000 g. The supernatant was discarded without dislodging the pellet. The sample tubes were inverted 5–10 times after the addition of 700 μL of ethanol (70%) and centrifuged for 1 min at 16,000 g. After discarding the supernatant, 700 μL of ethanol (95%) was added and the tubes were inverted 5–10 times followed by a centrifugation step for 1 min at 16,000 g. The supernatant was discarded and the tubes were kept upside down on a clean tissue paper to dry for 10–15 min. After drying, the pellets were re-suspended in 50 μL TE buffer (1 M Tris HCl pH 8.0; 0.5 M EDTA) and stored at −20 °C until further use.

2.3. Spectrophotometric determination of DNA concentration

The concentration of extracted DNA was obtained by measuring the absorbance at 260 nm. The purity was determined by calculating the ratio of absorbance at 260–280 nm using Gene quant RNA/DNA calculator (Amersham Biosciences, Germany).

2.4. Real time PCR amplification and detection

For real time PCR amplification using Light Cycler 1.5 instrument (Roche diagnostics, Mannheim, Germany), food proof® GMO screening kit (Biotecon diagnostics, Potsdam, Germany) was used according to the manufacturer’s instructions. The kit is optimized for the fluorimetric detection of 35S promoter of cauliflower mosaic virus (CaMV) and the 3’ untranslated region (terminator) of the nopaline synthase (Tnos) gene of Agrobacterium tumefaciens. A highly conserved plant specific gene sequence has been incorporated in the kit which serves as a control to check the efficiency of DNA extraction. Certified reference material (CRM) for event specific transgenes have been obtained from Institute for Reference Materials and Measurements (IRMM) and included as additional controls. PCR reaction was performed in glass capillaries in a total reaction volume of 20.0 μl containing 5 μl of template DNA (40 ng/μl), 2.0 μl of food proof 35S/Nos detection mix or plant gene detection mix, 2.0 μl of food proof GMO screening enzyme master mix and 11.0 μl of PCR grade water. Positive control from the kit and reaction control containing all the reaction mixture except template was included in each run. The cycling conditions included an initial pre-incubation step of 95 °C for 10 min followed by 45 cycles of denaturation at 95 °C for 20 s, annealing at 60 °C for 25 s and extension at 72 °C for 15 s. Data analysis was performed using Light Cycler data analysis software ver. 3.5.5 using fit point algorithm.

2.5. Amplifiability of sample DNA using plant specific control primers

The presence of soybean and or maize DNA and their amplifiability were confirmed using plant specific primers (Querci, Jermini, & den Eede, 2006) from soya (lectin gene) and maize (zein gene) after optimization using SYBRGreen detection channel in the Light cycler.

3. Results and discussion

The DNA concentration of the samples was ranging from 100 ng to 850 ng/μl and the purity was between 80 and 98% as determined by the spectrophotometer. The effect of PCR inhibitors were determined by a four-fold dilution series of DNA concentration intended to be used in the assay (Zel et al., 2008). To estimate the limit of detection (LOD), two different DNA concentrations (0.02 ng & 0.05 ng) of 0.1% GM soya and maize were analyzed in a total of 10 replicates from 5 different days of PCR analysis. Both concentrations showed 100% of detection for GM soya and maize.

The results obtained from the CTAB method and the Qiagen plant mini kit warrant the validity of the protocol that it may be
employed for DNA extraction from raw and processed food matrix. However, the yield and purity of DNA from CTAB method was higher when compared to Qiagen kit with an exception of Soy products for which Qiagen plant kit yielded better results. The samples originated from plant material produced an amplification curve for the plant gene as expected with an exception of two tomato puree samples. Although the concentrations of these samples were sufficient for PCR, DNA might have got fragmented while processing (Greiner, Konietzny, & Jany, 1997) resulting in negative results for the plant gene fragment and was considered invalid. In the present study, samples with Cq values greater than 40 was considered suspect because of the implied low efficiency and was not reported (Bustin et al., 2009). Out of 128 samples, sixteen samples were positive for plant gene, 35S promoter and Tnos terminator fragment suggesting the presence of GM foods in the UAE market (Table 1). About fifty percent of the positive samples were frozen pizza containing corn and/or soya as one of the ingredient. It was further confirmed by taxon-specific assay for lectin and zein gene for soya and maize respectively. The remaining eight positive samples were bread containing soya, tinned corn and soya milk. Although a noodle sample and frozen vegetable were also positive in the screening method, we were not able to confirm the source as they showed negative results for lectin and zein gene.

Amplification results of two processed samples showed positive results for plant and Tnos fragment and negative result for 35S promoter. Taxon-specific real time PCR of these samples showed the presence of maize (zein gene) suggesting the possibility of GA21 maize which has Tnos fragment without 35S promoter.

The negative results obtained from the samples entail either the absence of 35S promoter and Tnos fragment or the concentration of DNA present is lower than the detection limit of the assay (0.1%). One of the major problems encountered while testing processed sample is the amplification failure due to potential PCR inhibitors (Cazzola & Petruccelli, 2006). Therefore, inclusion of internal amplification control is pre-requisite for standardized methods (Hoorfar et al., 2003). In this case, although internal amplification control (IAC) was not included in the reaction mixture, taxon-specific control (plant gene) was included for each sample to verify the presence of PCR inhibitors in the sample. Furthermore, positive samples with an amplification curve for plant gene, 35S and/or Tnos sequences were further verified through species-specific primers from maize and soya to confirm the origin of GM foods.

The base line data obtained from the present study demonstrate the significance of surveillance program for the policy makers to assess the status of GM crops in the country. Furthermore adventitious presence of GM materials in non-GM grains is a concern for trading partners and requires periodic monitoring. In conclusion, GMO screening assay applied in this study confirms the presence of genetically modified food in the UAE market.

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References


