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Full Length Research Paper

Monitoring of genetically modified food in Saudi Arabia

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Growing global cultivation and trading of genetically modified (GM) crops has led to increasing complexity in managing identity preservation of diverse agriculture commodities in food chain. In Saudi Arabia, public has expressed desire of having informed choice in what they are eating. This is the first study conducted to survey for the presence of genetically modified food in the Saudi Arabian markets. A total of two hundred and two samples were tested. Twenty products were found to be positive: 16 samples, three samples of corn or corn products and one sample of potato. The ground meat samples were positive as a result of added soybean. Two of the three corn samples were pop corn and corn starch, of which both were positive for the presence of the 35S promoter only. The third corn sample was a product known in the market as mixed nuts, was positive for both the 35S promoter and (NOS) terminator. The sample of potato (which was positive) was positive for both the 35S promoter and (NOS) terminator.

Key words: Genetically modified organisms, diagnosis, real time polymerase chain reaction, food, survey, Saudi Arabia.

INTRODUCTION

At the time the global demand for grain is increasing, the world stocks-to-use ratio is declining. In parallel, shortage of water resources, continued global warming, and many instability factors are threatening grain production in many parts of the world. In addition, increasing demand for bio-fuel is gradually putting pressures on the supply and demand for food in the world. Under this situation, the production of genetically modified (GM) crops had increased since the start of full-scale commercialization of GM crops in 1996, as both industrial countries and developing countries have attempted to secure the desired amount of production and benefit for farmers. In fact, the global area of GM crops in 25 countries exceeded in about 134 million hectares in 2009 and is expected to spread even further in the nearest future (James, 2009), revealing that more GM food will arrive at our table.

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As a result of introducing GM food to the market, many countries, such as the European Union has set several regulations. The regulation encompasses (EC Council Directive 2001/18/EC, 2001): The deliberate release into the environment of genetically modified organisms (EC Regulations 1829, 2003); traceability and labeling of genetically modified organisms, and the traceability of food and feed products produced from genetically modified organisms and (EC Regulation No. 1830/2003) for marketing and labeling of products that contain over 0.9% of approved genetically modified organisms (GMOs (European Union 2001, 2003a and 2003b). Labeling regulations of GM products has also been introduced in many other countries such as Japan, Australia and New Zealand (Carter and Gruere, 2006). Labeling of GM products is very important to help the consumer decide on whether or not to use the GM food. Detection and quantification methods of GM food (Hernandez et al., 2005) were developed to label the food correctly. To comply with the legislation, reliable and accurate methods for the identification of GM food in

either raw materials or processed food products are required. Numerous analytical methods have been described so far. Most currently, Japan has developed and validated many detection methods for newly approved GM events. Moreover, time- and cost-effective detection methods are currently under development (Kitta et al., 2009).

Screening techniques are of great importance in routine GM food detection as they rapidly provide information about the presence or absence of a GM food. Today the polymerase chain reaction (PCR) technique is widely used for detecting GMOs and can be used for both screening and quantification (Holst-Jensen, 2003; Cankar et al., 2005). The screening assays are based on the detection of elements frequently used in genetic engineering. The majority of GM plants that have been introduced to the market were transformed with Cauliflower mosaic virus (CaMV) 35S promoter (p35S) and nos-terminator from *Agrobacterium tumefaciens* (T-nos) or kanamycin resistance marker genes (nptII). The predominant screening assays for genetically modified plants are based on detecting 35S promoter (p35S) and nos-terminator sequences using either conventional or real-time PCR (Ahmed, 2002; Holst-Jensen, 2003; Cankar et al., 2005; Oraby et al., 2005).

At present, real-time PCR is the most commonly used technology for qualitative and quantitative detection of GMO as the risk of carry over contamination in routine analysis is reduced (Miraglia et al., 2004; Lyon and Wittwer, 2009). The amount of products synthesized during PCR is measured in real-time by detection of fluorescence signal produced as a result of specific amplification. Real-time PCR requires a special thermal cycle and an addition of fluorescence probe. Real-time PCR with TaqMan chemistry has been used in various kinds of qualitative and quantitative detection methods for GM crops (Kuribara et al., 2002; Holst-Jensen et al., 2003).

TaqMan PCR provides higher specificity than conventional PCR due to the chemistry with TaqMan probes. These characteristics are advantageous for a universal detection platform. The validation studies on the detection methods have also been reported on the Web site, “Community Reference Laboratory for GM Food and Feed” (http://gmo-crl.jrc.it/default.html) of the European Commission. As a result of the frequent addition of newly approved GM lines, the ideal system for the detection of GM is easily updatable and customizable as the situation demands. In that regard, a universal detection platform was developed where many analyses can be performed in a single system for the detection of genetically modified crops (Mano et al., 2008).

The goal of the present study is to monitor the presence of GMO in food stuff on Saudi market using the commercial kit of Roche, USA, which is based on the detection of 35S promoter and NOS terminator as target sequences.

MATERIALS AND METHODS

Sample collection

Two hundred and two samples, mostly imported food, were randomly collected from Riyadh local markets. The samples comprised 50 types of corn seeds, flour, starch, pop corn, fresh sweet corn and baby corn; 36 types of seeds, pre-fried and frozen potatoes, 14 types of canned foods, 14 types of wheat seeds and flours, 32 types of frozen meat, 6 samples of tofu, soy flour, soy sauce and seeds, 16 samples of clover seeds, 10 samples of sorghum seeds, 24 samples of tomato seeds, paste and canned tomatoes. Certified reference materials consisting of dried soybean and maize with 0 and 1% GMO commercialized by Fluka Chemika were used as controls.

DNA extraction and quantification

Dried samples were homogenized, while wet samples were lyophilized and homogenized. After sample homogenization, DNA was extracted from 100 mg of duplicate samples using the DNeasy Plant Kit (Qiagen, Hilden, Germany) according to the protocol provided with the kit. The concentration and purity of the extracted DNA were measured by absorbance at 260 and 280 nm using spectrophotometer (Pharrmacia LKB, Ultraspec III, USA). DNA purity was estimated by measuring OD260/OD280 ratio. The concentration was calculated according to the following formula:

1.0 OD260 = 50 μg/ml DNA

Qualitative detection of genetic modification

PCR amplification was carried out in a capillary reaction tube containing 50 ng of the isolated DNA. The survey of GMO food was performed using LightCycler-GMO Screening kit from Roche Applied Science, USA. This kit based on the detection of two transgenic DNA elements, the 35S promoter and NOS terminator.

RESULTS AND DISCUSSION

Out of the 202 samples of food tested, twenty samples were positive (Table 1). These positive samples contained 16 ground meat. The source of the GM in these ground beef samples was soybean. The soy proteins are widely used by meat industries as additive in the production of emulsified and ground meat products. Brod and Arisi (2007) demonstrated the presence of GM soybean in meat additives. Out of thirty two meat additives tested, twenty-five samples gave a positive signal for the soybean lectin gene confirming the presence of soybean DNA, 15 samples gave a positive signal for specific roundup ready (RR) soybean. Thus, indicating the presence of genetically modified soybean.

Three of the four remaining positive samples were corn or corn products (out of 50 tested). These three were pop corn, corn starch, and a corn that was sold in the market under mixed nuts. Both pop corn, and corn starch samples had only 35S promoter. The third corn sample was positive for both the 35S promoter and NOS terminator. Total of 36 potato samples tested, only one

Table 1. Food samples and percentage of GMO positive food in Saudi market.

Items	Number of GMO samples	Total number of negative samples	% of GMO positive
Corn and corn products	50	3	6
Potato	36	1	2.77
Frozen minced meat	32	16	50%*
Tomato seeds and products	24	0	0
Clover seeds	16	0	0
Canned food	14	0	0
Wheat and wheat products	14	0	0
Sorghum seeds	10	0	0
Soybean products	6	6	0
Total number	202	26	9.9

*, the GM positive ground meat is due to the added soybean protein which is a common practice in meat products manufacture.

sample was positive. This sample was tested positive for both the 35S promoter and NOS terminator.

Several countries have conducted survey to monitor the presence of GMO. For example, in Egypt, out of 24 different food samples tested, three products were positive with 35S promoter and none with nos terminator (Oraby et al., 2005). In another study in Egypt, 40 samples of soybean and another 40 samples of maize products were randomly collected from both Cairo and Giza markets. The results showed that twenty percent of soy samples contained Roundup ReadyTM soybean; 15% of maize samples were positive for Bt176; and 12.5% positive for Bt11 maize (El-Sanhoty et al., 2002). In Malaysia, a study was conducted for the detection of genetically modified soybean in processed food in the market. Out of 85 samples tested, 18 were positive: 9 were raw bean, 8 were tofu, and one was tempe (a Malay food) (Abdullah et al., 2006). In Bavarian, Health and Food Safety Authority analyzed 63 corn and 19 rape-seed samples for GM components in 2007. In one rape seed sample, non-approved GM components below the limit of quantification (0.1%) were detected (Goerlich et al., 2008).

Further, a survey was conducted in Turkey to monitor the genetic modified soybean products (flour, raw bean, tofu, soy milk, soy sauce). The results showed the presence of GMO in almost all soybean products (Aril and Cakir, 2008). Another study in Turkey (Ertugrul et al., 2008) showed the presence of GMO content in some maize samples, however, GMO positive soybean was found in the majority of soybean products. In Serbia (Taski-Ajdukovic et al., 2008), fifty processed meat products were examined, twelve gave positive results with 35S promoter and all of them contained roundup ready soybean. In Spain, only two samples were tested positive out of 237 seed lots in 2006 and 132 in 2007 submitted to maize seed certification program (Sanz and Dominguez, 2008). Using 35S-P and NOS-T and ELISA methods, a study conducted in Moldova on cultivated and arketed corn, potato and tomato showed that non of the tested products were positive (Duca et al., 2008).

Similarly, a study conducted in the Czech Republic from 2002 to 2007 on the detection of GM foods on the market in 1164 samples indicated that most of the positive samples were mainly roundup ready soybean, 3 varieties of GM maize and one GM rice (Kyrova et al., 2008a). However, the same group showed no detection of transgene in papaya during their study in 2005 and 2006 (Kyrova et al., 2008b). In Canada, out of the thirty-five representative food products selected based on their commercial importance (corn, soybean and canola), about one-third of the samples were GM positives (Gobeil et al., 2008). In Botswana, commercially available processed food derived from soy and maize were analyzed for the presence of cauliflower mosaic virus 35S (CaMV35S) promoter and NOS-terminator sequence, during 2006 and 2007. The results indicated that transgenic sequences were present in some of the commercially available processed food products (Mokhawa, 2008). In Algeria, a preliminary screening was conducted on randomly selected 20 samples of crops and 20 food products for the presence of the transgenic 35S CaMV sequence. Results showed that some samples of crops and food products were GMO positive (Louanchi et al., 2008). In Serbia, a survey was conducted to specifically detect RR soybean. They found that RR soybean was found in 68 samples in 2003 and in 20 samples in 2004 (Nikolic et al., 2008).

The Saudi Arabian GMO labeling requirement is set to be 1% maximum threshold limit for defining a GM foodstuff. If a product contains one or more GM ingredients, a triangle should be drawn and in it the text should read "Contains Genetically Modified Product (s). The Saudi Arabian Ministry of Agriculture (MOA) banned imports of GM seeds in January 2004, and thus no GM crop is grown in the country. Both the MOA and the Saudi Ministry of Commerce and Industry (MOCI), respectively, allow imports of GM grain and plant/vegetable based processed foodstuffs as long as they are labeled (Mousa and Giles, 2005).

The compliance with the above regulations was examined. Two hundred and two samples were tested (Table 1). Out of these, total of 20 samples were positive. This means that the non-compliance with the Saudi regulations was 9.9%. A similar survey was conducted in Taiwan from 2002 to 2007 to test for non-compliance with regulations. Sample size of each year was 138, 230, 322, 402, 325 and 322, respectively. Non-compliance from 2002 to 2007 was 46, 44, 14, 2, 5 and 2%, respectively (Lin et al., 2008). The non-compliance in Saudi Arabia is in the lower end of the Taiwan market. This indicates the effectiveness of the strict regulation on GM by Saudi Government. To further asses the level of compliance with the Saudi legislation, there is a need for quantitative detection methods as a routine analysis for the enforcement of the introduced labeling threshold for GM in food products.

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