Detection of Unlabeled Genetically Modified Soybean in the Omani Market

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ABSTRACT: This study aimed to screen for products containing Genetically Modified (GM) food in the Omani market using detection methods for the presence of Roundup Ready Soybean, Bt176 and MON810 maize in food products and to quantify it in positive samples using real time Polymerase Chain Reaction (PCR). A total of 100 food samples were collected randomly from markets in Oman. Out of 59 samples, 8 (13.5%) were successfully amplified with the maize plant specific PCR. GM screening showed negative for all samples, which indicated low or no GM maize in the samples tested. Out of 57 soy containing samples, 40 (70%) were successfully amplified by the soybean plant specific PCR. Six samples out of the 40 (15%) were found positive for GM using P35S-cf3/P35S-cr4 and HA-nos118-f/HA-nos118-r, primer pairs and using GMO5/GMO9 and GMO7/GMO8 primer pairs for specific detection of Roundup Ready Soybean. Real time PCR (TaqMan™ system) was carried out for the positive Roundup Ready Soybean samples and results showed that 2 out of the positive GM soy samples contained more than 5%; a Soy Formula for Infants (imported) sample contained 21% GM soybean and raw soybean seeds (imported in bulk amounts and packed in Oman) contained 88% GM soybean. The results demonstrate for the first time the presence of GM-soy in food products in the Omani market, reinforcing the need for the use of qualitative and quantitative methods for GM detection in food products.

Keywords: Genetically modified food; GMO detection; PCR; Soybean.

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الكشف عن الصويا المعدلة جينيا والغير معرفة في السوق العماني

نبيلة الصادقية وعلياء الأنصارية

الملخص: الهدف من هذه الدراسة هو الكشف عن المواد المعدلة جينيا في المنتجات الغذائية المتواجدة في الأسواق العمانية, وذلك باتباع طرق جزيئيه معتمدة ودقيقة للكشف عن تواجد ثلاثة أنواع منها : نوع من حبوب الصويا المعدلة جينيا (Roundup Ready Soybean) ونوعين من الذرة الصفراء المعدلة جينيا (MON810, Bt176). وبالإضافة إلى ذلك فقد تم تقدير نسبه الصويا المعدلة جينيا في العينات الموجبة وذلك باستخدام تقنية Real time PCR. جمعت مئة عينة عشوائيا من الأسواق المحلية في السلطنة. بواسطه تفاعل البلمرة المتسلسل و كانت نسبه نجاح الكشف عن وجود الذرة في عينات المواد الغذائية التي تحتوي علي الذرة 13.5% (8 من 59) وبالنسبة إلى العينات التي تحتوي علي الصويا بلغت النسبة 70%(40 من 57). لم يتم الكشف عن أي محتوي معدل وراثيا بالنسبة لعينات المواد الغذائية التي تحتوي على الذرة, بينما أظهرت النتائج وجود صويا معدلة جينيا بنسبه 15% (6 من 40) بأستخدام الزوج التمهيدي P35S- cf3/P35S-cr4 و HA-nos118-f/HA-nos118-r و الزوج التمهيدي GMO5/GMO9 and GMO7/GMO8 للكشف عن نوع Roundup Ready Soybean من الصويا المعدلة جينيا. و قدرت نسبه محتوى الصويا المعدلة في العينات الموجبة بواسطة Real time PCR. ووجد أن اثنين من هذه العينات تحتويان على نسبة أكبر من 5% من الصويا المعدلة جينيا, أحداها لمسحوق حليب مستورد للأطفال وقدرت النسبة فيه ب 21% و الأخرى لحبوب صويا مستوردة بكميات كبيره ومعبأة في السلطنة وقدرت النسبة فيها ب 88%. وهذه النتائج تعزز وبشدة الحاجة إلى استخدام طرق الكشف عن المنتجات المعدلة جينيا ومشتقاتها نوعيا وكميا لمراقبه المواد المعدلة جينيا المستوردة والمتوافرة في الأسواق دون بيانات.

الكلمات المفتاحية: المواد المعدلة جينيا، المنتجات الغذائية، الكشف عن المواد المعدلة جينيا.

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1. Introduction

Technological breakthroughs in plant genetic engineering have enabled scientists to directly introduce novel genes into a variety of economically important crops, such as soybean, corn, cotton, etc. [1]. Genetically

modified organisms (GMO) are produced to provide higher yield and nutritional and economic benefits to consumers, such as longer shelf-life, enhanced flavour, reduced allergenicity and enhanced health or wellness attributes. The new generation of GM foods with enhanced quality attributes or nutritional benefits elicits much greater public acceptance than previous products [2].

There are certain benefits of GM food; a study on Bt-Corn found that it has significantly reduced levels of fumonisin (which is amycotoxin derived from Fusarium) when produced in a region where the European corn borer (ECB) is an important pest [3]. Women who consume a diet heavy in unprocessed corn or lightly processed corn (e.g., corn meal) contaminated with Fumonisin B1 or FB1 (the most prevalent members of a family of toxins known as fumonisin) are at significantly higher risk of having a baby with neural tube defects (NTDs) because FB1 interferes with the uptake of folate in maternal cells [3].

However, there are major concerns regarding GM foods, such as the risk of allergy in humans and the potential negative environmental effects of modified crops.

A study was carried out on crop-to-crop gene flow using farm scale sites of fodder maize (Zea mays L.) in the UK, and reported that gene flow was detected in 30% of the samples (40 out of 135) at 150 m from the GM source, and events of GM to non-GM gene flow were detected at distances up to and including 200 m from the GM source [4]. With increasing use of GM crops, expanding from 1.7 million hectares in 1996 to 44.2 million hectares in 2000, gene-flow is an important consideration in the contamination of conventionally or organically grown crops [5]. A further valid argument made about GM foods is that little is known about the long-term effects of GM crops on the environment, microorganisms, animals, and humans [6].

Therefore, there is a growing public demand for transparency and accountability in terms of GMOs in the market place [1]. Regulators and governments have come under pressure to strengthen labeling requirements for GM foods. More than 35 countries have developed some form of labeling requirement, including China (0% threshold), the European Union (EU) (0.9% threshold), Australia and New Zealand (1% threshold), South Korea (3% threshold) and Japan and Taiwan (5% threshold) [1]. In order to support legislation, reliable, accurate methods for identification of GMOs in raw materials and food products are required [5,7,8]. Many analytical methods are available, including PCR methods for screening and for qualitative and quantitative detection of GMOs [5,9,10,11]. A review of methods has been published [12].

Oman was one of the countries that signed the Cartagena Protocol on Biosafety at the Convention on Biological Diversity in 2000. GMOs are not grown in Oman and the overall attitude of Omani consumers to GMOs is negative, although they are generally of the opinion that the government should allow importation of GM food if clearly labeled. When respondents were questioned about clearly labeled GM foods, the majority of respondents (59%) were happy to see them imported, if clearly labeled (Unpublished results of PhD study). There is, therefore, a need to label GM products in Oman in order to fulfill consumer demand for clear labeling of GMOs, to overcome the lack of GMO detection in Oman and to meet the demands of the biosafety protocol. The Central Laboratories for Food and Water in Oman, which undertakes a range of analyses on food do not analyze for GMOs or GM foods [13].

However, there is a proposal, prepared by the Ministry of Environment and Climate Affairs in collaboration with academic institutions and relevant organizations, for a national biosafety framework that will support the implementation of the biosafety protocol.

In this study, DNA extraction of different food products was performed. Plant specific, screening of GMOs and specific detection for Roundup Ready Soybean, Bt-176 maize and MON810 maize were carried out. In addition, the amount of GM soy in positive samples using quantitative real time PCR was semi-quantified.

2. Materials and Methods

2.1 Sample Collection

A total of 100 food samples, including raw soybean, maize seeds and processed products containing soya and/or maize ingredients were collected during March - April 2008. Samples were collected randomly from four superstores in the capital area of Oman (Muscat). These stores are located in the districts of Al-Qurum, Madinat As'Sultan Qaboos, Al-Hail and Al-Khod. The samples analyzed were: 8 varieties of baby foods, 2 types of burgers, 3 types of cake mixes, 5 types of canned foods, 2 types of cereals, 3 types of chips (fries), 1 type of chocolate drink mix, 5 varieties of confectionary, 3 types of cookies, 2 types of cornflour, 4 varieties of corn puffs, 4 types of corn seeds, 5 types of dried noodles, 1 type of fish cake, 2 types of franks, 2 types of fruit yoghurts, 2 types of frozen meals, 6 types of meal mixes, 2 types of sauces, 1 type of seasoning mix, 3 types of soup mixes, 1 type of soya bread, 1 type of soya cream, 3 types of soya milk, 1 type of soya pellets, 4 types of soya seeds, 1 type of spring rolls, 7 types of sweet biscuits (cookies), 7 types of sweet mixes, 2 types of sweet rolls, 1 type of thick cream and 4 types of tofu. The information on the labels

of the food samples collected was checked, and it indicated that 59% of the samples collected contained maize and 57% contained soy.

Institute for Reference Materials and Measurements (IRMM)-certificated soybean and maize reference material was obtained commercially (Sigma-Aldrich, St. Louis, MO). For MON 810 Maize, CRM was IRMM-413, 77119 (0% GM), 79521 (0.5% GM) and 76182 (5% GM). For Bt-176 Maximizer Maize, CRM was IRMM-422R, 91528 (0% GM), 14724 (0.5% GM) and 19074 (5% GM). For Roundup Ready Soybean, CRM was IRMM-410S, 83063 (0% GM), 81751 (0.5% GM) and 93109 (5% GM).

2.2 DNA Extraction

DNA was extracted using the Cetyltrimethyl Ammonium Bromide (CTAB) method [11] with some modifications. The extraction buffer contained 1% CTAB, 1.4M NaCl, 0.1M Tris-HCl (pH 8), 0.02M EDTA (pH 8), 0.5% w/v PVP and 0.1% v/v β-Mercaptoethanol.

DNA from reference materials was extracted by the same method. DNA concentration was measured for all samples (100) and the 9 controls using a NanoDrop ND-000 Spectrophotometer. The concentration of DNA for PCR was diluted to 50 ng/µl.

2.3 Plant Specific PCR for Maize and Soybean

Due to the high risk of cross contamination of samples and reagents, individual PCR steps were performed separately in terms of physical space and equipment [5,11,13]. PCR reaction with water instead of DNA extract was carried out with every mix as a reagent control and, with every set of samples, a series of positive and negative controls were run to ensure the accuracy of the results [5,11].

To confirm the presence and quality of DNA extracted from maize and/or soybean containing samples, primers ZEIN3/ZEIN4 specific to the maize zein gene (ze 1, coding for a 10-kb protein) and GMO3/GMO4 specific to soybean lectin gene (le 1) were used [11].

The PCR master-mix was set up in a total volume of 50 µl using 26.25 µl MilliQ water, 2.5 µl Glycerol (50%), 5 µl 10x Buffer (of 1x concentration), 5µl $MgCl_2$ (2.5 mM, Applied Biosystems, USA), 5µl dNTPs (0.2mM, Promega, USA), 0.25µl Taq. polymerase (0.025U/µl Inno), 0.5 µl of each primer, (0.5 µM, Sigma, USA) and 5 µl of DNA (5 ng/µl). The PCR program for primer pair ZEIN 3 & 4 was: initial denaturation for 3 min at 95ºC, denaturation for 1 min at 96 ºC, annealing for 1 min at 57 ºC, extension for 1 min at 72 ºC, number of cycles 50, final extension for 3 min at 72 ºC. The PCR program for primer pair GMO 3 & 4 was: initial denaturation for 3 min at 95 ºC, denaturation for 30 sec. at 95 ºC, annealing for 30 sec at 63 ºC, extension for 30 sec at 72 ºC, number of cycles 40, final extension for 3 min at 72 ºC.

2.4 Screening for Promoters and Terminators

The detection of the 35S promoter and *nos* terminator by PCR was carried out for screening. The identification of one of these regulatory sequences in soybean or maize containing samples indicates GMO presence. In Roundup Ready Soybean, the identification of both 35S promoter and the *nos* terminator is possible, whereas only the 35S promoter is present in the Bt-176 and MON810 Maize [11].

The PCR master-mix was set up as detailed, with 0.5 µl of each primer, P35S-cf3 and P35S-cr4 for 35S and HA-*nos* 118-f and HA-*nos* 118-r for *nos* (0.5 µM, Sigma, USA) and 5 µl of DNA (5 ng/µl).

The PCR program for primer pairs P35S-cf3, P35S-cr4 and HA-*nos* 118-f, HA-*nos* 118-r was: initial denaturation for 3 min at 95 ºC, denaturation for 25 sec at 95 ºC, annealing for 30 sec at 62 ºC, extension for 45 sec at 72 ºC; number of cycles 50, final extension for 7 min at 72 ºC.

2.5 Detection of Roundup Ready Soybean

Primer pairs GMO5/GMO9 and GMO7/GMO8 were used for specific detection of Roundup Ready® Soybean [11].

The PCR master-mix was set up in a total volume of 25 µl with 0.1µl of each primer, GMO5 and GMO9 (0.5 µM, Sigma, USA) and 2 µl of DNA (5 ng/µl).

The PCR program for primer pair GMO5 and GMO9 was: initial denaturation for 3 min at 95 ºC, denaturation for 30 sec at 95 ºC, annealing for 30 sec at 60 ºC; extension for 40 sec at 72 ºC, number of cycles 25, final extension for 3 min at 72 ºC. After amplification, 0.5 µl of the previous PCR product was added to 24.5 µl of the master-mix for a second PCR (nested PCR) with 0.1µl of each primer GMO7 and GMO8 (0.5 µM, Sigma, USA).

The PCR program for primer pair GMO7 and GMO8 was: initial denaturation for 3 min at 95 ℃, denaturation for 30 sec at 95 ℃, annealing for 30 sec at 60 ℃, extension for 40 sec at 72 ℃, number of cycles 35, final extension for 3 min at 72 ℃.

2.6 Quantification of Roundup Ready Soybean using Real Time PCR

RT-PCR was carried out to determine the ratio of target GM DNA to the total of the species DNA. Applied Biosystem ABI PRISM ™ 7700 detector instrument (TaqMan^{TM}) was used. The primer pairs used for real time PCR were: reference gene primers and probe, Le-F, Le-R, Le-Probe and trans-gene primers and probe; RR-F, RR-R, RR-Probe [11].

2.7 Agarose Gel Electrophoresis

The extracted DNA was subjected to electrophoresis on a 1% agarose gel at a constant voltage (75 V for 15 min); PCR products were checked on a 2% agarose gel (75V for 30 min) and nested PCR on a 2.5% agarose gel (100V for 30 min) containing ethidium bromide in 1XTBE buffer. The visualization was performed under UV light and the images were captured with a digital camera (Casio, EX-Z1000) for documentation.

3. Results and Discussion

3.1 DNA Extraction

The modified CTAB method yielded a good amount of DNA for all controls and food samples. Most of the samples under UV radiation showed a clear heavily illuminated area of DNA. The DNA extraction method used was satisfactory for most of the products, which ranged from raw seeds to processed food. The quantity of DNA extracted ranged from 16.23 to 5454 ng/μl.

3.2 Plant Specific PCR for Maize and Soybean

Out of 57 samples containing soybean in their ingredients, 40 samples (70%) were positive for soybean plant specific PCR, and out of 59 samples containing maize in their ingredients (all were processed), 8 samples (13.5%) were positive for maize plant specific PCR. A study by Cardarelli *et al.* showed that the analysis of products containing maize seemed to be more difficult, probably due to the low concentration of maize ingredients or to some processing steps in food production that strongly contributed to the failure of DNA extraction [5]. In addition, the treatment during food production processes, such as heating, high pressure and the physical processing are known to affect the DNA content. This was found in a study on maize samples screened for GM maize, where the corn samples investigated contained corn starch, corn grits, hydrolyzed corn starch, or corn flour [8].

3.3 Screening for Promoters and Terminators

PCR was carried out for maize samples using primer pair P35S-cf3 and P35S-cr4. A band of 123bp indicates the presence of GM.

Six soybean samples out of 40 showed clear positive in both the 35S promoter and *nos* terminator reactions, 15% of soybean samples were GM soybean (Table 1). Although there were about the same number of samples containing maize (59) and soya (57), none of the maize-containing food were GM positive. The negative GM maize results in this study may indicate that Oman's source of maize is not a GM maize producer. However, having highly processed samples can potentially interfere with the PCR detection. This is clearly demonstrated by having only 5 samples detected out of 59 samples with the maize specific PCR.

3.4 Detection of Roundup Ready Soybean

For specific detection of Roundup Ready Soybean, standard controls, 2% GM and 5% GM, were used. The six samples, which were positive with both screening PCRs (35S and *nos*) were also positive for the specific PCR detection of Roundup Ready Soybean. One was raw soybean seeds and five were processed foods (Table 1).
In previous studies where the primer pair GMO5/GMO9 and GMO7/GMO8 were used in the nested PCR the sensitivity was 0.01% [14]. The results of this study agree with these findings because all six GM soybean samples checked for Roundup Ready® Soybean were positive.

3.5 Quantification of Roundup Ready Soybean Real Time PCR

Real time PCR (TaqMan™ system) was carried out for the six Roundup Ready soybean samples and the controls, Roundup Ready Soybean 0%, Roundup Ready Soybean 2%, Roundup Ready Soybean 5% and a DNA negative control sample.

Two of the samples contained higher amounts of GM than the 5% control. One sample, raw soybean seeds imported and packed in Oman, contained 88% GM soybean and the second sample, a Soy Formula for Infants, contained 21% GM soybean (Table 1). Neither sample was labeled as containing GM, although analysis showed that the GM ingredients exceeded the legal threshold (1%) level of GM content in a food product.

More of the samples could have contained GM, but with it failing to be detected due to method limitations, such as the quality of DNA extracted from highly processed food products possibly being affected by degradation processes. The process of DNA and protein detection faces difficulties when processed and highly refined food products are involved, e.g. starch, sugar or vegetable oils. Sometimes food products are subjected to extensive food processing stages which could affect their ingredients and GMO origin. This can result in the possibility of non-detectable GM DNA or proteins [15].

Table 1. Results of PCR for soybean samples

Product	Number of Samples	Soybean Plant Specific	Screening		Detection	RT PCR
			Promoter	Terminator	RR Soybean	GM %
Baby Food	8	5	1	1	1	21
Soya Seeds	4	4	1	1	1	88
Soya Pellets	1	1	0	0	0	0
Soya Milk	3	3	0	0	0	0
Soya Bread	1	1	0	0	0	0
Sweets	18	5	0	0	0	0
Biscuits	10	8	1	1	1	0
Tofu	4	4	0	0	0	0
Frozen Meals	5	1	1	1	1	0
Canned Meats	5	2	0	0	0	0
Burgers	2	2	2	2	2	0
Franks	2	2	0	0	0	0
Mixes	11	1	0	0	0	0
Noodles	5	1	0	0	0	0

4. Conclusion

Using PCR-based methods we detected and quantified the presence of GM foods in a collection of 100 samples from the market. The results demonstrate the availability of unlabeled GM soybean products containing higher percentages than the limit for labeling (1%) in the Omani food market. This indicates the need for better control and labeling of GM food products in Oman, to address public concerns about GM foods and their potential health and environmental risks, the consumer right to choose, and also to support the implementation of the recently proposed National Biosafety Framework for Oman.

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