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Detection approaches for genetically modified organisms in foods

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Abstract

This review examines the various detection strategies for genetically modified organisms (GMOs) in food products. It begins with a brief discussion of the issues related to the technology especially the risks and public concerns. An introduction to the biological aspects of the major GMOs then follows. The bulk of the review is concerned with the different approaches toward detection: (a) PCR-based methods such as real-time, duplex and multiplex, (b) the use of biosensors and microarrays, (c) the presence of commercially available kits, and (d) other methods such as electrophoresis and wavelength-dispersive X-ray fluorescence. Each of these methods is critically discussed and various applications are described.

Keywords: Polymerase chain reaction; Biosensors; Kits; Genetically modified organisms; Microarrays

1. Introduction

The term 'genetic engineering' covers several methods of manipulating genetic material and other terms commonly used to describe the field include genetic manipulation, recombinant DNA technology and in humans, gene therapy (Atkinson, 1998). It involves moving genes both within and/or between different species, with the latter being of greater interest. Organisms modified in this way are said to be 'transgenic' or 'genetically modified' (GMOs). In contrast to conventional breeding, genetic engineering does not involve the mixing of the entire gene complement of two or more individuals and the subsequent need to re-sort them to give only the desired change (Atkinson, 1998). Transgenic methods, however, do not work well in all cases but they have the potential to produce faster results than traditional approaches.

1.1. Benefits

GM plants can offer several benefits for agricultural practice, food quality, nutrition and health (Royal Society, 2003a). In 2000, the United Kingdom Royal Society along with six other national academies considered the role of GM plants in world agriculture. The report (Royal Society, 2000) concluded that commercially produced GM crops in the USA and Canada are designed to confer resistance to insect pests and to produce tolerance to specific herbicides. This can lead to a reduction in the use of pesticides. Additionally, GM plants can improve the nutritional qualities of crops and agronomic performance by targeting variables such as yield and stress tolerance. It has been proposed that GM technology can help meet the demand for food by an increasing world population with less impact on the environment (Royal Society, 2003b). Supporters of GM foods see the technology as powerful enough to increase the yield from existing food production and, also, to grow crops in those regions of the world where agricultural methodologies are not highly developed (Atkinson, 1998).

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1.2. Risks

As with all new technologies, potential risks exist and GM technology is no different. Two main issues are: (i) the transfer of the introduced genes to wild plants and non-GM crops and (ii) the indirect effects of the GM crops in the environment, e.g., effects on non-target insect and weed population and the possible development of resistant insects and weeds (Royal Society, 2003c). It is also possible that GM technology can lead to unpredicted harmful changes in the nutritional status of foods, although, this can also happen with conventional techniques. Finally, there is the possibility that the biodiversity of wildlife can be modified as a result of changes in the availability of food (Atkinson, 1998).

1.3. Public concerns

The use of genetically modified organisms raises several questions on the morality of the technology, the balance of risk in society, public involvement in decision making and the appropriateness of using patents in an area linked to life processes (Atkinson, 1998). As is well known, the public has been vocal with respect to the technology as applied to foods. In Europe, there have been more protests and discussion than in North America where the technology is more widely accepted.

At governmental levels, the USA has been against the EU moratorium on GM crops, introduced in 1999, mainly from the standpoint that scientific evidence shows the safety of these products. On the other hand, the EU insists that due to uncertainty surrounding the long-term effects of these crops, they are protecting the consumers (Yogendra, 2004). This clearly shows that very different views can arise from using the same scientific information. It has been suggested that these differences develop due to variation in public perception, social values, political culture, regulatory style and the capacity of consumers and producers to take collective action (Yogendra, 2004).

A moratorium on the commercial planting of GM crops is currently in effect in the UK. In 2000, the British Government announced a three-year research programme, the Farm-Scale Evaluation (FSEs), to study the effects on some species of wildlife, of the way weed-killers are used on herbicide resistant GM maize, oilseed rape and beet (Royal Society, 2003d). The results of the FSEs have now been published and they reveal significant differences in the effect on biodiversity when managing GM herbicide-tolerant (GMHT) crops as compared with conventional varieties (Royal Society, 2003e). Oilseed rape and sugar beet had a worse impact on farmland wildlife than conventional crops while for maize the situation was reversed (Coghlan, 2003). The researchers stress the results measured the impact of herbicides on wildlife rather than on the crops themselves. However, critics of GM crops are elated about the results as these could provide much needed data for world trade rules to ban the commercialisation of the two crops (Coghlan, 2003).

1.4. Aims and organisation of the paper

Since 1999, there have been increased consumer concerns over the use of GMOs in food products, especially in the EU countries. Foods of such a nature have been labelled 'Frankenstein' implying that consumption of these may lead to deformities of some form. Thus, in the intervening five years, there have been concerted efforts from scientists, consumer groups, farmers and supermarket personnel, to distinguish GM products from non-GM entities. One of the major ways in which this has been achieved was the development of rapid methods for the detection of GMOs in food products. This will form the basis of discussion in this review article.

New techniques for detecting small differences between transgenic and the parental or recipient plants are becoming available (e.g., proteomics) which could be useful in detecting unintentional harmful interactions (Royal Society, 2003a). Safety assessments can make use of techniques such as microarrays for detailed studies of mRNA expression, quantitative two-dimensional gel electrophoresis and mass spectrometry for protein analysis and metabolomic analyses to detect changes in metabolites (Royal Society, 2003a).

The rest of the review is organised into two main sections viz: (i) an introduction to biological aspects of the major GM foods and (ii) current methods of detection. The former is included to allow the reader to appreciate the methodologies involved in producing the GM food. Once this is clear, it will be easier to understand the methods used for detecting the GMOs.

2. Biological aspects of major GMOs

The novel traits of GMOs can vary widely and it will be useful to briefly consider biological aspects of the three most common GMOs: Bt corn and cotton, and glyphosate-resistant (GR) soybeans. All three were developed to deal with pests of a commercial crop but they differ in the nature of the pest and in their physiology. Bt corn and cotton were designed to kill lepidopteran pests, while GR soybeans allowed the application of glyphosate (Roundup™), a herbicide, directly to the soybean field. This kills all unwanted plants while leaving the soybeans unharmed (Nelson, 2001).

2.1. The biology of Bt corn (Nelson, 2001)

Bt corn hybrids contain genetic material from one of several strains of *Bacillus thuringiensis* (Bt). Bt is a nat-

urally occurring soil-borne bacterium which is widespread in nature. During spore formation, Bt produces crystal-like or 'Cry' proteins which, when ingested by susceptible larvae, act as poisons. Enzymes in the larvae activate the toxin, the Cry proteins bind to receptors in the intestine and rupture the cells. Insects stop feeding within 2 h of the first bite and, if enough toxin is digested, die in 2–3 days.

A specific Cry protein is toxic only to specific insects, and usually has no effect on mammals. These features make Bt insecticides useful and approximately 170 Cry proteins have been identified. Cry proteins toxic to Colorado potato beetle, corn earworm, European corn borer, mosquitoes and the cabbage looper have been produced.

Nelson (2001) has indicated that conventional Bt insecticides may be as toxic as synthetic insecticides, but their performance in the field is not always consistent. The toxic effect is lost quickly upon exposure to ultraviolet radiation, heat or dryness. Additionally, traditional application techniques can give incomplete coverage of feeding sites. These problems, however, are overcome by modifying a corn plant to produce its own Bt protein and the protein is present whenever larvae need to feed so there is no need for application.

2.2. Biology of Bt cotton

The most serious pest of cotton plants is the cotton bollworm or corn earworm (*Helicoverpa zea*) which is closely followed by the boll weevil and pink bollworms.

Bt cotton also contains genetic material from *B. thuringiensis* and the Bt gene transferred to cotton expresses the Cry protein Cry 1AC. The target insects are the cotton and pink bollworms and the tobacco budworm. Resistance to pyrethroid insecticides in the early 1990s was a serious problem which led to the development of the Bt cotton (Nelson, 2001).

2.3. Characteristics of glyphosate-resistant soybeans

Glyphosate (Roundup™) is a non-selective herbicide used on many food and non-food field crops. It is also effective on a variety of weeds. It has also been reported that the product has only mild effects on mammals but this is subject to controversy. The largest uses are for hay, soybeans and field corn (EPA, 2002).

Glyphosate controls weeds by inhibiting the enzyme EPSP synthase which is necessary for the growth of plants. GM crops contain the gene CP 4 EPSP synthase isolated from *Agrobacterium* sp. strain CP 4. The gene produces an enzyme which performs the functions of EPSP synthase but also is not inhibited by glyphosate. In 1996, Monsanto introduced the first glyphosate-resistant crop, Roundup Ready soybeans and this was followed by cotton, corn, beet and canola (EPA, 2002).

3. Detection approaches

In this section, the various methodologies which have been employed to analyse and/or detect GMOs in food products will be critically reviewed. The main categories of detection strategies are polymerase chain reaction (PCR)-based methods, DNA-based approaches (which may or may not involve the use of PCR), commercially available kits and other approaches including electrophoresis, biosensors and DNA microarrays.

3.1. PCR-based methods

In order to meet regulatory and consumer demand, several PCR-based methods have been developed to detect and quantify GMOs in food and feed. Pre-requisites for GMO detection include a minimum amount of the target gene and prior knowledge of the type of genetic modification, such as insect resistance traits, including controlling elements such as promoters and terminators (Gadani, Bindler, Pijnenburg, Rossi, & Zuber, 2000). DNA extraction and purification is a crucial step for the preparation of samples for PCR.

The unambiguous identification of a transgenic element in a food sample can be easily detected if information on the modified sequences is available (European Union, 2004). This can be possible by making use of PCR and its modified approaches such as PCR-ELISA and direct hybridisation with specific probes.

3.1.1. PCR-ELISA

Brunnert, Spener, and Borchers (2001) have reported a PCR-ELISA approach as a high-throughput method suitable for automation. It is based on the specific hybridisation of an immobilised, biotinylated PCR product (CaMV-35S promoter) with a digoxigenin-labelled internal probe suitable for colorimetric detection. This method excludes the need for time-consuming blotting procedures and for the use of hazardous ethidium bromide in gel staining. Detection of as little as 0.1 ng amplicon was possible in 2 h and RRS and soybean flour were analysed in the range 0.1–2.0%.

A ring-tested PCR-ELISA method for screening of the 35S promoter sequence in GM soyabeans has been modified to produce quantitative results (Vollenhofer, Burg, Schmidt, & Kroath, 1999). Additionally, multiplex PCR, biosensor technologies special gel system for amplicon characterisation (HA-yellow), direct hybridisation, nucleic acid sequence based amplification (NASBA) protein diagnostic methods, amplified fragment length polymorphism (AFLP) and fingerprinting techniques were studied. The researchers concluded that all of these methods may be feasible but that there are restrictions with respect to establishing standard procedures for quantitative assays compared with PCR methods.

In a similar approach, a PCR-ELISA method involving liquid-phase hybridisation (LPH) was used to detect GMPs in food. In this LPH-PCR ELISA, the biotinylated PCR product was hybridised with digoxigenin-labelled probes in the PCR reaction mixture immediately after the amplification (Liu et al., 2004). This was followed by enzyme conversion of the substrate to give optical density values which allowed for the detection of samples containing GMOs in different concentrations. This approach, which is not fully automated as yet for routine analysis, is a forward step in the development of a high-throughput screening method.

3.1.2. Use of SYBR Green I

SYBR Green chemistry combined with PCR is also becoming useful in the development of real-time detection of GM foods. In one approach, transgenic maize event GA 21 was detected by a combination of SYBR® Green I, Amplifluor™ and TaqMan® technologies (Hernandez, Esteve, Prat, & Pla, 2004). Maize GA 21 line has integrated several tandemly repeated copies of the r-act 5-enol-pyruvylshikimate-3-phosphate synthase construct used for transformation. A nucleotide sequence corresponding to the polylinked plasmid vector flanked by the r-act promoter and nopaline synthase 3′ terminator. GA 21 specific primers and probe were designed to target the vector-promoter junction region by amplification of a 72 bp DNA fragment. Quantification was optimised by using three real-time PCR methodologies viz. SYBR® Green I, Amplifluor™ and TaqMan®. All three were specific sensitive and reliable for identification and quantification of GA 21 DNA. The detection limit was 0.01% GA 21 which makes the approach suitable for routine GMO analysis. In another similar approach, the real-time detection of GM soya using lightcycler and ABI 7700 platforms with Taq Man®, Scorpion and SYBR Green I chemistries was reported (Terry et al., 2002). Various copy menus of a plasmid containing the soya lectin sequence were used to determine the sensitivity and reproducibility of the different technology combinations and to examine both the inter- and intra-machine variability. The genetically modified soya content of baked products containing known amounts of Roundup Ready soya was determined by detection of lectin and EPSPS transgene. The researchers concluded that the combination of TaqMan detection chemistry and the ABI 7700 platform was the best method of quantitative detection of GMOs with respect to precision and accuracy.

3.1.3. Real-time PCR

Real time PCR has become very useful in the quest towards achieving quantitative PCR methodologies for determination of GMO content in food products. This is a difficult process since the actual GMO content of standards can vary from lot to lot and commercial certified reference materials are only available if the range of 0–5%, which limits the working range (Pardigol, Guillet, & Poepping, 2003). Recombinant plasmid standards have been used as an alternate to commercial reference standards, but their production requires cloning and microbiological facilities. Apart from these problems, however, real-time quantitative PCR is probably the most accurate method for GMO quantification. It is readily able to determine the food requirement that products should not contain more than 1% of GMOs.

Validation of PCR methods for quantification of GM plants in food was carried out by three European food control laboratories (Hubner, Waiblinger, Pietsch, & Brodmann, 2001). The limit of quantitation (LOQ) of GMO-specific, real-time PCR was determined to be 30–50 target molecules which is dependent on the genome size of the target plant. The LOQ ranges from 0.02% for rice to 0.7% for wheat. Additionally, the precision of quantitative PCR detection varied from 10% to 30% RSD. As mentioned earlier, these researchers have also suggested that high priority must be given to international agreements and standardisation of certified reference materials. In another study, it has been shown that the accuracy of GMO quantification also depends on the category of transgenic kernels present in a mixture of maize cultivars, as the DNA content per mass unit varies significantly among different cultivars (Trifa & Zhang, 2004).

3.1.4. Duplex and multiplex PCR

Although real-time PCR is a powerful technique for testing of GMOs in foods, it has a few problems as it depends critically on the correct use of calibration and reference materials (Taverniers, van Bockstaele, & De Loose, 2004). At present, the choice of reference materials is being debated as the use of these can lead to errors in the estimation of DNA concentrations. To overcome these issues, Taverniers et al. (2004) have recently described two new real-time PCR methods for the detection of Roundup Ready soybean.

In these approaches, two types of plasmid DNA fragments are used as calibrators. Single-target plasmids (STPs) diluted in genomic DNA were used in one method while multiple-target plasmids (MTPs) containing both sequences in one molecule were used as calibrators in the other. It has been reported that both methods simultaneously detected a promoter 35S sequence as a GMO-specific target and a lectin gene sequence as an endogeneous reference target in a duplex PCR. Relative GMO percentages were estimated by two approaches: (a) a method based on direct comparison of measured CT values of both the GMO-specific target and the endogeneous target (∇CT method) and (b) a standard curve method which measured the absolute quantities of target copies on haploid genome equivalents. The authors reported that a duplex CT method with STP calibrators

performed as well as a similar method with genomic DNA calibrators from commercial certified reference materials. Also, good results were obtained with the standard curve method using MTP calibrators. Therefore, these approaches should find greater usefulness in the detection of GMOs in various products. However, the production of recombinant plasmids requires expensive cloning and microbiology facilities. To partially overcome this problem, it is possible to construct hybrid amplicon standards containing both transgene and reference gene targets in a tandem orientation on the same molecule (Pardigol et al., 2003).

The use of multiplex PCR systems using combined probes and primes targeted to sequences specific to various GMOs is becoming more important than standard PCR, which is proving to be insufficient for GMO-products. Spanish workers have developed a multiplex real-time PCR suitable for multiple GMO identification based on the use of SYBR Green I and the analysis of melting curves of the amplified products (Hernandez, Rodriguez-Lazaro, Esteve, Prat, & Pla, 2003). Different amplification products specific for Maximizer 176, Bt-11, MON 810 and GA 21 maize and for GTS 40-3-2 soybeans were obtained and identified by their T_m values. This multiplex approach showed a sensitivity of 0.1% in duplex reactions and is a useful alternative to real-time PCR based on sequence-specific probes, e.g., TaqMan chemistry.

3.1.5. Quantitative PCR

Zimmermann, Luthy, and Pauli (2000) described an event specific transgene detection in Bt-11 corn by quantitative PCR at the integration site. This GM corn is grown for the production of food and fodder. The insertion and expression of a 6.3 kb sequence containing a Bt-transgene coding for a synthetic cry IA (b) δ-endotoxin leads the Bt-11-corn to be resistant to lepidopteran insects. Conventional PCR approaches whereby unique sequences within the integrated transgenic regions are amplified can lead to the ambiguous results. Thus, the characterisation of the sequences of the 5′-site of the integrated transgenic sequence in Bt-11-corn genome using inverse PCR was investigated. By using one primer annealing exactly at the integration border between the transgenic construct and the corn genome and the other within vector sequence of the transgenic construct, a 207 bp sequence was amplified. A 22 bp fragment was inserted and the amplification product was then used as a competitor in quantitative PCR (QC-PCR). The researchers then calibrated the QC-PCR system to an equivalence point of 1% Bt-11-DNA with this method leading to unambiguous detection.

Five quantitative competitive (QC-PCR) and two real-time PCR methods for the detection of RRS or Bt-176 maize were tested in a ring trial in Switzerland (Pauli et al., 2001). For QC-PCR, the results included judgement of the GMO content by eye and calculation by soft-ware image analysis. Comparison of standard derivatives of the results by QC-PCR with those of real-time PCR showed no significant differences indicating that, at the 1% threshold, the precision of QC-PCR is similar to real-time PCR. The trial also showed that quality control in the production of certified reference materials for GMO analysis for accurate quantification.

3.1.6. Quantitation

Fig. 1 summarises the procedure for GMO testing. The enforcement of the Swiss Food Regulation and of the EU Novel Food Regulation is based on a PCR detection system specific for 35S promoter region originating from cauliflower mosaic virus. This 35S promoter is used in GM plants (Hubner, Studer, & Luthy, 1999). Although, the 35S-PCR detection system has undergone much testing, its detection limits varies from 100 pg to ng RRS-DNA between different analytical laboratories. It is also suited for distinguishing GMO mixtures due to its high sensitivity. In practice, the 35S-PCR technique allows detection of GMO contents of foods and raw materials in the range 0.01–0.1%. The Swiss government has introduced a threshold value of 1% GMO content as a basis for food labelling. This has led to the development of quantitative detection systems such as quantitative competitive PCR (QC-PCR), real-time PCR and ELISA systems. With ELISA, however, there can be protein denaturing during food processing. Quantitative

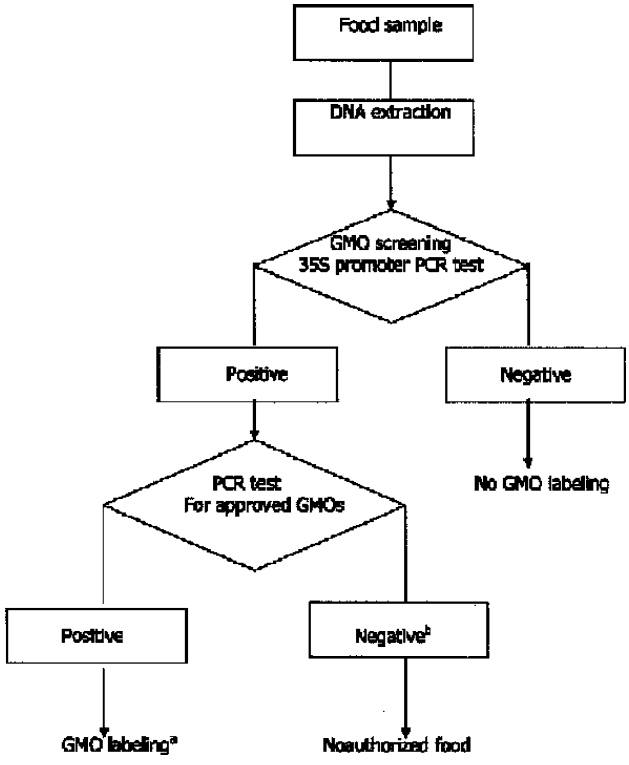

Fig. 1. Procedure for GMO testing of food.

PCR has the advantage of survival of DNA in most manufacturing processes.

Inter-laboratory differences were found to be smaller with the QC-PCR than with quantitative PCR mainly due to insufficient sample homogenisation. Furthermore, QC-PCR allows the threshold values for GMO labelling to be more readily determined (Hubner et al., 1999). Still, disadvantages can exist. The amount of DNA, which could be amplified, is affected by food processing techniques and can vary up to 5-fold. Thus, results need to be normalised by using plant-specific QC-PCR system. Also, DNA, which cannot be amplified, will affect all quantitative PCR detection systems.

3.1.7. General discussion

The high sensitivity and specificity of PCR allow it to be the first choice of laboratories interested in detecting GMOs in foods. The quality and quantity of target analyte will also influence the method of choice and again PCR methods are generally chosen (Holst-Jensen, Ronning, Lovseth, & Berdal, 2003). The choice of target sequence is the most important factor controlling the specificity of PCR method and it is normally part of the modified gene construct, e.g., promoter, terminator, a gene or a junction. New approaches include event-specific methods, which are very useful for quantification of GM content.

Methods are being developed for increasing the sensitivity of detection of PCR products. Fluorescence is the favoured signalling technology and several techniques rely on energy transfer between a fluorophore and a proximal quencher molecule (Whitcombe, Theaker, Guy, Brown, & Little, 1999). In these methods, dual-labelled probes hybridise to an amplicon and changes in the quenching of the fluorophore are detected. An advance in this approach is to use a primer with an integral tail which is used to probe an extension product of the primer. The probing of a target sequence is converted into a unimolecular event. This leads to considerable advantages with respect to kinetics, thermodynamics, assay design and probe reliability (Whitcombe et al., 1999).

Another important consideration when conducting PCR methods for detecting GMOs in foods is the reliability of food measurement. The Food Analysis Performance Scheme (FAPAS), based in the United Kingdom, has operated a proficiency testing scheme for GM food analyses. It has been found that there is a tendency for laboratories to overestimate the GM levels, for results obtained by PCR and ELISA to be significantly different and that data are skewed and not normally distributed until undergoing mathematical treatment (Powell & Owen, 2002).

One of the problems with PCR and other DNA-based techniques, is that they do not allow differentiation between GMOs and the natural occurrence of transgenic elements, such as 35S-promoter of cauliflower mosaic virus (CaMV) or the NOS-terminator of *Agrobacterium tumefaciens*. This may result in false-positive detection of GMOs (Wolf et al., 2000). Thus, it is critical to develop new approaches involving PCR for specific results.

In PCR-based methodologies, the crucial step is the isolation of DNA. As a result of several processing stages, the quality of the extracted DNA may be considerably reduced which may make GMO detection impossible. An example of this is the evaluation of the DNA quality of soy lecithin in margarines. DNA was isolated from margarines with different levels of lecithin by two extraction methods, one of which was the CTAB method. The amplification of soy DNA by PCR was difficult due to the complex DNA extraction from margarine and the low lecithin contents (Gryson, Messens, & Dewettink, 2003).

Finally, sampling procedures are of great importance towards the validation of analytical methods for GMO analysis. The largest single source of error in the analysis of grains is the sampling procedure. Sampling risks can be managed by using a diverter-type mechanical sampler and by choosing an appropriate sample size for analysis (Tanner, 2001).

3.2. Commercially available kits

There are numerous commercially available kits for the detection of genetically modified organisms. It is not the intention of this paper to focus on these and, therefore only the main features of some of the kits are summarised in Table 1. Further information is available from the respective companies' web-sites.

3.3. Biosensors and microarrays

Biosensors and microarrays are two emerging fields with enormous potential. Both are rapid, sensitive, specific and yield results in real-time. However, each has its own problems with biosensors proving difficult to commercialise due to inherent instability issues while microarrays yield a vast amount of data which are complex for analysis.

3.3.1. Biosensors

Mascini's group in Italy has been at the forefront in the development of biosensors for the detection of GMOs. In one instance, the group used a bulk acoustic ware affinity to achieve detection. A nucleic acid sensor for hybidisation was used for the detection of DNA target sequences in solution (Mannelli, Miunni, Tombelli, & Mascini, 2003a). The DNA probe was immobilised on the sensor surface while the target sequence was allowed to be free in solution and the hybridisation was followed in real-time. The probe sequences were internal

Table 1
Commercially available kits for GMOs (AOAC International, 2003)

Company	Name of kit	Primary matrices
Applied Biosystems	Taq Man® GMO 35S detection kit	Processed foods, flour, meal, seeds, grain, plant tissues
Biote Con Diagnostics (Germany)	Bt-176 maize	Corn
Envirologix	Quali Plate kit for Roundup Ready corn, Event 603 and corn	Roundup Ready corn, RR cotton
Hong Kong DNA Chips Ltd	GMO Watcher – 1.0	GM ingredients, unprocessed foods
Investigen Inc.	Commodity Check PCR based GMO detection kit	Soy, corn, potato, canola in raw, processed, intermediate food products
Promega Corporation	Wizard Magnetic DNA Purification system for food	Corn, soybean, cornmeal, cornstarch, soy flour, cornflakes
Dupont Qualicon	Bax® for screening Qualitative GMO	Food
Scil l Diagnostics Gene Scan	GMO *IdentKit* Liberty Link™ T 25 corn	Plant tissues, seeds, meals, flours, processed/ unprocessed foods and feeds, food additives
Scil Diagnostics/Gene Scan	GMO *Ident Kit* Roundup Ready™ soya	Plant tissues, seeds, meals, flours, processed/ unprocessed foods, feeds, unrefined oils
Teprel Bio Systems Ltd	Bio Kits DNA Extraction kit (GM foods)	Raw, processed or intermediate food products
Strategic Diagnostics	GMO Check Food Ingredient testing	Soybean flour

Source: http://www.aoac/testkits/gmo.html.

to the sequence of the 35S promoter and NOS terminator. Two different probe immobilisation methods were characterised to improve the performances of piezoelectric crystal DNA sensor for GMO detection: (a) thiol–dextran–streptavidin–biotin procedure and (b) thiol-derivatised probe and blocking thiol procedure. The system was optimised using synthetic oligonucleotides while probe immobilisation was monitored by a surface plasmon resonance (SPR) system.

This same group described the development of a DNA piezoelectric sensor based on the immobilisation of single-stranded DNA probes on to the surface quartz crystal microbalance (QCM) device. QCM devices are simple and may consist of a quartz surface to which gold electrodes are attached. Fig. 2 shows a typical QCM device. The system, which was identical to that described above, was applied to samples of plasmidic and genomic DNA isolated from pBI 121 plasmid, certified reference materials and real systems which were amplified by PCR (Mannelli, Miunni, Tombelli, & Mascini, 2003b). Analytical parameters such as sensitivity, reproducibility and lifetime were investigated and were found to lead to sensitive and specific detection of GMOs. This method has been proposed as a useful tool for screening of food samples.

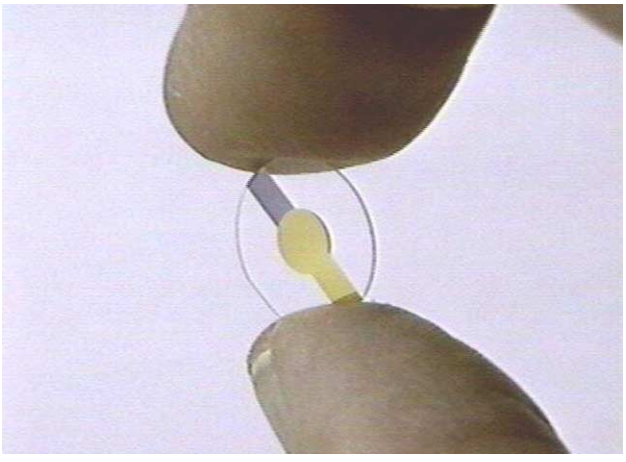

Fig. 2. Quartz crystal microbalance (QCM) device. (Reprinted by permission of Dr. Shakour Ghafouri, University of Toronto.)

Surface plasmon based-sensors are now starting to find use in detection by biospecific analysis (BIA). In one approach, biotinylated multiplex PCR products from non-transgenic maize as well as maize powders containing 0.5% and 2% genetically modified Bt-176 sequences were immobilised on different flow cells of a sensor chip (Feriotto, Gardenghi, Bianchi, & Gambari, 2003). After immobilisation, different oligonucleotides probes recognising maize zein and Bt-176 sequences were injected and the results were compared with Southern blot and real-time PCR analyses. The authors reported that sequential injections of Bt-176 and zein probes to sensor chip flow cells containing multiplex PCR products allow discrimination between PCR performed using maize genomic DNA containing 0.5% Bt-176 sequences and that performed using maize genomic DNA containing 2% Bt-176 sequences. It was concluded that SPR-based biosensors are comparable, in efficiency, to real-time PCR and more reliable than Southern blotting procedures. Additional advantages of an SPR system include: (a) the assay could be repeated several times on the same immobilised product after washing and regeneration and (b) by using stirred micro-cuvettes than flow-based sensor chips, costs and significantly reduced.

SPR-based biosensor technology was also used for the detection of Roundup ready soybean gene

sequences. Single-stranded biotinylated oligonucleotides containing lectin and Roundup Ready gene sequences were immobilised on sensor chips. The efficiency of hybridisation to different-length probes was determined. This was followed by a study of immobilised biotinylated PCR products from non-transgenic soybeans (containing only the lectin gene) and from genetically modified RRS (Feriotto, Borgatti, Mischiati, Bianchi, & Gambari, 2002). It was found that 13 and 15-mer oligonucleotides were suitable probes to detect RRS gene sequences while 11-mer probes were inefficient. All SPR-based formats were useful for the detection of RRS gene sequences in real-time formats without the need for radioactive protocols.

3.3.2. Microarrays and chip-based approaches

DNA microarrays exploit an ordered two-dimensional presentation of bio-recognition probe entities, fluorescence tagging of targets and scanning confocal imaging of the recognition-target complex or hybridisation product (Deisingh & Guiseppi-Elie, in press). Microarrays possess the advantages of throughput and speed of analysis which allow them to have potential for nucleic acid analyses. These systems are made from glass, silicon or plastic supports and are usually composed of thousands of reaction zones onto which individual oligonucleotides have been deposited. This will result in densities of up to 10^6 sites/cm^2 in a typical 1–2 cm^2 chip (Wang, 2000).

A proposal for the analysis of GMOs by the use of DNA arrays has been reported. As described, DNA microarrays can allow several DNA sequences to be analysed in parallel. This can prove useful in GMO analysis as more GMO traits are being approved, e.g., with maize, more than a dozen transgenic events are approved in the United States. DNA array technology will be utilised to simultaneously determine the presence or absence of the whole panel of relevant genetic modifications (Lauter, 2001). The use of arrays followed by the subsequent quantification of specific GMO positives will allow for rapid and economical screening of GMO events. However, it has to be noted that DNA microarrays generate lots of data which requires skilled personnel to interpret. This is probably one of the reasons for the lack of a concerted effort to use this technology as a routine method.

A commercially available microfluidic capillary electrophoresis system (Lab Chip™, Agilent Technologies) was used to detect GMO presence in food (Birch, Archard, Parkes, & McDowell, 2001). This work compared the traditional agarose slab gel electrophoresis method combined with digital image analysis with the Lab Chip™ system for the quantitative analysis of PCR products. The authors reported that the microfluidic system offered superior quantification, objectivity and ease of use.

Finally, a group of Chinese researchers has developed an accurate DNA chip method to quickly identify GMOs (Miao et al., 2003). Foreign elements such as promoters, terminators and selective marker genes in samples of maize, canola and cotton were detected. They have indicated that the method is comparable to those obtained from PCR.

3.4. Other methods

Other methods include the use of electrophoresis in tandem with other techniques and X-ray fluorescence.

3.4.1. Electrophoresis

A microfabricated, inexpensive, reversible glass capillary electophoresis chip and a laser-induced fluorescence system were developed for the DNA-based analysis of GMOs. The 35S promoter sequence of CaMV and the terminator of the NOS gene from *A. tumefaciens* were detected (Obeid, Christopoulos, & Ionnou, 2004). The detection of a GM soybean in the presence of unaltered soybean was used as a model system and lectin was also detected. The chip was made up of two glass plates (25 mm × 76 mm) which were thermally bonded to form a closed structure. Photomasks with cross-topology were prepared by using polymeric material rather than the standard chrome plates as these allow t to be more rapidly prepared. The widths of the injection and separation channels were 30 and 70 μm respectively with the effective separation length being 4.5 cm. The glass side was etched to a depth of 30 μM. The researchers reported that the separation and detection of PCR amplified sequences were completed in less than a minute with the limit of detection being 0.1% GMO content. It is interesting to note that only 25 ng of extracted DNA were required as starting material, this corresponding to just 20 genome copies of GM soybean (Obeid et al., 2004).

In another study using capillary electrophoresis with laser-induced fluorescence, Spanish investigators reported the quantitative of transgenic Bt event-176 maize (Garcia-Canas, Cifuentes, & Gonzalez, 2004). The method involves the use of co-amplification of specific DNA maize sequences with internal standards using competitive PCR (QC-PCR). The products of PCR are analysed using capillary gel electrophoresis with detection by laser-induced fluorescence (LIF). The combination of CGE and LIF allow the use of internal standards differing by only 10 bp from the original target fragments. According to the authors, this is probably the smallest size difference yet reported and should be seen as a major advancement in technology. Another common issue is that there are calibration problems involving the initial DNA concentration. In this paper, the authors overcame the difficulty by utilising a spectrophotometric procedure using ROX

reference dye which enhances the accuracy of the quantitative analysis of QC-PCR. Reproducibility of analysis times and corrected peak areas (measured as target/competitor PCR products ratio) were determined to be better than 0.91% and 1.93% (RSD, $n = 15$) respectively over three different days. Additionally, CGE-LIF provides better resolution and a signal: noise ratio improvement of about 700-fold over slab electrophoresis. This approach allows accurate quantitative of multiple GMOs in one run.

3.4.2. X-ray fluorescence

Wavelength-dispersive X-ray fluorescence (WD-XRF) has been proposed for the determination of phosphorus in GMO food samples (Jastrzebska, Brudka, Szymanski, & Szlyk, 2003). Materials tested included commercially available transgenic, unmodified soya-foods and common dairy products. A comparison of the WD-XRF and standard molybdenum blue methods was carried out. Matrix effects, which are common in XRF, were reduced by use of standard reference materials. The authors have suggested that the WD-XRF approach may be superior to the standard technique with respect to accuracy and that it may be used for routine food analysis. This is because XRF offers several advantages such as simple spectra, the method is non-destructive, analyses may be performed on any sample size and multi-element analyses are completed in a few minutes. However, it must be noted that the instrumentation is expensive and sensitivity is only in the ppm range.

4. Concluding remarks

This review considered the various approaches which have been used to detect the presence of genetically modified organisms in food products. Emphasis was placed on PCR methodologies as these are most widely used and several variations on the standard PCR have been proposed. PCR is highly sensitive and specific but it is not without its problems such as the lack of differentiation between GMOs and the natural occurrence of transgenic elements. These issues were discussed in detail in the relevant section. Several new methods are currently being used or are being developed to produce rapid detection methods. These include the use of biosensors, microarrays, chip-based systems and the use of capillary electrophoresis combined with laser-induced fluorescence. All of these were critically reviewed in this paper.

The main aim of developing these methods is to increase food safety especially with the furore over the presence of GMOs. All foods contain DNA which is ingested in reasonable quantities. In humans, dietary intakes of RNA and DNA are typically in the range of 0.1–1.0 g per day (Doerfler & Schubbert, 1997). Concerns over the presence of novel DNA in a GM food consumed must consider that this DNA would represent less than 1/250,000 of the total amount of DNA consumed. According to the World Health Organisation (WHO, 2000), considering this factor and the digestibility of dietary DNA, the probability of the transfer of genes from the GM plants to mammalian cells is very low. However, the possibility of this event occurring must still be considered. Many experiments have been conducted to evaluate the possibility of gene transfer, but generally the results have been inconclusive. Thus, it is very important to develop rapid, accurate methods of detection.

Recent developments in the industry are also noteworthy: Monsanto recently decided not to commercialise its Roundup Ready™ wheat while Bayer also dropped plans to commercialise Chardon LL™, its GM wheat, in the UK (Murphy, 2004). These decisions have been hailed as victories by the anti-GM movement while the companies involved indicate that it had to do with lack of commercial success. However, regardless of which direction the debates go, it will still be imperative to have an arsenal of rapid detection methods available. This is especially important from a regulatory standpoint where there is a need for detection methods that can distinguish between approved and non-approved GMOs and for the quantification of GMO content (European Union, 2003).

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