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TRACEABILITY AND DETECTION OF GENETICALLY MODIFIED ORGANISMS IN THE LABELLING OF FOOD PRODUCTION CHAIN. UE DIRECTIVES AND MOLECULAR APPROACHES

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Abstract

The application of modern biotechnology to food and plants is currently the focus of intense public and political debate with particular reference to the issue of food safety. This untenable situation has led to the completion by the European Union of a new regulatory framework for GM products, centred on the requirements of traceability and labelling.

Under this regulatory framework the significance of adequate molecular methods becomes more and more apparent. In this paper we will discuss about the EU legislation and we will stress the attention to the molecular techniques, either genomic and proteomic ones, indispensable for the GMOs detection.

Key words: Labelling, Traceability, Detection methods, Regulation, DNA analyses, Protein analyses.

Introduction

For centuries cross-breeding techniques have been used to modify or improve the quality, yield and taste characteristics of food. Those plants and animals with the most desirable characteristics, caused by naturally occurring variations in their genetic makeup, were chosen for food production and for breeding the next generations.

Now, with new technology, it is possible to identify and transfer particular characteristics of living organisms and alter them in a specific and direct way. By introducing a new segment of genetic material coming from other living organisms,

whether plant, animal or microbe, the resultant plant or animal is what is called “a genetically modified organism” or GMO.

Genetically modified organisms (GMOs) can be defined as organisms in which the genetic material (DNA) has been altered in a way that does not occur naturally by mating or natural recombination. The use of "recombinant DNA technology" or "genetic engineering" allows selected individual genes to be transferred from one organism into another, sometimes between non related species. The European Union defines Genetically modified organism (GMO) an organism in which genetic material (DNA/RNA) has been altered in a way that does not occur naturally by mating and/or natural recombination [European Council, (1990)].

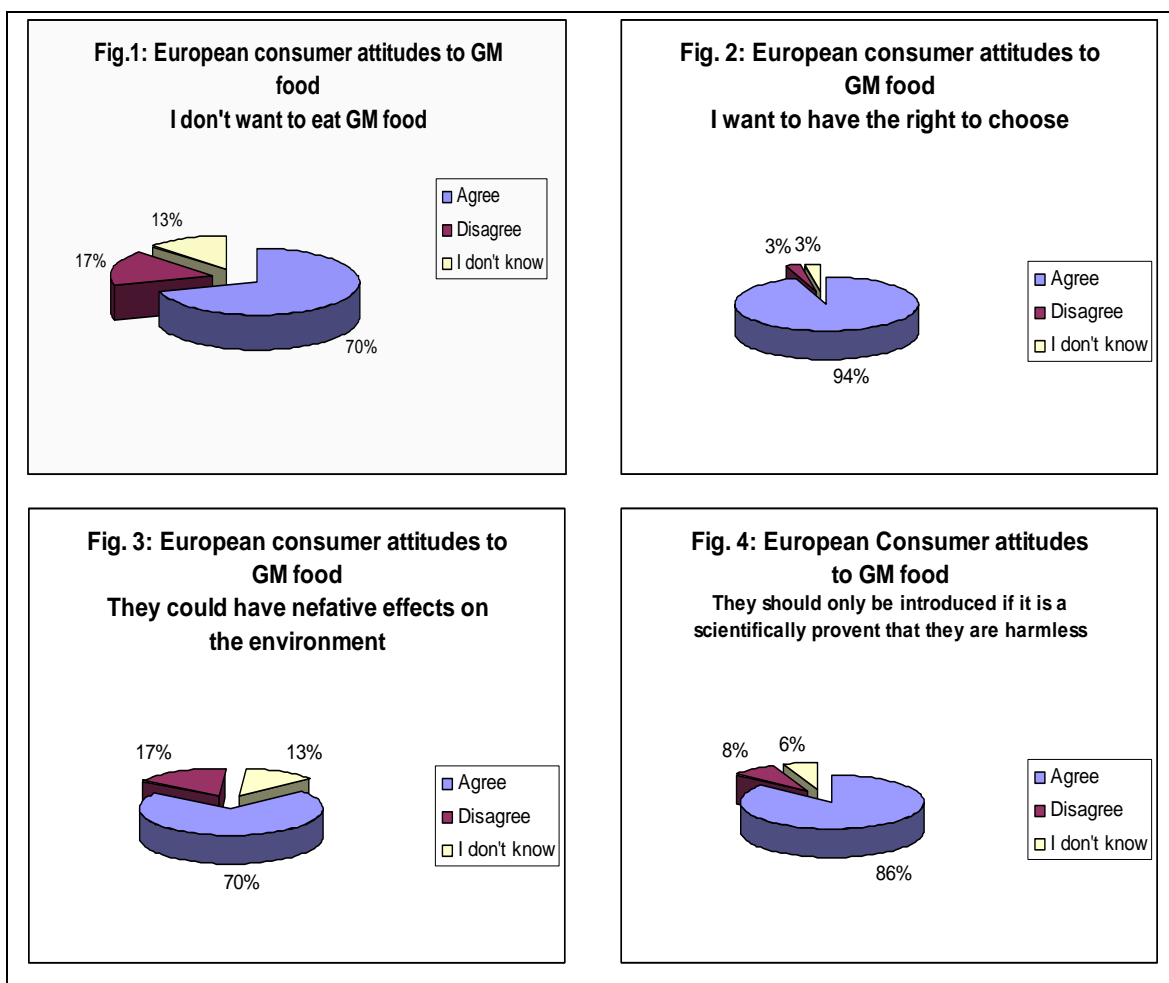
The deliberate release of genetically modified organisms into the environment and the application of modern biotechnology to food and plants is currently the focus of intense public and political debate with particular reference to the long-term effects on the environment and also the issue of food safety.

European consumers remain sceptical about GMOs, especially when it comes to their own foods (fig.1, fig.2, fig.3, fig.4) [European Opinion Research Group, (2001)].

Continued surveys of the European Unions Eurobarometer and other sources show a constant rejection of GM foods, even though confidence in benefits arising from genetic engineering technologies seems to increase in other areas.

Consumers are concerned about the long-term consequences of GM particularly given its irreversibility and are worried that enough is not yet known about the implications of its use. They expect there to be a rigorous approval procedure in place and they expect to be able to choose whether or not to accept the technology and its products.

For these reasons in Europe legislation on GMOs is in place since the early 1990s and has been extended and refined over time. This evolving process is set to continue.



Source: Eurobarometer 55.2, December 2001

Two new EU regulations, 1829/2003/EC [European Parliament and Council, (2003a)] and 1830/2003/EC [European Parliament and Council, (2003b)], dealing with the authorization of genetically modified food and feed, and the traceability and labelling of genetically modified organisms in food and feed products, have been published by the European Commission in November 2003.

These regulations will substantially change the rules and practicalities of labelling genetically modified organisms (GMOs) in products for human consumption and animal feed.

New labelling rules

The Regulation EC No 1829/2003 puts in place a centralised, uniform and transparent EU procedure for all applications for placing on the market, whether they concern the GMO itself or the food and feed products derived there from.

The new GM food and feed regulation provides a harmonised procedure for the scientific assessment and authorisation of GMOs and GM food and feed. The assessment procedure is centralised with the European Food Safety Authority (EFSA) taking responsibility for undertaking this process.

The Regulation requires labelling of all GM food and feed, which contain or consist of GMOs or are produced from or contain ingredients produced from GMOs regardless of the presence or absence of GM material in the final food or feed. This is an extension to the previous labelling rules which were only triggered by the demonstrable presence of GM material in the final product. With the new threshold, for example, the use of genetically Roundup Ready[®] Soya above the 0,9% threshold for production of refined oil requires labelling of the end product, even if the DNA has been completely destroyed during the transformation process. The same situation holds true for products like corn flakes, tomato ketchup, starch or lecithin. In such cases, evidence for the presence of GM crops in the production line can be achieved only by implementation of a traceability system (see next paragraph) monitoring the use of genetically modified crops throughout the entire food chain, from the raw material up to the final product available in retail outlets. Within the framework of the traceability system, the absence of GMOs above the labelling threshold needs to be thoroughly documented at all stages of the process. Details on the labelling rules can be found on the table 1.

The regulation does not cover food and feed produced 'with' a GMO. The determining criteria are whether or not material derived from the genetically modified source material is present in the food or the feed. Processing aids, which are only used during food or feed production process, are not covered by the definition of food or feed and therefore, are not included in the scope of this regulation, and therefore products produced with processing aids do not need to be labelled. For example there is an enzyme, or protein called chymosin, which is used to make cheese from milk. Prior to the development of the GM method, chymosin was obtained by scrapping calves stomachs. It is now produced in a much "purer form" from bacteria that have altered to produce large amounts of chymosin from natural sources. Since only the bacteria used to produce the chymosin and not the chymosin itself has been genetically modified then such products would still not need to be labelled under the new reforms. Food or feeds that are manufactured with the help of a GM processing

aid are also exempt from this regulation. As a result, products obtained from animals fed GM feed are not subject to authorisation or labelling requirements.

Table 1: Examples of labelling requirements under EC Regulation No. 1829/2003 for authorised GMOs (updated March 2004)			
GMO-type	Hypothetical Examples	Labelling under new regulation	Labelling under past regulation
GM plant	Chicory	yes	Yes
GM seed	Maize seeds	yes	yes
GM food	Maize, soybean sprouts, tomato	yes	yes
Food	Maize flour		
Food produced from GMOs	highly refined soya oil, glucose syrup from maize starch	yes	no
Food from animals fed GM animal feed	Meat, milk, eggs	no	no
Food produced with help from a GM enzyme	Cheese, bakery products produced with the help of amylase	no	no
Food additive/flavouring produced from GMOs	Highly filtered lecithin extracted from GM soybeans used in chocolate, Vitamin B2 (Riboflavin)	yes	no
GM feed	Maize	yes	yes
Feed produced from a GMO	Corn gluten feed, soybean meal	yes	no
Feed additive/flavouring produced from GMOs	Vitamin B2 (Riboflavin)	yes	no

Source: European Commission, 2002

The new labelling threshold has now been set to:

- 0,9% for GMOs having successfully passed all authorization stages including a full risk assessment and final approval by the respective national and European Food Safety Authority (EFSA);
- 0,5% tolerance for all those GMOs, for which the risk assessment has been finalized, but final approval for authorization in Europe has not yet been granted, so not yet authorised but that has a favourable assessment from an EU scientific committee or EFSA;
- zero tolerance has been adopted for GMOs for which the risk assessment is ongoing and those for which authorization is not applied for.

Products from numerous GMOs can legally be marketed in the EU¹. These are in particular:

- one GM soy and one GM maize approved under Directive 90/220/EEC prior to the entering into force of Regulation (EC) No 258/1997 on novel foods.
- processed foods derived inter alia from seven GM oilseed rape varieties, four GM maize varieties and oil from two GM cottonseed varieties, which have all been notified as substantially equivalent in accordance with Article 5 of Regulation (EC) No 258/1997 on novel foods.
- more recently, Bt 11 sweet corn and NK603 maize have been approved under Regulation (EC) No 97/258 on novel foods on 19 May and 26 October 2004 respectively.

Further applications for the placing on the market of food products have been introduced in accordance with the normal authorisation procedure provided for in Article 7 of Regulation (EC) No 258/1997 and the new Regulation (EC) No 1829/2003. More precisely, numerous GM foods are currently pending at different stages in the authorisation procedure. This mainly concerns products derived from GM maize, sugar beet and soybean².

The European Union's new regulation on food and feed labelling is the strictest in the world. International rules for the labelling of GM foods vary considerably between nations. Some countries are in the process of discussing legislation, some have had mandatory laws in place for several years, and others such as Canada have opted for a voluntary labelling regime (See table 2) [Agrifood Awareness Australia, (2004)]

These differences in national regulations and lack of international harmonization regarding labelling of foods derived from biotechnology could cause barriers to trade; the Codex Committee on Food Labelling is currently working on a standard for the labelling of GMO food and the *“proposed draft guidelines for the labelling of food obtained through certain techniques of genetic modification/genetic engineering:*

¹ For genetically modified (GM) food authorised in the EU under the Novel Food Regulation (EC) No. 258/97 see: http://europa.eu.int/comm/food/food/biotechnology/authorisation/258-97-ec_authorized_en.pdf
For GMOs authorised for feed use in the EU in accordance with Directives 90/220/EEC and 2001/18/EC see: http://europa.eu.int/comm/food/food/biotechnology/authorisation/2001-18-ec_authorized_en.pdf

² For applications for authorisation of genetically modified food and feed submitted under Regulation (EC) No 1829/2003 on genetically modified food and feed see: http://www.efsa.eu.int/science/gmo/gm_ff_applications/catindex_en.html

labelling provisions” [Codex Alimentarius Commission, (2004a)] will be discussed in the next meeting that would be held in Malaysia, from 9 to 13 May 2005.

Table2: International GMO labeling regulations and thresholds

Country	Labeling	% Threshold	Scheme *
European Union (25)	Mandatory	0.9%	GM
Russia	Mandatory	0.9%	GM
Australia / New Zealand	Mandatory	1.0%	GM
Brazil	Mandatory	1.0%	GM
China	Mandatory	1.0%	GM
Israel	Mandatory	1.0%	GM
Saudi Arabia	Mandatory	1.0%	GM
Switzerland	Mandatory	1.0%	GM
South Korea	Mandatory	3.0% ^a	GM
Indonesia	Mandatory	5.0%	GM
Taiwan	Mandatory	5.0%	GM
Thailand	Mandatory	5.0%	GM
Japan	Mandatory	5.0% ^a	GM
USA	Voluntary	5.0%	non-GM
Canada	Voluntary	5.0%	non-GM
South Africa	Proposed Voluntary	1.0%	non-GM
<p>*GM scheme requires identifying the existence of GM content. Non-OGM scheme labelling identifies the absence of GM content – e.g. "Does Not Contain GM Ingredients", "Free of GMOs" or “GMFree”. ^aTop three ingredients in Japan and top five ingredients in South Korea.</p>			

Source: Modified from Agrifood Awareness Australia, June 2004

Traceability

Products which consist of GMOs or which contain GMOs and food products derived from GMOs, which have been authorised, are subject to traceability requirements in application of Regulation (EC) No 1830/2003.

Traceability is not a new concept. In the 1994 the International Standard Organization in the ISO 8402 [International Standard Organization, (1994)] standard define it as “*the ability for the retrieval of the history and use or location of an article or an activity through a registered identification*”; in the ISO 9000:2000 [International Standard Organization, (2000)] traceability is defined as “*the ability to trace the history, application or location of that which is under consideration*”.

In the food context a clear definition of traceability is put forward at European level in the Regulation (EC) No 178/2002 [European Parliament, (2002)] where it is defined as “*the ability to trace and follow a food, feed, food-producing animal or substance intended to be, or expected to be incorporated into a food or feed, through all stages of production and distribution*”.

Codex Alimentarius, at its meeting in July 2004, in Geneva, proposed that the following definition of traceability be added to its standard: *“Traceability/Product tracing: the ability to follow the movement of a food through specified stage(s) of production, processing and distribution”* [Codex Alimentarius Commission, 2004b].

The traceability concept is not a new concept even for GMO sector, in fact the Directive 2001/18 clearly introduced the principle of traceability for GMOs, requiring that Member States ensure traceability at all stages of the placing on the market of GM products. In this regulatory framework, provisions for traceability are seen as a valuable tool for environmental and health protection and monitoring, while they are regarded as an important prerequisite for labelling, as well as for withdrawal in case of an unexpected adverse effect to human health or to the environment. However, the Directive does not provide details on definitions, target, and procedures for implementation of traceability in the GMO sector, but it calls for a horizontal Regulation on traceability to complement the Directive [Miraglia M., (2004)] These relevant aspects of traceability are detailed and clarified in the Regulation 1831/2003 that lays down a harmonised Community framework for tracing and identifying GMOs, as well as food products and feed derived from GMOs, at all stages of their placing on the market through the production and distribution chains.

According to the regulation operators placing on the market an authorised GMO are obliged to inform in writing receiving operators about the fact that the product contains or consists or is produced from GMOs, and of the unique identifiers assigned to these GMOs. This information must be transmitted to any subsequent operator receiving the product. Records of any such transactions must be kept by the operators for a period of five years.

As a prerequisite for its authorisation any GMO requires a "unique identifier", details of the concept of these unique identifiers have been established by the Commission [European Parliament, (2004)].

The traceability system should allow all the sector operators to rely on information from the previous operator(s). In general, the following reasons for the establishment of a traceability system for GMOs can be identified:

- to possibly withdraw products in the event of an unforeseen problem arising from consumption of material from GMO origin and if there is a risk to human health ;

- to facilitate the identification and monitoring of unintended and long-term effects on human health, where appropriate;
- to assist the control of labelling (even if traceability and labelling have different objectives they can be linked to complement one another. As an example, a traceability system could carry information to be used for labelling or in the other way, traceability for products derived from GMOs may facilitate control of labelling of such products) [Codex alimentarius Commission, (2001)].

The fundamental objective is to restore consumer trust through providing information and choice. Traceability of foods and food ingredients, including imported foods, also requires, however, the establishment of international systems allowing traceability of traded foods.

Establishment of an international system that allows to trace back to the origin and to understand the distribution of foods would depend on agreement on at least three of the system's elements:

- 1) each product must have a unique identifier (a bar code, lot identification number, or container identification marking in case of commodities);
- 2) guidance must be given on what specific information is recorded;
- 3) and all points in the production and distribution chain at which this information is recorded must be reliably linked.

Audits for verification of the implementation of the system are also required.

The concept and the meaning of traceability of GMOs is also discussed at the WHO/FAOs joint *Codex Alimentarius*, where an *Ad Hoc Intergovernmental Task Force On Foods Derived From Biotechnology* had been established in 1999 and presented its recommendations to the Meeting of the Codex Plenary in Rome (June 30 - July 7th 2003) [Codex Alimentarius Commission, (2003)]. No agreement has been reached so far on traceability, while an agreement on minimum standards for health risk assessment was adopted. These principles are to be based on pre-market assessment, performed on a case-by-case basis including an evaluation of both direct effects from the GMO and any unintended effects. Although these Codex principles would not have a binding effect on national legislation, they could be used as a reference in case of trade disputes. Present US regulations may not fully confirm with these standards, especially as regards pre-market testing.

Detection of GMOs in the food production chain

Prerequisites for the implementation of the new labelling and traceability provisions include the establishment of systems for documenting the distribution of individual GM crops in the food chain, as well as analytical methods for verification of this information.

A very important part of the accuracy of the quantitative detection of GM crops in diverse foods, including commodities and processed foods, depends to a large extent on the sampling protocol.

Since in most cases, GMOs are non-uniformly distributed in the bulk food, the variance of GM content across samples will likely represent the major contribution to the overall variance observed in quantitative detection. In general, sampling strategies have to take a wide variety of parameters into account, such as the nature of the sample for analysis, including the proportion of the different GM crops and non-GM crops and their distribution in the bulk. Consequences of error in sampling procedures and hence quantitative assessment of the GM content of foods or commodities can lead to false negatives and false positives: product lots above the GMO-content threshold may enter markets unlabelled, and product lots where adventitious contamination results in a GM content below the specified threshold may need to be labelled. Therefore, the adoption of reliable sampling procedures and the definition of the error related to the sampling procedures are very relevant for all parties involved [European Commission, (2004)].

Diverse sampling plans are currently available for bulk products with only some of them specifically designed for GM crops; The European Commission has now published Recommendation 2004/787/EC that is a technical guidance required under Article 9 of Regulation 1830/2003. It sets up a co-ordinated approach to inspections and control measures including sampling and testing (qualitative and quantitative) for GMOs, and food and feed material produced from GMOs, in products where EU marketing authorisation has been obtained [European Commission, (2004)].

Moreover, appropriate reference materials for positive and negative controls provide the basis for the validation of analytical procedures and for assessing the performance of methods and laboratories. Reference material should be independent of the analytical methods and should be focused on raw material or base ingredients rather than on finished foods. Each GMO requires specific reference material. Grains, altered DNA or expressed proteins have all been used as reference material. If grains

are used, they should realistically mimic real-life test material (i.e. have matrix effects and consistency similar to test grain samples, be of consistent quality over a long period of time, and must account for specific sample homogeneity, GMO content and stability) [Yates K., (1999)]. In a few cases, both genomic and plasmid DNA have been used as reference material; the former being more realistic in terms of matrix effect, whereas the latter is easier to prepare in large quantities and might provide greater consistency. In contrast to protein detection methods, in which a single standard can be settled on relatively easy, DNA-based methods are better served through combinations of several positive controls. The availability of reference materials is currently limited owing to concerns over intellectual property rights and costs [Serageldin I., (1999)]. The Institute of Reference Materials and Measurements at the Joint Research Center in Geel, Belgium, offer a limited number of reference materials [through Fluka (Buchs, Switzerland)] for modified soya, corn and maximizer maize (MM).

Either the sampling either the reference material are the basis of the method for a good and a entrust GMOs detection in food. The detection of GM crops represents a relatively new area of diagnostics; public and private sector engagement in this area of research has increased rapidly since the implementation of the first labelling requirements based on the detectability of recombinant DNA and proteins from GM crops. Rapid high-throughput methods for DNA and protein methods based on micro-array technologies are being developed. Prerequisites for the development of reliable detection methods are: access to information on all globally marketed GM crops, their inserted recombinant DNA sequence, and reference materials, as well as international co-ordination and interinstitutional ring-testing for the methods' validation. Methods for the detection of GM crops in commodity shipments or processed foods rely on identifying recombinant DNA or recombinant proteins (see Figure 2). Protein-based detection methods have been developed and validated only for very few GM crops. Principally, many methods that focus on fractionation, separation, and profiling of proteins and peptides, such as iso-electric focusing, affinity chromatography, and one- or two-dimensional separation approaches, might be applicable to the characterisation of GM crops when compared to the parental non-transgenic line.

Immunoassay technologies with antibodies are ideal for qualitative and quantitative detection of many types of proteins in complex matrices when the target analyte is known (Brett G.M., 1999). Both monoclonal (highly specific) and polyclonal (often

more sensitive) antibodies can be used depending on the amounts needed and the specificity of the detection system (e.g. antibodies to whole protein or specific peptide sequences), depending on the particular application, time allotted for testing and cost. On the basis of typical concentrations of transgenic material in plant tissue (>10 mg per tissue), the detection limits of protein immunoassays can predict the presence of modified proteins in the range of 1% GMOs (Stave J.W., 1999). Both western blot and enzyme-linked immunosorbant assay (ELISA) techniques have been used for the analysis of protein products of Monsanto's transgenic Roundup Ready soybean (RRS), which is resistant to the herbicide glyphosate and contains the gene encoding *Agrobacterium* spp. strain CP4-derived 5-enol-pyruvyl-shikimate-3-phosphate synthase (EPSPS) [Rogan G.J., (1999)]. Western blot analysis, however, is considered more suited to research applications than to routine testing and in general ELISA and Lateral Flow strip analyses are preferred. ELISA assumes more than one format: a microwell plate (or strip) format, and a coated tube format. The antibody-coated microwells are quantitative, highly sensitive, economical, provide high throughput and are ideal for quantitative high-volume laboratory analysis, provided the protein is not denatured. The typical run time for a plate assay is 90 min, and an optical plate reader determines concentration levels in the samples. Detection limits for CP4 EPSPS soybean protein was 0.25% for seeds and 1.4% for toasted meal [Yates, K., (1999)]. The antibody-coated tube format is suited for field-testing, with typical run times ranging from 15-30 min, and tubes can be read either visually or by an optical tube reader; results are qualitative. Because there is no quantitative internal standard within the assay, no extra information can be obtained concerning the presence of GMOs at the ingredient level in food.

A variation on ELISA, using strips rather than microtiter wells, led to development of lateral flow strip technology. Immobilized double antibodies, specific for the expressed protein, are coupled to a color reactant and incorporated into a nitrocellulose strip, which, when placed in a plastic eppendorf vial containing an extract from plant tissue harboring a transgenic protein, leads to an antibody sandwich with some of the antibody that is coupled to the colour reagent. This coloured sandwich flows to the other end of the strip through a porous membrane that contains two captured zones, one specific for the transgenic protein sandwich and another specific for untreated antibodies coupled to the colour reagent. The presence of only one (control) line on the membrane indicates a negative sample, and the presence of

two lines indicates a positive result (Fig. 2). The lateral flow format gives results in 5-10 min, is economical, more amenable to point-of-sale application, and is suitable as an initial screening method early in the food chain. These strips have been developed commercially to detect endotoxins expressed by the bacterium *Bacillus thuringiensis* that protect against insects, as in CryI(Ab) in corn plants, seeds and grain, in addition to CP4 EPSPS protein in soybean, canola, cotton and sugar beet [Lipton C.R., (2000)] [Fagan J., (2001)]. Commercially available lateral flow strips are currently limited to few biotechnology-derived protein-producing GM products, but strips that can simultaneously detect multiple proteins are being developed. In the near future, improvements in immunoassays are expected to occur via advances in antibody technology and improved instruments.

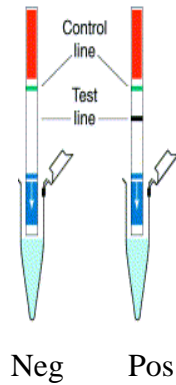
Unfortunately, the resolution is often not sufficient or resolved patterns are too complex to clearly distinguish a novel GM crop-derived protein from the protein pattern of its conventional counterpart. Several limitations are envisaged for quantitative determinations of protein-based methods. Since expression levels of introduced traits are tissue specific and developmentally regulated, protein levels in unknown samples hardly can be compared to those in the reference material used, and an accurate statement is only possible if sample matrices are identical to the reference material or if matched standard materials or standards that have been validated for the matrix are available. It seems therefore likely that the quantitative assessment of the GM crop component will be primarily done by DNA-based, rather than protein-based approaches.

DNA detection methods for GM foods rely on the complementarity of two strands of DNA double helix that hybridize in a sequence-specific manner. The DNA that has been engineered into a crop consists of several elements that govern its functioning. They are typically a promoter sequence, structural gene and a stop sequence for the gene. Although several techniques are available, two are commonly used: Southern blot and particularly PCR analyses [Sambrook J., Russel D., (2000)] [Ahmed F.E., (1995)]. Microarray-based technology for detecting gene expression is currently under development [Hertzberg M., (2001)]. At present, the most commonly used DNA based methods involve amplification of a specific DNA with the PCR technique. PCR exploits the specificity of DNA polymerase to allow the selective amplification of specific DNA segments occurring at low frequency in a complex mixture of other

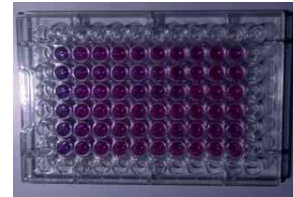
DNA sequences [Ahmed F.E., (1995)]. In a standard PCR test, two pairs of primers are used: (1) forward, sense or 5' → 3', and (2) reverse, antisense or 3' → 5'.

1. Protein-based GMO detection methods

Strip test

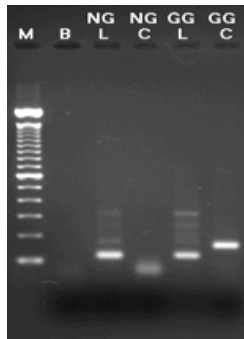


ELISA

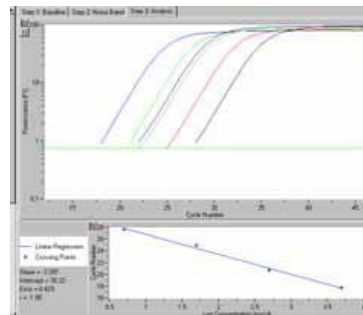


2. DNA-based GMO detection methods

Qualitative PCR



Quantitative PCR



Microarray

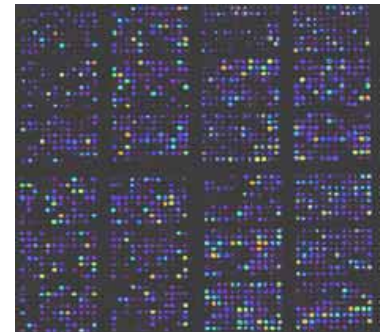


Figure 2. GMO detection methods.

These primers are designed to hybridize on opposite strands of the sequence of interest, and through a series of repetitive cycles, amplify the sequence between the primers millions of times. Amplified pieces can be subjected to agarose gel electrophoresis to separate amplified DNA according to size, although other separation methods, such as high performance liquid chromatography (HPLC) and capillary electrophoresis (CE), have been used [Ahmed F.E., (1995)] [De Palma A., (2001)]. Several food ingredients (e.g. soy, wheat, canola, potatoes, rice, maize, celery

and tomatoes) have been analyzed using PCR. A crucial rate-limiting step is DNA extraction and purification.

Detection limits are in the range of 20 pg to 10 ng of target DNA and 0.0001–1% of the mass fraction of GMOs [Yates, K., (1999)].

Real-time PCR is the most commonly used technology for (subsequent) quantification of the GM crop content. The amount of product synthesised during the PCR is measured in real-time by detection of the fluorescence signal produced as a result of the amplification. To circumvent some of the problems of conventional quantitative end-point PCR, a real-time Q-PCR was introduced [Heid C.A., (1996)]. In theory, production of PCR products should proceed exponentially. However, in practice it reaches a plateau between 30 and 40 cycles because certain reaction components become limiting. In conventional PCR, products of the reaction are measured at a single point in the reaction profile. Plotting the concentration of products present at this point as a function of the initial amount of DNA present in each of those reactions shows that proportionality between DNA concentration (dynamic range) and PCR products occurs over a limited range of DNA concentrations, leading to loss of precision in quantitation. However, it has been shown empirically that the concentration of DNA in real-time PCR reaction is proportional to PCR cycle number during the exponential phase of PCR (Ahmed F.E., 2000). Therefore, if the number of cycles it takes for a sample to reach the same point in its exponential growth curve is known, its precise initial DNA (then GMO) content can be determined. Real-time PCR also allows for detection of low copy DNA number. Several commercially available real-time PCR thermal cyclers automate the analytical procedure and allow cycle-by-cycle monitoring of reaction kinetics, permitting calculation of the target sequence concentration. A total of 179 food products containing GM (e.g. baby food and diet products, soy drinks and desserts, tofu and tofu products, cereals, noodles, fats, oils and condiments) were analyzed by TaqMan©. The method proved to be sensitive. Amplifiable soy DNA could not, however, be detected in fats, oils and condiments. ABI Prism 7700, employing TaqMan© in a relative quantitative PCR, detected 2 pg per gram of starting sample in 3h after DNA extraction [Vaitilingom M., (1999)]. Including a reference housekeeping gene as an internal standard, which will amplify in parallel with the gene of interest, provides quantitation in PCR-based assays.

The major procedures for detecting GMOs in food are summarized and compared in Table 3.

Table 3. Summary of methods that specifically detect OGM in food chain (Ahmed F.A., 2002).

Parameter	Protein based			DNA based		
	Western blot	Elisa	Lateral Flow strip	Southern blot	Qualitative PCR	Real Time PCR
Ease of use	Difficult	Moderate	Simple	Difficult	Difficult	Difficult
Needs special equipment	Yes	Yes	No	Yes	Yes	Yes
Sensitivity	High	High	High	Moderate	Very high	High
Duration	2d	30-90 min	10 min	6h	1.5d	1d
Cost/sample US\$	150	5	2	150	250	450
Gives quantitative results	No	Yes	No	No	No	Yes
Suitable for field test	No	Yes	Yes	No	No	No
Employed mainly in	Academic labs	Test facility	Field testing	Academic labs	Test facility	Test facility

Also, new methodologies for DNA-based detection of GM crops have been developed in the past few years, one of the most important developments being the use of the micro-array technology. This technique aims to eliminate the time-consuming gel electrophoresis step after the PCR, reducing the (considerable) risk of contamination during the handling of the samples at the same time. The main principle of the micro-array technology is miniaturisation. Standard molecular biological or other biochemical methods can be performed on a much large scale in much smaller volumes. This makes it possible to analyse samples not just for the presence of an individual or a selected group of transgenic or control genetic elements, but to extend the analysis to thousands of probes in a single hybridisation experiment. The basic idea is that (many) selected probes are bound spotwise in array format to a solid surface with each spot containing numerous copies of the probe. The array is subsequently hybridised with isolated DNA of the sample of interest that is labelled with a fluorescent marker. During the hybridisation phase the labelled fragments will associate with the spotted probes on the basis of complementary DNA sequences. The

larger the stretch of complementary sequences is, the stronger the bond will be. After the hybridisation phase, the remaining free labelled sequences, as well as the sequences that are only weakly attached to the probes, will be washed off and the array can subsequently be scanned for individual fluorescence intensity of each spot. Data analysis of the resulting patterns and relative intensities will reveal whether the patterns can be attributed to approved GM varieties. For protein-based detection methods, the microarray technology may also be an interesting alternative in (near) future times. In that case, specific antibodies or other types of selective proteins can be bound to the array and coupled to labelled proteins in the samples under investigation.

In recent years, mass spectrometrical methods based, for example, on the MALDI-TOF MS (matrix-assisted laser desorption/ionisation- time of flight mass spectroscopy) principle have gained more and more importance in analysis of larger biomolecules like proteins, but also oligonucleotides. In MALDI, the analyse is embedded in an UV absorbing matrix in vacuum on a carrier between electrodes. Subsequently ultraviolet laser light is applied. UV-energy is absorbed by the matrix and also carried over to the sample (polymer) such that it will be ionised. The ionised molecules move towards the oppositely charged electrode and enter the flight tube towards the detector. During the time of flight (TOF) until detection, the molecules are separated according to their mass to charge ratio. The technique has been successfully applied in genotyping of single nucleotide polymorphism (SNP) of genes. Since usually an amplification of the target by primer elongation is the preceding step, the analysis comprises of two steps where mass spectrometry is restricted to the final detection. Experience with GMO detection is not available yet, but a future application may be coupling of mass spectrometry with other techniques. Only few of the available detection methods have so far been validated in a collaborative trial according to international harmonised protocols (cf. EC-JRC, 2003). In future, methods will have to be harmonised internationally. Several European and worldwide collaborative trials have been organised and draft European standards by the Centre Européen de Normalisation (CEN) and international standards of the International Standards Organisation (ISO) are already available. The number of GM crops for which methods are available in these draft standards is very limited. In this context, the role of the European Network of GMO Laboratories will be crucial from an EU perspective.

A key issue in deliberations on international standardisation of methods will be the determination of criteria for method validation, method acceptance, and test reports. The starting point could be a currently constituted working group of Codex Alimentarius Commission (Codex Committee on Methods of Analysis and Sampling) where minimal requirements for the evaluation of data will be agreed. The recently adopted EU legislation on food and feed derived from GMOs addresses the need for reference materials and sequence data: applicants proposing to place a GMO on the market are required to supply reference materials and a detection method to the European Commission. However, the specifications on the types and quantities of materials required, the timeframe in which materials shall be made available, and the type(s) of detection methods requested are still being defined. At present, these issues are being discussed in various fora, including the European Network of GMO Laboratories. Validated methods to routinely detect or quantify the presence of unauthorised GM crops will not be available in the near future, especially since there is rarely access to sequence information and reference materials. To overcome this problem, deposition of sequence information and reference materials would need to be co-ordinated internationally.

At present, the lack of tools for detection of diverse GM crops released into the environment poses significant challenges for the food industry to comply with EU labelling legislation. Enforcement of the law is equally difficult. Furthermore, EU law requires separate registration of seeds in which two different traits obtained by genetic modification (such as insect resistance and herbicide tolerance) have been combined by breeding. Enforcement of this requirement would also require sampling detection methods that can distinguish between two traits that were stacked into one seed by breeding and two seeds of which each contains one trait. In the analysis of commodity shipments that may contain both types of seeds (single trait and stacked trait seeds), drawing such distinctions is impossible with current detection methods.

Regulatory compliance difficulties exasperate market risks from adoption of agricultural biotechnology, as food products that inadvertently contain unapproved GM crops (that were approved outside of the EU), or that have been inadvertently mislabelled, may need to be withdrawn from the market. Given uncertainties on who is liable and which of these types of damages are insurable, these laws have direct unfavourable repercussions on the adoption of agro-biotechnology in the agro-food chain.

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