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review

Molecular Markers for Food Traceability

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Summary

DNA analysis with molecular markers has opened a way to understand complex organism's genome. It is presently being widely applied across different fields, where food takes a preeminent position. Constant outbreaks of foodborne illnesses are increasing consumer's attention towards more detailed information related to what they are consuming. This overview reports on the areas where food traceability has been considered, and the problems that still remain to be bypassed in order to be widely applied. An outline of the most broadly used PCR-based methods for food traceability is described. Applications in the area of detection of genetically modified organisms, protected denomination of origin, allergenic and intolerance reactions are detailed in order to understand the dimension of the performed studies.

Key words: food traceability, DNA extraction, PCR-based methods, molecular markers, GMO

Introduction

Traceability/product tracing is defined by the Codex Alimentarius Commission as the ability to follow the movement of a food through specified stage(s) of its production, processing, and distribution (*1*). The same definition has been adopted by the European Union (EU) Regulation No 178/2002 including, besides food, 'feed, food-producing animals or substance intended to be, or expected to be, incorporated into a food or feed' (*2*,*3*).

The development of food traceability systems is becoming more demanding since consumers and producers are seeking more notable foods with high quality guarantee. As a consequence, food traceability is required by consumers and government organizations because it is a significant component of food safety. Consumers are interested in choosing what they are eating based on nutritional value and product origin, where labelling information has an important role (*4*). There is restricted legislation concerning food labelling, which has to be pursued, and according to each country's norms, it concerns several parameters, among them: (*i*) nutritional values, (*ii*) presence of genetically modified organisms (GMO), (*iii*) allergens (*e.g.* peanuts, milk, mustard or fish), and (*iv*) food additives.

GMO legislation varies among countries; according to some, any food containing material which consists of or is produced from GMO in a proportion higher than 0.9 % is obliged to be labelled (*5*), while other countries do not impose any labelling. Based on these considerations, it is important to have an efficient food control system capable of detecting GMO presence in countries where it is required.

Consumers have also increased their interest in food allergies and intolerances. Food allergies affect around 5–8 % of children and 1–2 % of adults, although self-reported food allergy is higher and runs at approx. 25 % of the population (*6*). The most effective way of preventing allergy episodes is through the avoidance of any allergen-containing food, which can only be achieved through a full information about the presence of any potential allergens in a given food product. Nevertheless, some food products are still not correctly labelled.

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There is great interest in gluten content in food, because many coeliac, an immune-mediated illness, patients do not tolerate cereals in their diet. Strict avoidance of gluten at levels that will elicit an adverse effect is the only means to prevent potentially serious reactions. Thus, consumers susceptible to coeliac disease need accurate, complete, and informative labels on food. Therefore, the Food Allergen Labeling and Consumer Protection Act (FALCPA) requires also that the Food and Drug Administration (FDA) promulgate a regulation to define and permit the use of the term 'gluten-free' on the food labels (*7*,*8*).

Food-producing companies consider traceability legislation as an additional production cost, but it could play an interesting commercial role in order to attract consumers' attention if it is directed to specific market niches linked to food safety and to high quality products with certified origin. Along with consumers' growing attention to these issues, each country is also interested in protecting its own quality products, increasing worldwide the regulation on certified products. The legislation, although tight, is still easy to circumvent, since most of the control measures used are ambiguous and lack the correct determination of the controlled parameters. As a result, the EU has recognized and supported the potential of differentiating quality products on a regional basis. In 1992, the EU Council Regulations No 2081/92 (*9*) and No 2082/92 (*10*) introduced an integrated framework for the protection of geographical indications and designations of origin for agricultural products and foodstuffs. Recently, both documents have been replaced by EU Regulations No 510/2006 (*11*) and No 509/2006 (*12*), respectively, and the EU Regulation No 1898/2006 (*13*) was added. The EU regulation allows the application of the following geographical indications to a food product: protected designation of origin (PDO), protected geographical indication (PGI) and traditional specialty guaranteed (TSG). The use of geographical indications allows producers to obtain market recognition and often a premium price. The false use of geographical indications by unauthorized parties is detrimental to consumers and legitimate producers. In order to fulfill all the requirements of the regulations mentioned above, the development of new and increasingly sensitive and accurate techniques for determining/detecting the presence of a particular component in food is highly desirable for consumers, producers, retailers and administrative authorities.

DNA has characteristics which make it an ideal biological tool: (*i*) it is present in most biological tissues, whereas proteins and other components may be tissue-specific, (*ii*) it can be amplified starting from very low quantities, and (*iii*) it is more stable than other molecules (*e.g.* proteins) towards physical and chemical procedures in processed food. Therefore, DNA is an interesting target for food control regarding traceability purposes. The DNA-based tests that have been more widely used in the authentication of species used in foodstuff production are real-time (RTi) and other polymerase chain reaction (PCR)-based methods, essentially due to the fact that they require a very small amount of sample, which can sometimes be a problem when using other detection methods.

Labelling control will increase in the near future, imposing the development of more accurate, cheaper and expedite systems for this purpose. In this way, new devices, as microchips, will have to be developed in order to control, in the same chip, several factors simultaneously (*e.g.* presence of GMO, allergens, and gluten). With the large genome sequencing programs and the development of chips with more information, it is expected that a system with multi-gene trace will exist, which will be able to trace all these occurrences in one single reaction.

DNA Extraction Protocols

Molecular marker technology can be applied for food traceability, although its wide application is limited mainly because DNA samples, suitable for PCR amplification procedures, are difficult to obtain. Some processed foods contain highly degraded DNA, which results from high temperature treatments and manipulation methods, and/or from the presence of PCR inhibitors. The DNA status may affect extensively the subsequent PCR amplification. A way to avoid these problems may be by improving DNA extraction protocols or PCR assay design and conditions (*14–16*).

DNA degradation is primarily linked to processes carried out at low pH and it increases significantly when performed in combination with thermal stress (*17–22*). Even more, DNA can be more fragmented when it is exposed to enzymatic hydrolysis (*23*,*24*) as in fermentation processes (*25*,*26*), or when it is subjected to grinding and milling processes (mechanical stress) (*27*), as it happens in olive oil extraction procedures (*28–30*).

Depending on the food matrices, the DNA extraction method needs to be optimized and scaled down until a suitable protocol is obtained. Several papers have been reported considering this issue, *i.e.* adapting DNA extraction protocols to food matrices (Table 1; *14*,*16*,*26*, *28*,*29*,*31–37*).

The extraction buffer most widely used in food products is based on hexadecyltrimethylammonium bromide (CTAB). However, every protocol has to be adapted according to each matrix. Nowadays, there are several DNA extracting commercial kits available on the market that have demonstrated to be appropriate for processed food (*14*,*21*,*38–42*). Nevertheless, these methods are still not always suitable for all food matrices that need to be adapted to each one in particular.

Apart from the DNA degradation, DNA extraction protocols have to account for the presence of PCR inhibitors (*15*). Several organic compounds found in food matrices, such as polysaccharides, fatty acids, polyphenols, oils and tannins (*43–46*) may inhibit/interfere with the PCR reaction. Standard protocols have been modified in order to avoid DNA contamination with these particular substances, combining specific extraction buffer with enzymatic treatments, isopropyl alcohol precipitations and several wash steps. During DNA extraction optimization, the purification steps seem to have an important role. In some cases, a simple step to avoid PCR inhibition can be achieved through a sample dilution, which is actually the most effective (*14*).

Table 1. DNA extraction protocols for different food matrices

Sample type	Extraction method	Ref.
biscuits	CTAB buffer	(*31*)
olive oil	CTAB buffer	(*28*)
	hexane	(*29*)
honey	CTAB buffer	(*32*)
must and wine	CTAB buffer and DNAzol®	(*16*,*26*, *33*)
flour-based products (biscuits, breakfast cereals, bread)	CTAB buffer	(*34*)
beer	CTAB buffer	(*34*)
rice-based drink	CTAB buffer	(*34*)
rice-based products (boiled rice, crackers and cakes)	SDS-based	(*35*)
refined vegetable oil	CTAB buffer	(*34*)
(sunflower, soya, maize)	NIAB and GENECLEAN®	(*14*)
chocolate	CTAB buffer	(*31*)
	Nucleon® phytopure kit	
soya milk	official Swiss method	(*36*)
potato and potato-derived products	CTAB buffer	(*37*)
	KingFisher® method	
	Wizard® method	

PCR-Based Methods

Microsatellites or single sequence repeats

Microsatellites are tandem repeated motifs of 1–6 bp, in tracts up to 10^2 bp, which have a frequent occurrence in prokaryotic and eukaryotic genomes. Single sequence repeats (SSRs) are distributed through the whole genome (although their distribution depends on the species and chromosome) and are flanked by highly conserved sequences (*47*). The high level of polymorphism is due to different number of repeats in the microsatellite region, therefore they can be easily and reproducibly detected by PCR. These markers are amenable to high throughput genotyping and have proven to be an extremely valuable tool for paternity analysis, construction of high density genome maps, mapping of useful genes, marker-assisted selection, and establishing genetic and evolutionary relationships (*48*). The detection of several alleles at a high frequency makes SSR markers an ideal tool for identifying specific species and in some cases one particular specimen in food. SSRs have been used to trace animal meat (*49–51*), olive oil (*30*,*52–57*), must and wines (*16*,*33*,*58–62*), blueberries (*63*), rice (*64*), cherry (*65*), tomato (*66*) and wheat (*67*,*68*), among others.

When dealing with food matrices, some constraints may appear concerning the application of molecular markers, mainly considering the reproducibility linked to the highly degraded DNA samples (*53*,*60*). Therefore, the use of SSR sequences that present lower allele size is recommended in order to achieve a more reliable result in food samples that present highly degraded DNA (*68–70*). Doveri and Lee (*14*) in their work with processed food verified that short fragments (less than 200 bp) amplified in all samples, while longer amplicons were dependent on the product and extraction methods.

Single nucleotide polymorphisms

Single nucleotide polymorphisms (SNPs) are the smallest unit of genetic variation and represent the most common type of sequence polymorphism in plant and animal genomes. SNP markers are biallelic (in diploids) providing an exact allele information. SNPs have been used to construct high-resolution genetic maps or to trace evolution, particularly in the human genome. SNP identification methods are based on expressed sequence tag (EST) data, on array analyses, amplicon resequencing, genome sequencing, and the use of next-generation sequencing technologies (*71*). When compared with other types of markers, such as restriction fragment length polymorphism (RFLPs), random amplified polymorphic DNA (RAPDs), amplified fragment length polymorphism (AFLPs) and SSRs, SNPs offer higher levels of genotyping throughput (*72*). SNP application for food traceability may offer several advantages, the most attractive one being the fact that they can be detected on a very small portion of DNA, which in the case of fragmented DNA may constitute an advantage. SNPs have been applied in food for traceability of olive oil (*29*,*53*) and meat (*73*). In the near future, microarrays based on SNP sequences may be widely applied for food traceability purposes.

Real-time PCR

Real-time PCR (RTi-PCR) is a powerful, specific, accurate, and sensitive technique for the quantification of nucleic acids by monitoring the increase of product through the fluorescence detection throughout the complete process (*34*). RTi-PCR allows the quantification of each particular constituent using extrapolation to a regression curve constructed with calibrators at known concentrations. The advantages of RTi-PCR include the reduction of time and artifacts, which is achieved by the elimination of gel electrophoresis, and the increase of specificity that is obtained by using probes.

Target sequences for plant RTi-PCR assays should be species-specific, conserved in different cultivars, present in low copy number, and should not be subjected to genetic manipulation (*34*). In the case of GMO detection, known genetically manipulated genes are targeted to detect and quantify the amount of contamination found.

There are several methods available for the development of RTi-PCR: (*i*) SYBR green (*74*,*75*), (*ii*) TaqMan (*74–76*), (*iii*) Amplifluor (*75*), (*iv*) molecular beacons (*74*), (*v*) minor groove binding (MGB) (*74*), (*vi*) Scorpion primers (*76*), and (*vii*) fluorescence (or Förster) resonance energy transfer (FRET) (*77*). Although several methods have been developed, the most commonly used is TaqMan (*78*). Doveri and Lee (*14*) developed crop-specific assays from primers designed using the 5S DNA spacers and amplified corresponding DNA samples using both conventional end-point and real-time PCRs. Furthermore, these assays enable the identification of maize, sunflower and soya in highly processed food matrices, mainly due to the high copy number of this specific region present. Pasqualone *et al.* (*79*) designed a RTi-PCR assay capable

of quantifying common wheat adulteration in semolina, allowing the detection of bread wheat in food products that should only have durum wheat like pasta and Altamura bread (PDO designation). Recently, a real-time PCR method has been developed for seafood products in order to authenticate European sole (*Solea solea*). The method was applied to 40 commercial samples to verify correct labelling, proving to be a useful tool for food label monitoring and regulation verification (*80*).

Other PCR-based methods

Several other PCR-based methods have been developed in order to have more discriminative markers for food control purposes. Ohtsubo and Nakamura (*35*) developed a set of 14 sequence-tagged sites (STS) that could discriminate among 60 dominant Japanese rice cultivars. They developed a primer set that was applicable for traceability purposes in several rice-based food products (boiled rice, crackers and cakes).

Application of PCR in the Food Sector

Genetically modified organisms

The EU legislation dictates the labelling of genetically modified food and feed products (*5*,*81*). According to the current directives, labelling is required for food products containing more than 0.9 % of authorized GMO. Quantification of transgenic DNA requires the analysis of species-specific reference genes that provide an estimation of the total amount of the DNA from that plant species; hence quantification can be calculated as GMO proportion. It is also important that these reference genes do not exhibit allelic variation, and ideally have a constant number of copies per haploid genome across different cultivars of the target species (*82*). Several species--specific reference genes have been obtained for the most relevant plant crops that have been submitted to genetic transformation. Usually, plant storage proteins are known to be highly species-specific and they are the target of various endogenous reference RTi-PCR assays (*e.g.* zein for maize, lectin for soybean, γ-hordein for barley) (*83*, *84*). However, several other unique genes have been used as reference; a brief summary is presented in Table 2 (*82*,*84–96*).

Table 2. Genes used as reference for a particular species in GMO determination

Gene	Target species	Ref.
γ-hordein	barley	(*84*)
ACC1		(*84*)
PKABA1		(*85*)
gos9	rice	(*84*)
oryzain β		
lectin		(*86*)
SPS		
helianthinin	sunflower	(*84*)
acetyl-CoA carboxylase	wheat	(*84*)
PKABA1		(*85*)
lectin	soya bean	(*87*)
le 1		(*88*)
β-actin		(*89*)
Sad1	cotton	(*90*,*91*)
Adh1	maize	(*92*)
invertase1		
zein		
Hmga		
acetyl-CoA carboxylase	rapeseed	(*82*)
Hmg I/Y		(*93*)
BnACCg8		
papain	papaya	(*94*)
CHY		(*95*)
LAT52	tomato	(*96*)

A classic GMO gene construct has at least three elements: (*i*) promoter element – start signal, (*ii*) gene of interest, and (*iii*) terminator element – stop signal. However, other elements can be present in the gene construct. Quantification of GMO content in food can be obtained by using promoter or terminator sequences, meaning that the target is not the event *per se*, which may generate false positives (*96*). However, this may give indication of the GMO presence mainly when the modification (gene of interest) is not known.

The first attempt of GMO food quantification was based on quantitative competitive PCR (QC-PCR). Nevertheless, with the development of RTi-PCR platforms, this method was replaced (*78*). In relation to the GMO analysis, both matrix-based and pure analytical reference material (RM) have been used (*24*,*97*,*98*).

Several GMO modifications have been targeted in most transformed crops. A list of major GMOs and PCR--based methods for their detection can be found in a recently published review concerning cotton, maize, papaya, pepper, potato, rapeseed (canola), rice, soya bean, squash, sugar beet, tomato and watermelon (*99*). However, improved methods have been developed for previously mentioned and/or not mentioned species regarding a consistent quantification assay such as: cotton (*90*), maize (*22*), soya bean (*22*,*100*), and wheat (*101*).

A real-time PCR method was developed for the differential detection and quantification of four Solanaceae species in GMO analysis: potato (*Solanum tuberosum* L.), tomato (*Solanum lycopersicum* L.), eggplant (*Solanum melongena* L.), and pepper (*Capsicum annuum* L.) using an endogenous reference gene, β-fructosidase, for the detection (*102*).

Querci *et al.* (*100*) developed a unique screening tool for the unequivocal identification of all currently EU-approved and unapproved GMOs known, which were established according to the Regulation (EC) No 2829/2003 (*5*). The 'real-time PCR-based ready-to-use multi-target analytical system for GMO detection' was applied to maize, cotton, rice, oilseed rape, soya bean, sugar beet and potato. The limit of detection (LOD) was determined to be at least 0.045 % expressed in haploid genome copies (*100*). Furthermore, this GMO detection system was successfully applied in 64 commercial maize chips (*103*).

PDO traceability – olive oil case study

PDO olive oil regions have been established by legislation to enhance the quality of this product, to ensure

both consumers' expectations and producers' profits. Virgin olive oil commands a premium segment of the market that attracts adulteration attempts with vegetable seed oil inclusion in their matrices (*104*). Several techniques based on olive oil composition (*e.g.* gas chromatography, and silica gel column chromatography) have been applied to detect these adulteration events (*105–108*). However, using the same type of methodology, some difficulties have been encountered in distinguishing olive cultivars in both drupe and olive oil samples, mainly because their characteristics are strongly influenced by environmental conditions (*109*).

DNA-based markers, which are independent from environmental conditions, have been widely applied to olive research (*110*). Nevertheless, several problems with homonymous and synonymous denominations have been pointed out in the literature in terms of olive germplasm, which may compromise a correct cultivar identification and influence the development of a reliable traceability system in olive. Apart from this, intra-varietal genetic variation has also been detected (*111,112*), which can complicate this DNA approach even more. Thus, it is urgent that a dependable olive database be generated, containing all available data, in order to have a better overview of the work that is needed in terms of germplasm evaluation. This database may define a set of markers that are discriminatory for traceability purposes.

Methods of DNA recovery from olive oil have been developed (*29,53,54*), and several molecular markers have been successfully applied for olive oil traceability. The techniques that have been applied in this context are: AFLP (*28,113–115*), RAPD (*28,30*), inter-simple sequence repeat (ISSR) (*30,116*), sequence characterized amplified region (SCAR) (*28,53,109,117*), SSR (*30,52,53,55,57,79,118*), SNP (*46*), and more recently, chloroplast DNA direct sequencing (*70,119*).

Recently, Giménez *et al.* (*70*) have used real-time SYBR Green-based PCR to detect amplification of DNA olive oil samples. The authors suggest that this method can be considered a useful tool in the development of molecular markers (primer combination selection) for olive oil authentication since it allows the inspection of PCR efficiency (*70*).

Food allergy and intolerance

Food allergies might be defined as adverse, immunomediated (IgE-mediated) reactions to food that can occur in intolerant individuals. The reaction may vary considerably from a mild urticaria to potentially lethal anaphylactic shock. The most common allergic reactions to food include cow's milk, crustaceans, hen's egg, fish, tree nuts (such as walnuts, pecans and almonds), peanuts, soya beans and wheat allergy (*120*). To safeguard the sensitized individuals, the legislation on food labelling has established clear guidelines in the EU, defined in the Directive 2007/68/EC (*121*), which requires mandatory labelling of 14 groups of allergenic food ingredients: cereals containing gluten, crustaceans, eggs, fish, peanuts, soya beans, milk, nuts, celery, mustard, sesame, sulphur dioxide and, more recently, lupine and molluscs. For allergic and intolerant persons, full information related to the potential presence of non-desired food components may be a key point of survival value (*122*). In many countries, food products must discriminate on their label the presence of potential contaminants along the food production chain of these specific products (*123*).

In principle, any molecule that is allergen-specific may serve as a marker of its presence in food. However, DNA and specific proteins are targeted for this purpose (*124*). DNA-based methods have provided reliable tools for detecting hidden allergens in a wide range of foods (*78,125*). Thus, several DNA-based methodologies have been developed for the detection of nut allergens in processed food such as: almond using RTi-PCR with SYBR® GreenER™ (*124,126,127*), hazelnut using TaqMan RTi-PCR (*128*); peanut using TaqMan RTi-PCR (*45*), macadamia nut using TaqMan RTi-PCR (*40*), and pecan nut using PCR with pecan-specific primers and TaqMan fluorescent probe (*129*). Most of these detection methods are based on the design of primers linked to allergenic genes, so they are directly targeting the origin of the problem.

Allergies to tomato, carrot and celery constitute a growing concern for food producers since their use has been increasing in various products. Recently, a DNA extraction method, a duplex PCR assay and RTi-PCR procedure have been developed to detect these particular allergens in commercial food products (*130*).

Cereals can trigger coeliac disease (gluten enteropathy). The proteins gliadin (wheat), secalin (rye) and hordein (barley) are described as causes of this disease (*131*). These proteins cause a characteristic damage of the small bowel mucosa. This adverse reaction is a lifelong illness, so persons with gluten enteropathy have to be on a gluten-free diet to avoid the use of cereals containing gluten (*132*). In order to be able to detect its presence in several food products, a PCR-based assay was developed targeting high molecular mass (HMM) glutenin in wheat, kamut, spelt and rye; gene *Hor3* in barley and gene encoding the 12S seed storage protein in oat (*132*). In association with these studies, RTi-PCR methods have been established for the specific discrimination of wheat, rye, barley, maize, almond and oats in several food samples, using melting curve analysis (*133–135*).

Meat sector

The meat market is often exposed to fraudulent practices concerning deviations from declared ingredients, mostly with the inclusion of cheaper meat. In order to control efficiently this market, accurate and robust methods are required regarding both fresh and processed meat. The use of PCR-based methods has demonstrated to be suitable for both types of meat samples (*136–138*). According to Dalvit *et al.* (*139*), traceability in the meat sector is divided into three major categories: (*i*) individual animal identification, (*ii*) genetic traceability of breed, and (*iii*) genetic traceability of species.

The importance of individual animal identification was raised when several animal disease outbreaks (*i.e.* foot and mouth and BSE diseases) appeared in the EU countries. Several security and quality issues were raised by consumers and importers, obliging the EU to regulate the labelling system (EU Regulation No 1825/2000, *140*). However, this system is based on paper document control and tags, which can be easily adulterated. In order to overcome this concern, animal DNA typing has been proposed as an alternative methodology. SSRs (*50,141, 142*) and SNPs (*73,143–145*) have been amongst the most widely applied molecular markers for this purpose.

In the EU, several animal PDO and PGI products are prepared using exclusively one animal breed. The detection of the presence of a specific breed in the products requires a previous knowledge of specific molecular markers capable of identifying the breed, and in the case of processed food, the application of these markers to different matrices. The ability to use SSR markers has allowed the identification of several animal breeds (*142*). These markers are available to be used in control of PDO and PGI certification. When dealing with highly processed food products, the molecular markers used need to target small fragments in order to overcome the high rate of DNA degradation present in those samples (*136*). The application of SNP markers can be a solution. Some work has been done in the authentication of Parmigiano Reggiano cheese, produced only with milk of Reggiana dairy cattle breed, using a coat-specific marker *MC1R* gene (*145*).

Regarding genetic traceability of species, the major concern is based on the use of cheaper meet in processed food. Some molecular markers have been developed to identify clearly different species of wild boars and Iberian and Duroc pigs (*146*), fish and seafood (reviewed by Rasmussen and Morrissey, *147*), chicken (*137*), Muscovy duck (*148*) and game birds (*149*). Most of the molecular markers developed for this purpose are based on mitochondrial sequences.

Recent research has been focusing on quick and reliable methods that will allow the analysis of multiple samples simultaneously. Recently, a commercial microarray-based tool has been developed (*150*). This system permits to screen 33 species (fish, birds, and mammals) in one test, using a reverse dot hybridisation technique on a DNA microarray that analyses the vertebrate mitochondrial cytochrome b gene. Fajardo *et al.* (*151*), using DNA1000 LabChip (Agilent Technologies, Santa Clara, CA, USA), were able to identify ten game and domestic meat samples in a single run, taking only 1–2 h to analyze the samples.

Conclusions

Food security and traceability are closely related. In the last decade, traceability initiatives have been in progress for products that have become of utmost importance in the agri-food export markets of numerous developing countries. New DNA-based diagnostic methodologies are being developed in order to increase consumer's confidence and to guarantee producer's profit in high quality food and beverages.

The main advantage of DNA-based technology is that it is not dependent on external conditions, which makes it reliable. However, there are still some bottlenecks that need to be improved in order for it to be widely applied, the first being the DNA recovery from the various food matrices available.

In the near future, DNA-based methodologies will be widely applied either by using PCR-based procedures as the ones described or using microchips with high throughput, able to detect several targets simultaneously. These chips will have to be continuously updated in order to contain the new generated markers for each target condition. Progress, both in sampling and detection methodologies, and in traceability strategies, strongly influences the potential for adequate implementation and fulfillment of legislation and labelling requirements.

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