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Artículo de investigación

Authentication of plant food products: Under the magnification of Botany Forensics

Autentificación de los alimentos de origen vegetal: ampliación de la Botánica Forense

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Abstract

Nowadays, food adulteration and counterfeiting are of primary concern to producers, retailers, and consumers. Therefore, the determination of product authenticity, the identification of foodstuff fraud and counterfeiting, and the misleading labeling are essential to assure all involved in the food supply chain. In the last few decades, several DNA-based techniques have become available to detect fraudulent practices. In this review, we cover the main topics associated with plant food traceability and molecular markers.

Keywords: Counterfeiting, forensic Botany, fraud, misleading labeling, molecular markers, plant food products.

Resumen

Hoy en día, la adulteración y falsificación de alimentos son de interés primordial para los productores, minoristas y consumidores. Por lo tanto, la determinación de la autenticidad del producto, la identificación del fraude, la falsificación de los productos alimenticios y el etiquetado engañoso, son esenciales para conocer todos los pasos en la cadena de suministro de alimentos. En las últimas décadas se han desarrollado varias técnicas basadas en ADN para detectar prácticas fraudulentas. En este trabajo revisamos los principales temas asociados con la trazabilidad de los alimentos y los marcadores moleculares.

Palabras clave: falsificación, plantas comestibles, botánica forense, etiquetado engañoso, fraude, marcadores moleculares.

INTRODUCTION

The consciousness of consumers in terms of food composition (Teletchea *et al.*, 2005) is widely increasing due to awareness of the latest food scares (such as contamination of sprouts with *Escherichia coli* serotype O104:H4), misconduct of some food producers, fashionable dietary preferences (such as the preference for organic products, vegetarianism), health concerns (such as peanuts, lactose or gluten for individuals with particular sensitivities or allergies), the inclusion of genetically modified organisms (GMOs) (Martins-Lopes *et al*., 2013), the worldwide circulation of food resulting in a mixture of local and foreigner products, and the increasing complexity of ingredients present in food products. Consumers depend on the description and/or labeling of food for a precise information to make educated choices concerning their diet and food purchasing (Woolfe & Primrose, 2004; Primrose *et al*., 2010). Nonetheless, labels frequently provide an incorrect and/or insufficient guarantee about the actual product contents being indispensable to recognize and/or validate the components of foodstuff, assuring producers, retailers, and consumers that illegal substitutions (Pascal & Mahe, 2001) were not practiced and that the labeling information is accurate.

So far, dozens of DNA marker-based methods have emerged and become remarkably useful for species and cultivar identification, in the context of forensic analyses, to assure food safety and quality (Liu *et al*., 2016). These genetic markers can differ regarding relevant features such as the level of polymorphism detected, locus specificity, genomic abundance, reproducibility, technical requirements, and time and cost limitations (Mondini & Pagnotta, 2015).

Restriction fragment length polymorphism (RFLP) was one of the first DNA markers to be developed for genetic studies. The main disadvantages are associated with the large DNA amount required, its low detection sensitivity, the complex experimental protocol for implementation, and the high cost associated which forbade its application in large-scale studies (Liu *et al.*, 2016).

The most frequently used molecular marker for plant analysis is the Random Amplified Polymorphic DNA (RAPD), first introduced in 1990 (Williams *et al*., 1990). The main advantages of the RAPD markers include (i) no *a priori* sequence knowledge, (ii) applicability in cases where limited amounts of DNA are accessible, (iii) efficiency and cost-effectiveness, and (iv) the same set of primers can be used in the analyses of several genomes of organisms (Hadrys *et al*., 1992).

Some years later, PCR-based markers, such as InterSimple Sequence Repeats (ISSR) (Zietkiewicz *et al.,* 1994) and Amplified Fragment Length Polymorphism (AFLP) (Vos et al., 1995), were introduced. Despite its potential, the use of these molecular markers is limited by some shortcomings. Some of these restrictions, as is reproducibility, seem to be less important for AFLP and ISSR than for RAPD (Zietkiewicz *et al.*, 1994; Vos *et al.*, 1995; Palacios *et al.*, 1999), probably due to the use of longer primers and higher annealing temperatures (Nybom, 2004). Partial DNA digestion could be responsible for some of the artifacts in AFLP analysis (Goulão *et al.,* 2001, Arnau *et al.,* 2003), being necessary to sample DNA at different stages of the growing season and from various organs (Nybom, 2004).

Simple sequence repeats (SSRs, STRs, or microsatellites) would be the markers of choice for genetic diversity studies (Downey & Iezzoni, 2000). The target sequences for these markers are highly abundant in the genome, highly polymorphic, are stable due to not being affected by environmental conditions, are easily and rapidly operated, and require small amounts of DNA (Liu *et al.,* 2016).

Single nucleotide polymorphisms (SNPs) are also of crucial importance since they can be detected even in very degraded and fragmented DNA due to the small size of the sequences (Martins-Lopes et al., 2013). Both SSR and SNPs are also prone to automation and portability of data between laboratories. Nevertheless, SNPs are frequently biallelic, and a large number of markers is required to obtain a high discrimination level or a reliable identification (Corrado, 2016).

Real-Time PCR (RT-PCR), a widely applied technique for food traceability, presents the advantage of quantifying each particular ingredient, providing an accurate composition of a given food product (Martins-Lopes *et al.*, 2013).

The recent advances in the next-generation sequencing platforms have become powerful tools to affordably and rapidly sequencing of genes, small genomes and metagenomes (Coghlan *et al.,* 2012; Wahler *et al.*, 2013; Sharma & Shrivastava, 2016,). Therefore, these high-throughput methodologies are becoming valuable tools for food traceability, being even possible to apply these markers to highly processed or degraded samples (Coghlan *et al.,* 2012).

Varietal identification

Classical methods of cultivar identification deeply rely on a set of morphological descriptors frequently difficult to evaluate and sensitive to both environmental conditions and production practices. The application of molecular markers overcomes some of the shortcomings associated with the classical methods. Several molecular methods have been created for the identification of cultivars, analysis of diversity, protection of patents, and nursery management. An overview of the molecular markers developed for varietal identification is presented in Table 1. Either as single ingredients or used in processed foods, the most well-known cases requiring varietal identification are associated with Basmati rice, potatoes, pome and stone fruits, and coffee and tea. Concerning the molecular marker, STR followed by AFLP and RAPDs are currently being used for such purpose.

Table 1. Molecular markers developed for varietal identification in important crops.
Tabla 1. Marcadores moleculares desarrollados para la identificación varietal en cultivos relevantes.

Species	Molecular Marker
Almonds (*Prunus dulcis*)	ISSR, RAPD, STR
Apple (*Malus* × *domestica*)	AFLP, ISSR, RAPD, RFLP, SNP, STR
Apricot (*Prunus armeniaca*)	AFLP, RFLP, STR
Asian plum (*Prunus salicina*)	RAPD, STR
Black cherry (*Prunus serotina*)	STR
Basmati rice (*Oriza sativa*)	AFLP, InDel, ISSR, QTL, RAPD, RT-PCR, STR
Coffee (*Coffea canephora*/*C. arabica*)	RFLP, RT-PCR, SNP, STR
European plum (*Prunus domestica*)	RAPD, STR
"Fava Santorinis" (*Lathyrus clymenum*)	ISSR, RAPD. RFLP
Mandarin (*Citrus reticulata*)	*trnT-trnL*, qPCR, RT-PCR
Nectarine (*Prunus persica* var. *nucipersica*)	AFLP
Orange (*Citrus sinensis*)	18S rDNA, ITS, *trnL*, *rbcL*, RT-PCR
Peach (*Prunus persica*)	RAPD, RFLP, SNP, SRAP, STR
Pear (*Pyrus* spp.)	18S rDNA, AFLP, ISSR, RAPD, SCAR, STR
Pomegranate (*Punica granatum*)	SCAR
Potato (*Solanum tuberosum*)	AFLP, ISSR, RAPD, STR
Sweet cherry (*Prunus avium*)	AFLP, STR
Tea (*Camellia sinensis*)	5S rDNA, *matK*, *rbcL*

Basmati rice

Basmati rice (*Oryza sativa* subsp.*indica*) is valued for its distinctive aroma and taste, its long thin grains and its unique cooking characteristics (Bhattacharjee *et al.*, 2002). Basmati rice varieties originated in the Indian and Pakistani foothills and had been exposed to centuries of cultivation and selection (Bligh, 2000). Such varieties produce a gastronomically superior grain; however, they present several disadvantageous agronomic traits, such as the lack of fertilizer response, sensitivity to the photoperiod, and difficulty in harvesting due to short plant height and a weak stem. To pledge these defects, Basmati varieties have been crossed with modern, improved varieties of long-grain rice, creating hybrid Basmati varieties. Both rustic and hybrid varieties are approved and considered as Basmati, but the rustic varieties fetch a higher price. Also, Moreover, some long-grain varieties of rice that morphologically resemble the Basmati variety, but without its characteristic properties (Khush & De la Cruz, 2002; Vemireddy *et al.,*

2015). Therefore, the differentiation between the various varieties of Basmati and other long-grain rice (Woolfe & Primrose, 2004) has become an imperative for food producers, food distributors, and consumers (Ganopoulos *et al.,* 2011).

Potatoes

Consumers and manufacturers want precise characteristics in potatoes (*Solanum tuberosum* subsp. *tuberosum*) for different gastronomic applications: potatoes used for salads must withstand cooking, for fries they should be crisp after frying, and for puree should be soft. Some potato varieties are known to have the desired characteristics. However, once the potato is processed, it is rather difficult to distinguish among varieties. This problem can be solved by identifying the genetic diversity of potato cultivars by their specific markers (Moisan-Thiery *et al.*, 2000, Rosa *et al.,* 2010).

Pome fruits

In temperate zones, apple (*Malus* × *domestica* Borkh.) is one of the most economically important fruit tree crop (Velasco *et al.,* 2010), available for consumers in an extraordinary number of commercial cultivars. Like in many other crops, a precise identification of the existing cultivars is crucial for breeding programs, patent protection, and nursery management (Goulão & Oliveira, 2001). The genus *Pyrus* includes at least 22 pear species. Among these, the European pear (*P. communis*) and Asian pear or nashi (*P. pyrifolia*) present interesting features for fruit production (Oliveira *et al.,* 1999). The existence of an exhaustive number of rootstocks, cultivars, and clones demonstrates the need for accurate identification, mainly to assure the patent protection of propagated material (Oliveira *et al.,* 1999).

Stone fruits

In the juice, jam, jelly, puré, and fruit preparation industries, fruit products with higher prices are occasionally adulterated with cheaper fruits to increase production profits (Popping *et al.*, 2005; Faria *et al.,* 2013,). This adulteration is difficult to detect and may also lead to a deterioration of the quality of the product (Fügel *et al.,* 2005). Moreover, the detection of fruit adulteration should also be extended to other products, such as yogurt, pudding, cream, fruit milk and ice-cream (Fügel et al., 2005). Therefore, several endeavors at finding suitable methods for authenticity control and determination of fruit content in fruit-based products have been undertaken (Fügel *et al.,* 2005).

Within the stone fruits, the *Prunus* genus includes several commercially important species, such as peaches (*P. persica*) and nectarines (*P. persica* var. *nucipersica*), apricots (*P. armeniaca*), European and Asian plums and prunes (*P. domestica* and *P. salicina*, respectively), and black (*P. serotina*), sweet (*P. avium*) and sour (*P. cerasus*) cherries, as well as almonds (*P. dulcis*) (Ahmad *et al.,* 2004). Within this genus, the botanical classification of species is often contentious, mainly due to the frequent interspecific hybridization, which results in the creation of various intermediate types which make particular species problematic to recognize (Dosba *et al.,* 1994; Baránek *et al.,* 2006).

Leguminosae

Worldwide, the Leguminosae family (alternately Fabaceae) is frequently considered one of the most important crops, constituting the second largest group of the food pyramid, after cereals (Madesis *et al.,* 2012). A common problem associated with grain legumes is the mixture of high-quality seeds of mixture of high-quality seeds of popular elite varieties (and price) or with seeds from other species with similar color and shape (Bosmali *et al.,* 2012). To the Protected Designation of Origin (PDO) "Fava Santorinis" (*Lathyrus clymenum*), also known as "fava" or "arakas'', are often added other legume products from other *Lathyrus* species (*L. cicera*, *L. ochrus*, and *L. sativus*), *Vicia* and *Pisum* species (Ganopoulos *et al.,* 2012). Also, lentils are often mixed with *Vicia* spp. The authenticity of "Fava Santorinis" is usually proven using ISSR (Belaid *et al.,* 2006), RFLP and RAPD molecular markers (Chtourou-Ghorbel *et al.*, 2001).

Coffee

Coffee, one of the most important world food commodities, is often a blend of *Coffea canephora* ("Robusta") and *C. arabica* ("Arabica") (Martel-

lossi *et al.,* 2005). Arabica coffee is frequently considered to have superior quality, attaining premium prices, mainly due to its finer flavor and better quality (Spaniolas et al., 2006, Trantakis *et al.,* 2012). Therefore, appropriated methods are needed, for both quality and economic reasons, to differentiate the two varieties, thereby ensuring coffee authenticity (Trantakis *et al.,* 2012). Conventionally, methods to distinguish the two coffee species have relied on the differences in the levels of several chemical compounds such as volatiles, amino acid enantiomers, metals, or caffeine, among others (Martellossi *et al.*, 2005, Trantakis *et al.*, 2012). However, in the last decade, DNA-based techniques have been developed to guarantee constant identity and also to prevent adulteration with both extraneous materials (such as cereals, coffee twigs) and low-grade varieties (Martellossi *et al.,* 2005, Trantakis *et al*., 2012).

Tea and infusions

Tea is and has always been one of the most popular beverages in the world. Nowadays, there are nearly 1500 different varieties of tea, offering a vast spectrum range of both taste and color, and presenting desirable physiologic activities and potential health benefits. According to the manufacturing process, teas are grouped into four major types: i) non-fermented white and green teas, produced by drying and steaming fresh tea leaves; ii) semi-fermented oolong tea, obtained by a partial fermentation of the fresh leaves before drying; iii) full-fermented red and black teas, produced by a post-harvest fresh leaf fermentation step before drying and steaming; and iv) post-fermented tea (*Pu-erh* tea) that undergoes a secondary fermentation and oxidation in open air (Stoeckle *et al.,* 2011, Daglia *et al.,* 2014). However, infusions prepared from a diversity plants other than *Camellia sinensis* and plant parts are also commonly and inadequately referred to as tea (Stoeckle *et al.,* 2011).

Fraud and counterfeiting

In the last few decades, food fraud, and economically motivated adulteration have become emerging risks, compromising food supply chains and gaining awareness of producers, manufacturers, processors, distributors, or retailers (Moore *et al.,* 2012). Food fraud includes aspects such as the thoughtful replacement, addition, altering, or misrepresentation of food, food ingredients, or food packaging, or false or misleading statements made about a product to improve economic gains (Spink & Moyer, 2011). Therefore, and despite its high impacts on public health, food fraud is frequently regarded as an economic crime. In the present work, we will review aspects associated with patent misappropriation, confirmation of Protected Designation of Origin (PDO) and Protected Geographical Indication (PGI). We will also focus procedures related with the addition, dilution, or extension of an authentic ingredient with an adulterant or mixture of adulterants as well as product mislabeling.

Strawberries

A well-known case of the lawsuit involving an Italian patented strawberry variety, 'Marmolada'®, registered in 1984 by the Consorzio Italiano Vivaisti (CIV), S. Giuseppe di Comacchio (Ferrara, Italy) was brought against farmers suspected of having reproduced and commercialized without permission. About one million strawberry plants were seized in a farm under suspicion to belong to the 'Marmolada'® variety and identified using RAPD technique (Congiu *et al.,* 2000).

Grapevine cultivars, grapes and wines

Nowadays, the wine market is turning towards the production and consumption of monovarietal wines. Therefore, grapevine (*Vitis vinifera*) identification is an imperative in this process, being crucial to control and certificate the plant material available to growers in the form of grafted woody canes. The growth of the scion and the quality of the grapes produced is determined by both the productive part of the plant (scion) and the rootstocks. Therefore, the genetic authentication of grapevine planting material constitutes an imperative to safeguard the viticulturist from fraudulent practices (such as misidentification, mislabeling, and counterfeit). Genetic authentication is also imperative in cases where the material subjected to intellectual property legislation (such as patents, trademarks, and contracts), protecting the owner of the rights from illicit propagation or

commercialization. The application of viticultural, winemaking and wine labeling regulations have been more rigorous in the Old World than in the New World. According to the production region, only specific cultivars are allowed in the vineyards, and the inclusion of others is restricted to legally defined percentages, consequently reinforcing the need for accurate cultivar identification. Also, wines are usually commercialized with labeling information regarding cultivar, cultivation area, and year of production. Therefore, wine legislation including origin and geographical indications, traditional terms, labeling and presentation of wine was implemented by the European Union. Wine quality categories are divided into PDO and PGI, being recurrent differences in regional specification according to the EU Regulation No 1151/2012 of the European Parliament and of the Council of 21 November 2012. While cultivar information in the labeling of wine is not compulsory by European legislation, it has become a significant factor of wine value in a market characterized by a ferocious competition. Once more, the accurate cultivar identification may act as a distinctive aspect that would benefit both producers and consumers (Santos *et al.,* 2014).

Olives and olive oil

Olive oils, important products due to their nutritional and commercial value, are obtained from drupes of the olive tree (*Olea europaea*) of several mixed or isolated cultivars. The olive oil organoleptic characteristics are attained by the combination of both weather conditions of a given region and cultivar(s) used for its production. As for wines, olive oils are also protected by EU appellations of PDO and PGI (Mafra *et al.*, 2008).

Traditional medicines

Nowadays, an increasing number of people frequently consume herbal medicines, mainly Oriental (Chinese, Indian, Thailand's, Tibetan) traditional medicines. These traditional drugs use medicinal plants (about 80%) as raw materials. In the most recent revision of the Chinese Pharmacopeia (2010), more than 4,600 species have been described as beneficial for human heath (Heubl, 2013). These medicines have been used for many centuries either to prevent or to treat diseases (Heubl, 2013, Ganie *et al.*, 2015). Also, in India, traditional medicine includes Ayurveda, Yoga, Naturopathy, Unani, and Siddha. Among them, Ayurveda, Siddha and Unani systems (ASU) use plants products as main drugs to alleviate various disorders (Revathy *et al.*, 2012).

However, these products are often contaminated or substituted with alternative plant species and fillers that are absent from the labels (Newmaster *et al.,* 2013). Therefore, pharmacists must be aware of purity, quality and safety of such products since unforeseen effects of many herbal products have been previously described in the literature (Boullata & Nace, 2000, Jayasinghe *et al.,* 2009, Liu *et al.,* 2009). Moreover, the inclusion of certain adulterants can also lead to intoxication (Yip *et al.*, 2007).

The unmistakable identification of the medical plants used constitutes a crucial step at the beginning of an extensive process of quality assurance (Colombo, 2014). Nevertheless, in powdered or otherwise processed plant materials, a traditional taxonomic system for plant species identification, based on diagnostic morphological features, cannot be typically applied (Mishra *et al.,* 2016). As such, several DNA-based methods have been applied for the identification of medicinal plants, which rely on the amplification of nuclear and chloroplast genes or hybridization with species-specific probes (Chatterjee *et al.,* 2015). Genomic fingerprinting is useful for the detection of sample homogeneity and presence of adulterants (Li *et al.*, 2011, Sucher & Carles, 2008). Several different PCR-based methods may be used to identify and authenticate these medicinal products derived from plant species (Table 2).

Table 2. Molecular markers developed for plant identification in fraud and counterfeiting cases.
Tabla 2. Marcadores moleculares desarrollados para la identificación de plantas en casos de fraude y falsificación.

Case	Species	Molecular Marker
Patent misappropriation	Strawberry (*Fragaria* x *ananasia* var. Marmolada)	RAPD
Discrimination of propagative material - Verification of EU appellations (PDO/PGI)	Grape (*Vitis vinifera*)	AFLP, STR
	Olive (*Olea europea*)	AFLP, RAPD, STR, SCAR, RT-PCR
Traditional and herbal medicines adulteration	Akebia (*Akebia* spp.)	Multiplex-PCR, STR, SCAR
	Alfafa (*Medicago sativa*)	ITS
	Artemisia (*Artemisia* spp.)	*psbA-trnH*
	Black cohosh (*Actaea racemosa*)	AFLP, RAPD
	Black cardamom (*Amomum* spp.)	*atpB-rbcL*, ITS, *matK*, *psbA-trnH*, *psbK-psbI*, *rbcL*, *rpoB*, RAPD, SNP
	Bupleurum spp.	ITS
	Brahmi (*Bacopa monnieri*)	SCAR
	Cat ginseng (*Actinidia macrosperma*)	RFLP
	Chirayat (*Swertia* spp.)	ITS, 5S rDNA, *matK*, *psbA-trnH*, *rbcL*, *rpl16*, AFLP, ISSR, RAPD
	Chi Shao (*Paeoniae rubra*)	ITS
	Chlorophytum spp.	RAPD
	Clematis (*Clematidis armandii*)	ITS
	Clematis spp.	ITS
	Danggui (*Angelica sinensi*)	ITS, 5S rDNA, 18S rDNA , RAPD
	Dendrobium spp.	ITS, ARMS, SNP, STR
	Dodder (*Cuscuta reflexa)*	RAPD , SCAR
	Gotu Kola (*Centella asiatica*)	ITS
	Drynaria fortunei	*trnL-trnF*, RT-PCR
	Embelia ribes	RAPD, SCAR
	Fallopia multiflora	*atpB-rbcL*
	Fritillaria spp.	ITS, RAPD, RFLP
	Gentiana macrophylla	RT-PCR
	Ginger (*Zingiber officinale*)	SCAR
	Ginkgo (*Ginkgo biloba*)	*matK*
	Huangqi (*Astragalus* spp.)	ITS, *coxI*, *matK*, *rbcL*, 5S rDNA, APPCR, RAPD, RFLP, SCAR
	Jack-in-the-pulpit (*Arisaema* spp.)	*matK*, *rbcL*
	Jinqian Baihua She (*Bungarus parvus*)	RFLP
	Lysimachia christinae	ITS
	Meconopsis impedita	*rps16*, ITS
	Nervilia fordii	ITS

Tabla 2. Traditional and herbal medicines adulteration. **(**Continuación).

	Panax spp.	ITS, 18S rDNA, *matK*, *rbcL*, AFLP, DAMD, ISSR, RAPD, RFLP, SCAR, SNP, STR
	Peking Spurge (*Euphorbia pekinensis*)	ITS
	Pinellia ternata	*matK*, *rbcL*, SCAR
	Phyllanthus spp.	*psbA-trnH*, RAPD, RFLP
	Punarnava (*Boerhavia diffusa*)	ITS, RFLP
	Qian-hu (*Peucedanum praeruptorum*)	ITS
	Red clover (*Trifolium pretense*)	ITS
	Rhei undulatai	SCAR
	Ruta graveolens	ITS, *rpoB*, *rpoC1*, RAPD
	Sabia parviflora	*matK*, *psbA-trnH*, *rbcL*-α
	Sal Leaved Desmodium (*Desmodium gangeticum*)	RAPD
	Schisandra spp.	ITS, Multiplex-PCR, SCAR
	Sedum spp.	ITS, *matK*, *psbA-trnH*, *rbcL*, STR
	Senna spp.	RAPD
	Sophorae tonkinensis	ITS
	Stemona spp.	*trnH-psbA*, RFLP
	Typhonium spp.	RAPD
	Valeriana spp.	ARMS
	Verbena officinalis	ITS, RAPD
	Viola yedoensis	ITS
	Withania somnifera	RAPD
Herbs and spices adulteration	Black pepper (*Piper nigrum*)	*rbcL*, *rpoC1*, *trnH-psbA*, RAPD, SCAR
	Basil (*Ocimum* spp.)	*matK*, *psbA-trnH*, *rbcL*, *rpoB*
	Cinnamon (*Cinnamomum verum*)	ITS, *matK*, *psbA-trnH*, *rbcL*, *trnL-trnF*, Multiplex-PCR
	Mentha (*Mentha piperita*, *M. aquatica*, *M. spicata*)	*matK*, *psbA-trnH*, *rbcL*, *rpoB*
	Origanum (*Origanum* spp.)	*matK*, *psbA-trnH*, *rbcL*, *rpoB*, RAPD, SCAR
	Paprika (*Capsicum annuum*)	ITS, ISSR, RAPD, SCAR, STR
	Rosemary (*Rosmarinus officinalis*)	*matK*, *rbcL*, *psbA-trnH*, *rpoB*
	Saffron (*Crocus sativus*)	ITS, *matK*, *psbA-trnH*, *rbcL*, ISSR, SCAR, RT-PCR
	Salvia (*Salvia rutilans*, *S. uliginosa*, *S. officinalis*)	*matK*, *rbcL*, *rpoB*, *trnH-psbA*
	Star anise (*Illicium verum*)	ITS, *matK*, *psbA-trnH*, *rbcL*
	Thyme (*Thymus vulgaris*)	*matK*, *psbA-trnH*, *rbcL*, *rpoB*
	Turmeric (*Curcuma longa*)	ITS, 18S rDNA, *matK*, *rbcL*, *trnK*, *trnS-trnfM*, ARMS, SNP, RAPD, ISSR

Tabla 2. Continuación.

Pasta and bread adulteration	Durum wheat (*Triticum durum*)	RT-PCR
	Lupine (*Lupinus* spp.)	RT-PCR
	Soybean (*Glycine max*)	RT-PCR
Fruit-derived products adulteration	Mandarin (*C. reticulate*)	RT-PCR
	Orange (*Citrus sinensis*)	*trnL*, *rbcL*
	Pomegranate (*Punica granatum*)	SCAR

Herbs and Spices

Herbs consist of the dried leaves from aromatic plants, while spices are dried parts, except leaves, of aromatic plants (Dal L'Asta, 2013). Therefore, for spice production, different parts of a plant such as the seed (mustard), fruit (pepper), floral parts (saffron), bark (cinnamon), root (horseradish), and rhizome (ginger), can be used (Focke et al., 2010). Both herbs and spices are frequently used worldwide for preservation enhancement, flavoring, seasoning, coloring and imparting aroma food (Srinivasan, 2005; Dhanya & Sasikumar, 2010; Dal L'Asta, 2013). Despite its role as food adjuvants, spices have also been recognized medicinal properties, being used in many traditional systems of medicine (Srinivasan, 2005). Due to their elevated economic and commercial interest, traded forms of spices are frequently subjected to admixing or substitution with cheaper and inferior substances.

Black pepper (fruits of *Piper nigrum*), the most widely used spice, is frequently adulterated with dried papaya seed (*Carica papaya* L.), wild *Piper* spp. (*P. attenuatum* and *P. galeatum*), dried fruits of *Lantana camara*, *Embelia ribes*, seeds of *Mirabilis jalapa*, and berries of *Schinus molle* (Dhanya, 2009, Dhanya et al., 2009, Dhanya & Sasikumar, 2010).

The Chinese star anise (fruits of *Illicium verum*), a commonly used spice for culinary, cosmetics and medicine purposes, is frequently adulterated with other *Illicium* species, such as *I. lanceolatum* and *I. anisatum*. These congeneric adulterants are responsible for the production of anisatin and sofrole that cause neurologic and gastrointestinal toxicities (MEIZI et al., 2012).

Paprika (fruits of *Capsicum annuum*), a widely used spice in all types of curried dishes (Raghavan, 2006), is frequently adulterated with dried and powdered fruits of 'Choti ber' (*Ziziphus nummularia*) (Dhanya *et al.,* 2011), dried red beet pulp (Schwein & Miller, 1967; Berke & Shieh, 2009), and almond shell dust (Berke & Shieh, 2009).

Saffron (stigmas of *Crocus sativus*), is one of the most valuable seasonings, being its market price among the highest in the food and flavoring sector (Marieschi et al., 2012). This spice is frequently adulterated with less expensive plant materials such as *Carthamus tinctorius*, *Calendula officinalis*, and *Arnica montana* flowers, *Bixa orellana* ground seeds, *Hemerocallis* sp. tepals, *Curcuma longa* powdered rhizomes, and *Crocus vernus* stigmas (Hagh-Nazari & Keifi, 2006; Kanti *et al.,* 2011). Renowned as the most expensive spice the saffron market price ranks among the highest in foods. In 2015, its value reached 20,000 €/kg or more in the case of particular PDO productions (Soffritti *et al.*, 2016).

Cinnamon (dried bark of *Cinnamomum verum*), an important tree spice indigenous to India and Sri Lanka, is frequently adulterated with *C. aromaticum*, a rougher, thicker, cheaper, and less aromatic bark with a bitter and burning flavor (Swetha *et al*., 2014).

Turmeric (rhizomes of *Curcuma longa*), generally used in Bangladeshi, Indian, and Pakistani cuisines, is frequently adulterated with other cheaper *Curcuma* species (Sen *et al.*, 1974; Mitra, 1975; Zwaving & Bos, 1992; Sasikumar, 2005; Sasikumar *et al.*, 2005). According to the spice considered, several methods have been developed for species identification and authentication as demonstrated in Table 2.

Pasta and Bread

The cereal composition is important to guarantee the quality and safety of food and feed. For instance, food intended for patients with celiac disease must be checked for contamination of different cereal species since storage proteins (gluten) can damage the small-intestinal mucosa of these patients (Terzi *et al*., 2003). A large proportion of the dried pasta products produced and commercialized in the European community use as raw material pure durum-wheat (*Triticum turgidum* subsp. *durum*) semolina, considered as superior quality wheat. Therefore, the use of *T. aestivum* (common wheat) or mixtures of both types of wheat is usually regarded as an adulteration. However, a maximum of 3% common wheat is allowed to tolerate for cross contamination during the agricultural processes. To identify and quantify non-durum wheat, real-time PCR using *T. aestivum* D-genome specific sequences and several STRs have been developed (Alary *et al.,* 2002, Bryan *et al.*, 1998, Pasqualone *et al.*, 2007, Terzi *et al.,* 2003). Similarly, bread crumbs were analyzed by RFLP to estimate the common wheat content of the flour (von Büren *et al.*, 2001) (Table 2).

Fruit-derived products

Orange (*Citrus sinensis*) juice is frequently adulterated with the admixture of lower pricy mandarin (*C. reticulata*) juice (Popping, 2007). Pomegranates (*Punica granatum*) are currently enjoying a huge interest, mainly due to its anthocyanin and polyphenol contents. It has been frequently detected the unlabeled addition of both anthocyanin-rich plants or cheaper plant material, such as maqui berries (*Aristotelia chilensis*), black chokeberry (*Aronia melanocarpa*), purple yam (*Dioscorea alata*), açaì (*Euterpe oleracea*), apple (*Malus* × *domestica*), black mulberry (*Morus nigra*), elderberry (*Sambucus nigra*), cranberry (*Vaccinium macrocarpon*), bilberry (*Vaccinium myrtillus*), and grapes (*V. vinifera*), as bulking and diluting agents in juices and herbal preparations to increase economic profits (Marieschi *et al.*, 2016).

Although these two examples illustrate the adulteration of fruit juices, the same principles could be applied to other fruit-derived products such as jams, jellies, ice-creams, yogurts and flavored milks. In table 2 are revised methods for the identification of citrus fruits in processed foods.

Insufficient and erroneous food labeling

Nowadays, consumers are well conscious of the importance of the exact and precise labeling of plant food products. An incomplete or erroneous labeling can have a serious impact on public health. Next, we focus some of the concerns related to this issue.

Allergens

According to the European Regulation (EU) No 1169/2011, from the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers, all allergens present in food must be indicated and highlighted in the food label, guaranteeing a high level of health protection for consumers and their right to information.

Food allergies constitute a significant health problem in developed countries, affecting children (8% of the population) and adult (2% of the population) (Poms et al., 2004), albeit it has been suggested that susceptibility decreases with age (Goodwin, 2004). Total allergen avoidance is rather difficult, due to the large variety of ingredients present in processed foods, and the presence of unlisted allergen (Zaya and Ashley, 2012). Thus, reliable methods for the detection and quantification of food allergens are essential to safeguard food labeling and to improve consumer security. Although the detection of potentially allergenic proteins is the widest method for allergen detection (Poms et al., 2004), DNA-based techniques can also be regarded as a marker for the presence of allergens (Mafra et al., 2008). Problematic foods can either be a part of the foodstuff or a cross-contaminant in processed foods (known as "hidden" allergens) (Goodwin, 2004; Mafra *et al.*, 2008). So far, more than 160 food ingredients have been reported as allergenic (Poms *et al*., 2004).

Almonds (*P. dulcis*), hazelnut (*Corylus avellana*), peanuts (*Arachis hypogaea*), and walnuts (*Juglans regia* fruits) are potential food allergens widely consumed as nuts, often used in bakery, and pastry as basic components of fillings in several cakes, confectionary products as filled chocolates, cereal muesli, ice-cream, and oils.

Celery (*Apium graveolens*) is extensively used as an ingredient in the food industry, in several products such as dried seasoning, dehydrated bouillons, sauces, sausages and ready-made meals (Hupfer *et al.*, 2007). White mustard (*Sinapis alba*), black mustard (*Brassica nigra*), and Indian or brown mustard (*Brassica juncea*) are frequently used as an additive in diverse food products and extensively used in food formulations for its pungency, thickening, stabilizing abilities, and other properties. Mustard is used in several product formulations, such as sauces, dressings, marinades, seasonings, and processed meat (Shim & Wanasundara, 2008). Soybean (*Glycine max*) present excellent foaming abilities, due to their water-binding properties soy proteins improve and maintain moistness and softness of the product, being used in several foodstuffs for different purposes.

Over the last years, several methods have been proposed for the simultaneous detection of several allergens using several DNA-based techniques, allowing the detection of several targets in a one-tube assay, and contributing to food security (Table 3).

Table 3. Molecular markers developed for cases of insufficient and erroneous food labeling.
Tabla 3. Marcadores moleculares desarrollados para etiquetado insuficiente y erróneo de alimentos.

Case	Species	Molecular Marker
Allergens	Almonds (*Prunus dulcis*)	Multiplex RT-PCR, RT-PCR
	Black mustard (*Brassica nigra*)	RT-PCR
	Brazil nut (*Bertholletia excelsa*)	*Ber e1*, RT-PCR
	Cashew (*Anacardium occidentale*)	Multiplex RT-PCR, RT-PCR
	Celery (*Apium graveolens*)	*mtlK*, Multiplex RT-PCR, RT-PCR
	Hazelnut (*Corylus avelana*)	PCR-Elisa, RT-PCR., Multiplex RT-PCR, Microarray, PNA-array
	Flaxseed (*Linum usitatissimum*)	RT-PCR
	Indian or brown mustard (*Brassica juncea*)	RT-PCR
	Lupine (*Lupinus* spp.)	RT-PCR
	Macadamia (*Macadamia intergrifolia*)	RT-PCR
	Peanuts (*Arachis hypogaea*)	PNA-array, RT-PCR, Multiplex RT-PCR
	Pecan (*Carya illinoinensis*)	RT-PCR
	Pistachio (*Pistacia vera*)	RT-PCR
	Poppy (*Papaver rhoeas*)	RT-PCR
	Sesame (*Sesamum indicum*)	RT-PCR, Multiplex RT-PCR
	Sunflower (*Helianthus annuus*)	RT-PCR
	Soybean (*Glycine max*)	*atpA*, RT-PCR, Multiplex RT-PCR
	Walnuts (*Juglans regia*)	*matK*, RT-PCR, Multiplex RT-PCR
	White mustard (*Sinapis alba*)	RT-PCR

Table 3. Continuación.

Genetically modified organisms	Canola (*Brassica* spp.)	RT-PCR, Multiplex RT-PCR, Microarray, LPA
	Common bean (*Phaseolus vulgaris*)	RT-PCR
	Cotton (*Gossypium hirsutum*)	Multiplex PCR, RT-PCR, Multiplex RT-PCR, Microarray, LPA
	Eggplant (*Solanum melongena*)	RT-PCR
	Maize (*Zea mays*)	Multiplex-PCR, q-PCR, Multiplex RT-PCR, Microarray, LPA, RT-PCR
	Papaya (*Carica papaya*)	Duplex-PCR, RT-PCR
	Pepper (*Capsicum annuum*)	Multiplex PCR, RT-PCR
	Potato (*Solanum tuberosum*)	RT-PCR, Multiplex RT-PCR, Microarray, LPA
	Rice (*Oryza sativa*)	RT-PCR, Multiplex RT-PC , Microarray, LPA
	Soybean (*Glycine max*)	Multiplex PCR, RT-PCR, Multiplex RT-PCR, PNA-array, Microarray, LPA
	Sugar beet (*Beta vulgaris*)	RT-PCR, Multiplex RT-PCR, Microarray, LPA
	Tomato (*Solanum lycopersicum*)	PCR, RT-PCR, Microarrays
	Wheat (*Triticum aestivum*)	RT-PCR
	Wheat (*Triticum durum*)	RT-PCR

Legend: *atpA*: alpha chain of adenosine triphosphate synthetase gene; *atpB-rbcL*: *atpB-rbcL* intergenic spacer; *Ber e1*: *Bertholletia excelsa* major allergen; *coxI*: cytochrome c oxidase subunit I gene; ITS: internal transcribed spacer region of nuclear ribosomal DNA; *matK*: maturase K gene; *mtlK*: mannitol dehydrogenase gene; *psbA-trnH*: *psbA-trnH* intergenic spacer; *psbK-psbI*: *psbK-psbI* intergenic spacer; *rbcL*: ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene; *rpoB*: β subunit of RNA polymerase gene; *rpoC1*: RNA polymerase beta' chain gene; *rpl16*: ribosomal protein L16 gene; *rps16*: ribosomal protein S16 gene; *trnH-trnL*: *trnH-trnL* intergenic spacer; *trnL-trnF*: *trnL-trnF* intergenic spacer; *trnS-trnfM*: *trnS- trnfM* intergenic spacer.

AFLP: amplified fragment length polymorphism; ISSR: inter simple sequence repeat; APPCR: polymerase chain reaction with arbitrary primer; ARMS: amplification-refractory mutation system; DAMD: Directed Amplification of Minisatellite-Region DNA; LPA: ligand-dependent probe amplification; PNA-array: PNA probes are analogues of oligonucleotides in which the sugar-phosphate backbone has been replaced by a pseudopeptide chain of N-aminoethylglycine monomers; q-PCR: quantitative PCR; QTL: quantitative trait locus; RAPD: random amplified polymorphic DNA; RFLP: restriction fragment length polymorphism; RT-PCR: real time PCR; SCAR: sequence characterized amplified regions; SNP: single nucleotide polymorphisms; SRAP: Sequence-related amplified polymorphism; STR: short tandem repeat, single tandem repeat or microsatellite.

Genetically modified organisms

Since the last decades, improvements in the field of biotechnology lead to profound transformations in agricultural systems, mainly by the introduction of genetically modified organisms (GMO) (Mafra *et al.*, 2008). Nowadays, more than 25 fruits and agricultural products have been modified by the introduction of new agronomic qualities or inhibition of constituent genes from different organisms. Among the genes introduced are those

which code for disease and pest resistance, herbicide tolerance, inhibition of ripening, increase of nutritional value, decrease toxins, and improve desirable characteristics (Elsanhoty *et al.,* 2013). The principal transgenic crops are herbicide and insecticide resistant soybean, maize, cotton, and canola. Other crops includes a variety of sweet potato resistant to a virus that could destroy most of the African harvest, and rice with increased iron and vitamins that may alleviate chronic malnutrition in Asian countries. There are bananas that genetically modified to vaccines against infectious diseases such as hepatitis B, fruit and nut trees that start producing years earlier, and plants that produce new plastics with unique properties (Bawa & Anilakumar, 2012). However, the cultivation of GMO raises several ethical questions, mainly due to the potential risks associated with GM technology, which includes the indirect effects of GM crops on the environment, biodiversity changes, and possible the development of resistant insects and tolerant weeds (Elsanhoty *et al.,* 2013). In fact, since the release of the first GM crops, mostly in Europe, an intense scientific and public debate concerning the safety issues of such products took place. As such, the EU have promulgated appropriate legislation concerning the compulsory labeling of food products containing more than 0.9% of authorized GMO (Mafra *et al.*, 2008).

Therefore, the detection of GMO crops has become a priming need to only assure both producers and consumers to choose products but also to comply with labeling regulations. The DNA-based PCR methods are most widely applicable and could be applied in unprocessed as well as highly processed foods. Two types of PCR can be applied for the GMO content examination: conventional PCR, to confirm the presence of GMO with the help of gel electrophoresis, and real-time PCR, to detect and quantify the GMO content (Table 3). The conventional approaches, mainly based on the detection of the sequence of a pre-selected target at a time, or on a restricted multiplexing, allowing the analyzed of only a limited number targets at once; being, therefore, inadequate to the actual testing requirements. As a consequence, in several countries, new approaches for the detection of GMOs have been authorized for commercial purposes. These approaches rely on a smart and accurate strategy for target selection, such as the use of high-throughput systems, platforms for the simultaneous detection of multiple targets, and algorithms allowing the conversion of diagnostic results into an indication of the presence of individual GMOs.

CONCLUSIONS

At present, the detection and identification of plant materials or remains in the food is mainly supported by several molecular approaches. A number of DNA fingerprinting techniques, have been developed to identify varieties, clones, and cultivars of a plant species. Also, several multiplex-PCR protocols have been used for the simultaneous detection of several allergens and GMOs in a one-tube assay, which contributes to increase food security.

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