DNA markers as a tool for genetic traceability of primary product in agri-food chains

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Abstract

The agri-food components of the *Made in Italy* are well known all over the world, therefore they may significantly contribute to the Italian economy. However, also owing to a large number of cases of improper labelling, the Italian agro-food industry faces an ever-increasing competition. For this reason, there is a decline of consumers' confidence towards food production systems and safety controls. To prevent erroneous classification of products and to protect consumers from false in-store information, it is important to develop and validate techniques that are able to detect mislabelling at any stage of the food-chain. This paper describes some examples of genetic traceability of primary products in some important plant food chains such as durum wheat, olive and tomato, based on DNA analysis both of raw material and of processed food (pasta, olive oil, and peeled tomato).

Introduction

In the agro-food world market the label *Made in Italy* is certainly very popular, but recent data show a decrease of the Italian performance on international markets (http://www.oecd.org/), probably due to the high competition of global market systems. Italian agro-food provides an important contribution to the National economy: a variety of typical products (*i.e.* wines, pasta, sauces, oils and gastronomic preparations), that have quality labels [*i.e.* Protected Designation of Origin (PDO) or Protected Geographical Indication (PGI)] and belong to consolidated cultural and culinary traditions (tomato *San Marzano*, apple *Annurca,* apricot of the Vesuvio, olive oil *Terra di Bari*, *Altamura bread* and many others) are popular abroad and give important contributions to the export of Italian agro-products. Currently, the number of food frauds is increasing and recent data suggest that the Italian products are the most affected, because of their good quality and well known fame (http://www.mdc.it/it/3715.html).

Markets globalization largely affects supply chain networks that, in consequence, became extremely complex to control. For example, although Italian agriculture has a long standing tradition in tomato production, presently the processed tomatoes are largely imported from China (42% of total imports in 2010; www.coldiretti.it). At the same time, there is a growing decline of consumers' confidence for food production systems and controls as well as for the credence attributes such as *healthy and safe* food chains. This is due to the progressive loss of contact between consumers and the food production systems and it is boosted by recent food crises: the cases of the Bovine Spongiform Encephalopathy (BSE) and of the contamination with dioxin or with mercury of animal feed and fish respectively. For these reasons there is an increasing demand for identification and labelling of food products (Ammendrup and Fussel, 2001; Caporale *et al.,* 2001; Carcea *et al.,* 2009; Wen *et al.,* 2010).

In this context, technologies able to trace primary products processed in food chains represent a key issue that is receiving growing attention both from producers and consumers for the relevant contributions they may offer in respect to fraud and mislabelling reports and their connections with the possibility to track the food geographic origin.

Traceability

The traceability is the ability to trace and follow a food, feed, an animal or substance intended to become part of a food or feed, through all stages of production, processing and distribution (EU regulation No. 178/2002). This definition is necessarily broad because food and feed are complex matrixes and traceability is a tool that should allow to track them, from row materials (*e.g.,* plant cultivars) to final processed products and beyond, up to market distribution and consumers. As a consequence, no traceability system is complete. Therefore different systems should be integrated to satisfy different requirements.

The important parameters that characterize a traceability system are breadth, depth, precision and running costs. Breadth relates to the

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Key words: molecular markers, SSR, AFLP, tomato, olive oil, durum wheat pasta.

Received for publication: 2 May 2012.
Accepted for publication: 24 August 2012.

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Licensee PAGEPress, Italy
Italian Journal of Agronomy 2012; 7:e45
doi:10.4081/ija.2012.e45

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amount of available information which should include key attributes (for example product or process characteristics). Depth describes how far, back or forward inside the food *netchain,* the system is able to capture the relevant information (for example, a traceability system for cultivar authentication should be able to authenticate the genetic material both back to cultivation and forward to processed food). Precision reflects the capability to provide information in a particular step of a food chain (*e.g.,* in cultivar authentication precision is the ability to identify contaminating genetic material). Running costs, that include the resources to set-up and run the system, should be in a range that would incentivize industries to activate traceability systems from which they could receive consistent benefits as they can improve the appropriate use and reliability of information, effectiveness and productivity of the organization.

The tracking method involves monitoring of stuff flow from the raw material to final sale. This can be essentially done by the manufacturer through a process of self-certification to be released, for example, after the measurement of specific physicochemical parameters that are supposed to be almost invariable during transition from raw material to final product. A company that is able to follow the entity flow and to monitor at any time raw material, semi-processed or processed products that are within the limits of its own responsibility, is a company able to streamline identification processes, to individuate causes of errors, to monitor the efficiency of each stage and of the overall process. In addition, the possibility to verify the geographic origin of the primary product increases the value of quality certification (such as PGI, PDO) (Rao *et al*., 2009a), supporting the development of local economies through the commercialization of typical food products.

For all the above mentioned considerations, we believe that the development of a highly comprehensive and efficient traceability system requires an integrated interdisciplinary approach of the different expertises active in the agro-food sciences, that allow to study the system from different points of views.

Genetic traceability

Genetic traceability, based on DNA analysis, refers to the ability of a system to identify the species or genotypes of food stuff components. This system is depth and precise as may allow to identify and trace food components along the *netchain*. For example it has been demonstrated that the DNA sequence of a single mitochondrial gene (in animals) or chloroplast gene (in plants) differs among species but is very much alike in individuals of the same species (Waugh, 2007; Lahaye *et al*., 2008). As a consequence, the nucleotide sequence polymorphism of these genes could be used as a *barcode*, theoretically able to identify every species (http://barcoding.si.edu/).

DNA barcoding in animals is typically based on the mitochondrial gene *cox1* sequence variations available in comprehensive databases. The system is presently routinely used for animal species identification and has successfully contributed towards food authentication. Species discrimination has been very successful in seafood industry (Nicolè *et al*., 2012) allowing to highlight frauds caused by substitution of expensive fish species by cheap one (Eugene *et al*., 2008; Filonzi *et al.,* 2010) as well as in meat industry (Chen *et al*., 2010). In plants a number of different chloroplast genes have been proposed, but there is not yet any universally accepted barcode (Lahaye *et al*., 2008). Nevertheless DNA barcoding proved effective in tracing out olive oil adulteration by canola and sunflower oil, not always easy to identify by using fatty acid analysis (Kumar *et al*., 2011).

For many plant species, the market price of an edible product is largely dependent on the cultivated varieties. Compared to animal samples, the correct identification of the cultivated plant variety requires a deeper level of genetic information as it often relies on intra-species genetic variability. The analysis of such variability at molecular level represents a reliable tool to identify DNA fingerprints of cultivated varieties. Application of DNA fingerprint in plant food traceability allows the authentication of cultivars in commercial edible products. DNA fingerprint may be determined through different types of molecular markers; the most commonly used markers are:

RFLP, Restriction Fragment Length Polymorphism (Van Ooijen *et al*., 1994; Sandbrink *et al*., 1995; Smulders *et al*., 1997);

RAPD, Randomly Amplified Polymorphic DNA (Stevens *et al*., 1995; Grandillo and Tanksley, 1996);

AFLP, Amplified Fragment Length polymorphism (Rao *et al*., 2009b; Rony *et al.*, 2009);

VNTR, Variable Number of Tandem Repeat, or minisatellites (Andreakis *et al*., 2004);

SSR, Simple Sequence Repeat, or microsatellites (Smulders *et al*., 1997; He *et al*., 2003, Corrado *et al*., 2009);

CAPS, Cleaved Amplified Polymorphic Sequence (Yang *et al*., 2004; Caramante *et al*., 2009);

COS, Conserved Ortholog Set (Fulton *et al*., 2002; Van Deynze *et al*., 2007; Labate *et al.,* 2009);

SNP, Single Nucleotide Polymorphism (Labate and Baldo, 2005);

In/Del, Insertion Deletion (Yang *et al*., 2004).

They are all characterized by significant discrimination power although technical feasibility and running costs may differ consistently. The direct identification of polymorphism at the DNA level provides a powerful tool for the authentication of row and processed food components as a pool of DNA sequences may univocally identify a genotype.

The application of DNA fingerprint to the identification, characterization and traceability of plant species and cultivars in food chains has been demonstrated in different studies. For example, Terzi and coworkers (2004) highlighted the possibility to identify wheat species used for pasta production through AFLP markers. Similarly, SSR polymorphism was able to discriminate apple varieties and to authenticate the cultivar *Annurca* in processed food such as apple purée and nectar (Melchiade *et al*., 2007).

Tomato traceability through Simple Sequence Repeat allelic profiles

As mentioned for animal food chains, also for plant food chains it happens that, due to economic interests, premium varieties are replaced with varieties of lower quality. For example, *San Marzano*, a traditional tomato local variety with DPO, is frequently substituted with different cultivars with similar fruit shape and size (Scarano *et al*., 2011). Recently, it has been reported that SSR alleles are stable in the tomato food chain (Caramante *et al*., 2010; Turci *et al*., 2010) and that SSR allelic profiles successfully trace tomato cultivars in peeled, diced and cherry canned tomatoes (Caramante *et al*., 2010). Moreover, it was shown that SSR fingerprinting is useful to evidence erroneous labelling of processed tomato, possible consequence of the failure of the internal traceability system in establishing correct associations between registration numbers and genetic identity of samples entering and exiting the industrial process (Caramante *et al*., 2010).

Olive oils traceability through DNA marker profiles

Olive (*Olea europea* L.) is one of the oldest and most important crops

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in the Mediterranean area. The database of the olive germplasm (http://www.oleadb.it) contains information on 5435 accessions, stored in more than 100 different collections. About two-thirds of the varieties are present in the Southern European countries. Unfortunately, the characterization of olive germplasm resources is complicated by the very large number of available accessions, not always properly classified, that originate several cases of synonymy or homonymy (Bartolini *et al*., 2005). In this scenario the evaluation of olive molecular diversity is particularly important both for discrimination of olive varieties and clones and for the valorisation and protection of high quality extra-virgin olive oils (Doveri *et al*., 2008; Baldoni *et al*., 2009; Rao *et al.,* 2009b; Rony *et al*., 2009).

Cultivar traceability in extra-virgin olive oils has been demonstrated by a number of papers (Busconi *et al*., 2003; Pasqualone *et al*., 2004; Pafundo *et al*., 2005; Muzzalupo *et al*., 2007; Pasqualone *et al*., 2007a; Consolandi *et al*., 2008; Montemurro *et al*., 2008; Alba *et al*., 2009) and recently the possibility to identify the varieties included in simple olive oil blends was also reported (Pasqualone *et al*., 2007b; Corrado *et al*., 2011). In addition, Montemurro and colleagues (2008) demonstrated the high discrimination power of AFLP markers in olive oils identification. These authors show that one AFLP primer combination, revealing 29 polymorphic bands, identifies ten extra virgin olive oils prepared in the laboratory from ten different Italian cultivars. The extension of this analysis to commercial monovarietal olive oils would validate the powerfulness of AFLP markers application in olive food industry.

The improvement of DNA-based methodology to authenticate varieties used for olive oil production represent an important requisite to certify and protect quality labels, against fraud and mislabelling.

Quantification of soft wheat adulteration in durum wheat-based foodstuffs by real-time PCR

Pasta is a traditional Italian product, made of durum wheat (*Triticum turgidum* L. Thell. subsp. *turgidum* convar. *durum* Desf. MK.). Currently, Italian legislation interdicts the production of pasta containing soft wheat (*Triticum aestivum* L. Thell. subsp. vulgare Vill. MK.). Only a maximum of 3% of *T. aestivum* can be tolerated to account for cross contamination during the agricultural process (DPR Reg. 187/2001). However, Italy allows import-export of pasta totally or partially prepared using *T. aestivum*. It is obvious that the composition of such product should be clearly labelled (Sonnante *et al.,* 2009). Consequently, there is a strong interest in the development of molecular methods able to detect soft wheat in pasta.

Monitoring the presence of soft wheat in durum wheat semolina and pasta preparations has always been of interest for Italian food industry and analytical methods used to discriminate between the two species were previously based on the analyses of protein fractions (Cantagalli, 1969; Garcia-Faure *et al*., 1969; Stevenson *et al.,* 1994). However, proteins have a reduced stability in comparison with nucleic acids especially in processed food as bakery products that are exposed to high temperature. Recently, a new method based on DNA screening for sequences localized in the D-genome, has been developed (Bryan *et al*., 1998; Alary *et al*., 2002; Arlorio *et al.,* 2003; Terzi *et al.,* 2003; Pasqualone *et al*., 2007c; Prins *et al.,* 2010). A microsatellite region mapping exclusively on the wheat D-genome proved to be able to detect and quantify soft wheat in durum wheat semolina and in some typical breads made in Southern Italy like *Pane di Altamura* and *Pane di Matera*, awarded with PDO and PGI marks, respectively that have to be prepared exclusively employing durum wheat (Pasqualone *et al*. 2007c; Sonnante *et al.,* 2009) .

Conclusions

Validation of product quality, and safety in agri-food sectors has become a priority. To meet this requirement the development of a highly comprehensive and efficient traceability system, that integrates multidisciplinary approaches, is highly desirable.

Genetic traceability based on DNA markers offers a valuable contribution for the identification of genetic material along the production chains, also because DNA is a molecular label difficult to remove or alter. It may restore consumers' confidence in respect of possible frauds and protect individual food choices as it can partially verify the information upon food labels.

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