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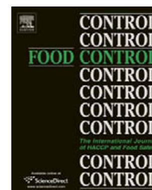

Evaluation of DNA extraction procedures for traceability of various tomato products

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A B S T R A C T

Globalization of food trade requires the development of integrated approaches, such as traceability of origin, quality and authenticity, to ensure food safety and consumers satisfaction. In this study, different genomic DNA extraction procedures were evaluated for their applicability to internal traceability of different products in the tomato food chain. Quality, quantity and amplificability by SSR markers of extracted DNA tallied the methods performances; times and costs were considered too. The results were processed with "fuzzy-logic" approach. "Wizard" (Promega) scored the best performance in methods final ranking. This work demonstrated the value of genomic methodologies for internal traceability of tomato-derived goods.

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1. Introduction

The strategic development of a food chain approach to food quality and safety must be considered within a global context that is constantly evolving in terms of normative and requirements. Globalisation in food trade needs in particular the development of a more integrated and preventive approach.

Expansion in international trade, highly integrated markets, more rapid adoption of new technologies, and an increased market concentration have important implications, both positive and negative, for the development of a food chain approach to food safety (FAO, 2003).

In particular, internal traceability has been indicated as a production action to improve reliability of labelling, to certify the origin and the quality of products on the market, and to prevent fraudulent or deceptive labelling (EC No 178/2002). The European Union has considered the use of high-quality raw material in food production as a prerequisite to obtain a genuine and safe product of adequate nutritional value (White Paper on Food Safety. COM/99/719); consequently internal traceability is assuming a particular relevance in the global process of traceability.

The requirement of internal traceability procedures in food production has stirred also a certain level of technological implementation (Di Bernardo, Del Gaudio, Galderisi, Cascino, & Cipollaro, 2007; Peano, Samson, Palmieri, Gulli, & Marmiroli, 2004). Methodologies based on genetic and molecular biology are acquiring great interest for their applicability to track a given item at any stage along the food supply chain, from "farm to the fork" (Di Bernardo, Galderisi, Cipollaro, & Cascino, 2005). Among these, PCR analysis allows the identification of traces of genomic DNA that may residue in a food matrix from the principal component and/or from contaminants (Marmiroli, Peano, & Maestri, 2003). The DNA extraction method can affect the PCR based analysis by: (i) the presence of PCR inhibitors in the food matrices, (ii) the excessive fragmentation of the DNA molecules, and (iii) the short average length of DNA fragments.

Quantity and quality of the extracted DNA are extremely sample-dependent. In fact the food matrix production, and its chemico-physical composition can introduce many degrees of variability into the DNA extraction methods and in the efficacy of the DNA amplification (Di Bernardo et al., 2007; Peano et al., 2004).

Moreover, a large number of plant species, including tomato, produce secondary metabolites such as phenolic compounds, tannins, flavonoids and alkaloids, whose presence in the extract can interfere with DNA analysis and inhibit its amplification (Di Bernardo et al., 2005). Processing at acid or alkaline pH may constitute

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important steps in any production chains, as well as in tomato, and may be the cause of hydrolytic degradation of DNA (Bauer, Weller, Hammes, & Hertel, 2003). In fact at acid pH purines are removed from the nucleic acid backbone as a result of the cleavage of *N*-glycosidic bonds (between deoxyribose residues and bases). Subsequently, the nearest 3′,5′-phosphodiester bonds are hydrolyzed leading to the dwindling of DNA strands (Anklam, Gadani, Heinze, Pijnenburg, & Van Den Eede, 2002). Temperature degrades DNA as well: tomato processing at high temperatures triggers the Maillard's reaction, the condensation of carbonyl groups of reducing sugars with primary amines. This reaction may result in extensive cross-linking of macromolecules, producing "ball"-shaped proteins and nucleic acids, which hamper DNA extraction and/or amplification.

In this study, molecular techniques based on DNA analyses suitable for traceability along the tomato (*Solanum lycopersicum* L.) food chain were considered. In particular, different DNA extraction procedures, based on either laboratory protocols or commercial kits, were tested on different matrices taken from the tomato food chain. The analysed samples were chosen for their complexity and for the technological treatment they have been subjected to: from fresh tomato to tomato sauce. Quantity and quality of the extracted DNA, amplification of the latter by simple sequence repeats (SSR) markers, costs incurred in carrying each procedure through and the time needed for the overall process were used to assess the total performance of each analysis using a mathematics approach that combines fuzzy logic and expert weights. The former parameters were utilised to select an optimal experimental procedure applicable to the different matrices at the lowest cost.

SSR analysis was carried out with eight tomato-specific primer pairs to compare the allelic patterns of DNA extracted from fresh tomatoes of ten different cultivars with those obtained using DNA extracted from processed products.

2. Materials and methods

2.1. Food matrices

The food matrices to be tested were chosen on the basis of their complexity: tomato fresh fruits, tomato sauce, tomato puree, tomato pulp, whole peeled *S. Marzano* PDO (Protected Designation of Origin) tomato, whole peeled tomato, tomato concentrate and "Arrabbiata sauce". The list of technological treatments these products have undergone is shown in Table 1. Samples of these products were bought in a supermarket; processed products were chosen within the product range of major international brands.

The tomato fresh fruits considered for the subsequent SSR analysis and allele comparison were collected from Parma farmers and are listed in Table 2.

2.2. DNA extraction

All samples were homogenised with Knifetec® 1095 (FOSS TECATOR AB, Hoganas, Sweden). DNA extraction was performed starting from 200 mg of homogenised material utilising several methods, adapted to plant tissues, each sample was extracted in three replicates.

Table 2
List of tomato varieties used for DNA extraction and SSR analysis.

Tomato variety	Seed company
Heinz 1900	Furia sementi (Parma, Italy)
Heinz 3406	Furia sementi (Parma, Italy)
Heinz 7204	Furia sementi (Parma, Italy)
Heinz 9144	Furia sementi (Parma, Italy)
Heinz 9478	Furia sementi (Parma, Italy)
Heinz 9997	Furia sementi (Parma, Italy)
Caliendo	Esasem (Verona, Italy)
Costuluto di Parma	Local variety (Parma, Italy)
Perfect Peel	Peotec (Parma, Italy)
Riccio Grosso	Local variety (Parma, Italy)

The methods tested, as reported in Table 3, were:

(i) *SDS based protocol*: performed by adding 400 μL of extraction buffer [10 mM Tris–HCl pH 8, 150 mM NaCl, 2 mM EDTA pH 8, 1% (w/v) SDS] in a 1.5 mL tube. The mixture was vortexed and incubated at 65 °C for 20 min. The extract was added with 250 μL of cold Phenol/Chloroform/Isoamyl Alcohol (25:24:1) and vortexed. The solution was centrifuged at 11,000*g* for 10 min at room temperature; the supernatant was transferred to a new tube and added with 400 μl of isopropanol at room temperature. After incubation for 20 min at −20 °C, DNA was spun down at 11,000*g* for 10 min and washed with 250 μL ethanol 70% (v/v) at room temperature. The pellet was dried at room temperature and DNA resuspended in 30 μL of TE [10 mM Tris–HCl, 1 M EDTA pH 8] with RNase (200 ng/μL) and incubated at 65 °C for 2 min. At this stage the sample was ready for use or cold storage.

(ii) *CTAB-based method*: the protocol is based on cetyltrimethylammonium bromide (CTAB) buffer and it was performed in our laboratory following the indications of Corbisier et al. (2007).

(iii) *Modified CTAB-based method*: this protocol is a modification of the protocol described in (ii), performed in our laboratory also according to some suggestions from Doyle and Doyle (1990). The main difference between these two methods was the DNA precipitation step: in the latter case only isopropanol was used, while in the former method a precipitation buffer was needed.

DNA extraction was also carried out with commercial Kits specifically developed for food matrices, following the manufacturer's instructions (Table 3) These methods were: (i) QIAamp DNA Stool Minikit (QIAGEN, Milano, Italy) (Tengel, Schuessler, Setzke, Balles, & Sprenger-Haussels, 2001), (ii) NucleoSpin Food (MACHEREY-NAGEL GmbH & Co. KG, Duren Neumman Neander, Duman, Germany), (Hupfer, Hotzel, Sachse, & Engel, 1998), (iii) ChargeSwitch

Table 1
List of the food matrices used for DNA extraction validation.

Products	Ingredients	Mechanical treatment	Thermal treatment	Chemical additives
Tomato	Fresh tomato	No	No	No
Sauces	Tomato sauce	Yes, softly	Yes	No
Puree	Tomato puree and salt	Yes, deep	Yes	No
Pulp	Chopped tomatoes, tomato paste and salt	Yes, softly	Yes	No
S. Marzano	San Marzano PDO peeled tomatoes and tomato juice	Yes, softly	Yes	No
Peeled	Peeled tomatoes and tomato juice	Yes, softly	Yes	No
Concentrate	Tomato paste and salt	Yes, deep	Yes, deep	No
Arrabbiata	Tomato sauce, tomato paste, salt, olive oil, capsicum, garlic, parsley and chili.	Yes	Yes, deep	Citric acid

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