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What do we think we eat? Single tracing method across foodstuff of animal origin found in Greek market

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ABSTRACT

In this study, analysis of 348 food products was performed based on a universal 16S rDNA marker. The purpose was to check whether the content of the product in particular species listed on the label corresponded with the actual composition of the product. All products were purchased from the local market and from national and international super-market chains and included dairy products and industrially processed packaged food from meat, poultry and fish. All products were grouped in seven groups: milk, food for pets, packaged yellow cheeses, packaged white cheeses, PDO cheeses, processed meats and frozen fish foodstuff. Mislabelled foods were found in all seven groups but the extent of adulteration differed between groups. The lowest percentage (15%) was found for packaged yellow cheeses whilst the highest (54%) for foods for pets. Mislabelling for milks was 26%, for packaged white cheeses 29%, for PDO cheeses 26%, for frozen fish products 35% and for processed meats 34%. These alarming findings, combined with those retrieved from the literature, raise significant concern in the monitoring methods employed for supervision worldwide. It is urgent for the authorities to address the adequacy of labelling, to improve and extent monitoring methods and redefine penalising policies against food fraud. On the other hand, the food industry should assume its responsibilities and establish its own accurate and extensive control and inspection mechanisms. Otherwise, there is a growing risk of losing the trust of consumers as well as a large percentage of profits.

1. Introduction

High profile accidents, regarding food products, such as the EU horsemeat scare, the case of halal products that were found to contain pork in South Africa, and more recently the traces of donkey DNA found in processed meat products had made the international headlines in recent months. A report released by the international organisation Oceana published after a 2-year investigation on the seafood fraud within the U.S. revealed that over one-third of the collected and analysed seafood samples were mislabelled, with 74% of sushi restaurants having the worst level of mislabelled fish (Oceana, 2014). These incidents raise important questions from a food safety and consumer protection perspective, especially regarding traceability and how authorities can ensure that the food we eat is indeed what it is labelled to be.

A definition, among others, of food fraud is the "deliberate substitution, addition, tampering, or misrepresentation of food, food ingredients, or food packaging, or false or misleading statements made about a product for economic gain" (Moore, Spink, & Lipp, 2012). Three common categories of food fraud are replacement, addition and removal. In the scientific literature the most common type of fraud reported, seen in 95% of the publications, is replacement, while addition and removal represented less than 5% and 1%, respectively, of the publications (Moore et al., 2012). Food replacement or substitution occurs when one tissues, breeds or species are sold as other tissues, breeds or species. However, although many of the publications are focusing on developing detection methods for food fraud, few publications are devoted to food fraud incidents.

Meat is normally subject to long production and distribution chains and as a food product can be adulterated in many different ways (e.g., by replacing the more expensive cuts with cheaper parts of the animal or with different species, by changing the country of origin, the breed and the way the animal has been reared to better meet the need of the customers etc) (Ballin, 2010). The term "seafood" is used to indicate edible aquatic life forms, such as fish, mollusks, crustaceans and echinoderms, available on the market as whole organisms, or as processed products. The increased demand for seafood, the globalisation of the market and the introduction of several new species in the market have made the control of seafood market more difficult (Barbuto et al., 2010). Dairy products are generally defined as foodstuff made from mammalian milk. Milk products are also subject to fraud especially when the products can be labelled with protected designation of origin (PDO) such as, among others, feta cheese [produced in Greece with

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sheep (*Ovis aries*) and goat *(Capra hircus)* milk] or mozzarella cheese [produced in certain regions of Italy with only with milk from water buffalo (*Bubalus bubalis*)]. The PDO label comes from the European Commission and was introduced to protect traditional foods. The fraud is committed when sheep and buffalo milks are replaced with cheaper milks in order to increase profit. Economic relevance, risk of allergies and religious practices are the main reasons for which an entire traceability system was developed to assess authenticity and adulteration of milk-derived products (Mafra, Ferreira, & Oliveira, 2008).

Regulation 178/2002/EC has provided a basis for consumers to make informed choices by preventing "fraudulent or deceptive practices," any "adulteration of food" and any other practices which may be misleading. These principles are confirmed and further specified in Regulation 1169/2011, which requires that the labelling and the methods used must not be such as could mislead the purchaser to a material degree, particularly to the characteristics of the foodstuff and, specifically, to its nature, identity, properties, composition, quantity, durability, origin or provenance, method of manufacture or production.

Molecular authentication or molecular traceability of meat (Valentini, Pompanon, & Taberlet, 2009) and crops (Galimberti et al., 2014), which is based on the PCR amplification of DNA, gave an important boost to detection methods that allow identification of different species in foodstuff and/or of the different components in processed food. In a recent study (Sarri et al., 2014), bioinformatics were used to design universal primers, targeting a short segment within the 16S rRNA gene. This segment was proven to be a good candidate for a rapid and accurate method to identify all kinds of tissue tested in both raw and processed samples in a wind range of species. In this study, we performed the analysis of 348 food products either as milks and cheeses or subjected to various cooking methods or technological processes, based on this 16S rDNA marker. All products were purchased from the local market and from national and international supermarket chains. The purpose was to check whether the content of the product in particular species listed on the label corresponded with the actual composition of the product.

2. Materials and methods

Three hundred and forty-eight processed food products were purchased from 2010 to 2013 from the local market and from national and international super-market chains in Central Greece (Region of Thessaly) (Table 1). Processed foodstuff included dairy products and industrially processed packaged food from meat, poultry and fish. All products were grouped in seven groups: milk, food for pets, packaged yellow cheeses, packaged white cheeses, PDO cheeses, processed meats and frozen fish foodstuff. The selected foodstuff constitute the majority of the products encountered in the Greek market in each category. All products are kept and preserved frozen in our laboratory.

Total DNA from each sample was extracted in triplicate for maximum reliability. As all of the samples were processed foodstuff, triplicates ensured that all species included would be represented. All solid samples were chopped with sterile surgical blade. DNA isolation from all samples was performed using PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA 92008, USA) according to the manufacturer's instructions. The set of universal primers used for PCR amplifications for 16S rDNA marker as well as reaction and cycling conditions were previously described (Sarri et al., 2014). To eliminate possible PCR artefacts leading to erroneous nucleotide substitutions for each specimen, besides the use of a proofreading polymerase, three PCR replications were also performed for each extraction and excellent reproducibility was observed.

The composition of each product for particular species was screened using the single-strand conformation polymorphism (SSCP) method. This method allows the detection of polymorphisms in short DNA segments due to mobility differences of single-stranded DNA fragments during electrophoresis in polyacrylamide gels (Orita, Iwahana, Kanazawa, Hayashi, & Sekiya, 1989). PCR amplifications of the 16S rDNA marker in a wide range of species and subsequent application of the SSCP method had shown completely different profiles for each species (Sarri et al., 2014). Furthermore, we performed an analysis of 92 samples artificially prepared, after grinding an admixture of an increasing number (up to five) of different species [chicken (*Gallus gallus domesticus*), turkey (*Meleagris gallopavo*), sheep (*O. aries*), pig (*Sus scrofa domesticus*) and beef (*Bos taurus*)]. Each admixture contained a combination of different species in different quantities. After PCR amplification of the 16S rDNA marker the SSCP method was capable of fully discriminating up to four species within an admixture regardless of the quantity of the species' meat even in highly asymmetric mixtures where the presence of the species in the mixture was the minimum (1%) (Sarri et al., 2014). SSCP was applied as follows: 5 μl of the PCR products was mixed with 10 μl of loading dye (95% v/v formamide, 10 mM NaOH, 0.05% w/v bromophenol blue, 0.05% w/v xylene cyanol), denatured at 95 °C for 6 min, cooled on ice and loaded onto a 10% polyacrylamide gel. The samples were electrophoresed in 0.5× TBE buffer at 220 V for 18–20 h at 4 °C. SSCP separations for each product always included previously typed DNA of each species that served as standards to ensure genotype scoring. The resulting bands were visualised by silver staining, according to Sambrook, Fritsch, and Maniatis (1989). In the cases where the SSCP profiles did not correspond to the basic profiles of the species under study (e.g., food for pets and fish products), PCR products were sequenced directly and bi-directionally by Macrogen Inc. Nucleotide sequences were compared against known available sequences retrieved from GenBank, using BLAST scores. All sequences examined scored 100% identity with the reference sequences.

Although no quantification of DNA was performed, extraction success was verified by successful PCR amplification. Consistently, the same SSCP profile in all three replicates ensured the PCR amplification success.

3. Results and discussion

Mislabelled foods, concerning substitutions and/or additions of tissues of one species with tissues from another species, were found in all seven groups of processed products that had been analysed during this study (Table 1). However, the extent of adulteration differed between groups (Fig. 1). The lowest percentage was found in packaged yellow cheeses while the highest in pet food. For the 47 analysed packaged yellow cheeses, the percentage of mislabelled products was 15%, and the problems were confined to few products with the addition of either goat or cow milk products labelled as sheep cheeses. For the 50 milk tested, 26% of the products were mislabelled. Among the 33 products labelled as "sheep milk," six also contained goat milk and six also goat and cow milk; of the seven products labelled as "goat milk," two also contained sheep milk. For the 35 packaged white cheeses, up to 29% of the products were mislabelled. In one product labelled as sheep and goat cheese there was a substitution with cow cheese, in four products labelled as goat cheese sheep milk was added; cow milk was traced in a product labelled as sheep cheese and in four products labelled as sheep and goat cheese no goat milk was present. For the 86 processed meats, the mislabelled products reached 34%. Sixteen cases of removal, seven cases of addition and five cases of the substitution of tissues of particular species were recorded compared to the initial statement in the label. Within the group of the 23 PDO cheeses 26% of the products were mislabelled. However, even with the additions and removals, all products that have been tested corresponded to the PDO norms established by the Hellenic Ministry of Rural Development & Food http://www.minagric.gr/images/stories/docs/agrotis/POP-PGE/cheese-low40.pdf. Thirty-five percent of the mislabelled cases within the group of the 31 frozen fish products concerned substitutions of species but also the misconception between commercial common name used and the scientific names of the species contained in sea foods. In Greece, the word "bakaliaros" is used without distinction for a number of