International Journal of Clinical Nutrition & Dietetics
Original Reports Open Access

Embracing the Next-Generation Technology in Food Industry

Ana Sofia Moreira, Inês Eulálio, Inês Gomes and Daniela Silva*
ALS Controlvet - Segurança Alimentar, S.A Tondela, Portugal

Abstract

In recent years there has been a significant increase in fraud cases in different food sectors. The complex nature of our globalized food supply chains and the economic motivation to provide cheaper food products have contributed to the prevalence of food fraud (20). In this regard, the European Parliament and the Council approved "Regulation (EC) No 178/2002" states the general principles and requirements of food law (General Food Law Regulation), stating that producers need to provide an appropriate declaration of ingredients, i.e. by naming species components of food products. In this context, verification of food products authenticity is nowadays one of the most requested requirements for distributors and consumers for reasons of transparency of labelling, ethnic origins, public health and economic transactions. The Next Generation Sequencing (NGS) technologies represent a turning point in the food conformity assessment field, particularly for species identification in matrixes composed of a blend of two or more species. In this study, NGS technologies were applied by testing its usefulness to the food ingredient traceability. DNA sequencing analysis of food products enables species detection in a completely unbiased manner, allowing the detection of fraud by mixing undeclared species. Using selected primers to amplify barcode genes, followed by high throughput DNA sequencing and complex tailored bioinformatics analysis, it is now possible to conduct a complete food ingredient screening analysis, even in complex mixtures and highly processed food items. With this cutting-edge approach, we developed a fast, safe and economically effective solution for species identification and fraud detection. In this study, we present the successful results of our internal NGS pipeline in commercial samples like fish, mammalian, birds, crustacean mollusk and vegetable products, apart from meeting the agro-food industry needs regarding food safety and consumer rights.

Publication History:

Received: April 19, 2021
Accepted: June 09, 2021
Published: June 11, 2021

Keywords:

NGS authenticity, Food industry, Fraud, Bioinformatics, Public health

Introduction

Consumer audience changed food landscape, which responds to the rise in veganism, a greater focus on allergens and demand for craft and artisan fare in home and in restaurants. Quality, safety of food and negative effects of bio-industrial production has become a concern for the today's consumer [1]. Despite many food quality control programs, the safety perception of consumers has decreased significantly [2]. Food sector has been rapidly internationalized, and it's location is no longer confined to local or regional supply. The products from the retailers and food industries come from all over the world, transforming the food industry towards an interconnected system with a large variety of complex relationships. Hence these developments, governments, both national and international are responding to this by imposing new legislation and regulations to ensure labelling transparency, ethnic origins, public health and economic transaction [3].Traceability has become an essential requirement to ensure the quality of food products that reach the market. Their implementation in the food industry involves the development of control systems of raw materials, from their entry into the chain of production to their marketing, ensuring the quality and reliability of food for both the producer and the consumer. Species identification based on morphological character are possible and even relatively easy where the manipulation is minimal usually those which are sold whole and without transformation processes, both fresh as chilled or frozen [4]. However, in other cases were external morphological characteristics are eliminated during the processing phase, identification is not possible. Currently most classification methods for species identification are mostly focused on the detection/amplification of a single DNA species based on PCR technology. On the other hand, if the analysis requires the ability to detect a wide variety of different species, would require testing for each specific kind, making it tedious, costly and not always applicable [5].

DNA barcoding is revolutionary to promote greater traceability and transparency, which is a molecular technique based on the DNA-level identification of differences that univocally characterize individual species. The potential of this technique increased with the merge of next generation sequencing (NGS) platforms speeding up the possibility of simultaneously analyzing multiple ingredients from complex matrixes [6]. NGS technology is based on the massive sequencing of DNA, with significant increase in the ability to obtain information of the sequences of individual molecules within a source of complex or degraded DNA. NGS encompasses both massively parallel and single-molecule sequencing, which provides short and long sequencing reads, respectively. The advantages of this technology include scalability, simplicity and less error prone. This technique is still economically viable and capable of producing results in real time. These are the following main types of new generation sequencing applications:(i) determination of the whole genome sequence of a single cultured isolate, which is commonly referred to as "whole genome sequencing" (WGS) and (ii) "target sequencing", directed sequencing of a determined genome region, among other. The target sequencing demonstrates a huge potential in the areas of food safety and control [7]. For this reason, the FDA is now exploring new regulatory approaches to ensure that NGS tests have adequate analytical performance of the complexity and enormous potential for useful information to generate this new technology. With this approach, we can rapidly identify the genomic information in a given sample [8]. Thus, NGS can be used against problems in food industry,

***Corresponding Author:** Dr. Daniela Silva, ALS Controlvet - Segurança Alimentar, S.A Tondela, Portugal; E-mail: daniela.silva@alsglobal.com

Citation: Moreira AS, Eulálio I, Gomes I, Silva D (2021) Embracing the Next-Generation Technology in Food Industry. Int J Clin Nutr Diet 7: 157. doi: https://doi.org/10.15344/2456-8171/2021/157

customize internal reporting based on top-of-the-line science and business practices. Hence, this study will demonstrate the efficiency of NGS-based DNA metabarcoding targeted to identify the species presented in food labels not only in with plant, meat, fish, crustacea species [9]. Regarding the fish, meat and crustacea analysis, this study considers high throughput sequencing of different fragments from barcoding genes namely Cytochrome oxidase I (COX I) and Cytochrome b (Cyt b). Whereas the plant approach considers different fragments from different barcoding genes namely Maturase K gene (MATK), Ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit gene (RBCL) and Internal Transcribed Spacer 1 (ITS1).

Materials and Methods

Sample preparation and analysis

Samples used in this type of analysis were purchased from local retailers. The authenticity of the samples was verified by Sanger sequencing of mitochondrial gene. The analysis of the sequences of specific organisms has as a target, mitochondrial genes for animal species (COI and Cytochrome B) and for vegetable species chloroplast genes such as (matK, rbcl and trnH-psbA). Apart from that, the present study also identifies a group of species by genes. The method is based on the high throughput sequencing different fragments from barcoding genes.

Analyzed samples were previously homogenized to have better reliability of the results. This step is necessary and applicable by grinding/milling. The amount necessary to be analyzed should be adjusted according to the instructions of the DNA extraction kit used, DNeasy® mericon® Food QIAcube® HT Protocol, Quiagen. The kit DNEasy Mericon 96 QIAcube HT Kit is used for the DNA purification of DNA from a variety of processed matrices, minimizing the transition from PCR inhibitors inherent in complex food samples. The DNeasy mericon 96 QIAcube HT Kit uses modified cetyltrimethylammonium bromide (CTAB) with proteinase K. To the 350µl samples, 350µL of CTAB buffer.

DNA extracted from the test samples are subjected to PCR for amplification of barcoding regions (developed internally). Three microliters DNA template was added to the PCR mix for a total volume of 25 µL. The PCR protocol includes an initial denaturation at 95°C for 2 min, followed by 45 cycles at 95°C for 30s, 48°C for 30 s, and 72°C for 30s. For terminal extension, the reaction was heated for 5 min at 72°C.

DNA library was created with equal amounts of mixed PCR products. The libraries were purified with AgentCourt® AMPure® XP beads considering manufactures instructions. Qubit™ dsDNA HS Assay Kit was used to quantify the final library. Purified amplicons were then used for the preparation of the amplicon libraries for subsequent NGS sequencing in the Ion torrent system (illustrated in Figure 1). Generically, purified amplicons are subjected to End Repair reactions followed by ligation of the sequencing adapters, and nick repair to complete the bond between adapters and inserts. Finally, the amplification of the libraries, the procedure is schematically illustrated below.

The sequences obtained from Chef™ System and Ion PGM™ Systems (Thermo Fisher Scientific Inc.) [4]. A specific bioinformatic analysis pipeline was specifically and internally constructed to process the large amount of sequences generated by the NGS technology which includes grouping identical sequences and matching with genetic data bases for species identification.

Specificity/selectivity

Primers were designed internally based on sequence alignment of the barcoding regions from species available in genetic databases. Conserved regions flanking variable regions were selected for primer design. Four different regions from barcoding genes were selected. The size of the amplified regions, was kept between 200 bp and 400 bp [10]. Primer coverage was analyzed "in silico" by evaluation of primers and probes using the tool National Center for Biotechnology Information (NCBI) Nucleotide Blast (https://blast.ncbi.nlm.nih.gov/Blast.cgi?PROGRAM=blastn&PAGE_TYPE=BlastSearch&LINK_LOC=blasthome).

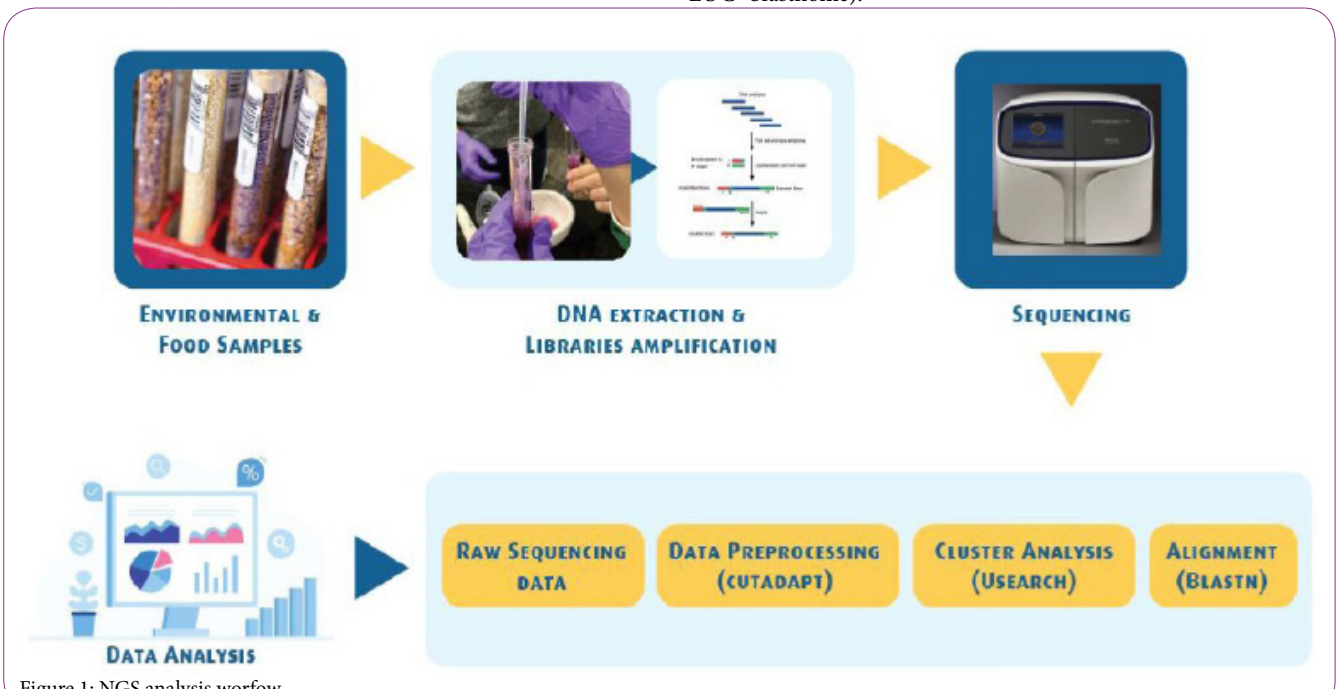

Figure 1: NGS analysis worfow.

Bioinformatic pipeline

Raw sequences were demultiplexed according to the Ion Torrent (Life Technologies) assigned to samples and saved in FASTQ files. The files are, then processed to trim low-quality bases from the end of the reads and filter out unsuitable ones. Reads < 200 were also removed. At present, there are many software programs for data quality preprocessing. To the library preparation we use Cutadapt [11], which is widely user, to remove adapters and identify the targeted fragments [12]. The resulted sequences were then clustered at 98% similarity and groups them together, using the USEARCH algorithm [13]. The sequences generated were then matched against the target database using the BLASTn search based on the MegaBLAST algorithm BLASTn parameters may be adjusted according to the different analysis. The BLASTn results were parsed to count how many times a species was identified in the read data, according to input parameters [14].

Results

Strategy food analysis

For the detection/identification of fish and meat species, 3 different fragments were selected from 2 mitochondrial genes namely the cytochrome c oxidase1 (COX 1) and cytochrome b gene (Cyt b). For the identification/detection of crustacea 3 different fragments were selected from cytochrome c oxidase1 (COX 1) gene and ribosomal RNA 16S gene [15].

However for the detection/identification of vegetable origin is only possible using different fragments of 3 different coding genes, namely Maturase K (MATK) gene, gene of the large subunit of ribulose-1,5-bifosfato carboxilase/oxigenase (RBCL) and internal transcript spacer 1 (ITS1The analysis presented on this study was designed to determine the adulteration of different types of species in simple and complex foodstuff by using next-generation DNA sequencing with bioinformatic analysis.

Sample analysis

This simple approach matches and identifies more than 20 different types of food labelling. The samples, which some of them were pure and others contained mixtures of different tissues, lead to the correct identification of the intended species. The BLASTn analysis produces results were the most significant results were the ones with the lowest E-value. The lowest E-value represents the most accurate matching during the blast analysis [16]. The BLASTn results were parsed to count how many times a specie was identified, according to the input parameters. The results of the clustered analysis were included has well. All species detected using DNA sequences obtained using the Next generation DNA sequencing (NGS) methodology could be identified to the species level to the threshold of 98% of similarity against a curated reference DNA library. We have analyzed 342 fish samples, 482 meat samples, 35 crustacea samples, 59 plant samples. Table 1 lists some of the species analyzed by target sequencing, with the %ID of each species.

The limit of detection was determined using mixtures of gDNA from different species in different DNA concentrations containing 10%, 1%, 0.5% 0.1% of the target species to identify. The obtained results are presented on Table 2. In all cases the obtained limit of detection was 0.5% of target specie in a mixture of other species (either from the same or different group of species).

Common name	Scientific name	Analysis Pipeline
Whiting Sea bass Atlantic cod Common dab Hake Blue whiting Common ling Smooth Hound Rainbow trout Atlantic pollock Atlantic salmon Violet warehou Sea bream Yellowfin tuna Atlantic horse mackerel Striped catfish Poor cod Pouting	Brotula barbata (98% ID), Dicentrarchus labrax (98% ID), Gadus morhua (98% ID), Limanda limanda (98% ID), Merluccius merluccius (98% ID) Micromesistius poutassou (98% ID) Molva molva, Mustelus punctulatus (98% ID) Oncorhynchus mykiss (98% ID) Pollachius pollachius (98% ID) Salmo salar (98% ID) Schedophilus velaini (98% ID) Sparus aurata (98% ID) Thunnus albacares (98% ID) Trachurus trachurus (98% ID) Pangasianodon (98% ID) Hypophthalmus (98% ID) Trisopterus minutus (98% ID) Trisopterus luscus (98% ID)	Fish
Duck Cow Goat Deer Horse Chicken Turkey Rabbit Lamb Pork Wild boar Quail Pheasant Partridge	Anas sp (98% ID) Bos Taurus (98% ID) Capra hircus (98% ID) Cervus elaphus (98% ID) Equus caballus (98% ID) Gallus gallus (98% ID) Meleagris gallopavo (98% ID) Oryctolagus cuniculus (98% ID) Ovis aires (98% ID) Sus scrofa (98% ID) Coturnix japonica (98% ID) Pahsianus colchicus (98% ID) Alectoris sp (98% ID)	Meat
Indian white prawn Jinga shrimp Greasyback shrimp Norway lobster Brown crab Goose neck barnacle European green crab Indian white prawn Western king prawn Pink spiny lobster Velvet crab Deep-water rose shrimp Giant tiger prawn	Penaeus indicus (98% ID) Metapenaeus affinis (98% ID) Metapenaeus ensis/ Metapenaeus Monoceros (98% ID) Nephrops norvegicus (98% ID) Cancer pagurus (98% ID) Pollicipes pollicipes (98% ID) Carcinus maenas (98% ID) Penaeus indicus (98% ID) Penaeus latisulcatus (98% ID) Palinurus mauritanicus (98% ID) Necora puber (98% ID) Parapenaeus longirostris (98% ID) Penaeus monodon (98% ID)	Crustacea
Celery Almond Peanut Oat Hazelnut Cashew Brazil Nut Common Walnut Pistachio Sesame Soy Lupine Pumpkin Garlic Apricot Eggplant Beet Broccoli Carrot Zucchini Spinach Raspberry	Apium graveolens (98% ID) Prunus dulcis (98% ID) Arachis hypoaea (98% ID) Avena sativa (98% ID) Corylus avellane (98% ID) Anacardium occidentale (98% ID) Bertholletia excelsa (98% ID) Juglans regia (98% ID) Pistacia vera (98% ID) Sesamum indicum (98% ID) Glycine max (98% ID) Lupinus albus (98% ID) Cucurbita pepo (98% ID) Allium sativum (98% ID) Prunus armeniaca (98% ID) Solanum melongena (98% ID) Beta vulgaris (98% ID) Brassica oleracea (98% ID) Daucus carota (98% ID) Cucurbita pepo (98% ID) Tetragonia tetragonoides (98% ID) Rubus idaeus (98% ID)	Plant

Table 1: Summary of the "pure" samples analyzed, with the respective analysis pipeline identified. Since the plant analysis, has a lot of pure samples, this table presents some species.

Mixtures species	Result	Analysis Pipeline
Pollachius pollachius Gadus morhua	Gadus morhua Pollachius pollachius	Fish
Salmo salar Trachurus trachurus	Trachurus sp Salmo salar	
Salmo salar Trachurus trachurus	Salmo salar Trachurus sp	
Thunnus albacares Salmo salar	Thunnus albacares Salmo salar	
Salmo salar Sardina pilchardus	Sardina pilchardus Salmo salar	
Trachurus trachurus Sardina pilchardus	Trachurus sp Sardina pilchardus	
Molva molva Pollachius virens	Molva molva Pollachius virens	
Pangasionodon hypophthalmus Salmo Salar	Pangasianodon hypophthalmus Salmo salar	
Reinhardtius hippoglossoides Polachius virens	Reinhardtius hippoglossoides Pollachius virens	
Microstomus kitt Clupea harengus	Microstomus kitt Clupea harengus	
Pollachius virens Trachurus trachurus Gadus ogac	Gadus macrocephalus/ogac Trachurus sp Pollachius virens	
Microstomus kitt Pleuronectes platessa Sus Scrofa	Microstomus kitt Sus scrofa Pleuronectes platessa	
Merluccius merluccius Salmo salar Limanda limanda	Merluccius merluccius Salmo salar Limanda limanda	
Thunus Albacares Schedophilus velaini Scomber scombrus	Thunnus albacares Schedophilus velaini Scomber scombrus	
Pollachius virens Merlangius merlangus Molva molva	Molva molva Pollachius virens Merlangius merlangus	
Sebastes sp Merluccius merluccius Salmo salar Limanda limanda Sardina Pilchardus	Sebastes sp Merluccius merluccius Salmo salar Limanda limanda Sardina pilchardus	
Duck; Chicken	Anas sp; Gallus gallus	Meat
Cow; Pork	Bos taurus; Sus scrofa	
Cow; Pork; Rabbit	Oryctolagus cuniculus; Sus scrofa; Bos taurus	
Cow; Pork; Rabbit; Chicken	Bos taurus; Sus scrofa; Oryctolagus cuniculus; Gallus gallus	
Chicken; Turkey; Quail; Pheasant; Duck	Gallus gallus; Meleagris gallopavo; Coturnix japónica; Pahsianus colchicus; Anas sp	
Penaeus indicus; Cancer pagurus	Penaeus indicus;Cancer pagurus	Crustacea
Penaeus indicus; Metapenaeus monoceros / Metapenaeus ensis	Penaeus indicus; Metapenaeus monoceros / Metapenaeus ensis	
Penaeus indicus; Necora puber	Penaeus indicus; Necora puber	
Penaeus indicus; Penaeus latisulcatus	Penaeus indicus; Penaeus latisulcatus	
Pollicipes pollicipes; Penaeus indicus	Pollicipes pollicipes; Penaeus indicus	
Penaeus indicus; Necora puber; Nephrops norvegicus	Penaeus indicus;Nephrops norvegicus;Necora puber	
Louisiana Crayfish (Procambarus clarkii); Escargot (Helix lucorum); Yellow Mustard (Sinapis alba) Soya	Procambarus clarkii	
Juglans regia; Macadamia integrifolia	Macadamia sp (inc Macadamia integrifólia);Juglans sp (inc Juglans regia)	Plant
Apium graveolens; Lupinus albus	Lupinus albus ;Apium graveolens	

Table 2: Summary of the mixture's species analyzed, considering the different pipeline analyzes.

Real Samples	Result	Analysis Pipeline
Cod with cream	Gadus morhua (Atlantic cod)	Fish
Tuna Flour	Katsuwonus pelamis (Bonito) ; Thunnus sp (Tuna)	
Salmon Flour	Thunnus sp ; Auxis rochei; Oncorhynchus sp; Scyliorhinus canicula; Salmo salar;	
Roe cod	Gadus morhua (Atlantic cod)	
Hake patties	Merluccius hubbsi (Argentina hake)	
Individual cod fillet	Gadus morhua (Atlantic cod)	
Codfish pastries	Gadus morhua (Atlantic cod)	
Hake fillets	Merluccius paradoxus (Namíbia hake);	
Merluccius capensis (South Africa hake)		
Salmon rolls	Salmo salar (Atlantic Salmon)	
Frozen cod pastries	Gadus morhua (Atlantic cod)	
Frozen Sea Delights	Trichiurus japonicus ; Atrobucca nibe); Sardinella longiceps (Sardinela); Sarda orientalis; Scomberomorus niphonius Decapterus maruadsi; Ilisha elongata; Trachurus sp.; Larimichthys polyacti;	
Deep-frozen cod pastries	Gadus ogac/macrocephalus;	
Gadus morhua		
Hake to boi	Merluccius capensis	
Delights of the sea	Theragra Chalcogramma	
Sea snacks	Theragra Chalcogramma	
Shredded cod	Gadus morhua	
Codfish slices	Gadus morhua	
Frozen shredded cod	Gadus morhua	
Hake medallions	Merluccius paradoxus ; Merluccius capensis	
Hake loins	Merluccius paradoxus;Merluccius capensis	
Hake patties	Merluccius hubbsi	
Seafood cocktail	Theregra Chalcogramma	
Spiritual Codfish	Gadus morhua	
Norway smoked tuna	Thunnus albacares	
Smoked salmon marinated norway	Salmo salar	
Hake fishblock	Merluccius productus	
Alheira caça	Sus scrofa (swine); Cervus elaphus (Deer)	Meat
Alheira caça chaves	Sus scrofa (Swine); Cervus elaphus (Deer);	
Alheira caça chaves	Sus scrofa (Swine); Cervus elaphus (Deer);Oryctolagus cuniculus (Rabbit);	
Alheira Mirandela	Sus scrofa (swine); Gallus gallus (chicken); Bos taurus (bovine)	
Duck rice	Anas sp (Duck);	
Bacon strips	Sus scrofa (Swine)	
Meat Samosas	Bos taurus (Bovine);	
Cheeseburger	Sus scrofa (Swine);	
Chouriça Ponte de Lima	Sus scrofa (Swine);	
Chouriça onion Ponte de Lima	Sus Scrofa (Swine)	
Chorizo	Sus scrofa (Swine)	
Chorizo wine	Sus scrofa (Swine)	
Moorish chorizo Ponte de Lima	Sus scrofa (swine)	
Iberian pork chorizo	Sus scrofa (swine)	
Black pig chorizo	Sus scrofa (Swine)	

Continue...

Black Chorizo Beira Alta	Sus scrofa (Swine)	
Black Chorizo Beira Litoral Quiaios	Sus scrofa (Swine)	
Chorizo Quiaios	Sus scrofa (Swine)	
Chorizo wine Beira Alta Seia	Sus scrofa (Swine)	
Meat croquettes	Sus scrofa (Swine); Bos taurus (Bovine)	
Meat pies	Bos taurus (Bovine); Sus Scrofa (Swine)	
Lamb meal	Ovis aries (Ovino); Sus scrofa (Swine); Bos taurus (Bovine); Gallus gallus (Chicken); Capra hircus (Goat)	
Poltry meal	Gallus gallus (chicken); Meleagris gallopavo (turkey); Anas platyrhynchos (Duck); Sus scrofa (Swine);	
Farinheira Beira Alta Seia	Sus scrofa (Suino)	
Farinheira Beira Baixa Fundão	Sus scrofa (Swine)	
Ham chicken	Gallus gallus (Chicken)	
Extra leg ham	Sus scrofa (Swine)	
Duck Foie Gras	Anas sp (Duck)	
Hot dog	Sus Scrofa (Suino)	
Bolognese Lasagna	Sus scrofa (Swine); Bos Taurus (Bovine)	
Stuffed squid (stuffing)	Sus scrofa (Swine)	
Pasta ravioli meat	Sus scrofa (Swine); Bos taurus (Bovine)	
Ham tortellini pasta	Sus scrofa (Swine)	
Goat lunch box	Capra hircus (Goat)	
Mini puffed hunting sausage	Sus scrofa (Swine); Oryctolagus cuniculus (Rabbit) Cervus elaphus (Deer)	
Mini meat croquettes	Sus scrofa (Swine); Bos Taurus (Bovine)	
Arganil black pudding	Sus scrofa (Swine)	
Mortadela	Sus scrofa (Swine);Gallus gallus (Chicken); Meleagris gallopavo (Turkey);	
Mortadella with olive	Sus scrofa (Swine); Bos Taurus (Bovine)	
Simple mortadella	Sus scrofa (Swine); Bos Taurus (Bovine)	
Duck Mousse	Anas sp (Duck)	
Duck Mousse with orange scent	Anas sp (Duck)	
Truffled Duck Mousse	Anas sp (Duck)	
Duck mousse with fine herbs	Cairina moschata (Wild duck); Gallus gallus (Chicken)	
Chicken breast nuggets	Gallus gallus (Chicken)	
Sirloin steak Beira Alta Seia	Sus scrofa (Swine)	
Paio loin Beira Alta Seia	Sus scrofa (Swine)	
Breaded turkey	Meleagris gallopavo (Turkey)	
Pate with mushrooms	Sus scrofa (Swine);Gallus gallus (Chicken)	
Pate with fine herbs	Sus Scrofa (Swine)	
Poultry with Green Pepper	Sus scrofa (Suino); Gallus gallus (Chicken); Cairina moschata (Wild Duck)	
Turkey breast	Meleagris gallopavo (Turkey)	
Turkey breast reduces in salt	Meleagris gallopavo (Turkey)	
Turkey breast	Meleagris gallopavo (Turkey)	
Turkey breast with herbs	Meleagris gallopavo (Turkey)	
Turkey breast slices	Meleagris gallopavo (Turkey)	
Smoked Turkey chest with herbs	Meleagris gallopavo (Turkey)	
Turkey chest wood oven	Meleagris gallopavo (Turkey)	

Continue...

Turkey breast wood oven	Meleagris gallopavo (Turkey)	
Fresh chicken pizza	Gallus gallus (Chicken)	
Fresh cheese / ham / mushrooms pizza	Sus scrofa (Swine)	
Pizza hawaiian wood oven	Sus scrofa (Swine)	
Roman pizza oven	Sus scrofa (Swine)	
Black pork ham	Sus scrofa (Swine)	
Bufala burrata cheese	Bos taurus (Bovine); Bubalus bubalis (Bufalo)	
Yellow cheese from Beira Baixa	Ovis aries (Sheep); Capra hircus (Goat)	
Azeitao Cheese	Ovis aries (sheep)	
Goat cheese	Capra hircus (Goat)	
Alentejo goat cheese	Capra hircus (Goat)	
Serra da Gardunha goat cheese	Capra hircus (Goat)	
Seia buttery sheep cheese	Ovis aries (sheep)	
Seia buttery sheep cheese	Ovis aries (Sheep)	
Seia buttery sheep cheese	Ovis aries (Sheep)	
Buttery cured sheep cheese	Ovis aries (Sheep)	
Rabaçal cheese	Capra hircus (Goat); Ovis aries (sheep)	
star saw cheese	Ovis aries (Sheep)	
Sheep cheese Seia	Ovis aries (sheep)	
DOP star saw cream cheese	Ovis aries (Sheep);Capra hircus (Goat)	
Salami slices	Sus scrofa (Swine)	
Salpicão Beira Arganil Coast	Sus scrofa (Swine)	
German sausage	Sus scrofa (Swine)	
Sausage poultry	Gallus gallus (chicken); Meleagris gallopavo (Turkey)	
Spicy BBQ Sausage	Sus scrofa (Swine)	
Hot dog sausage	Gallus gallus (Chicken); Meleagris gallopavo (Turkey); Sus scrofa (Swine)	
Sausages	Gallus gallus (Chicken); Sus scrofa (Swine); Meleagris gallopavo (Turkey)	
German sausages	Sus Scrofa (Swine)	
Tortellini with ham	Sus scrofa (Swine); Bos taurus (Bovine)	
	Parapenaeus longirostris	Crustacea
Linguini shrimp and watercress pesto	Penaeus monodon	
Patties Knife shrimp	Haliporoides triarthrus	
shrimp bread soup	Metapenaeus dobsoni; Metapenaeus monoceros/Metapenaeus ensis; Metapenaeus affinis; Metapenaeus brevicornis	
Swab Taken From Knife	Pandalus borealis	
Patties Knife shrimp	Haliporoides triarthrus Parapenaeopsis sp	
Brown crab	Cancer pagurus	
Knife shrimp (Haliporoides triarthrus)	Haliporoides triarthrus Parapenaeopsis	
Peri Peri	Capsicum sp. (Inc. Capsicum frutescens)	Plant
Red Fruits Tea	Camellia sp (inc Camellia sinensis)	
Citrus Infusion	Citrus sp (inc Citrus sinensis);Malus domestica; Glycyrrhiza sp (inc Glycyrrhiza glabra) (≥98% ID);Sambucus sp	
Lucia lima infusion	Aloysia citrodora; Bidens sp	

Continue...

Lemongrass infusion	Melissa officinalis	
Indian saffron	Curcuma sp. (inc Curcuma longa)	
Orange nectar	Citrus sp. (inc Citrussinensis)	
Potato sticks	Solanum tuberosum	
Pesto sauce alla genovesa	Ocimum basilicum;Arachis hypogaea;Anacardium occidentale Portulaca oleracea	
Leaf oregano	Origanum vulgare	
Gnocchi With Tomato And Spinach	Triticum sp (inclui Triticum aestivum), Spinacia sp. (inclui Spinacia oleracea), Solanum sp (inclui Solanum tuberosum), Allium sativum, Hordeum vulgare	
Cinnamon Stick	Cinnamomum sp (inc Cinnamomum verum)	
Vegetarian Meal w / Falafel Lebanese-Style	Capsicum annum; Cicer arietinum; Cucurbita pepo; Daucus sp. (inclui Daucus carota); Glycine max; Petroselinum crispum; Plantago ovata; Triticum sp. (inclui Triticum aestivum);	
Orange Nectar Algarve	Citrus sp. (inclui Citrus sinensis)	
Shovel Ham	Glycine soja	
Alheira	Triticum sp (inc Triticum aestivum); Glycine soja; Capsicum sp (inc Capsicum annuum)	
Fruit and Nut Cookies	Corylus sp. (inc Corylus avellana);Ostryopsis davidiana; Triticum sp. (Triticum aestivum)	
Roasted Pistachio	Pistacia vera	

Table 3: Summary with the results from the real samples analyzed.

Additionally, food samples, of complex composition were retrieve from the supermarket and analyzed using the developed methodologies. In all the analyzed samples, we were able to identify all the ingredients present on the label (Table 3) confirming that the methods developed are suitable to be put in use for food fraud detection.

Discussion

Species identification resorting to Sanger sequencing has been the gold standard method so far and has been proved a powerful tool. With the development of the new technologies namely the NGS technology based on massive DNA sequencing, with a significant increase in the ability to obtain information on the sequences of individual molecules within a complex or degraded DNA source. The advantages of this technology include scalability, simplicity, being less prone to errors, and even more economically viable with the eventual objective of obtaining results in real time. The number of applications NGS is unlimited. Although there are still many problems to solve, a priori the DNA of any organism can be sequenced. NGS technology can and is currently being applied directly to various sectors such as the clinical diagnosis, forensic analysis and demonstrates also having enormous potential in the areas of security and control to feed. For this reason, the FDA is now exploring new regulatory approaches to ensure that NGS tests have analytical performance suited to the complexity and enormous potential of generating information useful of this new technology.

Animal species identification has been based on a standardized genetic target with DNA barcoding. To the amplification of a target gene, "universal primers" were designed in conserved regions that surround hyper variable regions, with less than 400 bp [17]. With this study, we demonstrate the utility of target-based approach allergen food authentication for meat, fish, plants and vegetable products. Ion S5 system sequencing, is one of the high-throughput sequencing methods, which originated novel ways to detect allergen and control food authenticity.

From the bioinformatic analysis, the number of reads obtained from each species were not the same between the different approaches. This difference is due to PCR bias, sequencing artifacts and other technical challenges. Nevertheless, the developed methodology allows the identification of fish species, crustacea and plant origin allowing, in case they are presented to identify it and the specie, with a detected liming of 0.5%. All the development methodologies allow the species identification presented in samples at a specie level regarding fish and crustacea analysis. As for the plant analysis, the identification was only possible in some cases at the gender level, with a limit of detection of 0.5% (5000 ppm).Specially the mixed samples where several organisms from the same genus are present the analysis is more complex returning several species form the same genus, and in such cases a direct comparison with the product label must be performed in order to ensure the compliance.

Hence, the use of different fragments and improvement of lab techniques have strong chances of reducing the imprecision and resolution of NGS results. Some of them, present unexpected species, in some of the analyzed samples, this is due to sequencing errors or alignment errors. Although the quantification of DNA can be high sometimes, that amount can't be related to the amount of reads per samples, specially the mixed samples. Thus, the target DNA sequencing method continues to be a powerful tool for authentication and mislabeling detection in several products.

Adulteration in some products include many issues, such as the replacement of the primarily animal species by low-value animal or vegetable species, mislabeling of ingredients and the geographical origin, misunderstood processing methods and the geographical origin, undeclared processing methods, the addition of undeclared ingredients and non-meat components [10]. For samples with

processed meat and fish products, the inability to visually identify species in these products, coupled with price variations in different animal species, increases the probability of species substitution [18].

Overall, there are two types of food adulteration, the replacement of a species for another one due to financial issues, and unintentional adulteration due to accidental alteration.

Conclusion

The study presents a methodological tool for species identification in different types of samples. The results presented by the abundance of each species, can determine the amount of those in a sample and if, in fact, if they are presented or not. The target sequencing allows the rapid detection of mislabeling, fraud or accidental contaminations. Hence, this project delivers a single analysis using NGS technology, to detect the presence of a certain group or subgroup of the suggested analysis in this study, in foods and / or materials that are in contact with food. This technology allows the identification of all species present even in complex food matrixes with a level of detection of 0.5%.

This technology allows to accurately identify the species (vegetables / plants, fish, crustaceans) present, without the need to carry out various analyzes on a mixture of many ingredients, as well as allowing the detection of unanticipated food components, which was previously impossible. Besides that, this approach is viable in terms of cost-effective when used to analyze several samples and it can also detect DNA in low amounts.

Competing Interests

The authors declare that they have no competing interests.

References

1. Current challenges in the food and beverage industry.
2. Food fraud - an evolving crime with profit at its heart. New Food Magazine.
3. Trienekens J, Zuurbier P (2008) Quality and safety standards in the food industry, developments and challenges. Int J Prod Econ 113: 107-122.
4. Mayo B, Rachid CTCC, Alegría A, Leite AMO, Peixoto RS, et al. (2014) Impact of Next Generation Sequencing Techniques in Food Microbiology. Curr Genomics 15: 293-309.
5. Barbosa C, Nogueira S, Gadanho M, Chaves S (2019) Study on commercial spice and herb products using next-generation sequencing (NGS). J AOAC Int 102: 369-375.
6. Palumbo F, Scariolo F, Vannozzi A, Barcaccia G (2020) NGS-based barcoding with mini-COI gene target is useful for pet food market surveys aimed at mislabelling detection. Sci Rep 10: 1-8.
7. Jagadeesan B, Gerner-Smidt P, Allard MW, Leuillet S, Winkler A, et al. (2019) The use of next generation sequencing for improving food safety: Translation into practice. Food Microbiol 79: 96-115.
8. NGS in Food Safety: Seeing What Was Never Before Possible. FoodSafetyTech.
9. Roy S, Durso MB, Wald A, Nikiforov YE, Nikiforova MN, et al. (2014) SeqReporter: Automating next-generation sequencing result interpretation and reporting workflow in a clinical laboratory. J Mol Diagn 16: 11-22.
10. Espiñeira M, Santaclara FJ (2016) The Use of Molecular Biology Techniques in Food Traceability. Advances in Food Traceability Techniques and Technologies: Improving Quality throughout the Food Chain.
11. Saeidipour B, Bakhshi S (2013) The relationship between organizational culture and knowledge management, & their simultaneous effects on customer relation management. Adv Environ Biol 7: 2803-2809.
12. He B, Zhu R, Yang H, Lu Q, Wang W, et al. (2020) Assessing the Impact of Data Preprocessing on Analyzing Next Generation Sequencing Data. Front Bioeng Biotechnol 8: 817.
13. Edgar RC (2010) Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26: 2460-2461.
14. Chen Y, Ye W, Zhang Y, Xu Y (2015) High speed BLASTN: An accelerated MegaBLAST search tool. Nucleic Acids Res 43: 7762-7768.
15. Mohanta TK, Mishra AK, Khan A, Hashem A, Abd_Allah EF, et al. (2020) Gene Loss and Evolution of the Plastome. Genes 11: 1133.
16. Akbar A, Shakeel M, Al-Amad S, Akbar A, Ali AK, et al. (2021) A simple and sensitive NGS-based method for pork detection in complex food samples. Arab J Chem 14: 103124.
17. Frantzen CA, Kot W, Pedersen TB, Ardö YM, Broadbent JR, et al. (2017) Genomic characterization of dairy associated Leuconostoc species and diversity of leuconostocs in undefined mixed mesophilic starter cultures. Front Microbiol 8: 132.
18. Carvalho DC, Palhares RM, Drummond MG, Gadanho M (2017) Food metagenomics: Next generation sequencing identifies species mixtures and mislabeling within highly processed cod products. Food Control 80: 183-186.