Next Generation Sequencing for Food Authenticity, Traceability and Safety

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1. INTRODUCTION

Food authenticity and fraud are topics of high interest in the food industry and highly controlled by authorities. The complexity of the food supply chain is challenging the abilities of analytical tools used for traceability of ingredients for food production. The most common method to verify species substitution and species identification is Real Time PCR. However, PCR testing is limited by the number of targets that can be simultaneously identified and differentiated. This can be critical, especially when testing highly processed and complex food that often contain multiple different species.

The introduction of Next Generation Sequencing (NGS) into the food sector revolutionizes food authenticity testing. NGS untargeted approach enables accurate detection and differentiation of thousands of different species in each sample using DNA sequencing that is recognized as the most reliable method for species identification.

In the present study an innovative NGS-based approach was tested for meat, fish and spices/herbs species identification.

2. WORKFLOW

Amplification with SGS ALLspeciesID DNA analyser kits

DNA sequencing with Thermo Ion Torrent™ platform ION CHEF + S5

Data analysis with SGS ALLspeciesID software

- Short DNA fragments suitable for processed food
- 24-36h workflow
- Multiplex and meta-barcoding approach
- In silico analysis for DNA sequence comparison
- Curated Database with thousands of entries

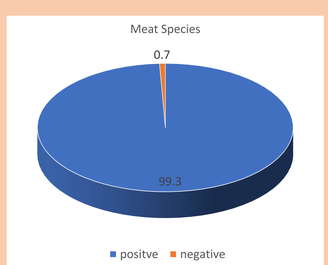
3. RESULTS

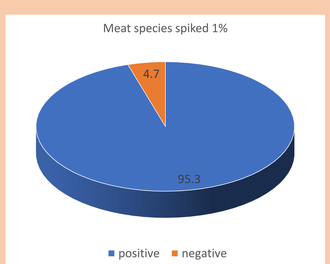
3.1 Meat species results

Number of samples tested: 148
Number of species tested: 49
Species mix: up to 3
Spiked level: 1%, 10%, 50%, 100%

Ovis_aries	Sheep
Capra_hircus	Goat
Lepus_capensis	Hare
Oryctolagus_cuniculus	Rabbit
Macropus_rufus	Kangaroo
Capreolus_capreolus	Roe Deer
Cervus_elaphus	Red Deer
Rangifer_tarandus	Reindeer
Antidorcas_marsupialis	Springbok
Equus_hemionus	Zebra
Lama_glama	Lama
Gallus_gallus	Chicken
Canis_familiaris	Dog
Bison_bison	Bison
Cervus_dama	Fallow Deer
Equus_caballus	Horse
Sus_scrofa	Pork
Bos_taurus	Beef
Meleagris galopavo	Turkey
Cairina moscata	Duck
Alopochen aegptiacus	Goose
Struthio camelus	Ostrich
Columba livia	Pigeon
Numida meleagris	Guinea fowl
Dromaius novaehollandiae	Emu

Tragelaphus strepsiceros	Kudu
Felix catus	Cat
Rattus norvegicus	Rat
Vulpes vulpes	Fox
Alces alces	Elk
Coturnix japonica	King quail
Bubalus bubalis	Buffalo
Camelus dromedarius	Camel
Crocodylus niloticus	Crocodile
Lophura inornata	Pheasant
Oryx leucoryx	Oryx gazella
Alcelaphus buselaphus	Gnu
Bos grunniens	Cattle Yak
Equus asinus	Donkey
Meles meles	Badger
Tragelaphus scriptus	Antilope
Corvus macrorhynchos	Daw
Mustela erminea	Weasel
Ondatra zibethicus	Muskrat
Anas sp	Mallard duck
Crocodylus siamensis	Crocodile
Phasianus colchicus	Pheasant
Alectoris chukar	Partridge
Aepyceros melampus	Impala



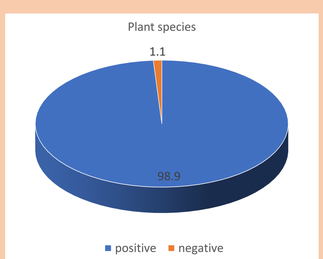


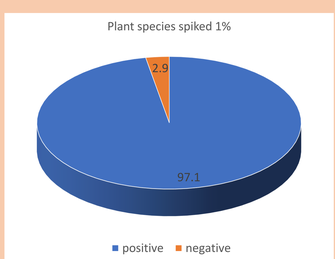
3.2 Plants species (spices/cereals) results

Number of samples tested: 347
Number of species tested: 39
Species mix: up to 5
Spiked level: 1%, 5%, 10%, 20%, 100%

Origanum sp.	Origanum
Allium schoenoprasum	Wild chives
Allium_sativum	Garlic
Anethum graveolens	Dill
Argemone sp	Prickly poppy
Avena sativa	Oat
Brassica napus	Rape
Capsicum annuum	Cayenne pepper
Carum carvi	Caraway
Ceratonia siliqua	St John's bread
Conium maculatum	Poison henlock
Coriandrum sativum	Coriander
Crocus sativus	Saffron
Cuminum_cyminum	Cumin
Curcuma longa	Turmeric
Elettaria cardamomum	Cardamom
Foeniculum vulgare	Sweet fennel
Glycine max	Soybean
Hordeum vulgare	Barley
Juniperus_communis	Juniper

Laurus_nobilis	Sweet bay
Manihot_esculenta	Cassava
Mentha spicata	Spearmint
Myristica fragrans	Nutmeg
Ocimum basilicum	Sweet basil
Oryza_sativa	Rice
Panicum miliaceum	Millet
Papaver somniferum	Opium poppy
Petroselinum crispum	Parsley
Pimpinella anisum	Anis
Piper nigrum	Black pepper
Rosmarinus_officinalis	Rosemary
Sesamum indicum	Sesame
Sinapis_alba	White mustard
Sorghum_bicolor	Sorghum
Thymus_vulgaris	Garden Thyme
Triticum_aestivum	Wheat
Triticum_durum	Durum wheat
Zingiber_officinale	Garden Ginger



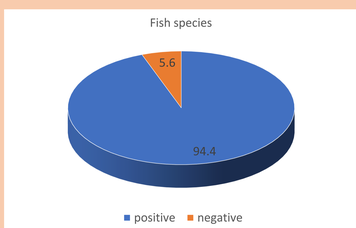


3.3. Fish species results

Number of samples tested: 78
Number of species tested: 26
Species mix: up to 2
Spiked level: 1%, 2%, 5%, !0%, 100%

Salmo salar	Atlantic Salmon
Thunnus albacares	Yellowfin tuna
Gadus morhua	Atlantic cod
Hippoglossus hippoglossus	Pacific halibut
Limanda limanda	Common dab
Merluccius merluccius	European hake
Melanogrammus aeglefinus	Haddock
Katsuwonus pelamis	Skipjack tuna
Thunnus alalunga	Albacore
Pleuronectes platessa	European plaice
Molva molva	Ling
Sander lucioperca	Pike-perch
Pollachius pollachius	Pollack

Trisopterus luscus	Norway pout
Cynoglossus senegalensis	Witch flounder
Oncorhynchus chrysogaster	Pink salmon
Lophius piscatorius	Angler
Oncorhynchus nerka	Sockeye salmon
Pangasianodon hypophthalmus	Silver carp
Scomber scombrus	Atlantic mackerel
Oncorhynchus gorbuscha	Pink salmon
Merluccius hubbsi	Argentine hake
Merluccius productus	North Pacific hake
Macruronus magellanicus	Patagonian grenadier
Merluccius gayi	South Pacific hake
Thunnus obesus	Bigeye tuna



4. CONCLUSIONS

- The NGS workflow could enable to identify all the species tested individually
- Identification of pure material at spiked levels higher than 5% was successfully obtained for more than 94% of the samples tested. A few negative results were obtained for some highly processed food products and mostly for fish-based samples.
- At the level of 1% spiking, more that 95% of the samples were correctly assigned to the species name.
- When combined, all targets could be analyzed simultaneously in a single NGS run which reduce significantly the NGS costs
- Based on these invitro analysis and on the in silico analysis of DNA sequences a database containing thousands of species was created.
- Extension of the databases is an on-going activity to include additional species