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FOOD AUTHENTICITY TESTING WITH NEXT-GENERATION SEQUENCING

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INTRODUCTION

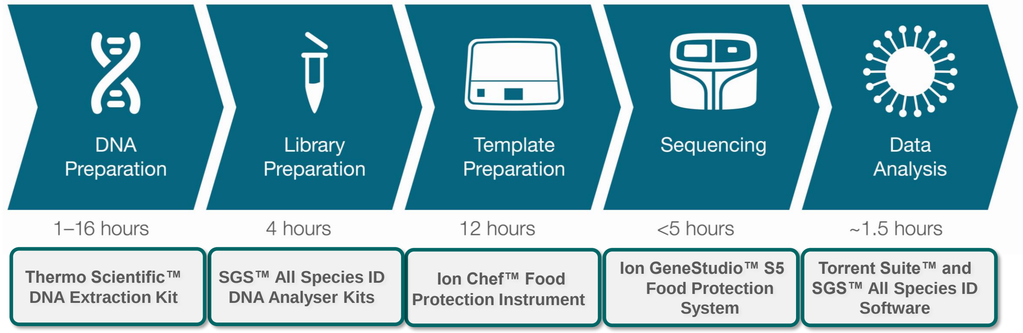
A study was executed in order to identify meat, fish and plant species in food products. The goal was to verify the compatibility of the methods included in the workflow and the identification of species from several different sample types.

- ✓ End-to-end workflow to identify all plant, fish and meat species within a food sample
- ✓ Samples from several food categories (ready-to-eat meals, fresh products, soups, ingredients, canned foods, etc)
- ✓ Comprehensive list of all species detected

MATERIALS AND METHODS

- ✓ **Homogenization** with Precellys® homogenization instrument (Bertin Instruments) to prepare a representative sample for DNA extraction.
- ✓ **GMO Extraction kit** (Thermo Fisher Scientific) with silica based spin-column technology was used to produce high-quality DNA for library preparation.
- ✓ **Libraries for sequencing** were prepared with SGS™ All Species Meat, Fish and Plant Analyser kits (Thermo Fisher Scientific). Unique barcodes (i.e. molecular tags) were added to each sample to enable sequencing and analysis of several samples within the same sequencing run.
- ✓ A fully automated **templating** reaction on the Ion Chef™ Food Protection instrument (Thermo Fisher Scientific) was performed to prepare the sample libraries for sequencing on the Ion Chips.
- ✓ **Sequencing** was performed on the Ion GeneStudio™ S5 Food Protection System (Thermo Fisher Scientific) relying on semi-conductor technology.
- ✓ Sequencing results were mapped against a database of species DNA of meat, fish and plant for **data analysis**. A comprehensive list of all species detected in a sample was generated by the SGS™ All Species ID software (Thermo Fisher Scientific).

WORKFLOW



FISH PRODUCTS

Product	Detected species	
Tuna (in water)	Skipjack tuna	✓
Tuna (in oil)	-	×
Mackerel in tomato sauce	Atlantic mackerel	✓
Canned Sardine	European pilchard	✓
Smoked salmon pizza	Atlantic salmon/Brown trout	✓
Fish rolls (roach, pollock)	Common roach, Pollock	✓
Crispy Cod files	-	×
Pollock with almond crust	Alaska pollock	✓
Fish and veggie patties	Atlantic salmon/Brown trout, Atlantic cod	✓
Salmon soup	Rainbow trout, Pollock	✓
White fish patties	Lake whitefish, Whitefish Pollock	✓
Lake fish patties	Common bream, Common dace, Ide, Ray-finned fish sp., Common roach, Bream, European perch	✓
Fish fingers	Atlantic cod, Haddock	✓
Smoked sprat	European sprat	✓
Salmon rolls	Atlantic salmon/Brown trout	✓
Rainbow trout strips	Rainbow trout	✓
Pickled herring	Atlantic herring	✓
Salmon loaf	Atlantic salmon/Brown trout, Pollock	✓
Frozen salmon cubes	Pink salmon	✓
Frozen Alaska Pollock	Alaska pollock	✓

MEAT PRODUCTS

Product	Detected species	
Canned pork & beef	Pork, Beef	✓
Canned ham	Pork	✓
Canned chicken	Chicken	✓
Sautéed reindeer	Reindeer	✓
Sautéed red deer	Red deer	✓
Vegetables and pork	Pork	✓
Ground pork & beef stick with cheese	Pork, Beef	✓
Pork & beef dumplings	Pork, Beef	✓
Smoked and sliced pork, turkey and chicken	Pork, Turkey, Chicken	✓
Beef soup	Beef	✓
Pork liverwurst	Pork	✓
Sliced ham	Pork	✓
Bratwurst (pork, beef)	Pork, Beef	✓
Sausage (Chicken, pork, beef, turkey)	Chicken, Pork, Beef	*
Ox meat chips	Beef	✓
Kebab meat	Beef	✓
Beef and pork patties	Beef, Pork	✓
Ground beef & pork patties	Beef, Pork	✓
Minced chicken	Chicken	✓
Low fat minced beef with chicken	Chicken, Beef	✓

PLANT PRODUCTS

Product	Detected species	
Bell pepper spice	Pepper	✓
Potato, onion and meat	Onion	*
Beef soup (Potato, carrot, parsley, swede, leek, rapeseed)	Wild carrot, Wild leek, Rapeseed, Parsley, Carrot, Carrot oil	*
Salmon soup (Potato, onion, celery, leek, carrot, dill, black pepper)	Onion, Dill/Fennel, Celery, Parsnips	*
Sweet & sour sauce (Tomato, onion, carrot, celery, green/red pepper, bamboo)	Onion, Pepper	*
Cinnamon	Cinnamon	✓
Chives powder	Onion/chives	✓
Coriander powder	Coriander	✓
Garlic powder	Garlic	✓
Grilling spice (Bell & black pepper, coriander, garlic, chili)	Onion, Garlic, Chili/Pepper	*
Pasta sauce (Onion, basil, garlic, parsley, oregano)	Onion/chives, Oat, Wild leek	*
Pesto sauce (Basil, cashew, garlic)	Basil, Cashew	*
Oregano	Oregano/Marjoram/Syrian oregano	✓
Tea	Tea plant	✓
Veggie dumplings (Wheat, potato, onion, dill, black pepper)	Rapeseed, Potato, Rye, Oat, Onion, Barley, Rapeseed, Dill/fennel	*
Lentil and pea sprouts	Peas	*
Smoked tofu	Soybean	✓
Wok veggies (Peas, beans, corn, soy bean, sunflower seeds, kidney beans, linseed)	Pea, Soybean, Common bean, sunflower, garlic, Corn, Parsley	*
Frozen peas, corn, bell pepper	Pea, Pepper, Corn	✓
French fries	Potato	✓

CONCLUSIONS

90% of the fish species and 95% of the meat species that were declared as an ingredient were detected. Some plant species were not detected with samples where multiple species were listed in the ingredients, but all declared spice species were detected in spice-type samples. Also, since the products tested are real food products there is no information about the concentration of the species labelled in each product and therefore this can impact on the successful detection of species that can be present at a very low level.

The system is able to analyze several sample types and targets (meat, fish and simple plant samples) within a single sequencing run, enabling shorter processing times with lower cost.

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Application of Next-Generation Sequencing to Food Authenticity Testing - Study of Adulterated Beef Samples Using Ion GeneStudio S5 Food Protection System

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ABSTRACT

Following the UK/EU Horse-meat issues of 2013, where a significant amount of horse DNA was found in a number of processed beef products there has been an increased need for routine non-targeted species detection methods. In recent years Next-Generation Sequencing (NGS) has been promoted as a useful technique to identify species present in samples containing a mixture of species. Very few studies have looked into application of processed meat products where DNA can be highly degraded. This study applies a commercial NGS system to a range of spiked meat product samples processed to industry standard conditions. The samples consisted of lean beef spiked with varying levels of pork and horse muscle was used to prepare raw, burger, canned meat and cottage pie sample types. Multiple DNA extracts were prepared from each sample type and NGS was performed using SGS™ All Species Meat Analysis kit in conjunction with Ion Chef™ Food Protection Instrument and Ion GeneStudio™ S5 Food Protection System. Results will be presented and relevance to food screening will be discussed.

INTRODUCTION

Next Generation Sequencing (NGS) has been introduced in recent years as a very powerful DNA-based method for species identification in food products. However, the use of NGS as a food protection tool requires the development of optimized, fit for purpose, workflow to ensure reliability of results and to maximize the advantages of this high throughput method. To take advantage of the non-targeted and massive sequencing output obtained by NGS a food protection specific end to end workflow has been developed to enable identification of meat, fish and plant species in food products.

In the present study the Thermo Scientific™ NGS Food Authenticity Workflow (Figure 1) for meat species identification is used for the detection of adulterated beef samples. An application which has particular relevance given the EU horse-meat issues of 2013 in which food advertised at containing beef where found to contain significant amounts of horse meat and to a lesser degree other undeclared meat such as pork. The resulting scandal highlighted a significant breakdown in the traceability of the food supply food chain and called into question brand integrity within the food industry.

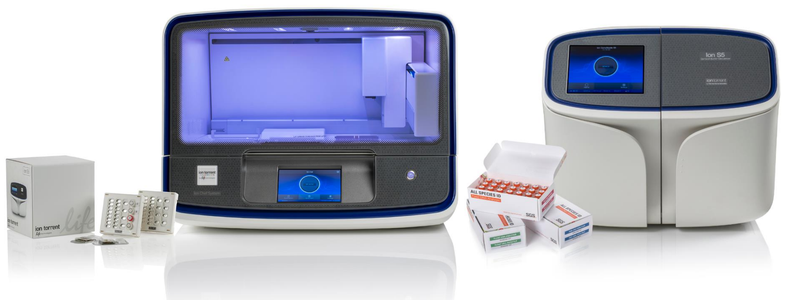

Figure 1. Ion Chef™ Food Protection Instrument, Ion GeneStudio™ Food Protection NGS System and SGS™ All Species ID Analyser Kits

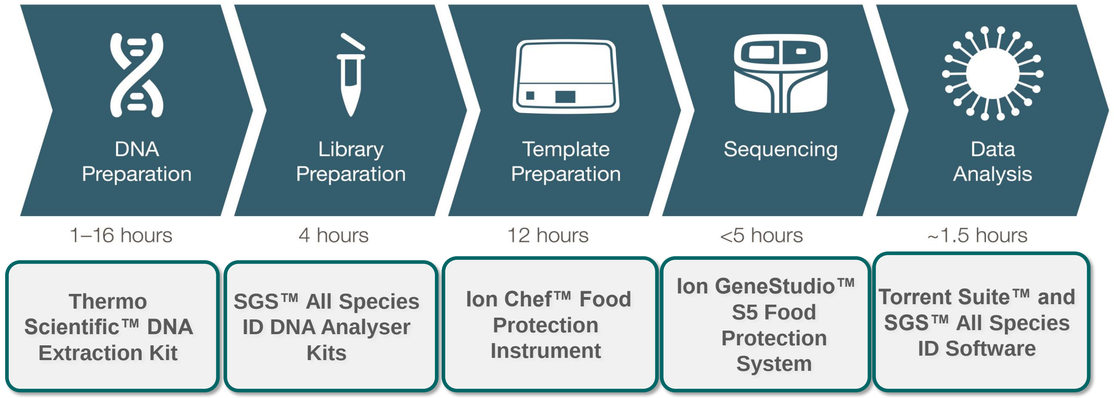

Figure 2. Thermo Scientific NGS Food Authenticity Workflow overview

MATERIALS AND METHODS

A number of samples of beef with different amounts of horse and pork meat were prepared and then subjected to different processing levels.

Samples were homogenized and a DNA extraction step performed on 200 mg sample. Libraries for sequencing were prepared using SGS™ All Species Meat Analyser kit (Thermo Fisher Scientific). Unique barcodes (i.e. molecular tags) were added to each sample to enable sequencing and analysis of several samples within the same sequencing run.

A fully automated templating reaction on the Ion Chef Food Protection instrument (Thermo Fisher Scientific) was performed to prepare the sample libraries for sequencing on the Ion Chips. Sequencing was performed on the Ion GeneStudio S5 Food Protection System (Thermo Fisher Scientific) relying on semi-conductor technology. Sequencing results were mapped against a database of species DNA of meat, fish and plant for data analysis. A comprehensive list of all species detected in a sample was generated by the SGS™ All Species ID software (Thermo Fisher Scientific).

RESULTS

The output from the Thermo Scientific NGS Food Authenticity Workflow was collated to show whether Horse or Pork meat was detected in the samples at the given contamination levels, as shown in Tables 1 & 2.

CONCLUSIONS

Results suggest that this NGS Food Authenticity Workflow is capable of detecting contamination down to 1% in processed samples although there may be some slight differences in sensitivities between species.
The results suggest that this system will not detect contamination at a level of 0.1% so may not be suitable in situations where there is a requirement for low level detection i.e. halal foods where levels down to 0.1% are often tested. Real-time PCR is used in targeted species detection and can detect down to 0.1%.

The method is suitable for screening and as an estimate of potential levels and should not be considered quantitative.

TRADEMARKS

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Horse %	Unprocessed (n=4)	Canned (n=4)	Burger (n=3)	Cottage Pie (n=3)	% Detection
10	4	4	3	3	**100**
2	4	4	3	3	**100**
1	2	1	0	2	**36**
0.1	0	0	0	0	**0**

Table 1. Percentage of horse contamination in mixed beef and horse samples

Pork %	Unprocessed (n=4)	Canned (n=4)	Burger (n=3)	Cottage Pie (n=3)	% Detection
10	4	4	3	3	**100**
2	4	4	3	3	**100**
1	4	3	3	3	**93**
0.1	0	0	0	0	**0**

Table 2. Percentage of pork contamination in mixed beef and pork samples

Campden BRI food and drink innovation

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Next Generation Sequencing for Detection of Meat, Fish and Plant Species in Pure and Mixed Species Samples

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ABSTRACT

In this study the technical experts from Thermo Fisher Scientific and SGS Molecular supported scientists at Nestle Research in the use of the Thermo Scientific™ NGS Food Authenticity Workflow (Figure 1) to test for meat, fish and spices/herbs species detection and identification at a variety of different spike levels (1% to 100%) and combinations of species (up to 5 different species combined into a sample).

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 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.

Figure 1. Left to right – Ion Chips and consumables, Ion Chef™ Instrument, SGS™ All Species ID Meat, Fish and Plant Analyser Kits and Ion GeneStudio™ S5 System

MATERIALS AND METHODS

All samples analyzed in this study were selected to include common species present in commercial food products. DNA was extracted as described below from different materials, including reference samples obtained from samples repositories and proficiency tests and commercial single species food products according to the label.

A total of 148 meat samples, 347 plant samples and 78 fish samples were tested.

Mixtures of species were produced by mixing DNAs to be tested with the NGS workflow proposed. Artificial DNA mixtures contained up to 5 species:
Meat DNAs up to 3 species
Fish DNAs up to 2 species
Plant DNAs up to 5 species

Additionally spiked samples where produced at different levels:
Meat spiked samples – 1%, 10% and 50%
Fish spiked samples – 1%, 2%, 5% and 10%
Plant spiked samples – 1%, 5%, 10% and 20%

MATERIALS AND METHODS

Thermo Scientific NGS Food Authenticity Workflow (See figure 1 for an overview of the NGS Food Authenticity Workflow steps and timings).
Homogenization: To prepare a representative portion of the sample homogenization using the Precellys™ homogenization instrument (Bertin Technologies) utilizing bead-beating technology was carried out.
DNA Extraction: The GMO Extraction Kit (Thermo Fisher Scientific) with silica based spin-column technology was used to produce high-quality DNA for library preparation.
DNA library preparation: DNA libraries were prepared using the SGS All Species Meat, Fish and Plant Analyser Kits (Thermo Fisher Scientific). Regions of interest were amplified using PCR with the DNA extractions of the samples sequencing adapters added. During library preparation unique barcodes (molecular tags) were added to each sample to enable sequencing and analysis of multiple samples in the same sequencing run.
Template preparation and Library pooling: After library preparation, a fully automated templating reaction on the Ion Chef™ Food Protection Instrument (Thermo Fisher Scientific) was performed to prepare the sample libraries for sequencing on the Ion Chips.
Sequencing: Performed on the Ion GeneStudio™ S5 Food Protection System (Thermo Fisher Scientific) DNA sequences were determined relying on semi-conductor based sequencing technology.
Data analysis: Results were mapped against the SGS® All Species ID Software, a database containing the DNA sequences of many thousands of meat, fish and plant species to provide an identification for all species detected in the samples.

Figure 1. Thermo Scientific NGS Food Authenticity Workflow overview

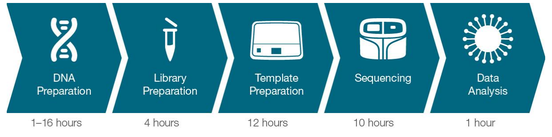

Table 1. List of meat species tested

Meat species name	Meat ommon name	Meat species name	Meat ommon name
Ovis aries	Sheep	*Tragelaphus strepsiceros*	Kudu
Capra hircus	Goat	*Felix catus*	Cat
Lepus capensis	Hare	*Rattus norvegicus*	Rat
Oryctolagus_cuniculus	Rabbit	*Vulpes vulpes*	Fox
Macropus rufus	Kangaroo	*Alces alces*	Elk
Capreolus capreolus	Roe Deer	*Coturnix japonica*	King quail
Cervus elaphus	Red Deer	*Bubalus bubalis*	Buffalo
Rangifer tarandus	Reindeer	*Camelus dromedarius*	Camel
Antidorcas marsupialis	Springbok	*Crocodylus niloticus*	Crocodile
Equus hemionus	Zebra	*Lophura inornata*	Pheasant
Lama glama	Lama	*Oryx leucoryx*	Oryx gazella
Gallus gallus	Chicken	*Alcelaphus buselaphus*	Gnu
Canis familiaris	Dog	*Bos grunniens*	Cattle Yak
Bison bison	Bison	*Equus asinus*	Donkey
Cervus dama	Fallow Deer	*Meles meles*	Badger
Equus caballus	Horse	*Tragelaphus scriptus*	Antilope
Sus scrofa	Pork	*Corvus macrorhynchos*	Daw
Bos taurus	Beef	*Mustela erminea*	Weasel
Meleagris galopavo	Turkey	*Ondatra zibethicus*	Muskrat
Cairina moscata	Duck	*Anas species*	Mallard duck
Alopochen aegptiacus	Goose	*Crocodylus siamensis*	Crocodile
Struthio camelus	Ostrich	*Phasianus colchicus*	Pheasant
Columba livia	Pigeon	*Alectoris chukar*	Partridge
Numida meleagris	Guinea fowl	*Aepyceros melampus*	Impala
Dromaius novaehollandiae	Emu		

Table 2. List of fish species tested

Fish species name	Fish common name	Fish species name	Fish common name
Salmo salar	Atlantic Salmon	*Trisopterus luscus*	Norway pout
Thunnus albacares	Yellowfin tuna	*Cynoglossus senegalensis*	Witch flounder
Gadus morhua	Atlantic cod	*Oncorhynchus chrysogaster*	Pink salmon
Hippoglossus hippoglossus	Pacific halibut	*Lophius piscatorius*	Angler
Limanda limanda	Common dab	*Oncorhynchus nerka*	Sockeye salmon
Merluccius merluccius	European hake	*Pangasianodon hypophthalmus*	Silver carp
Melanogrammus aeglefinus	Haddock	*Scomber scombrus*	Atlantic mackerel
Katsuwonus pelamis	Skipjack tuna	*Oncorhynchus gorbuscha*	Pink salmon
Thunnus alalunga	Albacore	*Merluccius hubbsi*	Argentine hake
Pleuronectes platessa	European plaice	*Merluccius productus*	North Pacific hake
Molva molva	Ling	*Macruronus magellanicus*	Patagonian grenadier
Sander lucioperca	Pike-perch	*Merluccius gayi*	South Pacific hake
Pollachius pollachius	Pollack	*Thunnus obesus*	Bigeye tuna

Table 3. List of plant species tested

Plant species name	Plant common name	Plant species name	Plant common name
Origanum species	Origanum	*Laurus_nobilis*	Sweet bay
Allium schoenoprasum	Wild chives	*Manihot_esculenta*	Cassava
Allium sativum	Garlic	*Mentha spicata*	Spearmint
Anethum graveolens	Dill	*Myristica fragrans*	Nutmeg
Argemone species	Prickly poppy	*Ocimum basilicum*	Sweet basil
Avena sativa	Oat	*Oryza_sativa*	Rice
Brassica napus	Rape	*Panicum miliaceum*	Millet
Capsicum annuum	Cayenne pepper	*Papaver somniferum*	Opium poppy
Carum carvi	Caraway	*Petroselinum crispum*	Parsley
Ceratonia siliqua	Carob	*Pimpinella anisum*	Anis
Conium maculatum	Poison henlock	*Piper nigrum*	Black pepper
Coriandrum sativum	Coriander	*Rosmarinus_officinalis*	Rosemary
Crocus sativus	Saffron	*Sesamum indicum*	Sesame
Cuminum_cyminum	Cumin	*Sinapis_alba*	White mustard
Curcuma longa	Turmeric	*Sorghum_bicolor*	Sorghum
Elettaria cardamomum	Cardamom	*Thymus_vulgaris*	Garden Thyme
Foeniculum vulgare	Sweet fennel	*Triticum_aestivum*	Wheat
Glycine max	Soybean	*Triticum_durum*	Durum wheat
Hordeum vulgare	Barley	*Zingiber_officinale*	Garden Ginger
Juniperus_communis	Juniper		

RESULTS

All pure (100%) meat, plant and fish species were detected and correctly identified.

For the meat samples spiked at 1% two out of 81 meat species were not detected (2.5%). These samples were both cooked beef spiked with 1% pork.

For all meat samples with spike level above 1%, all the species were correctly identified.

Of the ten fish samples spiked with the most common fish species at 1%, all were detected and correctly identified.

170 plant samples were spiked at 1%, 29 of these were not detected (17.1%)

All plant species were detected for the 46 plant samples spiked at 5%.

A few samples didn't originate results since no DNA could be obtained due to high sample processing .

CONCLUSIONS

The Thermo Scientific NGS Food Authenticity Workflow was shown to detect and correctly identify 100% of meat (n=49), fish (n=26) or plant (n= 39) species at a spike level of 5% or higher).

For meat samples at a spike level of 1%, 79/81 (97.5%) of the species were detected and correctly identified.

For fish samples at a spike level of 1%, 10/10 (100%) of the species were detected and correctly identified.

For plant samples at a spike level of 1%, 143/170 (82.9%) of the species were detected and correctly identified. At a level of 5% all plant species were detected.

Combining up to five species for plant, three species for meat or two species for fish samples had no effect on the detection or correct identification of the species present.

When combined, all targets could be analyzed simultaneously in a single NGS run which reduces NGS costs compared with having to carry out separate runs.

The workflow could differentiate very closely related species with important commercial impact like for Bigeye and Yellowfin tuna that are know to be very difficult to distinguish by DNA sequencing.

The workflow is defined to work with highly processed food (including canned food) by analysing very short DNA fragments. However products originating very low or no DNA can't be analysed.

The identification success of the workflow depends on the number of different species included in the databases. Nevertheless the current databases for meat and fish ID include many thousands of species entries that makes unlikely the absence of and ID result.

For plant ID, the present database is mostly focused on spices, herbs and cereals

At spike levels 1-5% all species were detected making the workflow appropriate for food species ID analysis.

TRADEMARKS/LICENSING

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Next Generation Sequencing (NGS) Workflow Applied to the Analysis of Commercial Spices and Herbs Products

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ABSTRACT

The use of DNA-based testing methods is increasing in the food sector. DNA analyses can be a helpful tool for analysis of many food products and can address some of the present concerns about adulteration and authenticity. Several analytical methods have been proposed to answer the specific topic of species composition in foods. Next Generation Sequencing (NGS) has been found to be a suitable tool for food analysis including spices, herbs, seasonings, etc. In the present study, we show how an internal NGS workflow was set up and tested for species composition in real food seasoning samples. NGS was used for testing several commercial samples of different spice and herb mixtures. The results obtained will be discussed based on the labeling of the products relative to the type of sample and species mixtures.

INTRODUCTION

Herbs and spices are common and important ingredients in a large variety of foods, beverages, supplements, medicines and cosmetics. Herbs are typically green-leaved plants used either fresh or in dried form and contain pleasant savory or aromatic properties. Spices are the dried parts of plants, often with bright or vibrant colors and usually collected from regions known for warmer climates. Herbs used in culinary or food applications are typically the leaves, flowers, or stems of plants (e.g., oregano and basil), whereas spices are composed of seeds, fruits, roots, barks, etc. (e.g., black pepper, cinnamon, and ginger). Widespread culinary use and the potential health and wellness benefits of herbal products including spices and herbs establish the importance of these ingredients in a major industry with many economic benefits. In 2009, it was estimated that the global market of herbs and spices was worth $2.97 billion, of which the European Union market accounted for 520 thousand tons with a value of €1.8 billion. Supply and demand is a fundamental economic principle that determines the price of all products. Because of the inherent value in some products, the food industry is very prone to product adulteration, mainly by deliberate substitution or addition of counterfeit food ingredients.

Next-generation sequencing (NGS) is an automated, high-throughput sequencing technology. For DNA sequencing with the aim of species identification and discrimination, NGS technology has been shown to be potent, reliable, and robust with high potential to be successfully applied to food, feed, and related plant materials. The massive data generated by NGS enables the sequencing of heterogeneous samples in a short time and a cost-effective way. Therefore, a single instrument can run multiple species from the same sample or multiple samples can be simultaneously sequenced.

We successfully show here how an internally developed NGS workflow is used to analyze and characterize the composition and authenticity of 66 samples of spices, herbs, seasoning products, and materials.

MATERIALS AND METHODS

To pre-homogenize the samples, each individual package was vigorously shaken for 15–30 s. Powdered samples did not need any homogenization after the shaking step. Dried fruits were homogenized with a blender until a powder was obtained (the entire sample). Dried leaves and herbs were homogenized with cryogrinding in a mill (liquid nitrogen cooled) until a powder was formed (8–10 g sample).

DNA extraction was performed using a commercial kit, NucleoSpin® Food kit (Macherey-Nagel), with the following alterations: cetyltrimethylammonium bromide (CTAB) buffer instead of the kit lysis buffer (CF), for polysaccharides elimination; 5 mg of polyvinylpolypyrrolidone (PVPP) added to the lysis step, for polyphenols removal.
The extracted DNA was amplified with the SGS™ All Species ID Plant DNA Analyser Kit following the instructions. The PCR products were mixed in equal amounts to create the DNA library that was purified with AgentCourt® AMPure® XP beads (Beckman Coulter) according to the manufacturer instructions. The final libraries were quantified with dsDNA BR Assay Kit using Invitrogen™ Qubit™ Fluorometer equipment (Thermo Fisher Scientific) and sequenced with Ion Chef™ Food Protection Instrument and Ion PGM™ System (Thermo Fisher Scientific) following the instructions.

The amount of DNA sequences generated by the DNA sequencer was very high (between some hundreds of thousands and millions of sequences). Therefore, the data analysis was performed with an internally developed software which contains a set of algorithms that will group the sequences by similarity and compare them with an internal DNA sequences database.

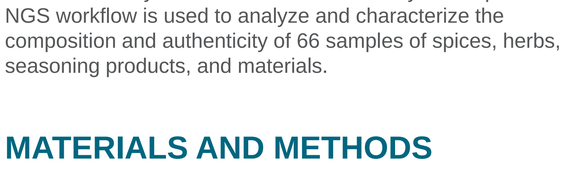

Figure 1. Overview of the complete workflow applied in this study.

RESULTS

Table 1. List of one-products used in the present study distributed by matrix type

Product	Matrix type	No. of samples
Basil	Dried leaves	2
Turmeric	Powder	4
Cumin	Powder	11
Oregano	Dried leaves	10
Pepper	Powder	7
	Dried seeds	6
Curry powder seasoning	Powder	11
Pasta seasoning mix	Powder	7
	Dried herbs	1
Meat seasoning mix	Powder	2
	Dried herbs	5

Forty samples that consist of one-ingredient only and 26 samples identified as mixtures were used in the study (Table 1). Powder products are the most common and the most prone to fraudulent practices, thus 63.6% of the samples used in this study were in a powder form. Samples in non-powder form include dried leaves (12 samples) or dried berries (6 samples) for one-ingredient matrices and dried herbs (6 samples) from samples characterized as mixtures. Thus, all 66 samples were successfully sequenced which demonstrates the suitability of the present DNA extraction method and PCR primer panel to food products and materials containing spice and herbs.

ANALYSIS OF ONE INGREDIENT PRODUCTS

Table 2. Number of one ingredient products with an accordant or discordant result between the declared species in the label and the ones identified by NGS

Product	Declared species	Matrix type	Accordant result	Discordant result	Total of samples
Basil	*Ocimum basilicum*	Dried leaves	0	2	2
Turmeric	*Curcuma longa*	Powder	0	4	4
Cumin	*Cuminum cyminum*	Powder	5	6	11
Oregano	*Oregano vulgare*	Dried leaves	0	10	10
Pepper	*Piper nigrum*	Powder	4	3	7
		Dried fruits	6	0	6

It was observed that all dried whole-fruit/berry samples gave an accordant species identification (Table 2). This observation is consistent with the idea of whole-herb/spice matrices are more difficult to adulterate since a visual confirmation would be possible.

The source of additional species identified in discordant results is not clear (Table 3). We cannot say with certainty they are the result of a fraudulent practice. Indeed, cross-contaminations can occur during the harvest, handling or processing of the ingredients and final product. Thus, a deeper look and understanding of the analyses and the species detected will be important to understand the true authenticity of a sample based on NGS test results collected in this manner.

Table 3. List of species identified in more than 50% of the samples with discordant results for each product

Product	Identified species	Common name	Possible source
Basil	*Ocimum basilicum**	basil	expected
	Convolvulus arvensis	field bindweed	field contaminant
	Corchorus olitorius	jute	contaminant, unknown
Turmeric	*Curcuma longa**	turmeric	expected
	Trigonella foenum-graecum	fenugreek	contaminant, unknown
	Cuminum cyminum	cumin	contaminant, unknown
	Capsicum annuum	chili pepper	contaminant, unknown
	Allium sativum	garlic	contaminant, unknown
	Coriandrum sativum	coriander	contaminant, unknown
Cumin	*Cuminum cyminum**	cumin	expected
	Polygonum aviculare	knotgrass	field contaminant
	Coriandrum sativum	coriander	contaminant, unknown
	Plantago sp.	plantain	field contaminant
Oregano	*Origanum vulgare**	oregano	expected
	Convolvulus arvensis	field bindweed	field contaminant
	Origanum majorana/ Origanum onites/ Origanum syriacum	sweet marjoram/ oregano/ Syrian oregano	field or processing contaminant
Pepper	*Piper nigrum**	black pepper	expected
	Schinus terebinthifolius	Brazilian peppertree	contaminant, unknown
	Capsicum annuum	cayenne pepper	contaminant, unknown

* Species declared on the label

ANALYSIS OF MIXTURES

Table 4. Number of mixture-based products with an accordant or discordant result between the declared species in the label and the ones identified by NGS

Product	Matrix type	Accordant result	Discordant result	Total of samples
Curry powder seasoning	Powder	3	8	11
Pasta seasoning mix	Powder	0	7	7
	Dried herbs	0	1	1
Meat seasoning mix	Powder	2	0	2
	Dried herbs	3	2	5

Table 5. List of all species identified in the samples analyzed for each mixture-based product

Product	Identified species	Common name	Source
Curry	*Coriandrum sativum*	coriander	expected
	Foeniculum vulgare	sweet fennel	expected
	Curcuma longa	turmeric	expected
	Trigonella foenum-graecum	fenugreek	expected
	Allium sativum	garlic	expected
	Sinapis alba/Brassica nigra	white and black mustard	expected
	Capsicum annuum	cayenne pepper	expected
	Anethum graveolens	dill	expected
	Cinnamomum sp.	cinnamon	expected
	Elettaria cardamomum	cardamom	expected
	Zingiber officinale	ginger	expected
	Fallopia convolvulus	black bindweed	field contaminant
	Cuminum cyminum	cumin	contaminant, unknown
	Thymus vulgaris	thyme	contaminant, unknown
	Origanum sp.	oregano/marjoram	contaminant, unknown
	Petroselinum crispum	parsley	contaminant, unknown
	Laurus nobilis	laurel	contaminant, unknown
	Carum carvi	caraway	contaminant, unknown
	Amomum sp./Aframomum sp.	includes true and false cardamom	contaminant, unknown
	Pimpinella anisum	aniseed	contaminant, unknown
	Convolvulus arvensis	field bindweed	field contaminant
	Helminthotheca echioides	bristly oxtongue	field contaminant
	Cuscuta campestris	field dodder	field contaminant
	Pastinaca sativa	parsnip	contaminant, unknown
	Helianthus annuus	sunflower	contaminant, unknown
	Centaurea diluta	lesser star-thistle	field contaminant
	Reseda luteola/Reseda lutea	yellow weed	field contaminant
	Amaranthus caudatus	foxtail amaranth	field contaminant
	Polygonum aviculare	common knotgrass	field contaminant
Pasta seasoning mix	*Capsicum annuum*	cayenne pepper	expected
	Allium sativum	garlic	expected
	Allium cepa	onion	expected
	Origanum sp.	oregano/marjoram	expected
	Pastinaca sativa	parsnip	expected
	Daucus carota	carrot	expected
	Levisticum officinale	lovage	expected
	Thymus vulgaris	thyme	expected
	Piper nigrum	black pepper	expected
	Citrus sp.	citrus fruits	expected
	Petroselinum crispum	parsley	expected
	Apium graveolens	celery	expected
	Coriandrum sativum	coriander	expected
	Cuminum cyminum	cumin	expected
	Origanum vulgare	oregano	expected
	Convolvulus arvensis	field bindweed	field contaminant
	Senna sp.	sennas	field contaminant
	Pimpinella anisum	anise	contaminant, unknown
	Carum carvi	caraway	contaminant, unknown
	Myrtus communis	myrtle	contaminant, unknown
	Sida cordifolia	flannel weed	field contaminant
	Satureja hortensis	summer savory	field contaminant
	Ocimum basilicum	basil	contaminant, unknown
	Lactuca sativa	lettuce	contaminant, unknown
	Amaranthus retroflexus	pigweed amaranth	field contaminant
	Corchorus olitorius	jute	field or processing contaminant
Meat seasoning mix	*Ocimum basilicum*	basil	expected
	Origanum sp.	oregano/marjoram	expected
	Artemisia dracunculus	tarragon	expected
	Rosmarinus officinalis	rosemary	expected
	Thymus vulgaris	thyme	expected
	Anthriscus cerefolium	chervil	expected
	Levisticum officinale	lovage	expected
	Allium sativum	garlic	expected
	Capsicum annuum	cayenne pepper	expected
	Coriandrum sativum	coriander	expected
	Citrus sp.	citrus fruits	expected
	Petroselinum crispum	parsley	expected
	Satureja montana	winter savory	field contaminant
	Convolvulus arvensis	field bindweed	field contaminant

All other mixture samples with discordant results showed no identification of declared species or, more species identifications than those declared were assigned (Table 4). In cases where species declared are not identified a possible cause is the inability for this workflow to detect ingredients in trace amounts in the sample. In addition, the diverse ingredients in the mixture sample(s) may have undergone different levels of processing leading to DNA degradation and consequently a lower contribution of viable DNA to the final extract of that particular ingredient. This combination of factors may explain some of the non-detected species identifications. The higher than expected number of species reported can also represent fraudulent practices or may simply be cross contamination during harvest, handling or processing of the product.

The analysis of the mixture-based products returned a high number of species as possibilities using our internal method (Table 5) suggesting the present workflow is suitable for both simple and more complex samples containing spices, herbs and similar plant materials. Indeed, NGS demonstrates a great advantage of possible multiple species identification from the same sample in a single instrument run while sequencing several other samples simultaneously.

CONCLUSIONS

NGS is a promising tool for authenticating many spices and herbs because:
(1) it is suitable for samples containing highly processed and degraded DNA,
(2) there is no need of a priori species information,
(3) is cost-effective when processing numerous samples, and
(4) it is possible to detect viable DNA in very low amounts.

We have shown that NGS can be successfully used in complex food matrixes containing spices and herbs. Limitations for the current NGS technology applied to plants including spices and herbs are the requirement of simple and fast bioinformatics tools for data analysis and more complete and reliable DNA reference databases. Overcoming these limitations will establish DNA and NGS as reliable technologies for authenticating spices, herbs and their related products.

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TRADEMARK STATEMENT

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