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

Amanda Manolis[2], Sofia Nogueira[1], Franck Pandiani[1] and Cristina Barbosa[1]
[1]SGS Molecular, Lisbon, Portugal; [2]Thermo Fisher Scientific, Basingstoke, UK

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.

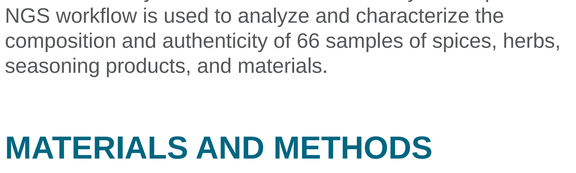

Figure 1. Overview of the complete workflow applied in this study.

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.

REFERENCES

Marieschi, M., Torelli, A., Poli, F., Sacchetti, G., & Bruni, R. (2009) J. Agric. Food Chem., 57: 1835–1840.

Black, C., Haughey, S. A., Chevallier, O. P., Galvin-King, P. & Elliott, C. T. (2016) Food Chem., 210: 551-557.

Parveen, I., Gafner, S., Techen, N., Murch, S. J. & Khan I. A. (2016) Planta Med, 82(14): 1225-1235.

Focke F, Haase I, & Fischer M. (2011) J Agric Food Chem, 59(2):513-520.

Reuter J., Spacek D. & Snyder M. (2016) Mol Cell, 58 (4): 586-597.

Barbosa, C., Nogueira, S., Gadanho, M., & Chaves, S. (2019) J. AOAC Int., 102 (2): 369-375.

TRADEMARK STATEMENT

LT2492A
September 2019