



## **New DNA-based Food Scanning Tool**

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## 1. Executive Summary

As part of routine official controls, the Food Safety Authority of Ireland (FSAI) has used targeted DNA analysis with considerable success to challenge the authenticity of various foods (FSAI, 2002, 2009, 2011, 2013). However, where the identity of all ingredients in a food product is unknown, the need to target specific animal or plant species limits the usefulness of this analytical tool. In order to overcome this constraint, the FSAI has successfully employed next generation DNA sequencing (NGS) technology to screen 45 plant-based foods and food supplements on the Irish market for the presence of undeclared plant species. This non-targeted screening technique identified 14 food products of interest which were then subjected to more established DNA and/or protein analyses for verification purposes. Two products were found to contain undeclared plant species while a third will require further analysis before a definitive conclusion can be reached.

## 2. Introduction

The presence of undeclared ingredients in a food may be the result of inadvertent contamination or deliberate adulteration, the latter primarily for financial gain. Regardless of the reason, the presence of undeclared ingredients in foods can pose a safety risk (e.g. food allergens) and mislead consumers with respect to the product they are purchasing.

Plant-based foods are readily available on the Irish market, with many of the ingredients originating outside of Ireland, particularly in relation to food supplements. The presence of relatively minor levels of contaminating plant material in a food product is not automatically a cause for alarm, especially considering the source of the ingredients as well as the nature of the production and processing methods used. However, reports of deliberate adulteration and substitution of plant-based foods have become more commonplace in recent years (Black *et al.* 2016).

The FSAI has used targeted DNA analysis to detect and quantify Genetically Modified Organisms (GMOs) in food, to find beef and porcine DNA in processed chicken filets and to detect the mislabelling of fish products on sale in Ireland (FSAI, 2002, 2009, 2011). In 2013, the FSAI was the first to identify the presence of significant amounts of horse meat in beef products, which subsequently became known as “the horsemeat scandal” (FSAI, 2013). That targeted FSAI project was initiated primarily on the basis of media and other reports about the clandestine movement of horses out of Ireland, along with some questions about the use of the horse passport and microchip traceability system in place at the time. The heightened EU awareness of food fraud following the horsemeat scandal has resulted in a greater interest in the use of laboratory analyses, particularly targeted DNA analysis to ensure the integrity of many meat and plant-based products. The analytical DNA technique of choice has been based

on the amplification by Polymerase Chain Reaction (PCR) of known DNA segments specific to particular animal or plant species. While PCR amplification is a powerful technique used routinely in several areas of scientific research and in criminal investigations, prior knowledge of the target species is essential and means that it has not been effectively used as a screening technique.

Next Generation Sequencing (NGS) is a technology that has been in development in recent years, with a number of characteristics separating it from the long established “Sanger” sequencing. The main discerning features of NGS include the ability to facilitate large scale, non-targeted parallel sequencing of whole genomes. In order to manage and interpret the voluminous amount of data generated, sophisticated bioinformatics software managed by specialist technicians is required to convert DNA sequence data into meaningful results. Although NGS has been proposed (Galvin-King *et al*, 2018, Han *et al*, 2018) as a means by which to screen food for the presence of undeclared plant and animal species, the effective use of non-targeted NGS has not been demonstrated in a practical situation until now.

### **3. Aim of the study**

The aim of this study was to determine if NGS could be useful as a non-targeted screening technique in order to challenge the authenticity of various plant-based food products on the Irish market.

### **4. Methods**

In April of 2017, the FSAI collected 45 different plant-based food products, including supplements (Annex I) on sale in health food shops and supermarkets in Ireland. The products were sent unopened in their original containers/packaging for DNA analysis to a selected commercial laboratory. The first phase of the project used non-targeted NGS techniques to detect and identify the DNA all plant species present in each product. Two NGS methods were employed, Oxford Nanopore MinION and Illumina MiSeq. The Illumina MiSeq technology was found to produce more reliable results and was selected as the method of choice. The NGS data was subsequently used to interrogate publically accessible DNA databases in order to identify the plant species detected. The food products identified as possibly containing undeclared plant species were then subjected to established DNA analysis (DNA sequencing, real-time PCR) and protein analysis (ELISA) in order to verify the initial NGS screening results.

### **5. Results**

Of the original 45 samples, 14 were selected for further examination based on the first phase Illumina NGS results. These initial results indicated that two samples may have contained plant material from the *Poaceae* family, which includes wheat, while another sample possibly contained a member of the *Betulaceae* family which includes hazelnut. Wheat contains gluten and along with hazelnut must be declared as an allergen when used to produce foods in the EU. As this was potentially a consumer safety issue, those samples were sent directly to the Public Analyst laboratory in Galway for ELISA analysis to determine if proteins from wheat and hazelnut were present. Gluten or hazelnut proteins were not detected in the relevant

samples. Of the 14 samples subjected to further analysis, four (Oregano, Chili Powder, Spicy Cajun Mix and Red Clover Blossoms) were confirmed as containing undeclared plant material (Table 1).

**Table 1: Targeted DNA analysis of 14 food products for undeclared plant species**

Commercial product	Undeclared ingredients
Cumin seeds	-
Sage	-
Chilli Powder Hot	Mexican oregano ( <i>Lippia graveolens</i> )
Black peppercorns	-
Oregano	Bindweed ( <i>Convolvulus arvensis</i> ) and Indian sweet-clover ( <i>Melilotus indicus</i> )
Chinese 5 spices	-
Herbal infusion-Moringa TeaTox	-
Chamomile Tea	-
Psyllium husks Fibre	-
Black Tea PuErh	-
Cajun Spice Mix	White mustard ( <i>Sinapis alba</i> )
Aloe Vera	-
Red Clover Blossoms	Oryzinae subtribe (includes Rice ( <i>Oryza sativa</i> ))
Astragalus	-

### ***Oregano***

Using targeted PCR and DNA sequencing, two undeclared plant species were detected in the Oregano (*Origanum vulgare*) sample. Field bindweed (*Convolvulus arvensis*) was present at an estimated 10.6% of the level of Oregano, while Indian sweet-clover (*Melilotus indicus*) was detected at an estimated 0.08% of the Oregano content.

### ***Chili Powder***

The targeted DNA analysis identified *Lippia graveolens* as the possible contaminant in the “Chili Powder Hot” product. However, “Oregano” was listed as an ingredient and as *Lippia graveolens* is also known as Mexican Oregano no further action was considered.

### ***Cajun Spice mix***

The “Cajun Spice Mix” was found to contain DNA from *Sinapis alba*, a member of the Brassicaceae (mustard) family of plants, at an estimated level of 5.4% in relation to the main listed ingredient (*Capsicum annuum*). *Sinapis alba* is also known as “white mustard”, the seeds of which are used to make mustard, an allergen that must be declared when used to produce or prepare a food. The “Cajun Spice Mix” that was analysed by NGS was purchased in 2017 and the presence of mustard was not declared on the packaging. As this was a potential safety issue, the sample was sent to a commercial laboratory for protein analysis (ELISA) to determine the presence of mustard. In order to explore the possibility that this

was a batch-specific problem, a different batch of the same “Cajun Spice Mix” was purchased in October of 2018 and sent for the same protein analysis. The 2018 product carried a precautionary label indicating that it “May contain mustard”. The presence of mustard protein was confirmed at >6000 mg/kg in both the 2017 and 2018 products. The presence of mustard allergen at this level would be considered indicative of it being an undeclared ingredient rather than a cross-contaminant and as such could pose a safety risk to people with a mustard allergy. Further analysis of this product is being carried out to determine if the packaging declarations in relation to mustard content are appropriate.

### ***Red Clover Blossoms***

“Red Clover Blossom” is on the market as a powdered food supplement in gelatine capsules. Using non-targeted NGS, the powder was found to possibly contain plant material from the *Poaceae* family which includes grasses and wheat. However, gluten was not detected in a sub-sample analysed by ELISA. DNA from red clover (*Trifolium pratense*) was not detected by the initial screening by NGS analysis or subsequent targeted DNA analysis. Targeted DNA analysis was only able to detect DNA from a plant in the rice family. The original “Red Clover Blossom” product and a different batch of the same product were sent to a different commercial laboratory for DNA analysis (meta-bar-coding) in order to identify and possibly quantify the presence of DNA from “Red Clover Blossom” and a rice-related plant. However, that laboratory failed to extract any amplifiable DNA from either product. Therefore it has not been possible to conclude on the exact content of this product.

## **6. Discussion and Conclusions**

This is the first reported use of non-targeted next generation sequencing technologies to detect the presence of undeclared plant ingredients in foods and food supplements. The results of this study demonstrate that non-targeted NGS can be a proactive screening tool with which to detect, identify and tentatively quantify undeclared plant and animal species in a food. It is important to remember that, for now at least, NGS is primarily a screening technique and any results of interest should be subjected to verification through more established analytical methods.

The undeclared presence of a food allergen (mustard) in a food product (Cajun Spice Mix) was independently verified by immunological analysis (ELISA). The estimated mustard protein level in the spice product (>6000 mg/kg) suggests that it was likely to be present as an undeclared ingredient rather than being the result of cross-contamination. The mustard declaration on the packaging appears to have changed since this project began which suggests that the manufacturer themselves had discovered that the product was incorrectly labelled and that mustard was present as an ingredient. Further analysis is being carried out on this product to ensure that the mustard declaration is appropriate and that consumers are protected.

Oregano products have been the subject of a number of surveys in recent years that suggested adulteration may be a problem (Black *et al.* 2016). Therefore, it was not unexpected to discover the presence of undeclared Bindweed and Indian sweet clover, the former at significant levels (10%). These results provide sufficient justification for a more broad-

ranging investigation of Oregano products in order to determine the extent of adulteration within this product category.

The results of the DNA analysis of Red Clover Blossom supplements were inconclusive. The primary commercial laboratory was able to extract DNA (albeit with some difficulty) in which they reproducibly identified a plant from the rice family. However, they could not detect DNA from Red Clover Blossom. In contrast, a secondary commercial laboratory failed to extract any amplifiable DNA from the same sample. The failure of one laboratory where another succeeds (even to a limited extent) is possibly a function of a laboratory's capacity to troubleshoot, particularly when problems arise with the extraction and purification of DNA. Technical difficulties aside, an alternative reason for these DNA results is that there is no Red Clover Blossom material present in this supplement. However, safely reaching such a conclusion will require further examination, possibly using a variety of analytical methods.

This two year study has proved that next generation sequencing has the capacity to screen a variety of plant-based foods for the presence of undeclared plant species. However, there is no technical reason that the same technology cannot also be used to carry out non-targeted analysis of foods of animal origin and therefore broaden its future role in the proactive non-targeted detection of food safety and food fraud issues.

## 7. References

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## Annex I

### Plant-based foods screened by next generation DNA sequencing (NGS) in 2017

Product Name		Product Name	
1	Ground Almonds	28	Herbal Infusion - Moringa TeaTox
2	Roasted Chopped Hazelnuts	29	Chamomile Tea
3	Cumin Seeds	30	Herbamare Original
4	Coriander Ground	31	Organic Tomato & Herb
5	Extra Spicy Cajun	32	Organic Spirulina
6	Thyme	33	Psyllium Husks Fibre
7	All Season Paprika & Pepper	34	Saw Palmetto Berry
8	Sage	35	Ginger Root
9	Chilli Powder Hot	36	Milk Thistle
10	Steak Herb & Spice Blend	37	Organic Green Tea
11	Turmeric	38	Black Tea PuErh
12	Black Peppercorns	39	Madras Curry Powder
13	Black & Red Pepper	40	Greens & Berries
14	Italian Herb Seasoning	41	Cajun Spice Mix
15	Oregano	42	Organic Wheatgrass Powder
16	Rosemary	43	Aloe Vera
17	Herbes de Provence	44	Red Clover Blossoms
18	Chinese 5 Spice	45	Astragalus
19	Chives		
20	Paprika		
21	Parsley Flat Leaf		
22	Mixed Herbs		
23	Bay Leaves		
24	Bouquet Garni		
25	Basil		
26	Milled Hemp		
27	Herbal Tea		