Mass sequencing for rapid identification of ingredients in processed foods

What precisely is in our food? Here, Miguel Angel Pardo from AZTI extolls the virtues of mass sequencing in bringing clarity to this vital subject...

FOOD AUTHENTICITY is a major concern for consumers and food producers, with the European Union regulation (EU) No 1169/2011 requiring that consumers should be appropriately informed about the food they consume. Dishonest or inaccurate product information at societal level can cause reputational damage to food companies and create trade difficulties that eventually negatively affects the competitiveness of the whole sector.

It is vital to achieve a high level of health protection and a trustworthy food chain that ensures businesses are transparent and safe for consumers and reduces fraudulent practices. Widespread food fraud is the substitution of one ingredient for a similar cheaper one. A prime example is the horse meat scandal uncovered in 2013, where beef was substituted with horse meat in highly processed meat products and sold on the European market. This scandal revealed the complexity of the food chain and illustrated that food products such as hamburgers, lasagnes, meat cakes, meatballs, stews, etc., are particularly susceptible to mislabelling owing to the ease with which ingredients mixtures can be fraudulently added or by cross-contamination in the processing plant.

Options for identifying fraud

Different analytical procedures can be used to identify such adulteration, including spectroscopic, chromatographic, proteomic and DNA-based approaches. In recent years, DNA-based methodologies have shown to be an ideal approach to address the identification of species in food authenticity, mainly due to the sensitivity, accuracy and DNA stability under stressed food processing conditions.

The use of the polymerase chain reaction (PCR) technique as a routine method also enhances the expansion of DNA-based tools in control laboratories for food authenticity. Most of these methods amplify specific areas of DNA, using PCR, which is subsequently analysed with species-specific fluorescent probes in real-time PCR and arrays, fragments analysis by electrophoresis and DNA barcoding, among others.

DNA barcoding has demonstrated to be a very useful authentication tool for species identification by Sanger sequencing. Sanger sequencing (known as first-generation sequencing) is based on electrophoretic separation of products produced in individual sequencing reactions. This is very useful but has some significant limitations, showing only the dominant component and failing to identify other species present in the sample, which is very common in processed and complex food products.

To solve this issue, next generation sequencing (NGS), or second-generation sequencing, is a more recent and promising tool for routine analysis of mixed species in complex food samples, which doesn't require any prior knowledge of the species included in the sample. Several NGS platforms, using a variety of different chemistries, have been launched for high-throughput DNA sequencing and some laboratories even offer some platforms as an external service to identify ingredients in complex food samples.

Unfortunately, NGS applications are slow (requiring days of analysis), expensive, and need well-equipped laboratories and bioinformatic skills to analyse the large amounts of sequence data. Moreover, it is not, as of yet, suitable for rapid assessment of the authenticity of raw materials and food commodities.

Meatballs, stews and hamburgers are particularly vulnerable to adulteration

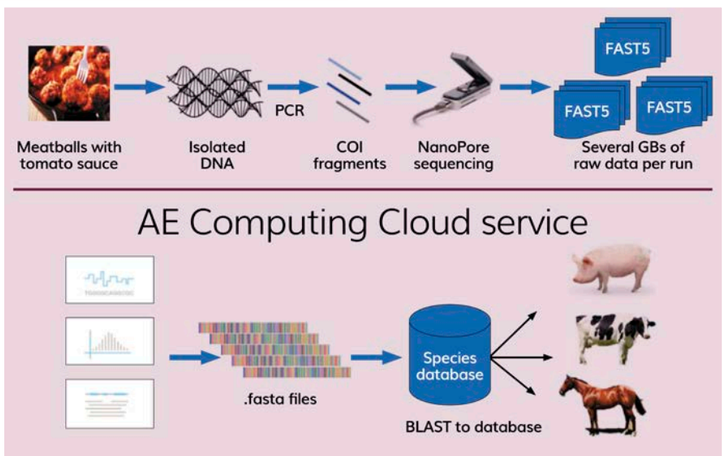
The most beneficial application of NGS for the food industry and consumers would be its incorporation into routine analytical authenticity checks at different levels of the food supply chain. In fact, third-generation sequencing (also known as long-read sequencing) is a class of DNA sequencing methods, currently under active development, that can be applied to solve this constraint since one of its key advantages includes portability and sequencing speed. The minimal sample pre-processing required, compared to second-generation sequencing, means that smaller sequencers can be designed.

A new tool on the block

The objective of the ongoing EIT-Food DNA Complex Project[1] is the application of the MinION™ small and portable third-generation sequencer (a four-inch-long USB-powered device containing an array of 512 sensors, commercialised by Oxford Nanopore Technologies), to monitor complex food products at retailer and/or producer sites. The hope is this will enable more frequent testing with a quicker turnaround time and at a lower price.

By testing more food products, we aim to proactively tackle fraud and reduce the number of reactive food recalls in store that cause loss of consumer trust. By testing more quickly, we can avoid blocking stocks of fresh products for a week, which often results in unnecessary food waste.

To meet this challenging objective, the project includes the optimisation of a rapid and simple protocol for the process of DNA isolation from complex food products, and the development of an innovative and user-friendly DNA-based identification workflow. This will include a curated DNA database and a computing cloud bioinformatic solution to detect the presence of not declared ingredients in complex food products, at real time, and directly in a retail environment. The selected complex food products are: (i) ready-to-eat meatballs (beef and pork) »



in tomato sauce and (ii) ready-to-eat fish stews (Atlantic cod and saithe; *Gadus morhua* and *Pollachius virens* respectively) with vegetables, scallops and prawns.

We have optimised a rapid DNA isolation protocol so that it takes just a few minutes. Basically, the food sample is completely homogenised with a blender and a small portion of mixture is solubilised with an extraction buffer after shaking for one minute. DNA solution is finally diluted in distilled water to reduce the presence of PCR inhibitors. This protocol has been successfully validated in-house in the laboratory after analysing 24 ready-to-eat meatballs and fish stew samples.

In all cases, the yield and quality of DNA obtained were comparable with that obtained from commercial kits that are routinely used in the laboratory. All DNA samples were successfully used as templates for PCR amplification of mitochondrial cytochrome oxidase I (for meat) and/or cytochrome b gene (for fish) for two hours. DNA amplified fragments were directly sequenced in a matter of hours, using the versatile Ligation Sequencing Kit through R9.4.1 flow cells with MinION™.

As a result, thousands of sequences were obtained and blasted against the curated DNA database developed in this project to obtain a specific and accurate identification of the ingredients. This database, including almost 200 seafood, fish and meat species, is part of the user-friendly DNA-based identification workflow that has been used in the project. This workflow aims to automate time- and labour-intensive bioinformatic processes and enable users to access data insights quickly and clearly by presenting powerful data visualisations.

Finally, the complete pipeline, including DNA isolation, PCR amplification and NGS analysis, can be achieved in just one working day, in contrast with the current NGS analytical services that need one week or more for testing.

Conclusion

To summarise, we have obtained a fast, cost-effective and user-friendly NGS pipeline based on the third-generation sequencing MinION™ device, for animal species identification in mixed food products to be directly applied in the food industry.

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Miguel, Food Quality, Safety and Identity Principal Researcher in charge of the Molecular Biology laboratory in AZTI (Member of Basque Research & Technology Alliance), has a PhD in Biochemistry and Molecular Biology. During the last 20 years, he has participated in numerous national and international research projects involved in the development of molecular identification methods, based on DNA analysis, for the identification of ingredients, micro-organisms, and parasites in food products to assure the identity and safety of the complete food chain. During his scientific career he has published 40 peer-reviewed scientific publications and more than 80 communications to congress, workshops and more. He is also co-author of four patents.

Reference

1. https://www.eitfood.eu/projects/dna-complex