LEARNING SERIES

FOOD AUTHENTICITY
TRACEABILITY & SAFETY

UNDERSTANDING DNA-BASED TEST METHODS

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in partnership with

FORUM

SGS

CONTENTS

I. EXECUTIVE SUMMARY

The increasing complexity of the food supply chain is challenging the industry to find ways to improve the traceability of ingredients used for food production. As DNA is one of the most universal molecules which can be used for species identification, DNA analysis is now being used extensively for food traceability.

Most DNA analysis methods in use today are based on targeted real-time Polymerase Chain Reaction (PCR) methods. However, the recent introduction of Next Generation Sequencing (NGS) into the food sector opened the door to an untargeted approach, one which is essential for addressing authenticity issues. With NGS, a single DNA analysis can assess the whole composition of a food product, whether the content is meat, fish, seafood, plants, microbes, or something else. There is currently no standardization of this method, however, extensive discussions and draft projects on the topic are being pursued by international standardization bodies, specifically the International Organization for Standardization (ISO).

In this paper, produced for The Consumer Goods Forum CGF and its End-to-End Value Chain Learning Series, we look at some of the pioneering work being done to introduce NGS for routine use in the food sector. A number of companies already use NGS, SGS included, for routine authenticity analysis, which provides clear evidence of the value of this method for traceability along the food production process. In the near future, it is expected that this technology will become common place for addressing authenticity and microbial safety issues.

II. THE GLOBAL FOOD INDUSTRY TODAY

Increasing need and demand for food products means that the global food industry is facing several new challenges. To meet demand, new approaches have been introduced for food production, such as farmed fish, and the introductions of new proteins like algae and insects. At the same time, the industry is working to meet a noticeable increase in demand for high quality and premium food products.

To address these challenges, the global food sector is developing and producing innovative and diversified food products to cover different market segments and to meet consumer expectations. The challenge facing stakeholders is to do this without compromising quality and safety. Additionally, it is important to differentiate products from the competition and meet consumer expectations.

TRACEABILITY

Today, the food industry is truly global, involving producers and manufacturers from around the world. Such complexity makes ensuring traceability of the entire supply chain, from primary production to the end-consumer product, truly challenging. The number of intermediaries and geographical locations involved in manufacturing processes creates a network that requires the most advanced traceability systems.

At the same time, the industry is trying to meet pressure from consumers to systematically reproduce the same kind of food products, sometimes with minor changes in terms of the final product.

Consumers base their decision to choose a food product from a specific brand on several factors. Final price and organoleptic characteristics are probably the major drivers of the food industry. Therefore, the industry's challenge is to create products with the best combination of these two criteria.

In order to achieve this and be competitive in a highly dynamic and competitive market, food producers must control their raw materials and obtain them at the lowest price. However, raw materials are also of interest to producers from a labeling perspective. All food products must display a label listing their ingredients. This is for legal reasons and because consumers expect to be able to not only check ingredients, but also to evaluate the product and compare it with similar products from other brands.

Consumer choice is highly dependent on a number of socio-economic factors such as culture, religion, health concerns, quality, and prices, among others. Increased requirements for product information and the globalization of the food industry have turned food safety and authenticity into major issues demanding specific authorities and regulations worldwide. Consumers want traceability.

III. INTERNATIONAL STANDARDS AND REGULATIONS

Food regulation and internationally recognized standards are the most challenging topics facing the food sector. These dovetail nicely with new demands for information on and requirements for food safety, traceability and authenticity. As a result, regulators are facing the introduction of new analytical methods.

Food authenticity is starting to be introduced to the regulatory arena. Recent food fraud scandals mean it is imperative that the industry can identify the food ingredients that compose each food product, whether it is meat, fish or plant-based. However, the more ingredients there are in a single food product the more complex the issue of traceability becomes, particularly if ingredients are sourced from different geographical origins, each with its own requirements. This means that the global food industry needs to adapt to the challenges presented by a dynamic and rapidly growing food market.

AUTHENTICITY

To address these challenges, DNA-based methods are being introduced in relation to authenticity at a regulatory/standardization level. To date, these methods have been used extensively for food safety purposes, mainly pathogen detection and genetically modified organisms (GMOs). Already, DNA-based methods are recognized as powerful, reliable tools in this field, and are used by official safety regulatory entities for epidemiological testing and characterization of specific pathogens including, but not exclusively, *Salmonella, Listeria*, and *Escherichia coli*.

Among DNA-based methods, DNA sequencing is considered the gold standard for species identification. In recent years, the development of high throughput sequencing methods, such as NGS, has heralded a new era in the food sector for traceability, safety and authenticity. This is evident at the standardization level as several working groups and projects are underway to propose new standards or technical specifications based on NGS.

ISO INVOLVEMENT

Recognized as the main entity for method standardization worldwide, the ISO is involved in discussions and working groups to devise and define the use of NGS methods for food topics including authenticity. At ISO technical committee (TC) 276, which deals with biotechnology, joint working groups are underway for the introduction of NGS into standardization. With regard to microbiological testing standards, a new working group (within ISO/TC 34 – focused on food products) was created in 2015 for the application of NGS for Whole Genome Sequencing (WGS) for microbiology typing and gene characterization. Additionally, new projects are ongoing in TC34 for the introduction of NGS for food authenticity purposes. In parallel, many national standardization entities are working on new standards for authenticity based on DNA sequencing, using the NGS method.

BARCODING

Probably the most well-known use of DNA sequencing for food authenticity is the DNA Barcoding strategy that is already in use by many regulatory entities in the sector. Perhaps one of the most widely used barcoding methods is the one for fish-based products, enabling fish species identification by regulatory bodies in the US and Europe. However, this method is not suitable for processed samples that contain multiple ingredients (species) as it only enables the identification of a unique species. Food products containing multiple species cannot be analyzed with this approach.

NEW STANDARDS

There is no ISO standard supporting any species identification method. However, SGS is currently involved in working groups at the ISO and AFNOR (Association Française de Normalization) level to produce new standards for species identification, where NGS will be introduced into those new standards.

Since no official worldwide standard exists for species identification, each laboratory can use a different method for authenticity evaluation. This is creating additional problems for the food industry.

IV. INDUSTRY CHALLENGES

In common with almost every industry, profitability is a major driver for food company sustainability. At present, many food retailers have their own brand and competition is fierce for these brands to guarantee good quality products at the lowest prices. This results in pressure all along the supply chain. A single change to just one ingredient can have a significant impact on the final product.

LAYERS OF REGULATION

At the same time, local legislation creates new challenges for the food industry. Local regulators respond to increased concern about anything that can impact human health, as well as issues of authenticity. This adds more layers of regulation to food markets. Furthermore, today's consumers are much more concerned about a product's ingredients. These concerns can be at different levels.

The first is probably a concern that they are paying for something that is not as labeled, or what they paid for – this is mainly a financial concern. However, additional consumer concerns can be about allergens, food intolerances, species protection and species sustainability, amongst others. Nutritional content is highly dependent on a product's ingredients, the full or partial substitution of any specific ingredient can impact this.

Any of these concerns can be highly damaging to a food brand and consumers can rapidly lose confidence.

ALLERGENS

NGS is a particularly powerful tool when it comes to allergens. Undeclared allergens are the most common reason for food product recalls. These are costly, both in financial and reputational impacts. Yet, the untargeted approach of NGS makes it significantly easier to identify the presence of any allergens and allow remedial action to be taken.

Although in recent years many chemical and DNA based methods have been introduced to solve the question of food authenticity, only the use of NGS in an untargeted approach is able to detect, in a sample with any DNA presence, all those species present. This is a significant development on methods like PCR which can only focus on a single, or small group of targets.

A more detailed description of the NGS method and its properties is described in the following sections of this paper.

For the industry, the challenge now is to use this type of untargeted method, as it is recognized by experts as the most appropriate. Draft ISO standards have already reached the first stage and in the interim many companies are already using NGS as a routine method for verifying food authenticity and identifying the species content of highly processed products.

V. DNA-BASED METHODS: STATE-OF-THE-ART

DNA-based analysis is has come to the fore in the last four years as a direct result of food safety and authenticity issues. Its use started with the need to control genetically modified (GM) food products. With the increasing number of new genetic events (detectable changes in DNA) released in recent years, the method of choice for both qualitative and quantitative analysis of genetically modified organisms (GMOs) has been real-time PCR.

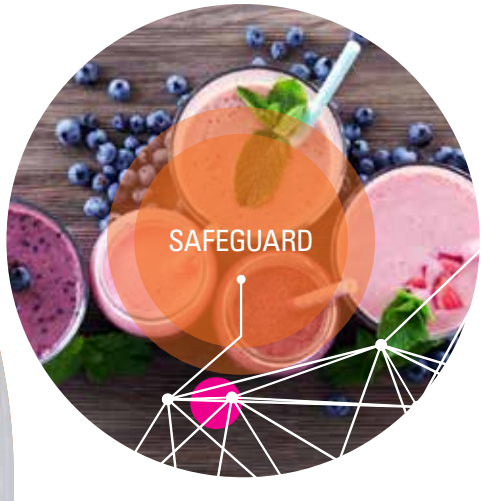
Since 2013, the use of DNA-based methods has been seen, in many cases, as a good alternative for identification of species, or as a complementary tool for other chemical analysis. This follows a number of food authenticity scandals, in Europe – horse meat substitution in beef meat products, in the US – herbal supplements and spice mixtures contaminated with substances including rice and beans, as well as traces of hazelnut, and in Australia – oregano spices adulterated with olive and sumac leaves.

However, aside from GMO analysis there is no ISO standard describing which method should be used for species identification. As a first approach, most laboratories dealing with authenticity issues employ real-time PCR methods targeting the most common species. This method is based on the amplification with artificial molecules (called primers) of specific DNA regions for each species to be detected. In the event that the targeted DNA is present then a fluorescence signal is produced. Today, most labs still use this targeted approach even with its very limited use for authenticity issues.

DNA-based methods, are limited by the need to obtain DNA fragments with the integrity needed to perform analysis. In some products, specifically those which have been highly processed, the DNA of ingredients can be highly fragmented and even absent. This is particularly true for highly processed foods where, typically, the DNA obtained after extraction is very low and highly fragmented. When DNA is highly fragmented it is essential to guarantee that the DNA based method used will enable the detection of DNA fragments as small as 100 base pairs (bp), or even lower.

The smaller the DNA fragment to be analyzed, the more difficult it is to differentiate between closely related species. The best strategy is to use a DNA sequencing method that obtains the full nucleotide (A, T, G, C) sequence of the target region to be analyzed. Real-time PCR's fluorescent signal is a limitation for the detection of species cross reactivity, and may produce false positive results, especially in complex food products containing multiple ingredients.

Experience tells us that when authenticity issues are involved a targeted approach is not suitable, as it will only deliver a result for the species targeted. If a product contains any additional species, besides those targeted by PCR analysis, no information is available. Therefore, the availability and use of an untargeted approach is of great significance and importance.



VI. NGS AS THE GOLD STANDARD FOR DNA-BASED SPECIES ID?

The NGS method is based on DNA analysis through DNA sequencing and produces millions of individual DNA sequences all grouped in a single file. With NGS different DNA sequences can be produced from different DNA templates without affecting the final result, unlike Sanger sequencing where sequencing different DNA templates simultaneously results in an undefined result. The Sanger method can therefore only be used for single species products.

Using appropriate software, the scope of NGS is virtually unlimited and it can be used on any kind of sample DNA, whether or not it contains different DNA sequences. This means that any kind of species can be detected, the analytical method is no longer focused on the detection of a limited number of species.

When a sample is analyzed the question is no longer: "Are species X, Y or Z present in the sample?" Using NGS the question is: "Which species are present in the sample?"

NGS analysis delivers millions of individual sequences making it possible to identify species in complex foods that contain multiple ingredients. This is because each ingredient will produce a single and unique DNA sequence. DNA sequences obtained by NGS are compared with databases and the results identify all species, including scientific names.

Currently, NGS is the only method that ensures the correct identification of species in complex foods, due to the characteristics described above. Therefore, use of this method is increasing and it is being applied by all major laboratories for food authenticity analysis. Due to the untargeted nature of this method even exotic species can be identified.

The advantages of this method include:

- Processed food samples containing multiple species can be analyzed
- The method is not based on the detection of specific species (pork, horse, etc.)
- The result obtained is a list of the species present in a sample (any species can be detected)
- The database used for identification contains thousands of species and is not limited to a short list containing the most common species
- The method employed is based on the most reliable method for identification of organisms – DNA sequencing
- The method enables relative quantification of the DNA species detected, with a quantification/ detection limit ranging from 0.0001% to 0.5%

HOW NGS WORKS

PCR amplicons obtained are sequenced using the NGS approach to obtain thousands of DNA sequences for each different species that are included in a sample. The amplicons used are very short (average 100 bp) to maximize success, even in highly processed food products, such as canned food and animal feed (170°C, 3 atm).

The DNA sequences obtained are compared with internal and/or external DNA databases and species identified. As NGS is an untargeted approach the final result is a list of all the species that are included in a sample.

For example, PCR analysis of a meat-based product (hamburger, lasagne, pizza) will tell you whether pork is present – yes, or no. With just one test, the SGS All Species ID DNA Analyzer based on NGS will tell you all the meat species that are present. Adding an NGS analysis of plant DNA, the meat based-product can also be screened for adulteration with plant material.

At < 0.1%, the Limit of Detection (LOD) for NGS is similar or even lower than that of real-time PCR.

Additionally, for DNA quantification, NGS also offers advantages over the real-time PCR approach. This is because universal primers are used in NGS for the different species in a food product, instead of the taxa-specific primers used for PCR that can cause bias during the amplification step. Although NGS is not yet used routinely for species DNA quantification, this method will also introduce value in terms of its reliability.

VII. THE FOOD DNA ANALYZER: AUTHENTICITY, TRACEABILITY AND SAFETY IN A SINGLE SHOT

During the last three years, a workflow based on NGS was developed to ensure its suitability for food analysis, namely to fulfill the following requirements:

- Optimal multiple gene regions to be analyzed – they must be as short as possible to be applicable to processed foods where DNA can be highly fragmented, but informative enough to provide a reliable species identification
- Universal primers that ensure an unbiased NGS library construction and cover as close as possible 100% of all known species
- Bioinformatics tools that allow the efficient and reliable analysis of a huge number of DNA sequences using suitable databases for identification. This process must be as simple and as quick as possible to avoid bottlenecks in analysis

For example, SGS routinely uses NGS for complex and processed food analysis, to assess both food authenticity and safety. A complete workflow that fulfills the requirements described above has been developed and enables, in a single NGS run, the identification of almost any kind of organism relevant to the food sector using an untargeted approach. These include, but are not restricted to, meat, fish, seafood, spices, herbs, fruits, cereals and microbes.

Based on DNA and bioinformatics expertise for species identification in highly complex and processed food products new tests are continuously under development to fulfill client needs. Depending on allergen lists in different countries, specific tests for allergenic species screening are available, including plant-based and animal-based allergenic species. Based on this screening, any food product can be tested for the presence of DNA of any kind of allergenic species, offering the market simple and cost-effective information about the presence of allergenic species.

SGS has also developed, and maintains, a NGS database of thousands of species (mammals, birds, reptiles, amphibians, seafood and plants). Additionally, safety issues can be addressed by NGS, namely for pathogen and spoilage microbial analysis. Available in SGS labs worldwide, this workflow makes NGS-based food species identification more accessible.

VIII. CASE STUDIES

SPICES

SUPPLIER SELECTION

A retailer evaluating suppliers to select the best spice producer, to source oregano-based products, knows that several studies have highlighted the presence of additional plant species in oregano samples. To evaluate the purity of oregano, NGS was used to evaluate the presence of additional plant species. NGS is the appropriate method to identify the full species composition in products potentially containing multiple species. Based on the NGS results a ranking could be performed for oregano suppliers.

COFFEE

ARABICA AND ROBUSTA COFFEE VARIETIES

Roasted coffee can be composed of two varieties, Arabica and/or Robusta, identifying which is which requires sampling and testing. However, due to the transformation processes for roasted coffee, the DNA is highly fragmented. The NGS method is suitable to assess the content of roasted coffee by obtaining data from the very short DNA sequences and using them for identification of the correct variety or to detect mixings. Additionally, NGS can be used to identify the presence of any other plant material in roasted coffee.

FISH

MULTI-SPECIES GROUPER FILLET

When identification was sought for a grouper fillet, using the DNA barcoding method for single species products (based on conventional Sanger sequencing) it could not produce a result because multiple fish DNA sequences were present. Subsequently, NGS was used and it identified four different species in the fillet. Based on the results obtained, and after checking the supply chain, it was concluded that the fillet was in fact a mass of fish which had been processed to create the same visual appearance as a single-species fillet. The multiple-species capability of NGS proved to be of major value for this product.

MICROBIOLOGY

PROBIOTIC DAIRY AND OTHER FERMENTED PRODUCTS

During the production of microbial based products, for both probiotics with bacteria and fermented products with yeasts, the correct assessment of the microbial inoculum used and the traceability of the microbes during the manufacturing process is essential. The correct microbial strain or mixed inoculum are essential to ensure the properties of the end product. To guarantee the correct composition NGS can be used for strain certification by Whole Genome Sequencing (WGS) or inoculum species certification using a species NGS metagenomics analysis.

IX. CONCLUSION

Among DNA based methods for food analysis, NGS is recognized as the most powerful. Its untargeted nature and flexibility is undoubtedly changing the way the food industry is looking to achieve traceability along the supply chain, in terms of ingredient identification from farm to fork.

Although no official standards are available for the NGS method, it is already widely used for complex food products, as it is the most suitable and offers an untargeted approach that is essential for establishing food authenticity.

Today the most common DNA-based method in use by many food laboratories remains Real-time PCR. However, it is likely that NGS will become common in the food analysis arena in the near future. The flexibility of NGS is one of its major advantages since all types of DNA present in a food product can be identified.

At the same time, standardization entities worldwide are looking at the science and the technology behind NGS to plan the way forward and create official standards.

NGS is well known in this sector as the DNA-based tool for microbial characterization and epidemiology. One of its best-known uses is for Whole Genome Sequencing which has been used extensively by authorities for pathogen strain characterization.

In this white paper, SGS has shown how NGS is routinely used to support clients in the identification of meat, fish, seafood, plants, and microbes. Such flexibility means that the same method can be used not only for authenticity, but also microbial safety and traceability across the manufacturing food process.

ABOUT THE CONSUMER GOODS FORUM

The Consumer Goods Forum ("CGF") is a global, parity-based industry network that is driven by its members to encourage the global adoption of practices and standards that serves the consumer goods industry worldwide. It brings together the CEOs and senior management of some 400 retailers, manufacturers, service providers, and other stakeholders across 70 countries, and it reflects the diversity of the industry in geography, size, product category and format.

Its member companies have combined sales of EUR 3.5 trillion and directly employ nearly 10 million people, with a further 90 million related jobs estimated along the value chain. It is governed by its Board of Directors, which comprises more than 50 manufacturer and retailer CEOs.

For more information, please visit: www.theconsumergoodsforum.com

ABOUT SGS

SGS is the world's leading inspection, verification, testing and certification company. SGS is recognized as the global benchmark for quality and integrity. With more than 95,000 employees, SGS operates a network of over 2,400 offices and laboratories around the world.

SGS helps enhance food safety and quality with a comprehensive and cost-effective set of control solutions including audits, certification, testing, inspection, technical solutions and training. These services can be stand alone or part of an integrated package of measures to assist your company in continuously improving the culture of food safety, quality and sustainable development. Enhancing processes, systems and skills is fundamental to your ongoing success and sustained growth. SGS enables you to continuously improve, transforming your services and value chain by increasing performance, managing risks, better meeting stakeholder requirements, and managing sustainability.

With a global presence, we have a history of successfully executing large-scale, complex international projects. SGS people speak the language, understand the culture of the local market and operate globally in a consistent, reliable and effective manner.

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WHEN YOU NEED TO BE SURE

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