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Meat Technology — Special Issue 66/3

www.meatcon.rs ▪ www.journalmeattechnology.com

UDK: 663/664:658.562.4

ID: 179289097

https://doi.org/10.18485/meattech.2025.66.3.4

Review Paper

From gene to table – DNA barcoding the backbone of next-generation food integrity and safety

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ARTICLE INFO

Keywords:
DNA barcoding
Food authenticity
Food safety
Molecular identification
Traceability
Food fraud

ABSTRACT

The authenticity and safety of food are critical issues in global supply chains, where mislabelling, adulteration and contamination continue to challenge food integrity, consumer-trust and safety, and public health. DNA barcoding, a molecular identification method based on short genetic sequences, is a powerful tool for species verification in a wide range of food products, including meat, fish, plant-based foods, and processed foods. This study explores the application of DNA barcoding with an emphasis on its role against food fraud, consumer protection and public health. Advances in barcoding technologies, coupled with accessible databases enable identification of species even in complex or degraded samples. Case examples illustrate its successful implementation in regulatory, industrial, and research settings. The integration of DNA barcoding into traceability systems supports a more transparent, accountable, and trustworthy food supply industry. As food systems evolve, DNA-based verification is poised to become a core component of next-generation food integrity strategies.

1. Introduction

Food quality and safety are critical issues in the increasing global food trade characterized by complex supply chains. Although consumers increasingly demand sustainable, transparent, and healthy food products — and despite stricter regulations and technological progress — food fraud remains a persistent issue. It poses risks to public health, causes economic damage, compromises religious dietary requirements, and may even contribute to the endangerment of protected species (*Li et al*., 2020; *Filonzi et al*., 2023). Food fraud involves deliberate mislabelling, species substitution, and exposure to toxins, pathogens and allergens (*Ortea et al.,* 2012) threatening consumer health and undermine consumer trust (*Dawan and Ahn*, 2022). Mislabelling rates across various food sectors including seafood, meat, botanicals, spices, and probiotics exceed on average by > 20% (*Gorini et al.,* 2023) to a maximum of >70% for certain food categories (*Stamatis et al.,* 2015). The most common type of food fraud is the replacement of a component with a similar cheaper one, followed by undeclared ingredients (*Stamatis et al.,* 2015).

Since the 1980s worldwide research strategies have been aiming to develop and improve

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Paper received September 10th 2025. Paper accepted September 15th 2025.
The paper was presented at the 63rd International Meat Industry Conference "Food for Thought: Innovations in Food and Nutrition" – Zlatibor, October 05th-08th 2025.
Published by Institute of Meat Hygiene and Technology – Belgrade, Serbia.

technologies to control authenticity and safety of foods (*Katerinopoulou et al*., 2020). Amongst the analytical tests used for food authentication DNA-based techniques are most effective. DNA barcoding, a molecular technique that uses short, standardized genetic markers to identify species (*Hebert et al.,* 2003), has become a cornerstone in addressing these challenges (*Kumar et al.,* 2009). Originally developed for taxonomic and biodiversity research, DNA barcoding has been adapted to food science to authenticate raw and processed food products, even when morphological characteristics are degraded or lost (*Dawn and Ahn*, 2022). The technique has evolved further with the integration of next-generation sequencing (NGS), enabling DNA metabarcoding—a high-throughput approach capable of detecting multiple species in complex mixtures (*Dobrovolny et al*., 2022). This has proven especially valuable in uncovering adulteration in meat products and composite foods. For example, a 2024 study applying metabarcoding to EU meat samples revealed numerous undeclared animal and plant species, providing evidence of hidden substitutions and labelling violations (*Mottola et al*, 2024). However, the field is not without its limitations. A 2024 systematic review of metabarcoding in animal-origin food authentication highlighted persistent issues such as inconsistent primer sets, platform-dependent biases, and the need for standardized quality control procedures before the method can be widely adopted for regulatory use (*Giusti et al*., 2024). In the seafood industry, DNA barcoding continues to reveal significant mislabelling — for instance, a 2021 Italian border-inspection study found 22.5 % mislabelling in imported seafood, with cephalopods mislabelled at 43.8 % and fish at 14.0 % — sometimes involving endangered or protected species (*Filonzi et al*., 2023; *Guardone et al*., 2021). The economic impact of food fraud remains uncertain, with global estimates ranging between $ 10 billion (*Robson et al*., 2020) to at least $ 65 billion (*Agetu*, 2020). In 2017 alone, authorities in Europe seized approximately 9,800 tonnes of non-authentic food (*Kendall et al*., 2019). These findings illustrate the global scale of the problem and the importance of molecular tools in enforcing food law and protecting endangered species.

This review provides a comprehensive overview of DNA barcoding and its technological advancements, with a focus on its role in ensuring food authenticity and traceability "from gene to table" (*Liu K et al*, 2022). Current applications across food categories are reviewed, regulatory perspectives are addressed, and emerging trends including portable sequencing and the integration with blockchain technology are examined (*Singh and Sharma*, 2023).

2. Principles and technological advancements in DNA barcoding

The selection of DNA barcoding markers depends on the target organism. For animals, the cytochrome c oxidase I (COI) remains the standard. In plants, mitochondrial genes evolve too slow, so plastid genes like *rbcL* and *matK* are used instead (*CBOL Plant Working Group*, 2009). For fungi, the internal transcribed spacer (ITS) region offers high discriminatory power (*Schoch et al.*, 2012).

The barcoding workflow typically begins with sample collection from food matrices (Figure 1). DNA is then extracted using commercial kits or CTAB-based protocols designed to overcome food-specific inhibitors like fats or tannins. Target regions are amplified via PCR, often using nested or multiplex strategies in complex mixtures (*Handy et al.*, 2011). Sequencing is performed through either traditional Sanger methods or whole-genome sequencing. Traditional Sanger sequencing, while reliable, is limited by low throughput and an inability to resolve multiple species in complex mixtures. These constraints have been overcome by the introduction of next-generation sequencing (NGS) technologies. This enables DNA metabarcoding—simultaneous identification of multiple species within a single mixed sample—making the technique particularly valuable for testing multi-ingredient and processed foods like sausages, herbal blends, and convenience meals.

In cases where DNA is degraded—such as in canned or heavily processed foods—shorter "mini-barcodes" (less than 200 base pairs) improve amplification success (*Stahl et al*., 2023). Mini-barcoding achieved identification rates above 90% across a range of difficult food matrices (*Stahl et al.,* 2023). Nevertheless, the shorter length of mini-barcodes may limit their ability to discriminate between closely related species, and issues such as misidentified specimens, sample confusion, or contamination can further compromise accuracy. However, advances in reference databases and bioinformatic methods have improved reliability, and combining multiple loci or integrating mini-barcodes with full-length markers allows robust species identification (*Galimberti et al.,* 2013; *Cheng et al*., 2023).

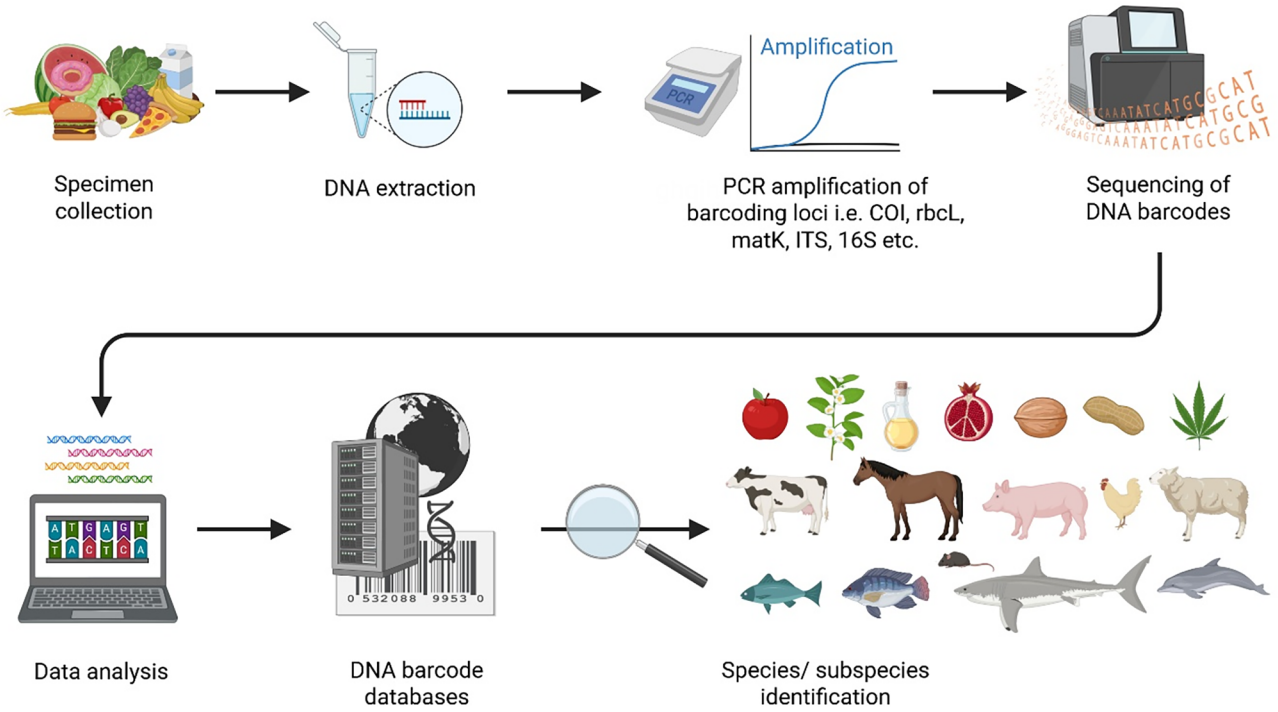

Figure 1. The principles of the DNA- barcoding technique (Created in BioRender. Ruppitsch W. (2025) https://BioRender.com/bwtr3ev»)

The effectiveness of these tools depends heavily on the quality of the reference databases used. Customized regional or institutional databases are increasingly being incorporated into barcoding workflows to complement public repositories and improve identification accuracy. Such tailored datasets, for example those developed for mammalian and poultry species authentication, allow laboratories to use relevant barcode sequences adapted to their validation needs (*Dobrovolny et al*., 2022). Two of the most common global databases are the Barcode of Life Data System (BOLD), which is curated specifically for barcoding and linked to voucher specimens (*Ratnasingham and Hebert,* 2007), and GenBank, which offers broader coverage but is less curated and more prone to misidentifications (*Nilsson et al.,* 2006). To enhance consistency and data interoperability, initiatives such as the FAIR data principles (Findable, Accessible, Interoperable, Reusable) have been introduced. These guidelines aim to improve database standardization and facilitate the integration of barcoding outputs into regulatory and traceability systems (*Wilkinson et al*., 2016). Hybrid strategies—such as cross-validating GenBank hits with BOLD entries or building in-house curated databases—are becoming increasingly common for ensuring accuracy in critical applications like regulatory inspections and certification.

Collectively, these technological advancements have pushed DNA barcoding from a niche laboratory method into a mainstream, scalable, and field-deployable tool with broad applications in food integrity and safety.

3. Applications in food integrity and safety

One of the most pressing concerns in global food markets is species mislabelling and substitution, which remains prevalent in seafood, meat, and plant-based products. For instance, studies in Thailand have shown that over 24% of seafood products were mislabelled, including cases where endangered species like *Thunnus maccoyii* (Southern bluefin tuna) were substituted for more common varieties (*Senathipath et al.,* 2024). DNA-based methods, including metabarcoding and DNA barcoding, have revealed the presence of undeclared animal species in a substantial proportion of meat products, highlighting widespread mislabelling and the utility of these molecular tools for verifying species composition and ensuring food authenticity (*Tantuan and Viljoen*, 2021; *Hellberg et al.,* 2017). In the plant sector, DNA markers such as *rbcL*, *matK*, and ITS2 have uncovered extensive adulteration in herbal supplements and traditional medicines (*Harris et al.,* 2024; *Chen et al.,* 2022). High-value oils, such

as olive oil from protected regions, can also benefit from DNA-based traceability tools, which help authenticate geographic origin and detect adulteration with lower-quality oils (*Agrimonti et al.,* 2011). DNA barcoding is also instrumental in detecting adulterants and contaminants that pose health risks to consumers. Even trace amounts of undeclared allergens like peanuts, soy, or crustaceans can trigger serious allergic reactions. DNA-based analyses have proven effective in identifying such allergens in processed foods and sauces, as well as banned substances like bushmeat or endangered species in imported products (*Staats et al*., 2016). Using metabarcoding techniques, numerous instances of undeclared animal DNA have been detected in products labelled as vegan or allergen-free, which can lead to product recalls and stricter regulatory oversight (*Giusti et al.,* 2023; *BeVeg*, 2023). The method also enables detection of toxic plant species that may be accidentally or fraudulently incorporated into teas, supplements, or traditional remedies—situations where visual inspection would be inadequate *(Sucher and Carles*, 2008).

Beyond authenticity and allergen control, DNA barcoding plays a growing role in verifying compliance with religious and ethical food certifications. Halal certification, for example, requires the exclusion of any porcine material; barcoding is routinely employed to test for pork DNA in meat, gelatine, and additives (*Shabani et al.*, 2015). Kosher regulations similarly restrict specific ingredients and combinations, and DNA-based tests can detect the presence of shellfish or non-kosher fish in processed foods (*Andronache et al.*, 2025). Even vegan-labelled products are increasingly scrutinized using metabarcoding to ensure they are free of trace animal DNA, especially in shared production facilities (*Srivathsan et al.,* 2021).

DNA barcoding is expanding into the realm of microbial food safety (Sabater et al.,2021). Although originally developed for multicellular organisms, adaptations using 16S rRNA and ITS rRNA sequencing now allow for the identification of bacterial, fungal, and parasitic contaminants. This approach has been applied to detect key pathogens such as *Salmonella*, *Listeria*, and *E. coli* in meat and dairy products, as well as toxigenic fungi like *Aspergillus* spp. and *Fusarium* spp. in cereals and spices (*Muñoz-Martinez et al.,* 2025; *Gonzalez-Escalona et al.,* 2017), and protozoan contaminants like Cryptosporidium spp. and Giardia spp. in water and fresh produce. DNA metabarcoding has been applied in Sardinian sheep cheese processing environments, identifying bacterial strains that could contribute to product contamination and spoilage, highlighting the utility of metabarcoding for monitoring microbial risks in cheese production (*Giagnoni et al*., 2025). Applications of DNA barcoding in food integrity and safety are shown in Table 1.

4. Regulatory and industrial perspective

Food fraud within the European Union is regulated under Regulation (EC) No. 178/2002 of the European Parliament. Despite significant efforts toward its prevention, access to reliable and publicly available information remains limited (*Brooks et al.,* 2021). European mechanisms such as the Rapid Alert System for Food and Feed (RASFF), established in 1979, and the EU Food Fraud Network,

Table 1. Applications of DNA barcoding in food integrity and safety

Application area	Description
Species mislabelling	Identification of incorrectly labelled or substituted species in meat, fish, etc.
Detection of allergens and contaminants	Detection of trace allergens (e.g., peanut, soy) and harmful substances.
Religious and ethical standards	Verification of the absence of prohibited ingredients in Halal, Kosher, and vegan products.
Microbial contamination	Identification of bacteria, fungi, and parasites relevant to food safety.
Authentication of plant-based products	Analysis of teas, supplements, and herbal blends for undeclared or toxic species.
Verification of declared composition	Detection of undeclared or illegal ingredients in complex processed foods.

launched in 2013, have improved data sharing and cross-border collaboration in addressing food fraud (*Brooks et al*., 2021). However, the complexity of international supply chains, combined with the highly complex nature of many food products, increases the probability that fraud remains undetected (*Manning & Monaghan*, 2019).

One contributing factor is that the Hazard Analysis and Critical Control Points (HACCP) system, while effective for food safety, was not designed for the detection of fraudulent practices (*Kowalska,* 2018). To address this gap, complementary frameworks such as Threat Analysis and Critical Control Points (TACCP) and Vulnerability Assessment and Critical Control Points (VACCP) are increasingly being adopted (*Brooks et al.,* 2021). The effectiveness of these systems, however, depends heavily on the application of advanced analytical techniques capable of verifying food authenticity and preventing adulterated products from entering the supply chain (*Manning & Soon*, 2016; *Fox et al.,* 2018).

Building on this foundation, DNA-based techniques such as DNA barcoding and metabarcoding are increasingly regarded as valuable tools to strengthen global food authenticity and traceability systems. In the EU, the European Food Safety Authority (EFSA) has acknowledged their scientific potential, particularly regarding identity verification of novel foods including plants, animals, fungi, and algae, but has not yet formally endorsed their widespread use (*Veveris,* 2024). Metabarcoding, for instance, shows particular promise in detecting undeclared species or complex plant compositions, though its regulatory application remains under development by EFSA and EU Member States (*Mottola et al*., 2024; *Giusti et al*, 2024).

In the United States, the Food and Drug Adminsitration (FDA) has integrated DNA-based methods into its seafood inspections and guidance materials to support regulatory compliance and ensure seafood authenticity (*Handy et al.,* 2011). These methods use standardized DNA sequences, such as those in the Regulatory Fish Encyclopedia, to detect mislabelled or substituted species throughout the supply chain, enhancing traceability, preventing fraud, and supporting consumer protection (*Haile et al.,* 2008). DNA barcoding represents a valuable tool that helps the FDA ensure the safety and integrity of the U.S. food supply (*Jones et al*., 2013).

Countries like Canada, Australia, and China also apply DNA barcoding in customs, Halal/Kosher certification, and herbal verification (*Fathima et al.,* 2024; *Aghayeva et al.,* 2021). Seafood processors use DNA barcoding to confirm species at each supply chain step, helping detect mislabelling and thus improve traceability (*Pardo et al*., 2020). The technology is also being embedded in blockchain platforms for meat and herbal supplements, linking product records to genetic proof (*Gorini et al*., 2023).

Globally, the Codex Alimentarius Commission has begun incorporating molecular tools into its food authenticity standards, encouraging harmonization. While Codex recognizes the importance of DNA-based methods for detection and identification of species and specific proteins in foods, their use is not yet mandatory, and implementation depends on national authorities (*Codex*, 2011). So far, FDA and the Joint Research Centre (JRC) of the European Commission have released detailed protocols, and Codex is evaluating molecular methods for inclusion in international guidelines, however standardized and validated protocols are still needed to ensure harmonized application across countries (*JRC*, 2024; *Codex*, 2023).

5. Challenges and limitations

Despite its advantages, DNA barcoding faces several limitations that hinder its universal application in food authenticity and safety. A major technical barrier is DNA degradation in processed foods. Thermal, chemical, or mechanical treatments often fragment DNA, making standard barcode regions like COI, *rbcL* or ITS difficult to amplify (*Meusnier et al*., 2008). Mini-barcodes (<200 bp) have been developed to overcome this, though they may lack full taxonomic resolution (*Shokralla et al*., 2015). Another critical issue is the incompleteness and inconsistency of reference databases (*Kartzinel et al*., 2025). Many food-relevant species are underrepresented, especially among plants, fungi, and minor seafood taxa. Misannotations in public databases such as GenBank can result in misidentifications, prompting researchers to cross-reference with BOLD or use curated in-house databases (*Ratnasingham and Hebert*, 2007).

A lack of global harmonization means results accepted in one region may not be valid in another (*Geary et al*., 2019). Data sovereignty is also a concern, particularly where genetic resources from indigenous regions are used without consent, raising compliance issues with the Nagoya Protocol (*Prathapan and Rajan*, 2020). Misidentification due to contaminated or incomplete data can lead to costly

recalls and reputational harm. Costs and infrastructure constraints remain a major hurdle in low- and middle-income countries. Although technologies have improved accessibility, expenses for reagents, limited lab capacity, and lack of trained staff are persistent challenges (*Yuan et al.,* 2025). Additionally, global databases are biased toward species from developed regions, limiting their relevance for foods originating from Africa, Asia, and Latin America. Greater investment in local databases, training, and technology transfer is essential for equitable global adoption (*Yuan et al*.,2025).

6. Future perspectives

DNA barcoding is evolving beyond species identification into a central tool for transparency, adaptability, and sustainability. Its convergence with blockchain, artificial intelligence (AI), and ecosystem monitoring opens new opportunities for traceability and risk management. Blockchain integration offers secure, tamper-proof traceability by linking DNA-verified species data to immutable digital records. Pilot projects in high-value sectors like seafood and organic meat have already connected QR codes on packaging to DNA-based authenticity logs (*Gröppel-Klein et al.,* 2023; *FAO*, 2022). This model could become standard for certified products like Halal or sustainably sourced goods. AI and machine learning are transforming how barcoding data is processed. These tools automate species classification, detect anomalies, and integrate genetic, geographic, and trade data to predict fraud and supply chain vulnerabilities (*Lokan et al.*, 2024). Barcoding also supports climate-resilient food systems by enabling rapid identification of shifting species and empowering small producers to validate products for competitive markets and it contributes to reduce food waste through improved sorting, labelling, and quality control (*Gorini et al.,* 2023). In this broader role, DNA barcoding is poised to become essential infrastructure for building ethical, transparent, and sustainable food systems worldwide.

Disclosure Statement: No potential conflict of interest was reported by the authors.

Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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