Review Article

Trends in Pharmaceutical Sciences and Technologies 2025: 11(3):211-224.

TIPS and Technol

A review and viewpoints on the application of DNA barcoding for the authentication of dairy products and the identification of contaminants

Sepideh Maghami[1†];Ph.D, Kiana Yousefipour[1†];Ph.D, Razieh Bagheri[1];Ph.D, Mohammad Javad Khoshnoud[1];Ph.D, Marzieh Rashedinia[1*];Ph.D

[1]Department of Pharmacology and Toxicology, School of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
[†]First Author

Abstract

Food adulteration, particularly in dairy products, poses significant economic and public health challenges worldwide. Milk and dairy products, valued for their nutritional content, are frequently targeted for fraudulent practices such as dilution, substitution, and contamination with foreign substances. Traditional methods for detecting adulteration, including physical, chemical, and biochemical analyses, often lack the specificity and sensitivity required for accurate authentication in complex or processed foods. DNA barcoding has emerged as a powerful molecular tool for species identification and the detection of adulterants and contaminants in dairy products. This review explores the principles and applications of DNA barcoding, highlighting its advantages over conventional approaches. DNA barcoding utilizes short, standardized genetic markers to uniquely identify plant and animal species, enabling the detection of both animal- and plant-based adulterants, as well as microbial contaminants and mycotoxins. The integration of advanced techniques such as real-time PCR and next-generation sequencing has further enhanced the sensitivity and reliability of DNA-based authentication. Case studies demonstrate the effectiveness of DNA barcoding in identifying species substitution, detecting the addition of vegetable oils, and tracing microbial contamination in milk and dairy products. Despite challenges such as DNA degradation in processed foods and the need for comprehensive reference databases, DNA barcoding offers a robust, rapid, and universally applicable solution for ensuring dairy product authenticity and safety. Continued advancements in molecular techniques and database development are expected to further strengthen the role of DNA barcoding in protecting consumers and supporting regulatory frameworks in the global food industry.

Keywords: DNA Barcoding, Food Authentication, Dairy Products, Food Contaminants.

Please cite this article as: Maghami S, Yousefipour K, Bagheri R, Khoshnoud MJ, Rashedinia M. A review and viewpoints on the application of DNA barcoding for the authentication of dairy products and the identification of contaminants. Trends in Pharmaceutical Sciences and Technologies. 2025;11(3):211-224. doi: 10.30476/tips.2025.107292.1301

1. Introduction

The globalization of food trade and rising demand for specific food varieties have resulted in increased in adulteration cases, particularly in the form of species substitution/ mixing with less expensive taxa. This phenomenon has had significant economic impacts and, in some cases, public health implications (1). In recent years, there has been increased attention to food quality and safety, as these factors greatly impact human health. Food adulteration is a concern for food quality and safety on a global scale, yet it is difficult

Corresponding Author: Marzieh Rashedinia, Department of Pharmacology and Toxicology, Faculty of Pharmacy, Shiraz University of Medical Sciences, Shiraz, Iran
E-mail: Rashedinia@sums.ac.ir

Received: 16/01/2025; Accepted: 01/03/2025

to detect (2). Oil, fish, honey, milk, dairy products, meat, grains, fruit juices, wine and alcoholic beverages, organic foods, spices, coffee, tea, and other highly processed meals are among the food ingredients that are frequently associated with food fraud (3).

Milk is a highly nutritious food because it contains proteins, carbohydrates, vitamins, minerals, and fats (4). However, it is often adulterated by the addition of synthetic chemicals and detergent powder, which can cause permanent harm (5). Milk fat is one of the most economically valuable edible fat commodities, and consequently, the dairy industry reports that adulteration of milk fat with cheaper foreign fats is a major concern (4). Milk adulteration is a common issue worldwide, leading to health hazards and significant economic losses, especially in developing countries with inadequate monitoring techniques. Therefore, studies on milk adulteration focus on identifying various foreign substances intentionally added to milk to increase volume or shelf life, thereby compromising nutrition, quality, and safety (6).

Milk is frequently diluted with water to increase its volume and subsequently reduce nutritional components such as protein and fat. Substances such as sugar (sucrose), starch, urea, detergents, formaldehyde, or even melamine may be added to mask this dilution. These adulterants could lead to a variety of health issues, from gastrointestinal diseases to renal failure and even death (7, 8).

Authenticity testing of milk and dairy products is required to protect consumers from fraudulent products, mislabeling, and health risks, as well as to avoid unfair competition in food industry. Milk-based products made from milk of a specific animal origin are considered healthier dairy products than others, leading to widespread consumer and food industry acceptance. As a result, these animal-derived products are extremely vulnerable to economic substitution with cheaper milk for profit. Furthermore, milk derived from specific animals (e.g., bovine) has been associated with allergenicity and other health issues, particularly among sensitive populations. Therefore, there is high demand for sensitive, accurate, and quantitative analytical methods for detecting milk adulteration (9).

Food authentication is becoming more common among food safety authorities, particularly in many developed countries, as a result of concerns raised by several high-profile cases of food mislabeling and substitution (10). Adulterated food is dangerous because: a) it may be toxic and harm one's health; b) it may deprive one of the nutrients needed to maintain good health; and c) it may cause intoxication or problems such as allergies in sensitive individuals (11).

Currently, adulteration in milk fat is a major concern. To assess the current situation in the commercial milk market, milk samples are analyzed for fatty acid (FA) and sterol profiles to detect potential adulteration using multivariate analysis (12).

DNA barcoding uses specific sequences of DNA to uniquely identify different species within food products and facilitates traceability and authentication of dairy product origins. This method has gained popularity in Europe and some parts of the world for protection of food authenticity and safeguarding consumers. It assists in identifying adulteration, like the replacement of expensive camel milk with inexpensive cow milk.

This is achieved by amplifying specific regions of mitochondrial DNA through sophisticated methods such as triplex real-time PCR, which can detect as little as 0.1% contamination in dairy and meat products (13, 14). Unlike traditional culture methods, which take days, DNA barcoding reduces analysis time to 1-2 days, assisting in detecting microbial contaminants in fat and oil products and dairy products (15).

With high sensitivity and speed, DNA barcoding can identify specific species in dairy products to detect microbial or other contaminants. Using DNA barcoding in conjunction with NGS, real-time PCR, and other complementary strategies provides thorough verification, including detection of contaminants; however, incomplete databases and poor sample preparation necessitate more comprehensive solutions for these challenges.

2. Adulterant Detection Methods

Currently, there are three basic strate-

gies for detecting adulteration: (1) detecting the presence of a foreign substance or marker in the commodity, (2) showing that a component deviates from its normal level, and (3) identifying a profile that is unlikely to occur naturally (11). Detecting adulteration is essential for ensuring the quality and purity of food, herbal medicines, pharmaceuticals, and other consumer products (16).

These methods utilize various methodological strategies, including physical, chemical, biochemical, and DNA-based molecular methods (17).

2.1. Physical methods

Physical adulteration analysis involves visual inspection or instrumental analysis to detect physical imperfections or contaminants in products. These methods are typically the first step in quality control, especially for botanical and herbal drugs. Some common techniques include:

- Microscopy (stereomicroscopy or compound microscopy): This technique identifies foreign plant materials, sand, or extraneous seeds.
- Spectroscopy (e.g., Near-Infrared Spectroscopy - NIR): Used to detect defects in physical composition or particle size.
- Colorimetry and image analysis: These methods examine the color profiles or morphology of powders or tablets.

Physical methods are particularly effective in distinguishing between similar-looking materials, such as natural saffron and dyed fibers. However, they have limitations, including the subjectivity of visual inspection and their inability to detect chemical or genetic adulterants (18).

2.2. Chemical and biochemical techniques

Chemical and biochemical methods are highly specific, allowing for the detection of particular compounds, impurities, or markers indicative of adulteration. Some examples of these techniques include:

- Chromatography (TLC, HPLC, GC): These methods are used to separate and identify chemical components. For instance, High-Performance Liquid Chromatography (HPLC) can detect synthetic drugs in herbal supplements.
- Mass Spectrometry (MS) and NMR Spectroscopy: These techniques analyze the chemical signatures of a product, enabling the detection of adulterants or synthetic ingredients.
- Enzyme-Linked Immunosorbent Assay (ELISA): This method detects specific proteins or allergens and is useful in identifying food adulteration.
- Chemical Fingerprinting: This technique provides a comprehensive profile of all the compounds in a sample and compares them with a standard reference.

These analytical methods are highly sensitive and are widely utilized in food authentication, pharmaceutical quality control, and herbal medicine analysis. However, one major drawback is that chemical profiles can naturally vary due to environmental conditions, which may result in false positives (19).

2.3. DNA-based Methods /Molecular Techniques

DNA-based techniques have revolutionized the detection of adulteration by enabling the identification of species-specific DNA, even in highly processed or powdered samples. The most common methods include:

- DNA Barcoding: This method uses standardized regions of DNA (such as COI, ITS2, rbcL, and matK) to identify different species. It is particularly effective in detecting mislabeling or substitution in herbal products and food items.
- PCR-based Techniques: Polymerase Chain Reaction (PCR) amplifies specific DNA regions to identify particular species or contaminants.
- Real-time PCR (qPCR) and Digital PCR: These quantitative techniques can iden-

tify even trace amounts of contamination.

• Next-Generation Sequencing (NGS): This method allows for the simultaneous identification of multiple species within a mixture, known as metabarcoding.

These methods are highly specific and can distinguish between closely related species. They are especially valuable for identifying herbal medicines, protecting wildlife, and detecting fraud in meat or dairy products. One limitation is that high-quality DNA is often required, which can be challenging to obtain from highly processed materials (20, 21).

3. DNA barcoding

DNA barcoding is a relatively new method for taxonomists and researchers in various fields. This tool enables the identification of plant and animal species and facilitates the use of genetic markers, such as polymerase chain reaction (PCR) for DNA amplification, alongside a relatively compact genetic makeup (22). It is regarded as a true identity card for different species, as they are highly polymorphic and uniformly distributed in species' genomes, although their functions remain largely unknown. DNA barcoding plays a crucial role in maintaining high product quality and consumer health (23).

The main principle underlying DNA barcoding is the amplification of homologous genes via PCR, followed by DNA sequencing, which identifies the DNA of various plant and animal species and verifies the authenticity of raw materials in both food and non-food products (24, 25). DNA barcoding is an innovative method of biological identification that utilizes relatively short genomic DNA fragments as species markers. Paul Hebert, a Canadian taxonomist, first proposed this technique in 2003. This method creates a universal barcode through DNA screening, followed by the establishment of a DNA barcode database and an identification platform, where the DNA data are analyzed and compared using bioinformatics to identify species (26).

Although DNA barcoding may take longer than some alternative techniques, it offers a universal approach to species identification, backed by a high level of genetic information (27). As a recent DNA-based identification method, DNA barcoding employs short stretches of standardized gene sequences from either the nuclear or organelle genome. It is a simple, quick, accurate, and cost-effective technique (28).

DNA barcoding is a well-established molecular tool for verifying the authenticity of food items. This sequencing-based method provides several significant benefits, including the ability to collect molecular data at relatively low analysis costs and the availability of extensive reference sequence libraries, such as those found in the BOLD database of the Barcode of Life (http://www.barcodeoflife.org). The success of DNA barcoding has gradually gained recognition from government authorities, who have proposed its official adoption for authentication purposes in certain food categories, such as fish-based products, by the US FDA. Moreover, new regulatory directives concerning food labeling, such as European Regulation N° 1169/2011 on food labeling and the provision of food information to consumers, will inevitably encourage national institutions to employ molecular DNA-based tools to tackle food authenticity and safety issues (1). DNA barcoding facilitates the identification of plants, animals, and fungi, leading to the employment of genetic markers and methods such as polymerase chain reaction (PCR) for DNA amplification, as well as the construction of relatively dense genetic maps that function as a true "identity card" for various species (29). Universal DNA barcodes hold immense potential as diagnostic markers for food authenticity and adulteration. Since they are conserved sequences, they can be amplified and analyzed across a wide range of taxa, eliminating the need to design specific assays for detecting each potential adulterant species. By utilizing barcodes derived from organelle

genomes, the issue of DNA degradation during food processing is minimized. Furthermore, interspecific polymorphisms within the barcode sequences allow for the determination of species composition in mixed samples (4).

3.1. The Role of Mitochondrial Genome Elements as Barcodes

In animals, the mitochondrial gene, specifically 500 base pair fragments of cytochrome oxidase subunit I (COI or COXI), is compared to 16S rRNA, another mitochondrial gene, or nuclear ribosomal DNA, making it a strong candidate for a DNA barcode marker (30). This suitability arises because recombination of mitochondrial DNA (mtDNA) is rare, thus preventing the usual shuffling of DNA sequences. Additionally, mtDNA is a haploid genome, which avoids sequencing complexities associated with heterozygous organisms (31).

3.2. DNA Barcodes for Raw Food Identification and Authentication

High-quality raw materials are necessary to produce dairy products with high nutritional value and good taste. The authenticity assessment of food often depends on the analysis of proteins and DNA sequences. Protein assay methods include immunological methods, and electrophoretic and chromatographic techniques such as HPLC and TLC. However, protein-based approaches are of limited value in the analysis of processed foods (32). In these cases, DNA-based methods are more effective and can also be used in various food materials. Furthermore, DNA provides more detailed information than proteins (33).

DNA barcoding is the latest technology as a universal tool for food traceability. The DNA barcode is a tool used to confirm the origin and quality of raw materials and detect adulteration (for example, by mixing vegetable fats instead of milk fat) that occurs in the industrial food chain (34).

DNA barcoding is a reliable and universally applicable method for identifying and authenticating raw foods. It relies on universal, short genetic tags—most commonly derived from mitochondrial or chloroplast DNA—to identify species with high accuracy. rbcL and matK are extensively used in plants, COI (cytochrome c oxidase I) in animals, and ITS (internal transcribed spacer) in fungi.

3.3. DNA Barcodes Uses in processed and raw foods

1. Traditional medicines and herbal products: DNA barcoding has been widely used to verify traditional herbal medicines and raw materials, ensuring correct species are used and excluding toxic or inactive substitutes (20).

2. Authentication of meat and seafood: Meat and seafood are particularly vulnerable to species substitution, a common method of adulteration. DNA barcoding ensures proper species identification, even in processed or cooked products. Wong and Hanner employed COI barcodes to verify mislabeling in sushi restaurants, revealing that more than 25% of the fish samples were mislabeled (35).

3. Dairy and animal products: DNA techniques are increasingly employed to authenticate the origin of dairy products and to detect adulteration with milk from other species. For example, real-time PCR and DNA barcoding can identify the presence of cow's milk in products labeled as goat or buffalo milk, ensuring accurate labeling and protecting consumers with allergies or dietary restrictions. These techniques are also effective in detecting fraudulent substitution in cheese, yogurt, and other processed dairy products (36-38).

4. Detection of plant-based adulterants: Spices are often adulterated with cheaper plant parts or morphologically similar species. DNA barcodes provide verification through genetic analysis. Research utilizing ITS-based DNA barcodes has reported substitution of turmeric or marigold with saffron (39, 40).

5. Processed foods: DNA barcoding remains effective even in highly processed foods, where traditional morphological or protein-based identification methods fail due to degradation. Short, robust DNA fragments can still be amplified and analyzed, allowing for the identification of species in cooked, canned, or otherwise processed foods (41, 42).

Raclariu *et al*. utilized metabarcoding to identify ingredients in blended supplements and herbal teas and detect undeclared adulterants and species (43).Table 1 summarizes the applications of DNA barcodes in raw and processed foods.

3.4. Advantages of DNA barcoding for food authentication

- High specificity and sensitivity: DNA barcoding can distinguish between closely related species, even in mixed or processed samples.
- Universal applicability: Standardized barcodes (e.g., COI for animals, rbcL/matK for plants) can be used across a wide range of taxa.
- Speed and cost-effectiveness: Modern PCR and sequencing technologies have reduced the time and cost required for analysis.
- Robustness: DNA is more stable than proteins or metabolites, making it suitable for processed foods.

3.5. Challenges and limitations

- DNA degradation: Highly processed foods may yield fragmented or low-quality DNA, complicating analysis.
- Database completeness: Accurate identification relies on comprehensive reference databases; gaps can limit effectiveness.
- Complex mixtures: Detecting minor components in complex food mixtures may require advanced techniques, such as next-generation sequencing (NGS).
- Legal and regulatory acceptance: While DNA barcoding is gaining official recognition, some regulatory frameworks may still require validation or standardization.

4. DNA barcode of dairy products

Milk and dairy products are essential components of human diets worldwide because of their high nutritional value. They provide important nutrients, including protein, calcium, and vitamins A, D, and B12, as well as various minerals. Generally defined as key food items produced from the milk of dairy animals, dairy products hold a special place in people's diets (61).

Table 1. Applications of DNA Barcoding in Raw and Processed Foods.

Food Category	Sample Product/Ingredient	Purpose of DNA Barcoding	Barcode Region / Method	Reference
Herbal Products	Echinacea, Ginseng, St. John's Wort	Authenticate plant species in supplements	rbcL, matK, ITS2	(20)
Seafood / Fish	Sushi, fillets, canned tuna	Detect species substitution/ mislabeling	COI (Cytochrome Oxidase I)	(35)
Meat Products	Minced meat, sausages	Detect undeclared or restricted meats	COI, 12S rRNA	(44-46)
Dairy Products	Cheese, milk powders	Identify species origin (e.g., cow, goat)	Species-specific primers (PCR, qPCR)	(47-49)
Spices & Condiments	Saffron, black pepper, turmeric	Detect dilution or substitution of spices	ITS, matK	(29, 50, 51)
Grains & Cereals	Rice, wheat, and millet	Verify authenticity and detect GMOs	rbcL, trnL, SSRs	(52-54)
Processed Foods	Protein bars, mixed herbal teas	Identify hidden or misrepresented ingredients	Metabarcoding (NGS), ITS, COI	(55-58)
Beverages	Herbal teas, juices	Confirm the botanical identity of source plants	matK, ITS2	(29, 59, 60)

However, milk and dairy products are often targeted for fraud, as they are valuable commodities in the food industry. This makes them susceptible to various types of adulteration, such as the addition of foreign substances to increase volume, alter composition, or enhance appearance. Such fraud can compromise the quality, safety, and nutritional value of dairy products, posing health risks to consumers. Considering the economic impact and potential allergy risks associated with these products, it is particularly important to develop techniques for assessing the authenticity and detecting adulteration in milk-derived foods. (62).

The use of molecular techniques to detect adulteration in dairy products has gained widespread acceptance, because DNA barcoding can identify the specific target sequence, and can detect and track the original raw materials. For example, this method can be used in complex matrices containing heterogeneous genomic DNA, such as milk. However, species-specific PCR is a reliable method for verifying the authenticity of dairy products (62, 63).

The applications of DNA barcodes in dairy products have been well documented, and the presence of plant DNA fragments from feed in raw milk and other dairy products has been reported in cases of dairy product adulteration. Molecular techniques such as DNA barcoding open new perspectives for the traceability of milk and dairy products (64, 65).

In general, accurate analysis is necessary to ensure the desired quality of dairy products. DNA barcoding techniques provide a reliable method for determining the composition and authenticity of raw milk.

4.1. Types of Adulteration and Contaminants in Dairy Products

4.1.1. Adulteration with Vegetable Oils

Vegetable oils, such as palm oil and soybean oil, are sometimes added to milk to increase fat content and reduce production costs. This practice is commonly found in the production of powdered milk and low-fat milk products. The addition of vegetable oils can alter the flavor, texture, and nutritional composition of the milk, and consuming them in large quantities may pose health risks (66).

DNA barcoding can be employed to detect the presence of plant species in milk. Specific primers designed for plant DNA (such as matK and rbcL) can identify the DNA of vegetable oils used for adulteration. Studies have shown that DNA barcoding can distinguish milk from different sources and identify the plant oils used, such as soybean or palm oil (29, 67).

4.1.2. Adulteration with Milk from Different Animal Species

Milk from various animal species, such as buffalo, goat, or sheep, may be mixed with cow's milk to reduce production costs. This can lead to problems, as it may trigger allergic reactions in sensitive individuals and result in misleading product labeling (37, 68).

DNA barcoding enables the identification of animal species in milk and dairy products. Specific genetic markers, like COI (Cytochrome C Oxidase I), are amplified to detect the species of origin. Research has demonstrated the effectiveness of barcoding in authenticating milk species and identifying adulterated products, such as goat milk mixed with cow's milk (41).

4.1.3. Microbial Contaminants and Mycotoxins

Milk can become microbiologically contaminated at various stages of production, storage, and transportation. Pathogenic microorganisms, such as Salmonella, E. coli, and Listeria, can contaminate milk, leading to foodborne illnesses. Additionally, mycotoxins—harmful compounds produced by fungi like Aspergillus and Penicillium—can contaminate dairy products, especially if feed is improperly stored (69). Consuming milk con-

Table 2. DNA Barcoding Applications in Dairy Adulteration and Contaminant Detection.

Type of Dairy Product	Adulteration / Contamination Studied	Method / Gene or Target Sequence	Advantage / % Detection	Problems & Limitations	Ref
Milk powder	Vegetable oil (palm, soybean)	PCR barcoding of rbcL, matK	Detects down to 0.5% (w/v) adulteration	The fat-rich matrix can inhibit PCR; plant DNA may be degraded	(67, 73)
Liquid cow's milk	Non-cow milk substitution (goat milk)	qPCR of mitochondrial cytochrome b	Quantifies ≥ 1% substitution (R^2 = 0.99)	Mitochondrial copy number variation requires precise calibration	(74, 75)
Bulk tank milk	Listeria monocytogenes (bacterial)	16S rRNA gene metabarcoding (NGS)	Detects low-level pathogens missed by culture	Does not provide absolute quantification; limited resolution below species level	(69, 76-78)
Dairy feed / Milk residue	Mycotoxin-producing fungi (Aspergillus, Penicillium)	ITS region metabarcoding (NGS)	Simultaneous detection of multiple toxigenic fungi	Complex bioinformatics cannot directly quantify toxin concentration	(70-72, 79, 80)
Dairy feed	Mycotoxigenic community profiling	PCR-DGGE fingerprinting of ITS	Differentiates closely related fungal species	Low sensitivity; fails to detect rare taxa; labor-intensive	(81-83)

taminated with mycotoxins can result in serious health issues, including liver damage, cancer, and immunosuppression (70).

DNA barcoding serves as a powerful tool for identifying the microbial species present in milk and dairy products. Specific primers can be used to detect fungal species responsible for mycotoxin contamination as well as bacterial pathogens. Studies have confirmed the application of barcoding in detecting Aspergillus spp. and Penicillium spp. in contaminated dairy products (Table 2) (71, 72).

5. Conclusion

DNA barcoding has revolutionized the authentication of dairy products and the detection of contaminants. Its ability to provide rapid, accurate, and reliable species identification makes it an invaluable tool for food safety, quality control, and consumer protection. As reference databases expand and molecular techniques advance, DNA barcoding will become even more integral to combating food fraud and ensuring the integrity of the global food supply.

However, challenges remain, including the need for comprehensive reference databases, improved methods for extracting DNA from highly processed foods, and the standardization of protocols across laboratories. Continued research and collaboration among scientists, regulatory agencies, and industry stakeholders are essential to fully realize the potential of DNA barcoding in food authentication.

Funding

The authors received not any financially support for this research or publication.

Conflict of Interest

The authors declare that they have no conflict of interest.

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